DGHM & VAAM 2024

7th Joint Microbiology & Infection Conference

2–5 June | Würzburg

Abstracts

www.dghm-vaam.de

© SimpLine/vector_v/Inna/Jefry Maviskho | stock.adobe

76th Annual Meeting of the DGHM

Annual Meeting of the VAAM

Content

Plenary Lecture 1
Invited Talk 3
Oral Presentation 11
- Archaea & Extremophiles 11
- Biotechnology & Synthetic Microbiology 12
- Diagnostic and Clinical Microbiology 19
- Epidemiology and Antimicrobial Resistance of Zoonotic Pathogens 27
- Environmental Microbiology & Processes 30
- Eukaryotic Pathogens 37
- Food Microbiology and -hygiene 40
- Gastrointestinal Infections 42
- Healthcare-Associated Infections and Pathogens: Prevention, Surveillance, Outbreaks and Antibiotic Stewardship 44
- Host-Associated Microbiomes and Microbe-Host Interactions 56
- Host-Pathogen Interactions and Clinics of Zoonotic Infections 59
- Infection Immunology 61
- Microbial Cell Biology 65
- Microbiology in the Digital Era 67
- Microbial Ecology & Evolution 70
- Molecular Infection Epidemiology and Prediction of Antimicrobial Resistance 76
- Microbial Metabolism & Biochemistry 80
- Microbial Pathogenicity 85
- Membranes and Transport 94
- National Reference Centers and Consiliary Laboratories 95
- Phages and Microbial Defense Systems 98
- RNA Biology 101
- Regulation & Small Proteins 103
- Secondary Metabolites and Natural Products 104
- Sensing, Signaling & Communicating Microbes 106
- Teaching Approaches & New Medical Approbation Regulations 107

Poster 107
- Archaea & Extremophiles 107
- Biotechnology & Synthetic Microbiology 111
- Diagnostic and Clinical Microbiology 130
- Epidemiology and Antimicrobial Resistance of Zoonotic Pathogens 147
- Environmental Microbiology & Processes 151
- Eukaryotic Pathogens 167
- Food Microbiology and -hygiene 172
- Gastrointestinal Infections 178
- Healthcare-Associated Infections and Pathogens: Prevention, Surveillance, Outbreaks and Antibiotic Stewardship 182
- Host-Associated Microbiomes and Microbe-Host Interactions 200
- Host-Pathogen Interactions and Clinics of Zoonotic Infections 216
- Infection Immunology 222
- Microbial Cell Biology 234
- Microbiology in the Digital Era 243
- Microbial Ecology & Evolution 247
- Molecular Infection Epidemiology and Prediction of Antimicrobial Resistance 255
- Microbial Metabolism & Biochemistry 259
- Microbial Pathogenicity 277
- Membranes and Transport 293
- National Reference Centers and Consiliary Laboratories 297
- Phages and Microbial Defense Systems 301
- RNA Biology 312
- Regulation & Small Proteins 318
- Single Cell Microbiology 323
- Secondary Metabolites and Natural Products 325
- Sensing, Signaling & Communicating Microbes 329
- Technical Hygiene, Environmental Sustainability Regarding the Climate Change 332
- Teaching Approaches & New Medical Approbation Regulations 333

Plenary Lecture

PL-001

How a plant pathogenic bacterium manipulates the plant

Prof. Ulla Bonas[1]
[1]*Dept. of Genetics, University of Halle, Halle/Saale, Germany*

Pathogenicity of most Gram-negative human and plant pathogenic bacteria depends on a type III secretion (T3S) system which injects effector proteins (T3Es) into the eukaryotic cell. We study the interaction between *Xanthomonas euvesicatoria* and its host plants pepper and tomato. The bacteria enter plant tissues via natural openings (stomata) or wounds and settle down in the intercellular spaces. This intimate contact induces expression of the bacterial T3S system, a nanomachine spanning both bacterial membranes, prolonged by a T3S pilus, a thin and hollow structure (in animal pathogens corresponding to the needle). The *Xanthomonas* T3S system injects more than 30 different T3Es into the plant cell, termed Avr (avirulence protein), if they were identified before the discovery of the T3S system, or Xop (Xanthomonas outer protein). The outcome of an infection depends on the genotype of both the plant and the bacterium. In susceptible plants, T3Es interfere with host cell processes to the benefit of the pathogen and allow its proliferation. In a resistant plant, a plant resistance gene mediates recognition of a "corresponding" T3E, often resulting in a hypersensitive response (HR), a rapid and localized programmed cell death restricting pathogen multiplication. T3Es from *Xanthomonas* exhibit different biochemical activities, e.g., as plant immunity suppressors, cell death inducers, ubiquitin ligase or transcription factor. Selected T3Es will be discussed.

PL-002

C-type lectins – key regulators of anti-fungal immunity

Prof. Gordon Brown[1]
[1]*MRC Centre for Medical Mycology, University of Exeter, Exeter, Great Britain*

The last few decades has seen a tremendous increase in our understanding of the mechanisms underlying the development of protective anti-microbial immunity. Key among these discoveries is the identification of pattern recognition receptors (or PRRs) expressed by immune cells, which recognise conserved microbial components. Recognition of these structures by PRRs, such as members of the C-type lectin receptor (CLR) family, triggers intracellular signalling cascades that initiate a variety of cellular and inflammatory responses, and induce the development of pathogen-specific adaptive immunity. We now understand that innate recognition by CLRs is essential for the development of protective antimicrobial immunity, but that they also play a key role in other aspects of immunity. In this presentation, I will present an up to date overview of the role of these receptors in immunity, with a particular focus on anti-fungal immunity, and highlight some aspects of ongoing work from my laboratory.

PL-003

Understanding the bacterial immune system

Prof. Rotem Sorek (Rehovot/IL)

The arms race between bacteria and phages led to the development of sophisticated anti-phage defense systems. A flurry of recent discoveries showed that the microbial pan-genome contains over 100 defense systems whose function was so far unexplored. The talk will present progress in understanding the mechanisms of action of new defense systems, will highlight cases in which bacterial defense from phage gave rise to key components in the eukaryotic innate immune system, and will demonstrate how phages evolved to overcome bacterial immunity.

PL-004

Fighting with phages: how epidemic *Vibrio cholerae* defends against viral attack

Prof. Kimberly Seed[1]
[1]*Dept. of Plant & Microbial Biology, University of California, Berkeley, USA*

The evolution of all forms of life is a history of the relentless conflict between hosts and parasites. Viral parasites of bacteria, known as phages, are key components of all ecosystems and profoundly influence the biology of their bacterial hosts. Phages select for bacteria that evade phage predation by deploying elaborate and mechanistically diverse defense systems, the full breadth of which is only beginning to be realized. Phages also drive the mobilization and dissemination of genetic material. Yet, despite the central role of phages in microbial evolution and ecology, molecular insight into the reciprocal dynamics of phage-bacterial adaptations in nature is lacking, particularly in clinical contexts. As a direct consequence, the discovery of phage-encoded defense inhibitors dramatically lags behind the known arsenal of bacterial defenses. The growing interest in using phages as targeted antibacterial agents to contend with rising antibiotic resistance further necessitates an understanding of phage-bacterial interactions in medically relevant contexts. In partnership with international collaborators, my lab has established a longitudinal collection focused on the diarrheal pathogen, *Vibrio cholerae*, and the lytic phages that prey on this pathogen as it causes disease in humans. Leveraging genomic and mechanistic approaches, we use this tractable platform to gain an in-depth understanding of how these interacting microbes coevolve within the context of human infection. We have discovered novel mechanisms of defense in epidemic *V. cholerae* mediated by parasitic mobile genetic elements and revealed phage-encoded mechanisms driving the diversification of such defense systems in epidemic *V. cholerae.*

PL-008

AI for Antibiotic Discovery

Prof. Cesar de la Fuente[1]
[1]*University of Pennsylvania, USA*

Computers can be programmed for superhuman pattern recognition of images and text; however, their application in biology and medicine is still in its infancy. In this talk, I will discuss our advances over the past half-decade, which are accelerating discoveries in the crucial and underinvested area of antibiotic discovery. We developed the first antibiotic designed by a computer with proven efficacy in preclinical animal models, demonstrating that machines and artificial intelligence (AI) could be used to design therapeutic molecules. Our algorithms have accelerated antibiotic discovery, and for the first time, we successfully mined the human proteome for antibiotics. Recently, we expanded our proteome-mining efforts to explore the proteomes of extinct species. Using AI, my lab discovered the first therapeutic molecules in extinct organisms, including Neanderthals and Denisovans, launching the field of molecular de-extinction. Collectively, our efforts have dramatically reduced the time needed to discover preclinical antibiotic candidates from

years to hours. I believe we are on the cusp of a new era in science where advances enabled by AI will help control antibiotic resistance, infectious disease outbreaks, and pandemics.

PL-012
Permafrost Microbes and Climate Change

Dr. Neslihan Tas[1]
[1]*Lawrence Berkeley National Laboratory*

This talk delves into the intricate life of microorganisms inhabiting permafrost environments, and the broader implications of climate change on these unique ecosystems. Permafrost, perennially frozen ground mainly found in polar regions, is a significant carbon reservoir, containing twice as much carbon as is currently in the atmosphere. Global warming is leading to the accelerated thawing of permafrost, posing a risk of releasing vast amounts of stored carbon into the atmosphere, which could further exacerbate global warming in a positive feedback loop. This talk will explore the current state of knowledge regarding Arctic soil and permafrost microbes, their metabolic processes, and the potential climate impacts of their activities as permafrost continues to thaw. Furthermore, the talk will highlight innovative research methodologies employed to study arctic microbial communities, offering insights into microbial diversity and functionality in response to changing environmental conditions.

PL-018
Microbial Theranostics – A Resistance Combatting Strategy

Prof. Robin Patel (Rochester, Minnesota/US)

Antimicrobial resistance is a major threat to human health globally and has changed the way medicine is practiced. Infections previously treated with oral antibiotics now require intravenous treatment, and, because whether an antimicrobial resistant bacterium may be causing infection is often unknown when therapy is initiated, unnecessarily broad-spectrum antibiotics are frequently prescribed, or alternatively, the prescribed regimen may not even treat the infection because of unrecognized underlying resistance. In addition, antibiotics are given to those who do not need them because they do not have a bacterial infection. Antibacterial resistance involves hundreds of microbial species, dozens of antibiotics, and many clinical syndromes. While development of new therapeutics and antimicrobial stewardship are needed, better diagnostics and appropriate use thereof need to be better incorporated.
The classic three-step diagnostic paradigm used in medicine, taught to students, and applied by medical professionals, involves asking whether a clinical presentation could be due to infection (step 1 – based on history, physical examination, initial tests), what the causative microorganism(s) might be (step 2 – based on culture, serologic testing, molecular testing for microorganisms), and finally, which treatment should be administered (step 3 – based on culture-based antimicrobial susceptibility testing). This approach, although intellectually interesting, is at once contributing to the antimicrobial resistance crisis and failing because of it. Creative use of technology can help; fortunately, we are in a technology revolution in terms of microbial diagnostics. There have been major advances in the application of proteomics, nucleic acid amplification tests and sequencing-based diagnostics, microbial imaging, microbial metabolomics, and advanced host response assessment for infectious diseases in recent years, and point-of-care diagnostics are transforming where testing can be done. Modern diagnostic tests can curb emergence of antimicrobial resistance by informing improved antibiotic use (a patient and societal benefit), leading to avoidance of unneeded testing and treatment (a patient benefit), decreasing transmission of infectious diseases (a societal benefit) and informing new discoveries and better delivery of healthcare (which will have future benefits).
This presentation will overview how, with improved diagnostic testing, medicine can undo the classic and slow diagnostic paradigm, using diagnostics that detect microorganisms and directly call out ideal therapy in a single step, so-called, microbial “theranostics”.

PL-019
Evolutionary history and adaptive landscape of the M. tuberculosis Beijing lineage

Prof. Thierry Wirth[1]
[1]*EPHE, PSL University, Paris, France; 2Institut de Systématique, Evolution, Biodiversité, ISYEB, Muséum national d’Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France.*

M. tuberculosis lineage 2 is one of the most widely dispersed lineages in the world, and is particularly associated with the spread of multi-resistant strains in Eurasia. However, the reasons for the success of Lineage 2 are only partially understood. My presentation will develop and underline the importance of an integrative approach to answer this question. Firstly, using Bayesian phylogenomics and demogenetics analyses, based on hundreds or thousands of complete genomes, I will illustrate the strength, spread and timing of major L2 epidemics, which highlight and confirm the dominance of this lineage both in terms of antibiotic resistance and epidemiological success. In addition, we will also see how major socio-economic events reinforce ongoing epidemics. In the second part, which focuses on the underlying mechanisms, I will summarize how predictive simulations, epidemicity indices, genome-wide association studies and, finally, laboratory fluctuation assays have enabled us to make progress on this subject.
For example, we were able to show that the collapse of the Soviet Union and, later, the Russian economic crisis were the main drivers of epidemics rebounds. L2 strains showed a significant ($P < 0.01$) pattern of accelerating mutation rate along the tree branches, and this on a scale of only 30 years. Furthermore, fluctuation assays showed that modern Central Asian lineage 2 strains acquire rifampicin resistance about twice as fast as ancestral L2 or Lineage 4 strains. The same applies to bedaquiline, for which basal Beijing strains outperform L4 strains. The fact that L2-derived strains have significantly higher mutation rates than other lineages is likely to enhance their adaptive landscape, as well as their propensity to acquire resistance/compensatory mutations.

PL-020
Exploring the diversity of antifungal drug tolerance

Judith Berman[1]
[1]*Tel Aviv University*

Antifungal drug tolerance and resistance are distinct responses to drugs that inhibit fungal growth. Intrinsic tolerance is a relatively stable property of each isolate characterized by a subpopulation of cells that grow in drug concentrations that inhibit the growth of the overall population. Strains with higher tolerance have a larger subpopulation of cells that can grow in the drug and, on average, those tolerant cells overcome cell cycle arrest

earlier than cells with lower tolerance levels. Intrinsic tolerance levels, which differ between *C. albicans* isolates, are influenced by growth conditions and stresses including temperature, pH, and osmotic stress, with different isolates affected by these conditions and stresses to different degrees. These intrinsic differences between tolerance in different strains are likely a function of the broad diversity of genetic backgrounds, and the many genes involved in the tolerance response. We are testing the hypothesis that antifungal drug tolerance requires a broad range of interdependent physiological processes that operate to different degrees in different isolates across the diversity of the *C. albicans* species.

Acquired tolerance emerges in newly drug-tolerant cells, usually as a consequence of drug selection. Heterotolerance is a transiently stable type of acquired tolerance usually conferred by the transient genome changes such as aneuploidy or copy number variations. It is thought that the increased level of genes that promote het extra copies of genes that affect drug responses. Relative drug concentration has a dramatic effect on the evolution of survival strategies: Hetero-tolerant colonies arise frequently (~1/1000 cells within a single liquid passage) in cultures exposed to supra-MIC drug concentrations, whereas heteroresistance (transiently acquired resistance) appears only after multiple passages at sub-MIC drug concentrations. Isolates with heterotolerance often carry one of several recurrent aneuploid chromosomes, alone or in combination with other chromosomes. We are leveraging a large collection (> 1500 isolates) of *C. albicans* to better understand how genetic, physiological and environmental factors affect the evolutionary trajectories and dynamics with which antifungal drug tolerance and resistance emerge and persist.

PL-022
Siderophores as drivers of bacterial communication, cooperation, and competition
Prof. Rolf Kümmerli[1]
[1]Department of Quantitative Biomedicine, University of Zürich

Bacterial siderophores are a chemically diverse group of secondary metabolites to scavenge iron from natural sources. Here, I show how secreted siderophores can induce various types of social interactions among bacteria. They can serve as signalling molecules to coordinate bacterial activities in clonal groups. As iron carriers, they can be cooperatively shared among cells that possess matching receptors for ferri-siderophore uptake. Finally, siderophores can be deployed as competitive agents to withhold iron from competitors with non-matching receptors. Using data from the single-cell to the microbiome level, I explain how these interactions guide evolutionary and ecological dynamics in bacterial communities and how siderophores can be harnessed for human interventions against pathogens.

PL-023
Cross-kingdom RNA trafficking between plants and fungal pathogens
Prof. Hailing Jin[1]
1Department of Microbiology and Plant Pathology, Center for Plant Cell Biology, Institute for Integrative Genome Biology, University of California, Riverside, CA 92521, USA

Small RNAs (sRNAs) are short non-coding RNAs that mediate gene silencing in a sequence-specific manner. My lab discovered that some sRNAs from eukaryotic pathogens, such as Botrytis cinerea, can be transported into host plant cells and suppress host immunity genes for successful infection (Weiberg et al., Science 2013). We further demonstrated that such cross-kingdom RNAi is bi-directional. Plants can also send sRNAs into pathogens using extracellular vesicles to silence fungal virulence genes as part of its immune responses (Cai et al., Science 2018). We found that plants have multiple classes of extracellular vesicles, and exosome is the major class responsible for sRNA delivery. We identified a group of RNA binding proteins that contribute to the selective sRNA loading into extracellular vesicles (He et al., Nature Plants, 2021). Recently, we show that fungal pathogen B. cinerea also utilizes extracellular vesicles to transport sRNAs, which can enter plant cells through clathrin-mediated endocytosis pathway (He et al., Nature Communications, 2023). Furthermore, we found that some plant mRNAs can also be secreted by extracellular vesicles and enter fungal cells to be translated for fungal inhibition (Wang et al., Cell Host & Microbe, 2024). Cross-kingdom RNA trafficking is broadly present between many interacting organisms.

Invited Talk

IT-004
Wastewater Based Epidemiology – Potentials for pandemic management
Susanne Lackner, Shelesh Agrawal, Kira Zachmann, Carrie Moore
Technical University of Darmstadt, Institute IWAR, Chair of Water and Environmental Biotechnology,

Wastewater-based epidemiology (WBE) can serve and support the public health sector by providing additional valuable indicators of infection incidence in communities. Though it was first used over 80 years ago to monitor Polio outbreaks, WBE experienced a resurgence after the onset of the SARS-CoV 2 pandemic, with global research efforts and collaborations improving sampling infrastructure and developing new analysis methods. Wastewater monitoring of SARS-CoV-2 has been shown to provide early warnings of increased incidence in communities and uncover variant occurrence profiles across time and geographic space. We have been able to expand the horizon of wastewater surveillance by leveraging the now well-established WBE infrastructures to monitor trends in the spread of other pathogens, including monkeypox and influenza. Due to the modular nature of wastewater infrastructure, surveying the emergence of many diseases and mutations is possible at multiple community levels (buildings, neighbourhoods, cities, airports, and individual flights), allowing for tailored public health responses. Next-generation sequencing of wastewater allows for whole or near whole genome resolution of the genetic assemblies of circulating bacterial and viral pathogens and antimicrobial resistance genes, providing a holistic understanding of pathogen dynamics within a community. Since the beginning of the COVID-19 pandemic, working with health authorities, we have been monitoring SARS-CoV-2 in wastewater at the National and European level in our lab, which has allowed us to observe the emergence of SARS-CoV-2 variants in wastewater shed region before reported in clinical data. We have also been successfully monitoring SARS-CoV-2 variants on incoming flights to the Frankfurt International Airport, as well as profiling variants in the surrounding urban communities. We are also looking at the impact of COVID-19 on circulating antimicrobial resistance, as it will provide information about whether the change in people's behaviour toward

disinfectants and hygiene practices impacted antimicrobial resistance. We are expanding our methods to profile other diseases in wastewater to further utilize the potential of WBE as an early monitoring system as a part of the One-Health concept.

IT-005

Advancing Visual Quality Controls in Medical Device Reprocessing: Exploring Innovative Technological Solutions

Dr. Marcus Grohmann

This presentation explores the domain of quality controls in the reprocessing of medical devices, shedding light on current practices, challenges, and avenues for improvement through innovative technological solutions. Medical device reprocessing is integral to ensuring patient safety, reducing healthcare costs, and promoting environmental sustainability. However, it also poses unique challenges related to efficacy, consistency, and regulatory compliance.

Current quality control practices, including visual inspection and manual cleaning verification, are vital but inherently limited by subjectivity and resource-intensive processes. This highlights the pressing need for innovative technological solutions to augment existing methods and address emerging challenges.

We explore the design of novel visual inspection systems integrating advanced image recognition technology to automatically identify medical devices, streamlining the inspection workflow, and reducing the risk of human error. Additionally, the design should provide visual assistance to personnel during the quality control process, guiding them through comprehensive checks of sterilizing trays to ensure proper arrangement and integrity of instruments. Practical considerations and challenges associated with the adoption of visual technologies, including integration with existing workflows should also be addressed.

By leveraging automation, advanced imaging technologies, and data analytics, healthcare facilities can enhance patient safety, streamline operations, and elevate the standard of care in medical device reprocessing. This emphasis on innovation fosters continuous improvement and sets the stage for future advancements in healthcare quality and safety.

IT-006

Studying the Impacts of Microplastics on Microbes in Agricultural Soil

Aileen Jakobs[1]*, Ryan Bartnick[2], Eva Lehndorff[2] and Tillmann Lueders[1]
[1]Chair of Ecological Microbiology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Germany
[2]Chair of Soil Ecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Germany

Terrestrial ecosystems are exposed to extensive pollution with microplastics (MP), with agricultural practices and unintentional dispersal especially impacting arable soils. A profound understanding of the interactions of MP with physical, chemical, and microbial factors in agricultural soils is critical to assess impacts on crop production. Our research, as part of the Collaborative Research Center 1357 “Microplastic” at the University of Bayreuth, aims to elaborate a comprehensive insight into the response of prokaryotic and fungal soil microbiota to MP pollution. We employ environmental samples, bench-scale microcosms, and controlled greenhouse experiments to determine MP-induced alterations in the overall taxonomic and functional diversity of soil- and plant-associated microbiota, as well as changes in soil biogeochemical parameters and processes. In soil microcosms, we show that soil microbes tend to be more clearly responsive to biodegradable vs. conventional plastics, but that effects vary with soil type. Next, maize (*Zea mays*) and strawberries (*Fragaria × ananassa*) were cultivated in soils amended with different types of conventional (LDPE, PET, PS) or biodegradable (PBAT) MP. The results reveal a distinct influence of plant hosts on prokaryotic soil microbiomes, largely overruling direct impacts of MP in both plant systems. However, variations in alpha diversity were evident, depending on the plant species, root proximity, and also on some, but not all plastic types. Interestingly, the degree of mycorrhization of maize plants was markedly reduced only for one polymer type. Our ongoing work aims to unravel the metabolic potential and functional context of microbial taxa most responsive to plastic amendment. We also discuss a polymer-specific approach towards extracting and characterizing environmental plastisphere microbiota, based on isopycnic density separation. Overall, our research within the CRC “Microplastic” advances the curent understanding of the intricate interplay between soil, soil microbes and microplastics as soil pollutant. This is an important contribution to the development of sustainable management strategies for soils and agroecosystems.

IT-007

The eco-corona on microplastic particles enhances their interactions with cells.

Anja Ramsperger[1,2], Holger Kress[2], Christian Laforsch[1]
[1] Chair of Animal Ecology I, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Germany
[2] Chair of Biological Physics, University of Bayreuth, Germany

Microplastic particles ubiquitously found in the environment are taken up by many organisms. Subsequently, microplastic particles can translocate from the primarily exposed organs, e.g., the gastrointestinal tract or the respiratory organs, into the tissues, likely through cellular internalization. The reasons for cellular internalization are still largely unknown since this has only been shown for specifically surface-functionalized particles. We show that environmentally exposed microplastic particles are internalized into macrophages significantly more often than pristine microplastic particles. We identified biomolecules and microbial biofilms forming an eco-corona on the surface of microplastic particles, suggesting that environmental exposure promotes the cellular internalization of microplastics. Subsequently, we thoroughly analyzed the eco-corona originating from freshwater and saltwater to understand its role in particle-cell interactions better. We found the zeta potential to be increased due to the coating with an eco-corona, alongside with structural biofilm distinctions, which can be one driving factor for enhanced particle-cell interaction. Our results help better understand the particle-cell interaction mechanisms of environmentally exposed microplastic particles. Cellular internalization is a key route by which microplastic particles translocate into tissues, which may cause toxicological effects that affect environmental and human health.

IT-010
Perspektiven der ärztlichen Ausbildung

*M. Frosch[1]
[1]*University Hospital Würzburg, Dekanat, Würzburg, Germany*

Ebenso wie die Medizin, muss sich auch das Medizinstudium kontinuierlich weiterentwickeln. Eine frühere Verzahnung der Fächer, eine stärkere Kompetenzorientierung, ein noch größerer Praxisbezug, eine frühe Verschränkung von Theorie und Praxis, die Stärkung der Allgemeinmedizin und die Stärkung der Wissenschaftlichkeit ist daher Teil der geplanten Reform der Ärztlichen Approbationsordnung, die die Qualität der Lehre in der Medizin verbessern und die Ausbildung der Medizinstudierenden besser an die Herausforderungen eines zukünftigen Gesundheitswesens anpassen soll.

IT-011
What can we learn from the Hamburg model curriculum for future medical teaching? An analysis and discussion.

*R. Machnik[1]
[1]*Mercuri Urval, Aptitude Testing, Hamburg, Germany*

Against the background of a possible change in the regulations towards the mandatory establishment of so-called medical model curriculums (Modellstudiengängen) in Germany, it is of particular importance to scientifically analyse the existing experiences for advantages and areas of development of such curricular concepts. I will therefore use the example of the already well established Hamburg model curriculum to present important findings and experiences with this form of curriculum there and then discuss implications for a possible future national design of modern medical teaching with the audience. The aim is, on the one hand, a fact-based neutral presentation and, on the other, a constructive and objective dialog.

IT-012
Studierendenauswahl in der Medizin

*W. Hampe[1]
[1]*University Hospital Hamburg-Eppendorf, Hamburg, Germany*

Da die Zahl der Bewerbenden die der Medizinstudienplätze bei weitem übersteigt, muss eine Auswahl erfolgen. Nach dem NC-Urteil des Bundesverfassungsgerichtes von 2017 wurden die Gesetze zur Studierendenauswahl geändert. Seit 2020 müssen die Fakultäten neben der Abiturnote auch die Ergebnisse eines Auswahltests sowie ein weiteres Kriterium zur Auswahl einsetzen. Welche Zusammenstellung von Auswahlkriterien findet die besten zukünftigen Medizinstudierenden? Vor- und Nachteile von kognitiven (z.B. Abiturnote oder Auswahltests wie TMS oder HAM-Nat zur Messung der fluiden und kristallinen Intelligenz) und sogenannten nicht-kognitiven Auswahlkriterien (z.B. Multiple Mini-Interviews oder Situational judgement Tests zur Messung sozialer Kompetenz) werden diskutiert.

IT-013
The undetectable enemy

*A. M. Aldejohann[1,2], A. Uribe Munoz[1], R. Martin[1,2], G. Walther[2], O. Kurzai[1,2]
[1]*Institute for hygiene and microbiology, Universität Würzburg, Würzburg, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology, National Reference Center for Invasive Fungal Infections, Jena, Germany*

Introduction

Mucormycosis is a rare as well as deadly invasive fungal infection. Uncontrolled diabetes, haematological malignancies and immunosuppression are important risk factors. High dose Amphotericin B and radical surgical resection are so far the only treatment options. We present a severe and fatal case of rhino-orbito-cerebral mucormycosis in a 11-year-old child, addressing important questions from the likelihood of clinical suspicion to the challenge of timely diagnosis. In addition, we elucidate the value of diagnostic approaches.

Case presentation

The girl suffered from an early isolated cerebral recurrence of a pB-ALL. Four days prior to admission the last block of chemotherapeutic agents accompanied by intrathecally prednisolone were administered. On day of admission, she presented with aggravating headache, painful abducens nerve palsy and anisocoria. Firstly, no significant radiological or ophthalmological correlate was found explaining the symptoms, so that prednisolone was started due to suspected vasculitis following ICU admission. After further clinical deterioration a second MRI scan revealed a prolonged occlusion of the left carotid artery, which was successfully stented in a neurosurgical intervention. However, during the next day the child presented with signs of central diabetes insipidus and pathological reflexes indicating severe cerebral dysfunction, which was confirmed by an emergency CT scan showing complete infarction of the left hemisphere with a progredient perfusion deficit and beginning tissue swelling. Due to the unfavourable progression best supportive care was initiated and the patient deceased on day two after admission. Pathological workup identified mycotic clods in all major cerebral arteries and retained serum blood material led to the final identification of *Lichtheimia corymbifera* by mucorales-specific PCR.

Conclusion

This case illustrates the rapidity and severity of *Mucorales* infection. It shows the importance of early clinical suspicion and the need of an aggressive laboratory testing algorithms. The stratification of risk factors and definition of red flags may be a future task fighting these infections.

IT-016
Microbial interactions at different levels of organization: A holistic approach from cultures to environmental omics

Sarahi Garcia[1,2]
[1]*Carl von Ossietzky Universität Oldenburg, Institute for Chemistry and Biology of the Marine Environment (ICBM), 26129 Oldenburg, Germany*
[2]*Stockholm University, Department of Ecology, Environment, and Plant Sciences, Science for Life Laboratory, 106 91 Stockholm, Sweden*

Natural microbial communities have a mixture of both complementary and (seemingly) redundant metabolic capacities that drive biogeochemical cycles. Moreover, much of the complementarity involves metabolically costly

compounds that promote not just their own survival and reproduction but can also support neighboring cells. Complementary metabolic interactions develop over an evolutionary time scale as microorganisms utilize metabolites released by other organisms in the same environment. We studied natural microbial communities using both a reductionist approach (dilution-mixed-cultures, a.k.a. model communities) and a holistic (metagenomics) approach. Model communities simplify the community while keeping some genotypic and nutritional diversity and consist of 2 or more microbial strains that would naturally co-occur. We inoculated thousands of model communities from environmental samples, resulting in different success rates depending on the media and the number of cells co-inoculated. Many of these cultures allowed for growing and studying previously uncultivated bacteria. Combined with sequencing techniques, we found complementary metabolic capacities in the genomes of the model community members as well as growth of consortium capable of specific functions, such as methane degradation. Moreover, the different levels of organization of microbial communities start to emerge, showing the intricate network of anabolic and catabolic interactions that play out in the natural environment.

IT-017

Genetic regulation of stress response in the model archaeon *Sulfolobus acidocaldarius*

Eveline Peeters[1]
[1]Research Group of Microbiology, Department of Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium

In this talk, current insights will be presented into the genetic regulation of stress response in *Sulfolobus acidocaldarius*, a thermoacidophilic model crenarchaeon thriving in volcanic hot springs. The impact of heat shock on the transcriptomic and proteomic landscape is highlighted, revealing prominent roles of both transcriptional and post-transcriptional/post-translational regulation. These findings underscore the prevalence of alternative mechanisms beyond classical transcription factor regulation in Crenarchaeota. Notably, the presence of RNA thermometer-like elements is proposed as one such mechanism, as exemplified by their identification in a gene encoding a thermosome subunit. Furthermore, the significance of chromatin restructuring, particularly mediated by proteins from the Lrs14 family, is emphasized. These abundant small non-specific DNA-binding proteins are hypothesized to be involved in various stress responses beyond heat shock, as well as exerting a control over pleiotropic physiological switches, such as the transition between planktonic and biofilm growth.

IT-018

Infection mechanism of tailed haloarchaeal viruses

Tessa Quax

Viruses by far outnumber cells and there is an ongoing arms race for survival. Archaea are infected by unusual viruses that are structurally very diverse and include those with unique capsid shapes such as a bottle, and viruses with cosmopolitan morphologies, such as head-tailed viruses. As the archaeal cell envelope is fundamentally different from that of bacteria and archaea, archaeal viruses face different challenges when traversing the cell envelope on their way in and out of the cell. We use haloarchaea and their viruses to study viral infection mechanisms. In my talk I will focus on the tailed haloarchaeal virus HFTV1 and present data on the molecular mechanisms underlying infection.

IT-027

What is a species and a strain? The data has shown us the answers!

Konstantinos T. Konstantinidis[1]
[1]Georgia Institute of Technology, Atlanta, GA, USA

Large scale surveys of natural microbial communities (metagenomics) or isolate genomes have revealed species clusters around 95% Average Nucleotide Identity (ANI) of shared genes. That is, members of the same species tend to show >95% ANI among themselves and <85% to members of other species with a clear scarcity (gap) of genome pairs showing between 85-95% ANI. We have recently reported a similar ANI gap within species, around 99.5% ANI, revealing that discrete, intraspecies units may also exist. We suggested referring to these units as genomovars (Rodriguez-R, mBio 2024), and to employ a higher ANI value (99.9%) and level of shared genes (>99% of total genes) to define strains (Viver, Nat. Comms. 2024). To further understand and model these patterns of diversity, however, the underlying genetic and/or ecological mechanisms that maintain discrete units at the species and intraspecies levels need to be elucidated. By analyzing closely related isolate genomes from the same or related samples we show that high ecological cohesiveness among the genomes, coupled to functionally and spatially (across the genome) unrestricted homologous recombination, likely underly these ANI units. Therefore, our results represent a departure compared to previous models of microbial speciation that attributed speciation to either recombination or ecological cohesiveness but not their combined effect.

IT-036

LUX-in-Space - the long and stony way to Low Earth Orbit

Petra Rettberg[1], Johanna Piepjohn[1], Corinna Panitz[2], Kristina Beblo-Vranesevic[1], Elke Rabbow[1]
[1] DLR, Institute of Aerospace Medicine, Astrobiology, Köln, Germany
[2] RWTH Aachen, Institute for Pharmacology and Toxicology, Aachen, Germany

Experiments conducted in space are a rarity, making the opportunity to perform one on the International Space Station (ISS) exceptionally thrilling. However, apart from having a significant scientific query to address and a hypothesis that can only be tested in space, there are other essential elements required for success - dedication, patience, and resilience. The experiment known as LUX-In-Space serves as an exemplary case study, highlighting the various stages involved in its preparation, the timeline, and its current progress.

In space, all organisms are exposed to and affected by space radiation and microgravity. Radiation and microgravity were identified as two of the five most important hazards for manned spaceflight. Therefore, the knowledge of biological space radiation effects as well as the impact of microgravity on enzymatic repair processes is mandatory for risk assessment, especially in view of long duration missions to Mars or permanently inhabited bases on the Moon.

The repair kinetics of radiation induced DNA damages will be investigated with the SOS-Lux Test. Bacteria serve as model organisms. They possess the same type of nucleotide excision repair as all other living organisms including humans. *Salmonella enterica subsp. enterica* cells are transformed with the pBR322-derived plasmid pPLS-1, carrying the promoterless lux operon of *Photobacterium leiognathi* as the reporter element controlled by a DNA

damage-dependent SOS promoter as sensor element. In response to exposure to radiation the SOS promoter is activated. Due to the genetic modification, the connected so-called lux genes are expressed, resulting in the emission of measurable bioluminescence proportional to the applied dose of radiation. The DNA repair kinetics are followed by bioluminescence and optical density measurements.
LUX-in-Space is the first space experiment where the whole series of events from DNA damage induction in metabolically active cells to the different steps of enzymatic repair will take place in real microgravity and the repair kinetics will be monitored *in situ* by optical measurements. The effects of microgravity will be clearly separated from other spaceflight factors by comparison with parallel samples on an onboard 1g centrifuge in the Biolab facility on the ISS and in a parallel ground control experiment with identical samples in flight-identical hardware.

IT-037

Planetary protection and ESA's bacterial strain collection

R. Pukall[1], C. Moissl-Eichinger[2], P. Rettberg[3]
[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany*
[2]*Diagnostic & Research Institute of Hygiene, Microbiology and Environmental Medicine, Medical University Graz, Graz*
[3]*German Aerospace Center (DLR), Institute for Aerospace Medicine, Radiation Biology, Cologne, Germany*

Introduction
Microbial examination of flight hardware and cleanrooms is a major focus of planetary protection activities to control bioburden and to analyze the complexity of the cleanroom microbiome.
Enriched isolates from samples collected during various missions, including ExoMars and JUICE, have been identified and deposited at the DSMZ to establish a special strain collection of bacterial species that can survive and adapt under extreme conditions. Cultures originally obtained from the International Space Station (ISS) or isolated within the Mars Analogues for Space Exploration project (MASE) complement the collection with additional strains.
The collection was founded by the European Space Agency (ESA) and is publicly accessible via the online strain catalog at the DSMZ and is also integrated into the BacDive database.
Objective
ESA has implemented the public culture collection to facilitate the distribution of bacterial isolates to the scientific community. This allows researchers to study specific properties of these microbes to gain insights into the survival and adaptation mechanisms in extreme environments.
Current status
Spacecraft are built in particle-controlled clean rooms. Particles can affect the function of spacecraft instruments, and for missions under planetary protection restrictions, biological contamination must be limited as much as possible. A clean room is an extreme environment with constant control of humidity and temperature, air filtering, and surface disinfection. Bioburden and diversity studies have been performed for several missions (Smart-1, Rosetta, Herschel, ExoMars and Juice) in various European clean rooms to validate biological contamination control and reduction procedures.
Swab and wipe samples were collected from clean room facilities and directly from spacecraft surfaces by our collaborators from DLR in Cologne and the University of Graz. Most isolates enriched from these samples have been identified and freeze dried at DSMZ for long-term storage. The collection currently contains about 900 isolates, with the predominant species belonging to the endospore forming genera *Bacillus*, *Paenibacillus*, *Peribacillus* and *Priestia*. Other representative species are from the genera *Acinetobacter*, *Stenotrophomonas*, *Micrococcus*, *Kocuria* and *Staphylococccus*. Selected isolates are now being genome sequenced to gain further insights and to refine its phylogenetic assignment.

IT-038

Unraveling Martian Microbial Survival Strategies: Insights from Salts, Stress Responses, and Habitability

Jacob Heinz[1], and the BRINES Team[2]
[1]*Research Group Astrobiology, Center for Astronomy and Astrophysics, Technische Universität Berlin, Germany*
[2]*Including all students (Camilo Mayr-Wolf, Florian Carlo Fischer, Elisa Soler, Anne Gries, Shivani Nundoo, Lea Kloss, Weronika Julia Lukas, Vita Rambags, Ksenia Malahov) involved in the DFG project "BRINES" (#455070607), and the research group leader Dirk Schulze-Makuch*

Introduction: The search for life on Mars involves understanding the habitability of its surface and near subsurface, particularly in relation to the presence of salts and their solutions, such as perchlorates (ClO_4^-), which were detected on the Red Planet but are rare on Earth. Studies of our research group aim to investigate the microbiological responses to Martian-relevant salts, focusing on their tolerances and stress reactions.

Goals: The primary objectives included determining the salt tolerances of various halophilic and halotolerant microorganisms, assessing their survivability under Mars-like conditions, and analyzing their stress responses.

Materials & Methods: Microorganisms, including model species and environmental isolates, were subjected to varying concentrations of sodium perchlorate ($NaClO_4$) and other salts to assess their tolerances. Mars-like environmental conditions were simulated in a chamber, and survival rates in Martian regolith mixed with different salts were measured. Salt stress responses were analyzed using microscopic, proteomic, and metabolomic techniques.

Results: Most halotolerant organisms exhibited higher $NaClO_4$ tolerances than obligatory halophiles, suggesting their adaptability to habitat-untypical salts. The halotolerant yeast *Debaryomyces hansenii* demonstrated the highest tolerance, leading to further investigations. Chlorates (ClO_3^-) were tolerated in significantly higher concentrations than perchlorates, suggesting an increased habitability potential for chlorate-rich areas on Mars. Proteomic analyses revealed perchlorate-specific stress responses, particularly in *D. hansenii*, indicating chaotropic stress as a significant factor.

Summary: Experiments in Mars-simulated conditions showed that Martian subsurface may offer a more hospitable environment than the surface, with chlorates enhancing habitability over perchlorates. Microbial stress responses to perchlorate, analyzed through morphological, proteomic, and metabolomic approaches, highlighted species-specific reactions, emphasizing the necessity of understanding adaptations to Martian conditions for future astrobiological research.

IT-039

Efficacy of antimicrobial copper surfaces under spaceflight conditions: Preliminary results of the BIOFILMS experiment

Katharina Siems[1], Daniel Wyn Müller[2], Aisha Ahmed[2], Rob Van Houdt[3], Rocco L. Mancinelli[4], Kristina Brix[5], Ralf Kautenburger[5], Jutta Krause[6], Marco Vukich[6], Rodrigo Coutinho De Almeida[7], Alessandra Tortora[8], Christian Roesch[9], Gudrun Holland[10], Michael Laue[10], Frank Mücklich[2] and Ralf Moeller[1]

[1] Department of Radiation Biology, Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne (Köln), Germany
[2] Department of Materials Science and Engineering, Saarland University, Saarbrücken, Germany
[3] Microbiology Unit, Belgian Nuclear Research Centre (SCK CEN), Mol, Belgium
[4] NASA Ames Research Center / Bay Area Environmental Research Institute, CA, USA
[5] Department of Inorganic Solid State Chemistry, Elemental Analysis, Saarland University, Saarbrücken, Germany.
[6] ESA, European Space Research and Technology Centre (ESTEC), Noordwijk, Netherlands
[7] Telespazio Belgium S.R.L. for ESA, European Space Research and Technology Centre (ESTEC), Noordwijk, Netherlands
[8] Kayser Italia Srl, Livorno, Italy
[9] Biotechnology Space Support Center (BIOTESC), Lucerne University of Applied Sciences and Arts, Lucerne, Switzerland
[10] Robert-Koch-Institute, Berlin, Germany

Biofilms are a significant challenge in environments such as hospitals and industrial facilities, but also in space environments. Microbial induced corrosion due to biofilm formation, biofouling and clogging of pipes are serious threats to the integrity of spacecraft. In addition, biofilms can harbor and act as reservoirs for opportunistic pathogenic microorganisms, which could pose a threat to astronaut health, especially since astronauts exhibit a compromised immune system during spaceflight. Antimicrobial surfaces are one of many mitigation strategies against microbial biofilm formation. Copper has been known for its antimicrobial properties since ancient times, and additional surface modification using Direct Laser Interference Patterning (DLIP) has been shown to increase antibacterial efficacy. However, the interplay between cells, their environment and surfaces under space conditions requires further investigation, as fluid dynamics, mass transport and sedimentation are different in microgravity. The BIOFILMS experiment was selected by the European Space Agency (ESA) to test antimicrobial copper-containing surfaces against bacterial adhesion and biofilm under reduced gravity conditions on the International Space Station (ISS). The BIOFILMS experiment included three flights to the ISS from August 2021 to March 2023, during which the bacterial model organisms *Staphylococcus capitis*, *Cupriavidus metallidurans*, and *Acinetobacter radioresistens* were incubated on various surfaces inside specially designed hardware to ensure a controlled environment. Preliminary results show no negative effect of reduced gravity on the antimicrobial efficacy of copper and no clear effect of gravity conditions on adhesion and biofilm formation on the reference stainless steel surfaces. The final results of the project will provide valuable insights into selecting suitable surfaces and functionalization techniques to reduce biofilm formation, increase safety, and improve the sustainability of space travel.

IT-040

PhotoSynthetics: Elucidating energy balancing mechanisms in cyanobacteria via metabolic engineering

Daniel C. Ducat[1]

[1] Department of Biochemistry & Molecular Biology, Michigan State University, USA

Photosynthetic microbes are increasingly under consideration as an alternative chassis for solar-driven synthesis of bioproducts. However, it remains unclear how rerouting carbon flux towards target metabolic products may impact the physiology of the engineered microbes. We have observed that cyanobacteria engineered to secrete a large amount of the carbon they fix in the form of a simple sugar, sucrose, exhibit features consistent with a relaxation of photosynthetic sink inhibition. Here, we summarize recent efforts within the lab to identify the regulatory and cellular changes that follow activation of a bioproduction pathway. We've identified 4 two-component signaling proteins that appear to have roles related to the process of energy sensing and balancing in the model cyanobacterium, *Synechococcus elongatus* PCC 7942. We discuss recent evidence that these proteins are involved in regulating the carbon fixation machinery in response to changing cellular demands, including in the organization of the carboxysome.

IT-041

Revealing the structure of the light-harvesting antenna and its photoprotected state

Maria Agustina Dominguez-Martin[1,2,3,*], Paul V. Sauer[4,5], Markus Sutter[1,2,3], Henning Kirst[2,3], David Bina[6,7], Basil J. Greber[3,4,&], Eva Nogales[3,4,5,8], Tomáš Polívka[6] & Cheryl A. Kerfeld[1,2,3,9]

[1] MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA
[2] Environmental Genomics and Systems Biology Divisions, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[3] Molecular Biophysics and Integrated Bioimaging Divisions, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[4] QB3 Institute, Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA.
[5] Howard Hughes Medical Institute, University of California, Berkeley, CA, USA.
[6] Faculty of Science, University of South Bohemia, Czech Republic.
[7] Biology Centre of the Czech Academy of Sciences, Czech Republic.
[8] Department of Molecular and Cellular Biology, University of California, Berkeley, CA, USA.
[9] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA
[(&)] Present address: Division of Structural Biology, Institute of Cancer Research, London, UK.
(*) Present address: Departamento de Bioquímica y Biología Molecular. Universidad de Córdoba (Spain).

Phycobilisomes (PBS) are the elaborated light-harvesting antennas in cyanobacteria. To balance the harvesting of light energy against the risks of photodamage, many cyanobacteria have evolved a photoprotective mechanism that relies on the interaction between a photoreceptor, the Orange Carotenoid Protein (OCP), and the PBS. Here we present four cryo-electron microscopy structures, with and without OCP, of the 6.2 MDa PBS from the model organism *Synechocystis* PCC 6803 at overall resolution 2.1-3.5 Å. The structures revealed the existence of three different conformational states of the antenna, two previously unknown, for the unquenched PBS. We found that two of the rods can switch conformation within the complex, suggestive of a potentially new type of regulation. We also discovered a novel linker protein, named ApcG, that binds to the membrane facing side of the PBS. In addition, the structure of the PBS-OCP complex shows four 34 kDa OCPs organized as two dimers quench the PBS. The complex also reveals for the first time, the structure of the active form of the OCP, revealing an ~60 Å displacement of its regulatory C-terminal domain. Finally, we elucidate energy transfer pathways based on structural and spectroscopic properties. These results provide detailed insights into the cyanobacterial light-harvesting and place a foundation for

future bioengineering applications.

IT-042

c-di-AMP signaling in cyanobacteria: a new paradigm in controlling cellular homeostasis

Khaled A. Selim[1*]

[1] *Microbiology/ Molecular Physiology of Prokaryotes, Institute of Biology, University of Freiburg, Schänzlestraße 1, 79104 Freiburg, Germany*

Because of their photosynthesis-dependent lifestyle, cyanobacteria evolved sophisticated regulatory mechanisms to adapt to oscillating day-night metabolic changes. How they coordinate the metabolic switch between autotrophic and glycogen-catabolic metabolism in light and darkness is poorly understood. Recently, we showed that the second messenger c-di-AMP is implicated in diurnal regulation[1]. To unravel the signaling functions of c-di-AMP, we identified the cyanobacterial c-di-AMP receptor proteins. The carbon-sensor protein SbtB was identified as a major c-di-AMP receptor[1]. We found that c-di-AMP–bound SbtB interacts with the glycogen-branching enzyme GlgB. Accordingly, both c-di-AMP-free (Δ*dacA*) and Δ*sbtB*–deficient mutants displayed impaired glycogen synthesis and nighttime survival[1]. To gain better understanding of cellular processes regulated by SbtB or c-di-AMP, we compared the metabolomic, transcriptomics, and proteomic landscapes of both mutants[2,3]. While our results indicate that the cellular roles of SbtB is restricted to carbon/glycogen metabolism[1-5], the Δ*dacA* lethality seems a result of dysregulation of multiple metabolic processes[2,3]. These processes include photosynthesis and redox regulation, which lead to elevated levels of intracellular ROS and glutathione[2]. Further, we showed an impact of c-di-AMP on transcription/transitional regulations, ion homeostasis, and especially on central carbon metabolism. Adding to its cellular functions catalog, for first time, we show physiologically that c-di-AMP signaling influences on nitrogen metabolism, implying new cellular roles for c-di-AMP in controlling nitrogen homeostasis[2]. Additionally, we found that the cyanobacterial pilus biogenesis and natural competence are regulated by c-di-AMP and show that the ComFB signaling protein is a novel c-di-AMP-receptor protein[6], widespread in bacterial phyla, and required for DNA uptake.

1. Selim KA, Haffner M, Burkhardt M, Mantovani O, Neumann N, Albrecht R, Seifert R, Krüger L, Stülke J, Hartmann MD, Hagemann M, Forchhammer K. Diurnal metabolic control in cyanobacteria requires perception of second messenger signaling molecule c-di-AMP by the carbon control protein SbtB. Sci Adv. 2021; 7(50):eabk0568.
2. Haffner M, Mantovani O, Spät P, Maček B, Hagemann M, Forchhammer K, Selim KA. Diurnal rhythm causes metabolic crises in the cyanobacterial mutants of c-di-AMP signalling cascade. BioRxiv 2023; doi: https://doi.org/10.1101/2023.11.14.567006.
3. Mantovani O, Reimann V, Haffner M, Herrmann FP, Selim KA, Forchhammer K, Hess WR, Hagemann M. The impact of the cyanobacterial carbon-regulator protein SbtB and of the second messengers cAMP and c-di-AMP on CO_2 -dependent gene expression. New Phytol. 2022; 234(5):1801-1816.
4. Selim KA, Haffner M, Mantovani O, Albrecht R, Zhu H, Hagemann M, Forchhammer K, Hartmann MD. Carbon signaling protein SbtB possesses atypical redox-regulated apyrase activity to facilitate regulation of bicarbonate transporter SbtA. PNAS. 2023; 120(8):e2205882120.
5. Selim KA, Haase F, Hartmann MD, Hagemann M, Forchhammer K. P_{II}-like signaling protein SbtB links cAMP sensing with cyanobacterial inorganic carbon response. PNAS. 2018; 115(21):E4861-E4869.
6. Samir S, Doello S, Zimmer E, Haffner M, Enkerlin AM, Müller T, Dengler L, Lambidis SP, Sivabalasarma S, Albers S-V, Selim KA. The second messenger c-di-AMP controls natural competence via ComFB signaling protein. BioRxiv 2023; doi: https://doi.org/10.1101/2023.11.27.568819

IT-043

Two interconnected circadian oscillators in one cyanobacterium

Anika Wiegard[b,1], Christin Köbler[a,1], Nicolas M. Schmelling[b,1], Alice Pawlowski[b,1], Gopal Pattanayak[c], Philipp Spät[d], Nina M. Scheurer[a], Kim N. Sebastian[a], Florian P. Stirba[b], Lutz C. Berwanger[b], Boris Maček[d], Michael Rust[c], Ilka M. Axmann[b,*], Annegret Wilde[a,*]

[a]*Institute of Biology III, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany;* [b]*Institute for Synthetic Microbiology, Biology Department, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany;* [c]*Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637,* [d]*Department of Quantitative Proteomics, Interfaculty Institute for Cell Biology, Eberhard Karls University Tübingen, 72076 Tübingen, Germany*

Cyanobacteria use a circadian clock to predict environmental daily cycles and organize a multitude of cellular processes in matching daily rhythms. In the clock model organism *Synechococcus elongatus* PCC 7942 the clock is composed of the three proteins KaiA, KaiB and KaiC. Some cyanobacteria express multiple diverged Kai proteins, which implies that circadian regulation might be more complex in those organisms. In *Synechocystis* sp. PCC 6803 three KaiB and KaiC proteins are present. We chose it as a representative to study the function and interplay of diverged clock proteins in an otherwise well characterized model organism (1- 4). Based on homology searches, it was believed that all cyanobacteria contain only one copy of KaiA, but we discovered a second KaiA protein (KaiA3) in *Synechocystis* (5). Only the C-terminal domain of this new chimeric KaiA3 protein resembles a KaiA domain. The N-terminal domain is similar to a NarL-type response regulator receiver domain. Mixing KaiA3, KaiB3 and KaiC3, we could reconstitute low amplitude, temperature compensated ~24h oscillations of KaiC3 phosphorylation in vitro. This demonstrates that KaiA3B3C3 form a bona fide circadian oscillator. In the cell, KaiC3 displayed 24h phosphorylation rhythms that were phase-locked with KaiC1 phosphorylation cycles. Circadian rhythms can be simply read out via online monitoring of the backscatter properties of cells (6). Studies of *kai* gene deletion strains indicated that the KaiA1B1C1 oscillator and KaiA3B3C3 oscillator are both required to maintain stable backscatter oscillations, which implies that two interconnected oscillators are present in *Synechocystis*. We are currently investigating the interplay of these two oscillators.

(1) Wiegard A, Dörrich AK, Deinzer HT, Beck C, Wilde A, Holtzendorff J, Axmann IM. Microbiology (Reading). 2013 May;159(Pt 5):948-958. doi: 10.1099/mic.0.065425-0.
(2) Beck C, Hertel S, Rediger A, Lehmann R, Wiegard A, Kölsch A, Heilmann B, Georg J, Hess WR, Axmann IM. Appl Environ Microbiol. 2014 Sep;80(17):5195-206. doi: 10.1128/AEM.01086-14.
(3) Schmelling NM, Lehmann R, Chaudhury P, Beck C, Albers SV, Axmann IM, Wiegard A. BMC Evol Biol. 2017 Jul 21;17(1):169. doi: 10.1186/s12862-017-0999-7. P

(4) Wiegard A, Köbler C, Oyama K, Dörrich AK, Azai C, Terauchi K, Wilde A, Axmann IM. J Bacteriol. 2020 Jan 29;202(4):e00478-19. doi: 10.1128/JB.00478-19.
(5) Köbler , Schmelling NM, Pawlowski A, Spät P, Scheurer NM, Sebastian K, Berwanger LC, Maček B, Wiegard A, Axmann IM, Wilde A. *preprint*, bioRxiv 2021.07.20.453058; doi: https://doi.org/10.1101/2021.07.20.453058
(6) Berwanger LC, Thumm N, Gholamipoor R, Wiegard A, Schlebusch J, Kollmann M, Axmann IM. *preprint*, bioRxiv 2023.09.26.559469; doi: https://doi.org/10.1101/2023.09.26.559469

IT-055

Legionella relative abundance in shower hose biofilms is associated with specific microbiome members

Alessio Cavallaro[1,2]*, Marco Gabrielli[1], Frederik Hammes[1]
[1]*Department of Environmental Microbiology, Eawag: Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland*
[2]*Department of Environmental Systems Science, Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, 8092 Zürich, Switzerland*

Legionella are natural inhabitants of building plumbing biofilms (e.g. shower hoses), where interactions with other microorganisms influence their survival, proliferation, and death. Their interactions with their surrounding microbiome remain poorly understood. Here, we investigated the associations of *Legionella* with prokaryotic and eukaryotic microbiomes in biofilm samples extracted from 85 shower hoses of a single residential building. We used specific ddPCR quantification as well as general 16S rRNA and 18S rRNA amplicon sequencing to characterize the shower hose microbiomes**.** Sequencing data revealed a complex prokaryotic and eukaryotic microbiome in all samples. *Legionella* spp. relative abundance in the biofilms ranged between 0 - 7.8%, of which only 0 - 0.46% was *L. pneumophila*. Our data suggests that some microbiome members were associated with high *Legionella* relative abundance (e.g., Chthonomonas, Vrihiamoeba), while others were associated with low *Legionella* relative abundance (e.g., Aquabacterium, Vannella). Interestingly, the data analysis also revealed high genetic variability in the 16S rRNA sequences assigned to the genus *Legionella* (30 unique Amplicon Sequence Variants detected). The correlations of the different *Legionella* variants with microbiome members showed distinct patterns, suggesting separate ecological niches potentially occupied by different *Legionella* species. This study provides insights into the ecology of *Legionella* with respect to: 1) the colonization of a high number of real shower hoses biofilm samples; 2) the ecological meaning of associations between *Legionella* and co-occurring prokaryotic/eukaryotic organisms; 3) the presence of multiple species of *Legionella* in building plumbing systems, and the potential of 16S rRNA based detection of *Legionella* diversity in the environment.

IT-056

Active thermophilic bacterial communities thrive in domestic hot-water boilers

Alexander K. T. Kirschner[a,f,h], Lena Campostrini[a,h], Mats Leifels[f,h], Hans Peter Füchslin[c], Claudia Kolm[f,h], Cheng Dan[i], Stefan Zimmermann[d], Vivian Hauss[d], Alexandre Guiller[d], Luigino Grasso[e], Adrian Shajkofci[e], Andreas H. Farnleitner[f,g,h], Thomas Egli[b]
[a] *Institute for Hygiene and Applied Immunology, Water Microbiology, Medical University of Vienna, A-1090 Vienna, Austria*
[b] *Microbes-in-Water GmbH, CH-8760 Feldmeilen, Switzerland*
[c] *Cantonal Laboratory Zurich, CH-8032 Zurich, Switzerland*
[d] *bNovate Technologies SA, CH-8045 Zurich, Switzerland*
[e] *bNovate Technologies SA, CH-1024 Ecublens, Switzerland*
[f] *Division of Water Quality and Health, Karl Landsteiner University, A-3500 Krems, Austria*
[g] *Institute for Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, A-1040 Vienna, Austria*
[h] *Interuniversity Cooperation Centre Water & Health, Austria*
[i] *Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore, Singapore*

Running cold and hot water in buildings is a widely established commodity. Except for legionellae, little attention has been given to the microbiology of domestic hot-water installations (DHWIs). In many countries, regulations consider hot water as heated drinking water that must comply (cold) drinking water (DW) standards. Only few reports exist on the microbiome in DHWIs. The lack of both, information and hygienic regulations, asks for a broad microbiological investigation of DHWI. Using flow cytometric (FCM) total cell count (TCC) determination, FCM-fingerprinting, and 16S rRNA-gene-based metagenomic analysis, the characteristics and composition of bacterial communities in hot water from associated boilers was studied in 14 inhouse DW installations in Switzerland and Austria. At all locations, 1.3 to 8-fold enhanced TCC were recorded in hot water compared to the cold DW. FCM-fingerprints of cold and corresponding hot water indicated different composition of cold- and hot-water microbiomes. Also, hot water in each boiler had its own individual FCM-fingerprint. 16S-rRNA-gene-based community analysis confirmed the marked differences in microbiome composition. Generally, bacterial diversity in cold DW was broad, that in hot water was restricted, with thermophilic strains from the families *Hydrogenophilaceae*, *Nitrosomonadaceae* and *Thermaceae* dominating. Batch growth assays, consisting of heated cold DW and inoculated with hot water, resulted in immediate cell growth, suggesting hot-water boilers as autonomously operating, thermophilic bioreactors. When cold DW was used as inoculum no significant growth was observed. The generation of assimilable organic carbon from dissolved organic carbon due to heating appeared to be the driver for growth of thermophilic microbial communities in hot-water systems. Despite consumers have been exposed to hot-water microbiota for a long time, with the exception of *Legionella*, no major pathogens have so far been associated with hot-water use. The role of harmless thermophiles and their interaction with potential human pathogens at elevated temperatures in DHWIs remains to be investigated (see Water Research 253 (2024): 121109).

IT-057

Some like it hot: how much heat do different *Legionella* species and bacteria in drinking water actually tolerate?

Andreas Nocker[1], Maja Stahl, Sarah Demuth
[1]*IWW – Institut für Wasserforschung gGmbH, Angewandte Mikrobiologie, Mühlheim an der Ruhr*

Our knowledge on bacteria in drinking water is mainly limited to cold water (potable water cold, PWC). Bacteria in hot water (potable water hot, PWH) are understudied as routine diagnostics does typically not include incubation of nutrient agar plates with PWH samples at elevated temperatures like e.g. 50°C or 60°C. These temperatures would however be needed to enable growth and detection of thermotolerant bacteria that do not form colonies at 22°C or 36°C. Recently the bacterial diversity of PWH received more attention. We established a flow cytometry-based method to rapidly assess the heat tolerance of bacteria subjected to a heat gradient. The indirect viability criteria comprised maintenance of (1) cellular integrity, (2) enzymatic activity and (3) intracellular homeostasis when challenged with an acidic pH. When

applied to different *Legionella* species, results demonstrated that the group of *Legionella* is highly diverse and that different *Legionella* differ greatly in their heat resistance. *Legionella micdadei* or representatives of *Legionella pneumophila* displayed a substantially greater resistance to heat than for example *Legionella anisa* or *Legionella dumoffii*. When applied to real water samples, bacteria in the hot water circulation displayed much greater thermotolerance than bacteria contained in cold drinking water. Also, the passage of PWC through a tube reactor kept at 36°C markedly increased the number of bacteria resistant to elevated temperatures. Apart from quickly assessing the heat tolerance of individual species or entire water microbiomes, the method is useful as a diagnostic guide when performing thermal disinfection and when being challenged with the question which temperature is needed and how long it should be maintained to achieve a satisfactory disinfection result.

Oral Presentation

Archaea & Extremophiles

OP-AE-001
Tracing the gaps - Unraveling initial steps in hexose metabolism in Saccharolobus solfataricus using an improved stable isotope labeling approach

*K. Mucha[1], J. Wolf[1], B. Siebers[2], M. Neumann-Schaal[1]
[1]*Leibniz Institut DSMZ, Brunswick, Germany*
[2]*Universtiy Duisburg-Essen, Essen, Germany*

Archaea are often found in harsh habitats withstanding for instance high temperatures, salinity and acidic or alkaline environments making them valuable models to understand life under extreme conditions. Their metabolism is characterized by unique and unusual metabolic pathways and enzymes that differ from bacteria and eukaryotes. Among the *Thermoproteota*, *Saccharolobus solfataricus* represents an important model organism for archaeal metabolism. It thrives under thermoacidophilic conditions with an optimum temperature of 80 °C and a pH value between 2 and 4. Additionally, it is characterized by its metabolic versatility, which allows it to grow on a wide variety of carbon sources. For some hexoses like D-glucose and D-galactose the central carbohydrate metabolism has been extensively investigated, revealing a modified branched Entner-Doudoroff (ED) pathway for degradation instead of the Embden-Meyerhof-Parnas (EMP) pathway commonly used by other organisms. However, the degradation of other hexoses, such as D-mannose and D-fructose, is poorly understood so far. An initial $^{13}C_6$-mannose spiking experiment indicated that the ED pathway is involved in D-mannose degradation. Due to the absence of homologues enzymes for known D-fructose degradation, a completely novel pathway is hypothesized. To unravel these metabolic blind spots, a GC-MS-based method has been optimized to intentionally slow down the metabolism of *S. solfataricus*. In this way, the metabolic flux of $^{13}C_6$-labeled D-mannose and D-fructose can be traced in the first steps of degradation, paving the way for future elucidation and mapping of the degradation pathway.

OP-AE-002
Translational coupling via termination-reinitiation in archaea and bacteria

*N. Vogel[1], M. Huber[1], M. Töpfer[1], J. Muralha Schweikert Farinha[1], J. Soppa[1]
[1]*Goethe-University Frankfurt/Main, Insitute for Molecular Bioscience, Frankfurt a. M., Germany*

Translational coupling occurs at many gene pairs of archaea and bacteria. Translational coupling means that translation of the downstream gene on a polycistronic transcript strictly depends on translation of the upstream gene. One specific mechanism of coupling is called termination-reinitiation (TeRe). In this case, the translating ribosome (at least the small subunit) remains on the mRNA after terminating translation of the upstream gene, and it directly continues translation by reinitiating at the downstream gene. This mechanism typically occurs at gene pairs that have overlapping stop/start codons or very small intergenic distances.

In previous studies we have shown that TeRe operates at several native gene pairs of *Haloferax volcanii* and *Escherichia coli*[1] and that efficient coupling required very short intergenic distances[2]. Translational efficiencies were quantified using reporter genes fused to native gene pairs, enzyme assays and northern blotting were used to determine protein and transcript levels. In the present study, we investigated whether local mRNA structures at gene overlaps in *E. coli* are required to inhibit novel initiation at the downstream genes and guarantee coupling. To this end, we generated several constructs with truncated or mutated gene pairs, with the aim of destabilizing local mRNA structures close to the overlaps. The effects of these genetic perturbations on the efficiencies of translation and coupling will be reported.

Additionally we created a random library with a 42 random nucleotide sequence directly upstream of the overlapping start/stop codon. In our two-reporter gene system, this library is used to select and screen for sequence motives that are important for efficient novel initiation at the downstream gene as well as translational coupling via TeRe.

Taken together, translational coupling via TeRe operates at many gene pairs in archaea and bacteria, and various aspects of the molecular mechanism could be characterized using native gene pairs fused to reporter genes.

[1] Huber et al. (2019) Nature Comm. 10: 4006.

[2] Huber, Vogel et al. (2023) Front Microbiol. 14: 1291523.

OP-AE-003
Characterization of Methanosarcina mazei Solo-Cas4

*L. Rentz[1], F. Gehlert[1], L. Hellwig[1], R. Schmitz-Streit[1]
[1]*Institut for Microbiology, Kiel, Germany*

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) system and its associated (Cas) proteins serve as a defensive mechanism in prokaryotes against foreign genetic elements like viruses, plasmids, or transposons. Thereby, Cas proteins play a key role in CRISPR/Cas systems by recognizing invading nucleic acids and inserting fragments of the foreign nucleic acids (spacer) into the CRISPR array to reminisce overcome future

infections. While most Cas proteins are encoded in the CRISPR locus, various *cas* genes exist that are located outside of CRISPR loci. The function of most of these stand-alone Cas proteins has yet not been investigated. *Methanosarcina mazei*, an anaerobic methanogenic archaeon, possesses two CRISPR loci together with an additional *cas4* gene that has been identified outside of the CRISPR loci encoding a so-called solo-Cas4 protein. The function and structure of this solo-Cas4 protein and the potential connection to the CRISPR/Cas system or a function beyond have not been examined so far. The widespread occurrence of solo-Cas4 proteins even in organisms lacking CRISPR/Cas systems as well as encoded in viral genomes strengthen the hypothesis that solo-Cas4 have a potential function outside the prokaryotic defense.

We heterologously expressed His-tagged SUMO fusion of solo-Cas4 and purified the tag-less protein. The purified solo-Cas4 protein showed a brown coloration and the characteristic absorption spectrum of a 4Fe4S-cluster with absorption peaks at approximately 330 nm and 420 nm. We further showed that *M. mazei* solo-Cas4 possesses a metal dependent exo- (from 3' to 5') and endo-nuclease activity against ssDNA substrates. The nuclease activity, as well as the 4Fe4S cluster, are characteristic features for Cas4 proteins. However, *M. mazei* solo-Cas4 showed only low sequence similarity to other Cas4 proteins. The detailed study of *M. mazei* solo-Cas4 may contribute to a broader understanding and elucidating a distinct role of solo-Cas4 proteins in archaea.

OP-AE-004

Plasmid segregation in Haloferax volcanii is mediated by a hybrid partitioning system

*K. Premrajka[1], U. Johnsen[1], H. Preidel[1], G. Giacomelli[1], F. Meyer[1], M. Bramkamp[1]
[1]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for General Microbiology, Kiel, Germany*

Proper segregation of the genome, a fundamental process across all domains of life has not been extensively studied in Archaea. Archaea show a high degree of variability in the organisation and copy number of chromosomes and plasmids. The archaeon *Haloferax volcanii* is a polyploid organism containing two large and two small plasmids as part of its genome. It is generally accepted that genomic segregation is carried out randomly due to its polyploidy.

However, we have identified multiple two-gene operons across its genome; one gene encoding a ParA like protein and the other encoding unknown proteins. One such operons in the large plasmid pHV3 contains the loci *hpaB* and *hpaA* (Haloarchaeal partitioning protein A/B). We show phylogenetic and functional similarities between the operon and the ParABS DNA segregation system from bacteria. The predicted structure and conserved domains in the sequence of HpaA suggest that it is a ParA-like protein having a bacterial origin, acquired via horizontal gene transfer. HpaB is a ParB analogue of archaeal origin capable of forming foci in the cytosol. HpaA is necessary for the proper spatial distribution of the HpaB foci. Single particle tracking shows that HpaB regulates the dynamics of HpaA. Comparative qPCR suggests HpaB is essential for maintaining the plasmid to chromosome ratio.

We thus propose a bacterial-archaeal hybrid plasmid segregating system in *H. volcanii* comprising of a repurposed bacterial protein (HpaA) and a customized archaeal DNA adapter (HpaB); the HpaAB system. We further explore how it could be especially necessary for two-dimensional segregation of its genome as *H. volcanii* cells form flat disks in middle to late logarithmic phase of growth. Regular spatial distribution of all genome copies ensures proper genome segregation irrespective of the plane of division, which has been shown to not be influenced by the location of DNA in *H. volcanii*. We suggest that segregation of its genome depends on a two-dimensional distribution of all its copies rather than a directional movement across the plane of division.

Biotechnology & Synthetic Microbiology

OP-BSM-001

Understanding microbially induced calcite formation by Bacilli for application in bio-based, green construction materials

M. Seidel[1], C. Hamley-Bennett[2], B. Reeksting[2], K. Paine[3], *S. Gebhard[1,2]
[1]*Johannes Gutenberg Universität Mainz, Institute of Molecular Physiology, Mainz, Germany*
[2]*University of Bath, Department of Life Sciences, Bath, United Kingdom*
[3]*University of Bath, Department of Architecture and Civil Engineering, Bath, United Kingdom*

The construction industry, particularly the use of cement-based materials, has become a concern amid growing unrest over climate issues. Cement production and use accounts for 8% of global anthropogenic CO2 emissions. One exciting approach to reduce the use of cement is to take advantage of Microbially Induced Calcite Precipitation (MICP). This describes the ability of some bacteria to form calcium carbonate minerals, specifically calcite, the main component of limestone. Application of spore-forming MICP-bacteria into concrete, for example, can be used to develop self-healing technologies that autonomously heal microcracks in structures and thus prevent further deterioration and the need for repairs. Other uses include consolidation of soils, or, more recently, the development of engineered living materials, where bacterial activity replaces the use of cement entirely, e.g. for the production of bricks.

A major drawback of current technologies is the use of expensive complex nutrients, which leads to high costs of the biobased materials, reducing economic feasibility. Optimisation, however, is hampered by a limited understanding of the molecular and physiological mechanisms of MICP. Here, we report on detailed profiling of environmental *Bacillus* sp. and close relatives to identify optimally suited MICP-strains. Exploration of the relationship between MICP and the metabolism of organic acids showed that acetate metabolism is a key driver of precipitation in some species, leading to rapid and almost complete precipitation of soluble calcium nitrate to insoluble calcite. Our findings further indicate that calcium homeostasis in bacteria may play a role in MICP. Detailed insights into how bacteria contribute to or even control the formation of calcite minerals opens the way for targeted design of economically viable self-healing or *de novo*-built construction materials. Bio-based cement alternatives could thus pave the road towards a greener, more sustainable infrastructure of the future.

OP-BSM-002
Designing a genetic switch for controlled magnetosome formation in Magnetospirillum gryphiswaldense
*A. Woller[1], D. Schüler[1]
[1]*University of Bayreuth, Lehrstuhl für Mikrobiologie, Bayreuth, Germany*

The alphaproteobacterium *Magnetospirillum gryphiswaldense* biosynthesizes magnetosomes, which consist of membrane-enveloped magnetite crystals aligned in linear chains. As one of only few tractable magnetic bacteria it has emerged as a widely used model for the study of prokaryotic organelle formation and the bioproduction of magnetic nanoparticles. However, analysis and engineering of magnetosome biogenesis has been hampered by the limited toolset for controlled gene expression in this bacterium.

Here, we investigated novel candidate promoters for strong and tunable gene expression. Transcription from a set of putative and known promoters from *M. gryphiswaldense* and other bacteria was explored in *M. gryphiswaldense* using the *luxABCDE* luciferase operon as reporter. The resulting bioluminescence was measured in the absence and presence of cognate repressors. Among all tested promoters, P*cym* and *cymR*, a promoter-repressor system derived from *Pseudomonas putida,* caused the highest expression of *lux* genes, thereby exceeding the activity of all currently used constitutive and inducible foreign and endogenous promoters in *M. gryphiswaldense* such as P*mamG*, P*lac* and P*tet*. Furthermore, P*cym* was tightly repressed in the absence of its inducer cumate, but could be rapidly induced at different cumate concentrations.

To explore *cymR*/P*cym* for the induced expression of single genes, we placed this system in front of *mamI, mamE, mamL, mamM, mamO, mamQ, mamB*, respectively, all encoding essential magnetosome proteins. Wildtype-like magnetosome formation could be restored in non-magnetic single deletion mutants in the presence of the inducer. By varying the conditions, we are currently exploring the system for the tuned (over)expression of entire accessory magnetosome gene clusters, such as *mms6, mamGFDC* and *mamXYZ*. This might enable the tuning of size, shape, and number of magnetosome crystals. In conclusion, this novel genetic tool is expected to be used for functional analysis and engineering of prokaryotic organelle formation, specifically to identify the influential parameters for controlled magnetosome biogenesis in *M. gryphiswaldense.*

OP-BSM-003
Genetic engineering of *Agrobacterium* increases production of the industrially useful polymer curdlan from apple waste
*M. McIntosh[1]
[1]*Justus Liebig University Giessen, Institute of Microbiology and Molecular Biology, Giessen, Germany*

Curdlan is a water-insoluble polymer that has structure and gelling properties that are useful in a wide variety of applications such as in medicine, cosmetics, packaging and the food and building industries. The capacity to produce curdlan has been detected in certain soil-dwelling bacteria of various phyla, although the role of curdlan in their survival remains unclear. One of the major limitations of wider use of curdlan in industry is the high cost of production during fermentation, partly because production involves specific nutritional requirements such as nitrogen limitation. For broader application in industry, the production of curdlan needs to be significantly cheaper and avoid competing with human nutritional resources such as glucose, sucrose or starch. Engineering of the industrially relevant curdlan-producing strain Agrobacterium sp. ATTC31749 is a promising approach that could decrease the cost of production so that agricultural waste flows could be used as fermentation substrates. Here, during investigations on curdlan production, it was found that curdlan was deposited as a capsule. Curiously, only a part of the bacterial population produced a curdlan capsule. This heterogeneous distribution appeared to be due to the activity of Pcrd, the native promoter responsible for the expression of the *crdASC* biosynthetic gene cluster. To improve curdlan production, Pcrd was replaced by a promoter (PphaP) from another *Alphaproteobacterium*, *Rhodobacter sphaeroides*. Compared to Pcrd, PphaP was stronger and only mildly affected by nitrogen levels. Consequently, PphaP dramatically boosted *crdASC* gene expression and curdlan production. Importantly, the genetic modification overrode the strict nitrogen depletion regulation that presents a hindrance for maximal curdlan production from complex media. With this biotechnological innovation, apple pomace was used as an example of agricultural waste for curdlan production and demonstrated excellent potential for achieving high yields using cheap substrates under relaxed fermentation conditions.

OP-BSM-004
Development of THERMOphilic microorganisms for SYNgas CONversion to biobased fuels and chemicals
*M. Basen[1], L. T. Angenent[2], R. Daniel[3], V. Müller[4]
[1]*University of Rostock, Microbiology, Rostock, Germany*
[2]*University of Tübingen, Tübingen, Germany*
[3]*Georg-August University Göttingen, Genomic and Applied Microbiology, Göttingen, Germany*
[4]*Johann Wolfgang Goethe-University, Molecular Microbiology and Bioenergetics, Frankfurt a. M., Germany*

The decreasing availability of fossil fuels and the consequences of climate change increase the demand for biofuels and bioproducts from renewable resources and waste within a circular economy. Syngas, which contains various ratios of CO_2, CO, and H_2, is generated as waste gas in industrial processes. Acetogenic bacteria convert syngas into value-added carbon-based chemicals. Given the high temperatures of these industrial gas streams, syngas conversions (fermentations) at temperatures between 50–80°C are attractive. Within the BMBF-funded consortium ThermoSynCon, four partners from different German universities bundled their expertise in a research consortium to establish a syngas fermentation production platform using new thermophilic isolates or engineered strains.

Within the project, we enriched and isolated new thermophilic acetogenic bacteria and created a strain collection of thermophilic acetogens. Small-scale growth studies and fermentation studies of the new isolates on H_2+CO_2, CO, and syngas were performed, and the isolates were characterized and compared in their performance to the few known thermophilic acetogens, including the promising species *T. kivui* (T_{OPT} 66°C) and *M. thermoacetica.* We improved the bioenergetics models for *T. kivui* and *M. thermoacetica* based on detailed *omics*, biochemical, physiological, and genetic studies. We developed bioreactor technologies for (syn)gas fermentation at elevated temperatures (50–80°C), optimized the process control, and evaluated the performance of *T. kivui*, and *M. thermoacetica*

in bioreactors. Genetic tools for *T. kivui* were improved, with the goal of gaining a fundamental understanding of its physiology and constructing superior strains to produce alcohols from syngas. We subsequently genetically engineered *T. kivui* for ethanol production and tested the strains in the bioreactor, achieving a 50-mM titer in a continuous bioreactor.

Overall, the project provided deep insights into the metabolism of thermophilic acetogens, and proof-of-concept results of syngas conversion to ethanol in a continuous bioreactor. The road is paved towards a thermophilic syngas fermentation platform!

OP-BSM-005
Novel multi-kingdom consortium for the production of antimicrobial caproic acid
*M. F. Bambace[1], K. Wiborg Jensen[1], K. S. Ng[1], A. Schneider[1], A. Marietou[1], C. Schwab[1]
[1]*Aarhus University, Department of Biological and Chemical Engineering, Aarhus, Denmark*

Microbial fermentation systems can be designed to produce specific short chain carboxylic acids. The strong antimicrobial caproic acid can be produced by strains of the strictly anaerobe *Clostridium kluyveri* by chain elongation using acetate and ethanol as electron donor. We hypothesized that multi-kingdom microbial consortium composed of *C. kluyveri*, the heterofermentative *Limosilactobacillus reuteri* and the yeast *Saccharomyces cerevisiae* leads to the production of caproic acid in presence of glucose and fructose.

Fermentations were performed in 400 ml bioreactors at 37°C and pH 6.8. We carried out first fed batch fermentation consisting of a bacterial consortium (BC) of *L. reuteri* and *C. kluyveri* with external addition of ethanol, and afterwards batch fermentations with a multi-kingdom consortium (MKC) of *L. reuteri*, *C. kluyveri* and *S. cerevisiae*. Substrate consumption and metabolite production were quantified by HPLC-RI, UHPLC-DAD and confirmed by 1H NMR, and the cells growth was monitored by qPCR. The minimum inhibitory dilution fold 50 % (MIDF50) value of the fermentates was tested against *Salmonella enterica* subsp. *enterica* CCM 4420. Fermentations were started with *L. reuteri* or *L. reuteri* with *S. cerevisiae* to initiate acetate and ethanol production. *C. kluyveri* was inoculated 24 h later in both BC and MKC.

During 12 days of fermentation, only *L. reuteri* increased in cell counts by 1.5±0.7 and 0.7±0.1 log cells/ml in BC and MKC, respectively. Acetic acid levels were highest (about 12-18 mM) between days 2-5. In the same period, the concentration of butyric acid increased, and after 5-6 days of fermentation caproic acid was detected. After 12 days, 25.3± 0.1 and 31.2±11.0 mM of caproic acid were produced by BC and MKC, respectively. In addition to caproic acid, the fermentates contained lactic acid (116-108 mM), butyric acid (4-6.8 mM), propionic acid (4.1-5.1 mM) and ethanol (9.7-10.2 mM). Fermentates produced by BC and MKC showed high inhibitory effects against *S. enterica*.

We designed a self-contained process to produce fermentates containing antimicrobial caproic acid by a novel multi-kingdom microbe consortium with potential in food preservation.

OP-BSM-006
Engineering of a sticky therapeutic microbe
*A. Chamas[1,2], G. Lackner[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute, Jena, Germany*
[2]*Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Jena, Germany*

Innovative strategies are needed in the permanent fight against microbial pathogenic infections. Utilization of antibiotics is limited by the spread of resistant strains, the slow rate of new antibiotics discoveries and the deleterious off-target effect of these antibiotics against our microbiome. Probiotic strains represent an appealing alternative to antibiotics but suffer from weak efficacy and a poorly understood mode of action. In an effort to combine the best of the two strategies, the concept of therapeutic microbes has been developed in the last years. Therapeutic microbes are commensal organisms genetically engineered to have an enhanced therapeutic effect and they can be designed to specifically combat a microbial pathogenic strain. Thanks to synthetic biology tools, these natural hosts of our microbiome can be tuned to produce bioactive molecules against a wide variety of pathogens *in situ*. For that purpose, they can be upgraded by the integration of genetic elements called modules allowing detection of a target or ensuring biocontrol of the modified organism. In our lab, we work on implementing a cell-cell adhesion module, i.e. allow the specific attachment of our therapeutic microbe to a microbial target. With this module, higher bioactive compound concentrations can be achieved near the target and the therapeutic potential of our microbe will be enhanced. In a first proof of principle experiment, we describe the engineering of an adhesion module for the specific binding of the probiotic *Escherichia coli* Nissle to the fungal pathogen *Candida albicans*. Through membrane display of diverse carbohydrate binding proteins, we can show tight adhesion of the bacteria to the fungi hyphae. Adhesion between two members of the microbiome would not only be interesting to treat microbial infections but could also help to better understand mutualistic or antagonistic relationships inside the microbiome. Moreover, alternative adhesion modules could be engineered to target abiotic surfaces or different parts of the human body, therefore opening the way for innovative medical applications.

OP-BSM-007
Styrene oxide Isomerase, a catalytic efficient enzyme acting as peroxidase, peroxygenase, and isomerase
*S. Kumaran[1], D. Tischler[1]
[1]*Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany*

Styrene oxide isomerase (SOI), an integral membrane protein in the upper styrene degradation pathway, which catalyses a rare chemistry of Meinwald rearrangement in converting epoxides into aldehyde by 1,2-hydride/methyl shift1. The stereo- and regio-selective conversion by SOI makes it appealing for biocatalysis. But the molecular mechanism of SOI was not studied so far. Our recent collaborative work on SOI, and the cryo-EM structure showed that the enzyme possesses heme as a cofactor and active site. Hence, the study aimed at understanding the mechanism as well as to uncover the potential of heme-based isomerase.

This study included screening of eight SOIs from different classes of bacteria and fungi, where all the enzymes were purified to its homogeneity and screened for specific activity of its natural substrate *(R/S)*-styrene oxide. Out of all the SOIs tested, the enzyme from *Zavarzinia compransoris* Z-1155 (*Zc*SOI) stood out in protein yield and activity which was further characterized for its stability and kinetics. The enzyme showed a turnover frequency of about 250 s^{-1} with a catalytic efficiency of 2.5×10^6 $s^{-1}M^{-1}$. The mutation and reduction study revealed that both tyrosine and heme are co-ordinating the substrate and required for effective catalysis. Owing to the fact that heme is the active site and reducible, the study was conducted to utilize *Zc*SOI as peroxygenase. The ABTS assay proved that *Zc*SOI can accepts H_2O_2 with kcat of 2.3 s^{-1}, hence the enzyme was used to achieve both epoxidation and isomerization of styrene to produce phenylacetaldehyde. The biotransformation of *Zc*SOI in presence of H_2O_2 showed about 10-15% conversion of styrene to phenylacetaldehyde after 2h, indicating that the enzyme functions as peroxygenases in addition to isomerase. This study opened up the new regime in the biocatalysis of industrially high valuable product production with single enzyme.

1. Xin, R. P., See, W. W. L., Yun, H., Li, X. R. & Li, Z. Enzyme-Catalyzed Meinwald Rearrangement with an Unusual Regioselective and Stereospecific 1,2-Methyl Shift. *Angewandte Chemie-International Edition* 61 (2022). https://doi.org/10.1002/anie.202204889

OP-BSM-008

Reprogramming a protein ligase for genetic code expansion

G. Gallo[1], A. Sieber[1], *J. Lassak[1]

[1]*Ludwig-Maximilians University Munich, Biologie / Mikrobielle Biochemie, Planegg, Germany*

Nature employs a limited and conservative set of amino acids to synthesize proteins. The ability to genetically encode an extended set of building blocks can be used in diverse applications, including approaches to study and control protein function as well as to design novel therapeutics. Non-natural amino acids (NAA) are co-translationally incorporated into proteins by orthogonal pairs consisting of aminoacyl-tRNA synthetase and cognate tRNA. However, the current repertoire can neither display the full natural diversity of NAAs and is especially limited for backbone modifications. Excitingly, we now succeeded in reverse engineering a protein ligase into a new-to-nature tRNA synthetase (aaRS-β) that can load tRNA with β-amino acids (βaa). Additionally, we derived a variant aarRS-α accepting α-amino acids that cannot be integrated into the genetic code by any other means including adavanced glycation endproducts (AGEs). Our discovery lays the foundation for understanding the role of AGEs in the development and pathogenesis of major diseases such as diabetes and atherosclerosis as well as in the process of aging. At the same time the co-translational incorporation of βaa into the nascent chain will allow the cost-effective production of antimicrobial peptides with high protease stability and increased activity against multidrug-resistant pathogens.

OP-BSM-009

Upcycling of PBAT to 4-coumaric acid by combined enzymatic hydrolysis and monomer conversion with engineered Pseudomonas taiwanensis

*L. Op de Hipt[1], Y. Ackermann[1], C. Siracusa[2], T. Polen[1], F. Quartinello[2], B. Wynands[1], G. Gübitz[2], N. Wierckx[1]

[1]*Research Center Juelich, IBG-1, Jülich, Germany*

[2]*ACIB GmbH, Graz, Austria*

Bioplastics present a very promising approach to help solving the plastic crisis. However, it is crucial to establish efficient recycling strategies for these materials to ensure circularity of the plastic economy. Microbial upcycling is an important option for the end of life of more complex polymers or blends as well as mixed plastic waste. We engineered the metabolism of the PBAT monomer 1,4-butanediol in an aromatic-producing *Pseudomonas taiwanensis* strain with the ultimate goal of upcycling PBAT to valuable aromatic compounds. Growth on 1,4-butanediol was achieved via adaptive laboratory evolution. Subsequent genome sequencing and reverse engineering revealed a mutation upstream of PVLB_10545 encoding a putative ethanol dehydrogenase as essential for growth on 1,4-butanediol. This dehydrogenase likely oxidizes 1,4-butanediol to 4-hydroxybutyrate thus substituting for PedE which is present in *P. putida* but not *P. taiwanensis* (1). Adding two further mutations enhanced growth on 1,4-butanediol to ALE-like growth. One of these mutations resulted in an amino acid substitution in a LysR family transcriptional regulator (PVLB_12690), the homologue of which was also mutated in *P. putida* evolved on 1,4-butanediol (1). After successful reverse engineering, the strain was utilized in combination with two other strains growing on the PBAT monomers adipate and terephthalate and the PBAT hydrolyzing enzyme HiC for a process intensification, in which PBAT was hydrolyzed and the monomers were upcycled to 4-coumarate within one reactor.

OP-BSM-010

Highly selective whole-cell 25-hydroxyvitamin D_3 synthesis using molybdenum-dependent C25-steroid dehydrogenase and cyclodextrin recycling

*D. Kosian[1], M. Willistein[1], R. Weßbecher[1], C. Eggers[1], M. Boll[1]

[1]*University of Freiburg, Institute of Biology II / Microbiology, Freiburg i. Br., Germany*

Vitamin D (VitD) deficiency, which is associated with several acute and chronic diseases, is prevalent in populations worldwide and has inspired researchers to develop strategies to increase serum VitD levels via VitD-fortified food and supplementation.[1] In this context, the physiologically active form in the human body, 25-OH-VitD_3, has been shown to be significantly more effective as a supplement.[2] Enzymatic systems to produce this circulating form of VitD_3 typically use oxygen-dependent P450 monooxygenases, but these enzymes often produce undesired by-products and require costly NAD(P)H.[3]

As an alternative, we have developed a VitD_3-hydroxylating whole-cell system using a water-dependent steroid C25 dehydrogenase (S25DH) from denitrifying *Sterolibacterium denitrificans* in the related *Thauera aromatica* K172 as heterologous production host. Using resting cells of the S25DH-producing whole-cell biocatalyst at high densities, potassium ferricyanide as electron acceptor, 2-hydroxypropyl-β-cyclodextrin (HPCD) as solubiliser and ethanol as co-solvent, we demonstrated the formation of up to 1.85 g L^{-1} within 50 h at a 100 mL scale, making this the highest published 25-OH-VitD_3 titer achieved to date. In addition, no common by-products such as the di-hydroxlated 1α,25-di-OH-VitD_3 were formed. We also showed that the VitD_3-solubilising HPCD can be recycled and used for up to

10 consecutive conversion cycles without a significant loss of quality or quantity, making the entire process economically feasible.[4]

1 Holick, M. et al., *Rev. Endocr. Metab. Disord.* 18, 153-165 (2017)
2 Bouillon, R. and Quesada-Gomez, J., *J. Steroid Biochem. Mol. Biol.* 228, 106248 (2023)
3 Wang, Z. et al., *Biotechnol. Biofuels* 15, 109 (2022)
4 Kosian, D. et al., *Microb. Cell Fact.* 23, 30 (2024)

OP-BSM-011
Photosystem I-Powered Hydrogen Production in Phormidium lacuna: A Green Solution for Climate-Friendly Fuel
*H. Hoang[1]
[1]*Karlsruhe Institute of Technology, JKIP - General Botany, Karlsruhe, Germany*

Ongoing global climate change, driven by greenhouse gas emissions and fossil resource exploitation, increases demand for renewable energy sources. Especially green H_2 is an emerging emission-free alternative. Biological solar H_2 is produced as a byproduct of photosynthesis by green algae and cyanobacteria via hydrogenases. H_2 production is electron-dependent and limited by the electron availability. However, recent studies have shown that coupling of the hydrogenase to the Photosystem I (PSI) ensures a spatial proximity to the FeS cluster of PSI subunit PsaC so that the electron flow can be channelled towards H_2 production[1,2].

The goal of this study is to establish efficient solar H_2 production in the filamentous and biofilm forming cyanobacterium *Phormidium lacuna.*

To achieve this, different types of hydrogenases from various organisms are coupled to a full-length or truncated version of PSI subunit PsaD from *P. lacuna* and genomically integrated via natural transformation[3] into *P. lacuna*. H_2 production of the mutants are then measured via gas chromatography or H_2 microsensor relative to the wild type *P. lacuna*. Via biochemical analyses the coupling of the hydrogenases to PsaD are verified.

Preliminary results from the H_2 measurements of a coupled NiFe-hydrogenase to a full-length version of PsaD, show no increase in H_2 production, although western blot analyses using an anti-PsaD antibody verify the presence of PsaD chimeras. Sequencing results of the mutants also show a correctly integrated hydrogenase gene.

Based on the results a truncated PsaD is coupled to various hydrogenases which might ensure a closer proximity to the FeS cluster and possibly a higher H_2 production yield respectively. Two possible truncated PsaD versions as well as two hydrogenases from Chlamydomonas reinhardtii and Cupriavidus necator are currently investigated.

1. Appel, J. *et al. Nat Energy* **5,** 458–467; DOI: 10.1038/s41560-020-0609-6 (2020).
2. Kanygin, A. *et al. Energy Environ. Sci.* **13,** 2903–2914; DOI: 10.1039/C9EE03859K (2020).
3. Nies, F. *et al. PLOS ONE* **15,** e0234440; DOI: 10.1371/journal.pone.0234440 (2020).

OP-BSM-012
Influence of expression vector design on the simultaneous heterologous production of cytochromes and rusticyanin from Acidithiobacillus ferrooxidans in Escherichia coli and Vibrio natriegens
*H. Fuchs[1], S. R. Ullrich[1], L. Pester[1], S. Hedrich[1]
[1]*Technische Universität Bergakademie Freiberg, Institute of Biosciences, Freiberg, Germany*

Heterologous protein production is often employed to accelerate protein characterization and interaction studies. This can be especially relevant for proteins from acidophiles since these microorganisms only produce low biomass. However, the production of proteins from acidophiles in neutrophilic hosts, e.g. *Escherichia coli*, often poses a great challenge. Correct protein folding and co-factor integration need to be achieved despite the difference in extracellular and periplasmic pH. The Gram-negative acidophile *Acidithiobacillus ferrooxidans* possesses the remarkable ability to transfer electrons through its outer membrane to exocellular iron. This requires an electron transfer chain that spans both membranes and the periplasm and consists of three c-type cytochromes and the copper redox protein rusticyanin. We aim to investigate the functionality of this exocellular electron transfer chain and reconstructed it in several *E. coli* expression strains and *Vibrio natriegens* Vmax X2. We previously reported that *E. coli* and *V. natriegens* were able to express the genes from a pET16b-based vector construct and translocated all proteins to the correct cell compartment. Recent experiments focused on the impact of the expression vector design on the production of our four proteins of interest. The genes were encoded on a pRSF-1b vector, significantly reducing plasmid size compared to the pET16b-based construct. Additionally, we investigated a pCOLADuet-1-based construct where the inner membrane c-type cytochrome Cyc2 is under the control of a separate T7 promoter. The use of the pCOLADuet-1-based construct improved the production of Cyc2 in *V. natriegens*, while the viability of the *E. coli* hosts was reduced. This indicates that *V. natriegens* might be an interesting chassis for the production of integral membrane proteins. Additionally, *V. natriegens* possess a distinct advantage over *E. coli* for the production of c-type cytochromes. It does not require the co-expression of the cytochrome c maturation genes from an additional plasmid under aerobic conditions.

OP-BSM-013
Novel CRISPR/Cas system for highly efficient genome editing in A. niger
*R. Heger[1], S. Vetter[1], P. Scholz[1], C. Zurek[1]
[1]*Akribion Genomics AG, Zwingenberg, Germany*

The filamentous fungus *Aspergillus niger* (*A. niger*) is known for its high capacity for protein secretion. CRISPR/Cas-based systems are currently being used to generate high-yielding *A. niger* production strains. Given the difficult patent situation for the CRISPR nucleases known and used so far, limiting commercial use, we identified new Cas nucleases for genome editing based on metagenomic DNA analyses from environmental samples. The generated dataset allowed us to identify several novel nucleases, so-called G-dase M, with little or no homology to Cas proteins from public databases. To obtain a proprietary CRISPR tool for strain development of *A. niger*, a previously characterized G-dase M nuclease was established for genome editing.

We have successfully shown that the selected G-dase M nuclease can be programmed to efficiently and precisely introduce DNA double-strand breaks into the *A. niger* genome. An AMA1-based plasmid was adapted for optimized G-dase M expression and tRNA-gRNA transcription in *A. niger*. The functionality of the novel CRISPR/G-dase M single plasmid system was evaluated by clone reduction assays for different chromosomal genes in the NHEJ-deficient *A. niger* host strain. Furthermore, CRISPR/G-dase M activity induced HDR-mediated gene-specific integration of a reporter gene into various chromosomal targets with an efficiency of up to 99 %. In addition, we have developed a system to reduce the metabolic burden of plasmid-based overexpression of the nuclease by uncoupling the expression of G-dase M and tRNA-gRNA. For this purpose, the G-dase M expression cassette was integrated into the genome of *A. niger*, while the regulatory elements for tRNA-gRNA transcription were provided on the AMA1-based plasmid. This strategy effectively reduced the metabolic burden caused by overexpression of the episomal expressed nuclease.

In summary, we have developed a highly efficient CRISPR system for genome editing in *A. niger* using the novel class 2 type V G-dase M nuclease. These results have shown the functionality of our proprietary CRISPR nuclease G-dase M in *A. niger* and will thus expand the molecular toolbox for genetic modification of filamentous fungi.

OP-BSM-014

A global search for high-susceptibility targets of programmable RNA antibiotics

*L. Popella[1], J. Jung[1], L. Barquist[2,3], J. Vogel[1,2,3]
[1]*Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, Germany*
[2]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*
[3]*Faculty of Medicine, University of Würzburg, Würzburg, Germany*

The growing emergence of multidrug-resistant bacterial pathogens urgently demands the development of alternative antibiotics. Antisense oligomers (ASOs), such as based on peptide nucleic acid (PNA), can exert potent bactericidal effects when designed to sequester the ribosome binding site of an mRNA of an essential gene. Over the years, various essential genes have been investigated for their vulnerability to PNA targeting in enteric bacteria, with the *acpP* gene encoding acyl carrier protein being considered the most susceptible PNA target. However, it remains unknown what makes for a potent PNA or PNA target. We present a systematic investigation of the efficacy of antisense PNAs for 293 selected essential genes, tested in both *E. coli* K12 and uropathogenic *E. coli* (UPEC). Our screen has produced 23 and 55 potent antibacterial PNAs, corresponding to 21 and 47 essential target genes, in K12 and UPEC, respectively. In addition, we discover both strain-independent and strain-specific antibacterial PNA activities. Bioinformatic analysis will enable us to decipher common traits of the identified most efficient PNA sequences and determine core features of target susceptibility. Further, characterization and testing of UPEC-specific PNAs on a broader panel of Enterobacteriaceae may help to generate species-specific antibacterials. Overall, this study gives new insights into sequence- and target gene-dependent differences in PNA susceptibility and provides information on putative universal predictors of PNA efficacy.

OP-BSM-015

Harnessing synthetic biology tools for chemical production in Lithoautotrophic Biorefineries

*P. Schoenmakers[1], A. Ihl[1], C. Tahiraj[1], R. Rad[2], I. Weickardt[3], S. Guillouet[3], U. P. Apfel[4,2], L. Lauterbach[1]
[1]*RWTH Aachen University, iAMB, Aachen, Germany*
[2]*Ruhr University Bochum, Inorganic Chemistry I, Bochum, Germany*
[3]*TBI Bio & Chemical Engineering, INSA, Toulouse, France*
[4]*Fraunhofer UMSICHT, Electrosynthesis, Bochum, Germany*

Introduction: *Cupriavidus necator* efficiently sequesters CO_2, utilizing H_2 to recycle cofactors through its native hydrogenases[1]. Advancing synthetic biology tools is crucial to redirect carbon to valuable products like *N*-heterocycles. Since *C. necator* is an obligate aerobe, autotrophic cultivation usually entails hazardous pressurized knallgas.

Goals: We developed synthetic biology tools and safe electro-fermentation methods for *C. necator*, enabling electricity-driven biotransformations and pioneering the development of novel pathways for *N*-heterocycle synthesis[2,3].

Methods: Our engineered *C. necator* features two genomic integrations: 1) rhamnose-inducible dCas9 for gene repression; 2) arabinose-inducible T7 RNA polymerase-cytidine deaminase fusion (MT7) for gene expression and pathway hypermutation. A newly developed reactor incorporating a biocompatible electrolyzer regenerates hydrogen *in-situ*[4], with integrated sensors for precise monitoring of dissolved- and headspace gasses.

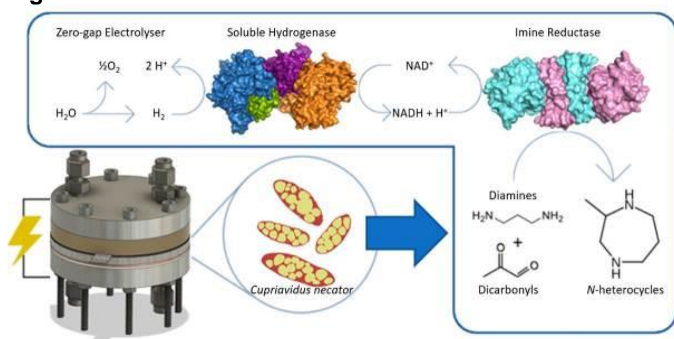
Results: Genomic integration of inducible MT7 and dCas9 systems regulated gene expression and enabled pathway evolution. These tools were applied to accelerate the development of new-to-nature pathways for *N*-heterocycle biosynthesis. Furthermore, an electro-fermentation reactor ensured safe and efficient synthesis of isopropanol, using a previously engineered strain[5], as a proof-of-concept.

Conclusions: Genomic integration of molecular tools significantly enhanced future *C. necator* strain development. Additionally, the developed electro-fermentation reactor was successfully implemented for isopropanol production. The utility of this reactor extends to newly developed strains for biosynthesis of *N*-heterocycles.

References:

1. Al-Shameri A., Siebert D. L., Sutiono S., **Lauterbach L.** & Sieber V. *Nat. Commun.* **14**, (2023).
2. 2. Al-Shameri A., Petrich M-C M., Puring K.j., Apfel U.-P., Nestl B.M., **Lauterbach L.**, *Chemie - Int. Ed.* **59**, 10929–10933 (2020).
3. 3. Al-Shameri A., Borlinghaus N., Schneller P., Nestl B.M., **Lauterbach L.**, *Green Chem.* **21**, 1396–1400 (2019).
4. 4. Rad, R. *et al. Cell Reports Phys. Sci.* **4**, 101526 (2023)
5. Grousseau E. *et al. Appl. Microbiol. Biotechnol.* **98**, 4277–4290 (2014).

Fig. 1



OP-BSM-016
Beyond rational : Independent biosensor-guided evolution of 100 strain variants and comparative analysis of their genomes

P. Baumann[1], M. Dal Molin[1], H. Aring[1], K. Krumbach[1], M. F. Müller[1], S. Noack[1], *J. Marienhagen[1]
[1]*Research Center Juelich , IBG-1: Biotechnology, Jülich, Germany*

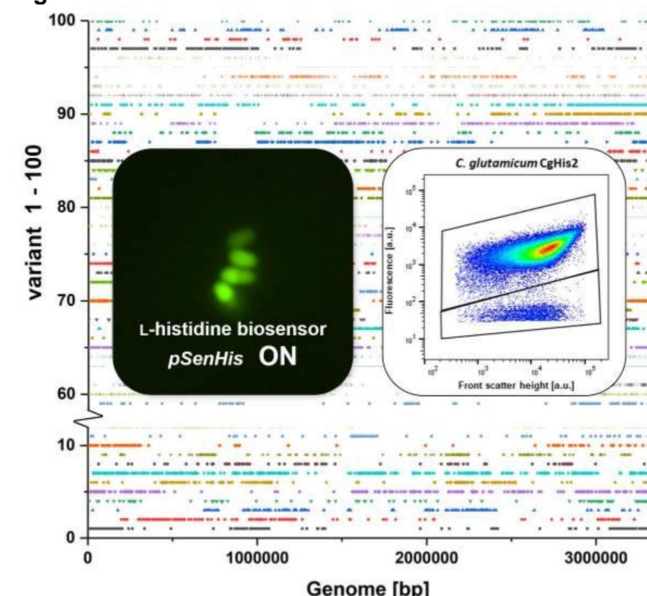
In contrast to rational metabolic engineering, classical strain development strongly relies on random mutagenesis and screening for the desired production phenotype. Nowadays, with the availability of biosensor-based FACS-screening strategies, these random approaches are coming back into fashion.

Here, we employ this technology in combination with comparative genome analyses to identify novel mutations contributing to product formation in the genome of a *Corynebacterium glutamicum* L-histidine producer. Since all known genetic targets contributing to L-histidine production have been already rationally engineered in this strain, identification of novel beneficial mutations can be regarded as challenging, as they might not be intuitively linkable to L-histidine biosynthesis. For the identification of 100 improved strain variants, we performed > 600 chemical mutagenesis, > 200 biosensor-based FACS-screenings, isolated > 50,000 variants with increased fluorescence, and characterized > 4,500 variants with regard to biomass formation and L-histidine production. Based on comparative genome analyses of these 100 variants accumulating 10-80% more L-histidine, we discovered several beneficial mutations. Combination of selected genetic modifications allowed for the construction of a strain variant characterized by a doubled L-histidine titer (29 mM) and product yield (0.13 C-mol C-mol^{-1}) in comparison to the starting variant.

This study may serve as a blueprint for the identification of novel beneficial mutations in microbial producers in a more systematic manner. This way, also previously unexplored genes or genes with previously unknown contribution to the respective production phenotype can be identified. We believe that this technology has a great potential to push industrial production strains towards maximum performance.

Baumann P.T., Dal Molin M., Aring H., Krumbach K., Müller M.-F., Vroling B., van Summeren-Wesenhagen P.V., Noack N., Marienhagen J. (2023). Beyond rational - biosensor-guided isolation of 100 independently evolved bacterial strain variants and comparative analysis of their genomes. *BMC Biology* 21:183. (https://doi.org/10.1186/s12915-023-01688-x)

Fig. 1



OP-BSM-017
A step-by-step roadmap involving physiological characterization, genomics, fluxomics, and tailored fermentation to empower Paracoccus pantotrophus DSM 2944 as a metabolically versatile SynBio chassis

*U. Pal1, T. Tiso1, D. Bachmann1, L. M. Blank1
1RWTH Aachen University, Institute of Applied Microbiology, Aachen, Germany

Introduction:

Paracoccus pantotrophus DSM 2944, a Gram-negative bacterium, was selected for its metabolic robustness and potential as a SynBio (synthetic biology) chassis due to its diverse substrate utilization including cheap and renewable feedstocks, CO2, and other C1 and C2 compounds coupled with the production of biopolymer polyhydroxyalkanoates (PHAs).

Goals:

The study aims to transform *P. pantotrophus* DSM 2944 into a proficient SynBio chassis through in-depth physiological, metabolic, computational, and phylogenetic studies coupled with industrially related bioreactor scale-up.

Results:

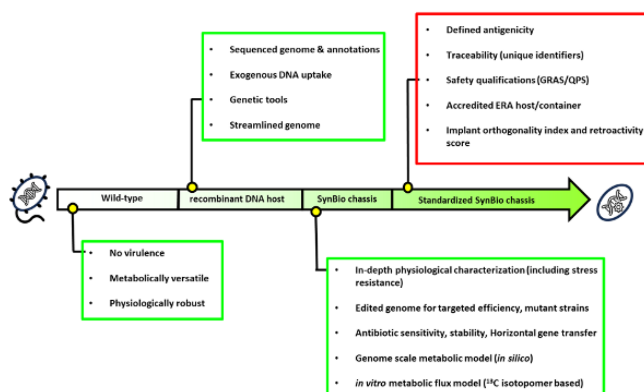
To investigate the suitability as a new chassis organism, extensive studies exploring substrate utilization and tolerance, optimal growth temperature, pH values, and phylogenetic studies were performed in *P. pantotrophus* DSM 2944. Moreover, to elucidate the strain"s metabolic flux in vivo, an in-depth analysis of flux distributions was also using 13C labeled glucose. The flux map showed that *P. pantotrophus* DSM 2944 prefers the pentose phosphate and Entner-Doudoroff pathways over glycolysis offering a surplus co-factor regeneration coupled with energy generation. Furthermore, to facilitate *in-silico* simulation of an organism"s growth and metabolism, the genome-scale metabolic model was constructed and validated with experimental data. A genetic toolbox was also designed comprising promoters with tunable strengths, gene integration and deletion strategies, and compatible origins of replication. Highlights

include improved growth on the non-native carbon-source terephthalic acid along with native carbon-source ethylene glycol, the two monomers from polyethylene terephthalate (PET). Finally, tailor-made DO-based fermentation strategies were established, showcasing the applicability of the strain in bioreactors regarding PHA production.

Conclusions:

Through the implementation of the chassis roadmap and comprehensive research, *P. pantotrophus* DSM 2944 emerges as a promising SynBio chassis. Its metabolic versatility and engineered capabilities highlight its potential in advancing sustainable bioeconomy initiatives, paving the way for a greener future.

Fig. 1



OP-BSM-018

Utilizing synthetic yeast chromosomes to map and phenotype large scale-deletions in high-throughput

*T. A. Lindeboom1, D. Schindler1, M. Sánchez Olmos1
1Max-Planck Institute for Terrestrial Microbiology, RG Schindler, Marburg, Germany

Question: The Synthetic Yeast Genome Project (Sc2.0) aims to produce the first synthetic eukaryotic genome. All synthetic chromosomes have been synthesised and characterised in individual strains. The sixteen synthetic chromosomes and the tRNA neochromosome are being consolidated in a single cell.1 All chromosomes have been redesigned according to strict principles. One notable feature is the insertion of symmetrical loxP sites downstream of almost every non-essential gene. Upon activation of Cre recombinase, this leads to highly complex structural variations, turning a single genotype into a population. Here we present our strategy and technology for using synthetic yeast to systematically study the effects of large-scale deletions, complementing our knowledge of single gene deletion libraries.

Methods: The loxP sites are 34 nucleotides in length. We use this as a landing pad to randomly integrate a modified marker by homologous recombination (HR). The resulting population is transformed with a plasmid overexpressing a system to introduce a double-strand break in the marker. The DNA is degraded in vivo until HR occurs at loxP sites. Counter selection ensures that only candidates that have lost the marker are obtained. We use laboratory automation to select and characterise large numbers of candidates. Based on our high-throughput, low-cost end-point genotyping, we can rapidly identify the deletion size for each strain.2,3 This is followed by phenotyping of the relevant strains.

Results: We have developed and established a strategy for targeted introduction of large-scale deletions into synthetic yeast chromosomes. The large deletions will be characterised using molecular and phenotyping techniques to understand their impact on cell viability.

References

- (1) Richardson S, et al. (2017) Design of a synthetic yeast genome. Science, 355(6329), 1040-1044.
- (2) Mitchell LA, et al. (2015) qPCRTag Analysis--A high throughput, real time PCR assay for Sc2.0 genotyping. J Vis Exp 25:(99):e52941.
- (3) Lindeboom TA, Sánchez Olmos MdC, et al. (2022) L-SCRaMbLE creates large-scale genome rearrangements in synthetic Sc2.0 chromosomes. bioRxiv.

Diagnostic and Clinical Microbiology

OP-DCM-001

Accuracy of a fluorescence flow cytometer (Sysmex XN-31) for diagnosing malaria: two years of experience in a tertiary care hospital

*H. L. Verhasselt[1], K. Krämer[2], D. Schmidt[1], J. Buer[1], P. M. Rath[1]
[1]*University of Duisburg-Essen, University Hospital Essen, Institute of Medical Microbiology, Essen, Germany*
[2]*MVZ Dr. Stein and colleagues, Laboratory Mönchengladbach, Mönchengladbach, Germany*

Question: Early and accurate diagnosis is essential for effective management of malaria. In non-endemic areas, laboratory staff may lack experience with malaria and fail to detect parasites when examining blood smears microscopically. Non-microscopy based methods like immunochromatographic assays, PCR and more recently fluorescence flow cytometry using Sysmex XN-31 (Sysmex Deutschland GmbH, Norderstedt) has been registered to support malaria diagnosis. XN-31 is fully automated with a limit of detection of 20 parasites/µl. We assessed the diagnostic accuracy of the XN-31 within a prospective study for malaria diagnosis compared to microscopy and PCR. Methods: 203 blood samples were analysed by XN-31, thin blood film after automatic Giemsa staining with Aerospray Pro (Kreienbaum GmbH, Langenfeld) and RealStar Malaria Screen & Type PCR Kit 1.0 (altona Diagnostics GmbH, Hamburg). An immunchromatographic assay was also performed but results were not taken into account in ths study. In order to assess interlaboratory performance of XN-31, a small subset of samples (n=14) was evaluated in a second laboratory. Results: Over two years, 137 negative and 70 malaria positive blood samples were analysed initially and 145 follow-up samples. Compared to microscopy, XN-31 showed high sensitivity (98.4%), specificity (100%) as well as excellent negative predictive value (NPV, 99,3%) and positive predictive value (PPV, 100%) for the initial diagnosis of malaria. Agreement was high with Cohen`s kappa of 0.988. In comparison to PCR, values were similar: sensitivity 92.6%, specificity 100%, NPV 96%, PPV 100% and high agreement (Cohen`s kappa 0.941). In five cases, the XN-31 result was not available. XN-31 separated *P. falciparum* from non-falciparum successfully and parasitaemia determination was performed within one minute. Interlaboratory comparison showed high reproducibility of XN-31. Conclusions: Malaria diagnosis can be improved in non-endemic areas by use of XN-31 combined with microscopy

and PCR. Parasite load is provided in significant shorter time by the device making diagnosis of malaria faster. Especially in follow-up samples microscopy can likely be replaced by XN-31.

OP-DCM-002

Development and diagnostic accuracy field testing in Sudan of a novel serodiagnostic POC for Visceral Veishmaniasis

*U. Steinhoff[1], R. Mahdavi[1], E. Abass[2]

[1]*Philipps University Marburg, Medical Microbiology and Hospital Hygiene, Marburg, Germany*

[2]*Imam Abdulrahman Bin Faisal University, Clinical Laboratory Science Department, Dammam, Saudi Arabia*

Introduction

Visceral leishmaniasis (VL) is the most severe form of leishmaniasis leading to death if not properly diagnosed and treated. Reliable diagnosis of infected patients and reservoir animals (dogs) is vital for controlling the spread of leishmaniasis. East-African countries suffer from a very high incidence of VL and although several diagnostic tests are available, point of care diagnosis (POC) continues to be a big challenge in these countries due to low sensitivity and specificity in these countries.

Goal

The aim was to develop a reliable, stable and easy to handle POC test that significantly improves the VL-diagnostic efficiency in East-Africa and other VL-endemic countries in both, humans and dogs.

Material & Methods

Bioinformatic and biochemical analysis of B-cell epitopes of kinesins from different East African VL-strains lead to the identification of a new diagnostic kinesin antigen (rKLi8.3) with high B-cell affinity. A novel cloning and expression strategy was developed to express rKLi8.3 for the production of a new and improved lateral flow test (LFT) POC.

Results

Prospective comparative field-testing of the rKLi8.3 LFT with the standard rK39 LFT in Sudan (Gedaref) on 107 VL suspects revealed 98.7% sensitivity and 100% specificity. No cross-reactivity was observed in patients with malaria, typhoid, brucellosis and tuberculosis and trypanosomiasis. Retrospective and comparative testing of the rK39 and rKLi8.3 ELISAs on 300 sera from Sudanese patients and controls revealed a sensitivity of 93.2% to 97.2% and a specificity of 93.6% to 99.2%, respectively. Similar results were observed in VL-patients from Brazil. In contrast to rK39, the rKLi8.3 based ELISA and LFT showed no cross-reactivity with African and American Trypanosomiasis. Similar results were also obtained in VL-infected dogs (CanL) in Brazil and Croatia.

Summary

rKLi8.3 based LFTs offer substantially improved diagnostic efficiency for VL in East Africa and other endemic areas, compared to currently available sero-diagnostic tests. Further, rKLi8.3 LFT are easy to handle and very heat stable, thus excellent for the use in tropical and remote endemic areas.

OP-DCM-003

Detection of Francisella tularensis holarctica specific SNP by a qPCR using T-Blocker technology

*A. Don Mallawarathy[1], *H. von Buttlar[1]

[1]*Bundeswehr Institute of Microbiology, Bacteriology and Toxinology, München, Germany*

Francisella tularensis is a Gram-negative bacterium causing Tularemia. While the high pathogenic subspecies *Francisella tularensis tularensis* has been isolated nearly exclusively in Northern-America the moderate pathogenic subspecies *Francisella tularensis holarctica* is distributed over the complete northern hemisphere. Especially, *Francisella tularensis tularensis* has a high potential to be misused as biowarfare agent. Therefore, the assessment of the subspecies of *Francisella tularensis* is of great importance for patient´s prognosis or as an indication of intentional exposition if Francisella tularensis tularensis is found outside of Northern-America.

To discriminate *Francisella tularensis holarctica* from other Francisella species and subspecies a SNP polymorphism in the 23s rRNA gene can be used. Besides probe based qPCR systems SNP can be detected by T-Blocker PCR. This technology uses forward primers that end at the SNP position. This is followed by a blocking-primer that binds, if the SNP is not complemetary to the forward primer and prevents amplification. So the amplification is less effective and by comparing ct values of T-Blocker qPCR for both alleles the allele can be distinguished. The set up and validation of this T-Blocker qPCR assay to confirm the occurence of *Francisella tularensis holarctica* in a sample is presented.

OP-DCM-004

A head-to-head comparison of three MALDI-TOF mass spectrometry systems with 16S rRNA gene sequencing

*K. Dichtl[1], I. Klugherz[1], H. Greimel[1], J. Luxner[1], J. Köberl[1], S. Friedl[1], I. Steinmetz[1], E. Leitner[1]

[1]*Medical University of Graz, Diagnostic and Research Institute for Hygiene, Microbiology and Environmental Medicine, Graz, Austria*

Background: Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized diagnostics in bacteriology. To date, virtually all MALDI-TOF MS based diagnostics is carried out using systems of only two manufacturers, i.e., Bruker Daltonics (MALDI biotyper system) and bioMérieux (VITEK MS system). Recently, a new competitor (Zybio) launched the novel EXS2600 MALDI-TOF MS device. This study aimed to evaluate the performance of the three test systems by comparing the results to 16S rRNA gene sequencing.

Methods: A set of 356 previously collected difficult-to-identify bacterial isolates was tested in parallel with the three systems relying only on the direct smear method and simple formic acid extraction.

Results: MALDI Biotyper, EXS2600, and Vitek MS yielded valid results for 98.6 %, 94.4 %, and 93.3 % of all isolates, respectively. Of all valid results, agreement with sequencing data was achieved in 98.9 % (MALDI Biotyper), 98.5 % (EXS2600), and 99.7 % (Vitek MS). Misidentification rates

were 0 %, 2.6 %, and 1.1 % for MALDI Biotyper, EXS2600, and Vitek MS, respectively, when only the isolates with valid measurements at single species level were considered. Minor differences concerning the handling were observed between the respective devices and software systems.

Conclusion: The three systems provided comparable results. Our data demonstrate that all systems are suitable for the use in medical diagnostic laboratories.

OP-DCM-005

Discrimination of Acinetobacter baumannii from Acinetobacter nosocomialis using lipid extractions and subsequent rapid MALDI-TOF MS analysis in negative ion mode

*M. Czaja[1,2], S. N. Richter[3], I. D. Nix[3], K. Sparbier[3], B. Oberheitmann[3], M. Kostrzewa[3], A. B. Pranada[1]
[1]*MVZ Dr. Eberhard & Partner Dortmund, Department of Medical Microbiology, Dortmund, Germany*
[2]*Hochschule Biberach University of Applied Science, Biberach, Germany*
[3]*Bruker Daltonics GmbH & Co. KG , Bremen, Germany*

Introduction: Precise species identification is crucial, especially for infection control. Due to its accuracy, MALDI-TOF MS has become a standard identification method in many microbiology laboratories. Nevertheless, for species with very similar proteomic spectra differentiation capability is limited, e.g. between *Acinetobacter baumannii* and *A. nosocomialis.* Using MALDI in negative ion mode with lipids as target might overcome this limitation.

Goals: Here, we investigated lipid extracts for the discrimination of *A. baumannii* from *A. nosocomialis* using a MALDI-TOF MS-based rapid workflow in negative ion mode.

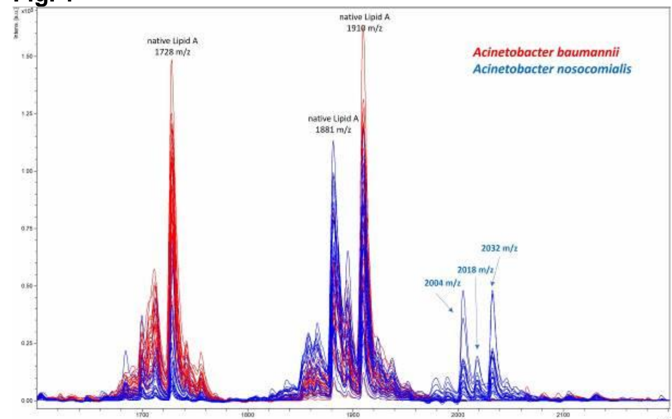
Materials & Methods: Heat-inactivated isolates of genetically characterized *A. baumannii* (n=30) and *A. nosocomialis* (n=25) including each type strain, were processed with the MBT Lipid Xtract Kit (Bruker Daltonics, Bremen, Germany) MALDI spectra of the obtained lipid extracts were acquired with an MBT Sirius mass spectrometer (Bruker Daltonics) in negative ion mode and analysed using FlexAnalysis software.

Results: The lipid spectra of *A. baumannii* and *A. nosocomialis* reproducibly showed each specific peaks at m/z 1728, 1881 and 1910 with different intensities, corresponding to lipid A molecules of the outer membrane. *A. baumannii* showed high intensities for the two peaks at m/z 1728 and 1910 while *A. nosocomialis* showed high intensities at m/z 1881 instead. Three specific peaks at m/z 2004, 2018 and 2032 were only present in *A. nosocomialis* spectra, which could be used for species discrimination (Fig. 1).

In total, 55 spectra were analyzed. The discriminating peaks showed a consistent appearance amongst the different strains of both species. The presence of the peaks at m/z 2004, 2018 and 2032 and simultaneously high intensities of the peak at m/z 1881 discriminated *A. nosocomialis* from *A. baumannii.* In total 54/55 (98%) strains were correctly identified using these specific lipid markers.

Summary: Differentiating lipid markers could be used in a decision tree analysis to discriminate the two species *A. baumannii* and *A. nosocomialis* reliably and quickly. Further studies will have to be carried out to establish an algorithm for clinical diagnostics.

Fig. 1



OP-DCM-006

Evaluation of FT-IR spectroscopy and machine learning for the discrimination of Escherichia coli and Shigella spp.

*M. Cordovana1, N. Mauder1, A. B. Pranada2, F. Pankok3, U. Loederstaedt3, S. Scheithauer3, D. Dekker4, A. E. Zautner5, W. Geißdörfer6, J. Overhoff7, M. Werner7, A. Wille7, H. Frickmann8,9
1Bruker Daltonics GmbH & Co. KG , Bremen, Germany
2MVZ Dr. Eberhard & Partner Dortmund, Department of Medical Microbiology, Dortmund, Germany
3University Medical Center, Georg-August University Göttingen, Department of Infection Control and Infectious Diseases, Göttingen, Germany
4Bernhard Nocht Institute for Tropical Medicine, The One Health Bacteriology Group, Hamburg, Germany
5Otto von Guericke University Magdeburg, Health Campus Immunology, Infectiology and Inflammation (GCI3), Medical Faculty and Center for Health and Medical Prevention (CHaMP), Magdeburg, Germany
6University Hospital Erlangen, Institute of Microbiology – Clinical Microbiology, Immunology and Hygiene, Erlangen, Germany
7City of Hamburg, Institute for Hygiene and Environment, Hamburg, Germany
8Bundeswehr Hospital Hamburg, Department of Microbiology and Hospital Hygiene, Hamburg, Germany
9University Clinics Rostock, Institute of Medical Microbiology, Rostock, Germany

Background. The differentiation of *Escherichia coli* (Ec) from *Shigella* spp., the identification of *Shigella* at species level, as well as the discrimination of Ec at serotype level to delineate pathogenic lineages, are challenging with common routine methods. They require serological/genomic typing approaches, which present some disadvantages in terms of costs, ease-of-use and applicability in routine settings. In this study, we evaluated the discriminative power of Fourier-Transform Infrared (FT-IR) spectroscopy to distinguish *E. coli* isolates at serotype level and to delineate *E. coli* from *Shigella* species.

Material/Methods. 225 genomically or serologically typed strains (n=132 Ec belonging to 71 serotypes, n=93 *Shigella* spp.) were investigated. FT-IR analysis was performed applying the IR Biotyper® system (IRBT - Bruker Daltonics, Bremen, Germany) following the manufacturer"s instructions. Exploratory data analysis was performed by principal component analysis (PCA) and linear discriminant analysis (LDA). Machine learning was applied to create an automated

classifier for the delineation of *E. coli* from *Shigella* spp., using the algorithms included in the IRBT software. One hundred and twenty-three isolates were used to build the training set, representing all 75 groups (Ec serotypes + the 4 *Shigella* species). The remaining 102 isolates were used as a testing set.

Results. Exploratory data analysis showed that IRBT clustering is correlated with the *E. coli* O-H serotypes and the *Shigella* species. PCA/LDA showed that the *E. coli* serotypes and the 4 *Shigella* species are separable. The classifier showed an accuracy of 99% (101/102), with one O157:H7 isolate misclassified as *S. sonnei*.

Conclusion. IR Biotyping showed the potential of delineating *E. coli* at serotype level, and of discriminating *E. coli* from *Shigella* spp., demonstrating its potential suitability for infection control, public health and epidemiological assessments. Further investigation is underway to confirm and strengthen these promising preliminary results.

OP-DCM-007

Metagenomic analysis of cell-free DNA from whole blood specimens of patients with suspected infections: performance and therapeutic impact in clinical routine

J. Esse[1], J. Träger[1], S. Krause[2], L. Herbst[3], R. Strauß[4], P. Morhart[5], N. Naumann-Bartsch[5], M. Chada[5], I. Castellanos[6], *J. Held[1]
[1]*University Hospital Erlangen, Mikrobiologisches Institut, Erlangen, Germany*
[2]*University Hospital Erlangen, Medical Clinic 5, Erlangen, Germany*
[3]*University Hospital Erlangen, Medical Clinic 4, Erlangen, Germany*
[4]*University Hospital Erlangen, Medical Clinic 1, Erlangen, Germany*
[5]*University Hospital Erlangen, Pediatric and adolescent clinic, Erlangen, Germany*
[6]*University Hospital Erlangen, Anesthesiology clinic, Erlangen, Germany*

Background: Sensitivity of blood culture analysis is limited especially in cases of existing antibiotic therapy and some pathogens, like *Aspergillus* spp., do not grow in blood cultures at all. Metagenomic analysis of cell-free DNA (cfDNA) from whole blood has the potential to compensate for the disadvantages of blood culture diagnostics.

Methods: The study was conducted over 3 months at the University Hospital Erlangen. Adult and pediatric patients with suspected infections were included. cfDNA from whole blood was analysed by metagenomic next-generation sequencing with an Illumina NextSeq instrument. Sequences were analysed with the DISQVER® platform (Noscendo GmbH, Germany), comprising a CE-IVD-labelled software algorithm and a curated database. In parallel, a minimum of two blood cultures were analysed.

Results: 203 samples of 156 patients (25 pediatric, 131 adult) were analysed. 12 samples (5.9%) were excluded from metagenomic analysis due to technical reasons. Mean time from sampling to result of metagenomic analysis (including logistics) was 3 days. A total of 160 pathogens were detected (1-10 pathogens per sample, mean 2). The positivity rate was 42.4%. Detected pathogens were 103 bacteria, 51 viruses, 4 fungi and 2 parasites. The most common bacteria were Enterobacteriaceae (n=31), Enterococci (n=16) and Staphylococci (n=11). The most common viruses were Epstein-Barr-virus (n=15), Herpesvirus-6B (n=10) and Cytomegalovirus (n=9). *Mycobacterium avium*, *Legionella pneumophila*, *Tropheryma whipplei, Rhizomucor pusillus, and Leishmania infantum* were detected in one sample each. Parallel blood cultures were positive in 11.2%. However, 62% of patients received antibiotic therapy at the time of blood culture sampling. The bacterial pathogens detected by metagenomic analysis were confirmed by classical microbiological diagnostics in 47% of cases. The analysis of the clinical data and the impact of the metagenomic analysis on anti-infective therapy are currently being investigated.

Conclusions: Metagenomic analysis of cfDNA with the DISQVER® platform found a pathogen in a significant number of samples in which classical microbiological diagnostics were negative.

OP-DCM-008

Can machine learning algorithms predict negative urine cultures using flow cytometry routine data?

A. Brenner[1], J. Esser[2], F. Schuler[3], J. Varghese[1], *F. Schaumburg[3]
[1]*University of Münster, Institute of Medical Informatics, Münster, Germany*
[2]*University Hospital Münster, Central Facility for Laboratory Medicine, Münster, Germany*
[3]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

Introduction

Urine samples are among the most frequent samples analysed in microbiology laboratories, while a large proportion are culture-negative. The aim of this study was to test whether culture-negative samples can be predicted from routine flow cytometric data and how reliable prediction algorithms are over time.

Materials & Methods

In 2023, 1325 urine samples from the University Hospital Münster were used for a training dataset (n=1032) and three independent test datasets (n=93-100 samples) that were collected three months apart. Predictors from flow cytometry were total counts per µl of bacteria, erythrocytes, yeast-like-cells, hyaline casts, crystals, leukocytes, squamous epithelial cells, non-hyaline casts and non-squamous epithelial cells in addition to type of urine sample as well as age and sex of the patient. Two different labels were applied (positive culture defined as any bacterial growth [yes/no] and detection of a clinically relevant uropathogenic species [yes, no]). Three classifiers (decision tree, random forest classifier, CatBoost) were cross-validated on the training dataset with 5 folds in order to select an optimized model with at least 95% sensitivity and maximum negative predictive value (NPV). Finally, the optimized model was trained on the complete training dataset and then evaluated on the three independent test sets.

Results

In total, 72.5% (960/1325) samples were culture positive with a predominance of *Escherichia coli* (n=295). In all three test sets the classifier predicted a negative culture with a moderate "balanced accuracy" (63-69%), "sensitivity" (94-97%) and "specificity" (31-44%). The test performance of the prediction algorithms was stable over a period of six months. Depending on the tested samples, the negative predictive value was 73-85%.

Summary

The machine learning algorithms in our study population had a moderate NPV to rule out culture negative isolates. Additional clinical parameters (e.g. pH) might improve the test performance.

OP-DCM-009
Prospective evaluation of the SeptiCyte® Rapid assay for the identification of systemic infectious diseases in patients with suspected sepsis

*S. Benthien[1], C. Ucarer[2], V. Mazuru[2], C. Zeiner[2], G. Danziger[2], P. Lepper[2], *S. L. Becker[1]
[1]*Saarland University, Institute of Medical Microbiology and Hygiene, Homburg, Germany*
[2]*Saarland University, Department of Internal Medicine, Homburg, Germany*

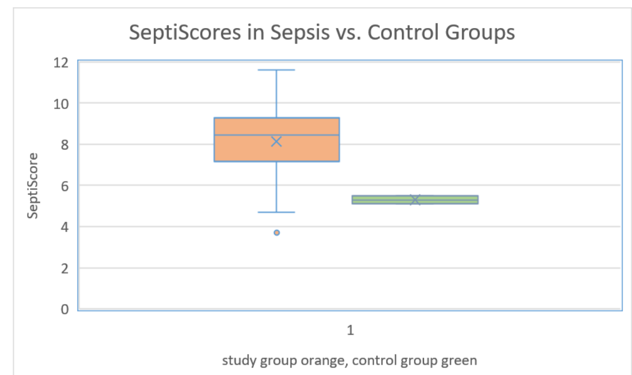
Question: Sepsis is a pathophysiologically complex condition with severe implications for patients and health care systems. In patients with suspected sepsis, the differentation between an infectious and non-infectious aetiology is challenging. Against this background, the SeptiCyte® Rapid assay was developed: This test detects two specific mRNA transcripts (PLA2G7 and PLAC8) that are part of the host"s immune response and that are differentially expressed during the early phase of systemic infection. While this test is commercially available, real-life clinical performance data are scarce.

Methods: Since November 2022, we prospectively enrolled patients with the clinical suspicion of sepsis (defined as an increase in sequential organ failure assessment (SOFA) score of ≥2 within 24 hours) and with antiinfective therapy for ≤ 48 hours on one ICU. Blood samples from the patients were collected using PAXgene RNA tubes and subjected to the SeptiCyte® Rapid assay. The test result is provided as a so-called SeptiScore, ranging from 0-15, with values ≥ 7.4 indicating a high suspicion of systemic infection. In parallel, samples from non-septic control patients from the same ICU were also analysed. Clinicians were blinded to the results.

Results: Until 30 January 2024, a total 50 patients were recruited, of which 36 belonged to the study group. 56% of the patients were male and the mean age was 65.1 years. In the preliminary dataset, symptomatic patients had an average SOFA score of 9.6. In 21/36 of symptomatic patients, a probable causative pathogen was detected, most frequently *Escherichia coli.* The average SeptiScore among clinically septic patients was considerably higher than in control patients (8.7 vs. 4.8, see also Figure 1). In patients with suspected sepsis, those with an identified causative agent had slightly higher scores than those without (9.0 vs. 8.2).

Conclusions: The SeptiScore Rapid assay was higher in patients with suspected sepsis than in control patients. Patient recruitment and further analysis are ongoing to evaluate whether the SeptiScore accurately differentiates those individuals with an infectious aetiology from those with other causes.

Fig. 1



OP-DCM-010
Performance evaluation of machine-assisted interpretation of Gram stains from positive blood cultures

*C. Walter[1], C. Weissert[2], E. Gizewski[3], I. Burckhardt[1], H. Mannsperger[3], S. Hänselmann[3], W. Busch[3], O. Nolte[2], S. Zimmermann[1]
[1]*University Hospital Heidelberg, Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg, Germany*
[2]*Centre for Laboratory Medicine, Division of Human Microbiology, St. Gallen, Switzerland*
[3]*MetaSystems Hard & Software GmbH, Altlussheim, Germany*

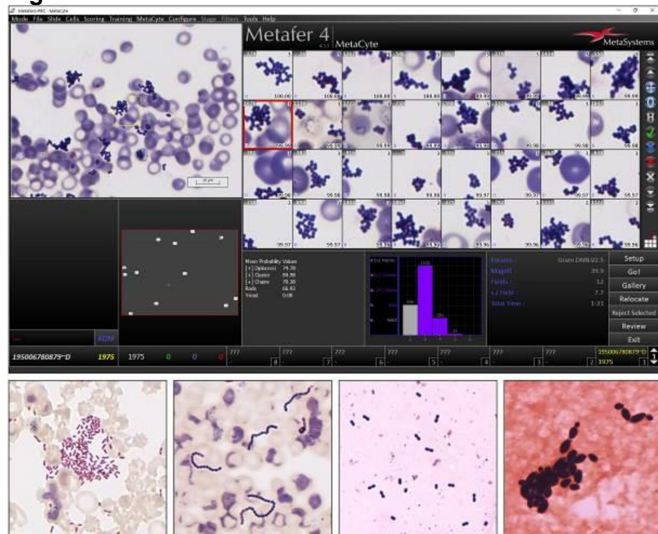
Background: Bloodstream infections are an important cause of severe morbidity and mortality. Manual microscopy of Gram stains from positive blood cultures (PBC) is pivotal in diagnosing bloodstream infections but remains labor-intensive, time-consuming, and subjective. The objective of the present study was to evaluate the potential of a scan and analysis system that combines fully automated digital microscopy with deep convolutional neural networks (CNN) to assist Gram stain interpretation from PBCs for routine laboratory use.

Methods: The CNN was trained to categorize images of Gram stains based on staining and morphology into seven different classes: negative/false-positive, gram-positive cocci in pairs (GPCP), gram-positive cocci in clusters (GPCCL), gram-positive cocci in chains (GPCC), rod-shaped bacilli (RSB), yeasts, and polymicrobial specimens (FIG1 exemplary image of the graphical user interface). 1555 Gram-stained slides of PBCs were scanned, pre-classified, and reviewed by medical professionals. The assisted Gram stain results were compared to manual microscopy as well as cultural species identification by MALDI-TOF MS as the corresponding reference standard. Furthermore, site-to-site reproducibility between three sites, repeatability, and limit of detection (LOD) were determined.

Results: Comparison of assisted Gram stain interpretation to manual microscopy yielded sensitivity and specificity of 95.8% / 98.0% (GPCCL), 87.6% / 99.3% (GPCP/GPCC), 97.4% / 97.8% (RSB), 83.3% / 99.3% (yeasts) and 87.0% / 98.5% (negative/false-positive), respectively. The comparison of assisted Gram stain interpretation to MALDI-TOF MS yielded similar results as the previous comparison. In the analytical performance study, assisted interpretation showed excellent reproducibility and repeatability. With the determined LOD of 10^5 CFU/ml, any microorganism in PBCs should be detectable.

Conclusion: The application has demonstrated its ability to classify microorganisms based on their Gram reaction, arrangement, and morphology. It has shown potential for implementation in clinical laboratories in the future. Furthermore, digital review allows off-site image interpretation.

Fig. 1



OP-DCM-011

Combination of molecular imaging with microbiome analysis: infection landscapes in clinical samples

Z. Xiong1,2, L. Kursawe3, S. Julia2,3, G. Fiedler2,3, A. Wießner2,3, K. Sarbandi3, E. Bednaric4, D. Theegarten4, A. Moter3,5, *J. Kikhney2,3

1Charité - University Medicine Berlin, Biofilmcenter, Institute for Microbiology, Infectious Diseases and Immunology, Berlin, Germany
2MoKi Analytics GmbH, Berlin, Germany
3Charité - University Medicine Berlin, Biofilmcenter, Institute for Microbiology, Infectious Diseases and Immunology, Berlin, Germany
4University Hospital Essen, Institute of Pathology, Essen, Germany
5Moter Diagnostics, Berlin, Germany

Question

Next-generation sequencing (NGS) is a powerful tool, which is broadly used for microbiome analysis. Its use for diagnostic applications, however, is hampered by technical challenges, such as lack of sensitivity and risk of contamination. Therefore, data might be difficult to interpret in the clinical setting. Whereas NGS allows the overall assessment of species involved, Fluorescence in situ hybridization (FISH) shows the abundance and localization of specific microbial populations. This way, key microbial species, which are directly associated with the host epithelial surfaces or invasive within the host tissue, can be identified. However, for proper FISH probe selection, prior knowledge on potential microbial candidates is required.

Methods

We developed MG-FISH (Microbiome-guided fluorescence in situ hybridization), which synergistically combines FISH with NGS out of consecutive tissue sections. Consequently, microorganisms (bacteria and fungi) are both identified and localized. We used MG-FISH to identify the bacterial species involved in a case of an aspirated, endoscopically removed peanut from a 78-year-old patient.

Results

We amplified and sequenced microbial DNA out of methacrylate-embedded tissue sections and identified *Staphylococcus, Streptococcus, Veillonella dispar* and *Actinomyces* as most abundant microorganisms. Using specific FISH probes for the respective target species, we visualized, quantified, and localized the microorganisms.

Conclusions

MG-FISH gives microbiome data a spatial dimension *in situ*: It allows quantification and distribution of the microorganisms involved, localization at and in the tissue, and identification of key pathogens. This will further the use of NGS for diagnostic applications.

OP-DCM-012

Antibiotic resistance and bacteriological profile of pathogens causing neonatal sepsis in Tanzania. A systematic review with meta-analysis

*D. Msanga[1], S. Mshana[2], O. Kurzai[3], C. Hartel[4]

[1]Catholic University of Health and Allied Sciences, Pediatric and Child Health, Mwanza, Tanzania, United Republic Of
[2]Catholic University of Health and Allied Sciences, Microbiology and immunology, Mwanza, Tanzania, United Republic Of
[3]Institute of hygiene and microbiology, Microbiology, Würzburg, Germany
[4]University Würzburg, paediatric and child health, Würzburg, Germany

Neonatal sepsis is the leading cause of neonatal mortality in Low- and Middle-Income countries (LMICs), where 99% of global neonatal mortality occurs. We performed a systematic review and meta-analysis to determine bacteriological profile, antibiotic susceptibility of pathogen and outcome of neonatal sepsis in Tanzania.

Methods: A systematic literature search on online databases such as PubMed/ Medline, Embase, Cochrane, google scholar and web of science for peer reviewed publications from year 2010 until May 2023 was done. The data were meta-analysed using STATA version 14 and risk ratios (RR) was used as the measure of the effect size with 95% CI.

Results: A total of 14 studies with 4077 neonates was included in the review. Overall, the pathogens with the most resistance were *Staphylococcus haemolyticus* (24%), *Staphylococcus aureus* (9.5%) and *Klebsiella pneumoniae* (7.9%). The overall mortality rate was 18%. The antibiotics used as second line were slightly more susceptible than the first line antibiotics: ampicillin and ciprofloxacin (RR= 1.195, 95%CI 1.121 - 1.269), ampicillin and cefotaxime (RR= 1.257, 95%CI 1.136 - 1.378) and ampicillin and ceftriaxone (RR= 1.250, 95%CI 1.184 - 1.316). We also noted comparable susceptibility between the antibiotics of the specific regimen: ampicillin and gentamicin (RR= 1.062, 95%CI 1.003 - 1.121), ceftriaxone and cefotaxime (RR= 1.052, 95%CI 0.866 - 1.237), meropenem and amikacin (RR= 1.481, 95%CI 1.241 - 1.722) and meropenem and piperacillin tazobactam (RR= 0.771, 95%CI 0.598 - 0.944). There was considerable heterogeneity among all analyses which might have resulted from the different regions where the studies were conducted

and the majority of studies were from tertiary hospital settings, which may overestimate the burden of AMR.

There is a high prevalence of AMR and neonatal sepsis mortality rate. There is no statistical significance between resistance in the first line regimen of ampicillin and gentamicin with comparable performance of antibiotics in the second line regimen. There is a need for revisiting the antibiotic guidelines of the management of neonatal sepsis in Tanzania especially in tertiary hospitals.

OP-DCM-013

Genetic Characteristics, Virulence Factors, and Clonality of ESBL-Producing *E. coli* and *K. pneumoniae*: a Comparative Study During and After National Action Plan on Antimicrobial Resistance in Mwanza, Tanzania.

*V. Silago[1], K. Oravcova[1], L. Matthews[1], S. Mshana[1], J. Seni[1], H. Claus[1]
[1]*Catholic University of Health and Allied Sciences, Microbiology and Immunology, Mwanza, Tanzania, United Republic Of*

Introduction: Antimicrobial resistance (AMR) poses a significant global health threat, necessitating regional-specific continuous surveillance to understand its dynamics and implications.

Goals: To respond to the changing landscape of AMR, a cross-sectional hospital-based study was conducted in two periods: June 2019 – June 2020, during the first Tanzania National Action Plan on AMR (NAP-AMR), and March – July 2023, after its completion.

Materials and Methods: Patients" sociodemographic and clinical data were collected by pre-tested structured questionnaires. A total of 2648 and 1929 samples (blood, urine, and pus) collected during and after NAP-AMR respectively, were cultured to isolate bacterial pathogens; identification was based on VITEK MS and 16S rDNA Sanger sequencing, and VITEK 2 was used to determine susceptibility towards antibiotic agents. Next-generation sequencing on the Illumina NextSeq 550 platform was used for sequencing ESBL *E. coli* (n=22) and ESBL *K. pneumoniae* (n=23) out of 64 and 79 ESBL strains, respectively isolated during NAP-AMR.

Results: ESBL-producing *E. coli* and *K. pneumoniae* phenotypes increased significantly after NAP-AMR [53.4%(134/268) vs. 73.8%(175/237), p<0.0001]. Specific increase for ESBL-*E. coli* [41.3%(64/155) vs. 61.7%(74/120), p=0.0084] and ESBL-*K. pneumoniae* [69.9%(79/113) vs. 85.5%(100/117), p=0.0057] was remarkable. *E. coli* ST 648 (40.9%, 9/22) and *K. pneumoniae* ST 280 (23.1%, 6/23) were predominant. The IncFII plasmid-replicon was predominantly found in both *E. coli* (68.2%, 15/22) and *K. pneumoniae* (65.2%, 15/23). The $bla_{CTX\text{-}M\text{-}15}$ gene was found in 81.5% (18/22) *E. coli* and all 23 *K. pneumoniae*. The virulence genes *yfc*V, *fyu*A, *sit*A, and *tra*T were found in 68.2%, 72.7%, 72.7%, and 77.3% of *E. coli* respectively while the virulence genes *acr*A/B, *ent*A/B/C/E, *fep*A-D/G, *fim*A-I/K, and *mrk*A-D/F/H-J were found in all *K. pneumoniae*.

Summary: This study underscores the escalating threat of highly virulent strains of ESBL-producing *E. coli* and *K. pneumoniae* causing infections. The study advocates for the stringent implementation of IPC measures to curb the spread of antibiotic-resistant pathogens and associated public health risks.

OP-DCM-014

Antimicrobial resistance surveillance of invasive Bacteroides fragilis isolated from blood, Europe, 2022

*M. Buhl[1], U. S. Justesen[2]
[1]*Institute for Clinical Hygiene, Nürnberg, Germany*
[2]*Odense University Hospital, Odense, Denmark*

Question

Bacteroides fragilis is the most frequent cause of anaerobic bacteraemia. Although recent data suggest a rise in resistance to antibiotics commonly recommended for therapy of this and other anaerobic bacteria, surveillance on antimicrobial resistance (AMR) remains limited due to a lack of both data availability and comparability. However, a newly introduced standardised method for antimicrobial susceptibility testing (AST) of anaerobic bacteria has made larger scale surveillance possible for the first time. Thus, a European multicentre study was initiated to investigate phenotypic AMR of *Bacteroides fragilis* isolates from bacteraemia across Europe in 2022.

Methods

Clinical microbiology laboratories in Europe were invited to contribute results of antimicrobial susceptibility testing (AST) for blood culture isolates identified as *Bacteroides fragilis* (per unique patient per year of observation (2022)). Antimicrobial susceptibility testing was performed locally by all participating laboratories in a prospective or retrospective way, using the EUCAST disc diffusion method on Fastidious Anaerobe Agar with 5% mechanically defibrinated horse blood (FAA-HB).

Results

A total of 16 European countries (14 EU plus Switzerland and Israel) reported 449 unique isolates of *Bacteroides fragilis* from blood for which AST had been performed (out of a total 602 unique isolates). Clindamycin showed the highest resistance rates (20.9%, range 0 - 63.6%), followed by piperacillin-tazobactam (11.1%, 0 - 54.5%), meropenem (13.4%, 0 - 45.5%), and metronidazole (3.1%, 0 - 40.0%), all with wide variation between countries.

Conclusions

Considering the regional differences (unexpectedly high resistance rates in some countries particularly in Southern Europe) and the mean resistance rates across Europe (above 10% for three of the four anti-anaerobic antibiotics included), both local AST of clinically relevant isolates of *Bacteroides fragilis* and continued surveillance on an international level is warranted.

OP-DCM-015

Assessing mecillinam resistance in E. coli strains causing UTI: A comparative evaluation of phenotypic testing methods

*S. Hauswaldt[1], L. Prange[2], S. Weikert-Asbeck[1], L. M. Göpel[1], S. Boutin[1], D. Nurjadi[1]

[1]University of Lübeck, Infectious Diseases and Microbiology, Lübeck, Germany
[2]University of Lübeck, Lübeck, Germany

Question

Mecillinam is recommended by German guidelines as a first-line treatment for uncomplicated urinary tract infections (uUTI). However, comprehensive data on antibiotic susceptibility are lacking due to the rare demand for microbiological diagnostics for uUTIs as well as the challenge of performing susceptibility testing with agar dilution as the reference method. We aim to evaluate various phenotypic methods for reliably predicting mecillinam resistance in *E. coli,* the most common UTI pathogen.

Methods

40 *E. coli* strains from routine urine cultures were selected, including 20 randomly chosen and 20 with elevated or resistant MIC to mecillinam based on Vitek testing with Ast-N371 (BioMérieux). 5 methods were compared in triplicates: agar dilution, Vitek Ast-N371, agar gradient diffusion (AGD), disk diffusion, and broth microdilution conducted in two different media—Müller-Hinton Broth (MHB) and artificial urine (AU). Categorial agreement was assessed based on the median, using EUCAST susceptibility breakpoints (MIC ≤8 mg/L, zone diameter ≥15 mm). Major errors (ME) were defined as false resistance and very major errors (VME) as false susceptibility compared to the reference method.

Results

Agar dilution categorized 28 strains as susceptible and 12 as resistant, with 6 near the breakpoint (8 mg/L or 16 mg/L). Categorial agreement to the reference method was 90% for disk diffusion, 83% for Vitek, 78% for AGD, 63% for MHB and 80% for AU. AGD had the most VME (n = 10), generally underestimating MIC values, while disk diffusion was the most reliable method with no ME and 4 VME. Notably, 3 of the 4 VME resulted in strains with an MIC of 16 mg/L. Microdilution methods, including Vitek, generally demonstrated a low reliability, with decreased reproducibility and more ME/ VME compared to disk diffusion. Only 2/12 resistant strains and 13/28 susceptible strains showed categorial agreement in all employed methods.

Conclusion

Mecillinam resistance testing remains challenging, with routine methods yielding unreliable results. The next phase of the study aims to elucidate the underlying genes associated with mecillinam resistance.

OP-DCM-016
Epidemiology of carbapenemases in Morganella spp., Serratia spp. and Providencia spp.

J. Noster[1], L. Schaffarczyk[2], J. Sattler[3], S. Göttig[4], Y. Stelzer[1], S. G. Gatermann[5], *A. Hamprecht[1]
[1]University of Oldenburg, Institute for Medical Microbiology and Virology, Oldenburg, Germany
[2]University of Oldenburg, Institut für medizinische Mikrobiologie u. Virologie, Oldenburg, Germany
[3]University Hospital Cologne, Institut für med. Mikrobiologie, Immunologie u. Hygiene, Köln, Germany
[4]Goethe University Frankfurt/Main, Institute for Medical Microbiology and Infection Control, Frankfurt a. M., Germany
[5]Ruhr University Bochum, Department of Medical Microbiology, Bochum, Germany

Introduction

Morganella spp., *Serratia* spp. and *Providencia* spp. (MSP) are Enterobacterales that cause infections of the urinary tract, wounds and bloodstream among others. Currently, the epidemiology of carbapenemases in MSP is largely unknown.

Goals

To determine the epidemiology of carbapenemases in MSP and performance of diagnostic assays.

Materials & Methods

In total, 131 clinical carbapenemase-producing MSP patient isolates were included (*Serratia marcescens*, n=107; *Morganella morganii*, n=13; *Providencia* spp., n=11). MSP were isolated from clinical samples at the University Hospitals Cologne, Frankfurt and the German National Reference Centre for multidrug-resistant Gram-negative bacteria. Susceptibility was determined by broth microdilution (BMD). Carbapenemase production was assessed by modified zinc-supplemented carbapenem inactivation method (mzCIM). Growth on carbapenem agar (mSuperCarba) was additionally determined. Resistance genes were analysed by whole genome sequencing.

Results

OXA-48-like were the most frequent carbapenemases (n=112; 85.5%), followed by KPC (n=10; 7.6%), IMP (n=6; 4.5%) and GES (n=3; 2.3%). KPC and GES were only detected in *S. marcescens*. Interestingly, only 29 isolates (22.1%) co-produced an ESBL, with CTX-M-15 being the most frequent (n=21). In contrast, genes coding for AmpC beta-lactamases were detected in 92 isolates (70.2%). Despite carbapenemase production, susceptibility (S/I) was observed for piperacillin-tazobactam in one isolate, ceftazidime in 61, ertapenem in 10 and meropenem in 42 isolates. All isolates were correctly identified as carbapenemase-producers by mzCIM, but only 120 grew on the selective carbapenem agar.

Summary

Carbapenemase production in MSP was mostly observed in *S. marcescens* and OXA-48-like were by far the most common carbapenemases. Further carbapenemases and ESBL production were less frequent compared to other Enterobacterales species. Despite carbapenemase production, MSP isolates frequently tested susceptible for ceftazidime and meropenem, which could result in insufficient detection in the routine microbiology laboratory.

OP-DCM-017
Performance of mycobacterium growth indicator tube incubated at 30°C for detection of mycobacteria in CF

and tissue samples compared to conventional growth conditions of liquid and solid media

*J. Kehrmann[1], A. L. Stumpf[1], A. Dragaqina[1], J. Buer[2]
[1]*University Hospital Essen, University of Duisburg-Essen, Institute of Medical Microbiology, Essen, Germany*
[2]*University Hospital Essen, Institute of Medical Microbiology, Essen, Germany*

Background

Mycobacterial culture using mycobacterium growth indicator tube (MGIT) liquid culture incubated at 36°C has a superior performance for mycobacteria detection compared to solid media. For cystic fibrosis (CF) patients and tissue samples incubation at 30°C may additionally improve performance. We evaluate MGIT incubated at 30°C compared to MGIT 36°C and solid media incubated at 36°C and 30°C in samples from CF patients and tissue samples.

Methods

All samples (n=1446) of CF patients and tissue samples submitted for mycobacterial diagnostics between February 2021 and September 2023 to the University Medicine Essen were inoculated on MGIT liquid medium (Becton Dickinson), Lowenstein Jensen (LJ) and Stonebrink solid media with PACT (Oxoid, Wesel, Germany) and were incubated at both temperatures, 30°C and 36°C. MGIT cultured at 36°C were incubated in a Bactec MGIT 960TM device (Becton Dickinson) for 6 weeks, all other cultures were incubated for 8 weeks. MGIT 30°C cultures were incubated in a 30°C incubator and analysed for growth using MicroMGIT reader (BD). Ziehl-Neelsen staining was primarily performed for all positive cultures to confirm mycobacterial growth. Identification of mycobacteria was primarily performed using GenoType CM, AS or NTM-DR (Hain Lifescience).

Results

Of 1446 samples, a total of 187 mycobacterial isolates were cultured. The highest detection rate was from MGIT 30°C with 86.1% (161 of 187 mycobacterial isolates). MGIT 36°C recovered 59.8% (112 of 187), LJ 36°C 48,1% (90), Stonebrink 36°C 50.8% (95), LJ 30°C 50.3% (94) and Stonebrink 30°C 55.1% (103) of grown isolates. MGIT 30°C detected 34 mycobacterial isolates that were not recovered by any other culture condition. MGIT 30°C detected the highest number of isolates for *M. abscessus* complex (39), *M. chelonae* (6), *M. chimaera* (20), *M. fortuitum* (1), *M. gordonae* (21), *M. intermedium* (1), *M. mucogenicum* (3), *M. simiae* complex (1), M. spec. (6), *M. nebraskense* (1) and *M. tuberculosis* (23).

Conclusions

For CF patients and tissue samples, MGIT cultivated at 30°C for eight weeks improves performance of mycobacterial culture.

Epidemiology and Antimicrobial Resistance of Zoonotic Pathogens

OP-EAZP-001

Characterization of Klebsiella species from slaughterhouses in Edo State, Nigeria: Insights from phenotypic and whole-genome analysis

*A. Beshiru[1,2], E. O. Igbinosa[1,3], S. Al Dahouk[4], R. Dieckmann[1,4], S. Neuhaus[1]
[1]*German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany*
[2]*Western Delta University, Department of Microbiology, Oghara, Nigeria*
[3]*University of Benin, Department of Microbiology, Benin City, Nigeria*
[4]*German Environment Agency, Department of Environmental Hygiene, Berlin, Germany*

Background

Slaughterhouses are known reservoirs for pathogenic bacteria, and elicit food safety apprehensions. *Klebsiella* species, recognized opportunistic pathogens, may occur on meat surfaces and require a thorough understanding of their slaughterhouse characteristics for accurate assessment of associated transmission and human health risks. This study investigated phenotypic and genomic features of *Klebsiella* isolates, shedding light on antimicrobial resistance (AMR) and potential virulence factors, offering valuable insights for public health considerations.

Methodology

Before and after disinfection, surface samples from slaughterhouse underwent *Klebsiella* species isolation and identification via MALDI-TOF. Antimicrobial and biocide susceptibilities were ascertained for relevant substances, evaluating both minimum inhibitory concentration (MIC) and additionally minimum bactericidal concentration for biocides. Isolates with high biocide MICs were subjected to efflux pump inhibition testing. Further, *Klebsiella* underwent screening for biofilm formation capabilities. Whole genome sequencing (WGS) was employed to explore genetic diversity and potential traits contributing to persistence.

Results

The majority of recovered isolates was identified as *K. pneumoniae* (*n*=105). Additional species included *K. aerogenes* (*n*=6), *K. quasipneumoniae* (*n*=7), and *K. variicola* (*n*=13). Biocide susceptibility varied, with two isolates showing triclosan resistance compared to in-use concentrations. Efflux pump inhibition resulted in increased susceptibility to octenidine dihydrochloride and didecyldimethylammonium chloride. Out of the total isolates, 31 *K. pneumoniae* (23.7%) exhibited multidrug resistance. Two *K. pneumoniae* isolates were extended-spectrum β-lactamase producers. Biofilm formation was prevalent, with stronger biofilm forming ability post-disinfection. WGS revealed a broad genetic diversity, encompassing AMR, virulence, biocide, and biofilm genes.

Conclusions

Our findings highlight various public health relevant characteristics of *Klebsiella* isolates from slaughterhouses, emphasizing diverse AMR profiles and genetic traits.

OP-EAZP-002

Cefiderocol resistance on the rise: Emergence of resistant Enterobacterales in the One Health context

*E. Eger[1], M. Schwabe[1], T. Homeier-Bachmann[2], A. A. Sylverken[3,4], K. Becker[5], H. Fickenscher[6], A. Krumbholz[6,7], D. Nurjadi[8], S. E. Heiden[1], K. Schaufler[1,9]
[1]*Helmholtz Institute for One Health/Helmholtz Centre for Infection Research, Epidemiology and Ecology of Antimicrobial Resistance, Greifswald, Germany*
[2]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Institute of Epidemiology, Greifswald, Germany*
[3]*Kwame Nkrumah University of Science and Technology, Theoretical and Applied Biology, Kumasi, Ghana*
[4]*Kwame Nkrumah University of Science and Technology, Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana*
[5]*University Medicine Greifswald, Friedrich Loeffler-Institute of Medical Microbiology, Greifswald, Germany*
[6]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute of Infection Medicine, Kiel, Germany*
[7]*Labor Dr. Krause und Kollegen MVZ GmbH, Kiel, Germany*
[8]*University of Lübeck, Infectious Diseases and Microbiology, Lübeck, Germany*
[9]*University Medicine Greifswald, Greifswald, Germany*

Enterobacterales, e.g., *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP), stand out for their ability to evolve and acquire resistance to last-line antibiotics such as carbapenems, contributing to difficult-to-treat infections. Cefiderocol (FDC) was developed to combat such multidrug-resistant (MDR) strains by using a mechanism that bypasses common resistance mechanisms. Unfortunately, as with most new drugs, resistance to FDC has been reported with increasing frequency, always associated with hospitalization and antibiotic therapy. However, little is known about the prevalence of FDC-resistant EC and KP beyond the hospital walls. Our studies aimed to fill this gap by investigating the presence of these pathogens in a One Health context.

We sampled surface water from different sites in a Western European (Germany) and a Sub-Saharan African (Ghana) setting. Bacteria were initially selected for their MDR phenotype, whole genome sequenced and subsequently tested for FDC resistance. In addition, strains from various human, animal, and environmental sources from our strain collection were systematically tested for FDC resistance.

We collected a total of 116 MDR EC and 28 MDR KP. EC strains with phenotypic resistance to FDC were present in both sets of water samples. Interestingly, typical mutations described in the literature as resistance markers (e.g., *ompC*) were not confirmed by genomic analysis. Knock-in and knock-out studies are ongoing. In addition, we identified FDC-resistant, convergent KP strains of sequence type 307 in our strain collection that were isolated during a clonal outbreak in Germany that preceded the clinical use of FDC. Of note, the underlying mutation type in the *cirA* gene has not yet been described in this context.

Our research has revealed the emergence of FDC-resistant strains in surface waters in Germany and Ghana. More importantly, FDC resistance is not a recent phenomenon and may have predated the clinical use. These findings highlight the need for comprehensive One Health studies that integrate genomic and epidemiologic data to effectivly monitor and manage antimicrobial resistance at the human-animal-environmental interface.

OP-EAZP-003

Low zoonotic potential but multi-drug resistance in Streptococcus suis isolates from healthy and diseased birds

*M. Dresen[1,2], G. G. R. Murray[3], P. Valentin-Weigand[1], M. Fulde[4], L. Weinert[2]
[1]*University of Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany*
[2]*University of Cambridge, Department of Veterinary Medicine, Cambridge, United Kingdom*
[3]*University College London, Department of Genetics, Evolution and Environment, London, United Kingdom*
[4]*Free University of Berlin, Department of Veterinary Medicine, Institute of Microbiology and Epizootics, Berlin, Germany*

Streptococcus suis is a respiratory commensal of pigs, with some lineages causing serious swine disease and zoonotic disease in humans. In recent years, *S. suis* has also been isolated from cats, dogs, cattle, sheep, wild boars, and different bird species, including chicken. It is generally assumed that, as in humans, these infections are due to "spillovers" from pigs, but no genomic investigation of respective isolates has yet been performed. This project aimed at investigating the zoonotic potential of these isolates by employing bioinformatics analyses with a special emphasis on host adaptation, virulence, and antimicrobial resistance.

Over the last six years, we collected and whole-genome sequenced *S. suis* samples from birds in the veterinary diagnostics department of the Freie Universität Berlin. Most of these birds were diseased, and *S. suis* was isolated among other pathogens. We combined these samples with published genomes of Vietnamese healthy chicken isolates and compared them to *S. suis* lineages isolated from pigs, wild boars, and human. In addition, to examine potential survival advantages of bird isolates in birds, we performed growth experiments in chicken vs. pig blood.

All the bird isolates clustered phylogenetically within a group of largely commensal isolates of pigs, and distinct from the lineages responsible for most zoonoses, lacking most known virulence genes. Using genome-wide association studies, we detected several unique mobile genetic elements specific to bird isolates, suggestive of *S. suis* adaptation to birds. Concordantly, *S. suis* bird isolates showed a better survival in chicken than in porcine blood, although this effect was not significant. Furthermore, bird isolates had a larger genome size than closely related pig isolates. Finally, our analyses revealed that bird isolates were often multi-drug resistant.

In summary, our results imply that *S. suis* could be persisting in bird populations independently of pigs, at least for short periods of time, but that birds are unlikely to be a source of zoonotic infection in humans. Our study also suggests a potential role for antibiotic resistance in host switching.

OP-EAZP-004

Single nucleotide polymorphisms (SNPs) in dacB/ampD alter population plasticity of gene amplification based heteroresistance

*J. Kupke[1], J. Brombach[1], S. Wolf[2], L. Thrukonda[2], F. Ghazisaeedi[1], D. Hanke[1], T. Semmler[2], K. Tedin[1], N. Nordholt[3], F. Schreiber[4], U. K. Steiner[5], A. Lübke-Becker[1,6], M. Fulde[1,6]
[1]*Free University of Berlin, Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Berlin, Germany*
[2]*Robert Koch Institute (RKI), MF1-Genome Competence Centre, Berlin, Germany*
[3]*Federal Institute for Materials Research and Testing (BAM),*

Department of Materials and the Environment, Division of Biodeterioration and Reference Organisms (4.1), Berlin, Germany
[4]Federal Institute for Materials Research and Testing (BAM), Department of Materials and the Environment (Dpt. 4), Berlin, Germany
[5]Free University of Berlin, Institute of Biology, Evolutionary Demography, Berlin, Germany
[6]Free University of Berlin, Veterinary Centre for Resistance Research (TZR), Berlin, Germany

Introduction Heteroresistance (HR) describes the ability of a subpopulation to grow in the presence of inhibitory antibiotic concentrations. We found HR to ceftazidime (CAZ) in a clinical *Enterobacter cloacae* complex (ECC) strain (IMT49658).

Material & Methods We performed extensive phenotypic (population analysis profiles, stability analysis of resistance, ScanLag) and molecular microbiological techniques (qRT-PCR, whole genome sequencing, raw read analysis) in order to show the plasticity and mechanism of HR in this ECC strain. We re-investigated the genome and phenotype of IMT 49658 after long-term evolution in 32 µg/ml CAZ and after exposure to 128 µg/ml CAZ in plates.

Goals By revealing mechanism and evolution of HR to conventional resistance, we aim for a better detection and treatment of bacterial heteroresistant infections.

Results WGS detected a plasmidal gene amplification with β-lactamase ampC *bla*DHA-1. qRT-PCR showed a high genomic copy number of *bla*DHA-1 in resistant subpopulations, decreasing when they reverted to susceptibility. Gene amplifications varied in single cells of one colony (raw read analysis). Resistant subpopulations showed heterogeneous lag times in ScanLag. After evolving ECC for 21 days in CAZ, we discovered a SNP in *dacB* and *ampD*, encoding for a stop codon. These mutants displayed low amplification levels but resistance in disk diffusion and homogenous lag times. The *dacB* mutant was more frequent than the *ampD* mutant on plates with 128 µg/ml CAZ.

Summary Long-term evolution in antibiotic niches drives the emergence of new resistant mutants, balancing the fitness costs of e.g., gene amplifications. Comprehension of the transition from HR to resistance is inevitable for successful treatment of bacterial infections.

OP-EAZP-005
Phage regulation and persister cell formation upon ciprofloxacin treatment via the toxin TisB in Salmonella Typhimurium

*S. Braetz[1], P. Schwerk[1], N. Figueroa-Bossi[2], K. Tedin[1], M. Fulde[1]
[1]Free University of Berlin, Institut für Mikrobiologie und Tierseuchen, Berlin, Germany
[2]Institut de Biologie Intégrative de la Cellule (I2BC), Gif-sur-Yvette, France

Introduction

Persisters are drug-tolerant cells that survive bactericidal concentrations of antibiotics, therefore playing an important role in clinical therapy failure [1]. Mechanisms influencing bacterial survival during treatment with antibiotics include the activity of bacterial toxins, induction of prophages, and activation of stress responses [2, 3].

Material/method

We examined the effects of a *tisB* toxin deletion mutant in *Salmonella* Typhimurium on the ATP concentrations during treatment with four-fold the MIC of ciprofloxacin. Furthermore, we also examined the TisB-dependent induction of the prophages and their impact on persister cell formation comparing a phage-free variant of the wild type strain in a Δ*tisB* background.

Results

Deletion of *tisB* significantly increased ATP concentration and SOS response when exposed to ciprofloxacin, with a reduction in the formation of persistent cells. Interestingly, a 90-fold reduction in bacterial survival was observed four hours after challenging the phage-carrying Δ*tisB* strain with ciprofloxacin. In contrast, deletion of all prophages in the *tisB* background slowed initial killing and had a less significant impact on drug persistence.

Discussion

The SOS response is controlled by RecA, which requires ATP for its activity. We suggest that TisB inhibits ATP formation, and interferes with DNA repair responses. This significantly reduces the SOS response-dependent prophage induction and thus increases persister cell formation.

1. Brauner, A., et al., *Distinguishing between resistance, tolerance and persistence to antibiotic treatment.* Nat Rev Microbiol, 2016. **14**(5): p. 320-30.
2. Dorr, T., M. Vulic, and K. Lewis, *Ciprofloxacin causes persister formation by inducing the TisB toxin in Escherichia coli.* PLoS Biol, 2010. **8**(2): p. e1000317.
3. Braetz, S., et al., *Prophage Gifsy-1 Induction in Salmonella enterica Serovar Typhimurium Reduces Persister Cell Formation after Ciprofloxacin Exposure.* Microbiol Spectr, 2023: p. e0187423.

OP-EAZP-006
Phylogenomic analyses identifies dog-human sharing of genetically closely related Escherichia coli, including carbapenemase OXA-244 producing and colistin resistant strains

*E. Ngbede[1,2], V. Junker[1], B. Kolte[1,3], U. Nübel[1,3,4]
[1]Leibniz Institute DSMZ -German Collection of Microorganisms and Cell Cultures, Braunschweig, Microbial Genome Research, Brunswick, Germany
[2]Federal University of Agriculture Makurdi, Department of Veterinary Microbiology, Makurdi, Nigeria
[3]Technical University of Braunschweig, Institute of Microbiology, Brunswick, Germany
[4]German Center for Infection Research, Brunswick, Germany

Introduction: In Nigeria, a large proportion of dogs are free roaming or semi-restricted; a practice that brings them in close contact with potentially virulent and antibiotic resistant bacteria in the community. We investigated the role of dogs as community reservoir for dissemination of *Escherichia coli* resistant to "high priority critically important antimicrobials" in Makurdi, North central Nigeria.

Methods: We subjected a total of 137 isolates to whole genome sequencing and uploaded the reads unto the

EnteroBase server which facilitates genome assembly, core and whole genome based phylogenomic analyses. Genome assemblies were queried for resistance determinants using AMRFinder while phenotypic expression of resistance was assessed based on the antibiotic minimum inhibitory concentration (MIC).

Results: Hierarchical clustering analyses of core genome multilocus sequence types (cgMLST) revealed a genetically diverse population of 34 clonal complexes (CC) predominated by CC10. We identified the *blaOXA244* cabarpenemase gene in two isolates and the plasmid mediated colistin resistance gene variants: *mcr-1.22, mcr-10.1,* with the former located on an IncX4 plasmid. Multiple strains harbored extended spectrum β-lactamases (ESBL) genes predominantly *blaCTX-M-15* (n=50) and *blaCTX-M-55* (n=6). Isolates expressed cefotaxime and colistin resistance and a meropenem MIC of 0.125 µg/ml, respectively. Comparative phylogenomic analyses revealed close genetic relationship at HC5 (pairwise genetic distance of ≤ 5 cgMLST allelic difference) between canine isolates and human strains recovered from the same geographic location in Nigeria, suggestive of potential human-dog exchange of strains. Additional closely related genomes were detected among sequence data publicly available from EnteroBase.

Conclusion: Our findings show that dogs in developing countries are an important reservoir for potential community dissemination of colistin resistant, ESBL and carbapenemase producing *E. coli*, reechoing concerns for zoonotic and bidirectional spread.

Environmental Microbiology & Processes

OP-EMP-001

Metagenomics of the open ocean Plastisphere: a novel microbial niche with unique functional potential

*E. Borchert[1], S. Lips[2], M. Schmitt-Jansen[2], U. Hentschel[1]
[1]*GEOMAR Helmholtz-Centre for Ocean Research Kie, Marine Symbioses, Kiel, Germany*
[2]*Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany*

Introduction

Plastic pollution in the oceans has become a major concern and focus of research in the last decades. Research so far has focused on the distribution of plastics and the taxonomic composition of the microbes living on plastic particles (termed Plastisphere). However, a functional understanding of the Plastisphere is lacking and thus its impact on global ecosystem functioning is unknown.

Goals

The aim of this study was to examine the functional potential and genetic adaptation of the open ocean Plastisphere based on a comparative metagenomics study with the surrounding seawater microbiome.

Materials & Methods

Plastic particles were collected from the North Atlantic and North Pacific Garbage patch on research cruises in summer 2019. Metagenomic DNA samples were extracted and sequenced. The resulting sequencing data was differentially binned into bacterial metagenome-assembled genomes (MAGs). The MAGs were used for Hidden Markov Model profiling utilizing public KEGG profiles of metabolic pathways, nitrogen cycling and others relevant for a surface-attached lifestyle in the oligotrophic open ocean.

Results

More than 1700 Plastisphere MAGs were reconstructed and compared to >450 corresponding seawater MAGs from the Tara Oceans project. The Plastisphere displayed a distinct microbiome that is similar across ocean basins and different to the surrounding seawater on the taxonomic class level. The Plastisphere further contains a higher genomic potential for coping with UV induced stress, as well as for diverse carbon utilization pathways, nitrogen fixation and uptake of essential elements (Ni, Po).

Summary

The open ocean Plastisphere is a complex assemblage of auto- and heterotrophic bacteria which appears to select for bacteria capable to cope with different stressors as well as having high metabolic flexibility. Our metagenomic study highlights that the open ocean Plastisphere displays a distinct functional profile, enabling microbes to thrive in an oligotrophic ocean.

OP-EMP-002

Plastic mineralization by the sooty-mold fungus Capnodium coffeae demonstrated by isotope-tracing and -probing approaches

S. Rohrbach[1], G. Gkoutselis[2], A. Mauel[3], N. Telli[2], J. Senker[3], A. Ho[1], G. Rambold[2], *M. A. Horn[1]
[1]*Leibniz University Hannover, Institute of Microbiology, Hannover, Germany*
[2]*University of Bayreuth, Department of Mycology, Bayreuth, Germany*
[3]*University of Bayreuth, Inorganic Chemistry III, Bayreuth, Germany*

Plastic pollution represents an increasing environmental burden. Modification of plastic surfaces and degradation by microbes contributes to the removal of plastic from the environment. However, plastic mineralizing organisms are rare, and often mis-classified as such, probably due to the lack of standards. We hypothesize that hydrophobic, waxy surfaces found on tropic leaves that mimic plastic surfaces in terms of hydrophobicity are reservoirs of plastic degrading microbes and developed a standard procedure to unambiguously demonstrate plastic mineralization [1]. A strain of the sooty-mold fungus *Capnodium coffeae* was isolated and tested on media amended with different plastics resulting in enhanced growth with polystyrene and other common plastics. *Capnodium coffeae* was incubated together with ^{13}C-labelled polystyrene and $^{13}CO_2$ emissions were followed via cavity ring-down spectroscopy. A continuous increase of polystyrene derived $^{13}CO_2$ was observed in the headspace indicating polystyrene mineralization. Phospholipid fatty acids stable isotope probing indicated incorporation of polystyrene ^{13}C into the fungal biomass and substantiated the polystyrene mineralizing capabilities of *C. coffeae*. Thus, we successfully isolated a polystyrene mineralizing fungus, and provide a

strategy for the assessment of various microbial plastic metabolizers.

[1] Rohrbach, S., Gkoutselis, G., Mauel, A., Telli, N., Senker, J., Ho, A., G. Rambold and M.A. Horn. 2024. Setting new standards: Multiphasic analysis of microplastic mineralization by fungi. Chemosphere 349: 141025. DOI: 10.1016/j.chemosphere.2023.141025

OP-EMP-003
Interactions Between High Load of a Bio-based and Biodegradable Plastic and Nitrogen Fertilizer Affect Plant Biomass and Health: A Case Study with Fusarium solani and Mung Bean (Vigna radiata L.)

S. Scheid[1], K. Juncheed[2], B. Tanunchai[3,4,1], S. F. M. Wahdan[1], F.

Bio-based and biodegradable plastics such as mulching films are widely used in agricultural field sites. However, there are limited studies of their impact on plant development and health even though an important soil-borne plant pathogen *F. solani* has been reported to associate with various types of bio-based and biodegradable plastics, especially polybutylene succinate-co-adipate (PBSA). To evaluate the influence of PBSA amendment in soils on plant development and health, *F. solani* and mung bean (*V. radiata*) were used as models in a modified petri-dish test using soil suspensions. Mung bean seeds were incubated in suspensions with two dilutions (high vs. low dilution with low vs. high PBSA amendment) of soils preincubated 1 year with PBSA under different treatments (combinations of N fertilizer (ammonium sulfate) and PBSA load) in the modified petri dish test. Plant development and disease incidence were recorded with both microscopic and molecular techniques (specific PCR and Illumina amplicon sequencing). Treatment with PBSA and N fertilizer in non-sterile soil suspensions strongly increased the disease caused by *F. solani* on *V. radiata* at both low and high soil dilution. At high soil dilution, the F. solani disease incident was 67.5% while at the low dilution the disease incidence reached 92.5%. In contrast, in treatments PBSA but without N fertilizer, non *F. solani* disease was observed. Apart from *F. solani* infection, other soil fungi can also infect the mung bean seedlings, especially at low soil dilution levels. Nevertheless, based on this short-term study, we found no evidence that PBSA alone can significantly increase the overall disease incidence.

OP-EMP-004
Microbial response in benthic alkalinity enhancement experiments

*R. Bährle[1], S. Böhnke-Brandt[1], M. Fuhr[2], A. W. Dale[2], S. Geilert[3], M. Perner[1]
[1]*GEOMAR Helmholtz-Centre for Ocean Research Kie, Geomicrobiology, Kiel, Germany*
[2]*GEOMAR Helmholtz-Centre for Ocean Research Kie, Benthic Biochemsitry, Kiel, Germany*
[3]*Utrecht University, Geochemsitry, Utrecht, Netherlands*

Ocean alkalinity enhancement (OAE) has been proposed as a technique for actively reducing atmospheric carbon dioxide levels. One of the tools for OAE is enhanced benthic weathering via addition of alkaline minerals such as dunite or calcite to surface sediments. This study investigates the effects of mineral additions on the benthic microbial community and possible feedbacks related to shifts in the microbial community. For this purpose, dunite and calcite were added to organic-rich sediments originating from the Baltic Sea (Eckernförde Bight) under fully oxic and under low oxygen conditions. Based on RNA-profiling the microbial communities shifted in all of these incubation experiments over time. At the beginning of the incubation experiments *Beggiatoaceae* were highly abundant in most surface sediments but decreased significantly over the course of the different experiments. Under fully oxic conditions, the microbial community shifts showed no clear relationship with the type of mineral added. In contrast, the oxygen low incubation experiments demonstrated an increase of cable bacteria after adding calcite to the sediments. This could be related to cable bacteria possessing a carbonic anhydrase. This enzyme transforms carbonate into carbon dioxide that is then converted into biomass. Possessing such an enzyme could be an advantage to grow under oxygen low conditions in the presence of calcite. The shift towards cable bacteria is in line with the measured pH profiles, typically associated with cable bacteria activity, where the pH is lowered in the deeper sediment horizons. This is the first study demonstrating that artificial benthic weathering using calcite promotes cable bacteria activity under low oxygen conditions.

OP-EMP-005
Survival and rapid resuscitation permit limited productivity in desert soil microbial communities

S. Imminger[1], D. Meier[1,2], A. Schintlmeister[1], A. Legin[1], J. Schnecker[1], A. Richter[1], O. Gillor[3], S. Eichorst[1], *D. Woebken[1]
[1]*University of Vienna, Department of Microbiology and Ecosystem Science, Wien, Austria*
[2]*University of Bayreuth, Bayreuth Center of Ecology and Environmental Research (BayCEER), Bayreuth, Germany*
[3]*Ben Gurion University of the Negev, Blaustein Institutes for Desert Research, Sde Boker, Israel*

Despite drylands constituting the largest terrestrial biome, key information such as microbial productivity in desert soil is lacking. However, rapid growth would be needed to maintain the microbial community, if, as proposed, desiccation and rehydration via rain causes widespread cell death due to osmotic stress. Further, major microbially-mediated ecosystem processes are confined to rare and short periods of rain, but it is unknown how desert soil microorganisms maintain ecosystem processes in such narrow activity time. We addressed these knowledge gaps on rain-mediated microbial resuscitation and associated activities in biological soil crusts from the Negev Desert, Israel. We investigated changes in gene transcription of individual microbial populations and single-cell biomass generation during a controlled rehydration experiment. Simulating rain with $^{2}H_2O$ followed by single-cell NanoSIMS, we showed that the desert biocrust microbial community is characterized by limited productivity, considering median replication times of 6 to 19 days and restricted number of days in the Negev Desert allowing growth. Genome-resolved metatranscriptomics revealed that nearly all microbial populations resuscitate within minutes after simulated rain, independent of taxonomy or encoded physiology. We observed distinct temporal phases of cellular processes, beginning with DNA repair powered by storage compound oxidation and only later followed by uptake of external carbon sources and resumption of main metabolism. Together, our data reveal a community that makes optimal use of short activity phases by fast and universal resuscitation enabling the maintenance of key ecosystem functions. This community is highly adapted to surviving rapid changes in soil moisture and solute concentrations, resulting in high persistence that balances limited productivity. These findings have important

implications considering preservation of soil microbial communities that are critical for soil stabilization.

Fig. 1

OP-EMP-006

Anammox and DNRA dominate anaerobic nitrogen transformation in carbonate-rock and sandstone aquifers

*M. Herrmann[1,2], R. Nafeh[1], B. Thamdrup[3], M. Krüger[1], C. E. Wegner[1], K. Küsel[1,2]
[1]*Friedrich Schiller University Jena, Institute of Biodiversity, Aquatic Geomicrobiology, Jena, Germany*
[2]*German Centre for Integrative Biodiversity Research (iDiv) , Leipzig, Germany*
[3]*University of Southern Denmark, Nordcee, Department of Biology, Odense, Denmark*

Microbially driven anaerobic nitrogen transformation processes play an important role in groundwater biogeochemical cycling. However, the contribution of processes other than denitrification, such as anaerobic ammonia oxidation (anammox) or dissimilatory nitrate reduction to ammonium (DNRA), and the overall influence of geological settings, have received little attention so far. We hypothesize that groundwaters from two contrasting geological settings – carbonate rock (Hainich Critical Zone Exploratory) and sandstone aquifers (Saale-Elster-Sandstone Observatory) in Thuringia (Germany) – differ in the dominant anaerobic nitrogen transformation pathways, the preferred electron donors for nitrate reduction, and the respective microbial communities. ^{15}N-based approaches revealed that anammox contributed about 80% to total nitrogen loss in suboxic carbonate-rock groundwater with maximum rates of 9.8 nmol N_2 L^{-1} d, while DNRA was the dominant nitrate reducing process in sandstone groundwater (12.8 nmol N L^{-1} d^{-1}). Despite a high genetic potential for denitrification, measured activities remained below 0.6 nmol N_2 L^{-1} d^{-1} across all groundwater wells of the two locations. Microcosm experiments conducted over 70 days showed that nitrate reducing communities from the sandstone groundwater responded more rapidly to the addition of organic carbon compounds or hydrogen than those from carbonate-rock groundwater. In sandstone groundwater microcosms, incubation with both types of electron donors resulted in an increase in *nirS* and *nrfA* genes encoding NO-forming nitrite reductase (denitrification) and ammonia-forming nitrite reductase (DNRA), respectively, by two orders of magnitude. In contrast, microorganisms with the genetic potential for DNRA were not stimulated in carbonate-rock groundwater microcosms. Our findings suggest that the geological setting strongly influences preferential nitrogen transformation pathways and electron donor usage in groundwater. DNRA could play an important role in nitrate reduction in groundwater from the sandstone aquifers, potentially supported by hydrogen and organic carbon compounds.

OP-EMP-007

Investigating manganese-oxidizing microbial biofilms in a historic copper mine of Upper Franconia

*D. Meier[1], C. Mechela[1], F. Beulig[1,2], M. Obst[3], T. Lueders[1]
[1]*University of Bayreuth, Ecological Microbiology, Bayreuth, Germany*
[2]*Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark*
[3]*University of Bayreuth, Experimental Biogeochemistry, Bayreuth, Germany*

Redox cycling of iron (Fe) and manganese (Mn) can be an important source of energy for microorganisms in subsurface habitats. While Fe oxidation is well known to support autotrophic growth, the first chemolithoautotrophic Mn-oxidizing organism was only recently enriched from tap water. Truly estimating the distribution and importance of chemolitho(auto)trophic Mn-oxidation remains a major challenge for environmental microbiology. Here, we report on microbial biofilms from a historic mine in Northern Bavaria, which may well represent a natural habitat for autotrophic Mn-oxidizers.

The biofilms are enriched in Mn oxides, with Mn making up to 40% of the biofilms" dry weight. Identification of Mn(II), Mn(IV) and the labile Mn(III) in microbial cells via scanning transmission X-ray microscopy coupled to near-edge x-ray absorption fine structure spectroscopy suggested an ongoing biological Mn oxidation in situ. Characteristic Mn oxide nodules with microbial cells attached were also found in aerobic enrichment cultures set up in minimal media. Lectin staining and confocal laser-scan microscopy showed abundant extracellular polymeric structures coating the mineral particles. Cell numbers and morphologies, as well as taxonomic composition of the microbial communities differed between different types of biofilms and different mining tunnels. From metagenomes of biofilms and mine water, we reconstructed 132 metagenome-assembled genomes (MAGs). Intriguingly, nearly half of the MAGs encoded multi-heme cytochromes indicative of extracellular electron transfer, a necessary capability for electron exchange with a mineral surface. Most of the potential autotrophs MAGs contained multi-heme cytochromes and constituted up to 23% of the community. This further supports our hypothesis that metal-cycling microorganisms belonging to uncultured families of Gammaproteobacteria, Nitrospirota, and Acidobacteriota may be the major primary producers in these biofilms. Based on taxonomic affiliation of the MAGs, the biofilms could be seen as a natural enrichment of Mn-cycling freshwater microorganisms and could help us understand the role of Mn in microbial freshwater ecosystems.

OP-EMP-008

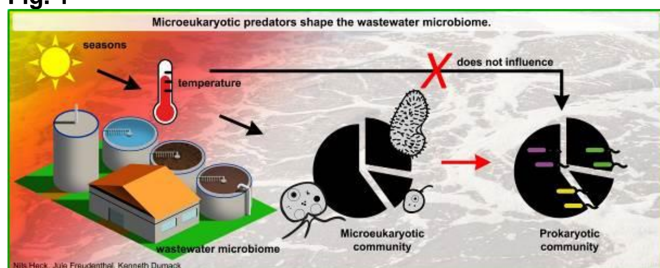
Predatory and parasitic microeukaryotes in wastewater treatment plants - diversity & function

*K. Dumack[1], N. Heck[1], J. Freudenthal[1], N. Pohl[1], M. Solbach[1], M. Bonkowski[1]
[1]*University of Cologne, Zoology, Köln, Germany*

Microeukaryotes, in particular protists, have been relatively overlooked in wastewater treatment studies due to methodological biases favoring prokaryotes. Applying metatranscriptomics has enabled us to address these biases, and in this presentation, I will provide a summary of four recent studies examining the entire wastewater microbiome, encompassing prokaryotes, fungi, protists, and microscopic metazoa (Heck et al. 2023, Freudenthal et al. 2022, Pohl et al. 2021, Solbach et al. 2021).

Our primary focus has been on exploring biotic interactions within wastewater, leading to several noteworthy findings: 1. Microeukaryotic predators shape the prokaryotic community composition and thus are integral for well-performed wastewater treatment. 2. Gut parasites among microeukaryotes exhibit heightened activity in wastewater but undergo effective reduction during denitrification, likely influenced by microbial predation. 3. The so-far overlooked, but most abundant wastewater protist worldwide, *Rhogostoma minus*, hosts Legionellales and potentially functions as a vector for Legionnaire's disease in humans. These findings underscore the significance of investigating microbial eukaryotes in wastewater, now made feasible with advanced tools.

Fig. 1



OP-EMP-009
Reduction of pathogens and (antibiotic-resistant) bacteria in advanced sewage treatment by membrane bioreactor systems and retention soil filters

*S. M. Essert[1], N. Zacharias[1], C. Lüchtefeld[1], T. Kistemann[1], N. T. Mutters[1], A. Ahring[2], D. Seiger[2], C. Schreiber[1]
[1]*University Hospital Bonn, GeoHealth Centre, IHPH – Institute for Hygiene and Public Health, Medical Faculty, Bonn, Germany*
[2]*Erftverband, Wastewater techniques/water, Bergheim, Germany*

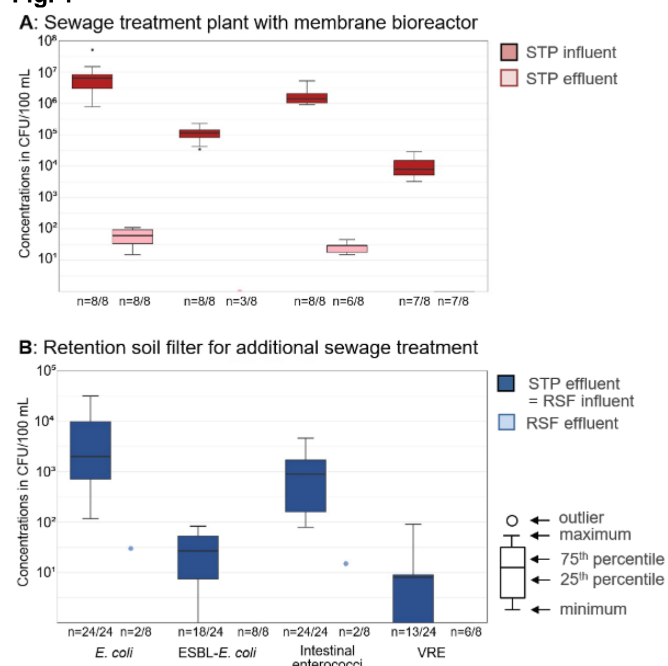
Sewage treatment plants (STPs) are important punctual sources of antimicrobial resistance spread, including antibiotic-resistant bacteria (ARB) [1]. Thus, an advanced wastewater treatment gained importance in order to decrease microbial load from the discharge of STPs, which enter freshwater systems.

In this study, two STPs with membrane bioreactor systems (MBRs) as advanced technologies in the activated sludge process were examined for their reduction performance for hygienically relevant pathogens and ARB. A retention soil filter (RSF) was tested at another STP as an additional treatment stage after conventional wastewater treatment. The reduction of ESBL (extended spectrum β-lactamase)-producing bacteria and VRE (vancomycin-resistant enterococci) as well as accompanying pathogens are in focus of this study. The MBRs and the RSF are running each on municipal STPs in Germany, operated by the Erftverband. Somatic coliphages, *Clostridium* (*perfringens*), *E. coli*, intestinal enterococci, target species of ESBL-producing bacteria (*E. coli*, *Klebsiella* spp., *Enterococcus* spp., *Citrobacter* spp., *Pseudomonas* (*aeruginosa*), *Acinetobacter* (*baumannii*)) and VRE were analyzed by cultural detection.

The purification performances of the tested species in the STPs with MBRs showed a reduction of 1-2 log10 units of colony-forming units (CFU/100 mL) higher than STPs with conventional wastewater treatment (Fig. 1 A). The RSF showed a reduction performance for e.g. ESBL-*E. coli* and *E. coli* of 1.2 and 3.4 log10 units (CFU/100 mL; mean values). ARB could only be detected in rare cases (Fig. 1 B). In total, both RSF and MBRs showed high potential in reduction of ARB and other hygienically relevant parameter. Thus, the treatment techniques offer benefits in reducing pathogens and preventing the dissemination of resistance into the environment.

1. Sib, E., Lenz-Plet, F., Barabasch, V., Klanke, U., Savin, M., Hembach, N., Schallenberg, A., Kehl, K., Albert, C. and Gajdiss, C. Bacteria isolated from hospital, municipal and slaughterhouse wastewaters show characteristic, different resistance profiles. *Science of The Total Environment,* **2020**, *746*, 140894, doi:10.1016/j.scitotenv.2020.140894.

Fig. 1



OP-EMP-010
Fertilization with microalgae-biomass from wastewater treatment ponds can transmit fecal indicator bacteria into the agricultural system and has specific effects on the root microbiome composition of wheat plants

*E. Ollo1, F. Mueckschel2, H. Velten3, U. Theilen3, P. Kämpfer1, M. Frei4, S. P. Glaeser1
1Justus-Liebig University Gießen, Institute of Applied Microbiology, Giessen, Germany
2Justus-Liebig Universität Gießen, Institute of Plant Nutrition, Giessen, Germany
3University of Applied Sciences Mittelhessen, Urban water management and anaerobic process engineering, Giessen, Germany
4Justus-Liebig-University Gießen, Department of Agronomy and Crop Physiology, Giessen, Germany

High rate algal ponds (HRAPs) are effective alternative wastewater treatment systems for sustainable nutrient recycling. The nutrient rich microalgae biomass (MB) can be used as efficient agricultural biofertilizer to replace mineral fertilizers.

Complex effects are expected for the plant microbiome due to MB absorbed organic compounds, pollutants and attached bacteria including wastewater derived pathogens and antibiotic resistant bacteria. We studied the release of potential pathogenic, antimicrobial resistant and biocide tolerant bacteria and effects on community structure of the plant microbiome after fertilization with dried MB.

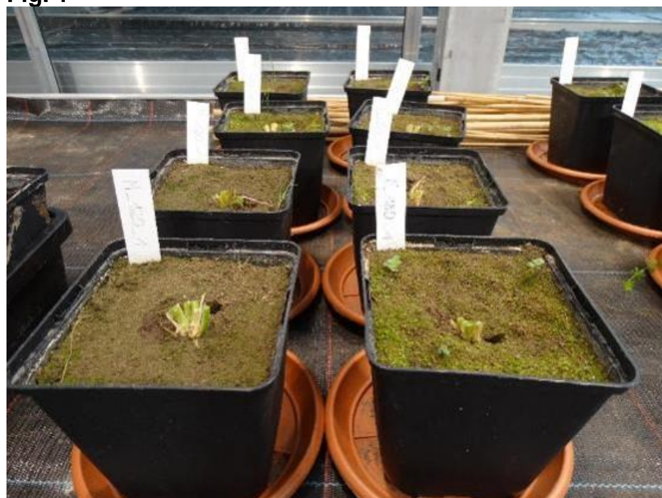
Wheat plants in a greenhouse pot experiment were fertilized either with dried MB or mineral fertilizer supplying 60, 120, and 180 kg ha-1 N-equivalents. The root microbiome of fertilized plants was compared to that of non-fertilized plants.

Cultivation dependent methods detected *E. coli,* ESBL-*E. coli,* enterococci and biocide tolerant bacteria. Total DNA was extracted from rhizospheres and roots for *16S rRNA* gene amplicon sequencing and quantification of bacteria, fungi, nitrogen cycling and antibiotic resistant genes (ARG). Compared to control plants, both fertilizers supported plant growth and yield (Mückschel et al. 2023). *E. coli*, ESBL-*E. coli* and enterococci were only cultured from MB and roots of MB-fertilized plants but not from potting soil and wheat seedlings used for the experiment. Biocide-tolerant *Pseudomonas* were cultured from all samples, while biocide tolerant *E. coli*, *Klebsiella* and *Enterobacter* were only cultured after MB application.

While the abundance of root colonizing bacteria, fungi and N cycling genes showed no significant difference after MB and mineral fertilizer application, clear differences were obtained for the diversities and composition of the rhizosphere and root microbiome. Plant colonization with MB derived bacteria was thereby indicated. In addition, higher abundance of ARGs was obtained after MB application.

Our data showed that fertilizations with HRAP derived MB has complex effects on agricultural ecosystems and still requires more detailed multidisciplinary research.

Fig. 1



OP-EMP-011
A molecular approach on understanding seasonal effects on methane-related microbial processes in permafrost-affected soils from West Greenland

*C. Bruhn1, P. Gasimova1, C. Vogt2, C. Knoblauch2,3, T. Peplau4, P. Liebmann4, G. Guggenberger4, J. O. Melchert5, S. Liebner1,6
1Helmholtz Centre Potsdam - German Research Centre for Geosciences, Geomicrobiology, Potsdam, Germany
2Universtiy Hamburg , Institute of Soil Sciences, Hamburg, Germany
3Universtiy Hamburg , Center for Earth System Research and Sustainability, Hamburg, Germany
4Leibniz University Hannover, Institute of Earth System Sciences, Soil Sciences, Hannover, Germany
5University of Cologne, Institute for Geology and Mineralogy, Köln, Germany
6University of Potsdam, Institute of Biochemistry and Biology, Potsdam, Germany

The warming Arctic is subjecting permafrost soils, which cover approximately 15 % of the northern land area, to drastically changing conditions. Thawing permafrost enables formerly inactive microbes to produce or oxidize methane (CH_4), a potent greenhouse gas. We present first data on seasonal observations of the microbial soil metagenome along two tundra soil moisture gradients on Disko Island, West Greenland.

Initial studies have been performed at a time of maximum active layer thickness in September 2022 and during snowmelt in June/July 2023. We show through metabarcoding (16S rRNA gene), that known methane oxidizers (methanotrophs) ubiquitously occurred at low relative levels throughout the whole soil community in September 2022, generally accounting for <1.5 % of all ASVs. During snowmelt in June/July 2023, methanotrophs appeared absent from all surface samples which were at dry to intermediately dry plots. They were mostly detectable below the active thawed layer (below ~10 cm). Methane producing archaea (methanogens) were predominantly present at the wet sites and at deeper depths – as it was expected based on anaerobic incubation experiments with the same samples. Similar to methanotrophs, they were much more prevalent at the thaw maximum in September than during snowmelt in June/July, adding up to almost ten times the amount of detected methanogens in June/July in their relative abundance.

Data from two additional field trips at times of the maximum active layer thickness (September 2023) and completely frozen and snow-covered soil (April 2024) will enable further insights into the seasonal variation of methane-related microbial communities. This data set will also be complemented by whole-genome metagenomics, adding information on seasonal changes in methane production and oxidation pathways. A final qPCR approach will add quantitative information to the metabarcoding-derived data. This way, we will be able to help understand the microbial functioning underlying CH_4-oxidation and production in different seasons, which in turn will be important for constraining the Arctic CH_4 budget, specifically during the shoulder seasons.

OP-EMP-012
Unveiling Microbial Adaptations to High CO2 Environments: Metagenomic Insights from the Eger Rift (Czech Republic)

*V. J. Cevik[1], D. Lipus[2], J. Kallmeyer[2], B. Usadel[1,3], A. Kranz[1]
[1]*FZJ, IBG-4, Jülich, Germany*
[2]*GFZ, Section Geomicrobiology, Potsdam, Germany*
[3]*Faculty of Mathematics and Life Sciences, Heinrich Heine*

University Düsseldorf, Institute for Biological Data Science & Cluster of Excellence on Plant Sciences (CEPLAS), Düsseldorf, Germany

Introduction
High CO_2 environments, such as the Hartoušov mofette system in Central Europe"s Eger Rift, harbor diverse microbial communities critical for understanding carbon cycling and extreme condition adaptation[1]. Metagenomic analysis has emerged as a powerful tool for combing the genetic makeup and metabolic capabilities of these microbial communities.

Goals
In this study, our objective was to characterize the microbial composition in the CO_2-rich environment at the Eger Rift site. Additionally, we analyzed their metabolic potential, focusing on carboxylation reactions involved in fixing CO_2 in cellular material.

Methods
Drill core and water samples were collected from various depths within a drill hole at the Eger Rift site and subjected to metagenomic sequencing. Taxonomic profiles were determined using Kaiju and Kraken2. Metagenome-Assembled Genomes (MAGs) were generated for single organisms, and gene prediction (Prodigal) and functional annotation (eggNOG-mapper) were performed.

Results
Metagenomic data analysis unveiled a diverse taxonomic landscape of bacteria and archaea in the high CO_2 environment of the Eger Rift. Predominant phyla in drill core samples included Pseudomonadota and Cyanobacteria, while Firmicutes, Pseudomonadota, and Bacteroidetes dominated water samples. Two high-quality MAGs were retrieved from the metagenomic samples. One MAG could be identified as the genome of an archaeon, *Methanobacterium paludis*, which shows an estimated average nucleotide identity of 89.84% and 93.62% with the published reference organisms. The other MAG was affiliated with the genus Proteiniphilum. Within these MAGs, the presence of diverse carbon fixation pathways, including key enzymatic steps and metabolic routes, was identified.

Summary
Our study extensively explored taxonomic diversity, functional potential, and genetic adaptations in the CO_2-rich Hartoušov mofette system. These insights into carbon fixation pathways hold promise for CO_2-based biotechnological advancements.

References
1. Liu, Q. et al. Microbial Signatures in Deep CO_2-Saturated Miocene Sediments of the Active Hartoušov Mofette System (NW Czech Republic). *Front. Microbiol.* 11, (2020)

OP-EMP-013
Discovering microbial communities within the deep biosphere of arid and humid ecosystems.
*L. Horstmann[1,2], D. Lipus[2], T. Friedl[1], R. Oses[3], D. Wagner[2,4]
[1]*Georg-August University Göttingen, Department Experimental Phycology and Culture Collection of Algae (EPSAG), Albrecht-von-Haller-Institute for Plant Sciences, Göttingen, Germany*
[2]*GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Section Geomicrobiology, Potsdam, Germany*
[3]*Universidad de Atacama, Centro Regional de Investigación y Desarrollo Sustentable de Atacama (CRIDESAT), Copiapó, Chile*
[4]*University of Potsdam, Insitute of Geosciences, Potsdam, Germany*

The deep biosphere is one of the most extensive and enduring habitats, comprising a significant fraction of Earth"s total biomass. Microbes inhabiting the pore space of sedimentary and igneous rocks in the subsurface encounter various challenges, including anoxic, xeric, and oligotrophic conditions. The initially organic-free igneous systems are nutrient-limited. Thus, they depend on biomass input from the surface through fluid transport along tectonic fractures. Little is known about processes in the deep subsurface of arid landscapes, where surface water input is extremely limited.

Our study provides insights into the deep biosphere of arid (Pan de Azúcar) and humid (Nahuelbuta) ecosystems along the coast of Chile. We analyzed microbial communities from up to 55 m deep subsurface granite rocks using 16S rRNA gene amplicon and shotgun metagenomics sequencing. By integrating DNA-based taxonomic and fluorescein diacetate hydrolytic activity (FDA) data, we identified a diverse deep biosphere microbial community beneath both the humid and arid surface conditions showing a potentially higher activity level compared to shallow desert soil communities. This community was characterised mainly by ubiquitous heterotrophic bacteria assigned to *Pseudarthrobacter*, *Janthinobacterium*, and *Pseudomonas*, regardless of surface climate. However, rare taxa affiliated with common chemolithoautotrophs, e.g., *Thiobacillus, Sulfuriverula, and Sulfuricurvum,* were only observed in the arid subsurface of Pan de Azúcar. This indicates an increased adaption to nutrient-limiting conditions in the arid desert deep biosphere. Functional analyses revealed sulfur and carbon monoxide as the most likely alternative electron donors, while no potential evidence for hydrogenotrophy or iron cycling was detected in both climates.

We discovered a diverse and potentially active deep biosphere inhabiting granitic rocks, a system partially influenced by climate conditions on Earth's surface. This contributes to the knowledge about energy acquisition in deep biosphere ecosystems and its interconnection with surface conditions.

OP-EMP-014
Cell shape modification as novel buoyancy control mechanism of marine Roseobacter bacteria
*M. Staack[1], C. Jogler[1], N. Kallscheuer[1], M. C. F. van Teeseling[1]
[1]*Friedrich Schiller University Jena, Microbial Interactions, Jena, Germany*

The *Roseobacter* group (class *Alphaproteobacteria)*- accounts for up to 25% of the bacterial community in marine habitats. Members of this group are physiologically versatile, which can be regarded as prerequisite for an adaptation to different ecological niches. In a constantly changing environment, they can exploit transient nutrient pulses and thereby successfully compete against microorganisms inhabiting the same habitat. Morphological heterogeneity is another common trait in the *Roseobacter* group. Morphotypes range from rod- shaped single cells of variable length to multicellular rosettes and filaments. For *Dinoroseobacter shibae* it has been shown that cell elongation is regulated by quorum sensing[1].

Here, we present evidence that for *Phaeobacter inhibens* DSM 17395, a common model organism of the *Roseobacter* group and successful colonizer of marine surfaces, there are triggers for cell elongation- decoupled from studied quorum sensing mechanisms. Using time-lapse microscopy

experiments and quantitative image analysis we show that *P. inhibens* undergoes morphological changes in response to the presence of yeast and algal extracts. We demonstrate that the longitudinal cell elongation increases the buoyancy of the bacterial cells. The phenotype was still observed for a *P. inhibens* mutant strains devoid of the cell cycle ranscriptional regulator gene *ctrA*. Removing the eukaryotic trigger by medium exchange results in an immediate reinitiation of cell division and reversal of the elongated cell morphotype back to rod- shaped single cells and rosettes. We hypothesize that *P. inhibens* uses cell shape modifications to control buoyancy in a nutrient-rich zone and to enlargen the surface are for attachment.

[1] D. Patzelt, H. Wang, I. Buchholz, M. Rohde, L. Gröbe, S. Pradella, A. Neumann, S. Schulz, S. Heyber, K. Münch, R. Münch, D. Jahn, I. Wagner-Döbler, J. Tomasch, You are what you talk: quorum sensing induces individual morphologies and cell division modes in *Dinoroseobacter shibae*, *The ISME Journal*, 7 (2013) 2274-2286.

OP-EMP-015

(Pan)genomic analysis of two Rhodococcus isolates and their role in phenolic compound degradation

*S. Benning[1], K. Pritsch[2], V. Radl[3], R. Siani[1], Z. Wang[1], M. Schloter[1,4]
[1]*Helmholtz Center Munich, Comparative Microbiome Analysis, Neuherberg, Germany*
[2]*Helmholtz Center Munich, Research Unit for Environmental Simulations, Neuherberg, Germany*
[3]*Helmholtz Center Munich, Neuherberg, Germany*
[4]*Technical University Munich, Chair of Environmental Microbiology, München, Germany*

Introduction: Members of the genus *Rhodococcus* are known for their ability to degrade a wide range of aromatic compounds such as phenolic exudates from plants. They have a high potential to adapt to different habitats due to their linear plasmids and large genome size.

Goals and Methods: We used comparative genomics to investigate the genomic properties of two newly described *Rhodococcus* strains (*R. pseudokoreensis* R79T and *R. koreensis* R85) isolated from apple rhizosphere and compared their traits to other members of the genus. Of particular interest was their ability to degrade phenolic substances as part of a multidisciplinary approach to mitigate apple replant disease (ARD).

Results: The pan-genome of 109 high-quality *Rhodococcus* genomes was open with a small core (1.3 %), consisting of genes responsible for basic cell functions. The range of genome sizes was immense (3.7 Mbp to 10.9 Mbp). Genomes of strains associated with hosts were generally smaller than those of environmental isolates, that displayed exceptionally large genome sizes. Due to the large genomic differences, we support taxonomic reclassification of certain rhodococci to new genera. Furthermore, we found several strains to be taxonomically incorrectly assigned or being probably undescribed species. Taxonomic affiliation was the most important factor in predicting the genetic content and clustering of the genomes. In addition, we found genes that differentiated between strains based on habitat. This could potentially be used for targeted isolation and screening for desired traits. All members of the genus *Rhodococcus* had at least one gene involved in benzoate degradation, whereas biphenyl degradation was mainly restricted to strains closely phylogenetically related to our isolates.

Summary: The genomic features displayed by our isolates suggest a potential application in the reduction of phenolic exudates of plants in soil. The approximately 40% of genes that remain unclassified in larger *Rhodococcus* genomes, particularly those from environmental isolates, require further investigation to explore the metabolic potential of this genus.

OP-EMP-016

Elucidating the upper pathway in anaerobic phenanthrene degradation

*N. Samak[1], R. Meckenstock[1]
[1]*Faculty of Chemistry, University of Duisburg-Essen, Environmental Microbiology and Biotechnology, Essen, Germany*

Polycyclic aromatic hydrocarbons (PAHs) threaten human lives since they are carcinogenic. They are widely distributed in the environment and oil. However, oxygen is rapidly depleted in water-saturated sediments containing PAHs, making anaerobic microorganisms responsible for biodegradation. So far, only the anaerobic degradation pathway of naphthalene as a model compound has been studied in more detail but the anaerobic degradation of larger PAHs such as phenanthrene is still unknown.

The anaerobic degradation of phenanthrene starts with the activation of the compound via carboxylation yielding 2-phenanthroic acid. An aryl-CoA ligase was confirmed to be responsible of the subsequent conversion of the produced 2-phenanthroic acid to 2-phenanthroyl-CoA.

Here, we studied putative following reduction reactions targeting the aromatic rings of phenanthrene to overcome the high resonance energy. We elucidated the function of four newly identified type III aryl-CoA reductases from the TRIP1 enrichment culture involved in anaerobic phenanthrene degradation. The corresponding genes were heterologously expressed in *Escherichia coli*, the enzymes purified, and their catalytic activity toward 2-phenanthroyl-CoA was confirmed by Liquid Chromatography–Mass Spectrometry and UV-vis spectroscopy. The oxygen-sensitive, ATP-independent enzymes reduced 2-phenanthroyl-CoA to decahydro-2-phenanthroyl-CoA with dithionite-reduced methyl viologen as electron donor. The reactions occurred in consecutive two-electron reduction steps each catalyzed by one enzyme. The four enzymes together could also reduce the last benzyl-ring of the former phenantroyl-CoA, which is thermodynamically very challenging. The four enzymes belong to the old yellow enzyme family, which contain the flavin cofactors flavin mononucleotide and flavin adenine dinucleotide. Iron content analysis and structural homology modeling confirmed that the four enzymes contain an iron-sulfur cluster which mediates electron transfer. In conclusion, we demonstrated that the ATP-independent NADH-Flavin oxidoreductases are responsible for phenanthrene ring reduction and belong to the recently discovered type III aryl-CoA reductases.

OP-EMP-017

Harnessing the potential of microbes: Identification and implementation of a new Pseudomonas strain for use in the field of industrial precious metal recycling

*E. M. Gabor[1], M. Gauert[1], A. Levesque[2], A. Blatter[2]
[1]*BRAIN Biotech AG, Zwingenberg, Germany*
[2]*PX Group, Research and Innovation, La Chaux de Fonds, Switzerland*

The challenges posed by climate change and the scarcity of resources call for new, sustainable production processes. In the field of precious metal recovery, research into the use of cyanogenic microorganisms has been ongoing for decades, however, without reaching sufficient technical maturity. At BRAIN Biotech, we have isolated a novel *Pseudomonas* strain (*Pseudomonas metallosolvens*) that exhibits particularly high metal tolerance, robustness and productivity in biocyanide synthesis, while being BSL-1 classified. This allows it to be used in industrial processes for the extraction of precious metals. Employing design of experiment (DOE) approaches, both the cultivation and extraction conditions for precious metals were optimized and a storage-stable formulation of the cells was developed for use as industrial starter cultures in dedicated recycling processes. Targeted bioprocess development has made it possible to scale up biocyanidation from laboratory scale to pilot scale (2 x 750 L) and to implement processing of gold-bearing ashes with promising results. Various influencing variables were taken into account, ranging from composition and physico-chemical accessibility of precious metals in different material classes to biologically relevant parameters such as oxygen transfer rate, pH, temperature and the effect of shear forces. Thanks to the interdisciplinary collaboration between chemists, microbiologists and process engineers, it was possible to scale up this complex process of biocyanidation to a relevant scale and to set up a mobile pilot demonstrator for further process development.

OP-EMP-018

Bioleaching of nickel and cobalt from laterite ores in Brazil: A reactor-based approach

*S. A. Hetz[1], A. Schippers[1]
[1]*Federal Institute for Geosciences and Natural Resources, Geo, Hannover, Germany*

Laterite ore deposits in Brazil and other tropical countries house substantial nickel and cobalt resources, as well as other vital raw materials. Traditional methods for extracting nickel and cobalt, such as pyrometallurgy or high-pressure acid leaching, come with significant downsides, including high energy usage, reagent expenses, and the necessity for costly equipment. To tackle these challenges, the German-Brazilian project BioProLat aims for an integrated, low-energy, and environmentally friendly biohydrometallurgical process for extracting metals from Brazilian laterite ores.

The process involves harnessing acidophilic bacteria that use sulfur as an electron donor, linking sulfur oxidation to the reduction of ferric iron. This transformative mechanism converts insoluble metal compounds into water-soluble forms. Consequently, sulfuric acid is produced, creating the essential acidic conditions to maintain the solubility of iron and other metals. Laboratory-scale bioreactor experiments were done with varying parameters such as pH, temperature, and the selection of a suitable bacterial consortium for optimal bioleaching of nickel and cobalt. Notably, aerobic bioleaching of laterite with a consortium of *Acidithiobacillus thiooxidans* strains achieved a maximal extraction of 85% cobalt and 85 % nickel from a laterite ore sample. Comprehensive mineralogical and geochemical analyses were conducted to identify mineral phases susceptible to bioleaching and estimate the proportions of cobalt and nickel released from various mineral phases. The overarching objective is to upscale the optimized process, transforming untapped ores and limonite stockpiles into valuable resources while unlocking new reserves of raw materials through enhanced metal recovery from existing mines, including economic and environmental evaluation.

OP-EMP-019

Use of microorganisms in peat-free and peat-reduced substrates.

*R. T. Proma[1], K. Burow[2], P. Franken[1,2]
[1]*Friedrich Schiller University Jena, Jena, Germany*
[2]*Erfurt University of Applied Sciences, Research Centre for Horticultural Crops (FGK), Erfurt, Germany*

Conventional horticultural practices often rely on the use of peat as a primary component in growing media. However, due to the environmental repercussions associated with peat extraction and its limited availability, a critical need has been prompted to explore sustainable alternatives. This study, a part of the ToPGa research project "Development and evaluation of peat-reduced production systems in horticulture", focuses on addressing challenges related to nitrogen (N) immobilization, substrate compaction and optimizing plant growth in cultivation systems that minimize or eliminate peat usage by using microorganisms. A comprehensive understanding of microorganisms and their influence on substrate quality could transform horticultural practices, presenting a more environmentally conscious approach while concurrently improving plant health and productivity. First, the impact of various substrates on plant growth was examined. Eight distinct substrate mixtures, comprising peat, green compost, wood fiber, nettle fiber, digestate, loess/loam, and perlite were used. Both sterile and unsterile treatments were applied to all substrates. *Petunia hybrida* cv. 'Mitchell' and *Ocimum basilicum* were chosen as the plant species. The plants' phenotypic characteristics were observed, and the substrates were analyzed for their chemical properties at the experiment's commencement and conclusion. After six weeks, both plant species exhibited superior growth in a sterile, peat-free substrate composed of 50% green compost and 35% wood fiber, as well as in a substrate mixture of 50% peat, 25% green compost, and 25% digestate, irrespective of sterility. In the subsequent phase, diverse microorganisms will be introduced to different substrate mixtures to augment substrate quality and assess their effects on plant growth development. Various plant growth-promoting rhizobacteria (PGPR), *Schizophyllum commune*, arbuscular mycorrhizal fungi (AMF) and Dark Septate Endophytes (DSEs) will be employed, leveraging their unique qualities for counteracting N-immobilization and substrate compaction. This aims to establish and enhance microbial consortia designs for future studies.

Eukaryotic Pathogens

OP-EP-001

Candida auris Transmission in Germany

*A. M. Aldejohann[1,2], N. Thielemann[1], R. Martin[1,2], O. Kurzai[1,2]
[1]*University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[2]*Leibniz Institut für Naturstoff-Forschung und Infektionsbiologie, Nationales Referenzzentrum für Invasive Pilzinfektionen (NRZMyk), Jena, Germany*

Introduction

Candida auris is an emerging fungal pathogen, which is resilient to environmental stress and rapidly develops

antifungal drug resistance. Unlike most other *Candida* species, *C. auris* causes nosocomial transmissions and infections leading to severe hospital outbreaks. However, in comparison to other European countries, the number of reported *C. auris* infections in Germany is still considered low.

Goals

We analysed fungal isolates and patient data from *C. auris* cases reported to the German National Reference Center for Invasive Fungal Infections (NRZMyk) to (i) monitor *C. auris* epidemiology in Germany (ii) record and classify possible/ probable transmission events (iii) to gain insights into colonisation and infection dynamics.

Materials & Methods

Strains were genetically and phenotypically characterized and subdivided into clades. Whole genome sequencing was performed on selected isolates recording the diversity between closely related isolates from patient-to-patient transmission events. Antifungal drug susceptibility was tested with broth microdilution according to EUCAST or other commercially available assays.

Results

Whin the last two years (2022/23) the number of *C. auris* primary isolates increased over six-fold from 12 to 77 cases. The majority (66) belonged to Clade I. Non outbreak related clinical data were available for 28 and signs of invasive infection were suspected in 13 cases. 49 isolates were assigned to overall 3 suspected events of transmission. Of these, two events were rapidly contained affecting 5 patients in total. However, the so far biggest recorded outbreak in Germany had an impact on 44 patients and over 4 different university hospital units. Whole genome sequencing revealed close relationships between the patients strains and thus proofed transmission.

Summary

We observed a further rise of *C. auris* cases in the last years. Increased awareness for colonization and infection with *C. auris* is required for a timely development of appropriate strategies regarding containment and surveillance. This will also contribute to a better understanding of patient-to-patient transmissions.

OP-EP-002
Single-cell force spectroscopy of Giardia duodenalis trophozoites revealed a unique attachment mode compared to other eukaryotic cells

*G. Gunaratnam[1], *B. Wieland[1], R. Leisering[2], J. Dudek[3], N. Miosge[3], S. L. Becker[1], M. Bischoff[1], S. C. Dawson[4], M. Hannig[3], K. Jacobs[5], C. Klotz[2], T. Aebischer[2], P. Jung[1]
[1]*Saarland University, Institute of Medical Microbiology and Hygiene, Homburg, Germany*
[2]*Robert-Koch-Institute, Department of Infectious Diseases, Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany*
[3]*Saarland University, Clinic of Operative Dentistry and Periodontology, Homburg, Germany*
[4]*University of California Davis, Department of Microbiology and Molecular Genetics, Davis, CA, United States*
[5]*Saarland University, Experimental Physics, Saarbrücken, Germany*

Introduction: The unicellular parasite *Giardia duodenalis* is the causative agent of giardiasis, a gastrointestinal disease with global spread. Disease initiation is provoked by adhesion between *G. duodenalis* and the human intestinal epithelium. A unique microtubule-based attachment organelle, the cup-shaped ventral disc, facilitates this process. However, the detailed physical function of the ventral disc is still debated. In this study, we investigate adhesion forces involved during attachment of single *G. duodenalis* trophozoites, in comparison to adhesion mechanisms of the opportunistic pathogen *Candida albicans* and spreading human keratinocytes.

Material & Methods: Single cell force spectroscopy, based on fluidic force microscopy (FluidFM) was used to investigate the adhesion parameters of *G. duodenalis* trophozoites adhering to a flat glass surfaces. Comparison were made with the aforementioned cell types.

Results: Force-distance curves displayed novel and so far undescribed characteristics for a microorganism, namely, gradual force increase on the pulled trophozoite, with localization of adhesion force shortly before cell detachment length. Adhesion forces reached 7.7 ± 4.2 nN at 1 μm/s pulling speed. Importantly, this unique force pattern was different from the saw-tooth pattern of *Candida albicans* and from the long cell interaction length of spreading keratinocytes, both considered for comparison in this study. Furthermore, the attachment mode of *G. duodenalis* trophozoites was mechanically resilient to tensile forces when pulling speeds were increased to 10 μm/s while adhesion forces rose to approximately 28.7 nN.

Conclusions: Comparative force spectroscopy revealed novel and unique retract force curve characteristics for attached *G. duodenalis* trophozoites, suggesting a ligand-independent suction or clutching mechanism that substantially differs from other well-described microbial adhesion strategies.

OP-EP-003
Multi-reporter: fluorescent reporter for temporal detection of cell death in infection

*M. Katsipoulaki[1], V. Trümper[1], B. Hube[1], S. Brunke[1]
[1]*Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie Hans-Knöll-Institut, Microbial Pathogenicity Mechanisms, Jena, Germany*

Most microbial pathogens, including bacteria, fungi, and viruses, can lead to the death of their host cells. Host cells, however, die in a multitude of different ways. Some are inflammatory, such as pyroptosis and necroptosis, and some are not, such as apoptosis. The currently available tools for cell stress and death detection are limited and cannot easily distinguish between the diverse cellular events leading to cell death.

Therefore, we are creating fluorescent reporters for cellular stress and different cell death pathways such as apoptosis, pyroptosis or necroptosis, which allow real-time detection by fluorescent microscopy. Cell lines are created via lentiviral transduction, which also allows multiple transductions into a single cell line to create multi-pathway reporters. Cell lines include THP-1 macrophages and C2BBe1 intestinal epithelial cells. We have created fluorescent reporters for redox status (stress), using a roGFP2 fluorophore, and apoptosis (cell death), using a bimolecular fluorescence complementation

(BiFC) reporter for live detection of caspase 3/7 activation. Another fluorescent reporter for live detection of pyroptosis, *via* visualization of Gasdermin-D oligomerization, is being validated. As all reporters work with non-overlapping spectra, we aim to create a single "multi-reporter" cell line, which will enable us to detect simultaneously different types of cell stress and death pathways.

This reporter system will allow to monitor cellular stress and to differentiate the different host cell death pathways that are active during infection. Importantly, it will be possible to follow the state of the host cells in a temporally and spatially resolved manner. Our new reporter will thereby help to answer many of the open questions on host-pathogen interactions during infection.

OP-EP-004

Reconstitution of an essential metabolic pathway of Plasmodium falciparum in Escherichia coli, facilitating functional and inhibitor screens

O. A. Akuh[1], D. Maus[1], M. Blume[1], K. Saliba[1], *F. Seeber[1]
[1]*Australian National University, ANU College of Science, Canberra, Australia*

Introduction: New and highly selective drug targets for the malaria-causing parasite *Plasmodium falciparum* are still needed. The ferredoxin redox system (Fd-FNR), located in a plant-derived organelle called apicoplast, is essential for parasite survival due to its involvement in the methylerythritol phosphate pathway (MEP) for isoprenoid biosynthesis. However, *in vitro* screens for inhibitors are challenging due to the labile nature of the recombinant iron-sulfur proteins involved.

Goal: To develop an *in situ* model in *E. coli* which reports essential functions of PfFd and its downstream electron acceptors (i.e. the terminal enzymes of the MEP pathway, shared by both organisms) as growth/intermediate/no growth.

Materials & Methods: Deletion of the two essential genes of *E. coli,* flavodoxin (EcFldA) and MEP enzyme EcIspH, and inducible expression of the respective *P. falciparum* proteins (PfFd, PfFd reductase (PfFNR) and PfIspH), followed standard procedures.

Results: We report for the first time a double mutant of *E. coli* FldA and IspH. It is dependent on the mevalonate bypass system which provides the essential metabolite IPP. Removing mevalonate makes the strain entirely dependent on the simultaneous expression of plasmid-encoded PfFd, PfFNR, PfIspH, which documents that the three parasite proteins are required functionally active to replace the respective endogenous *E. coli* proteins. This system will allow initial functional tests to be performed. As a proof of concept, several amino acids of PfFd suspected to be involved in the interaction with PfIspH were individually mutated and the respective redox function could be evaluated by simple growth assays. Metabolomic studies are under way to characterize the mutants' consequences on the MEP.

Summary: We describe an engineered *E. coli* strain dependent on three *P. falciparum* proteins, PfFd, PfNR and the terminal MEP enzyme PfIspH, which will be very useful for functional as well as drug screening purposes of this essential parasite pathway before findings are validated in the parasite.

OP-EP-005

Mechanisms of Candida albicans intracellular filamentation in human macrophages leading to peptide toxin secretion and escape

*J. Sonnberger[1], L. Denner[2], L. Kasper[2], T. Lange[2], S. Brunke[2], B. Hube[2,3,4]
[1]*Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie Hans-Knöll-Institut, Microbial Pathogenicity Mechanisms, Jena, Germany*
[2]*Leibniz-Institut für Naturstoffforschung und Infektionsbiologie Hans-Knöll-Institut, Microbial Pathogenicity Mechanisms, Jena, Germany*
[3]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*
[4]*Friedrich Schiller University Jena, Cluster of Excellence Balance of the Microverse, Jena, Germany*

Phagocytes are essential for the initial immune response to infections with the opportunistic fungal pathogen *Candida albicans*. While the fungus is efficiently phagocytosed by macrophages, it also possesses strategies to survive inside phagosomes and to escape from these phagocytes within hours.

Rapid initiation of filamentation inside the phagosome is a critical step for *C. albicans* escape since subsequently, filamentation leads to exertion of physical forces and drives damage to the macrophage. Filamentation also occurs in conjunction with the production of the peptide toxin candidalysin. After phagosomal escape, the toxin contributes to host cell damage, activation of caspase-1, and the NLRP3 inflammasome leading to the release of IL-1β. However, candidalysin is mostly dispensable for the induction of caspase-1-dependent pyroptosis. This undermines the importance of understanding how filamentation is initiated in the host phagosome.

This key process is likely triggered by the amino acid arginine. Under phagosome-mimetic conditions such as acidic pH and nutrient deprivation, addition of arginine and the related amino acids ornithine and proline selectively induces filamentation. This is the case in both arginine-prototrophic and -auxotrophic strains. During infection with human primary monocyte-derived macrophages, the same strains exhibit similar filamentation and escape rates and induce comparable levels of damage. These data support the hypothesis that fungal arginine biosynthesis is not required for induction of intraphagosomal filamentation, but rather host-derived arginine. Our initial data suggests that an amino acid transporter expressed by macrophages localizes to the phagosome, yielding a potential source for this signal.

In summary, host-cell escape requires a combination of hyphal extension and candidalysin production. In ongoing work, we are investigating the role of fungal arginine catabolism on the escape of *C. albicans*, especially with regard to the importance of arginine in macrophage polarization and the function of candidalysin in macrophage cell death.

OP-EP-006
Using a human airway organoid-derived monolayer co-culture model to study Cryptococcus neoformans and other fungal infections

*S. Reusch[1], E. Korsch[1], C. Schwister[1], T. Aebischer[1], C. Klotz[1], V. Rickerts[1]
[1]*Robert Koch-Institute, Department of Infectious Diseases, Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany*

Introduction

Cryptococci are environmental fungi that cause localized or disseminated infections. The WHO considers *C.neoformans* to have the highest need for research among fungal pathogens. Studies on pathogenicity are mainly based on animal models or immune cells. However, to understand the pathomechanisms associated with the transition from pulmonary colonization to tissue infection, complementary models are required. We are using a human lung infection model to investigate parameters of early cryptococcal infection and epithelial transition.

Methods

We used well-characterized human airway-organoid derived monolayers (ODMs) from nasal and bronchial tissue and co-cultured them with *C.neoformans* (VNI) and *C.gattii* (VGII). Barrier function of ODMs was probed by measuring the transepithelial electrical resistance (TEER). We quantified internalization of fungi and fungal viability by immunofluorescence and colony forming unit assays. Fungal morphology was assessed using ink stainings and image analysis.

Results

Human ODMs could be co-cultured with different cryptococcal strains and TEER measurements revealed no breakdown of barrier function, even after prolonged time intervals. Rare events of fungal internalization by airway epithelial cells were detected. Fungi remained viable during co-culture and were able to further proliferate. Quantification of cryptococcal morphology revealed a shift from uniform cells towards distinct phenotypes, including micro- and giant cells.

Discussion

The established co-culture model provides a suitable *in vitro* model to study host-cryptococcal interactions. Quantification of TEER indicate no impact of different cryptococcal strains on the barrier function of airway epithelial cells, despite the fact that both fungal strains show different disease phenotypes in humans. However, the model revealed that fungi undergo morphological changes, indicating that they adapt different phenotypes as a consequence of epithelial cell presence. Changes in morphology have been observed in *in vivo*-studies and are associated with pathogenic potential. Thus, the co-culture model can be used in the future for risk assessment of different fungal pathogens.

OP-EP-007
Plasmodium falciparum utilizes proteins of the complement and fibrinolysis pathway for red blood cell invasion

*A. Byrd[1], S. Ayoub[1], T. Reiß[1], G. Pradel[1]
[1]*RWTH Aachen University, Cellular and Applied Infection Biology, Aachen, Germany*

The intracellular parasite *Plasmodium falciparum* causes malaria tropica, the severest form of malaria in the WHO African region. With over 619,000 deaths in 2022, malaria is still one of the deadliest diseases worldwide. The symptomatic phase includes fevers, head- and body aches, and diarrhea. In erythrocytes, the parasite develops via the ring, then trophozoite stages into mature schizonts, which form up to 32 daughter merozoites (MZs) that are released and invade new red blood cells (RBCs). In the bloodstream, the extracellular MZs are susceptible to the human complement system. To avoid complement-mediated lysis, the parasites bind to the complement inhibitor factor H (FH) or to plasminogen, a protein in the fibrinolysis system. There is currently little information on how these proteins influence MZ invasion of RBCs. Therefore, we performed MZ invasion assays to investigate how proteins of the FH family, plasminogen, and other key alternative pathway proteins influence their entry into RBCs. We found that in the presence of C3b but also under FHR1-depleted conditions, the numbers of intraerythrocytic parasites increased. Furthermore, under plasminogen-deficient conditions, RBC infection by MZs was reduced, while the addition of plasminogen led to increased invasion rates independent of active complement. In conclusion, we hypothesize that MZs utilize proteins of the complement and the fibrinolysis system to facilitate erythrocyte attachment for efficient and successful invasion and intraerythrocytic replication.

Food Microbiology and -hygiene

OP-FMH-001
Biocontrol of pseudomonas biofilms in dairy farming environments using bacteriophages

*F. Hille[1], S. Gieschler-Lübbehüsen[1], E. Brinks[1], C. Franz[1]
[1]*Max Rubner-Institute, Microbiology and Biotechnology, Kiel, Germany*

Introduction

Pseudomonads are a genus of ubiquitous Gram-negative bacteria that encompasses species with major impact on food spoilage, especially in the dairy industry. Several physiological properties enable them to persist in production plants and increase the risk of food spoilage: *Pseudomonas* bacteria are psychrophile and outgrow other bacteria in cool conditions that are often present during storage of fresh produce. Moreover, they form biofilms, allowing them to persist in food plants and efficiently resist sanitation measures. Lastly, a major problem especially in the dairy industry is the ability of some *Pseudomonas* species to produce heat-stable proteases, which can withstand procedures like ultra-high-temperature treatment of raw milk, causing the decay of milk proteins even after sterilization, and thus leading to premature spoilage of the milk. In order to eliminate *Pseudomonas* species relevant for milk spoilage, we explore the application of bacteriophages (viruses that infect bacteria) to eradicate persistent biofilms and limit the contamination of milk with heat-stable proteases.

Goals

The goal of this study is to identify potent phages that are active against a broad spectrum of relevant spoilage species like *P. proteolytica*, *P. lactis* and *P. protegens*. The combined use of those phages in a cocktail will be utilized to eradicate *Pseudomonas* biofilms, which are grown under practically relevant conditions.

Results

A total of 29 phages were isolated. The majority (25) belonged to the *Caudoviricetes* class, which was expected as it is most prevalent in nature. Within that class, the majority of the isolated phages has not been described in literature. The host range varied significantly and some phages infected as much as 19 host strains, making them interesting candidates for biofilm inhibition. Their efficiency in the eradication of biofilms will be evaluated in coming experiments.

Summary

The use of phages as biocontrol agents against *Pseudomonas* biofilms is explored in this study and includes the identification of phages infecting relevant bacterial strains and the evaluation of their efficiency to degrade their host"s biofilms.

OP-FMH-002

Impact of primary production conditions on the microbiome of German bulk tank milk

*M. Wenning[1], A. Siebert[2], G. Fiedler[3], H. G. Walte[3], K. Hofmann[2], S. Gieschler-Lübbehüsen[3], G. Lücking[2], C. Böhnlein[3], C. Franz[3], S. Scherer[2]

[1]*Bavarian Health and Food Safety Authority, Oberschleißheim, Germany*

[2]*Technical University of Munich, Freising, Germany*

[3]*Max-Rubner Institute, Kiel, Germany*

Raw milk gets contaminated with a large variety of microorganisms during the milking process, e.g. via the teats of the udder or the milking equipment. Not much is known, however, how and to which extent the different conditions during primary production influence the composition of the microbiome. It is therefore difficult to choose the right measure for beneficially influencing the microbiological quality of bulk tank milk.

The aim of this study was the comprehensive evaluation of the microbial community composition of raw milk from 350 farms throughout Germany and their farm specific influencing factors. 16S rRNA gene amplicon sequencing was performed to map the microbiome and information on conditions of housing, milking process and other factors were collected by questionnaires. The analysis focused in particular on the diversity of the microbiome, the genera responsible for the main variance and a correlation of production conditions with abundance of particular genera in the microbiome.

The raw milk microbiome is influenced by a multitude of factors, which is reflected by a very heterogeneous distribution of bacterial numbers and microbial diversity. Outliers were detected for each factor analysed, however, there were also correlations observed. An automated milking system was associated with an increased microbial count and organic production conditions with a significantly higher diversity and lower microbial counts as conventional production.

The large variance in the data, though, suggests that there are complex relations between factors and possible other determining factors not evaluated so far that may also impact the microbial composition. More studies focusing on a more specific and narrow choice of parameters are needed to better understand the interrelations of different farm managing practices in milk production.

OP-FMH-003

Attachment of Listeria monocytogenes to corn salad.

*T. Hoffmann[1], A. Weiß[1]

[1]*Universtiy Hamburg, Foodmicrobiology, Hamburg, Germany*

Question *L. monocytogenes* is one of the most important foodborne pathogens because of the high hospitalization and mortality rate. Little is known about how exactly *L. monocytogenes* attaches to corn salad, one of the most prevalent fresh produce. Pathogenicity is highly serovar dependent giving rise to the question if there is a link between colonization and virulence. After contamination, attachment and biofilm formation poses a serious risk because it allows the bacteria to persist over long time periods. Therefore, the aim of this study is to investigate the attachment and colonization of *L. monocytogenes* on corn salad as well as its ability to form biofilm and to answer the question which genes are involved in these processes.

Methods Three strains of *L. monocytogenes* strains isolated from plant foods as well as from clinical samples were first characterized genotypically using whole genome sequencing (WGS). Strain NCTC 10587 was fluorescently labelled using the chromosomally integrating plasmid vector pAD1. Confocal laser scanning microscopy of the fluorescently labelled strain was used to confirm attachment to leaf surfaces, scanning electron microscopy (SEM) was used to track attachment at the single cell level. To quantify the biofilm formation, *in vitro* crystal violet assays were performed in triplicate in three different media at two temperatures for 72 h.

Results WGS of the three *L. monocytogenes* strains confirmed the presence of major virulence factors. Fluorescence microscopy was performed with infected corn salad leaves 48 h post infection. SEM imaging showed *L. monocytogenes* mainly colonizing the stomata of corn salad and no biofilm formation was confirmed on the surrounding areas. Contrary, crystal violet assays showed that biofilm formation was highest in Luria Bertani medium at 22°C after 12 to 14 h.

Conclusion The results of this study suggest a colonization of the corn salad stomata without biofilm formation on the leaf surfaces, even though the strains are capable of biofilm formation *in vitro*. Further studies need to elucidate the relevance of these findings for consumer safety.

OP-FMH-004

Parameters influencing water kefir grain growth dynamics by image analysis

*P. Bethge[1], B. Schmalfuss[2], C. Marashdeh[2], T. Henle[2], T. Mascher[1]

[1]*Technical University of Dresden, Chair of General Microbiology, Dresden, Germany*

[2]Technische Universität Dresden, Chair of Food Chemistry, Dresden, Germany

Water kefir is a fermented beverage that is produced by adding water kefir grains to a sucrose solution with dried fruits. The approximately 1-10 mm large grains consist of a bacterial exopolysaccharide matrix and are mainly colonised by lactic acid bacteria, acetic acid bacteria and yeasts. The production depends on the successful cultivation and propagation of the existing grains: During fermentation cycles, the mass of the grains increases, but growth dynamics between the start and end points of fermentation and the parameters that can influence this process have not yet been analysed. Monitoring growth is potentially relevant for the industrial production of water kefir, as no defined starter cultures are available. Our aim was therefore to establish a method for the continuous documentation of grain growth and thus enable a kinetic description of the involved processes.
We developed a documentation system that takes high quality pictures, which are then further processed with Fiji image-analysis software. Kefir grains were selected by size and incubated in standardized water kefir medium at room temperature for 72 h. The number of and increase in white pixels in the pictures against a black background represent a measure of the two-dimensional grain size and growth, respectively.
The developed method enables the visualization of grain growth curves as a function of white pixels over fermentation time. These are comparable to classic bacterial growth curves, as they exhibit lag, exponential and stationary phases. The influence of the number of starter grains and their size could be evaluated based on curve progression and total growth. It was found that an increasing number of grains and larger grain sizes have a negative effect on the overall growth rate. However, the exponential and stationary growth phases tend to be reached faster as the number of grains increases. In ongoing experiments, the influence of the sugar content and the type of water in the medium is being investigated.

Gastrointestinal Infections

OP-GI-001
A culture-free whole-genome sequencing approach for rapid detection and characterization of Helicobacter pylori in gastric biopsies
*F. Ailloud[1,2], G. Pfaffinger[2], E. Weiss[2], R. Vasapolli[1,3], C. Schulz[1,3], P. Malfertheiner[3], S. Suerbaum[1,2]
[1]*German Center for Infection Research, Partner Site Munich, München, Germany*
[2]*Max von Pettenkofer-Institut, Medical Microbiology and Hospital Epidemiology, München, Germany*
[3]*Ludwig-Maximilians University, Department of Gastroenterology, München, Germany*

Helicobacter pylori is a gastric pathogen with a high worldwide prevalence and a significant clinical burden. Chronic infection leads to chronic gastritis and can progress to other disorders such as ulcers, MALT lymphoma or gastric adenocarcinoma. The emergence of multi-resistant strains is responsible for increasing failure rates of first-line combination therapies and the WHO classified *H. pylori* as a high priority organism for the development of novel antibiotics in 2017. Current clinical guidelines for the eradication of *H. pylori* typically recommend antibiotic susceptibility testing only after unsuccessful first-line treatments. However, culture-based susceptibility testing of *H. pylori* can take up to two weeks and thus empirical drug therapies are generally started before results are available.

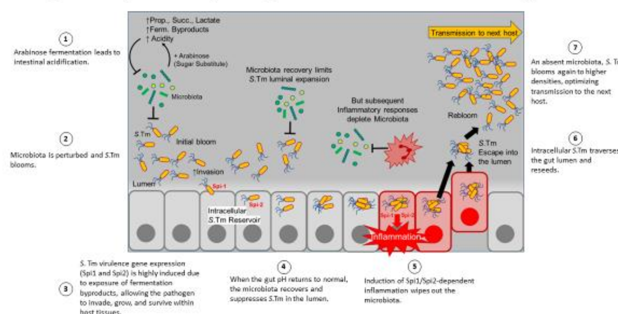
To address this clinical situation, we developed a microbial enrichment pipeline allowing us to sequence whole *H. pylori* genomes from DNA directly extracted from gastric biopsies obtained during routine endoscopy. The pipeline comprises multiple rounds of enrichment with native genomic DNA, sequencing libraries and real-time Nanopore sequencing. Using this approach, we were able to obtain complete *H. pylori* genomes with 5-10x coverage, representing a 50 to 100-fold enrichment of the low-abundance bacterial DNA within gastric tissue samples. The resulting genomic data can then be used for further characterization relevant to clinical diagnosis or public health surveillance. In particular, we performed a genotype-based analysis to predict susceptibility to clinically relevant antibiotics and obtained accuracies ranging from 93% (clarithromycin) to 62% (metronidazole).

In summary, our approach allows for quick determination of antibiotic susceptibility in *H. pylori* which can ultimately be implemented as a rapid diagnostic test to optimize therapeutic efficiency and curtail the development of antibiotic resistance in this pathogen.

OP-GI-002
Ingestion of sugar substitutes promotes gut colonization and virulence in salmonella typhimurium
*B. Nguyen[1], W. D. Hardt[1]
[1]*ETHZ, D-BIOL, Zürich, Switzerland*

Consumed by millions of people to manage blood glucose and body weight, sugar substitutes may have unintended side effects. Here, we found that the ingestion of arabinose, lactulose, maltitol, erythritol, and sorbitol, which can be substituted for table sugar, reduces colonization resistance against the common food borne pathogen, Salmonella Typhimurium and promotes its virulence. In mice, consumption of these sugars upon infection led to gut colonization by the pathogen characterized by high fecal shedding, a disseminated systemic infection and enteropathy. Although the gut microbiota can utilize these sugars, it is perturbed by intestinal acidification resulting from accumulating microbiota-derived fermentation byproducts, subsequently permitting pathogen colonization. In addition, Salmonella virulence gene expression is induced, which further enhances infection and intestinal disease. Moreover, the gut environment altered by these sugar substitutes selects for fully virulent, invasive *Salmonella*, as avirulent mutants are highly attenuated for growth in the gut. Interestingly, the diet can alter the pathogen's tissue tropism along the intestinal tract. These findings highlight that sugar substitutes in diets may pose a risk for enteric infections and offer a new model for studying how diet influences pathogen behavior and disease outcomes.

Fig. 1



OP-GI-003

The natural product chlorotonil A spares the microbiota, preserves colonization resistance and prevents relapsing Clostridioides difficile infection

*T. M. Fuchs1, T. Strowig2
1Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Institut für Molekulare Pathogenese, Jena, Germany
2Helmholtz Center for Infection Research, Microbial Immune Regulation, Brunswick, Germany

Clostridioides difficile infections (CDI) remain a major healthcare problem due to high rates of recurrent infections (rCDI). The treatment with broad-spectrum antibiotics often contributes to (r)CDI by sustained damage of the microbiota-mediated colonization resistance. Thus, the development of novel antibiotic candidates with distinct antimicrobial activity profiles against (r)CDI is of high public health interest to combat *C. difficile* infections.

Within the frame of InfectControl 2020, we investigated the antimicrobial effects of a natural product compound, chlorotonil A (ChA) on *C. difficile*1. The compound *in vitro* showed bactericidal activity at concentrations comparable to those of standard antibiotics on a *C. difficile* strains. In a pilot ChA feeding experiment with pigs, we observed only minor effects on the overall composition of the gut microbiota, whereas the absolute abundance of the genera *Terrisporobacter* and *Clostridium* was significantly reduced. A transcriptome analysis of *C. difficile* revealed the disturbance and re-programming of key cellular functions even at sub-MIC concentrations of ChA.

The notable microbiota-sparing property of ChA was confirmed in non-infected mice. In a CDI mouse model, ChA strikingly outperformed vancomycin in protecting mice against relapsing CDI. Subsequent experiments to characterize differences in anti-microbial activity revealed that ChA in contrast to vancomycin results in faster clearance of *C. difficile* vegetative cells and spores from the gut, a finding correlates with the faster recovery of the microbiota of ChA-treated *C. difficile*-infected mice. We also noted an unexpected direct activity of chlorotonils towards *C. difficile* spores, preventing their germination even after the removal of the antibiotic from the media. Together, chlorotonils act at similar concentrations on both vegetative cells and spores, defining a unique antimicrobial profile compared to existing antibiotics. Chlorotonils constitute a highly interesting compound family for the development as novel antibacterial agents to break the vicious cycle of rCDI.

1Bublitz *et al.* (2023). Cell Host & Microbe. doi: 10.1016/j.chom.2023.04.003.

OP-GI-004

SARS-CoV-2 tropism to intestine but not gastric epithelial cells is defined by limited ACE2 expression

M. Pauzuolis[1], D. Fatykhova[2], B. Zühlke[3], T. Schwecke[3], M. Neyazi[1], C. Aguilar[1], S. Dökel[4], M. Ralser[3], A. Hocke[2], C. Krempl[5], *S. Bartfeld[6]
[1]*Julius-Maximilians-University of Würzburg, Research Center for Infectious Diseases, Würzburg, Germany*
[2]*Charité - University Medicine Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany*
[3]*Charité - University Medicine Berlin, Institute of Biochemistry, Berlin, Germany*
[4]*Free University of Berlin, Institute of Veterinary Pathology, Berlin, Germany*
[5]*Julius-Maximilians-University of Würzburg, Institute for Virology and Immunobiology, Würzburg, Germany*
[6]*Technische Universität Berlin, Der Simulierte Mensch, Berlin, Germany*

Severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) infection primarily affects the lung but can also cause gastrointestinal symptoms. *In vitro* experiments confirmed that SARS-CoV-2 robustly infects intestinal epithelium. However, data on infection of adult gastric epithelium is sparse and a side-by-side comparison of the infection in the major segments of the gastrointestinal tract is lacking. We provide this direct comparison in organoid-derived monolayers and demonstrate that SARS-CoV-2 robustly infects intestinal epithelium, while gastric epithelium is resistant to infection. RNA sequencing and proteome analysis pointed to ACE2 as critical factor, and indeed, ectopic expression of ACE2 increased susceptibility of gastric organoid-derived monolayers to SARS-CoV-2. ACE2 expression pattern in patient gastrointestinal biopsies mirror SARS-CoV-2 infection levels in monolayers. Thus, local ACE2 expression limits SARS-CoV-2 expression in the GI tract to the intestine, suggesting that the intestine, but not the stomach is likely to be important in viral replication and possibly transmission.

OP-GI-005

Organoid Models to Investigate Single Nucleotide Polymorphisms Associated with Enterotoxigenic Escherichia coli Resistance in Pigs

A. Ritchie[1], B. van der Hee[1], *J. Wells[1]
[1]*Wageningen University and Research, Host-Microbe Interactomics, Wageningen, Netherlands*

Enterotoxigenic *Escherichia coli* F18 (ETEC F18) is a major cause of oedema disease and severe post-weaning diarrhoea in the pig industry and antibiotics are frequently used to control spread of disease. Resistance to ETEC F18 infections have been associated with a single nucleotide polymorphism (SNP) at amino acid position 307 in the alpha (1,2) fructosyltransferase-1 (FUT1) gene, changing an adenine to a guanine. Transformed cell lines fail to represent the cellular complexity and functionality of the epithelium and primary cells are relatively short-lived and pose other technical challenges. Our group is using pluripotent and tissue stem cell-derived organoids as new tools for the study of human and animal infections. The goal of this research was to use of intestinal organoids to investigate the mechanisms genetic resistance to infection with Enterotoxigenic *Escherichia coli* F18 and potential trade-offs on intestinal epithelial barrier function.

Ileum organoids were generated from resistant AA and susceptible (AG and GG) genotypes and used for ETEC adherence assays. As expected ETEC F18 bacteria

exhibited around 10-fold higher adherence to ileum organoid monolayers with the AG and GG genotype than the AA genotype. Cas9-mediated deleted of Fed F gene encoding the pilin adhesion subunit in ETEC F18 led to loss of adhesion confirming the adhesion was indeed mediated via the pilin. Staining of intestinal organoids with susceptible and resistant genotypes with fluorescently conjugated UEA-1, a lectin binding to *a*1,2 linked fucose, showed loss of surface staining in the AA genotype but not on the GG genotype supporting the hypothesis that the AA genotype at leads to loss of FUT1 catalytic function. However, expression of FUT1 was also significantly lower in AA genotype compared to the GG genotype raising the possibility that the resistance SNP may also affect transcriptional regulation. Results will also be presented on the effect of the AA genotype on MUC-2 expression and the potential consequences for intestinal homeostasis.

OP-GI-006
Role of the CFTR in pathogenesis of Giardia duodenalis infections

*A. Müller[1], T. Aebischer[1], C. Klotz[1]
[1]Robert Koch-Institute, Unit 16: Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany

Introduction

As a widespread protozoal parasite, *Giardia duodenalis* is a common cause for malabsorption and diarrhea. Intestinal epithelial barrier defects are frequently observed in patients affected by the multifactorial disease giardiasis. While the exact pathomechanisms remain unknown, recent findings suggest a novel chain of events amounting in epithelial barrier breakdown, which starts with a disturbed ion homeostasis during the early infection phase. Amongst intestinal ion transporters, the anion channel CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) has been shown to be severely affected by *Giardia*, i.e. resulting in reduced protein expression and function.

Goals

We aim to shed light on the putative role of the CFTR channel in *G. duodenalis* infections by investigating its impact on pathogenesis at different infection stages.

Materials & Methods

Using CRISPR/Cas9-technology, intestinal organoids from a healthy, CFTR wildtype donor were converted into CFTR-impaired CF-mimicking mutants. For comparison of CFTR-mutant and wildtype, the respective isogenic organoids were cultured in a compartmentalized transwell system, infected with *G. duodenalis* and the host response was analyzed at transcriptome level using single-cell RNA-sequencing (scRNAseq).

Results

scRNAseq experiments confirm a robust transcriptional downregulation of the CFTR ion transporter upon infection. Transcriptomic changes in intestinal organoids hint towards a severely altered host lipid metabolism and mitochondrial dysfunction in the late infection stage, which is significantly more pronounced in CFTR-impaired organoids.

Summary

Due to the significant downregulation of the CFTR ion channel during *G. duodenalis* infections and its pathogenic role in a variety of different diseases, we hypothesized that CFTR non-functionality may play a greater role in giardiasis pathogenesis. Experiments using CFTR-impaired organoids indeed suggest an increased susceptibility to lipid imbalances and mitochondrial dysfunction downstream of functional CFTR impairment.

Healthcare-Associated Infections and Pathogens: Prevention, Surveillance, Outbreaks and Antibiotic Stewardship

OP-HAIP-001
A natural focus of Francisella tularensis holarctica

L. Chitimia-Dobler[1], G. Dobler[1], M. Böhmer[2], H. Sill[3], M. Antwerpen[3], *H. von Buttlar[4]
[1]Bundeswehr Institute of Microbiology, Viruses and intracellular Bacteria, München, Germany
[2]Bavarian Health and Food Safety Authority, Oberschleißheim, Germany
[3]Bundeswehr Institute of Microbiology, Forensics and Microbial Genomics, München, Germany
[4]Bundeswehr Institute of Microbiology, Bacteriology and Toxinology, München, Germany

Francisella tularensis, the causative agent of Tularemia, is a Gram-negative bacterium that can be transmitted by aerosol, contact with mucous membranes or by the alimentary route. Most common in Germany and Europe is the vector-borne infection. While in Scandinavia mosquitoes are the dominating vector, in Germany most infections originate from tick-borne transmission. As for other vector borne diseases, i.e. Tick-Borne Encephalitis Virus, the occurence of natural foci is known, where a single strain of the pathogen circulates over time, indicated by the high genetic stability of isolates over time.

When a case of tularemia associated with a tick bite in Munich occured, we started to analyse ticks from this area for *Francisella tularensis*. Isolation of the bacterium was conducted with all samples positive in *Francisella tularensis holarctica* specific PCR. For this a selective medium comprising high cystein and iron content combined with antibiotics to eliminate companion bacteria was used. All isolates were tested for antibiotic susceptibility and showed no resistance against classes of antibiotics with therapeutical relevance for Tularemia. Moreover, NGS was performed and whole genomes compared to data from *Francisella tularensis holarctica* from Germany, Austria and Switzerland. All strains belong to Clade 33 of *Francisella tularensis holarctica.* Compared to other strains of *Francisella tularensis holarctica* the isolates only differ in very few single nucleotide polymorphisms. This low diversity was seen not only in isolates from the same year but for all isolates from 2022 to 2023. The sampling of the respective area will be further continued to monitor the behaviour of *Francisella tularensis holarctica* in a natural focus.

OP-HAIP-002
Transmission analysis of carbapenemase-negative multidrug-resistant Pseudomonas aeruginosa in critical

care settings based on whole genome sequencing and epidemiological data

*J. K. Jasuja[1], E. M. Klupp[1], P. M. Maurer[1], M. Christner[2], M. Aepfelbacher[2], J. Knobloch[1]
[1]*University Hospital Hamburg-Eppendorf, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Arbeitsbereich Krankenhaushygiene, Hamburg, Germany*
[2]*University Hospital Hamburg-Eppendorf, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Hamburg, Germany*

Introduction Carbapenemase-producing *Pseudomonas aeruginosa* lead to a higher mortality rate and have a higher risk of transmission. Less is known about the transmission risk of non-carbapenemase producing multidrug-resistant *P. aeruginosa.* Therefore, in this study the potential transmission risk of non-carbapenemase producing multidrug-resistant *P. aeruginosa* were analysed by whole genome sequencing.

Methods Between 07/2017 and 03/2023 screening and clinical *P. aeruginosa* isolates characterized as 3MRGN were included from inpatients in non-paediatric oncological wards and intensive care units at a tertiary care hospital. Additionally, all isolates characterised as 4MRGN were included. Identification and antimicrobial susceptibility testing (AST) was done with MALDI-TOF and Vitek2, respectively. Isolates carrying a carabapenemase, as confirmed from sequenced genomes by the ResFinder pipeline (Center for Genomic Epidemiology, Copenhagen, Denmark), were excluded from the cohort. Core genome Multi Locus Sequence Typing (cgMLST) was performed for all isolates that met the inclusion criteria. Clusters were identified with SeqSphere+ software (Ridom, Germany). Non-carbapenemase producing multidrug-resistant *P. aeruginosa* isolates with ≤55 different alleles in cgMLST target genes were considered as possibly related. Potential transmissions were analysed on prior colonisation and aligned to epidemiological data.

Results A total of 135 non-carbapenemase producing multidrug-resistant *P. aeruginosa* were collected out of which 35 isolates (25.9%) of 32 patients were highly related. 19 patients (61.2%) were colonised and/or infected beforehand with a pan-susceptible *P. aeruginosa*. Seven genetically related clusters were identified. Despite highly related sequencing data there was rarely an epidemiological link. Solely one patient-to-patient transmission event of 3MRGN isolates could not be excluded by epidemiological data.

Summary Molecular analysis could hardly be linked to epidemiological data in the transmission of non-carbapenemase producing multidrug-resistant *P. aeruginosa* in critical care settings. Further data is required to critically evaluate isolation measures.

OP-HAIP-003
Tracking clonal and plasmid transmissions in colistin and carbapenem resistant Klebsiella pneumoniae

I. Akintayo[1], M. Siroglavic[2], M. Budia Silva[1], H. Ghosh[1], H. Grundmann[1], A. Budimir[2], *S. Reuter[1]
[1]*University Hospital Freiburg, Institut für Infektionsprävention und Krankenhaushygiene, Freiburg i. Br., Germany*
[2]*University Hospital Centre Zagreb, Department of Clinical and Molecular Microbiology, Zagreb, Croatia*

Infections caused by multidrug resistant bacteria are a major threat to public health, and their potential transmission events are therefore monitored in hospital settings. In contrast, the surveillance of mobile genetic elements that facilitate the spread of antimicrobial resistance genes have been challenging given their often mosaic composition. In this study, we tracked both clonal and plasmid transmission in colistin and carbapenem-resistant *K. pneumoniae* (ColR-CRKP) strains using short and long read sequencing technologies. First, we observed three clonal transmissions within two sequence types (ST15 and ST392), all of which contained IncL plasmids as well as the *bla*$_{NDM-1}$ carbapenemase, however, these were not always co-located. We found that one IncL plasmid containing *bla*$_{NDM-1}$ had been transferred between one ST392 cluster and the ST15 cluster, and that the promiscuous IncL plasmid carrying *bla*$_{OXA-48}$ was likely shared between the second ST392 cluster and a singleton of the same ST. The *bla*$_{NDM-1}$ gene of these last isolates was carried on incHI2. We found 0-11 SNP differences within the clonal outbreaks over a timeframe of 15 weeks. Between the plasmids within the clonal transmissions, we found 0-2 SNP differences, and 0-2 SNPs in the transfer within and between clusters and STs, with only an insertion of an additional *insB* insertion element in ST15. This shows high stability of the plasmids both within clonal transmissions as well as upon transfer to same or different STs. Taken together, despite the fact that IncL as well as *bla*$_{NDM-1}$ were common in this collection, we found a total of four different plasmids and a network of plasmid transfers, which we were only able to track applying both short and long read sequencing. This emphasizes the need to also investigate plasmid-mediated transmission which could be another key epidemiological link and useful for effective containment of outbreaks.

Fig. 1

OP-HAIP-004
Analysis of plasmid transmissions in a single hospital using a real-time plasmid transmission detection pipeline

*N. Scherff[1,2], T. Weniger[2], J. Rothgänger[2], A. Mellmann[1], D. Harmsen[2,3]
[1]*Institute of Hygiene, University Hospital Münster, Münster, Germany*
[2]*Ridom GmbH, Münster, Germany*
[3]*Department of Periodontology and Operative Dentistry, University Hospital Münster, Münster, Germany*

Introduction: Horizontal plasmid transmissions play a major role in the dissemination of antimicrobial resistance. Therefore, a new real-time plasmid transmission detection pipeline was implemented in SeqSphere+ (Ridom GmbH, Muenster, Germany). We evaluated this pipeline using published data of carbapenemase-producing isolates from a UK hospital (Roberts *et al.* Microb. Genomics 2023).

Goals: Our aim was to evaluate different pipeline parameters and to quantify the frequency of transmission events.

Methods: Mash databases for all plasmids were created with sketch sizes 1,000 and 10,000. Clustering was performed with a distance threshold of 0.001. Additional clustering with a size correction was applied for both sketch sizes. Here, the Mash distance was lowered by 0.0003 per 1% size difference. For size differences >40%, the uncorrected value was taken to account for multimer formation. Discrepancies between plasmid clusters resulting from these four approaches were assessed by analysing the genomic differences using Quast. Transmissions of integrative mobile genetic elements (iMGE) were analysed using CGE MobileElementFinder. Potential transmission events were counted based on the clusters found with sketch size 10,000 and size correction.

Results: Only one additional plasmid pair was found with sketch size 10,000, which was also found when size correction was applied to sketch size 1,000. With size correction (sketch size 10,000), another 3 clusters contained additional plasmids. According to Quast, these had 13, 5, and 2 genomic differences, respectively. In total, 44 potential transmission events were counted. Of these, 12 were clonal, 18 single plasmid transmissions, and 14 co-transmissions. Fifteen samples transferred more than one plasmid, 9 to the same and 6 to different receiving samples. Two samples were involved in a clonal and an additional plasmid transmission. No transmissions of iMGE were detected.

Conclusions: For analyses of plasmid transmissions using Mash, a sketch size of 1,000 is sufficient if the distance is corrected for size differences. Plasmid transmissions in hospitals could be twice as common as clonal transmissions.

OP-HAIP-005

What is the enhanced hospital hygiene impact of sequencing data in tracking nosocomial transmissions?A genomic study utilizing long- and short-read technologies

*N. H. Leder[1], M. Cristofolini[2], S. Ehrenberg[1], M. Lohde[1], C. Brandt[1,3], F. Kipp[1], C. Stein[1]

[1]*University Hospital Jena, Institute for Infection Medicine and Hospital Hygiene, Jena, Germany*

[2]*BG Bergmannstrost Hospital, Hospital hygiene, Halle (Saale), Germany*

[3]*Centre for Applied Research, InfectoGnostics Research Campus, Jena, Germany*

Introduction Carbapenem-resistant Enterobacterales (CPE) pose great concerns in healthcare. Prevention efforts focus on patients with spatiotemporal connections carrying the same bacterial species of CPE. However, the extent of transmissions through environmental reservoirs and cross-species plasmid transfer in hospitals remains unknown. Whole genomic sequencing (WGS) offers a more in-depth insight into the genomic relationship on core-genome and plasmid levels (1). But this technology is not yet part of routine workflows.

Goals Using VIM-CPE as an example, we investigated the benefit of WGS in analyzing plasmids and exploring bacterial relationships outside of hospital outbreak events.

Materials & Methods We included inpatient episodes from 2018 to 2021 involving patients who tested positive for *bla*VIM CPE anytime during their admission. Employing short- and long-read technology, we combined WGS data on core-genome and plasmid levels with epidemiological and patient movement data, to analyze genomically related cases of VIM-CPE and detect potential transmission routes.

Results We included 43 cases from 38 patients. The core-genome analysis identified 26 cases belonging to four clusters of highly related isolates, indicating clonal spread. Two plasmids were present in multiple isolates of different species. Putative transmission events on both genetic levels were mainly indirect and occurred over a long period with the ICU being a key location. Nearly half of the transmission events were plasmid-mediated.

Summary Our findings challenge the dogma of spatiotemporal connection and urge a broader investigation into temporal connections when genomic data suggests relatedness. We emphasize considering plasmid transfer alongside bacterial relatedness in infection prevention efforts concerning CPE. By understanding these factors, we can enhance infection prevention strategies and effectively combat the spread of CPE in the hospital.

1. Orlek A, Stoesser N, Anjum MF, Doumith M, Ellington MJ, Peto T, et al. Plasmid Classification in an Era of Whole-Genome Sequencing: Application in Studies of Antibiotic Resistance Epidemiology. Front Microbiol. 2017;8:182.

OP-HAIP-006

Typing of Pseudomonas aeruginosa clinical outbreaks by Fourier transform infrared spectroscopy

N. Mauder[1], M. Cordovana[1], T. Burgwinkel[2,3], R. Arazo del Pino[2,3], K. Xanthopoulou[2,3], A. F. Wendel[4], *P. G. Higgins[2,3]

[1]*Bruker Daltonics GmbH & Co. KG, Bremen, Germany*

[2]*University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*

[3]*German Center for Infection Research, Partner site Bonn-Cologne, Köln, Germany*

[4]*Cologne Merheim Medical Centre, Institute of Hygiene, Köln, Germany*

Introduction. *Pseudomonas aeruginosa* (PA) is an opportunistic pathogen, involved in hospital-acquired infection as well as in community patients with cystic fibrosis, or impaired immune system. PA poses a serious public health threat, due to its high prevalence and intrinsic and increasing acquired antibiotic resistance. Understanding, tracking, and ending the transmission of PA isolates is a big challenge for infection control. Fourier transform infrared spectroscopy (FTIRS) enables microbial typing on different intraspecies levels, relying on the unique FTIRS spectrum of each bacterial strain, which represents a specific fingerprint signature.

Methods. Seventy-four previously genotyped (cgMLST Ridom SeqSphere+) PA isolates were included in this study, including ST395 (n=13) and ST253 (n=6) obtained in the context of 2 outbreaks(1,2). Isolates were analyzed by the FTIRS-based IR Biotyper® system (IRBT; Bruker Daltonics, Germany). The isolates were cultivated on MHA at 37°C for 24±2 h, in three independent biological replicates. IR spectra were acquired from dried spots of bacterial suspensions in ethanol solution on the IR Biotyper sample plate. Exploratory analysis for clustering was performed by Hierarchical Cluster Analysis (HCA). The accuracy of the IR Biotyper system to detect clonality was evaluated by comparison with WGS

results, by Adjusted Rand (AR) and Adjusted Wallace (AW) indices.

Results. HCA showed that IRBT discriminated the isolates in 43 clusters, 6 of them including more than one isolate, and 37 singletons (**Figure 1)**. IRBT partitioning was in very good concordance with cgMLST clustering (AR=0.85, 0.71-0.99 CI; AW=0.99, 0.98-1.00 CI).

Conclusions. IR Biotyper proved to be a novel, reliable method to reveal clonality among PA isolates and could thus represent a faster, easier, and more cost-effective alternative to molecular methods in the context of hospital hygiene, but also food quality assessment and control. Its ease of use and short analytical and handling time could allow to propose this method as a reliable real-time tool.

Fig. 1

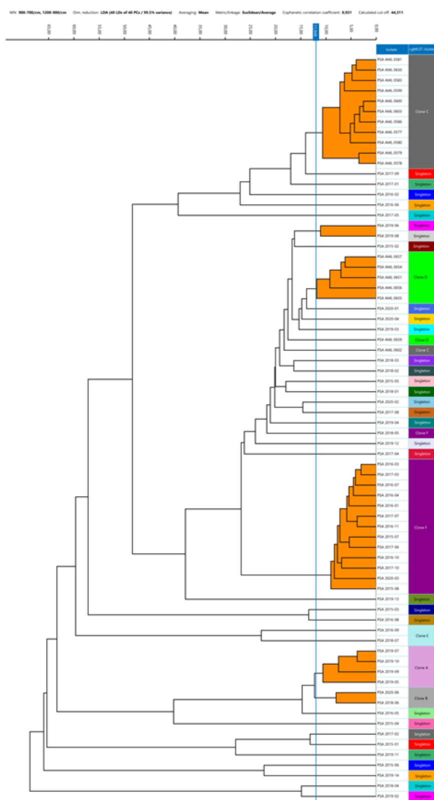


Fig. 1. HCA results. In the columns on the right side of the figure, from left to right, isolate and cgMLST cluster designation. The left part of the figure shows the IRBT clusters. Each isolate is represented in the dendrogram by its average spectrum (which is the average of the 9 spectra deriving from the three independent cultures, each one measured in three technical replicates).

OP-HAIP-007
Ralstonia pickettii bloodstream infections due to contaminated saline solution – a global problem?

*M. Krone1, V. Rauschenberger1,2, V. Blaschke1,2, H. Claus2, O. Kurzai2, S. Kampmeier1,2
1University Hospital Würzburg, Infection Control and Antimicrobial Stewardship Unit, Würzburg, Germany
2University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany

Question

Ralstonia pickettii is a gram-negative rod which may cause invasive infections due to the use of contaminated liquid medical products.

Methods

After *R. pickettii* was detected in blood cultures and a stem cell product from three patients in a tertiary-care hospital in Germany, environmental sampling was performed and subsequently whole genome sequencing (WGS) of the three patient isolates and two water isolates from the environment.

Results

Core Genome Multi Locus Sequence Typing (cgMLST) analysis showed that the three patient isolates were closely related and there was a large number of allele differences to the environmental isolates. The genomic comparison of the patients" isolates with a *R. pickettii* strain from an outbreak in Australia suspected to be caused by contaminated saline produced in India revealed only few differences.

Conclusions

Our data and information point towards an ongoing risk by medical products contaminated with *R. pickettii* potentially distributed worldwide. Identification of medical products applied to patients with *R. pickettii* infections and timely whole genome sequencing of all available *R. pickettii* isolates may help to identify the source of this potentially global outbreak.

OP-HAIP-008
Influence of antibiotics on microbial communities in hospital drain biofilms and their resistance profile

*N. van Leuven[1,2], A. Dicks[1], R. Lucassen[1], P. Braß[3], C. Consortium[4], A. Lipski[2], D. Bockmühl[1]
[1]*Rhine Waal University of Applied Sciences, Hygiene & Microbiology, Kleve, Germany*
[2]*University of Bonn, Bonn, Germany*
[3]*Helios Clinic Krefeld, Hospital hygiene, Krefeld, Germany*
[4]*Combat Consortium, Cardiff, United Kingdom*

Introduction & Aim

Since biofilms are known to contain resistant bacterial species and many hospital-acquired infections are believed to be biofilm-associated, their occurrence in hospitals is critical. As drains get in contact with antimicrobial residues like antibiotics, these may act as resistance drivers. Class 1 integrons as a multi-resistance marker allow for monitoring changes in resistances, checking for integrase gene *intI*1.

Our research aimed to investigate the impact of antibiotics on the composition of hospital drain biofilms and their genetic and phenotypic resistance profile based on consumption data of different wards and experimental approaches.

Methods

20 bathroom sink biofilms were sampled from 4 wards of a hospital. The cell count of viable cells, *Enterobacteriaceae* and yeasts/moulds was determined. Additionally, relevant pathogens were isolated, identified and analyzed for resistances with VITEK®2 compact system and by Epsilometer-testing. *intI*1-prevalence was measured by qPCR. Further testing took place by adding different antibiotics to the media during biofilm growth. After DNA extraction, changes of DNA in a high resolution melting analysis (HRMA) and the *intI*1 prevalence were evaluated.

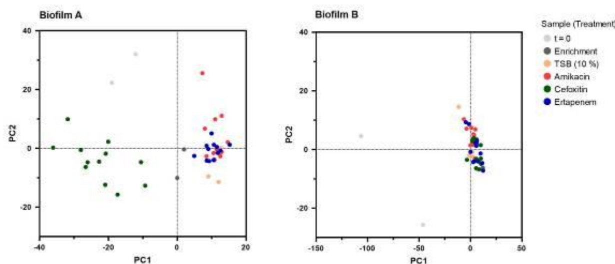
Results

Our data show that cell counts of the wards were not significantly different. MIC-values for antibiotics obtained by VITEK®2 and Epsilometer-testing showed no correlation to the administration of recommended daily doses (RDD)/100 bed days. In contrast, the median *intI* prevalence correlated with the RDD. While adding sub-inhibitory concentrations of antibiotics during growth, differences in HRMA were observed depending on the biofilm and antibiotic (Fig.1). Moreover, shifts in HRMA data correlated with changes in *intI*1 prevalences.

Summary

Our approach gave an insight into correlations between resistance values and the RDD of hospital wards. Expected pathogens were found. Genetic resistance markers correlated with the amount of prescribed antibiotics, although phenotypes didn´t. Thus, a selective pressure by sub-inhibitory antibiotic concentrations were found to cause community shifts accompanied by changes in the *intI*1 prevalence.

Fig. 1



OP-HAIP-009
Microbiome translocation and within-patient evolution promotes opportunistic, nosocomial infections

M. Fenk[1], S. Petros[2], B. Pasieka[2], *F. M. Key[1]
1Max Planck Institute for Infection Biology, Evolutionary Pathogenomics, Berlin, Germany
2University Hospital Leipzig, Internal ICU, Leipzig, Germany

Alarmingly, many commensal bacteria that peacefully reside in the human microbiome are also able to cause acute opportunistic infections. Thought to be triggered randomly in patients with impaired immunity, intriguing evidence suggests that within-host evolution can promote infection. The major barrier to studying the progression of opportunistic pathogens from carriage to acute infections is the challenge of sampling human patients prospectively. Here we test whether adaptive *de novo* mutations in asymptomatically colonizing opportunistic pathogen lineages facilitate translocation within the patient's body and promote acute infections. Therefore, we have enrolled 14 critically ill patients who are longitudinally sampled at four microbiome niches before the onset of healthcare-associated infection (HAI). Among four patients, we observe twelve HAIs caused by ten different opportunistic pathogens. Using culture-based sequencing we address within-patient evolution by generating over 600 whole genomes of the pathogen populations to characterize acquisition timing, spatial translocation, and whether adaptive *de novo* mutations promote acute infection. We find that in 70% of HAI cases the pathogen lineages can be identified within the microbiome prior to or at the time of disease onset. Moreover, using a molecular clock we infer that the pathogen lineages are regularly acquired already before hospitalization of the patient. In a single patient we observe a short-lived mutation in a fimbriae gene associated with HAI. Phenotypic tests reveal the mutation upregulates fimbriae production increasing cell adhesion and leads to higher virulence in a Drosophila infection model. In summary, our prospective approach allowed us to explore the hidden evolutionary paths leading up to HAI and underlines the importance to investigate microbiome dynamics at a population-wide whole genome level during disease.

OP-HAIP-010
WGS-based characterization of putative hypervirulent Klebsiella pneumoniae identified in a tertiary care hospital in Germany

*B. Neumann[1], F. Aurnhammer[2], L. Marr[1], T. Kohl[3], S. Niemann[3], A. Rath[4], B. Kieninger[4], W. Schneider-Brachert[4], J. Steinmann[1]
[1]Paracelsus Medical University, Institute for Clinical Hygiene, Medical Microbiology and Clinical Infectiology, Nürnberg, Germany
[2]Paracelsus Medical University, Institute of Clinical Hygiene, Medical Microbiology and Infectiology, Nürnberg, Germany
[3]Research Center Borstel, Molecular and Experimental Mycobacteriology, Borstel, Germany
[4]University of Regensburg, Abteilung für Krankenhaushygiene und Infektiologie, Regensburg, Germany

Introduction: Hypervirulent *Klebsiella pneumoniae* strains (hvKp), in contrast to classical *K. pneumoniae* strains, can cause invasive community-acquired infections in healthy patients of all ages. In this study, *K. pneumoniae* isolates from routine microbiological diagnostics were tested via string-test for hypermucoviscous phenotype and PCR for virulence genes to screen for hvKp, which were further analyzed by whole-genome sequencing (WGS).

Goals: The prevalence of "hidden" hypervirulent hvKp, including non-invasive strains and their population structure should be analyzed in a tertiary-care hospital in Southern Germany.

Materials/methods: For the study period 1.5 years, all *K. pneumoniae* isolates were string-tested. A multiplex PCR for hvKp genes was applied to all string-test positive isolates. PCR-positive isolates were subjected to WGS to determine genotyping and phylogeny.

Results: A total of 10.9% (331/3044) isolates with hmKp phenotype were detected by string-test. The patients" age rangend from 0 to 95 years, with a mean of 69 years. In total, 13.3% (44/331) isolates were tested positive by PCR for genes associated with hvKp. cgMLST revealed that 41.5% of sequenced isolates belonged to international hvKp clonal lineages ST23/K1 with high virulence scores and close phylogenetic relationships. In contrast, 22.6% of isolates belonged to the ST86/K2 with lower virulence scores. Isolates of liver abscesses (7.5%) belonged to ST23, ST25 and ST268, but without phylogenetic relatedness to other isolates.

Summary: We identified hypervirulent *K. pneumoniae* within the study period with an overall prevalence of 1.4%. No transmissions were identified. Isolates were assigned to the international hvKp lineage ST23/K1 and lineage ST86, bearing the potential to spread further in the community. The known association of multidrug-resistance ST86, was not observed in this study. Presented cases of liver abscesses seem to represent individual occurrences, so far. In

conclusion, the screening of hmKp phenotypes in routine diagnostics seems to be a suitable surveillance method. Further, the population size and structure of "hidden" putative hvKp were more complex than expected.

OP-HAIP-011

Genetic analysis of carbapenem resistant Acinetobacter baumannii isolates associated with the war in Ukraine

*J. Eisfeld[1], N. Pfennigwerth[1], S. G. Gatermann[1]
[1]*Ruhr University Bochum, Department of Medical Microbiology, Bochum, Germany*

Question

The worldwide spread and increase of multidrug-resistant Gramnegative bacteria represents a serious threat to public health. However, the prevalence of resistance mechanisms may differ between different regions or countries. In case of Ukraine, high AMR proportions in Gram-negative bacterial isolates have been reported in 2021, including carbapenem resistant *Acinetobacter* spp. (73%) and carbapenem resistant *Klebsiella pneumoniae* (64%)1. Recently, an increase in NDM-1 and NDM-1/OXA-48-producing *K. pneumoniae* in Germany was observed that derived at least partially from patients with prior exposure in Ukraine2.

Here, we report genetic surveillance of carbapenem resistant *A. baumannii* isolates in Germany from patients with prior exposure in Ukraine.

Methods

Carbapenemase detection was performed at the German National Refence Laboratory for multidrug-resistant Gram-negative bacteria (NRC) by modified Hodge test, combined disk test with EDTA and PCR for carbapenemase genes. Whole-cell DNA was subjected to WGS on an Illumina MiSeq platform with 2 x 300 bp paired end reads and subsequent genome assembly and typing was conducted using Ridom SeqSphere.

Results

In total, 58 *A. baumannii* isolates from patients with prior exposure in Ukraine were sequenced. Most of the isolates produced the carbapenemases OXA-23 or OXA-72. The most prevalent sequence types (STs) were ST2 for OXA-23 positive isolates and ST78 for OXA-72 positive isolates. Comparison via core genome MLST (cgMLST) revealed several clusters distributed all over Germany.

Conclusions

We report genetic analysis of carbapenemase producing *A. baumannii* isolates from patients with prior exposure in Ukraine. The distribution of closely related isolates all over Germany suggests that transmission may have occurred during hospitalization in Ukraine or transfer to Germany.

OP-HAIP-012

Full-length 16S rRNA gene nanopore sequencing for bacterial identification in routine diagnostics

*S. Posadas-Cantera[1], M. T. Badr[1], G. Häcker[1]
[1]*University of Freiburg, Institute of Medical Microbiology and Hygiene, Medical Center, Freiburg i. Br., Germany*

Introduction: Culture-negative bacterial infections can result from various factors, including prior antibiotic therapy, slow-growing bacteria, or extended transport times. In such cases, 16S rRNA gene Sanger sequencing can be employed to identify uncultured bacteria. However, Sanger sequencing is limited to monobacterial infections and may require significant time for results. Advances in sequencing technologies, such as nanopore sequencing, offer potential benefits for species identification in polybacterial infections, expanded species identification, and in-house sequencing capabilities.

Goals: The primary objectives of this study were to assess the accuracy and efficacy of full-length 16S rRNA gene nanopore sequencing in bacterial species identification, comparing it to the established Sanger sequencing method. Additionally, we aimed to evaluate whether nanopore sequencing could provide enhanced resolution for specific microbial species.

Materials & Methods: Forty samples, cultured and sequenced with Sanger in routine diagnostics, were selected for this comparative study. Subsequently, these samples underwent nanopore sequencing, and the results were compared among the three methods.

Results: Our findings demonstrated a high level of concordance between Sanger and nanopore sequencing for species identification, including the resolution of mixed sequences in polybacterial infections. Nanopore sequencing successfully identified certain species that would have remained undetected using Sanger sequencing.

Summary: In conclusion, this study highlights nanopore sequencing as a potentially valuable tool, especially in cases of culture-negative infections and critically ill patients where urgent bacteriological diagnosis is crucial. Further exploration and validation of nanopore sequencing in larger cohorts should be pursued to comprehensively assess its utility and integration into routine diagnostic workflows.

OP-HAIP-013

It is time for genome-oriented management of outbreaks involving vancomycin-resistant enterococci – the different stories told by different levels of characterization

*A. Rath[1], J. Hahn[2], M. Grube[2], A. Caplunik-Pratsch[1], A. Eichner[1], J. Fritsch[1], W. Schneider-Brachert[1], B. Kieninger[1]
[1]*University Hospital Regensburg, Department of Infection Prevention and Infectious Diseases, Regensburg, Germany*
[2]*University Hospital Regensburg, Department for Internal Medicine III, Regensburg, Germany*

Background

Genome-oriented outbreak management (OM) is still uncommon in routine infection control. The strain dynamics of vancomycin-resistant *Enterococcus faecium* (VRE), however, often complicate the distinction between real- and pseudo-outbreaks. This project analyzes how the same outbreak situations unravel, and how high the efficiency of OM is, when using strain-typing methods with increasing resolution of genetic data.

Methods

In 2022, prolonged VRE outbreaks affected 47 patients at our oncology department. OM interventions were done twice (June and October), dividing the events into three phases. 47 isolates from 44 patients (including two isolates with different *van*-genotypes for three patients) were collected during January 2022-February 2023, and characterized using MALDI-ToF, in-house *vanAB*-PCR, and short-read whole-genome sequencing (WGS). Subsequently, the different levels of characterization were used to evaluate the outbreak dynamics and OM efficiency.

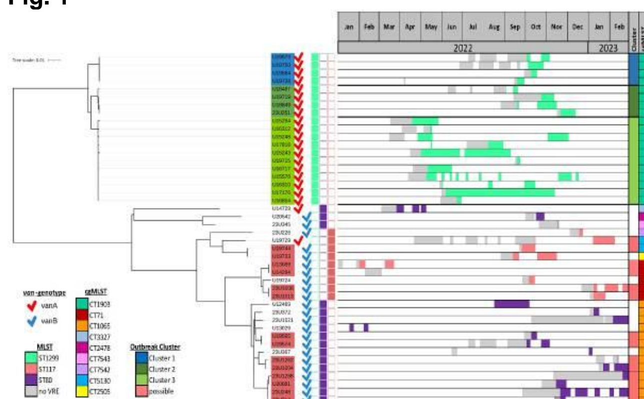
Results

Epidemiological data suggests that the VRE outbreak during Phase 1 was terminated by OM, whereas it did not affect Phase 2. While WGS confirmed a monoclonal outbreak of ST1299/CT1903/*vanA* during Phase 1, Phase 2 was caused by a polyclonal outbreak. ST1299/CT1903/*vanA* was also most common among VRE/*vanA* during Phase 2, and two isolates were closely related to isolates from Phase 1. The prolonged hospitalization of patients from Phase 1 appears to be the most probable reason. The remaining ST1299/CT1903/*vanA* isolates are split in two clusters, suggesting VRE introduction on two further occasions. Although clusters of VRE/*vanB* were also identified during Phase 2, these were small-sized. The spread of both VRE/*vanA* and VRE/*vanB* outbreak strains in Phase 2 was also promptly interrupted by OM according to WGS data and cgMLST. The outbreak suspicion in Phase 3 was not confirmed.

Conclusions

Our genome-oriented approach to infection control clearly demonstrates that the success of OM for VRE is feasible, but can only be evaluated using WGS. Without this, the extent of clinical outbreaks can be grossly overestimated during routine investigations, whereas OM efficiency is falsely questioned.

Fig. 1



OP-HAIP-014
Competition between vancomycin-resistant enterococci of different sequence types

*V. Rauschenberger1, V. Blaschke[1], H. Claus[1], S. Kampmeier[1,2]
1University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany
2University Hospital Würzburg, Infection Control and Antimicrobial Stewardship Unit, Würzburg, Germany

Introduction

Vancomycin-resistant enterococci (VRE) are multi-drug-resistant organisms that cause severe nosocomial infections. VRE of sequence types (ST) ST80 and ST117 experience an upcoming prevalence in screening and clinical isolates in Germany. Since this national increase cannot be explained by transmission events, we hypothesize that VRE of these STs either have selective advantages or harbor an increased virulence potential resulting in enhanced numbers of infections. This study aims at identifying ST-specific phenotypic differences of VRE and to investigate whether ST117 and ST80 VRE dominate VRE of other ST *in vitro*.

Materials & Methods

VRE isolated from patients of a tertiary care hospital in 2021-2023 were subjected to whole genome sequencing and compared using a core genome Multilocus Sequencing Typing (cgMLST) approach. Biofilm production was investigated by crystal violet assay. Competition of VRE with different STs was analyzed in a Spot Killing Assay.

Results

Most frequently, VRE of ST117 and ST80 but also rarer ST2032, ST2481 and ST18 were identified. VRE of ST117 and ST80 showed a significant higher biofilm production compared to ST2481 and ST18 (ST2481: $p = 0.006$; $p = 0.0002$; ST18: $p = 0.001$, $p = 2.9x10\text{-}7$, respectively). Only one of two ST2032 produced biofilm. Spot Killing Assays resulted in inhibited growth of ST2481 and ST18 VRE after spotting ST117 and ST80 VRE or their respective supernatants.

Summary

In this study, we identified two explanations for the current trend of increasing VRE STs 117 and 80 in Germany. ST117 and ST80 VRE strains and their supernatants lyse VRE of other STs *in vitro*, indicating a possible intra-host selective advantage. Additionally, biofilm production of these predominant STs is enhanced compared to others, probably facilitating survival of these strains in the patient environment.

OP-HAIP-015
Our first-time infection prevention and control experience with Candia auris at a burn intensive care unit

*C. Baier[1], A. Klos[1], L. Knegendorf[1], L. Sedlacek[1], S. Ziesing[1], T. Dieck[2], V. März[2], K. Dastagir[2], D. Schlüter[1], P. M. Vogt[2], E. Ebadi[1]
[1]*Hannover Medical School, Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany*
[2]*Hannover Medical School, Department of Plastic, Aesthetic, Hand and Reconstructive Surgery, Hannover, Germany*

Introduction

Candida auris (*Ca*) is an increasing infectious disease and infection prevention and control (IPC) challenge in recent years [1]. Its potential to spread in hospitals is feared. We present the IPC management of a *Ca* positive patient in a burn intensive care unit (BICU).

Materials

We describe the IPC measures which were inspired by existing recommendations [2]. We also highlight open aspects of IPC management that have arisen for us in practice.

Results

The pre-hospitalised patient was transferred from Ukraine and admitted to the BICU. In accordance with internal IPC standards, the patient was pre-emptively isolated and a screening for multidrug-resistant bacteria took place. This revealed several carbapenem-resistant Gram-negative bacteria, VRE and MRSA. The patient was cared for under strict IPC standards including PPE for staff and 1:1 care whenever possible. On day 13 of the stay, *Ca* was detected in a wound material. Immediately, the patient and all other patients on the BICU were screened for *Ca* (nose/throat, axillae, groin, rectal, respiratory secretions, catheter urine, wounds; initially with regular malt extract agar). CHROMagar™ Candida Plus was introduced *ad hoc* in the microbiological laboratory. The ward contact patients were screened every 3 days initially and then once a week. Ward disinfection was enhanced and repeated on-site audits took place. After the *Ca* patient was not more on the ward long term follow-up screening was implemented.

Summary

For the first time, a patient with *Ca* was treated in our BICU. Due to close co-operation between the clinic, IPC and microbiology, comprehensive screening was quickly established. To date, no transmission has been detected, to which the pre-emptive isolation and the high IPC standards of the BICU have contributed. An important aspect is to clarify which groups should be screened for *Ca* on admission.

References

[1] Aldejohann AM et al. Rise in Candida Auris Cases and First Nosocomial Transmissions in Germany. doi: 10.3238/arztebl.m2023.0047

[2] Aldejohann AM et al. Expert recommendations for prevention and management of Candida auris transmission. doi: 10.1111/myc.13445

OP-HAIP-016

Molecular typing of methicillin resistant Staphylococcus aureus (MRSA) in patients with mutual exposure reveals low proportion of transmission events.

*M. Kaase[1], D. Wolf[1], F. Pankok[1], M. Bornmann-Schrader[1], W. Leszczynski[1], A. Dudakova[2], O. Bader[2], U. Groß[2], S. Scheithauer[1], M. H. Schulze[1]
[1]*University Medical Center Göttingen, Department for Infection Control and Infectious Diseases, Göttingen, Germany*
[2]*University Medical Center Göttingen, Institute of Medical Microbiology and Virology, Göttingen, Germany*

Question: If patients with newly detected MRSA during their hospital stay had epidemiological exposure in the same room or on the same ward to other patients with hitherto unknown MRSA colonization, it is tempting to assume a transmission event. However, a previous unknown MRSA colonization could as well explain the above constellation, especially in a setting without universal screening on admission.

Methods: Data on patient admission, transfer and discharge were linked with microbiological lab data in a Jupyter Notebook using common Python packages to determine pairs of patients with MRSA colonization that could possibly be explained by mutual exposure either in the same patient room or on the same ward. Molecular typing by whole genome sequencing and cgMLST analysis was used to determine clonal relatedness.

Results: Between 2017 and 2020 five pairs of MRSA patients with first detection later than two days after admission and overlapping stay in the same room were evaluable, of whom two had the same clone according to cgMLST typing. The respective numbers for patient pairs with overlapping stay on the same ward and available isolates were six out of 47 pairs. Of the latter patients, 2.1% had a negative MRSA screening within the previous 14 days. Complex types accounting for transmissions were CT1426, CT1422, CT32066, CT30303, CT31532.

Conclusion: Even in MRSA patient pairs with a plausible epidemiological link the majority was not due to a transmission event between them in a setting without universal screening on admission. Our results help to estimate the pretest probability for transmission events, which might be useful when typing results are lacking or not yet available.

OP-HAIP-017

Genomic reconstruction of an azole-resistant Candida parapsilosis outbreak and the creation of a multilocus sequence typing scheme

P. Brassington[1], F. R. Klefisch[2], B. Graf[3], R. Pfüller[4], O. Kurzai[5], G. Walther[5], *A. Barber[1]
[1]*University of Jena, Fungal Informatics, Jena, Germany*
[2]*Paulinen Hospital, Berlin, Germany*
[3]*Labor Berlin, Berlin, Germany*
[4]*MVZ Hauptstadtlabor, Berlin, Germany*
[5]*National Reference Center for Invasive Fungal Infections, Würzburg, Germany*

Background

Fluconazole-resistant *Candida parapsilosis* has emerged as a significant healthcare-associated pathogen with a propensity to spread patient-to-patient and cause nosocomial outbreaks, similar to *Candida auris*. This study investigates a prolonged outbreak of fluconazole-resistant *C. parapsilosis* across multiple years and healthcare centers in Germany.

Methods

We employed whole-genome sequencing of isolates from the outbreak, other regions within Germany, and compared them with isolates from a global distribution to understand the molecular epidemiology of this outbreak. Additionally, we used the genomic dataset of 258 samples to identify loci with high discriminatory power to establish the first multi-locus sequence typing (MLST) strategy for *C. parapsilosis*.

Results

A clonal, azole-resistant strain of *C. parapsilosis* was observed causing invasive infections over multiple years and in multiple hospitals within the outbreak city. Including this outbreak clone, we identified three distinct *ERG11* Y132F azole-resistant lineages in Germany, marking the first

description of this azole-resistance in the country and its endemic status. Using the novel MLST strategy, isolates were categorized into 31 sequence types, proving the utility of the typing scheme for genetic epidemiology and outbreak investigations as a rapid alternative to whole genome sequencing. Temporal and genomic reconstruction of the outbreak indicated that transfer of patients between healthcare facilities was likely responsible for the persistent reimportation of the drug-resistant clone and subsequent person-to-person transmission.

Conclusions

This research underscores the importance of monitoring of *C. parapsilosis* epidemiology, not only in Germany but globally. The emergence of azole-resistant lineages necessitates continuous surveillance and rigorous infection control measures. By combining large-scale genomic epidemiology and introducing a novel typing method, our study offers valuable insights into the management of emerging healthcare-associated pathogens, with direct implications for public health and clinical practice.

OP-HAIP-018

Time-to event analyses for hospital stay: Nosocomial infections influence the length of stay for infections caused by respiratory viruses

*A. Ambrosch[1], F. Wang[2], F. Klawonn[2]
[1]Barmherzige Brüder Regensburg, Ins. für Labormedizin, Mikrobiologie und Krankenhaushygiene, Regensburg, Germany
[2]Helmholtz Center for Infection Research, Brunswick, Germany

Nosocomial transmission of respiratory viruses can contribute to increased morbidity and mortality and should therefore be prevented by preventive measures. In the present study, the extent to which nosocomial transmission influences the length of hospitalisation of patients was systematically investigated

Methods: In a retrospective monocentric analysis over 5 years, all hospitalised patients with evidence of influenza A, B, RSV and SARS-CoV-2 (original strain / Omicron) were included. Demographic data (age, sex), risk factors (COPD, diabetes, kidney desease, heart disease, cancer) and clinical course were recorded. Definitions were used to differentiate between nosocomial and community-acquired (CA) infections. A restricted mean survival time (RMST) (survival event is normal discharge) was used to calculate the influence of variables on the length of hospitalisation; the effects of each variable were compared by comparing Hazard Ratio (HR) and 95% CI. Deceased patients were excluded for the RMST / HR because of the bias problem on hospital stay.

Results: A total of 2269 patients with specific virus detection were included, 263 of whom were defined as nosocomial. The hospitalisation times for patients with nosocomial infections compared to CA infections were significantly higher at 23.4 vs. 10.4 days (p < 0.001) (differentiated by specific virus detection: Infl. A: 20.5 vs. 9.2 (p < 0.001) / Infl. B 17.9. vs. 8.2 (< 0.001) / RSV 24.6 vs. 10.7 (< 0.001) / SARS-CoV-2 26.4 vs. 15.5 (0.497) / Omicron 25.5. vs. 9.64 (< 0.001)). For patients with evidence of influenza A / B, RSV, SARS-CoV-2 and Omicron, the effect of a nosocomial infection on the length of stay was significant (HR = 0.52 (SARS-CoV-2) and HR < 0.5). Other factors in individual virus detections with a significant influence on the length of hospitalisation were age, diabetes and a bacterial superinfection.

Conclusion: Nosocomial infections have a significant impact on the length of stay and discharge of patients with specific viral infections. Preventive measures must therefore enforced in the management of patients with respiratory infections in order to prevent nosocomial infections.

OP-HAIP-019

Usage of oral contraceptives among healthcare workers: impact on COVID-19 booster vaccine immunogenicity

I. Wagenhäuser[1,2], J. Reusch[1,2], J. Mees[1,3], L. B. Krone[4,5,6,7], C. Curtaz[8], T. T. Lam[9], A. Schubert-Unkmeir[9], A. Frey[2], L. Dölken[10], O. Kurzai[9,11], S. Frantz[2], A. Wöckel[8], A. Gabel[1], *M. Krone[1,9], N. Petri[2]
[1]University Hospital Würzburg, Infection Control and Antimicrobial Stewardship Unit, Würzburg, Germany
[2]University Hospital Würzburg, Department of Internal Medicine I, Würzburg, Germany
[3]University Hospital Würzburg, Pediatric Rheumatology/Special Immunology, Würzburg, Germany
[4]University of Oxford, Department of Physiology, Anatomy and Genetics, Oxford, United Kingdom
[5]University of Oxford, Sir Jules Thorn Sleep and Circadian Neuroscience Institute, Oxford, United Kingdom
[6]Universtiy Bern, Universitäre Psychiatrische Dienste, Bern, Switzerland
[7]Universtiy Bern, Zentrum für Experimentelle Neurologie, Bern, Switzerland
[8]University Hospital Würzburg, Department of Obstetrics and Gynaecology, Würzburg, Germany
[9]University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany
[10]University of Würzburg, Institute for Virology and Immunobiology, Würzburg, Germany
[11]Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute, Jena, Germany

Question

Oral contraceptives profoundly impact various physiological functions, including the immune response. However, the influence of oral contraceptives (OC) on the immunogenicity of vaccinations in general, and in particular the COVID-19 vaccination, is largely unknown. This study explored the effects of OC on the immune response following COVID-19 booster vaccinations.

Methods

From 18 November 2021 to 13 October 2023 the immunogenicity of the third COVID-19 vaccination as booster among 711 female healthcare workers (HCWs) between 18-50 years was assessed as part of the CoVacSer study. Individuals on long-term OC medication were compared to those not taking OCs. Blood serum samples, along with a comprehensive study questionnaire on sociodemographic data, were collected before (pre) and after (post) a third COVID-19 vaccination. Anti-SARS-CoV-2-Spike IgG levels were measured using the SERION ELISA agile SARS-CoV-2 IgG.

Results

19.7% (140/711) reported taking OC regularly. Univariate analysis comparing the groups with and without OC medication, found no differences in Anti-SARS-CoV-2-Spike IgG titres after the third COVID-19 vaccination (p=0.48). Yet, in the univariate analysis the relative increase of IgG levels

after the third vaccination was significantly higher in cohort without OC (p=0.02). However, the linear regression model considering the factors smoking, age, BMI, COVID-19 booster vaccine, SARS-CoV-2 infection history and household size did not reveal a significant effect of OC medication on the IgG increase.

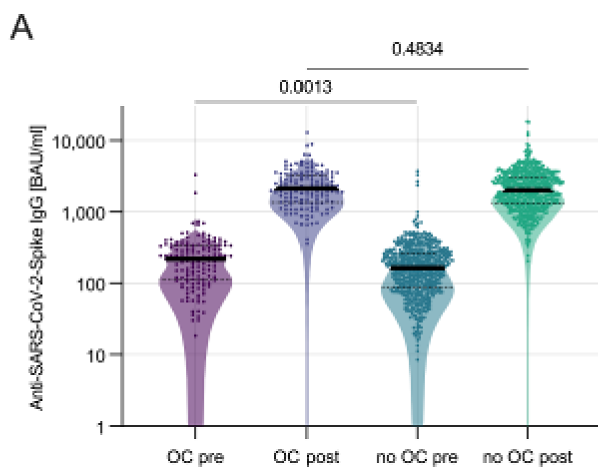
Figure 1: Anti-SARS-CoV-2-Spike IgG levels related to the third COVID-19 vaccination separated by OC

BAU/ml = Binding Antibody Units per millilitre

Conclusions

Despite the generally known interaction of OC intake and the immune response in women, our study was the first to investigate the influence of OC on the immunogenicity specifically for COVID-19 vaccination. Despite the large sample size of our study, we found no effect of OCs on antibody titres providing strong evidence that OCs have no influence on the immunogenicity of COVID-19 booster vaccinations.

Fig. 1



OP-HAIP-020
Risk factors for SARS-CoV-2 acquisition among vaccinated individuals in nosocomial outbreaks - a case-control study

H. Goodarzi[1], E. Lemke[1], S. Wolke[1], F. Schwab[1], B. Schlosser[1], M. Paul[1], A. Brodzinski[1], G. B. Fucini[2], B. Kohlmorgen[1], S. Jendrossek[1], S. Aghdassi[1], B. Piening[1], C. Geffers[2], M. Wiese-Posselt[1], *L. Denkel[2]
[1]*Charité - University Medicine Berlin, Institute of Hygiene and Environmental Medicine, Berlin, Germany*
[2]*Charité - University Medicine Berlin, Institut of Hygiene and Environmental Medicine, Berlin, Germany*

Introduction: Despite the widespread deployment of SARS-CoV-2 vaccines, breakthrough infections continue to pose challenges, particularly in healthcare settings. Nosocomial outbreaks remain a significant concern, warranting an in-depth investigation into the risk factors associated with SARS-CoV-2 acquisition among vaccinated individuals.

Goals: To identify risk factors for SARS-CoV-2 acquisition among vaccinated patients and healthcare providers in nosocomial outbreaks at Charité hospitals in Berlin, Germany, in 2022.

Materials & Methods: In this retrospective case-control study, we investigated SARS-CoV-2 acquisition in 114 nosocomial SARS-CoV-2 outbreaks notified to the healthcare authorities in 2022. Cases included 587 patients and 385 staff members who tested positive for SARS-CoV-2 during the outbreaks. Controls (not tested positive for SARS-CoV-2), were matched (1:1) to cases based on their patient/staff status and the time shared with a case on the ward 14 days before the case tested positive. All participants were fully vaccinated and completed a questionnaire regarding potential risk or protective factors for SARS-CoV-2 acquisition. Univariable analysis and multivariable logistic regression models were used.

Results: In total, 271 cases and 271 controls participated in the study. Our findings indicate that cases were more likely to be immunized > 3 months ago compared with controls (77.5% versus 68.6%, p= 0.02). Cases more often suffered from diabetes (15.2% versus 9.0%, p = 0.03) and less often had a previous SARS-CoV-2 infection (6.7 versus 15.7%, p < 0.01). Multivariable logistic models identified previous SARS-CoV-2 infection as an independent protective factor (OR=0.25, 95% CI: 0.11- 0.53). Independent risk factors were immunization > 3 months ago (OR=1.6, 95% CI: 1.05 – 2.7) and diabetes (OR =2.26, 95% CI: 1.13 – 4.72).

Summary: Our analyses suggest that certain primary diseases and an immunization older than 3 months were associated with increased risks of breakthrough infections in healthcare settings. However, a previous SARS-CoV-2 infection may have a protective effect. These findings represent preliminary results and warrant further validation.

OP-HAIP-021
Influence of vaccine administrations on dominance intervals of SARS-CoV-2 variants – A modelling observational study of regional German data 2021 - 2023

*H. Kaba[1], N. Srivastava[1], F. Hartkopf[2], J. A. Bucio Garcia[1], M. Kleines[3], F. C. Bange[4], T. Eckmanns[5], S. Scheithauer[6]
[1]*University Medical Center Göttingen, Infection Control & Infectious Diseases, Division of Healthcare Research, Göttingen, Germany*
[2]*RKI, Berlin, Germany*
[3]*RWTH Aachen University, Labordiagnostisches Zentrum, Virologie/Serologie, Aachen, Germany*
[4]*Hannover Medical School, Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany*
[5]*Robert Koch Institute, FG 37: Nosokomiale Infektionen, Surveillance von Antibiotikaresistenz und -verbrauch, Berlin, Germany*
[6]*University Medical Centre Göttingen (UMG), Infection Control and Infectious Diseases, Göttingen, Germany*

Introduction

The emergence of omicron SARS-CoV-2 resulted in the displacement of delta and later displacement events between omicron variants. However, patterns of dominance intervals varied geographically within and between countries.

Goals

Starting from the hypothesis that any single vaccination influences pandemic developments, we investigated whether vaccination densities influenced dominance intervals of the most prevalent variants in 16 German federal states (BL).

Materials & Methods

Our weekly data (01/2021–01/2023) consisted of >1.1M SARS-CoV-2 sequences from DESH, IMS-SC2 and GISAID. Duplicates were removed as were sequences with a N content of ≥95% and weeks with n<10 reported sequences. Displacement occurred when a given cluster made ≥50% of all sequences (tp). We calculated the time-to-displacement interval (TTD) for omicron (1.1.529* variants) displacing delta (1.617.2*), BA.2 (1.1.529.2*) displacing BA.1 (1.1.529.1*) and BA.5 (1.1.529.5*) displacing BA.2. TTD was the interval [weeks] between the onset (first report) and tp. The displaced variant cluster was dubbed "predecessor", and the new dominant cluster "successor". We used Cox Regression to model TTD in 48 independent displacement events (3x16 BL), controlling for selected covariates (Fig. 1).

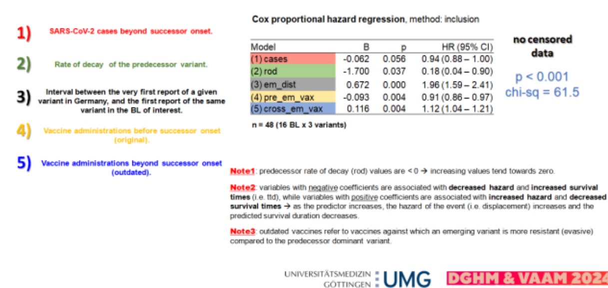
Results

Our model proposed a 12% [4; 21%] CI-95% increased risk of displacement for every outdated vaccine administration per 100 population. In contrast, every original vaccine administration per 100 population led to a 9% [3; 14%] decreased displacement risk (Fig. 1). The effect of low outdated vaccine density was most apparent in the delta-omicron event (mean ΔTTD 2.5 weeks [-0.6; 5.6] 95%-CI, log-rank test p=0.05), in contrast to BA.1-BA.2 (0.3, p=0.75) and BA.2-BA.5 (0.8, p=0.35).

Summary

Vaccine densities at BL-level influenced TTD in two ways. Vaccines administered before omicron onset helped delaying the displacement of delta. When the same vaccines became outdated due to omicron onset (immune evasive), they helped to accelerate delta displacement by omicron. The effect size dropped in following intra-omicron displacement events. Further research is needed to investigate causality.

Fig. 1



Model	B	p	HR (95% CI)
(1) cases	-0.062	0.056	0.94 (0.88 – 1.00)
(2) rod	-1.700	0.037	0.18 (0.04 – 0.90)
(3) em_dist	0.672	0.000	1.96 (1.59 – 2.41)
(4) pre_em_vax	-0.093	0.004	0.91 (0.86 – 0.97)
(5) cross_em_vax	0.116	0.004	1.12 (1.04 – 1.21)

OP-HAIP-022

Leveraging mobility data during a pandemic to implement a targeted sampling strategy for surveillance

*R. Spott[1], M. W. Pletz[2,3], C. Fleischmann-Struzek[2,3], A. Kimmig[2], C. Hadlich[4], M. Hauert[4], M. Lohde[1], M. Jundzill[1], M. Marquet[1], P. Dickmann[5], R. Schüchner[6], M. Hölzer[7], D. Kühnert[8,9], C. Brandt[1]
[1]Institute for Infectious Diseases and Infection Control, Jena University Hospital, Cloud Computing and Sequencing group, Jena, Germany
[2]Institute for Infectious Diseases and Infection Control, Jena University Hospital, Jena, Germany
[3]Center for Sepsis Control and Care, Jena University Hospital/Friedrich Schiller University Jena, Jena, Germany
[4]SMA Development GmbH - epicinsights Agentur für Künstliche Intelligenz und Big Data Analytics, Jena, Germany
[5]Department of Anaesthesiology and Intensive Care, Jena University Hospital, Jena, Germany
[6]Thuringian State Authority for Consumer Protection, Bad Langensalza, Germany
[7]Methodology and Research Infrastructure, Genome Competence Center (MF1), Robert Koch Institute, Berlin, Germany
[8]Robert Koch Institute, Centre for Artificial Intelligence in Public Health Research, Berlin, Germany
[9]Transmission, Infection, Diversification and Evolution Group, Max Planck Institute for Geoanthropology, Jena, Germany

Question

Efficient surveillance strategies are essential to keep track of the rapid developments during a pandemic. We investigated the potential of combining mobile service data and fine-granular metadata (such as postal codes and genomic data) to advance integrated genomic surveillance of the SARS-CoV-2 pandemic in the federal state of Thuringia, Germany.

Methods

We received anonymized and aggregated mobile service data from T-systems, containing circa 200 Mio data points from 2020 to 2021. Additionally, we sequenced over 6,500 SARS-CoV-2 Alpha genomes (B.1.1.7) across seven months within Thuringia while collecting patients' isolation dates and postal codes. Our dataset is complemented by over 66,000 publicly available German Alpha genomes. The dataset was screened for mutational clusters, and by combining the samples' time and location data with the mobile service data, the spread of identified clusters in Thuringia was analyzed.

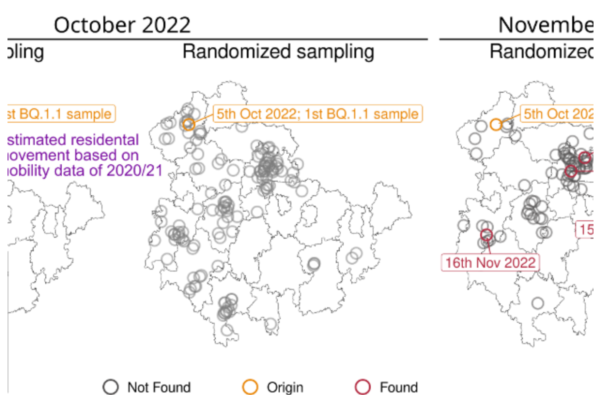
Results

We could track the existence and spread of nine persistent mutation variants within the Alpha lineage. Seven formed separate phylogenetic clusters with different spreading patterns in Thuringia, of which two contained an additional sub-cluster, totaling nine Thuringian clusters. Our data suggests that mobile service data can indicate these clusters' spread and highlight a potential sampling bias, especially of low-prevalence variants. Thereby, mobile service data can be used either retrospectively to assess surveillance coverage and efficiency from already collected data or to actively guide part of a surveillance sampling process to districts where these variants are expected to emerge. The latter concept proved successful as we introduced a mobility-guided sampling strategy to track the low-prevalence Omicron sublineage BQ.1.1.

Conclusions

The combination of mobile service data and SARS-CoV-2 surveillance by genome sequencing is a valuable tool for more targeted and responsive surveillance.

Fig. 1



e mobility-guided sampling of the Omicron sublineage BQ.1.1 in Thuringia
ipling (surveillance) in October 2022. For clarity, the surveillance results in Nov
reading progress of BQ.1.1. Circles reflect the location of each sample (based or
:t identified BQ.1.1 sample; Red circle: Additionally identified BQ.1.1 sample; Gre
rrows show the eight residential movements with the most participants from th
entified BQ.1.1 sample (orange circle). The mobile service data were extracted fr
ples; Randomized Sampling - October: 132 samples; Randomized Sampling - No

OP-HAIP-023
Evaluating multiple machine-learning algorithms as surveillance-related tools based on the example of the hospitalization rate 2.0 during the SARS-CoV-2 Pandemic

*M. Misailovski[1,2], N. Srivastava[1], S. Blaschke[3], R. Strobl[4,5], H. Kaba[1], M. Berens[1], A. Beste[1], M. Kaase[1], A. Leha[6], D. J. Chackalacka[l1], J. Chrampanis[1], J. M. Kosub[1], U. Groß[7], A. Fischer[8], S. Scheithauer[1]
[1]*University Medical Center Göttingen, Department of Infection control and Infection Diseases, Göttingen, Germany*
[2]*University Medical Center Göttingen, Department of Geriatrics, Göttingen, Germany*
[3]*University Medical Center Göttingen, Emergency Department, Göttingen, Germany*
[4]*LMU Munich, Institute for Medical Information Processing, Biometrics and Epidemiology, München, Germany*
[5]*University Hospital, LMU Munich, German Center for Vertigo and Balance Disorders, München, Germany*
[6]*Department of Medical Statistics, University Medical Center Göttingen, Göttingen, Germany*
7University Medical Center Göttingen, Institute for Medical Microbiology and Virology, Göttingen, Germany
[8]*University Medical Center Göttingen, Institute for Clinical Chemistry, Göttingen, Germany*

Introduction: While machine learning algorithms are gaining popularity in clinical research, their feasibility as surveillance-related tools is still a work-in-progress.

Goals: To address this, we conducted a comprehensive evaluation of different machine learning models as survey-related tools for their performance on the example of the so-called Hospitalization Rate 2.0, which aims to differentiate cases admitted due to COVID-19 from incidental SARS-CoV-2 positive cases.

Methods: Single-center characterization of 345 patients hospitalized with a positive SARS-CoV-2 PCR test were included in the model development (Jan. 2022- June 2022). Outcomes were defined as: Primary case (admitted due to COVID-19) and incidental case (admitted for another reason). A total of 6 models were applied including 3 linear models (Logistic Regression (LR)), Linear Discriminant Analysis (LDA), Support Vector Classification (SVC)) and 3 non-linear models (K-Nearest Neighbors (KNN), Random Forest (RF), Extreme Gradient Boosting (XGboost)). Models were then evaluated according to the "Area Under the Curve" of the "Receiver Operating Characteristic" (AUC-ROC), accuracy, precision, recall, F1-score, sensitivity, and specificity.

Results: Among the linear classifiers, LR showed the best performance over all metrics (accuracy (0.75), precision (0.74), recall (0.75), F1-score (0.74) and sensitivity (0.75)), followed by LDA and SVC which had better specificity values (0.83 and 0.86 respectively). From the non-linear classifiers, RF was superior to KNN in terms of accuracy, precision, recall, F1 score and sensitivity, while KNN had higher specificity. Of all the applied classifiers, the XGBoost model had the strongest discriminative ability with the highest accuracy (0.80), sensitivity (0.92), precision (0.82), recall (0.78) and F1 score (0.79).[sg1]

Summary: Our results indicated that the XGBoost model has better capability and accuracy than the other models applied for identifying primary cases. Implementation of such models as surveillance-related tools could be the very first step to count primary cases, and furthermore optimize resource allocation and hospital bed occupancy.

OP-HAIP-024
Shine like a diamond: Rapid ATP measurements to evaluate the efficacy of different cleaning methods for worn spectacles.

*N. Sultana Lupin[1], B. Fritz[1], S. Wahl[2], M. Egert[1]
[1]*Institute of Precision Medicine, Furtwangen University, Villingen-Schwenningen, Germany*
[2]*ZEISS Vision Science Lab, Institute for Ophthalmic Research, Eberhard-Karls-University, Tübingen, Germany*

Spectacles can harbor various types of microorganisms, predominantly bacteria, which pose potential health risks to spectacle wearers as well as to persons regularly working with worn spectacles, such as opticians [1-3]. ATP measurement represents a rapid and widely used method in hygiene to analyses the organic contamination of surfaces. However, clear correlations with microbial contents on the investigated surfaces are not always provable. Here, we examined the suitability of rapid ATP measurements to assess the efficacy of different cleaning methods for spectacle surfaces. Moreover, we explored the potential correlation with microbial germ counts. To do so, we swab-sampled worn spectacles at our university laboratory, and subsequent ATP bioluminescence assays with a handheld device were used to assess the level of contamination.

Six popular cleaning methods were evaluated for their ability to reduce the level of contamination using ten worn spectacles, respectively. Microfiber cloths were the most effective, reducing ATP levels by 93.2%. Alcohol-free wipes followed closely, with a 92.0% decrease. In addition, we investigated the correlation between germ counts and ATP content, which revealed no significant correlation for aerobic cultivation ($p = 0.36$), but a statistically significant correlation for anaerobic conditions ($p = 0.025$). Higher CFU values correlated positively with higher ATP levels, suggesting that ATP measurements can rapidly indicate microbial contamination. Our previous studies have shown that spectacles and similar surfaces are indeed colonized by significant shares of (aerotolerant) anaerobic bacteria [2,3]. In summary, ATP measurements are a suitable method to rapidly measure the contamination of spectacles and demonstrate the efficacy of cleaning measures under non-laboratory conditions, such as eyewear stores.

[1] Fritz, B. et al. (2018). PLoS ONE 13(11): e0207238. doi.org/10.1371/journal.pone.0207238
[2] Fritz B. et al. (2020). Sci Rep. 10(1): 5577. doi.org/10.1038/s41598-020-62186-6.
[3] Fritz, B. et al. (2020). J. Clin. Med. 9(5): 1572. doi.org/10.3390/jcm9051572

Host-Associated Microbiomes and Microbe-Host Interactions

OP-HAMI-001
Cross-kingdom RNA interference between plants and infecting microbes

*A. Weiberg[1]
[1]*LMU Munich, Martinsried, Germany*

Cross-kingdom RNA interference (RNAi) is an emerging field in plant-microbe research. The fungal pathogen *Botrytis cinerea* sends small RNAs into plant hosts to promote infection. These fungal small RNAs bind to the plant`s own Argonaute (AGO)/RNA-induced silencing complex (RISC) to silence plant defense-related genes (Weiberg et al., 2013).

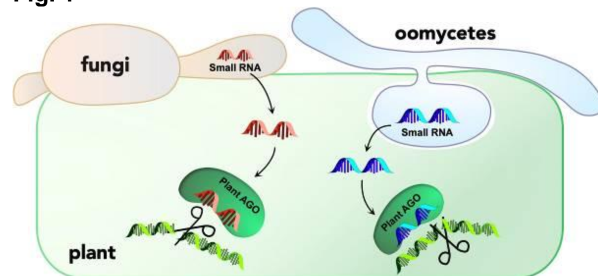
Amazingly, cross-kingdom RNAi is a virulence strategy of plant pathogens belonging the class of oomycetes (Dunker et al., 2020), which are phylogenetically very distinct to fungi. We performed plant AGO co-immunopurification coupled to small RNA deep sequencing that revealed dozens of oomycete small RNAs manipulating plant host gene expression. In this context, we developed a novel GFP-based switch-on cross-kingdom RNAi reporter that we expressed in plants. With this tool, we visualized for the first time the onset and cross-kingdom RNAi spreading in the infected plant tissue by life imaging (Cheng et al., 2023). We isolated pathogen extracellular vesicles (EVs) carrying small RNAs and applied these to plant leaves. The pathogen EVs were capable to induce cross-kingdom RNAi in plants suggesting EVs are one route of small RNA transport from microbes into plants during infection.

Cheng, A.P., Lederer, B., Oberkofler, L., Huang, L., Johnson, N.R., Platten, F., Dunker, F., Tisserant, C., and **Weiberg, A.** (2023). A fungal RNA-dependent RNA polymerase is a novel player in plant infection and cross-kingdom RNA interference. PLoS Pathog **19,** e1011885.

Dunker, F., Trutzenberg, A., Rothenpieler, J.S., Kuhn, S., Prols, R., Schreiber, T., Tissier, A., Kemen, A., Kemen, E., Huckelhoven, R., and **Weiberg, A.** (2020). Oomycete small RNAs bind to the plant RNA-induced silencing complex for virulence. Elife **9,** e56096.

Weiberg, A., Wang, M., Lin, F.M., Zhao, H., Zhang, Z., Kaloshian, I., Huang, H.D., and Jin, H. (2013). Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science **342,** 118-123.

Fig. 1



OP-HAMI-002
Non-antibiotic drugs break colonization resistance against pathogenic Gammaproteobacteria

*A. Grießhammer[1,2,3], J. de la Cuesta-Zuluaga[1,2,3], T. Zahir[1,2,3], P. Müller[1,2,3], C. Gekeler[1,2,3], H. Chang[4], K. Schmitt[1,2,3], C. Planker[1,2,3], E. Bohn[2,3], T. H. Nguyen[5], K. C. Huang[5,6,7], L. Maier[1,2,3]
[1]*University Hospital Tuebingen, M3 Research Center, Tübingen, Germany*
[2]*University Tübingen, Medical Microbiology and Hygiene, Tübingen, Germany*
[3]*University of Tübingen, Cluster of Excellence "Controlling Microbes to Fight infections", Tübingen, Germany*
[4]*European Molecular Biology Laboratory, Genome Biology, Heidelberg, Germany*
[5]*Standford University, Department of Bioengineering, Standford, CA, United States*
[6]*Standford University School of Medicine, Department of Microbiology and Immunology, Standford, CA, United States*
[7]*Chan Zuckerberg Biohub, San Francisco, CA, United States*

Introduction: Drugs have been shown to be a critical determinant of inter-individual differences in the composition of the human gut microbiome, which is true not only for antibiotics but also for non-antibiotic drugs. We hypothesized that by altering the composition of the microbiome, non-antibiotic drugs open niches and facilitate pathogen colonization in a similar way to antibiotics.

Methods: We compared the susceptibility of commensal and pathogenic bacteria to non-antibiotic drugs and found that pathogens have higher drug resistance, which could favor their expansion after treatment. Drugs were selected that inhibited a broad spectrum of commensals while sparing pathogenic *Gammaproteobacteria.* We then developed the synthetic model community Com20 to screen for drug-microbiome interactions that increase the risk of enteropathogenic infections. To increase microbial complexity, we performed the same experiments with human stool-derived microbial communities. For drug candidates with particularly strong phenotypes, we tested their ability to disrupt colonization resistance and increase the infection risk for *Salmonella* Typhimurium (*S.* Tm) in gnotobiotic and SPF mice.

Results and Conclusion: Approximately 35% of the >50 drugs from different therapeutic classes we tested increased the abundance of *S.* Tm in synthetic and human stool-derived communities. This was due to direct effects of non-antibiotics on individual commensals, altered microbial interactions within communities and the potential of *S. T*m to exploit different metabolic niches. Non-antibiotic drugs that favored *S.* Tm expansion *in vitro* also promoted other enteric pathogens and increased *S.* Tm loads in gnotobiotic and conventional mice. Our work allows the systematic evaluation and identification of non-antibiotic drugs that break colonization resistance and increase the risk of

infection with enteropathogenic bacteria. These findings may inform future strategies to control pathogen proliferation and to assess individual microbiota-drug-pathogen risks.

OP-HAMI-003
Neurotransmitter-producing commensal bacteria at the interface of host metabolism

*S. Rahmdel[1,2], E. Liberini[1,2], C. Struse[1], H. Brüggemann[3], A. Luqman[4], F. Götz[1,2]
[1]*Tübingen University, Microbial Genetics, Tübingen, Germany*
[2]*Excellence Cluster 2124 'CMFI', Tübingen, Germany*
[3]*Aarhus University, Department of Biomedicine, Aarhus, Denmark*
[4]*University Würzburg, Molecular Infection Biology, Würzburg, Germany*

Introduction. Trace amines (TAs) are neuro-monoamines produced by bacteria and humans mainly by decarboxylation of aromatic amino acids (AAAs). Bacterial AAA decarboxylases (AADCs) are generally specific in substrate preference. Staphylococcal AADC (SadA) is, however, non-specific and decarboxylates tryptophan, tyrosine and phenylalanine to tryptamine, tyramine, and phenethylamine, respectively. It is also able to convert dihydroxy phenylalanine and 5-hydroxytryptophan to dopamine and serotonin. **Goals.** What are the potential interactions between TA-producing commensal bacteria and the host cells? **Materials & methods.** The skin microbiome of healthy subjects was examined using shotgun metagenomic and culture-based analyses. The investigation of bacterial adherence and internalization was carried out using the HT-29 cell line. The *in vitro* and *in vivo* effects of TAs and TA-producing bacteria on wound healing assays were evaluated in HaCaT cells and in murine model. **Results.** Metagenomic analysis of the skin microbiota revealed a wide distribution of SadA homologs among at least 7 different phyla, remarkably within the Firmicutes phylum. In culture-based analysis, the AADC activity was found in isolates other than *Staphylococcus* sp. including *Cutibacterium acnes*, *Corynebacterium* sp., and *Bacillus* sp.. *Staphylococcus epidermidis* and *S. pseudintermedius* secrete TAs which trigger their adherence and internalization into human cells by activation of the α2-adrenergic receptor (AR). TAs could accelerate wound healing by antagonizing the b2-AR in keratinocytes which was confirmed *in vivo* by an application on the wound bed of TAs or a TA-producing *S. epidermidis* strain. As TAs can activate the wide distributed TA-associated receptors (TAARs) at nanomolar affinities, it is not unexpected to have their wide-range presynaptic "amphetamine-like" effects all over body and brain which we are currently investigating using Parkinson"s and Alzheimer"s mouse models. **Conclusion.** TA-producing bacteria are ubiquitous in human microbiota which interact with host metabolism through direct stimulation of TAARs. Further studies are required to address more aspects of this interaction.

OP-HAMI-004
The endosymbiont Spiroplasma poulsonii increases Drosophila melanogaster resistance to pathogens by enhancing iron sequestration and melanization

M. Serra Canales[1], A. Hrdina[1], A. Arias-Rojas[1], D. Frahm[1], *I. Iatsenko[1]
[1]*Max Planck Institute for Infection Biology, Genetics of Host-Microbe Interactions, Berlin, Germany*

Facultative endosymbiotic bacteria, such as *Wolbachia* and *Spiroplasma* species, are commonly found in association with insects and can dramatically alter their host physiology. Many endosymbionts are defensive and protect their hosts against parasites or pathogens. Despite the widespread nature of defensive insect symbioses and their importance for the ecology and evolution of insects, the mechanisms of symbiont-mediated host protection remain poorly characterized. Here, we utilized the fruit fly *Drosophila melanogaster* and its facultative endosymbiont *Spiroplasma poulsonii* to characterize the mechanisms underlying symbiont-mediated host protection against bacterial and fungal pathogens. Our results indicate a variable effect of *S. poulsonii* on infection outcome, with endosymbiont-harbouring flies being more resistant to *Rhyzopus oryzae*, *Staphylococcus aureus,* and *Providencia alcalifaciens*, but more sensitive or as sensitive as endosymbiont-free flies to the infections with *Pseudomonas* species. Further focusing on the protective effect, we identified Transferrin-mediated iron sequestration induced by *Spiroplasma* as being crucial for the defense against *R. oryzae* and *P. alcalifaciens*. In case of *S. aureus*, enhanced melanization in *Spiroplasma*-harbouring flies plays a major role in the protection. Both iron sequestration and melanization induced by *Spiroplasma* require the host immune sensor protease Persephone, suggesting a role of proteases secreted by the symbiont in the activation of host defense reactions. Hence, our work reveals a broader defensive range of *Spiroplasma* than previously appreciated and adds nutritional immunity and melanization to the defensive arsenal of symbionts. We propose that the mechanisms we have identified here may be of broader significance and could apply to other endosymbionts, particularly to *Wolbachia*, and potentially explain their protective properties.

OP-HAMI-005
The octocoral microbiome: from community structure and function to novel chitinases for the circular blue bioeconomy

*T. Keller-Costa[1], J. F. Almeida[1], D. M. G. da Silva[1], F. R. Pedrosa[1], U. Nunes da Rocha[2], M. Â. Taipa[1], R. Costa[1]
[1]*Instituto Superior Técnico, University of Lisbon, Institute for Bioengineering and Biosciences, Department of Bioengineering, Lisbon, Portugal*
[2]*Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany*

Octocorals are an integral part of benthic marine ecosystems, increasing habitat complexity and biodiversity. They associate with diverse microorganisms, including micro-eukaryotes, prokaryotes, and viruses. Our previous research on temperate octocorals showed that their microbiome is distinct from the environmental surroundings, host genus-specific, and undergoes complex structural changes under dysbiosis [1]. However, the role of microbial symbionts that populate octocorals is still poorly understood. To shed light on their metabolic capacities, we examined 66 high-quality metagenome-assembled genomes (MAGs), spanning 30 prokaryotic species, retrieved from microbial metagenomes of three temperate octocoral species and seawater [2]. Symbionts affiliated with *Endozoicomonadaceae*, *Candidatus* Thioglobaceae, and *Metamycoplasmataceae,* among others. Phylogenomics showed that the *Endozoicomonadaceae* MAGs represent a novel genus unique to temperate octocorals, denoted *Candidatus* Gorgonimonas. These symbionts harboured chitinase and chitin-binging protein-encoding genes, indicating that they hydrolyse the most abundant polysaccharide in the ocean. Subsequent examination of >40 genomes of cultured and uncultured *Endozoicomonadaceae* strains demonstrated that chitinases and other genes involved in chitin degradation are widespread in the

Endozoicomonadaceae family, suggesting that these symbionts play important roles in chitin turnover in marine invertebrates and benthic ecosystems [3]. Since Candidatus *Gorgonimonas* bacteria remain unculturable, we employed synthetic biology and heterologous expression to harness their enzymes. Two novel, active and mesophilic chitinases from octocoral symbionts were successfully produced, holding promise for upcycling of seafood waste as chitin(ase)-derived products find applications in multiple sectors, from biomedicine over the food industry to agriculture.

[1] Keller-Costa et al., 2021, Microbiome, 9
https://doi.org/10.1186/s40168-021-01031-y
[2] Keller-Costa et al., 2022, Microbiome, 10
https://doi.org/10.1186/s40168-022-01343-7
[3] da Silva,….,& Keller-Costa 2023 ISME COMMUN, 3
https://doi.org/10.1038/s43705-023-00316-7

OP-HAMI-006

The ability of Staphylococcus aureus to proliferate within nasal communities is strain-specific and dependent on resource availability

*L. Camus[1,2], J. Franz[1], J. J. Power[1], M. Navarro Díaz[2,3], A. Lange[1,2], D. Gerlach[4], S. Heilbronner[1,2,4,5]
[1]*Interfaculty Institute of Microbiology and Infection Medicine, Heilbronner Lab, Tübingen, Germany*
[2]*Cluster of Excellence EXC2124 Controlling Microbes to Fight Infections, Tübingen, Germany*
[3]*Interfaculty Institute of Microbiology and Infection Medicine, Link Lab, Tübingen, Germany*
[4]*Ludwig-Maximilians University Munich, Faculty of Biology, Microbiology, München, Germany*
[5]*German Center for Infection Research, Tübingen, Germany*

Introduction

The asymptomatic nasal colonization of *Staphylococcus aureus* increases the risk of infection and concerns approximatively 30% of the population. The underlying molecular principles and especially the role of the endogenous microbiota in this carriage pattern are poorly understood. We hypothesize that, depending on their composition and origin, nasal bacterial communities can promote or inhibit the proliferation of *S. aureus* within nares.

Goals

Characterize how *S. aureus* interacts with nasal commensals and communities from different volunteers.

Methods

Three nasal communities of different composition and *S. aureus* carriage level were reconstituted by isolating the nasal bacterial species of healthy volunteers. Using these communities and the corresponding resident strains of *S. aureus*, in addition to a reference *S. aureus* strain, we performed (i) pairwise interactions experiments between individual strains, (ii) in vitro co-cultivations between *S. aureus* and synthetic communities and (iii) in vivo nasal co-colonization assays in gnotobiotic mice. Bacterial proliferation, community structure and metabolomics profiles were monitored.

Results

Two of the tested communities showed predominantly negative pairwise interactions *in vitro* and inhibited all *S. aureus* strains regardless of their origin. Instead, the last community was dominated by cooperative interactions, and the corresponding synthetic consortia promoted the survival of *S. aureus*. This effect appeared to be specific to the *S. aureus* nasal strain that was co-isolated with the community and promoted in poor medium, suggesting the existence of co-adaptation mechanisms for resource acquisition. First *in vivo* experiments indicate that, as observed *in vitro*, this cooperative community does not interfere with nasal colonization of the reference *S. aureus* strain.

Conclusions

Nasal communities display different interaction patterns that can either support or hinder *S. aureus* proliferation. The strain-specific nature of these interactions could explain why certain communities are invaded by this pathogen, and could inform strategies for preventing nasal colonization by *S. aureus*.

OP-HAMI-007

Promoting gut decolonization of multi-drug resistant bacteria via the microbiome

*M. Wende[1], L. Osbelt[1], L. Eisenhard[1], T. R. Lesker[1], U. Muthukumarasamy[1], A. A. Bielecka[1], M. Galardini[1,2], T. Strowig[1]
[1]*Helmholtz Center for Infection Research, Microbial Immune Regulation, Brunswick, Germany*
[2]*Twincore, Hannover, Germany*

The fight against multi-drug resistant Enterobacteriaceae (MDR-E) has been declared as a high priority by the WHO. Colonization of the human gut with MDR-E, including MDR *E. coli,* is associated with an increased risk of infection and dissemination within the community. Probiotics developed to selectively decolonize the microbiota of carriers from MDR strains are a promising treatment alternative. We hypothesize that the human gut is a great resource for probiotics, which show the potential to selectively decolonize MDR-E. As a novel resource for the identification of potentially probiotic bacteria, a strain collection of Enterobacteriaceae was generated from 630 donors from four cohorts. As it is of great interest to screen as many strains as possible due to the high genetic diversity of bacterial isolates, we established an *ex vivo* assay to identify strains with protective properties. Specifically, candidate probiotics and a MDR *E. coli* strain were spiked into cecum content of mice representing the nutritional environment *in vivo*. Furthermore, we developed *in vivo* models for gut (de)colonization of MDR-E and characterized the decolonization properties of specific probiotic candidates. For this approach we selected a panel of commensal isolates which displayed different competitive abilities in the *ex vivo* assay and observed decolonization of MDR *E. coli* after precolonization with competitive isolates. To gain mechanistic insights into the competition between protective isolates and the MDR *E. coli* strain, we conducted growth curves and competition assays in minimal media supplemented with a single carbon source. We observed a general fitness advantage of the protective strain and reduced CFUs of the MDR *E. coli* strain after co-cultivation in minimal media with specific carbon sources. We could further demonstrate the competition for carbon sources *in vivo*. For promising candidates we intend to use loss-of-function genetic screens for genes, which may be crucial for the protective effect. We also want to identify potential

cooperation partners and microbiome signatures, which are relevant for the protective effect.

OP-HAMI-008
Exploring the Genus Peltigera: A Metagenomic Perspective on Lichen Biology
*V. Rosenthal[1], M. Heinen[2], L. Hüttebräuker[3], C. Kelly[4], F. Altegoer[2], M. Pauly[3], B. Thomma[4], M. Feldbrügge[2], B. Usadel[5,6]
[1]*Institute for Biological Data Science, Faculty of Mathematics and Natural Science, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[2]*Insitute for Microbiology,Faculty of Mathematics and Natural Sciences, Heinrich- Heine University Düsseldorf, Düsseldorf, Germany*
[3]*Institute of Plant Cell Biology and Biotechnology, Faculty of Mathematics and Natural Sciences, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany*
[4]*Institute of Plant Sciences AG Thomma, University of Cologne, Köln, Germany*
[5]*Institute of Bio- and Geosciences IBG-4, Bioinformatics, BioSC, CEPLAS, Forschungszentrum Jülich, Jülich, Germany*
[6]*Heinrich Heine University Düsseldorf, Institute of Biological Data Science, CEPLAS, Faculty of Mathematics and Natural Sciences, Düsseldorf, Germany*

Many fungi engage in symbiotic relationships that span from pathogenic and commensalistic to mutualistic interactions. Lichens are one of the most successful examples of complex mutualistic symbiotic systems, comprising an association predominantly between fungal (mycobiont) and one or more photosynthetic organism(s) (photobiont), including green algae and/or cyanobacteria. Additionally, diverse fungal and bacterial communities occur in the lichen association. Lichens can serve as long term air pollution indicators and 50% of lichen species exhibit antibiotic properties, with many metabolites holding promise as potential antibiotic agent.

The genus *Peltigera* is widespread across several continents and serves as a representative of cyanolichens, through its symbiotic association with a cyanobacterial photobiont of the genus *Nostoc*. Although lichens have been studied for at least 200 years, they remain poorly understood, despite the increasing interest in their properties. This is largely reflected in the absence in reliable genomic reference data until recently, as lichen genomic characterization is taking off.

To address this issue, here we present several metagenome assemblies of the lichen genus *Peltiger*a found in North-Rhine Westphalia, Germany. These genomic assemblies of both photo- and mycobiont were derived using advanced long-read sequencing methodologies. Our analysis shows that the mycobiont genomes are near complete in terms of gene content as measured by BUSCO analysis. Moreover, our metagenomic analyses uncovered additional organisms associated within the lichen community, which will be reported.

Our study offers the possibility to gain new insights into the intricate genomic characteristics of lichens, advancing our understanding of these remarkable organisms and make a significant contribution to the upcoming golden age of lichen research.

Keywords: Lichen, Metagenome, Symbiosis, Oxford Nanopore Sequencing

Host-Pathogen Interactions and Clinics of Zoonotic Infections

OP-HPIZ-001
The combination of microfluidic cell infection with TraDIS reveals potential adherence factors of Streptococcus canis
*A. Kopenhagen[1], M. Steinert[1], M. Katsburg[2], E. Aubry[2], S. Wolf[3], T. Semmler[3], M. Fulde[2], S. Bergmann[1]
[1]*Technical University of Braunschweig, Institut für Mikrobiologie, Brunswick, Germany*
[2]*Free University of Berlin, Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Berlin, Germany*
[3]*Robert Koch Institute, Genome Competence Center, Berlin, Germany*

Introduction: *Streptococcus canis (S. canis)* is an animal pathogen and causes zoonotic infections such as septicemia in humans. In previous studies, the M-protein of *S. canis* has been characterized as a bacterial surface receptor. *S. canis* is also known to produce a pore-forming toxin targeting the membrane of eukaryotic cells. Apart from these studies, little is known about other factors directly contributing to cell adherence and bacterial invasion into various host niches.

Goals: The aim of this study is to identify and characterize novel adherence factors that play a role in the pathogenesis of systemic infections, particularly in bloodstream infections.

Materials & Methods: For bacterial infection analyses of human primary endothelial cells (EC) under physiological conditions defining bloodstream infections, a microfluidic system was used to apply a defined shear stress. Bacterial infection was quantified and visualized microscopically after differential immunofluorescence staining followed by confocal laser scanning microscopy. A transposon-mediated mutagenesis library of a clinical *S. canis* isolate was generated, representing a high genome coverage. During the cell culture infection process, a differential selection of adherent and non-adherent transposants enabled an efficient transposon directed insertion-site sequencing (TraDIS).

Results: *S. canis* bacteria effectively adhere to EC even at high shear force levels during flow infection. A serial selection process of transposants successfully differentiates between a recovered pool of adherent and of non-adherent bacteria. Bioinformatical analyses of sequence pools resulted in identification of several putative factors contributing to bacterial adherence and cell damage.

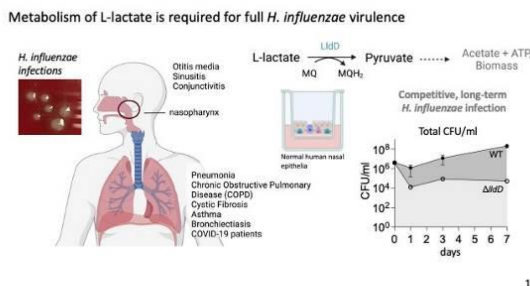
Summary: The combination of microfluidic infection of endothelial cells with the TraDIS technique proved to be ideally suited for the analysis of *S. canis* infections in the vascular system and for the search for new potential adherence factors.

OP-HPIZ-002
"Metabolic upcycling" – insights into the metabolic underpinning of Haemophilus influenzae persistence in human airways
*U. Kappler[1], J. Hosmer[1], M. Nasreen[1], R. Dhouib[1], H. J. Schirra[2], E. Fantino[3], P. Sly[3], A. G. McEwan[1]

[1]The University of Queensland, School of Chemistry and Molecular Biosciences, St Lucia, Australia
[2]The University of Queensland, Centre for Advanced Imaging, St Lucia, Australia
[3]The University of Queensland, Child Health Research Centre, South Brisbane, Australia

Haemophilus influenzae (Hi) infections are associated with recurring acute exacerbations of chronic respiratory diseases in children and adults including otitis media, pneumonia, chronic obstructive pulmonary disease and asthma. Physiological investigations show that persistence and recurrence of Hi infections are closely linked to Hi metabolic properties, where preferred growth substrates are aligned to the metabolome of human airway epithelial surfaces and include lactate, pentoses, and nucleosides, but not glucose that is typically used for studies of Hi growth *in vitro*. Enzymatic and physiological investigations revealed that utilization of lactate, the preferred Hi carbon source, required the LldD L-lactate dehydrogenase (conservation: 98.8% of strains), but not the two redox-balancing D-lactate dehydrogenases Dld and LdhA. Utilization of preferred substrates was directly linked to Hi infection and persistence. When unable to utilize L-lactate or forced to rely on salvaged guanine, Hi extra- and intra-cellular persistence was reduced in a murine model of lung infection and in normal human nasal epithelia, with up to 3000-fold attenuation observed in competitive infections. In contrast, D-lactate dehydrogenase mutants only showed a very slight reduction compared to the wild-type strain. Interestingly, acetate, the major Hi metabolic end-product, had anti-inflammatory effects on cultured human tissue cells in the presence of live but not heat-killed Hi, suggesting that metabolic endproducts also influence Hi-host interactions. Our work provides significant new insights into the critical role of metabolism for Hi persistence in contact with host cells and reveals for the first time the immunomodulatory potential of Hi metabolites.

Fig. 1



OP-HPIZ-003

Establishing a human *in vitro* 3D gut-on-a-chip model to study enterohemorrhagic *Escherichia coli* infection

*J. Treffon[1], A. Mellmann[1], P. Berger[1]
[1]University Hospital Münster, Institute of Hygiene, Münster, Germany

Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) are pathogenic bacteria that can cause severe intestinal infections and the hemolytic uremic syndrome. To study EHEC infections, cell culture systems or animal models can be applied. As none of the currently available animal models fully reflects EHEC pathogenesis in humans, human 3D cell culture systems mimicking *in vivo* conditions are a promising technology for monitoring EHEC infection.

Goals

We intend to establish and characterize an easy-to-use 3D cell culture model comprising four different cell types that mimics the human gut and is suitable for studying EHEC infections.

Materials & Methods

Caco-2 alone or together with HT29-MTX intestinal epithelial cells were seeded against extracellular matrix (ECM) in an OrganoPlate® 3-lane 40 (Mimetas). Cells were cultivated under flow conditions until leak-tight intestinal tubules were formed. Infection experiments with bacterial culture supernatant for 24 h or bacteria for 3 h were carried out using the 2011 outbreak strain EHEC O104:H4. Prior and after infection, barrier integrity was assessed by monitoring the translocation of fluorophore-labeled dextrans from tubule lumen to ECM channel.

Results

Leak-tight intestinal tubules were formed after six days of cultivation. Infection of tubules with culture supernatant from EHEC O104:H4 wild type, but not from its Shiga toxin 2 (Stx2) phage-cured derivative, induced epithelial barrier disruption, reflecting the known cytotoxic effect of Stx2. Additionally, infection experiments with bacteria revealed that an EHEC O104:H4 *rpoS*-mutant with similar Stx2 levels, but enhanced fimbriae and flagellin production compared to the wild type, caused a more severe tubule barrier disruption, indicating synergistic effects of Stx2 and these virulence factors.

Summary

So far, we established an easy-to-handle 3D bi-culture model suitable for studying EHEC infections *in vitro*. To fully picture host defense and EHEC disease pathology, we are currently implementing macrophages and neutrophils into the 3D cell culture system.

OP-HPIZ-004

Characterization of egress mechanisms of Coxiella burnetii

*J. Schulze-Luehrmann[1], E. M. Liebler-Tenorio[2], A. Felipe-López[1], A. Lührmann[1]
[1]University Hospital Erlangen, Microbiology Institute, Erlangen, Germany
[2]Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Federal Research Institute for Animal Health, Jena, Germany

Intracellular bacteria evolved mechanisms to invade host cells, establish an intracellular niche allowing survival and replication and exit the host cell after completion of the replication cycle to infect new target cells. *Coxiella burnetii* (*C.b.*) is a Gram-negative, obligate intracellular pathogen and the causative agent of the zoonotic disease Q fever. Resident alveolar macrophages are the first target cells, but *C.b.* spread to other organs and cell types. While we have information about the initial step of *C.b.* uptake and the maturation process of the *C. burnetii*-containing vacuole (CCV), it is not well studied how *C.b.* spreads, i.e. how it exits its host cell to enter new target cells. Understanding this essential step will be crucial to develop novel strategies to prevent *C.b.* from spreading and, thus, disease.

Here, we used human endothelial EA.hy926 cells and epithelial HeLa cells, as well as mouse fibroblasts lacking the lysosomal associated membrane proteins 1 and 2 (LAMP-1/-2) to study the escape of intracellular *C. burnetii* with live cell imaging, confocal laser scanning microscopy, electron microscopy, a trans-well system, and by establishing a FACS-based spreading assay.

We could demonstrate that *C.b.* inhibits host cell apoptosis early during infection, but induces and/or increases apoptosis at later stages of infection. Egress depends on previously established large bacteria-filled vacuoles and a functional intrinsic apoptotic cascade. The released bacteria are not enclosed by a host cell membrane and can infect and replicate in new target cells. However, in addition to cell death, lysosomal exocytosis might be involved, as egress is inhibited in LAMP-1/-2 deficient cells.

In summary, our data argue that *C. burnetii* egress in a non-synchronous way at late stages of infection. Apoptosis-induction is important for *C.b.* egress, but other pathways such as lysosomal exocytosis most likely contribute.

OP-HPIZ-005
The role of the RGD motif of streptococcal protein IdeC in Streptococcus canis infection

*S. Walsh[1], A. M. Lapschies[1], M. Müsken[2], M. Rohde[2], S. Bergmann[3], M. Fulde[1]
[1]*Free University of Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*
[2]*Helmholtz Center for Infection Research, Brunswick, Germany*
[3]*Technical University of Braunschweig, Institute for Microbiology, Brunswick, Germany*

Introduction

Streptococcus canis is a Lancefield group-G opportunistic pathogen that colonises the mucosal surfaces and skin of the host, predominantly affecting cats and dogs. *S. canis* is a zoonotic pathogen with human infections being observed more recently, mainly caused by contact with companion animals.

Different virulence factors, e.g. proteases, enable colonization, survival and replication during pathogenesis of streptococci. *In silico* analysis has shown that the IgG-specific protease of *S. canis* termed IdeC is a secreted protein containing a signal sequence but no membrane anchor. IdeC is suggested to act with a similar mechanism to the IdeS protein of *Streptococcus pyogenes*, a secreted cysteine kinase that cleaves the IgG molecule between the hinge and CH2 region. The IdeC protein also has an Arg-Gly-Asp (RGD) motif, an important integrin binding motif. Bacterial proteins containing RGD motifs have been implicated in adhesion and invasion of host cells. This along with the ability of IdeC to bind back to the bacterial surface after secretion suggest a possible second function of IdeC in adhesion.

Goals

This project is focused on elucidating the functional role of the *S. canis* protein IdeC. Specifically, what role this protein may play in mediating host-pathogen interactions.

Materials & Methods

Fluorescent latex beads coated in recombinant IdeC protein are used to assess the interaction of IdeC with epithelial and endothelial cells. Further, a recombinant protein with RGD replaced with RGE has been produced to test the role of the motif in any interaction observed.

Results

Based on fluorescence microscopy analysis, IdeC coated latex beads displayed increased interaction with both epithelial and endothelial cells when compared to IdeC_RGE and BSA controls. Electron microscopy indicates that IdeC coated beads may be internalised however, a mechanism is yet to be determined.

Summary

Here we present evidence that the streptococcal protein IdeC may have a second function, involving bacterial attachment and invasion into host cells during infection.

Infection Immunology

OP-II-001
Activin A levels are raised during tuberculosis and blockade of the activin signaling axis influences experimental M. tuberculosis infection

*N. E. Nieuwenhuizen[1,2], G. Nouailles[3], J. S. Sutherland[4], J. Zyla[5], A. H. Pasternack[6], J. Heyckendorf[7], B. C. Frye[8], K. Höhne[8], U. Zedler[2], S. Bandermann[2], U. Abu Abed[2], V. Brinkmann[2], B. Gutbier[3], M. Witzenrath[3,9,10], N. Suttorp[3,9,10], G. Zissel[8], C. Lange[11,12,13,14], O. Ritvos[6], S. H. E. Kaufmann[2,15,16]
[1]*Julius-Maximilians-Universität Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[2]*Max Planck Institute for Infection Biology, Berlin, Germany*
[3]*Charité - University Medicine Berlin, Berlin, Germany*
[4]*Medical Research Council Unit The Gambia, Fajara, The Gambia*
[5]*Silesian University of Technology, Gliwice, Germany*
[6]*University of Helsinki, Helsinki, Finland*
[7]*University Hospital Schleswig-Holstein, Kiel, Germany*
[8]*Medical Center – University of Freiburg, Freiburg i. Br., Germany*
[9]*CAPNETZ STIFTUNG, Hannover, Germany*
[10]*German Center for Lung Research (DZL), Giessen, Germany*
[11]*Research Center Borstel, Borstel, Germany*
[12]*German Center for Infection Research, Borstel, Germany*
[13]*University of Lübeck, Lübeck, Germany*
[14]*Baylor College of Medicine and Texas Children´s Hospital, Houston, TX, United States*
[15]*Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany*
[16]*Hagler Institute for Advanced Study, Texas A&M University, College Station, TX, United States*

Introduction: The TGF-beta superfamily member activin A is an important immunomodulatory cytokine, but few studies have examined its roles in infectious diseases. Activin A has been implicated in lung pathophysiology, driving us to examine whether it is increased in tuberculosis (TB) and pneumonia, as well as sarcoidosis, a granulomatous inflammatory disease of unknown aetiology that mainly affects the lungs.

Goals: We aimed to determine whether serum activin A levels are increased during pulmonary TB, pneumonia and

sarcoidosis. Furthermore, we aimed to investigate whether the activin signaling axis plays a role in experimental TB.

Materials & Methods: Serum activin A levels were measured in patients with TB, pneumonia (including bacterial and viral pneumonia), and sarcoidosis. A soluble activin type IIB receptor fused to human IgG1 Fc (sActRIIB-Fc) was used to inhibit the activin signaling axis in a murine TB model.

Results: In two independent cohorts of TB patients from Gambia and Germany, serum activin A levels were increased during active TB, and levels were associated with increased X-ray scores. In the Gambian cohort, Activin A levels were strongly increased in patients with active TB compared to healthy household contacts, and were reduced in most patients by six months of therapy. Many pneumonia patients also had elevated activin A levels, but activin A levels remained low in sarcoidosis patients. Administration of a soluble ActRIIB-Fc complex to *M. tuberculosis*-infected mice decreased lung bacterial loads, altered cytokine profiles and increased numbers of CD103+ T cells. The decreased frequencies of CD103+ T cells corresponded with downregulated T-bet expression in both CD4 and CD8 T cells, without changes to TGF-beta expression.

Summary: Activin A shows potential as a useful cytokine biomarker for diagnosis of active TB. The activin signalling axis appears to play a functional role during murine TB.

OP-II-002
Elucidating the role of IL4/IL13 signaling in tuberculous granuloma formation

*M. Herbert[1], M. R. Cronan[1]
[1]*Max Planck Institute for Infection Biology, In vivo cell biology of infection, Berlin, Germany*

Tuberculous granulomas are highly organized structures that form upon infection with a member of the *Mycobacterium tuberculosis* complex (MTBC) or close relatives like *M. marinum*. Granulomas act as a double-edged sword: they contain infection but can also enable bacterial expansion and limit antibiotic penetration. The central scaffold of the granuloma is a layer of epithelioid macrophages that shields the necrotic core, harboring the bulk of the bacterial burden away from immune cells and antibiotics that struggle to penetrate this structure. The transcription factor STAT6 has been shown to be vital for the epithelioid transformation of macrophages and necrotic granuloma formation through upstream IL4R signaling (Cronan et al. *Cell* 2021). Here, we investigate the cellular reservoirs of IL4 and IL13 within the granuloma and the contribution of each of these cytokines to granuloma formation and mycobacterial pathogenesis.

Goal: To delineate the role of IL4 and IL13 signaling in to the development and architecture of tuberculous granulomas, and assess these pathways as novel adjunct host-directed therapies.

We employed a zebrafish (*Danio rerio*)-*M. marinum* infection model, which closely mimics human tuberculosis pathology. Genetically modified zebrafish were used to disrupt IL4, IL13 or IL4/IL13 signaling. Histological and immunofluorescence staining were used to monitor granuloma organization and the induction and spatial distribution of IL4/IL13 throughout chronic mycobacterial infection. The functional outcomes of altered IL4/IL13 signaling were assessed by bacterial number within these hosts.

We expect to identify the significance of IL4/IL13 signaling within the granuloma and the role of the signals in the overall trajectory of mycobacterial infection. These findings will be critical to understanding the immunological mechanisms governing granuloma formation. As granulomas act as a substantial barrier to the effective treatment of mycobacterial infection, these insights could offer novel treatment approaches through modulation of granuloma formation and function.

OP-II-003
Type 2 conventional dendritic cells play a critical role in the disease outcome of Leishmania mexicana infection, but not in Leishmania major infection

*T. Gold[1], H. Sebald[1], N. Tochoedo[2], D. Dudziak[2,3], C. Bogdan[1,4], C. Lehmann[2], U. Schleicher[1,4]
[1]*Institute of Clinical Microbiology, Immunology and Hygiene; University Hospital of Erlangen, Erlangen, Germany*
[2]*Department of Dermatology, Laboratory of DC-Biology, Universitätsklinikum Erlangen, Erlangen, Germany*
[3]*University Hospital Jena, Institute of Immunology, Jena, Germany*
[4]*FAU Profile Center Immunomedicine (FAU-IMED), FAU Erlangen-Nürnberg, Erlangen, Germany*

Depending on the parasite species, cutaneous leishmaniasis (CL) in C57BL/6 mice differs in the disease outcome. *Leishmania (L). major* infection usually shows a strong Th1-driven interferon (IFN)γ response and subsequent production of leishmanicidal nitric oxide, limiting parasite growth and skin pathology, whereas *L. mexicana* infection ends up in chronic skin lesions due to a mixed IFNγ and interleukin (IL)-10 Th response. As Th responses are triggered by myeloid antigen-presenting cells, we hypothesized that differences in the infection and/or functionality of myeloid subpopulations at the site of infection control disease progression in self-healing versus chronic CL.

Using fluorescently labeled *Leishmania* and flow cytometry, we identified dendritic cells (DCs), monocytes, macrophages and granulocytes as *Leishmania* host cells in the skin early after infection. Dermal cDC2 showed higher infection rates than cDC1 in both infections. Single-cell RNA sequencing during early *L. major* and *L. mexicana* infection revealed that there is a distinct Cxcl9-positive macrophage population present in the draining lymph nodes of *L. mexicana*-infected mice, but not of *L. major*-infected animals after 24 hrs of infection. Other myeloid cell subclusters in skin and lymph nodes after 24 and 120 hrs of infection were similar for both parasites. To study the role of cDC2s *in vivo*, we analyzed Irf4ΔItgax mice that have reduced levels of cDC2 in their lymphoid organs. *L. major*-infected cDC2-deficient mice and wild-type (WT) littermates showed a comparable, self-healing course of disease. In contrast, *L. mexicana* infection caused the expected chronic skin lesions in WT controls, whereas *L. mexicana*-infected Irf4ΔItgax mice developed only mild pathology and resolved the disease. Quantitative PCR analysis of the infected tissue of these animals revealed a shift towards a stronger Th1 response and a subsequent dampening of Th2 related gene expression. Thus, cDC2 exert a hitherto unknown detrimental effect in chronic CL. Ongoing studies aim to unravel the disease-promoting mechanism of cDC2.

OP-II-004
Neutrophils sensitize Klebsiella pneumoniae for antibiotic-mediated bacterial killing

*N. Tassi[1], B. Kerscher[1], Y. Pfeifer[2], G. Werner[2], I. Bekeredjian-Ding[1,3]

[1]Paul-Ehrlich Institute, Microbiology, Langen, Germany
[2]Robert-Koch-Institut, Wernigerode, Germany
[3]University of Marburg, Institute for Medical Microbiology and Hospital Hygiene, Marburg, Germany

Introduction: Multidrug-resistant bacteria such as *K. pneumoniae* represent important health threats due inefficacy of antibiotics. First line innate immune defense through neutrophils plays a crucial role in the resolution of infections. However, the contribution of the immune system to effective antibiotic therapy remains unappreciated and hard-to-measure.

Goals: Here we hypothesized that neutrophils might enhance the effectiveness of antibiotic treatment against antibiotic-resistant *K. pneumoniae*.

Methods: For this study, the Robert-Koch-Institut provided clinical isolates *of K. pneumoniae.* AST was performed and interpreted according to EUCAST criteria. Isolates were selected based on MICs. Neutrophils were isolated from healthy volunteers (ethics approval #2021-253). Phagocytosis was assessed with gentamycin protection assay. IL-8 was measured by ELISA. Expression of CD62L, CD66b and CD63 was analyzed by flow cytometry. Serum resistance was assessed in 50% normal human serum.

Results: Neutrophils failed to kill clinical isolates of *K. pneumoniae.* However, their presence synergistically increased the effect of meropenem and cefotaxime on bacterial killing. This effect was specific for cell wall-active antibiotics, e.g. 3rd generation cephalosporins and carbapenems and was not obtained with tigecycline. It was further dependent on the strain and on the respective MIC. Of note, the synergy of neutrophils and antibiotics enables killing of bacteria tested as intermediate and resistant. It did not correlate with susceptibility to complement-mediated killing nor expression of activation markers on neutrophils. Moreover, most strains were not phagocytosed, suggesting an extracellular mechanism underlying the observed synergy.

Summary: Although *K. pneumoniae* was resistant to neutrophil killing, our data suggest that these cells can play a role in control of the infection and bacterial killing in the presence of cell wall-targeting antibiotics. Further experiments will elucidate the cellular events supporting *K. pneumoniae* killing.

Funding: European Commission Innovative Training Network (ITN) Training towards Innovative Personalized Antibiotic Therapy (TIPAT).

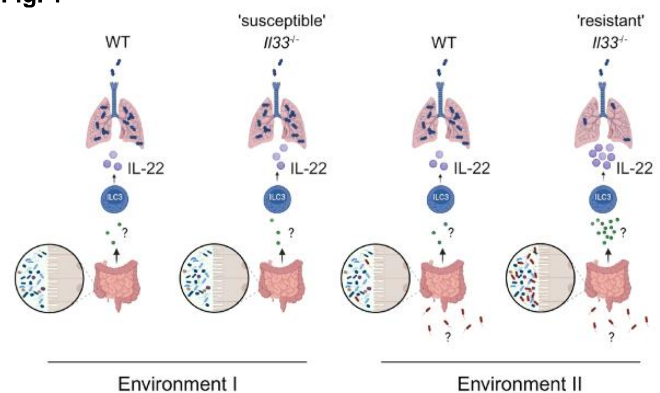
OP-II-005

IL-33 controls IL-22-dependent antibacterial defense by modulating the microbiota

*I. Röwekamp[1], L. Maschirow[1], A. Rabes[1], F. Fiocca Vernengo[1], S. Caesar[1], G. A. Heinz[2], M. Löhning[2], M. F. Mashreghi[2], G. Nouailles[1], N. Suttorp[1], S. M. Wienhold[1], L. Yao[1], M. Milek[1], A. Fagundes Fonseca[1], D. Bruder[3], M. Witzenrath[1], J. D. Boehme[3], M. Puzianowska-Kuznicka[4], D. Beule[1], M. Heimesaat[1], C. S. N. Klose[1], A. Diefenbach[1], B. Opitz[1]
[1]Charité - University Medicine Berlin, Berlin, Germany
[2] German Rheumatism Research Center Berlin, Berlin, Germany
[3]Helmholtz Institute for RNA-based Infection Research, Brunswick, Germany
[4]Mossakowski Medical Research Institute, Warsaw, Germany

IL-22 plays a critical role in defending against mucosal infections, but how IL-22 production is regulated is incompletely understood. Here, we show that mice lacking IL-33 or its receptor ST2 (IL-1RL1) were more resistant to *Streptococcus pneumoniae* lung infection than wild-type animals, and that single nucleotide polymorphisms in *IL33* and *IL1RL1* were associated with pneumococcal pneumonia in humans. The effect of IL-33 on *S. pneumoniae* infection was mediated by negative regulation of IL-22 production in innate lymphoid cells (ILCs), but independent of ILC2s as well as IL-4 and IL-13 signaling. Moreover, IL-33"s influence on antibacterial defense was dependent on housing conditions of the mice, and mediated by the modulatory effect of IL-33 on the microbiota. Collectively, we provide insight into the bidirectional crosstalk between the innate immune system and the microbiota. We identify a mechanism, dependent on both genetic and environmental factors, that impacts the efficacy of antibacterial immune defense and thus susceptibility to pneumonia.

Fig. 1



OP-II-006

Deubiquitinating enzyme OTUB1 is essential for intracellular control of *Salmonella* Typhimurium in macrophages during infection.

*I. Sagar[1], S. Beyer[1], N. Gopala Krishna[1], D. Schlüter[1]
[1]Hannover Medical School, Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany

Objectives: The bacterium *Salmonella* Typhimurium (STm) causes non-typhoidal gastrointestinal tract disease upon ingestion of contaminated food or water. STm first invades intestinal epithelial cells and then infects basolaterally located macrophages. Macrophages play an important role in the control of the pathogen but also serve as a niche for intracellular bacterial growth. Invasion and replication within the macrophages trigger activation of pro-inflammatory signaling pathways, which are essential for anti-bacterial responses. These pathways are tightly regulated by posttranslational modifications including ubiquitination/deubiquitination. The deubiquitinating enzyme (DUB) OTUB1 is one such versatile DUB, which preferentially cleaves K48 ubiquitin chains from substrates or can bind to E2 ubiquitin-conjugating enzymes to prevent the ubiquitination of the substrate and thereby regulate signaling. The objective of this project is to study the role of OTUB1 in macrophages during Salmonella infection.

Methods: We generated conditional OTUB1-deficient mice (LysM-Cre OTUB1$^{fl/fl}$) with deletion of OTUB1 in macrophages. LysM-Cre OTUB1$^{fl/fl}$ and control OTUB1$^{fl/fl}$ mice were orally infected with SL1344 wild-type STm. For *in vitro* infection, OTUB1-deficient and -competent bone marrow-derived macrophages (BMDM) were used.

Results: Upon oral infection with STm, LysM-Cre OTUB1$^{fl/fl}$ mice harbored higher bacterial load and showed increased inflammation in the spleen and liver compared to the OTUB1$^{fl/fl}$ mice. In concurrence, OTUB1-deficient BMDMs were unable to control STm in vitro. The impaired control of Salmonella in-OTUB1-deficient BMDM was paralleled by impaired activation of protective pro-inflammatory NF-κB and MAPK signaling and decreased production of anti-bacterial nitric oxide (NO). Mechanistically, OTUB1 removes the K48-like ubiquitin chain, stabilizes the E2-conjugating enzymes UBC13 and UBCH5c, and thereby fosters NF-KB and MAPK-mediated production of antibacterial NO in macrophages.

Conclusions: These data identify OTUB1 as a critical regulator of UBC13 and UBCH5c mediated anti-STm response in macrophages.

OP-II-007

Temporal single-cell RNA sequencing captures macrophage reprogramming by Salmonella

*C. Toussaint[1], P. Hill[2], S. Helaine[3], A. E. Saliba[1]

[1]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*

[2]*Imperial College, Center for Bacterial Resistance Biology, London, United Kingdom*

[3]*Harvard Medical School, Department of Microbiology, Boston, MA, United States*

While macrophages are well-equipped to track and kill foreign invaders, they paradoxically constitute a replicative niche for several intracellular pathogens. For instance, the intracellular Gram-negative bacteria Salmonella enterica serovar Typhimurium can turn infected macrophages into a favourable intracellular niche by secreting bacterial proteins effectors within the host cytoplasm. Typically, macrophages induce a pro-inflammatory program (coined M1) upon bacterial invasion to control and kill the bacteria. However, Salmonella can reverse macrophage polarization towards an anti-inflammatory state (so-called M2) permissive to bacterial survival and replication. The understanding of the transcriptional cascade leading to this reprogramming on the macrophage side remains unknown. Here, we leveraged temporal single-cell RNA-sequencing and RNA metabolic labelling to resolve the transcriptomic response of mouse bone marrow-derived macrophages during the first hours following Salmonella challenge.

The initial and stereotypical response driving infected macrophages to M1 polarization was characterized by the pathogen detection and the strong induction of NF-κB signaling. Heterogeneity emerged in the macrophage population at 6 hours following infection, with some infected cells switching to the M2 phenotype. This critical point determined the final polarization state of macrophages. The M2 macrophage population was marked by a clear dampening of the initial inflammatory response and the induction of specific gene modules, concurrently to the activation of the Salmonella pathogenicity island 2 (SPI2). We identified transcription factors marking the initiation of the reprogramming trajectory and correlated to the M2 polarization even at later timepoints. Overall, our work highlights a weak point in macrophage wiring exploited by a pathogen to overcome cellular immunity and induce host cell reprogramming.

OP-II-008

Dietary L-arginine supplementation cures chronic L. mexicana infection

*U. Schleicher[1], *B. Rai[1], L. Chunguang[2], A. Debus[1], M. Kunz[3], M. Jeninga[1], M. Rauh[4], C. Daniel[4,5], C. Bogdan[1]

[1]*University Hospital Erlangen, Microbiology Institute- Clinical Microbiology, Hygiene and Immunology, Erlangen, Germany*

[2]*University Hospital Jena, Institute of Immunology, Jena, Germany*

[3]*Friedrich-Alexander-University Erlangen-Nürnberg, Medical Informatics, Erlangen, Germany*

[4]*University Hospital Erlangen, Paediatric Clinic, Erlangen, Germany*

[5]*University Hospital Erlangen, Department of Nephropathology, Erlangen, Germany*

Control of *Leishmania* (*L.*) requires IFNγ-dependent type 2 nitric oxide (NO) synthase (NOS2), an enzyme that converts L-arginine into citrulline and NO. NOS2 activity is counteracted by arginase (ARG) 1 and 2, which are induced by Th2 cytokines and cleave L-arginine into urea and ornithine, a precursor of polyamines necessary for cell proliferation. Recently, we observed that the expression of ARG1 steadily increased in *L. mexicana*-infected BALB/c and C57BL/6 mice during disease manifestation. Here, we studied the functional role of host cell arginases during *L. mexicana* induced chronic cutaneous leishmaniasis (CL).

L.mexicana infected C57BL/6 WT mice developed non-healing chronic CL, whereas mice with deletion of ARG1 in monocytes and macrophages (Arg1 ΔCx3cr1) exhibited a strongly reduced pathology and ultimately resolved their skin lesions despite parasite persistence. Mice lacking IL-10 in CD4+ T cells (Il10ΔCd4) revealed IL-10 as a factor inducing ARG1 during infection. Flow cytometry and scRNAseq analyses of skin lesions showed an enrichment of dermal myeloid subpopulations in WT mice, including a dominant cluster of ARG1+NOS2+ inflammatory macrophages that developed from recruited monocytes in an ARG1- and IFNγ-dependent manner. These macrophages expressed high levels of CXCL9/10, thereby entertaining a self-perpetuating cycle of inflammation, and served as a replicative niche for the parasites, because ARG1 restricted NOS2-mediated killing due to substrate competition. Additionally, the accumulation of ARG1+NOS2+ macrophages led to a long-lasting depletion of L-arginine in the skin as measured by metabolomics, which was not observed in ARG1-deficient healer mice. Based on this finding we treated infected mice with dietary L-arginine via the drinking water. Oral supplementation with L-arginine was able to prevent chronic CL in a prophylactic setting. Most strikingly, infected sick WT mice could be cured by the L-arginine treatment in a therapeutic setting.

Thus, metabolic reprogramming by L-arginine can resolve chronic skin inflammation in *L. mexicana* infection, which is induced and maintained by an ARG1-dependent pathogenic differentiation of monocytes.

OP-II-009

Dendritic cell specific deletion of OTUD7b protects mice from experimental cerebral malaria

*K. Harit[1], R. Bhattacharjee[1], K. Matuschewski[1], J. Becker[2], U. Kalinke[2], D. Schlüter[3], N. Gopala Krishna[3]

[1]Humboldt university, Institute of biology, Berlin, Germany
[2]Institute for Experimental Infection Research, TWINCORE, Hannover, Germany
[3]Institute for Medical Microbiology and Hospital Hygeine, MHH, Hannover, Germany

Cerebral malaria (CM) is a severe life-threatening neurological complication of human malaria. Studies in murine experimental cerebral malaria (ECM) have shown that dendritic cells (DCs) prime CD8+ T cells, which migrate to the brain and cause disturbance of the blood-brain-barrier (BBB). The intrinsic mechanisms leading to DC survival permitting the development of CD8+ T cell-mediated immunopathology are yet incompletely understood. The pleiotropic cytokine TNF, which is highly expressed in both CM and ECM, regulates both cell survival and cell death depending on the ubiquitination status of signaling molecules. However, our molecular understanding of the highly dynamic processes of ubiquitination and deubiquitination is still fragmentary. Here, we newly identified that the ovarian tumor deubiquitinating enzyme 7b (OTUD7b) prevents TNF-induced apoptosis of DCs, thereby enabling efficient priming of pathogen-specific CD8+ T cells and development of ECM. Mechanistically, OTUD7b stabilizes TRAF2 in both human and murine DCs by preventing its K48-ubiquitination and proteasomal degradation. TRAF2 in turn facilitates K63-linked polyubiquitination of RIPK1, which mediates (i) activation of NF-kB and MAP kinases, (ii) production of IL-12, and (iii) expression of anti-apoptotic molecules cFLIP and Bcl-xL. Consequently, mice with DC-specific OTUD7b-deficiency were protected from ECM due to DC apoptosis and a failure to induce CD8+ T cell-mediated brain pathology, Taken together our study identifies OTUD7b as a molecular switch deciding on DC survival and death in both human and murine DC and qualifies it as a potential therapeutic target to manipulate DC responses.

Microbial Cell Biology

OP-MCB-001
Long as I can see the light – Control of cyanobacterial phototaxis orientation by the Tax1 system

Y. Han[1], *J. Hammerl[1,2], S. Engel[1], F. E. Flemming[1], N. Schürgers[1], A. Wilde[1]
[1]University of Freiburg, Institute of Biology 3, Molecular Genetics, Freiburg i. Br., Germany
[2]University of Freiburg, Spemann Graduate School of Biology and Medicine (SGBM), Freiburg i. Br., Germany

In order to seek out optimal photosynthetic conditions, cyanobacteria exhibit phototaxis, navigating either towards or away from a light source via polar activation of type IV pili (T4P). The Tax1 system is a chemotaxis-like signal transduction pathway that governs a switch in cell polarity, which is crucial for positive phototaxis in *Synechocystis* sp. PCC 6803. The system consists of a blue/green light sensor, PixJ, which controls the histidine kinase PixL and two CheY-like response regulators, PixG and PixH. However, the molecular mechanisms by which Tax1 regulates polar pilus assembly and thereby phototactic orientation are poorly understood. Here, we investigated the phosphotransfer between PixL and its cognate response regulators *in vitro* and analyzed the localization and function of wild-type and phosphorylation-deficient PixG and PixH during phototactic motility. We demonstrated that both PixG and PixH are phosphorylated by PixL but have different roles in the regulation of phototaxis. Only phosphorylated PixG interacts with the T4P motor protein PilB1 and localizes to the leading cell pole under directional light, thereby promoting positive phototaxis. In contrast, PixH acts as a phosphate sink, reducing PixG phosphorylation and inhibiting positive phototaxis. In addition, we showed that the C-terminal receiver domain of PixL is essential for positive phototaxis, and modulates the kinase activity of PixL. Our findings reveal the molecular basis of positive phototaxis regulation by the Tax1 system and provide insights into the division of labor between PatA-type and CheY-like response regulators in cyanobacterial chemotaxis-like systems. Furthermore, these findings highlight similarities in the regulation of movement direction during twitching motility in phototactic and chemotactic bacteria.

OP-MCB-002
Insights into the Flagellar Motility System in Magnetotactic Bacteria

*D. Pfeiffer[1]
[1]University of Bayreuth, Department of Microbiology, Bayreuth, Germany

Magnetotactic bacteria (MTB) possess the remarkable ability to navigate by utilizing the geomagnetic field. This magnetic navigation arises from a combination of passive magnetic alignment, mediated by intracellular chains of magnetic organelles (magnetosomes), and active motility driven by flagella, associated with chemotactic responses, particularly aerotaxis. Magneto-aerotaxis is believed to facilitate orientation within aquatic habitats towards preferred growth-favoring oxygen concentrations. Despite MTB exhibiting motility patterns that are highly distinct from well-studied non-magnetotactic model bacteria (including a directionally biased North- or South-seeking motility towards the poles of the geomagnetic field), the underlying molecular mechanisms have remained largely unstudied. To gain insights into these mechanisms, I implemented high-speed live-cell imaging of fluorescently labeled flagella in the genetically tractable alphaproteobacterium *Magnetospirillum gryphiswaldense*, initially reported to possess one flagellum at each of its cell poles. Here, I propose that flagellation in *M. gryphiswaldense* is not as strictly bipolar as reported previously. Moreover, I will demonstrate how different configurations of the flagella control the movement and directional reversals of *M. gryphiswaldense*, including the wrapping and pushing of individual flagella. In the near future, visualizing rotating flagella in *M. gryphiswaldense* will facilitate further investigations into the complex motility behavior in MTB and allow us to unravel the interplay between aerotactic sensing, polar flagellation, and directionally biased motility in magnetic fields. Additionally, a comprehensive understanding of the biophysical aspects governing flagellar motility in magnetospirilla could potentially find application in the construction of bio-inspired microrobots guided and steered by magnetic forces.

OP-MCB-003
Factors controlling in vivo phosphorylation of ReoM, an important player in control of peptidoglycan biosynthesis

*P. Rothe[1], S. Wamp[1], L. Rosemeyer[1], J. Rismondo[2,3], J. Döllinger[4], S. Halbedel[1,5]
[1]Robert Koch Institute, Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany
[2]Institute of Microbiology and Genetics, GZMB, Georg-August University Göttingen, Department of General Microbiology, Göttingen, Germany
[3]Imperial College London, Section of Molecular Microbiology and

Medical Research Council Centre for Molecular Bacteriology and Infection, London, United Kingdom
[4]Robert Koch Institute, ZBS6 Proteomics and spectroscopy, Berlin, Germany
[5]Otto von Guericke University Magdeburg, Institute for Medical Microbiology and Hospital Hygiene, Magdeburg, Germany

Listeria monocytogenes, a Gr+ bacterium, is characterised by a thick cell wall consisting mainly of peptidoglycan (PG). The cell wall counteracts the high internal turgor pressure and ensures viability. To fully understand PG biosynthesis and identify potential pharmaceutical targets, we are studying a regulatory pathway that controls PG production in *L. monocytogenes* and related Gr+ bacteria[1,2]. This pathway controls the proteolytic degradation of MurA, the enzyme that catalyses the first committed step of PG biosynthesis. MurA is degraded by the protease ClpCP in the presence of unphosphorylated ReoM. However, when ReoM is phosphorylated by the PASTA-domain containing protein serine/threonine kinase PrkA, the interaction of ReoM with MurA is inhibited and MurA degradation is prevented. The phosphorylation can be reversed by the phosphatase PrpC. In this way, PrkA and PrpC are involved in the decision whether PG biosynthesis is switched on or off.

We here have studied the phosphorylation profile of ReoM in living *L. monocytogenes* cells to clarify under which growth conditions ReoM is phosphorylated and thus PG biosynthesis activated, respectively.

Our results show that ReoM is fully phosphorylated under most tested laboratory growth conditions. However, we also found factors that prevented the phosphorylation of ReoM as, for example, ReoM phosphorylation was reduced in a mutant lacking the divisome gene *gpsB*. Artificial overexpression of MurA exerted a negative feedback on ReoM phosphorylation and this required a direct contact between ReoM and MurA, as well as MurA enzymatic activity and substrate binding.

These findings indicate that MurA and PrkA compete for ReoM as their shared interaction partner and suggest that the involved proteins constitute a safety valve mechanism ensuring controlled degradation of excess MurA to align the MurA amounts and the rate of PG biosynthesis with nutrient supply and growth.

[1]Wamp *et al*. (2020) *Elife*, *9*, e56048

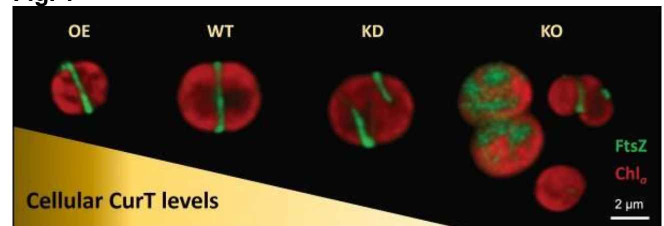
[2]Wamp *et al.* (2022) *PLoS Pathogens*, *18*(3), e1010406

OP-MCB-004
Cyanobacterial CurT proteins are involved in cell division.
*M. Dann[1]
[1]*Technical University of Darmstadt, Biology, Darmstadt, Germany*

Cyanobacteria are in large characterized by their capacity for aerobic photolithoautotrophic growth. The latter is driven by oxygenic photosynthesis whose molecular machinery resides within a specialized intracellular membrane system termed "thylakoid" in all but the most early-branching cyanobacterial species. Thylakoid membranes have undergone severe morphological changes during cyanobacterial evolution and the transition into endosymbiotic plastids. Here, the CurT/CURT1-like transmembrane proteins constitute a key determinant of thylakoid architecture in both cyanobacteria and the green lineage of algae and land plants. The evolutionary origin of this protein family, however, remains highly elusive, with the appearance of "fully-formed" CurT-like proteins roughly coinciding with the evolution of thylakoids themselves.

Microscopic analyses reveal a number of hitherto undescribed defects is cell division and thylakoid partitioning in *curT*-depleted mutants of *Synechocystis* sp. PCC 6803 which may provide the first relevant cue towards the origin of these fascinating proteins. CurT-depleted *Synechocystis* sp. PCC 6803 cells show impaired Z-ring formation, while a filamentous phenotype can be observed in *Synechococcus* sp. PCC 7942 *curT* mutants, all of which suggests an (original) involvement of CurT in cell division. Through phylogenetic analyses we identified a candidate for a distant sister clade to cyanobacterial/plastid CurT/CURT1 within the inherently thylakoid-less *Firmicutes* phylum. With *Firmicutes* and cyanobacteria being closely related phyla with overlapping cell division features, CurT/CURT1-like proteins having been originally obtained through horizontal gene transfer and stemming from a non-photosynthetic context becomes conceivable. Initially aiming at unravelling a tentative secondary function of CurT/CURT1 in cyanobacteria, we are now confident to have uncovered the evolutionary origins of these prominent photosynthesis-associated proteins in Gram-positive-like cell division.

Fig. 1



OP-MCB-005
Membrane binding triggers MinD ATPase activity in *Bacillus subtilis*
*H. Feddersen[1], M. Bramkamp[1]
[1]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for General Microbiology AG Bramkamp, Kiel, Germany*

Bacterial cell division relies on a complex protein network, and since most rod-shaped bacteria divide at midcell, correct placement of the division apparatus is crucial. One of the most studied division-site selection systems is the Min system, best understood in *Escherichia coli*, where defects lead to aberrant cell division and thus **min**icells. The active component MinC is localized through MinD, a Walker-type ATPase that dimerizes and interacts with the membrane upon ATP binding. The third protein MinE stimulates MinD ATPase activity, breaking the dimer and releasing it from the membrane, leading to a pole-to-pole oscillation. In contrast, *Bacillus subtilis* localizes MinD through MinJ and DivIVA to regions of negative curvature without oscillation.

Previously, we described a role of the Min system in *B. subtilis* in divisome disassembly (1) and high MinD dynamics to allow translocation to new septa (2). Here, we aimed to investigate how MinD membrane interaction is controlled and how ATPase activity is triggered in *B. subtilis*.

We employed in-vitro and in-vivo methods, including biochemical analysis of purified MinD and ATPase mutants as well as single-particle tracking (SPT) of MinD fusions.

Our findings suggest that MinD ATPase activity is solely stimulated by lipid-membrane interaction, while a membrane-binding mutant (I260E) is inactive. Bio-layer interferometry (BLI) with immobilized liposomes confirmed binding of monomeric MinD mutants (G12V, K16A) independent of ATP or dimerization, while a trapped-dimer mutant (D40A) showed reduced dissociation. SPT affirmed these findings, indicating similar shifts in mobility and dwelling times.

In summary, these results support the hypothesis that frequent MinD membrane association and rapid dissociation upon dimerization generates a sharp protein gradient in *B. subtilis*, without the requirement for a functional MinE homolog. Quick cycling of MinD in dense areas aided by local MinJ recruitment establishes a dynamic gradient, ensuring correct Min component localization throughout the cell cycle.

1. van Baarle S, Bramkamp M. 2010. PLoS One 5:e9850.
2. Feddersen H, Wurthner L, Frey E, Bramkamp M. 2021. mBio 12.

OP-MCB-006
Biogenesis of magnetic organelles

A. Paulus[1], *R. Uebe[1]
[1]Universtiy Bayreuth, Mikrobiologie, Bayreuth, Germany

The biogenesis of membranous cell organelles relies on extensive membrane remodeling and precise spatio-temporal targeting of proteins. While these processes are well characterized in eukaryotes, their molecular-level understanding in prokaryotes, which typically form organelles of lower complexity, still remains limited.

Here we analyzed the biogenesis of magnetosomes, membrane-enclosed magnetite nanoparticles used for magnetic navigation, as a model system for prokaryotic organelle formation. Using phenotypic assessments, proteome profiling, protein interaction studies, and fluorescence microscopy in *Magnetospirillum gryphiswaldense*, we identified three so-far uncharacterized proteins of the HOTT protein family that are crucial for the late stages of magnetosome membrane biogenesis. Since we also identified a remote homology between the HOTT protein family and chloroplast protein translocation systems, our findings suggest a novel mechanism of organelle biogenesis in prokaryotes. Moreover, analyses of subcellular localization patterns of GFP-labelled HOTT proteins in different mutant backgrounds are not consistent with the currently accepted model of magnetosome biogenesis. Thus, we propose a new spatiotemporal model for magnetosome biogenesis.

Microbiology in the Digital Era

OP-MDE-001
LLM-based question answering for the infectious disease domain

A. Bondarenko[1], H. Scells[2], M. Potthast[2], *A. Viehweger[1]
[1]University Hospital Leipzig, Medical Microbiology and Virology, Leipzig, Germany
[2]University Leipzig, Intelligent Language Technologies, Leipzig, Germany

Introduction

Using inappropriate antibiotics can quickly lead to antimicrobial resistance and infections that are difficult to treat. However, the capacity for training expert medical personnel is very limited, especially in developing countries. To ameliorate this gap, we develop a personalized AI-supported tutor for antibiotic usage training, with a chatbot as one of its core components.

Methodology

At the heart of the chatbot is a set of large language models (LLMs). However, LLMs are known to "hallucinate", i.e., generate factually incorrect information. To tackle this, we use a retrieval augmented generation (RAG) approach that restricts the LLM response to the evidence context retrieved from a curated corpus of credible data. In our case, we collected an intentionally diverse set of media from guidelines and (review) articles to podcasts and blog posts across several levels of evidence quality.

Our retriever combines lexical retrieval models (e.g., BM25), re-ranking based on the question–document semantic similarity, and topical routing. The routing filters out retrieved documents whose modeled topic mismatches the question topic (e.g., "skin infection").

To tackle complex questions (e.g., "Which empiric therapy should be used for erysipelas in a patient with kidney failure and diabetes mellitus type 2?"), we implement question decomposition that creates a set of shorter queries (e.g., "empiric therapy for erysipelas", "therapy with kidney failure", etc.).

Furthermore, we add the answer fact verification and source evidence citation modules. The fact verification module ensures that the generated answer"s statements are actually supported by the retrieved evidence, and evidence citation allows the user to verify the statements by inspecting the evidence sources.

Summary

We briefly describe our ongoing work on developing an AI-supported chatbot that assists in the rational selection of antibiotic therapy. The chatbot architecture combines RAG with fact verification and evidence citation modules. The planned evaluation will reveal existing drawbacks and allow us to address them.

Fig. 1

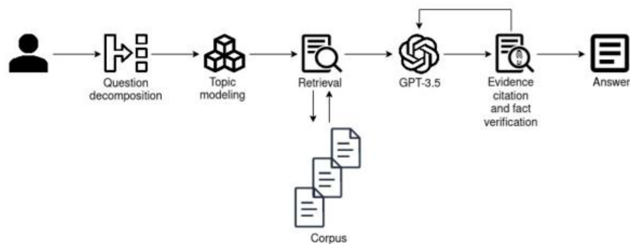


Figure: Overall chatbot architecture.

OP-MDE-002

StrainRegistry – A central registry for microbial strains

*I. Schober[1], A. Lissin[1], J. F. Witte[2], R. Pukall[3], B. Abt[2], M. Pester[3], Y. Mast[4], J. Overmann[2], L. C. Reimer[1]

[1]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Bioinformatics, IT and Databases, Brunswick, Germany*

[2]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Microbial Ecology and Diversity Research, Brunswick, Germany*

[3]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Microorganisms, Brunswick, Germany*

[4]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Bioresources for Bioeconomy and Health Research, Brunswick, Germany*

Increasingly, large microbiome studies generate considerable numbers of isolates. Deposition of these isolates to culture collections is not only in the interest of open science but is also required to formally name the many new taxa usually found among them. This presents a challenge to both researchers and biological resource centers. While the former wish to receive culture collection numbers as soon as possible to use as persistent identifiers in publications, the latter do not have the capacity to process these sizable collections simultaneously.

Here we present StrainRegistry, a novel strain registration and deposition management service connected to the StrainInfo database (https://straininfo.dsmz.de). It allows microbiologists to register strains with strain identity information to be published as strain entries in the StrainInfo database. These can be linked with culture collection numbers, sequences and publications as they become available. Each strain entry receives a persistent StrainInfo identifier and its respective DOI. While this cannot replace deposition in a culture collection and the rigorous quality checks needed, it will allow for consistent referencing of the strain from the very outset.

StrainRegistry also aims to simplify the deposition process by giving researchers the option to submit further meta- and deposition data, which are not published, but can directly be shared with a participating culture collection through the StrainRegistry deposition manager. This user interface visualizes the deposition status of each strain and facilitates communication between the depositor and the responsible culture collection curator, who can view strain information, demand further data, request shipping of biological material and change strains statuses.

This procedure is being developed in collaboration with the curators of the DSMZ to simplify bulk deposition at the DSMZ and with the ambition to engage other culture collections and standardize microbial strain deposition worldwide.

OP-MDE-003

Detecting interpretable patterns in estuarine-coastal microbial communities using methods from computational linguistics and Machine Learning

*A. S. Kujat[1,2], C. Glackin[2], C. Hassenrück[2], L. Vogel[2], E. Zschaubitz[2], S. Lüdtke[1], M. Labrenz[2], T. Sperlea[2]

[1]*University of Rostock, Institute for Visual and Analytic Computing/ Marine Data Science, Rostock, Germany*

[2]*Leibniz Institute for Baltic Sea Research (IOW), Biological Oceanography, Rostock, Germany*

Aquatic bacterial communities are integral components of earths ecosystems. Investigating temporal and spatial community patterns provide insights into the dynamics of the entire ecosystem. To uncover such patterns, next-generation sequencing methods provide the necessary data. But these data sets are usually compositional, extremely high-dimensional and exhibit non-linear relationships, which makes them difficult to understand and to analyse using statistical methods.

To circumvent these problems and to identify understandable compositional patterns in their temporal and spatial dynamics, we applied Topic Modeling (TM), a method originally from computational linguistics, to the microbial communities of the Warnow estuary and the Baltic Sea coast. This environment is characterized by strongly fluctuating environmental conditions and a steep salinity gradient and was sampled in high spatio-temporal resolution between April 2022 and April 2023.

More specifically, we compared several TM algorithms as well as various data preprocessing methods in their ability to capture ecological relationships using a Random Forest-based machine learning approach. This way, we could verify how much information was retained through TM while minimizing the dimensionality of the feature space as much as possible.

Using this approach, we identified subcommunities (corresponding to topics) specific to freshwater and brackish environments and specific seasonal phases. These subcommunities can be linked across primer sets and, therefore, the domains of life. In the estuary, we are able to link the retention time of specific subcommunities to weather and hydrodynamical dynamics.

Based on these results, we conclude that TM achieves a dimensional reduction of microbiome datasets that significantly increases their comprehensibility. Taking advantage of structural similarities between linguistic and microbiome datasets, TM proves to be a powerful tool especially for the analysis of microbial communities in dynamic habitats.

OP-MDE-004

Decoding Antibiotic Resistance: OprD Allels in Pseudomonas aeruginosa and their Impact on Carbapenem Resistance

*J. K. Warweitzky[1], J. Erdmann[1], S. Häußler[1,2]

[1]*Twincore - Centre for Experimental and Clinical Infection Research, Institute of Molecular Bacteriology, Hannover, Germany*

[2]*Helmholtz Center for Infection Research, Institute of Molecular Bacteriology, Brunswick, Germany*

Pseudomonas aeruginosa, commonly isolated from hospitalized patients, poses a significant therapeutic challenge due to its intrinsic and acquired antibiotic resistance. Carbapenems, crucial in treatment, often fail to eradicate the pathogen due to resistance associated with mutations in the outer membrane porin D (OprD). This study seeks to elucidate the impact of distinct OprD alleles (amino acid sequences) on carbapenem resistance, aiming to establish OprD as a reliable genetic predictor.

A dataset comprising 4,875 clinical isolates collected unbiased from a German and a Danish hospital, underwent susceptibility testing against imipenem and meropenem and whole genome sequencing. The analyzed isolates contained 192 distinct OprD alleles, with the most common variant occurring in 20.7 % of the isolates. Of the 4,875 isolates 46.7 % decoded an OprD sequence of conserved-length (441-446 amino acids). There were in total 41 different conserved-length Oprd variants. The fact that a clinical *P. aeruginosa* isolates does not contain a conserved-length allele showed unexpectedly high accuracy in predicting the antimicrobial resistance for both imipenem (sensitivity: 0.731; specificity: 0.962) and meropenem (0.764; 0.934). Further analysis focused on common OprD variants that were found in at least 10 clinical isolates (30 OprD variants) and revealed significant differences in their individual impact on carbapenem resistance. Interestingly, there are also variants that are exclusively predictive for antimicrobial resistance of either imipenem or meropenem.

The results again underline that OprD is the most common driver of carbapenem resistance *in P. aeruginosa* in terms of non-acquired resistance and it is the first time that OprD alleles have been analyzed in >1,000 isolates. We found that inactivation of OprD is usually caused by an early stop codon or a frameshift mutation and rarely by single amino acid exchanges.

OP-MDE-005

Modeling microbial communities using biochemical resource allocation analysis

*R. Höper[1], R. Steuer[1]
[1]*Humboldt University of Berlin, theoretical biology, Berlin, Germany*

Introduction:
Oxygenic photosynthesis, originating in cyanobacteria, is a critical biological process that drives primary production in most ecosystems. While we understand cyanobacterial growth dynamics in axenic laboratory cultures, most microorganisms evolved in interconnected ecosystems, and understanding their physiology requires taking their evolution within these dynamic ecosystems into account. Goals: Our objective is to understand the emergence of interactions between photo- and heterotrophic microorganisms using computational models grounded in biochemical resource allocation analysis. We aim to delineate the prerequisites and energetic trade-offs governing cooperation, division of labor, and nutrient cycles in microbial communities. Materials & Methods:
Building on previous advancements, we make use of high-quality quantitative computational models of microbial growth and resource allocation. Specifically, we simulate co-cultures of photo- and heterotrophic organisms, where each microbial partner maximizes its growth rate, allowing us to explore whether interactions and dependencies emerge. Results: Using a novel computational framework, we simulate interactions between photo- and heterotrophic microorganisms. By examining the costs and benefits of these interactions, we outline a plausible evolutionary pathway for the emergence of metabolic dependencies between these microorganisms in marine environments. We show that co-cultures can result in long-term stable cultures with increased productivity compared to axenic growth. Summary:
The perspective of cellular resource allocation offers a unique opportunity to understand the constraints and energetic trade-offs that govern the emergence of dependencies between photo- and heterotrophic microorganisms.

OP-MDE-006

Development of an integrated genomic surveillance (IGS) of public health relevant pathogens in Germany

*T. Semmler[1], H. Buck[2], N. Litzba[2], O. Hamouda[2], M. Mielke[3]
[1]*Robert Koch Institute, MF1 - Genome Competence, Berlin, Germany*
[2]*Robert Koch Institute, Dept 3 - Infectious Disease Epidemiology, Berlin, Germany*
[3]*Robert Koch Institute, Dept 1 - Infectious Diseases, Berlin, Germany*

Integrated genomic surveillance (IGS) is an effective public health strategy for the surveillance of infectious pathogens, which is becoming increasingly important worldwide. IGS combines the results of DNA sequencing and bioinformatics genome analyses of pathogen isolates from patient samples with epidemiological information from the reporting system and further data.

The main objective of IGS is to rapidly detect spread of infectious agents and to identify links, even between agents detected at different times and locations. The combination of genomic pathogen sequence data with selected epidemiological information on infected individuals allows outbreaks and transmission chains to be identified quickly and targeted interventions to contain the outbreak to be initiated at an early stage. In addition, the IGS offers the possibility of continuous detection and surveillance of pathogen variants with changing characteristics (e.g. transmissibility, virulence, AMR). The IGS also makes an important contribution to the decision-making process of public health authorities.

Two projects funded by the Federal Ministry of Health are currently underway with the aim to establish an IGS at the RKI in cooperation with the National Reference Centres (NRZ) and the Consiliary Laboratories (KL) as well as other stakeholders such as the health authorities of the federal states and the Network University Medicine (NUM). The necessary processes and an efficient infrastructure will be developed and established for the long-term implementation of the IGS according to §13 of the Infection Protection Act.

The necessary framework conditions for the systematic sequencing of infectious pathogens in the corresponding NRZ/KL have been created. The expansion of the German Electronic Reporting and Information system (DEMIS) for the transmission of genomic data is underway, as is the establishment of a high-performance IT infrastructure for the genome analysis, linking with epidemiological data, visualisation and provision of the information to stakeholders.

The so far established IGS nucleus should be stringently developed into a comprehensive sustainable Public Health Instrument in Germany.

Fig. 1

Microbial Ecology & Evolution

OP-MEE-001
The seeds of Plantago lanceolata comprise a stable core microbiome along a plant richness gradient.

*Y. Pinheiro Alves de Souza[1,2], Y. Huang[3,4], W. Weisser[2], M. Schloter[1,2], S. Schulz[1]
[1]*Helmholtz Center Munich, Institute of Comparative Microbiome Analysis, Neuherberg, Germany*
[2]*Technical University of Munich, TUM School of Life Sciences, Freising, Germany*
[3]*German Centre for Integrative Biodiversity Research (iDiv) , Leipzig, Germany*
[4]*University of Leipzig, Institute of Biology, Experimental Interaction Ecology, Leipzig, Germany*

Introduction

Research has demonstrated the critical role of diversity in ensuring the stability and resilience of a community in the face of environmental disturbances. This stability may be achieved through increasing metabolic complementarity and redundancy, which ensures the smooth functioning of different physiological processes at different trophic levels.

Goals

We investigated the effects of biodiversity on the diversity and composition of the microbiome of *P. lanceolata* seed, addressing the question whether the species richness of the surrounding plant community can influence the recruitment of bacteria from the parental plant to its seeds.

Materials and Methods

To access the effects of increasing biodiversity we made use of a well-established biodiversity experiment, The Jena Experiment (https://the-jena-experiment.de/), established on the floodplain of the Saale River, in Jena, Germany. There, biodiversity is manipulated in the form of a plant species richness gradient ranging from monoculture to 60 species. We sampled blossoms from *P. lanceolata* individuals grown on plots with different plant richness (monoculture, 4, 8 and 16 species). Seeds were surface sterilized to remove seed associated microbes and DNA extraction was performed. Metabarcoding and NGS sequencing was used to assess bacterial diversity in the seed materials.

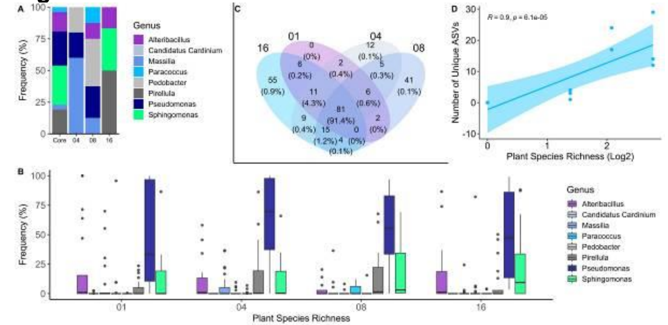
Results

Our data identified that endophytic microbiome composition of *P. lanceolata* seeds comprises a rather stable core microbiome, consisting of *Pseudomonas rhizosphere*, *Sphingomonas faeni* and *Pirellula spp.* However, we also identified unique taxa that only occur at certain diversity levels, such as *Pedobacter spp.* which was exclusively found on the plots with 4 species richness. Furthermore, we found the number of unique Amplicon Sequence Variants increase with plant diversity.

Summary

Our study nicely demonstrates that despite a rather stable core microbiome along the plant diversity gradient, plant diversity had an influence on the structure of the seed microbiome of *P. lanceoata*. The data has being acepted for publication in the Envriomental Microbiome Journal in January/2024.

Fig. 1



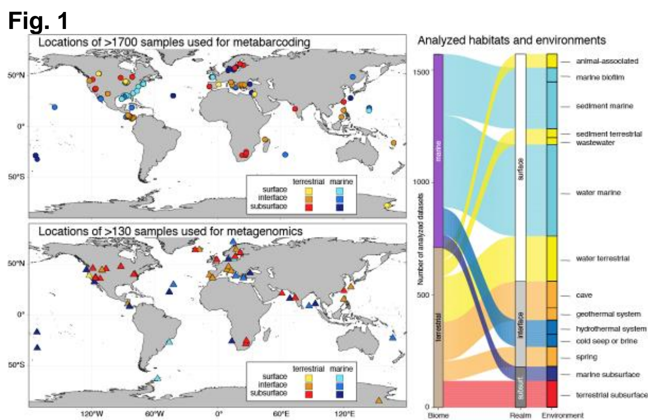
OP-MEE-002
A global atlas of subsurface microbiomes reveals phylogenetic novelty, large scale biodiversity gradients, and a marine-terrestrial divide.

*S. E. Ruff[1], I. Hrabe de Angelis[2], M. Mullis[3], J. Payet[4], C. Magnabosco[5], K. Lloyd[6], C. Sheik[7], A. Steen[6], A. Shipunova[1], A. Morozov[1], B. Kiel Reese[3], J. Bradley[8], J. Huber[9], A. J. Probst[10], H. Morrison[1], M. Sogin[1], J. Ladau[11], F. Colwell[4]
[1]*Marine Biological Laboratory, Ecosystems Center, Woods Hole, MA, United States*
[2]*Max Planck Institute for Chemistry, Mainz, Germany*
[3]*University of South Alabama, Mobile, AL, United States*
[4]*Oregon State University, Corvallis, OR, United States*
[5]*ETH Zürich, Zürich, Switzerland*
[6]*University of Tennessee, Knoxville, TN, United States*
[7]*University of Minnesota Duluth, Duluth, MN, United States*
[8]*Queen Mary University, London, United Kingdom*
[9]*Woods Hole Oceanographic Institution, Woods Hole, MA, United States*
[10]*University of Duisburg-Essen, Essen, Germany*
[11]*University of California, San Francisco, CA, United States*

Marine and terrestrial subsurface environments represent one of the largest habitats for microbial life on Earth. Despite the global importance of microbial communities for biogeochemical cycling and biodiversity, the differences in diversity and composition between marine and terrestrial as well as between surface and subsurface microbiomes remain unclear. We analyzed 523 archaeal and 1211 bacterial 16S rRNA gene amplicon datasets and 136 metagenomes from globally distributed environments, including lakes, estuaries, caves, hot springs, aquifers, coasts, hydrothermal vents, cold seeps, and the subseafloor. All samples were sequenced on the same sequencer using the same chemistry and workflows to minimize bias and ensure comparability. Archaeal and bacterial richness, evenness and composition fundamentally differed between marine and terrestrial microbiomes, revealing a marine-terrestrial divide that mirrors patterns in plant and animal diversity. We further

show that marine microbiomes are more phylogenetically diverse than terrestrial microbiomes. Within marine and terrestrial biomes, we find substantial community overlap between surface and subsurface environments suggesting a global diversity continuum rather than a discrete subsurface biosphere. Subsurface archaeal diversity far exceeded that of surface ecosystems suggesting that the subsurface holds a remarkable and largely underestimated fraction of Earth"s archaeal diversity. We identify specific archaeal and bacterial clades predominantly found in marine (e.g., *Lokiarchaeia*, *Caldatribacteria*) and terrestrial (e.g., *Hadarchaeia*, *Firmicutes*) subsurface ecosystems. Our analyses of metagenome-derived 16S rRNA genes and ribosomal protein S3 genes support the observed trends and findings. None of the included subsurface ecosystems appeared to be exhaustively sampled, indicating that considerable microbial biodiversity and metabolic capabilities remain to be discovered. This work improves our understanding of global-scale microbial ecology and biogeography.

Fig. 1



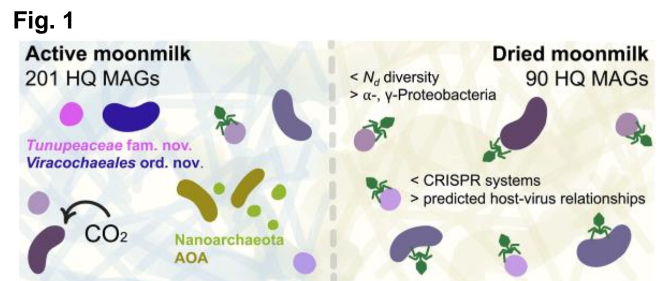
OP-MEE-003

A microbial oasis in karstic caves: a metagenomic view of active moonmilk microbiomes

*A. R. Soares[1,2], S. Eßer[1], J. Starke[1], K. Kaur[1], J. Banholzer[1], H. Jantschke[3], R. Straub[3], L. Rothe[2], A. J. Probst[1,2]
[1]*University of Duisburg-Essen, Faculty of Chemistry, Essen, Germany*
[2]*University of Duisburg-Essen, Center for Water and Environmental Research, Essen, Germany*
[3]*Landesverband für Höhlen- und Karstforschung Baden-Württemberg e.V. - LHK, Stuttgart, Germany*

Karstic caves are extreme critical zone habitats that remain underexplored mostly by virtue of the logistical difficulties in accessing them. However, despite the absence of sunlight and nutrients, microbial life is prevalent in karstic caves and is posed to play significant roles in the dissolution and re-precipitation of CaCO3, therefore aiding and guiding the formation of primary speleothems such as stalactites, but also of secondary speleothems, which include moonmilk. Previous investigations using cultivation, prokaryotic marker gene and stable isotope approaches indicated that the microbiomes of this "cream-cheese-like" (Barton & Jurado, 2007) secondary speleothem have the potential to fix atmospheric greenhouse gases such as CO2 and CH4. Here, we present metagenomes generated for active and dried moonmilk, for the first time allowing inferences of microbial function in this microenvironment from high-quality MAGs. Active moonmilk harbored several archaeal MAGs of phyla Thermoproteota and Nanoarchaeota that were not detected in dried moonmilk. Other MAGs uniquely detected in active moonmilk presented metabolic pathways indicative of carbon fixation potential via the pentose phosphate pathway. Predicted antiviral systems in active moonmilk MAGs were diverse, but largely exclusive of CRISPR-Cas systems, suggesting viral infection in this microenvironment may be limited by the unique chemistry of its inner carbonate matrix. The complex microbial ecology of active moonmilk resulted in the naming of two bacterial genera: Viracochaeum gen. nov. (Viracochaeales ord. nov., of Zixibacteria class MSB-5A5) and Tunupaeum gen. nov. (Tunupaeaceae fam. nov., Paceibacteria, Patescibacteria). Moonmilk is a common encrusted feature of karstic cave walls for which the first MAGs hereby recovered have exhibited diverse microbial communities of relevance to Earth"s carbon cycling.

Fig. 1



OP-MEE-004

Membrane changes during syntrophic growth of an archaeal/bacterial consortium: a model for eukaryogenesis

*K. Fiege[1], A. Abdala Asbun[1], J. Engelmann[1], L. Villanueva[1]
[1]*Royal Netherlands Institute for Sea Research, Marine Microbiology & Biogeochemistry, 't Horntje (Texel), Netherlands*

The first eukaryotic cell is assumed to have arisen from a symbiosis of an archaeal cell, likely an Asgard archaeon, and a bacterial partner. As an early stage of eukaryogenesis a syntrophic interaction between the partners has been suggested. Yet, little is known about the role of cell-to-cell contacts for the emergence of eukaryotes. Due to the lack of cultivated and genetically tractable Asgard archaea or closely relevant lineages, model systems of syntrophic interacting microorganisms can help to shed light on how cell-to-cell interactions between different species arose. Here, we specifically focus on determining which membrane lipids and proteins are involved in cell-to-cell interactions. To this end, we use syntrophic cocultures of the sulfate-reducing bacterium *Desulfovibrio vulgaris* and the methanogenic archaeon *Methanococcus maripaludi*s. In addition to an ancestor coculture, evolved cocultures after 300 & 1000 generations [1] were being analyzed by transcriptomics and proteomics to identify differentially expressed proteins connected to cell-to-cell interactions with a focus on membrane proteins. These analyses are further complemented with lipid analyses to determine changes in the cell membrane of the syntrophic coculture partners as a response to changes in the membrane proteins. To visualize cell interactions fluorescence microscopy was used. The combination of methods revealed membrane changes based on lipids and proteins between mono cultures and syntrophically grown cultures. Furthermore, comparing different coculture generations showed how the cell membrane of both species adapt to syntrophic growth. The observed differences indicate that membrane changes might have already occurred in early stages of eukaryogenesis. In summary, the in-depth analysis of a model syntrophic co-culture will provide clues on how interdomain cell-to-cell

interactions lead to the emergence of the first eukaryotic cell and which role early archaea had in it.

[1] Turkarslan *et al*., (2021) The ISME Journal 15(8):2233-2247.

OP-MEE-005
Ecology, ecosystem services and evolution of freshwater ammonia oxidizing archaea

*M. Pester[1], D. K. Ngugi[1], K. Kitzinger[2]
[1]*Leibniz Institute DSMZ, Department of Microorganisms, Brunswick, Germany*
[2]*Max Planck Institute for marine microbiology, Bremen, Germany*

Large planktonic population of ammonia oxidizing archaea (AOA) inhabit deep oligotrophic lakes, resembling the situation in the open ocean. They are thought to be the key ammonia oxidizers in these freshwater reservoirs and as such responsible for the rate-limiting step in nitrification. We followed this archaeal population in one of Europe"s largest lakes, Lake Constance, using metagenomics and metatranscriptomics combined with stable isotope-based activity measurements. An abundant (8–39% of picoplankton) and transcriptionally active archaeal ecotype dominated the nitrifying community. It represented a freshwater-specific species: "*Candidatus* Nitrosopumilus limneticus". Its biomass corresponded to 12% of carbon stored in phytoplankton over the year´s cycle. *Ca*. N. limneticus populations were driving potential ammonia oxidation rates of 6.0 ± 0.9 nmol l^{-1} d^{-1}. At the ecosystem level, this translates to a maximum capacity of archaea-driven nitrification of 1760 metric tons of N-ammonia per year or 11% of N-biomass produced annually by phytoplankton[1]. We extended this analysis by a global assessment of lacustrine AOA diversity. Using continental-scale metagenomics, we show that AOA species diversity in freshwater systems is remarkably low compared to marine environments. We further show that *Ca.* Nitrosopumilus limneticus is ubiquitous and genotypically static in various large European lakes where it evolved 13 million years ago. We find that extensive proteome remodeling was a key innovation for freshwater colonization of AOA[2]. These findings reveal the genetic diversity and adaptive mechanisms of a keystone species that has survived clonally in lakes for millennia.

[1]Klotz et al., 2022. ISME J. 16:1647-1656
[2]Ngugi et al., 2023. Science Advances. 9: eadc9392

OP-MEE-006
Diversity of far-red light photoacclimation responses in cyanobacteria

*D. Nürnberg[1,2]
[1]*Free University of Berlin, Berlin, Germany*
[2]*Dahlem Centre of Plant Sciences, Berlin, Germany*

The discovery of cyanobacteria capable of harvesting far-red light has changed the paradigm that oxygenic photosynthesis is only driven by visible light and exclusively by chlorophyll *a*. There are two known types of far-red photosynthesis. Firstly, a constitutive adaptation that uses a majority of chlorophyll *d,* which is restricted to a single genus (*Acaryochloris*). Moreover, an acclimation response, known as Far-Red Light Photoacclimation (FaRLiP), which uses chlorophyll *f* and is present in phylogenetically diverse cyanobacteria (1). FaRLiP involves the extensive remodelling of the photosynthetic machinery, via a cluster of approximately 19 *genes coding for* paralogous subunits of Photosystem I, Photosystem II, phycobilisomes and master control elements. Here, I will highlight the similarities and differences of FaRLiP among cyanobacteria on a cell, membrane, protein and DNA level by using bioinformatics, biochemical and biophysical methods. Our study focuses on cyanobacteria of the genus "*Chroococcidiopsis*" (2), as well as the phylogenetically early-branching group of "*Halomicronema*/*Nodosilineales*". The latter group are especially underrepresented. We could increase the number of FaRLiP cyanobacteria among them by using stringent far-red cultivation methods on samples from the hypersaline environment of the Sebkha Ooum Dba (Morocco). Furthermore, a strain was discovered that only contains a partial FaRLiP cluster, without genes for a far-red PSI variant, but with a normal growth behaviour under far-red light (3). This raises the question of the minimal requirements for FaRLiP.

(1) Antonaru LA, Cardona T, Larkum AWD and Nürnberg DJ (2020). *ISME J* 14, 2275–2287.
(2) Antonaru LA, Selinger VM,... and Nürnberg DJ (2023). *ISME Commun* 3, 113.
(3) Billi D, Napoli A,… and Nürnberg DJ (2022). *Front Microbiol* 13:933404.

OP-MEE-007
Phage-host dynamics during bacterial blooms in the coastal ocean

*N. Bartlau[1], F. Kreutter[1], M. F. Polz[1]
[1]*University of Vienna, Division of Microbial Ecology, Centre for Microbiology and Environmental Systems Science, Wien, Austria*

Phages influence microbial communities by their predatory activity and potential for horizontal gene transfer. Interactions between phages and their bacterial hosts are mediated by a combination of specific attachment, and host defense as well as phage counter defense mechanisms, all of which determine whether a phage can infect a particular host. However, how these complex mechanisms underlying potential interactions play out in the wild remains poorly understood. Here we reveal phage-host dynamics in a marine coastal environment using metagenomic analysis of the cellular fraction of a 93-day daily time series and show, as a general feature, that short host blooms are succeeded by equally short phage blooms across phylogenetically diverse bacteria. To further investigate such dynamics, we characterized a *Vibrio* bloom by bacterial culturing and phage isolation. The bloom of *Vibrionaceae* spans over nine days and is dominated in the first three days by *V. splendidus* while on the following days *V. cyclitrophicus* relative abundance also increases. Coincident with the bacterial bloom, an increase of *Vibrionaceae* infecting phages occurred. To further investigate if the susceptibility of bacterial hosts to phages changes over time, a cross-infection assay with 171 *Vibrio cyclitrophicus* and 76 diverse *V. cyclitrophicus* phages was performed. Results show that on average a third of the bacterial isolates were susceptible to at least one phage, but susceptibility prior to an increase in abundance of the host was doubled. This indicates a change in the host population and evolution on very short timescales. Further the infection dynamics during the bloom show that a diverse set of phages responds suggesting that a phage cocktail rather than single phages may play a role in limiting the bacterial bloom.

OP-MEE-008

Prophages can break down microbiome-specific disease barriers

*C. Wendling[1,2], Z. Bailey[1], R. Sampedro[1], M. Boumasmoud[1]
[1]*ETH Zürich, Zürich, Switzerland*
[2]*Ludwig-Maximilians University Munich, Medical Microbiology, München, Germany*

Background and hypothesis: Prophages play a crucial role in shaping infectious disease dynamics. They can for instance carry toxin genes or kill competing phage-susceptible gut commensals. In the gut microbiome, innate resistance to pathogen colonization and subsequent infection is a vital function. We predict that disruptions to the gut microbiota, caused by extensive lysis of phages released from invading pathogens, can lead to increased pathogen colonization success and worsen disease outcomes.

Methods:To test this hypothesis, we developed a novel high-through put in vivo infection model using *Galleria mellonella* larvae, into which we inoculated human-microbiome-like communities. Subsequently, we infected the larvae with *Salmonella Typhimurium*, either in the form of a lysogen carrying the broad-host range prophage P22 or without P22.

Results and Conclusion: While human-microbiome-like communities protected larvae from invading *Salmonella*, we found that *Salmonella* containing P22 exhibited enhanced invasion success of the larvae which translated into higher larval mortality. Survival data correlated with elevated *Salmonella* levels and free P22 virions, alongside a reduced number of resident *E. coli* commensals. This suggests, that invading *Salmonella* lysogens release P22 as weapons that kill niche competitors thereby enhancing pathogen invasion success. Importantly, we find that these findings are microbiome-specific, highlighting the intricate interplay between prophages and microbiome compositions in understanding infectious disease dynamics.

OP-MEE-009

Presence of Bdellovibrio bacteriovorus increases lifespan in C. elegans nematodes by altering microbiota dynamics

*J. Wülbern[1], L. Hansen[1], K. Rathjen[1], H. Schulenburg[1], J. Johnke[1]
[1]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Kiel, Germany*

In most ecosystems, a diverse microbiota contributes to stability - a principle applicable to host organisms. This is grounded in the enhanced functional redundancy associated with diversity, providing support during disturbances and mitigating dysbiosis risks. Predators like *Bdellovibrio bacteriovorus* influence community dynamics by creating niches. Indeed, *B. bacteriovorus* presence is associated with higher microbiota diversity of various hosts[1]. However, identifying a causal relationship between predator presence, community dynamics, and host fitness requires controlled laboratory experiments using a suitable host, such as the nematode *Caenorhabditis elegans*.

This study aims to assess the probiotic potential of two *B. bacteriovorus* strains to influence microbiota diversity and composition using a *C. elegans* reference microbiota (CeMbio)[2].

Exposure to *B. bacteriovorus* induces changes in CeMbio structure, in both the nematode"s gut and the surrounding medium. Moreover, *B. bacteriovorus* MYbb2 (broad prey range), but not MYbb4 (narrow prey range), presence significantly increases *C. elegans"* mean lifespan. Interestingly, the microbiota of nematodes exposed to MYbb2 demonstrates increased relative abundances of *Pseudomonas* and *Ochrobactrum*. Members of these genera may benefit the nematode by providing bacterial vitamin B12 according to whole genome predictions[3] and the results of a *Pacdh-1p::gfp* nematode reporter strain screen. Additionally, B12-supplementation of nematodes exposed to non-B12-producing CeMbio strains caused a significant increase in *C. elegans* longevity.

These findings underscore the intricate, context-dependent relationship between *B. bacteriovorus*, microbiota structure, and *C. elegans'* life-history traits, urging further analyses for a deeper understanding of these interactions and the impact of prey specificity in *B. bacteriovorus*.

[1] Johnke J. et al., Microb Ecol (2020)
[2] Dirksen P. et al., BMC Biol (2016)
[3] Zimmerman J. et al., ISME (2020)

OP-MEE-010

Effects of bacterial nitrogen transfer on fungal growth in nitrogen-deprived environments

*P. C. Anuforo[1], D. Schlosser[1], H. Harms[1], L. Wick[1]
[1]*Helmholtz center for environmental research UFZ, Department of Applied Microbial Ecology, Leipzig, Germany*

Biotransformation of organic contaminants is often impeded by a lack of nutrients relevant for growth and metabolic (co-metabolic) activity of fungal and bacterial degraders. While a growing number of studies have addressed the promoting role of fungal mycelia in bacterial activity and contaminant turnover, less is known on bacterial effects potentially supporting fungi in their functional interaction in the soil mycosphere (the zone surrounding and affected by hyphae). For instance, fungi are unable to fix atmospheric nitrogen and, hence, partly rely on the partnership with nitrogen-fixing bacteria. Here, we hypothesise that nitrogen-fixing bacteria in the mycosphere provide nitrogen to fungi to support growth and potential contaminant degradation in nitrogen-deprived environments. To challenge our assumption, we tested the impact of N-fixing soil bacteria (i.e. polycyclic aromatic hydrocarbon (PAH) degrading Pseudomonas sp SK1, Sphingomonas sp. *LH162,* and non-PAH degrading *Azotobacter vinelandii* B671) on growth of contaminant degrading basidiomycetes *Coprionopsis cinerea* and *Pleurotus ostreatus* using N-free agarose plates and liquid media at ambient air conditions. Relative to bacteria-free controls, bacterial-fungal co-cultures showed significantly increased fungal growth suggesting a fungal uptake and utilization of fixed nitrogen. Using stable isotope probing approaches, current work quantifies dynamic biomass development and concomitant N and C-flows between *Azotobacter vinelandii* B671 and *Coprinopsis cinerea*, and its impact on biodegradation of soil contaminants such as PAH.

OP-MEE-011

Evolution across environmental nutrient concentrations: How microbes evolve and maintain low half-saturation for growth rate

*J. W. Fink[1,2], N. Held[3,4,5], M. Manhart[1,6,2]
[1]*Rutgers University, Center for Advanced Biotechnology and Medicine, Piscataway, NJ, United States*
[2]*ETH Zurich, Institute of Integrative Biology, Zürich, Switzerland*
[3]*University of Southern California, Department of Biological*

Sciences, Los Angeles, CA, United States
[4]Swiss Federal Institute of Aquatic Research, Department of Environmental Microbiology, Dübendorf, Switzerland
[5]ETH Zurich, Institute of Biogeochemistry and Pollutant Dynamics, Zürich, Switzerland
[6]Rutgers University, Department of Biochemistry and Molecular Biology, Piscataway, NJ, United States

Microbes grow faster when they have more of a limiting nutrient. The relationship between nutrient concentration and population growth rate is critical to predict how members of microbial communities respond to environmental perturbations. Models of this response, such as the widely used Monod model, are generally characterized by a maximum growth rate and a half-saturation concentration of the resource. What values should we expect for these half-saturation concentrations, and how should they depend on the resource concentration in nature?

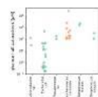
We survey growth response data across a wide range of resources and microbial taxa, including many phytoplankton. We find that the half-saturation concentrations vary across orders of magnitude, even for the same organism and resource. To explain this variation, we develop an evolutionary model to show that demographic fluctuations (genetic drift) can constrain the adaptation of half-saturation concentrations. We find that this effect fundamentally differs depending on the type of population dynamics: Populations undergoing periodic bottlenecks of fixed size will adapt their half-saturation concentrations in proportion to the environmental resource concentrations, but populations undergoing periodic dilutions of fixed size will evolve half-saturation concentrations that are largely decoupled from the environmental concentrations.

Our model provides testable predictions on how the ability for low concentration growth is distributed across microbial habitats and along resource gradients, but it also reveals how an evolved half-saturation concentration may not reflect the organism"s environment. In particular, this explains how taxa in resource-rich environments can still evolve fast growth at low resource concentrations.

Fig. 1

Conclusions from our analysis of Monod growth traits across resources and microbial taxa (new database)

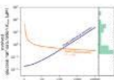
- Microbial species have different ability for low-concentration growth (half-saturation K).



- Environment shapes half-saturation concentration over evolutionary time, but this is **modified by the population size** at bottleneck.

$$K_{\mathrm{evo}} \propto \frac{R_0}{N_e}$$

- The evolved half-saturation concentration is **not a biomarker** for environmental nutrient concentrations.



OP-MEE-012
Evolution on a microscale: Empowering adaptation and selection through a microfluidic chip

*C. Stolle[1], C. M. Niemeyer[1], K. S. Rabe[1]
[1]Karlsruhe Institute for Technology, Institute for Biological Interfaces 1 (IBG 1), Eggenstein-Leopoldshafen, Germany

Introduction

Adaptive laboratory evolution (ALE) is a key tool to investigate fundamental questions about the processes underlying the evolution of life and how microbial populations adapt to their environments, such as the emergence of antibiotic resistance. Microfluidics have fundamentally changed the field of microbial ALE as it can mimic naturally occurring microenvironments promoting the growth of biofilms alongside planktonic cells.[1]

Goals

A microfluidic ALE chip device that features adjustable, spatially defined physico-chemical stressor gradients should be developed employing an in-flow gradient aligned parallel to the flow direction to enhance the robustness of ALE process including the efficient on-chip screening of the complete entire cell population.

Materials & Methods

The evo.S (*e*volution *u*nder *s*tress) microfluidic chip was designed using CAD and fabricated from PDMS (polydimethylsiloxane) and used for the adaptation of microbial cells to stressors in a customizable stressor concentration gradient.[2]

Results

The controlled generation of different gradient profiles across the interconnected compartments leads to adaptation of *Escherichia coli* to the presence of antibiotics and revealed the chip's capacity to differentiate between persistence and resistance. Importantly, this approach was effectively employed for the discovery of previously unknown mutations conferring resistance to nalidixic acid in *E. coli.* Recently, the chip was modified to successfully adapt the thermophilic organism *Thermus thermophilus* to kanamycin.

Summary

The evo.S chip enhances the occurrence of mutations, resulting in the generation of stress-resistant strains. This kind of miniaturized chip-based ALE offers crucial insights into the mechanism of antibiotic resistance and can be applied to adapt virtually any microorganism.

References

[1] Zoheir, A. E., Stolle, C., Rabe, K. S., *Appl. Microbiol. Biotechnol.* 2024, 108, 162.
[2] Zoheir, A. E., Späth, G. P., Niemeyer, C. M., Rabe, K. S., *Small* 2021, 17, 2007166.

OP-MEE-013
Tracking the Dynamics of Interacting Microbial Populations

*J. Ching Kuma Mbanghanih[1], N. Verdon[1], A. Zander[1], M. Mauri[1], S. Vareschi[1], T. Malycheva[2], R. Herbst[3], S. Schuster[2], P. Stallforth[3], R. Allen[1]

[1]Friedrich Schiller University Jena, Institut für Mikrobiologie, Jena, Germany
[2]Friedrich Schiller University Jena, Matthias Schleiden Institute, Jena, Germany
[3]Friedrich Schiller University Jena, Leibniz Institute for Natural Product Research and Infection Biology Knöll Institute, Jena, Germany

Microbes exhibit diverse interactions including predation, symbiosis, and competition. These interactions impact the dynamics of microbial populations and therefore shape ecological outcomes within microbial communities. This study focuses on the interactions between *Dictyostelium discoideum* (Dicty) and bacteria. Dicty is a social amoeba that feeds on soil bacteria by phagocytosis and develops into multicellular aggregates and forms fruiting bodies in the absence of a food source. It has been reported that some Pseudomonads produce toxic compounds that prevent amoebal predation. We hypothesize that the dynamics of these interactions are largely dependent on the population sizes and spatial distribution of the interacting microbes. We seek to understand predatory, foraging, and defence behaviours between Dicty and different bacteria species.

We developed experimental setups that allow microscopic imaging of the interaction between Dicty and bacteria in liquid suspension and on agar. The images are analyzed using ImageJ/Mtrack/Labkit and MATLAB. The results obtained will ultimately be compared with mathematical models.

By combining population dynamics experiments with mathematical models, we intend to provide a better insight into how interactions mediated by natural products can affect the balance of microbial ecosystems. This understanding would help in the development of solutions to ecological, agricultural, clinical, or environmental problems, as well as the advancement of natural product research.

OP-MEE-014
Context dependency of community ecology and keystone species in a synthetic gut bacterial community

*A. S. Weiss[1], L. Niedermeier[2], A. von Strempel[2], A. G. Burrichter[2], C. Meng[3], K. Kleigrewe[3], C. Lincetto[4], J. Hübner[4], B. Stecher[2]
[1]ETH Zürich, Department of Environmental Systems Sciences, Dübendorf, Germany
[2]Ludwig-Maximilians University, Max von Pettenkofer Institute, München, Germany
[3]Technical University of Munich, Bavarian Center for Biomolecular Mass Spectrometry, Freising, Germany
[4]Ludwig-Maximilians University, Division of Paediatric Infectious Diseases, Dr. von Hauner Children's Hospital, München, Germany

Microbe-microbe interactions are critical for gut microbiome function. A challenging task to understand health and disease-related microbiome signatures is to move beyond descriptive community-level profiling towards disentangling microbial interaction networks. Using a synthetic gut bacterial community (OMM^{12}), we aimed to study the role of individual members in community assembly, identify putative keystone species and test their influence across different environments. Single-species dropout experiments revealed that bacterial strain relationships strongly vary not only in different regions of the murine gut, but also across several standard culture media. Mechanisms involved in environment-dependent keystone functions in vitro included exclusive access to polysaccharides as well as bacteriocin production. Further, *Bacteroides caecimuris* and *Blautia coccoides* were found to play keystone roles in gnotobiotic mice by impacting community composition, the metabolic landscape and inflammatory responses. In summary, this work highlights the strong interdependency between bacterial community ecology and the biotic and abiotic environment. Our results question the concept of universal keystone species in the gastrointestinal ecosystem and underline the context-dependency of both, keystone functions and bacterial interaction networks.

OP-MEE-015
Evolution of flagellar assembly regulation in host-associated bacteria: a case study on plant commensal Acidovorax delafieldii isolates

*R. Siani[1], Y. Si[2], S. K. Thaqi[1], G. Stabl[2], C. Gutjahr[2], M. Schloter[1,3]
[1]Technical University of Munich, Chair of Environmental Microbiology, Neuherberg, Germany
[2]Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany
[3]Helmholtz Center Munich, Comparative Microbiome Analysis, München, Germany

In host-associated bacteria, benefits of traits common in their free-living counterparts, such as flagellar motility, can be offset by energetic costs and the risk of triggering immune responses. While the conserved and immunogenic flagellin gene is often lost, we expect other genes of the flagellar assembly pathway to undergo diversifying selection, in particular the master regulator flhDC. FlhDC has been shown to integrate environmental signals and regulate processes other than flagellar assembly, including virulence of pathogenic bacteria. We were also interested in how loss of flhC would affect the transcriptional landscape of two closely related flhC+ and flhC- Acidovorax delafieldii strains, isolated from Lotus japonicus.

Firstly, we mined 1839 reference Proteobacteria genomes for elements of the flagellar assembly pathway using hidden Markov models. Quantity and quality of each sequence were analyzed in a phylogenetic framework, comparing statistics across environmental and host-associated strains. We constructed knock-out and revertant mutants for the flhC+ isolate and tested their motility. We cultivated the subjects in media spiked with crude extracts from Lotus japonicus, axenic or infected by Rhizophagus irregularis, an arbuscular mycorrhizal fungus. After sequencing the transcriptome, we analyzed differential expression and regulation of ecologically relevant genes and studied differences in transcriptional networks topology.

We detected lower prevalence, number of copies and scores for most flagellar assembly genes of host-associated strains. However, flhDC shows similar prevalence in both groups, but lower scores in host-associated strains. This suggests that mutation might be more beneficial than loss. As a case study, flhC- co-expression networks show widespread deregulation, with effects on several plant-associated traits and an altered transcriptional balance between type II and VI secretion systems.

Our study provides insights into evolutionary dynamics of the flagella and examines a loss of regulation scenario, showing how effects on the phenotype can extend beyond motility, indirectly affecting host-microbe-microbe relationships.

OP-MEE-016
Influence of nutrient richness on Escherichia coli growth dynamics, cellular stress response and denovo emergence of resistance to rifampicin.

A. Kumar Shaw[1], L. M. Karnbach[1], A. Zander[1], M. L. Enghardt[1], M. Mauri[1,2], S. Tavaddod[3], *V. Srinivasan[1,2], R. Allen[1,4,2]
[1]*Friedrich Schiller University Jena, Department of Biological Sciences, Jena, Germany*
[2]*Friedrich Schiller University Jena, Cluster of Excellence Balance of the Microverse, Jena, Germany*
[3]*Leibniz Institute of Photonic Technology, Jena, Germany*
[4]*University of Edinburgh, School of Physics and Astronomy, Edinburgh, United Kingdom*

Introduction: Emergence of antibiotic resistance is a major threat to global health, limiting the available options for treating infectious diseases. External factors such as nutrient richness or chemical composition of the media can influence the antibiotic stress response, survival, cellular adaptations, population dynamics and adaptive evolution of antibiotic tolerance and resistance in bacteria.

Goals: To investigate the influence of nutrient richness on *Escherichia coli* (*E. coli*) population growth dynamics and cellular stress response to rifampicin. Probability estimation and genetic analysis of the adaptive evolution of rifampicin resistance in *E. coli*.

Materials and methods: We investigated *E. coli* (BW25113) rifampicin susceptibility under nutrient rich and poor conditions (Neidhardt's MOPS rich or minimal medium, with added glucose) by quantitatively determining the population growth dynamics, frequency of pre-existing rifampicin resistance and *denovo* emergence of resistance, live cell microscopy and genetic sequencing of ancestral and evolved strains.

Results: Our investigation revealed contrasting growth dynamics on rich and poor media near the minimum inhibitory concentration of rifampicin. Under the nutrient poor condition, rifampicin led to reduced growth rate, whereas in the nutrient rich condition an extended lag phase was observed followed by stochastic exit from the lag phase, which also corresponded to the emergence of phenotypic rifampicin resistance. Further investigation revealed that the resistance emerged *denovo* under rifampicin treatment in the nutrient rich condition. We further investigated the cellular heterogeneity and genetic variations in the rifampicin resistance determining region of *rpoB* in the *denovo* rifampicin resistant strains.

Conclusions: Nutrient richness resulted in increased rifampicin extended lag phase, cellular heterogeneity and *denovo*emergence of rifampicin resistance in *E. coli*.

OP-MEE-017
The Hidden Virosphere likely drives the microbial community and their functions in the pristine groundwater

*A. A. Pratama[1,2], O. Pérez-Carrascal[3,4], M. B. Sullivan[5,1,2,6], K. Küsel[3,4]
[1]*Center of Microbiome Science, The Ohio State University, Columbus, OH, United States*
[2]*National Science Foundation EMERGE Biology Integration Institute, Columbus, OH, United States*
[3]*The Friedrich Schiller University Jena, Aquatic Geomicrobiology, Jena, Germany*
[4]*Cluster of Excellence Balance of the Microverse, Jena, Germany*
[5]*The Ohio State University, Microbiology, Columbus, OH, United States*
[6]*The Ohio State University, Department of Civil, Environmental and Geodetic Engineering, Columbus, OH, United States*

The ocean contains 1010 virus-like particles (VLP) per liter, vastly outnumbering host cells. Viruses significantly affect nutrient cycling by lysing 20-40% of bacteria daily, reprogramming host cells and promoting horizontal gene transfer (1029 genes/day). Playing a vital role in carbon fluxes, including carbon transport to the deep sea, contributing to marine ecosystem stability amid climate change. Pristine groundwater is a vital drinking water source, yet we lack an understanding of its viral ecosystem. We examine virus diversity, ecological importance, and functional interactions in a two-year study of seven wells within a pristine groundwater system along a hillslope transect. From ~1.3 terabases of metagenomics data, we identified >7 million virus contigs, resulting in 257,814 and 82,807 virus operational taxonomic units (vOTUs) of ≥5 kb or ≥10 kb, respectively, representing a ~22-fold increase compared to publicly available groundwater vOTUs (n=3,584, ≥10 kb). Taxonomic analysis revealed that 99% of Hainich groundwater viruses were species-level unique, even when compared to a global ocean dataset. Approximately 81% could be taxonomically classified, with 99% belonging to the Caudoviricetes class (tailed phages). Ecological analysis using read mapping demonstrated site-specific endemism in virus communities, evidenced by strong grouping based on the sampled groundwater wells (p-value <0.001). Our host prediction analysis found that 88% of viruses infect 78% of microbes (1,275 MAGs) from the same sample, including ecologically important groups like Pastecibacteria (81%) and Proteobacteria (74%). Additionally, about 5% of viruses may reprogram ~38% of host pathways through auxiliary metabolic genes (5,883 AMGs found, 24 times more than the GOV v.1). For instance, GAPDH (K00134), which breaks down glucose for energy and carbon. Furthermore, additionally Nitrite Reductase (K15876) and Sulfate Adenylyltransferase (K00957) help groundwater microbes thrive in oxygen-depleted conditions. Overall, this research offers valuable insights into groundwater virus communities and the mitigation of human impacts on groundwater resources.

Molecular Infection Epidemiology and Prediction of Antimicrobial Resistance

OP-MIPA-001
HyDRA - Identifying recently acquired antibiotic resistance genes using machine learning

*J. Welling[1], S. Imangaliyev[1], S. Magin[1], J. Kehrmann[2], F. Meyer[1], I. Kraiselburd[1]
[1]*University Hospital Essen, Institute for Artificial Intelligence in Medicine, Essen, Germany*
[2]*University Hospital Essen, Institute for Medical Microbiology, Essen, Germany*

The spread of antibiotic resistance poses a significant threat to humanity [1]. Rapid and accurate detection of antibiotic resistance is required to determine an appropriate antibiotic therapy. Current methods are solely based on cultivation and therefore only test a limited number of antibiotics.

Our aim is to produce a detailed resistogram that will support the physicians to find a suitable treatment and render therapy more effective.

For this purpose we are developing a fully automated Snakemake [2] workflow for reproducible genome analysis, called HyDRA - Hybrid De novo assembly for Resistance Analysis. This analysis is based on a combination of short and long reads generated by Illumina and Oxford Nanopore sequencing of bacterial isolates. These reads are used for a hybrid genome assembly, which then is screened for known antibiotic resistance genes (ARGs) using a well curated ARG specific database. In order to additionally identify newly acquired and hitherto unknown ARGs we are currently working on a machine learning classifier to reliably detect recently horizontally transferred genes.

The best classifier so far was trained on E. coli K12 genome data from HGT-DB [3] and achieved credible results for different E. coli test data. However, this model does not work well for other tested organisms (see Fig.1). We are working on improving this classifier and creating more classifiers specialized on other bacteria. Furthermore, we are extending the workflow to metagenomics sequencing data to work directly with patient samples and skip the time-consuming cultivation.

Conclusively, this workflow will rapidly provide the physicians detailed information to select an appropriate antibiotic treatment. This should prevent the unnecessary use of broad-spectrum antibiotics and thus help fight the emergence of antibiotic resistance.

[1] ECDC "Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual epidemiological report for 2022", 2023
[2] J. Koster and S. Rahmann, Bioinformatics, 2012, doi: 10.1093/bioinformatics/bts480
[3] S. Garcia-Vallve et al., Nucleic Acids Res, 2003, doi: 10.1093/nar/gkg004

Fig. 1

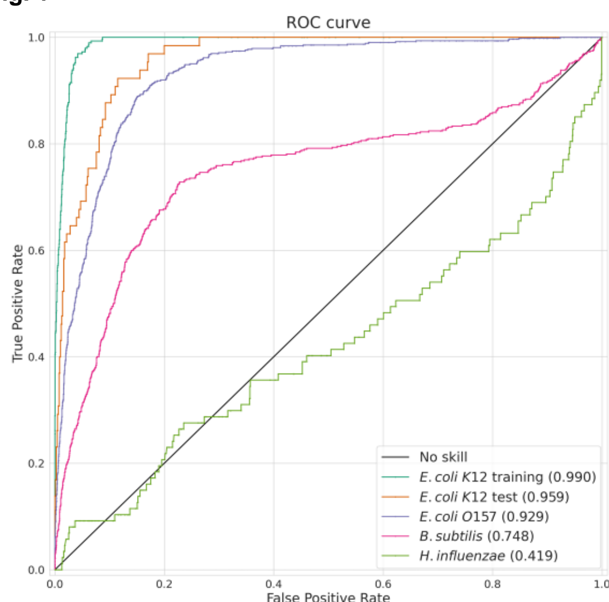


Fig. 1. Receiver Operating Characteristic (ROC) curves of the prediction results for four different genomes. Model evaluation based on ROC AUC (Area Under Curve) values, see brackets.

OP-MIPA-002

Phylogenomics and genome-wide profiling to revisit mutational resistance to ampicillin and cefotaxime in Haemophilus influenzae

M. Diricks[1], *S. Petersen[1], L. Bartels[1], T. T. Lam[2], H. Claus[2], M. P. Bajanca-Lavado[3], S. Hauswaldt[4], R. Stolze[1], O. J. Vázquez[1], C. Utpatel[1], S. Niemann[1], J. Rupp[4], I. Wohlers[1], M. Merker[1]
[1]*Research Center Borstel, Borstel, Germany*
[2]*Institute for Hygiene and Microbiology, Würzburg, Germany*
[3]*Haemophilus influenzae Reference Laboratory, Lisbon, Portugal*
[4]*University of Lübeck, Lübeck, Germany*

Background:

Haemophilus influenzae, an opportunistic bacterial pathogen, can lead to severe respiratory tract infections, septicemia, and meningitis. The emergence of β-lactamase-negative ampicillin-resistant (BLNAR) strains and difficulties in correlating genotypic (gBLNAR) and phenotypic resistance complicate empirical treatment and patient management.

Methods:

We conducted a systematic meta-analysis of previously identified *H. influenzae* gBLNAR groups characterized by distinct substitution patterns in penicillin-binding protein 3 (PBP3), and analyzed their associations with ampicillin and cefotaxime resistance. In addition, we used phylogenomics and a genome-wide association study (GWAS) to identify novel mutations implicated in resistance in a public global cohort (n=555), and a new clinical cohort from three European centers (n=322), respectively.

Results:

Isolates characterized as gBLNAR groups II and III were associated with ampicillin resistance ($p<0.03$), while only group III+/III-like+ isolates were associated with cefotaxime resistance ($p<0.03$). However, group II isolates had low specificities (≤ 71%) to rule in resistance against ampicillin, challenging the currently endorsed clinical breakpoint for its ability to distinguish between gBLNAR and susceptible wildtype isolates. Known and newly identified PBP3 substitutions often occur step-wise and show patterns of positive selection, and convergent evolution in the two independent cohorts. Furthermore, we discovered new genes and novel combinations of PBP3 substitutions associated with ampicillin resistance.

Conclusion:

This study reveals new insights on molecular ampicillin resistance determinants in β-lactamase-negative *H. influenzae* isolates. We emphasize an artifact with the currently applied clinical breakpoints for ampicillin. Further investigations, including treatment outcome data, are crucial to understand the impact of bacterial genome variations on susceptibility to cefotaxime and other cephalosporins.

OP-MIPA-003

A molecular cluster of multidrug-resistant Mycobacterium tuberculosis strains among patients arriving in Germany from the Ukraine

*V. Mohr[1,2], L. Bös[3], S. Andres[2], T. Kohl[1,2], M. Kuhns[2], W. Haas[3], I. Friesen[2], S. Kröger[3], S. Niemann[1,2,4]

[1]*Research Center Borstel, Molecular and Experimental Mycobacteriology, Borstel, Germany*
[2]*Research Center Borstel, National Tuberculosis Reference Laboratory, Borstel, Germany*
[3]*Robert Koch Institute, Respiratory Infections Unit, Dpt. of Infectious Disease Epidemiology, Berlin, Germany*
[4]*German Center for Infection Research, Partner site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany*

Introduction

Emerging multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) create significant challenges for TB control, particularly in Eastern European countries such as the Ukraine. International spread of highly resistant *Mycobacterium tuberculosis* complex (Mtbc) strains potentially impacts the MDR TB epidemiology in low incidence countries in Europe.

Materials & Method

Since 2020, WGS of Mtbc strains was carried out as part of the integrated molecular surveillance of TB (IMS-TB) by the Robert Koch Institute (RKI) and the National Reference Center (NRC) for Mycobacteria. Including retrospective data (years 1993 – 2019), whole genome sequencing (WGS) data of more than 11,000 Mtbc strains from Germany, containing more than 5,000 datasets from 2020 to 2023, are available at the NRC. Genome sequencing data were used for phylogenetic strain classifications, genomic resistance predictions based on the extended WHO catalogue (2021) and allele-based core genome cluster analysis using a threshold of 12 Alleles.

Results

Among the dataset, 67 rifampicin resistant (RR) lineage 2 (Beijing) Mtbc strains were identified between 2000 and 2023 (analyses for 2023 ongoing) based on their particular mutation profile (e.g. *embB* Y334H mutation) that also show a close genomic relationship with a pairwise distance of 16. Since the beginning of the war in Ukraine, 13 isolates were detected in 2022 and 9 in 2023. Out of all isolates, 35 were RR/MDR, 28 pre-XDR (MDR plus fluoroquinolone resistance), and four XDR. Cluster analysis with a threshold of 5 alleles revealed substructures with 7 clusters ranging in size from 2 to 22 isolates. Similarity analysis linked all isolates to a previously described MDR strain detected in Ukraine. Of the 22 isolates from 2022 and 2023, 16 cases were born in Ukraine, while 6 were born in other countries outside of Germany.

Summary

Our data suggest that the emergence of the described highly resistant MDR Mtbc isolates in Germany is linked to migration from Ukraine. Prospective surveillance is crucial to monitor potential long-term impacts on the MDR TB epidemiology in Germany.

OP-MIPA-004
Deciphering the secondary resistome of Methicillin-Resistant Staphylococcus aureus (MRSA) to Beta-lactams - a Multi-Omics Approach
*N. Abdelmalek[1], S. Waheed Yousief[1], A. Tanca[1], J. Elmerdahl Olsen[1,2], B. Paglietti[1]
[1]*University of Sassari, Department of Biomedical Sciences, Sassari, Italy*
[2]*University of Copenhagen, Department of Veterinary and Animal Sciences, Copenhagen, Denmark*

Introduction: Antimicrobial resistance (AMR) is a significant global health issue, impacting both human and livestock populations. Methicillin-resistant Staphylococcus aureus (MRSA), a common multidrug-resistant bacterium, has been identified as a high-priority pathogen for the development of new antibiotics. The approach of restoring susceptibility to existing antimicrobials presents a promising strategy in the battle against AMR, as the full expression of resistance relies on auxiliary enzymes.

Goal: This study aims to identify the auxiliary genes and proteins involved in the resistance of MRSA to oxacillin, which could potentially serve as targets for re-establishing susceptibility.

Materials and Methods: Using Transposon-Directed Insertion site Sequencing (TraDIS), we screened a large MRSA transposon mutant library post-exposure to subinhibitory concentrations of oxacillin and cefazolin. The output reads were analyzed via the BioTradis pipeline. Subsequently, label-free quantitative proteomics was performed on the antibiotic-exposed cultures, the mass spectrometer data was processed using Proteome Discoverer, and a comprehensive statistical analysis was conducted with Perseus v1.6.

Results: Our high-throughput transposon mutagenesis assay identified a set of conditionally essential genes potentially contributing to beta-lactam resistance. Subsequently, Post-antimicrobial exposure proteomics analysis highlighted significant alterations in protein expression, notably within the methionine and D-alanylation pathways of wall teichoic acid, which are linked to peptidoglycan integrity. Our findings underscore a limited overlap between TraDIS and proteomics results.

Conclusion: Our investigation sheds light on the identification of auxiliary genes and proteins linked to beta-lactam resistance in MRSA. By merging large-scale transposon mutagenesis and proteomics, we provide valuable data about the secondary resistome and potential targets for tackling antimicrobial resistance. These insights set the stage for devising novel strategies to reinstate MRSA"s susceptibility to existing antimicrobials, addressing the pressing global health issue of AMR.

OP-MIPA-005
ST1299/vanA is the new kid on the block! Analysis of the explosive regional expansion of a new strain of vancomycin-resistant Enterococcus faecium
*A. Rath[1], B. Kieninger[1], N. Mirzaliyeva[1], G. Werner[2], J. Bender[2], M. Fischer[2], A. Cabal-Rosel[3], W. Ruppitsch[4], M. Halabi[5], A. Hörtenhuber[6], W. Prammer[6], Y. Salaheddin[6], H. Kerschner[7], R. Hartl[7], M. Ehrenschwender[8], A. Ambrosch[8], H. Seth-Smith[9], L. J. Klages[10], A. Caplunik-Pratsch[1], A. Eichner[1], J. Fritsch[1], W. Schneider-Brachert[1]
[1]*University Hospital Regensburg, Department of Infection Prevention and Infectious Diseases, Regensburg, Germany*
[2]*Robert Koch Institute, 2Department of Infectious Diseases, Division of Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany*
[3]*AGES - Austrian Agency for Health and Food Safety, Division for Public Health, Wien, Austria*
[4]*AGES - Austrian Agency for Health and Food SafetyInstitute for Medical Microbiology & Hygiene,, Division for Public Health, Graz,*

Austria
[5]Barmherzigen Schwestern Ried Hospital, Institute for Clinical Pathology, Microbiology and Molecular Diagnostics, Ried, Austria
[6]Pyhrn-Eisenwurzen Clinical Centre Kirchdorf Steyr, Institute of Pathology, Upper Austrian Health Holding GmbH, Steyr, Austria
[7]National Reference Center for Antimicrobial Resistance, Institute for Hygiene, Microbiology and Tropical Medicine, Ordensklinikum Linz Elisabethinen, Linz, Austria
[8]Hospital of the Merciful Brothers, Institute of Laboratory Medicine, Microbiology and Infection Prevention, Regensburg, Germany
[9]University of Zurich, Institute for Medical Microbiology, Zürich, Switzerland
[10]Bielefeld University, Center for Biotechnology (CeBiTec), Bielefeld, Germany

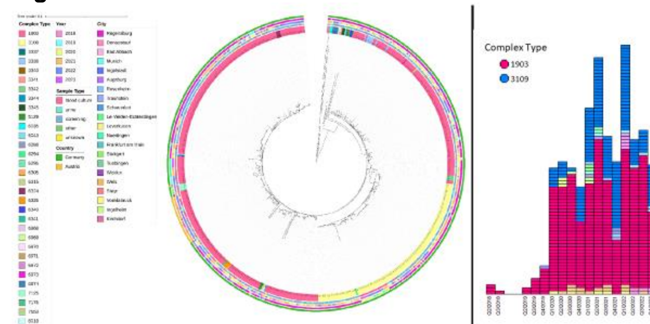
Introduction: Understanding dynamics and spreading patterns of novel vancomycin-resistant enterococci (VRE) strains could be the key to stopping their worldwide spread. The occurrence and explosive expansion of the novel strain ST1299 in Germany and Upper Austria gives us the opportunity to gain a more detailed insight.

Methods: The surveillance at a tertiary care hospital in southern Germany includes identification by mass spectrometry and vanA/B-PCR, then whole-genome sequencing (WGS) of at least one VRE isolate per *van*-genotype, patient and year since 2004. Isolates were identified by database inquiry for "ST1299", followed by selection of one isolate per patient and year. Further ST1299 isolate sources are 1) a further tertiary care hospital in Regensburg, 2) the National Reference Center for Enterococci, and 2.) outbreak isolates from Upper Austria.

Results: From 2018 - 2022, 622 VRE isolates were assigned to ST1299 with 100% *vanA*-genotype (488 Regensburg County, 98 Germany other, 34 Upper Austria). In Regensburg most isolates were detected in rectal swabs (58.4%), urine (24.0%) or blood cultures (5.3%). The first four isolates were detected in 2018, but explosive expansion started April 2019 substantiated by an outbreak in a COVID-19 quarantine department. Thereafter spread was accelerated by several outbreaks throughout the hospital. ST1299 numbers culminated in January-March 2022 (overall 79 isolates), whereas the most common complex type (CT) was ST1299/CT1903 (67.3%). This strain also reached Upper Austria by December 2021, where it caused four hospital outbreaks with up to 25 patients. ST1299/CT3109, the second most common CT (24.0%), was only detected starting 2020, but had a similarly dynamic expansion and even surpassed ST1299/CT1903 in Regensburg during Q1/2022. Overall, only 54 isolates were assigned to other CTs. Currently, further data acquisition on strain dynamics and clinical outbreaks is still in progress.

Conclusion: Since its first appearance in 2018, the strain ST1299 has proven highly transmissible, and has managed to expand throughout southeastern Germany and Upper Austria.

Fig. 1



OP-MIPA-006
How to define an infection cluster? - cluster dynamics of *S.* Typhimurium in Germany.

*M. Pietsch[1], S. Simon[1], E. Trost[1], A. Meinen[2], L. Giese[2], M. C. Lamparter[3], J. Fischer[3], A. Flieger[1]
[1]Robert Koch Institute, Unit for Enteropathogenic Bacteria and Legionella (FG11) and National Reference Centre (NRC) for Salmonella and other bacterial enteric pathogens, Wernigerode, Germany
[2]Robert Koch Institute, Unit for Gastrointestinal Infections, Zoonoses and Tropical Infections (FG35), Berlin, Germany
[3]German Federal Institute for Risk Assessment, Department Biological Safety and National Reference Laboratory for Salmonella (NRL), Berlin, Germany

Introduction: In Germany, salmonellosis is the second most frequently reported bacterial diarrheal disease and the zoonotic *Salmonella* cause many regional and multinational food-borne outbreaks. Among the reported *Salmonella* serovars in Germany, *S.* Typhimurium is the 2nd most common, responsible for 20-30% of cases each year and therefore has become one of the prioritized organisms for a comprehensive genome-based surveillance.

Goals: In this study we analyzed *S.* Typhimurium infection clusters epidemiologically and technically, in order to asses different cluster types, cluster plausibility of an epidemiological link, cross-sectoral relevance, and best suitable thresholds for cluster definition.

Methods: 2020-2023, the NRC for *Salmonella* received 14,252 unique clinical *Salmonella* isolates, of which 4,111 were *S.* Typhimurium (28.8% of all received isolates). Bioinformatic analysis of raw sequencing data and subsequent cgMLST (3.002 alleles) and cluster analysis were performed using Ridom SeqSphere$^+$. *Salmonella* isolates of animal or food origin, matching clinical *Salmonella* clusters, were obtained from the NRL for *Salmonella* at the German Federal Institute for Risk Assessment.

Results: From 2020-2023, a total of 3,320 *S.* Typhimurium isolates were analyzed, covering 61% of received isolates in 2020 up to 97.5% in 2023. 1,205 isolates were attributed into curated clusters, resulting in 81 clusters of variable size (4-97 isolates per cluster). Plausibility of an epidemiological link has been assessed for these clusters. Matching with isolates of non-human origin was possible in many cases. However, automatically assignment of clusters using variable threshold settings led to expanded and deviating clusters, highlighting the need for carefully selected and constantly reviewed cluster definitions.

Summary: Intensified genome-based surveillance of *S.* Typhimurium led to an increase in cluster observations and

cluster size. The number of clusters, their growth kinetics and regional distribution varied between different years of analysis and depended on the ratio of sequenced/received isolates and are strongly influenced by cluster definitions and detection methods.

Microbial Metabolism & Biochemistry

OP-MMB-001
Microbial Sulfur and Persulfide Oxygenases

*A. Kletzin[1], P. Rühl[2], C. Frazão[3]
[1]*Technische Universität Darmstadt, Mikrobiologie, Darmstadt, Germany*
[2]*Paul-Ehrlich-Institute, Langen, Germany*
[3]*ITQB, Oeiras, Portugal*

Introduction: Iron-containing persulfide dioxygenases (PDO) catalyze the oxidation of glutathione persulfide (GSSH) to sulfite and GSH. They are abundant in Bacteria and mitochondria.Sulfur oxygenase reductases (SOR) catalyze an O2-dependent elemental sulfur (S^0) dismutation to sulfite and H_2S. They occur in chemolithotrophic sulfur oxidizers and are structurally unrelated to PDOs. Both enzymes contain mononuclear non-heme Fe active sites, each with two His and one carboxylate ligands. Here, we compare structural and spectroscopic properties of the *Ac*PDO from *Acidithiobacillus caldus* and three different SORs from Bacteria and Archaea in order to better understand the reaction mechanisms.

Results: GSSH binds to the PDO active site in a cleft at the enzyme's surface with the iron at its bottom. Incubation with GSSH resulted in the formation of S-Fe charge transfer absorption spectra; which was not observed in the SORs. Modeling of GSSH binding to the *Ac*PDO crystal structure suggested that a transient persulfide-peroxo intermediate is formed, which is resolved by proton shuffling with a second coordination sphere His. Cysteines do not participate in catalysis, a disulfide present at the *Ac*PDO surface stabilizes its C-terminus.

In contrast, SORs have a spacious and deeply embedded active site cavity with a narrow entrance pore comprising 3 conserved cysteines. X-ray crystallography of SORs showed that each of the cysteines could be persulfurated and that SOR mutants can become stuck in that state. Free and/or cysteine-bound polysulfide chains seem to be binding to Fe with the terminal S atom. WE suggest that sulfane S dismutation occurs, when the Fe-bound peroxo oxygen attacks the penultimate sulfane S atom of the chain, thus splitting off the terminal sulfide, while the oxidation steps proceeds in a similar way as in PDO.

Conclusion: The results suggest that the glutathione moiety positions the persulfide for terminal sulfane sulfur oxidation at the open PDO active site, whereas the closed cavity of the SORs is required to keep the more mobile free persulfides trapped in the vicinity of the Fe atom in order to get S^0 dismutation to work.

OP-MMB-002
Investigating the regulation of the carbon switch in central metabolism of Synechocystis sp. PCC 6803

*R. S. Ojha[1], L. Shen[1], C. Peraglie[1], C. Bräsen[1], B. Siebers[1]
[1]*University of Duisburg-Essen, Molecular Enzymetechnology and Biochemistry, Essen, Germany*

Dependent on the light/nutrient conditions *Synechocystis* is able to grow auto-, hetero-, and mixotrophically for which the reversibility of sugar metabolism particularly the Embden-Meyerhof-Parnas pathway (EMP) is essential. To switch between anabolic and catabolic direction sophisticated regulation is required and also the interplay of e.g. EMP and Calvin cycle for CO_2 fixation must be tightly balanced. The antagonistic PFK and FBPase operate exclusively in the catabolic or anabolic direction of the EMP, respectively, and PFK is the classical control point of glycolysis. Two paralogous copies of both enzymes are present in *Synechocystis.* Furthermore, the reversible phosphoglucose isomerase (PGI) has been discussed to direct fluxes between EMP and CBB under different growth conditions. However, the control points and the function and regulatory capacities of the enzymes are not established in *Synechocystis.*

Herein, the regulatory functions of the PFK and FBPase isoenzymes as well as the PGI were analysed. *Pfk-A1*, *Pfk-A2*, *Fbpase1* and *Pgi* from *Synechocystis* were overproduced in *E. coli,* purified and characterized with respect to substrate specificity, kinetic and regulatory properties.

Both PFKs are ADP dependent, thereby defining a new class of ADP-dependent PFK-A superfamily. PFK-A1 is inhibited by 3-phosphoglycerate (3PG), and PFK2 by ATP. FBPase1 is not regulated by various effectors. The PGI displays a preference for the gluconeogenic direction and is inhibited by erythrose 4-phosphate (E4P).

Our investigations reveal that both PFKs are active under conditions of low energy-charge, facilitating the breakdown of glycogen/D-glucose. The inhibition of PFK-A1 and PFK-A2 by 3PG and ATP, respectively, enables rapid generation of CO_2 acceptor molecule. The sustained FBPase1 activity in presence of various effectors suggests its housekeeping role to enable reversibility of the EMP pathway in response to substrate availability/growth conditions. E4P enables PGI function as a valve to balance the non-oxidative pentose phosphate pathway intermediates and glycogen synthesis.

1. Feng *et al.*, FEBS J (2014) 281.3: 916-926
2. Schulze *et al.*, Microb Cell Fact (2022); 21:699

OP-MMB-003
A hexameric NADP+-reducing hydrogenase from Moorella thermoacetica: First insights into a unique hydrogenase

*F. Rosenbaum[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: *Moorella thermoacetica* reduces CO_2 with H_2 as reductant *via* the Wood-Ljungdahl pathway (WLP). The enzymes of the WLP in *M. thermoacetica* require NADH, NADPH and reduced ferredoxin as reductant. Whereas an electron-bifurcating, ferredoxin- and NAD^+-reducing hydrogenase HydABC had been described, the enzyme that reduces $NADP^+$ remained to be identified. A likely candidate is the HydABCDEF hydrogenase encoded by *M. thermoacetica.*

Goals: In order to clarify the electric connectivity between the oxidative and reductive branches in acetogenesis in *M. thermoacetica*, we attempted to isolate the $NADP^+$-reducing hydrogenase. Since cells grow poorly on H_2 + CO_2 and the activity was not present in glucose-grown cells we first identified conditions under which the enzyme was produced and cell mass could be obtained easily.

Materials & Methods: Growth experiments, enzyme purification, enzyme assays, genome-wide expression analyses and bioinformatic analysis were performed.

Results: Cell-free extract of H_2 + CO_2-grown cells catalyzed H_2-dependent $NADP^+$ reduction whereas this activity was not observed in glucose-grown cells. Cells grown on glucose + DMSO also produced the $NADP^+$-reducing hydrogenase. We have purified the hydrogenase to apparent homogeneity from cells grown on glucose + DMSO. The enzyme had six subunits encoded by *hydABCDEF,* contained 58 mol iron and one mol FMN. The enzyme reduced methyl viologen with H_2 as reductant and of the physiological acceptors tested only $NADP^+$ was reduced. Electron bifurcation to pyridine nucleotides <u>and</u> ferredoxin was not observed. Hydrogen-dependent $NADP^+$ reduction was optimal at pH 8 and 60°C; the specific activity was 8.5 U/mg and the K_m for $NADP^+$ was 0.086 mM.

Summary:

The $NADP^+$-dependent hydrogenase HydABCDEF from *M. thermoacetica* has been purified and characterized. Despite its similarity to the electron-bifurcating hydrogenase HydABC, the HydABCDEF hydrogenase did not bifurcate electrons and $NADP^+$ is the only physiological electron acceptor. The enzyme provides the WLP with NADPH.

OP-MMB-004

Exploring the genomic landscape: identification of aminoglycoside fitness-genes in resistant Escherichia coli

*S. M. Wellner[1], M. S. A. Alobaidallah[2,3], X. Fei[1], A. Herrero-Fresno[4], J. E. Olsen[1]

[1]*University of Copenhagen, Veterinary and Animal Sciences, Frederiksberg, Denmark*

[2]*King Saud bin Abdulaziz University for Health Sciences, Clinical Laboratory Sciences, Jeddah, Saudi Arabia*

[3]*King Abdullah International Medical Research Center, Jeddah, Saudi Arabia*

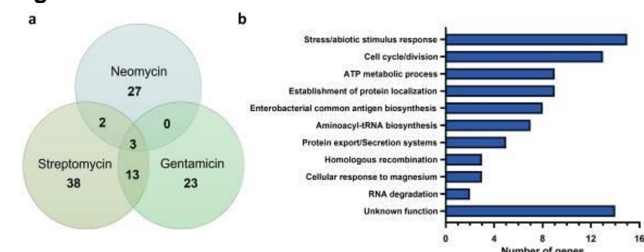
[4]*Universidade da Santiago de Compostela, Biochemistry and Molecular Biology, Lugo, Spain*

Aminoglycosides resistance is common worldwide in pathogenic bacteria, and helper-drugs are urgently needed for this class of antimicrobials. The objective of this study was to identify genes that are advantageous for growth of *E. coli* in the presence of aminoglycosides, as these genes may represent targets for helper-drugs. We generated three highly saturated transposon mutant libraries in *E. coli* MG1655 containing streptomycin (*aph(3"")-Ib/aph(6)-Id*), gentamicin (*aac(3)-IV*), or neomycin (*aph(3"")-Ia*) resistance gene(s). Transposon Directed Insertion-site Sequencing (TraDIS) revealed 106 genes, where transposon insertions caused reduced fitness in the presence of one or more of the antibiotics. Fifty-six, 39 and 32 genes were identified as fitness-genes under streptomycin, gentamicin and neomycin stress (Fig. 1 A). ATP-synthesis, cell division, and stress response genes dominated among streptomycin and gentamicin fitness-genes, while the neomycin fitness-genes were associated with enterobacterial common antigen biosynthesis or magnesium sensing/transport (Fig 1 B). To confirm the importance of the fitness-genes, the genes/gene clusters *minCDE*, *hflCK*, *clsA* and *cpxR* were deleted among the streptomycin and gentamicin fitness-genes, and *phoPQ*, *wecA*, *lpp* and *pal* among the neomycin resistance ones. Phenotypic characterization including growth curves, MIC testing and a novel sequencing-based competition approach revealed that all deletion mutants exhibited an attenuated growth in the presence of the corresponding aminoglycoside(s). In summary, we report a set of aminoglycoside fitness-genes which may be putative targets for helper-drugs. Moreover, our study has expanded our understanding of changes that occur in aminoglycoside-resistant *E. coli* in response to antibiotic exposure.

Figure 1: Aminoglycoside fitness-genes (**a**) and their functional classification (**b**) in *E. coli* MG1655 in the presence of STREP, GEN or NEO.

This INNOTARGETS project has received funding from the European Union"s Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement number 956154.

Fig. 1



OP-MMB-005

In situ electrophysiological profiling of Shewanella oneidensis MR-1 in a novel single-chamber microfluidic BES featuring a transparent gas diffusion anode

*Z. Saghir[1], L. Cristiani[1], J. O. Gath[1], F. Kemper[2], S. Schwinde[2], M. A. Rosenbaum[1]

[1]*Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e. V. Hans-Knöll-Institut, Bio pilot plant, Jena, Germany*

[2]*Fraunhofer Institute for Applied Optics and Precision Engineering IOF, Jena, Germany*

Oxygen plays a crucial role in the operation of bioelectrochemical systems (BESs). Not all microorganisms and bioprocesses thrive in oxygen-rich environments; however, it is crucial for many in supporting their growth and metabolism. Oxygen also competes with an anode as an electron acceptor in certain systems[1]. In conventional BES setups, spatial segregation of planktonic and biofilm forming communities occur when oxygen is supplied via headspace or sparging, complicating the physiological investigations. Moreover, visualizing electroactive biofilms in BES *in vivo* is challenging, as microbial characterization typically occurs post-experiment.

This study aimed at development of a microfluidic BES offering the unique advantage of real time biofilm growth visualization, even while dynamically switching between oxygen concentration (composition ranging from 0.1% to 21% v/v) and an anode as electron acceptors. This was achieved by a channel-type microfluidic gas diffusion layer, separated from the BES anode by a transparent

Polydimethylsiloxane (PDMS) membrane, enabling controlled aeration (or anaerobic) conditions at the BES anode. To verify the accurate gas dosing function of the PDMS membrane and validate the overall infrastructure in the Micro-BES, we investigated the aerobic and anodic metabolism of the model electroactive microorganism *Shewanella oneidensis* MR-1 in the Micro-BES. An electrochemical, secondary metabolite and mediator analysis was performed. Additionally, a fluorescently labelled ATP reporter[2] strain of *S. oneidensis* was used to record different growth phases of the electroactive biofilm.

The successful construction of a 0.3 ml BES was presented, allowing for online imaging under diverse operational conditions, including variations in oxygen concentration, working electrode potentials, electrode configurations and mediator concentrations. This comprehensive approach facilitated a thorough functional validation of the system and gave new options for physiological insight into the model electroactive bacterium *S. oneidensis*.

1. Lu, M., et al. (2017). Biotechnology and bioengineering **114**(1): 96-105
2. Deng, Y., et al. (2021). BMC biology **19**(1): 101

OP-MMB-006

Exploring the physiological and metabolic effects of rpsL mutations in Streptomyces albidoflavus J1074

*V. M. Tseduliak[1], O. Koshla[1], B. Ostash[1], A. Luzhetskyy[2], S. Matsumoto[3], Y. Ohtsubo[3], Y. Nagata[3]

[1]*Ivan Franko National University of Lviv, Genetics and Biotechnology, Lviv, Ukraine*

[2]*Saarland University, Saarbrücken, Germany*

[3]*Tohoku University, Sendai, Japan*

Introduction

Well-known antibiotic-producing genus *Streptomyces* remains a source of hidden chemical diversity. The introduction of point mutations in the *rpsL* gene for r-protein S12 is one of the tools that has shown great potential for manipulating the metabolism of these bacteria. One of the currently most popular heterologous expression hosts, *Streptomyces albidoflavus* J1074, was chosen to study the effect of the engineered rpsL mutations (1).

Goals This work aims to study the properties of *rpsL* mutants K88E, K88R, and R94G.

Methods Scanning electron microscopy; CFU, dry biomass, protein content measurement in TSB medium; fermentation in R5 and SDB media and subsequent antibiotic extraction; c-di-GMP extraction from TSB; HPLC-MS; bioactivity assays; qPCR; RNA-seq.

Results Strains *S. albidoflavus* K88E and K88R exhibited altered morphology and a reduced number of sporulating hyphae compared to SAM2 and R94G. Similar levels of total protein accumulation and CFU counts were observed in all strains. However, the dry biomass weight of the original strain SAM2 was significantly increased. The c-di-GMP level, a key regulator of specialized metabolite production, was notably reduced in the K88R mutant, and all strains showed a decrease in the expression of genes for c-di-GMP synthesis. K88E and R94G produced, on average, more specialized metabolites than SAM2 and K88R, as confirmed by bioassays and HPLC-MS analysis of extracts.

Summary The *rpsL* mutants exhibit distinct morphological and growth differences, highlighting the pleiotropic effects of missense mutations within genes for ribosomal proteins. To the best of our knowledge, our work for the first time links *rpsL* mutations to c-di-GMP metabolism in streptomycetes. This offers fresh insight into the mechanisms of pleiotropicity of *rpsL* mutations.

References 1. Lopatniuk M, Myronovskyi M, Nottebrock A, Busche T, Kalinowski J, Ostash B, et al. Effect of "ribosome engineering" on the transcription level and production of S. albus indigenous secondary metabolites. Applied Microbiology and Biotechnology. 2019 Sep 19;103(17):7097–110.

OP-MMB-007

Unexpected diversity of autotrophic carbon fixation pathways in Desulfovibrionales

*J. Borges[1], L. Schumann[1], T. Steiner[2], S. König[3], W. Eisenreich[2], I. A. Berg[1]

[1]*University of Münster, Institute for Molecular Microbiology and Biotechnology, Münster, Germany*

[2]*Technische Universität München, Bavarian NMR Center-Structural Membrane Biochemistry, München, Germany*

[3]*University of Münster, Core Unit Proteomics, Münster, Germany*

Autotrophic CO_2 fixation is the most essential biosynthetic process and the basis of primary production. Today, seven pathways of inorganic carbon assimilation are known. This diversity of autotrophy represents the diversity of microorganisms and their ecological niches, whereas autotrophic pathways are often specific to particular taxonomic groups. The order *Desulfovibrionales* consists mainly of anaerobic sulfate-reducing bacteria, some of which are autotrophic. Our analysis of *Pseudodesulfovibrio profundus* (*Desulfovibrionaceae*) genome showed the presence of the genes for the key enzymes of the Calvin-Benson cycle. We were able to confirm that this bacterium is capable of growing autotrophically, while enzyme assays and proteomic analysis revealed that the bacterium does indeed use the Calvin-Benson cycle. A related *Desulfovibrio desulfuricans* (*Desulfovibrionaceae*) was shown to fix CO_2 via the recently described reductive glycine pathway [1]. Here, we found that the acetyl phosphate formed in this pathway is further metabolized via phosphate acetyltransferase and not through acetate kinase/acetyl-CoA synthetase reactions, as it was initially proposed based on proteomics data [1].

Furthermore, we found that halophilic *Desulfovermiculus halophilus* (*Desulfohalobiaceae*) uses the Wood-Ljundgdahl (WL) pathway for growth. We have further analyzed the efficiency of the autotrophic metabolism as a function of biomass production versus H_2S production and found the highest efficiency in *D. desulfuricans*, while *P. profundus* was the least efficient species. The efficiency of carbon fixation in *D. halophilus* using the WL pathway was lower than that of *D. desulfuricans*, probably due to the energetic costs of adaptation to halophilic conditions. Our analysis revealed considerable diversity of autotrophic metabolism in *Desulfovibrionales*. The evolution of autotrophic pathways in this bacterial group will be discussed.

[1] Sánchez-Andrea *et al.*, *Nat Comm* 11:5090 (2020)

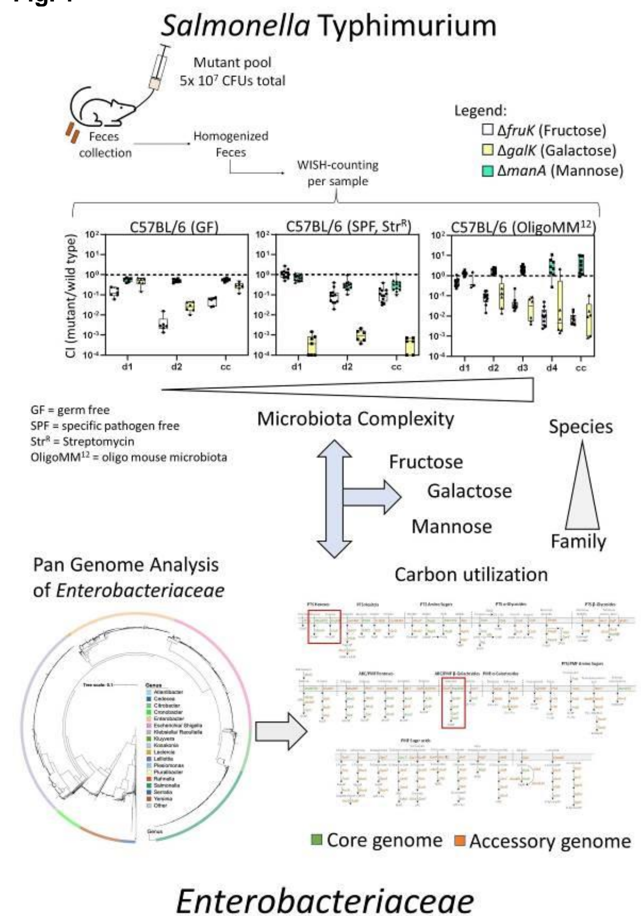
OP-MMB-008
Carbon utilization of Salmonella Typhimurium in different mouse models and on the family level of Enterobacteriaceae

*C. Schubert[1], W. D. Hardt[1], N. Näpflin[2], C. van Mering[2]
[1]*ETH Zürich, Institute of Microbiology, Zürich, Switzerland*
[2]*University of Zürich, Department of Molecular Life Sciences, Zürich, Switzerland*

Salmonella Typhimurium (*S.* Tm) is a common foodborne pathogen and a well-established pathogenic Enterobacterium to study colonization and infection of mammalian hosts. *S.* Tm invades the gastrointestinal tract and causes inflammation, which drastically changes the gut luminal environment, providing *S.* Tm with inorganic electron acceptors to bloom and outcompete the resident microbiota (1). However, it is largely unknown which carbon sources *S.* Tm utilizes at the onset of invasion. For this reason, mutant pools representing the metabolic capacity of *S.* Tm specifically sugar degradation were constructed, with each mutant labeled with a wild-type isogenic standardized hybrid (WISH) tag (2). The WISH tag is unique for each mutant and can be quantified by qPCR or Illumina sequencing, allowing us to study the fitness of several mutants. To modulate niche competition, the mutant pool will be studied in different mouse models and challenged in the presence of different competitors. Host physiology, e.g., colonization resistance and microbiota composition are known to impact *S.* Tm colonization (3). In a complementary approach, we looked at the family level of *Enterobacteriaceae* and identified the core and the accessory genome. Our focus was specifically on carbon utilization, leading to the identification of a core metabolic network centered around simple sugars and mixed acid fermentation. This observation aligns seamlessly with our *in vivo* data concerning *S.* Tm. Our investigation promises to uncover crucial insights into the significant metabolic pathways governing the initial growth of *S.* Tm. Additionally, we aim to identify shared metabolic traits within the family of *Enterobacteriaceae* that are imperative for invading mammalian hosts. By combining both species and family-level approaches, we aspire to unravel key metabolic characteristics inherent to *Enterobacteriaceae*.

(1) Rogers, A. W., Tsolis, R. M., & Bäumler, A. J. (2021). *Microbiol. Mol. Biol. Rev*, *85*(1), e00027-19.
(2) Daniel, B. *et al*, (2024). *Nat. Microbiol*, accepted.
(3) Herzog, M. K. M., ... & Hardt, W. D. (2023). *Gut microbes*, *15*(1), 2172667.

Fig. 1



OP-MMB-009
Unveiling diverse strategies: glucose-1,6-bisphosphate synthesis in bacteria

*J. Alford[1], M. Borisova-Mayer[1], C. Mayer[1], K. Forchhammer[1]
[1]*Eberhard Karls University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Organismic Interactions, Tübingen, Germany*

Glucose-1,6-bisphosphate (Glc-1,6-BP) is a key regulator of central carbon metabolism. While the origin of Glc-1,6-BP in vertebrates is well investigated, the existence of a bacterial Glc-1,6-BP synthase was confirmed only recently. Investigation of the photoautotrophic cyanobacterium *Synechocystis* revealed a cryptic secondary phosphoglucomutase (*Syn*PGM2) acting as a Glc-1,6-BP synthase. Despite displaying limited phosphoglucomutase (PGM) activity, *Syn*PGM2 efficiently converts fructose-1,6-bisphosphate and glucose-1-phosphate/glucose-6-phosphate to Glc-1,6-BP. *Bacteroides salyersiae* encodes a homolog of *Syn*PGM2, catalyzing the same Glc-1,6-BP synthesis reaction. Since both enzymes belong to the same conserved domain (CD) αPHM subfamily, it was concluded that bacterial Glc-1,6-BP is specifically formed by members of the cd05800 subfamily [1]. Remarkably, the *B. salyersiae* enzyme is annotated as the primary PGM, suggesting that heterotrophic bacteria might not use a secondary specialized enzyme for Glc-1,6-BP synthesis.

Here, we aim to ascertain if heterotrophic bacteria exclusively utilize their main PGM for Glc-1,6-BP synthesis and if Glc-1,6-BP synthesis is limited to PGM of the cd05800 subfamily. For this, heterologously expressed PGM enzymes

of various heterotrophic bacteria and several CD subfamilies were screened for Glc-1,6-BP synthase activity via coupled enzyme assays and HPLC-MS analysis.

Our results show that Glc-1,6-BP synthesis extends beyond the cd5800 subfamily and can also be found within the cd05801, cd05799 and cd03089 subfamilies. Additionally, only some heterotrophic bacteria like *Yersinia enterocolitica* and *Enterococcus faecium* employ their primary PGM for Glc-1,6-BP synthesis. Others, like *Escherichia coli,* possess a secondary Glc-1,6-BP-producing PGM. While Glc-1,6-BP was mainly formed via the *Syn*PGM2 reaction mechanism, the *Enterococcus faecium* and *Yersinia enterocolitica* PGM could also perform the reaction of the mammalian Glc-1,6-BP synthase. In summary, we could show that bacteria employ diverse strategies to produce the same essential regulator.

[1] Neumann, N., S. Friz, and K. Forchhammer,2022. mBio, **13** (4): p. e01469-01422.

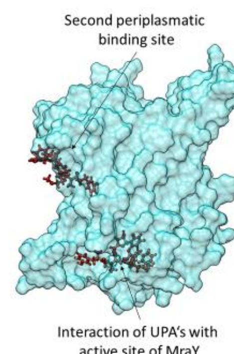
OP-MMB-010
MraY from Pseudomonas aeruginosa is inhibited by uridyl-peptide antibiotics
*J. M. Daniel[1], M. Arts[1], R. Sonnengrün[2], P. H. Koutsandrea[2], H. Groß[2], B. Gust[2], T. Schneider[1]
[1]*University clinic Bonn, Institute for pharmaceutical biology, Bonn, Germany*
[2]*University Tübingen, Institute for pharmaceutical biology, Tübingen, Germany*

Introduction
Antibiotic resistance is one of the most serious health threats and the therapeutic options to treat infections caused by multidrug-resistant strains are seriously compromised. Especially for infections caused by *Pseudomonas aeruginosa* isolates which have emerged resistance towards all carbapenems, aminoglycosides, and fluoroquinolones, novel antibiotics with new targets or unprecedented mechanisms of action are urgently needed. Integral component of the drug development process is the analysis of the mechanism of action of an antibiotic, as well as identification of the molecular target. Without this detailed knowledge, rational drug design is strongly hampered. Uridyl-peptide antibiotics (UPA) are a promising group of nucleoside natural products, which show potent activity against *P. aeruginosa*. An important molecular target of this compound class is the phospho-N-acetylmuramoyl-pentapeptide-transferase MraY, which catalyses the transfer of phospho-MurNAc pentapeptide to the membrane-standing lipid carrier undecaprenyl phosphate resulting in the formation of Lipid I.

Methods & Results
Using a fluorometric assay with the heterologously expressed MraY from *P. aeruginosa*, eight UPA's were characterized, and all showed IC_{50} values in the nanomolar range. Further, a second periplasmatic binding site was identified and confirmed by using site-directed mutagenesis resulting in significant elevation of the IC_{50} compared to the wildtype MraY, making UPA's the first antibiotics with a dual mode of action against the MraY of *P. aeruginosa*. To corroborate the data, a bioinformatical approach was used, by calculating the ΔG of the wildtype MraY and the mutants with AutoDock Vina. The theoretical binding showed very similar results to the biological data. Additionally, we could identify LPS synthesis of *Pseudomonas* as a new target of the UPA's, by establishing an *in vitro* assay with wbpL of *P. aeuruginosa*.

Conclusion
We could further characterize UPA's by the identification of a second periplasmatic binding site and a new mode of action by inhibiting LPS biosynthesis, making UPA's a promising new antibotic class.

Fig. 1



OP-MMB-011
Staphylococcus aureus and skin infection: A proteomic journey through metabolic pathways
*D. Al Nahhas[1], S. Pisanu[1], P. Guerra[2], J. E. Olsen[2], S. Uzzau[3], D. Pagnozzi[4]
[1]*Porto Conte Ricerche, Alghero, Italy*
[2]*University of Copenhagen, Sassari, Denmark*
[3]*University of Sassari, Sassari, Italy*
[4]*Porto Conte Ricerche, Alghero, Italy*

Objectives

Skin infections by methicillin-resistant *Staphylococcus aureus* (MRSA) present significant challenges due to their virulence and antibiotic resistance. This study aims to characterize the proteome variation of *S. aureus* during skin infection to identify new therapeutic targets.

Methods

Using in vivo (mouse model) and 3D in vitro skin models, we studied the proteome of *S. aureus* strains ST398 and JE2 (USA300) during skin infection. Mice were infected with 10^7 CFU mid-exponential phase cultures of MRSA onto a skin lesion by scrapping the dorsal back part, and skin samples were collected at 2- and 4- days post-infection for bacterial count and protein extraction. The 3D in vitro model, simulating human skin, was infected with 10^8 CFU of the same MRSA strains. After 2 days, we collected and extracted proteins from the tissues for analysis via shotgun proteomics, employing a Q-Exactive mass spectrometer. The data from this analysis were processed using SEQUEST-HT in Proteome Discoverer software. We applied a TMT labeling approach for the in vivo model and a label-free (LFQ) approach for the in vitro model.

Results and conclusion

KEGG mapping tool identified pathways involved in infection in both models, including glycolysis and gluconeogenesis, pentose phosphate pathway, and pyruvate metabolism, while

glycine serine and threonine metabolism pathway was seen solely in the in vivo model. In addition, we attempted to understand strain-specific adaptations to the infection, highlighting an increase in the arginine biosynthesis pathway observed in JE2 in both tissue models, along with differences in *S. aureus* infection protein expression between the two strains, such as the upregulation of femX and femB genes in JE2 which are involved in peptidoglycan biosynthesis.

This research enhances our understanding of protein expression levels during skin infection. By broadening our comprehension of *Staphylococcus aureus* biology, our work paves the way for developing targeted therapeutic interventions, thereby offering a meaningful contribution to the fight against antimicrobial resistance

---

OP-MMB-012

Underground metabolism in the gut: Degradation of Nε-carboxymethyllysine (CML) in Escherichia coli

*E. Aveta[1], J. Mehler[1], K. I. Behringer[2], N. Gericke[1], M. Walczak[3], P. Vougioukas[3], M. Hellwig[3], J. Lassak[1]
[1]*Ludwig-Maximilians University Munich, Biology, Microbiology, München, Germany*
[2]*Technical University of Braunschweig, Institute of Food Chemistry, Brunswick, Germany*
[3]*Technical University of Dresden, Special Food Chemistry, Dresden, Germany*

Amino acids undergo numerous of enzymatic or non-enzymatic post-translational modifications. *Nε*-Carboxymethyllysine (CML) results from the condensation of a reducing sugar with the free amino group of lysine, in the so called Maillard reaction or glycation (Maillard *et al*., 1912). This non-enzymatic event is responsible for the flavour and colour of thermally processed foods. Up to 11.3 mg of CML per kg of protein are ingested daily by humans, but the limited absorption in the gastrointestinal tract suggests a major role in its degradation by colonic bacteria (Delgado-Andrade *et al*., 2012, Lassak *et al*., 2023). When exposing *E. coli* to CML the predominant degradation product is carboxymethylcadaverine (Hellwig *et al*., 2019). However, no enzyme in the CML metabolism is known to date. We have now unveiled an unexpected high promiscuity of the ornithine decarboxylase SpeC toward many lysine derivatives including CML. The enzyme is the first in an underground metabolism, enabling *E. coli* to utilize CML as sole nitrogen source. We further discovered that proton consumption in the CML decarboxylation reaction helps to counteract the mild acid pH in the colon. Investigating the molecular "players" of CML metabolism in *E. coli* will help understand how glycated amino acids influence the ability of gut bacteria to thrive and compete, and gain insight about the effect those compounds and their degradation products have on our health.

Microbial Pathogenicity

OP-MP-001

A Novel Stx-phage-encoded RNA Methyltransferase Regulates Spontaneous Prophage Induction and STEC Virulence by Blocking Shiga Toxin Inactivation of Translation

*G. Koudelka[1], C. Gong[1]
[1]*University at Buffalo, Biological Sciences, Buffalo, NY, United States*

Shiga toxin (Stx) released by Stx-producing E. coli (STEC), can cause life-threatening illness. The synthesis and release of Stx requires induction of Stx-encoding prophage present in all STEC genomes. The STEC strains PA2 and PA8 harbor nearly identical Stx-encoding prophages, yet exhibit differing spontaneous induction frequencies and virulence.

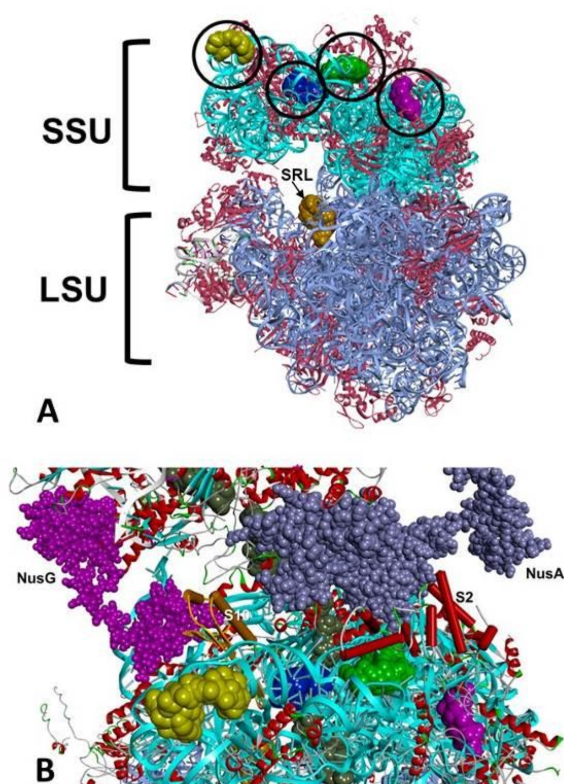
ϕPA2 and ϕPA8 each bear an IS629 insertion element but in different locations. This element lies in an intergenic region in ϕPA8. In ϕPA2, it disrupts a gene predicted to encode a DNA adenine methyltransferase (M.EcoPA8). Expressing wild-type M.EcoPA8 in STEC::ϕPA2 restores both prophage induction frequency and virulence to STEC::ϕPA8 levels, but an active site mutant does not. Thus M.EcoPA8 methyltransferase activity governs prophage spontaneous induction and STEC virulence.

Full, specific *in vitro* methyltransferase activity of M.EcoPA8 requires complexation with a second protein, PNB-2. Sequential deletion of the genes encoding these proteins leads to concomitant, progressive reductions in spontaneous induction and STEC virulence, showing that these phenotypes are interconnected.

We found that despite its annotation as a DNA adenine Mtase, the M.EcoPA8-PNB-2 holoenzyme specifically methylates 16S rRNA, not DNA. A ΔM.ecoPA8 Δpnb-2 STEC strain has fewer ribosomes than the M.EcoPA8-PNB-2 replete STEC strain. The translation activity of extracts made from a ΔM.EcoPA8 ΔPNB-2 STEC strain is lower than that of extracts from STEC::ϕPA8 or from a strain without any Stx-encoding prophage. Significantly, translation extracts from M.EcoPA8 replete STEC exhibit greater resistance to Stx-mediated inactivation compared to STEC strains lacking M.EcoPA8. Collectively, these findings suggest that M.EcoPA8-mediated 16S rRNA methylation safeguards ribosomes from Stx inactivation, enhancing prophage and Stx production, resulting in PA8's enhanced virulence.

16S rRNA sequencing identified 4 putative methylation sites all mapping onto the RNA polymerase-contacting face of the 30S ribosome subunit in the expressome. We suggest M.EcoPA8 -PNB-2 16S rRNA methylation protects the ribosome from Stx inactivation by stabilizing this complex.

Fig. 1



OP-MP-002

The role of MerA in the defense against the thiol-specific oxidant hypothiocyanous acid (HOSCN) in Staphylococcus aureus

*L. V. Vu[1], P. Weiland[2], T. Busche[3], F. Schnaufer[4], J. Kalinowski[3], G. Bange[2], H. Antelmann[1]
[1]*Free University of Berlin, Institut für Biologie-Mikrobiologie, Berlin, Germany*
[2]*Philipps-University Marburg, Center for Synthetic Microbiology (SYNMIKRO) and Department of Chemistry, Marburg, Germany*
[3]*Center for Biotechnology, Bielefeld University, Bielefeld, Germany*
[4]*Free University of Berlin, Institute for Biology-Microbiology, Berlin, Germany*

Introduction: *Staphylococcus aureus* colonizes the skin and the airways of the healthy human population, but can also lead to life-threatening infections. During colonization and phagocytosis by neutrophils, *S. aureus* encounters the oxidant HOSCN. **Goals:** Here, we aimed to characterize the defense mechanisms against HOSCN in *S. aureus*. **Materials & Methods:** RNA-seq analyses, redox biosensor measurements, mutant phenotypes and crystal structure analysis were used to investigate the HOSCN defense mechanisms. **Results:** In the transcriptome, HOSCN caused a strong thiol-specific oxidative, electrophile and metal stress responses as well as protein damage in *S. aureus*, as indicated by the upregulation of the HypR, TetR1, PerR, QsrR, MhqR, CstR, CsoR, CzrA, AgrA, HrcA and CtsR regulons. Brx-roGFP2 biosensor measurements and Western blot analyses revealed a reversible oxidative shift of the bacillithiol redox potential (*E*BSH) and increased *S*-bacillithiolations under HOSCN in *S. aureus*, supporting its thiol-specific mode of action [1]. Using phenotype analyses, the HOSCN reductase MerA was shown to confer the highest resistance towards HOSCN stress in *S. aureus* [1, 2]. The crystal structure of the MerA dimer revealed its function as group I flavin disulfide reductase, with the FAD cofactor close to the C43XXXXC48 motif, which interacts with the H427XXXXE432 motif of the opposing subunit, facilitating deprotonation of the active site Cys43 in the HOSCN reduction cycle [2]. **Summary:** HOSCN causes a thiol-specific stress response, *E*BSH changes and *S*-bacillithiolation in *S. aureus.* MerA functions as HOSCN reductase and main protection mechanism against neutrophil oxidants.

References:

[1] Loi VV, Busche T, Schnaufer F, Kalinowski J, Antelmann H. 2023. The neutrophil oxidant hypothiocyanous acid causes a thiol-specific stress response and an oxidative shift of the bacillithiol redox potential in *Staphylococcus aureus*. Microbiol Spectr. 11:e0325223
[2] Shearer HL, Loi VV, Weiland P, Bange G, Altegoer F, Hampton MB, Antelmann H, Dickerhof N. 2023. MerA functions as a hypothiocyanous acid reductase and defense mechanism in *Staphylococcus aureus*. Mol Microbiol. 119:456-470.

OP-MP-003

Type III secretion of transmembrane proteins and the case of the Salmonella´s chaperone, SscB

*S. Pais[1], S. Schroth[1], J. Joiner[2], P. Fauser[1], M. Hartmann[2], S. Wagner[1]
[1]*Eberhard Karls University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Tübingen, Germany*
[2]*Max Planck Institute of Developmental Biology, Tübingen, Germany*

Type III secretion (T3S) systems are present in Gram-negative bacteria and consist of a needle-like structure that creates a direct channel for the delivery of bacterial proteins into host cells, ultimately promoting bacterial survival. Among the proteins delivered are those containing transmembrane domains (TMD). These can interact with a T3S chaperone (T3SC), which protects the more hydrophobic TMDs from erroneous recognition and targeting to bacterial membranes. This interaction should be critical for an efficient and correct T3S. Here, we aimed to unveil the key features and processes that underlie the T3S of TMD-effectors.

In *Salmonella*, SscB/SseF are a chaperone/TMD-effector pair. Biophysical characterization of purified SscB and analysis of the predicted structure showed that SscB is structurally similar to T3SCs that bind translocators (TMD-proteins). Interestingly, a consensus amino acid sequence, which is conserved on the translocators of *Salmonella* and other bacterial species, was identified in SseF but also in the TMD-effector SseG. SscB interacts and promotes secretion of SseF, as seen previously, but also can interact and aid the secretion of SseG. We showed that mutagenesis of the consensus amino acid sequence abolished T3S of SseF and SseG. Next, it was observed that SscB and SseF co-stabilized but SseG was stable without the presence of the chaperone. This suggests that efficient SscB/SseF interaction is more critical than that of SscB/SseG. Moreover, since these three proteins are encoded adjacently in an operon, *sscB*-*sseF*-*sseG*, we have analyzed the effects of changing gene order on T3S. A decrease in the T3S of SseF was observed when *sscB* was relocated downstream of *sseF*.

Overall, SscB shares features with chaperones of translocators, which challenges the current classification of

T3SC. This suggests that the structure of the T3SC depends on the structural characteristics of their interacting partners rather than their timing of secretion. The gene order is important for efficient targeting to T3S, by possibly allowing for a more rapid interaction of SscB/SseF, which leads to protein co-stabilization and protection of the TMD of SseF.

OP-MP-004
Antigenic variation impacts gonococcal lifestyle and antibiotic tolerance by modulating interbacterial forces
*S. Kraus-Römer[1], I. Wielert[1], T. E. Volkmann[1], P. G. Higgins[2], L. Craig[3], B. Maier[1]
[1]*University of Cologne, Institute for Biological Physics, Köln, Germany*
[2]*University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*
[3]*Simon Fraser University, Department of Molecular Biology and Biochemistry, Burnaby, Canada*

Type 4 pili (T4P) are multifunctional filaments involved in adhesion, surface motility, biofilm formation, and horizontal gene transfer. These extracellular polymers are surface-exposed and, therefore, act as antigens. The human pathogen *Neisseria gonorrhoeae* uses pilin antigenic variation to escape immune surveillance, yet it is unclear how antigenic variation impacts other functions of T4P. Here, we addressed this question by replacing the major pilin of a laboratory strain of *N. gonorrhoeae* with pilins from clinical isolates. Structural predictions reveal filament features that vary from one strain to the next, with the potential to impact pilus:pilus interactions. Using a combination of laser tweezers, electron microscopy, and advanced image analysis, we explore the phenotypic consequences of these structural changes. We reveal that strains differing only in their major pilin sequence vary substantially in their attractive forces, which we attribute to variations in the stereochemistry of the T4P filament. In liquid culture, strongly interacting bacteria form microcolonies while weakly interacting bacteria retain a planktonic lifestyle. We show that lifestyle strongly affects growth kinetics and antibiotic tolerance. In the absence of external stresses, planktonic bacteria grow faster than aggregating bacteria. In the presence of the antibiotics ceftriaxone or ciprofloxacin, the killing kinetics indicate strongly increased tolerance of aggregating strains. We propose that pilin antigenic variation produces a mixed population containing variants optimized for growth, colonization, or survivability under external stress. Different environments select different variants, ensuring the survival and reproduction of the population as a whole.

OP-MP-005
Analysis of the emerging global pathogen Stenotrophomonas maltophilia in single vs. multispecies biofilms
*I. Alio[1], R. Moll[1], M. Gudzuhn[1], T. Hoffmann[1], H. Rohde[2], U. Mamat[3], U. Schaible[3], K. Papenfort[4], M. Alawi[2], R. Thünauer[5], W. R. Streit[6]
[1]*Universtiy Hamburg, Microbiology and biotechnology, Hamburg, Germany*
[2]*University Hospital Hamburg-Eppendorf, Hamburg, Germany*
[3]*Research Center Borstel, Borstel, Germany*
[4]*Friedrich Schiller University Jena, Jena, Germany*
[5]*Center for Structural Systems Biology (CSSB), Hamburg, Germany*
[6]*Universtiy Hamburg , Institute for microbiology and biotechnology , Virology und Hygiene, Hamburg, Germany*

Stenotrophomonas maltophilia is an opportunistic pathogen causing various infections. Its prevalence has increased, especially in cystic fibrosis patients, and its multidrug resistance poses challenges in treatment. The bacterium forms multispecies biofilms with other pathogens and is known for its substantial role in pathogenicity in infected lungs

Our goal is to understand the biofilm characteristics of *S. maltophilia* in single und mixed species biofilms as well as the differential gene expression patterns within these biofilms

In this study over 200 clinical isolates of *S. maltophilia* as well as chromosomally labeled fluorescent strains of *S. maltophilia* K279a, *P. aeruginosa* PA01, *S. aureus* SH1000 and *C. albicans* SC5314 were used. Biofilms were formed in flow and static settings and were used for RNA seq analysis. Additionally, reporter fusion constructs of selected genes were generated and were further analyzed.

Results and conclusion

Our analysis revealed a strain-specific variability in biofilm formation and architecture among *S.maltophilia* isolates. RNA seq. analysis of *S. maltophilia* isolates identified shared and strain-specific genes, with iron uptake being a key factor in biofilm metabolism. LSM imaging showed that species interactions affect the structural composition of multispecies biofilms leading to a layer formation. *S maltophilia* showed alteration in lactate metabolism , Propionate degradation and a switch in cytochrome oxidases in our mixed biofilm models.

The expression of virulence factors, QS signaling and cyclic diGMP was decreased in PA01 in coculture with K279a.

Our data show that isolates of *S. maltophilia* are highly diverse on a phenotypic and genotypic level. Common genes were identified to play a crucial role in the biofilm formation of *S. maltophilia*. LSM imaging has shown a distinct distribution and layer formation of different species within mixed species biofilms. RNA seq analysis and the application of reporter fusion constructs showed specific and different expression patterns for each species as compared to single species biofilms, suggesting that each species acknowledge and respond to the presence of others in these biofilms.

OP-MP-006
Expression and activation control of the T6SS4 machinery in Yersinia pseudotuberculosis
*A. Kerwien[1], C. Köster[1], P. Dersch[1], A. S. Herbrüggen[1]
[1]*Institute of Infectiology, Münster, Germany*

The type VI secretion system (T6SS) is a contact dependent protein delivery system of Gram-negative bacteria to intoxicate prokaryotic or eukaryotic cells. The enteropathogen *Yersinia pseudotuberculosis* (*Ypstb*) encodes four complete clusters (T6SS1-4) with potentially different functions controlled by intricate, independent regulatory pathways. Under laboratory conditions, only T6SS4 is mildly expressed which makes thorough analysis of each system difficult. The recent identification of the T6SS4 activator RovC allows a more detailed investigation of the regulation of this cluster.

Flow cytometry was used to analyse T6SS4 expression on a single cell level. We observed heterogenous expression of T6SS4 (T6SS4+ and T6SS4- subpopulations) within a *Ypstb* population, which strongly depends on temperature and growth phase. Heterogeneous expression is not only limited

to T6SS4, as also *rovC* is heterogeneously expressed, indicating an unknown additional transcriptional regulator upstream of *rovC*. Although all T6SS4 genes are under the control of the same promoter, qRT-PCR revealed different amounts of gene transcripts within the cluster, suggesting additional post-transcriptional pathways. However, expression of T6SS4 compounds alone does not result in a firing event. Analysis with fluorescence microscopy could show that several additional triggers such as high cell density and osmotic pressure are required to activate the T6SS4.

Our preliminary data show a tight regulation on transcriptional, post-transcriptional and post-translational levels, that differ from already known pathways in other organisms. However, as little is known about the T6SSs of *Ypstb* so far, further research is required to understand their function and the purpose of this heterogeneous expression.

OP-MP-007
Hydrogen peroxide is responsible for the cytotoxic effects of *Streptococcus pneumoniae* on primary microglia in the absence of pneumolysin

*D. Schaaf[1], F. Jennert[1], P. Valentin-Weigand[1], T. P. Kohler[2], S. Hammerschmidt[2], R. Nau[3], D. Häusler[3], J. Seele[3]
[1]*University of Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany*
[2]*Greifswald University, Department of Molecular Genetics and Infection Biology, Greifswald, Germany*
[3]*University Medical Center Göttingen, Department of Neuropathology, Göttingen, Germany*

Streptococcus pneumoniae is the most common causative agent of bacterial meningitis in humans worldwide with a high morbidity and mortality rate, especially in young children and older persons. One of the best-described virulence factors of *S. pneumoniae* is the pore-forming toxin pneumolysin (PLY), which can harm neurons and microglia by lytic pore formation and by triggering an excessive inflammation in the brain. Another important virulence factor of *S. pneumoniae* is hydrogen peroxide (H_2O_2), which is mainly produced by the enzyme pyruvate oxidase (SpxB) and has also cytotoxic effects on neurons and microglia. Microglia are the resident macrophages in the brain and are responsible for the elimination of microbes and other antigens as well as for the mediation of neuroinflammation and other cellular responses.

To investigate the role of PLY and H_2O_2 during pneumococcal meningitis, we infected primary murine microglia and, for comparison, bone marrow-derived macrophages (BMDM) from mice with *S. pneumoniae* wild-type strain D39 as well as its isogenic mutant strains deficient for the capsule, PLY, and SpxB, respectively. After infection, we analyzed survival of the phagocytes by fluorescence microscopy, bacterial growth by quantitative plating, and phagocytosis by an antibiotic protection assay. In addition, we determined cytotoxic effects of H_2O_2 and recombinant PLY by fluorescence microscopy or lactate dehydrogenase assay, respectively.

We found that microglia were killed during pneumococcal infection even in the absence of PLY, whereas BMDM survived. Treatment with recombinant PLY showed that very high concentrations were needed to harm the phagocytes but these concentrations were not reached during *in vitro* pneumococcal infection. In contrast, exogenously added catalase abolished the cytotoxic effects of H_2O_2 towards microglia and BMDM, and infection with pneumococci deficient for SpxB resulted in reduced killing of microglia.

To conclude, H_2O_2 produced by pneumococci was able to cause microglial cell death in the absence of PLY, whereas PLY was not released in sufficient concentrations to damage microglia under these experimental conditions.

OP-MP-008
Extracellular detoxification of host-derived antimicrobial fatty acids by Staphylococcus aureus

*A. Kengmo Tchoupa[1,2], A. M. A. Elsherbini[1,2], X. Fu[3], J. Camus[1,2], O. Ghaneme[1], X. Hu[1], L. Seibert[1], M. A. Böcker[1], M. Lebtig[1], S. Papadopoulos Lambidis[1], B. Schittek[2,4], D. Kretschmer[1,2], M. Lämmerhofer[3], A. Peschel[1,2]
[1]*University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Infection Biology Department, Tübingen, Germany*
[2]*University of Tübingen, Cluster of Excellence "Controlling Microbes to Fight Infections", Tübingen, Germany*
[3]*University of Tübingen, Institute of Pharmaceutical Sciences, Tübingen, Germany*
[4]*University Hospital Tübingen, Dermatology Department, Tübingen, Germany*

Long-chain fatty acids with antimicrobial properties are abundant on the skin and mucosal surfaces, where they are essential to restrict the proliferation of opportunistic pathogens such as *Staphylococcus aureus*. These antimicrobial fatty acids (AFAs) elicit bacterial adaptation strategies, which have yet to be fully elucidated. Characterizing mechanisms used by *S. aureus* to resist AFAs could open new avenues to prevent pathogen colonization, which is the overarching goal of our research program. For instance, we have used mutagenesis, recombinant proteins, click-chemistry, high-performance thin layer chromatography and ultra-high performance liquid chromatography-electrospray ionization-tandem mass spectrometry to unveil *S. aureus* lipase Lip2 as a resistance factor against AFAs. Lip2 detoxifies AFAs via esterification with cholesterol. Lip2-dependent AFA-detoxification was apparent during planktonic growth and biofilm formation. Importantly, Lip2 is also secreted into the extracellular milieu as a major component of *S. aureus* membrane vesicles (MVs). In a previous study, we have demonstrated that these hydrophobic MVs act as decoys to bind AFAs and protect bacteria from AFA toxicity. Our newfound role of Lip2 strongly suggests that MVs could serve as platform to sequester AFAs, which are then converted *in situ* into innocuous fatty acid esters by Lip2. Indeed, Lipase-proficient MVs complement a lipase-deficient mutant and enable the bacterium to grow in the presence of otherwise toxic AFA concentrations. Besides, our genomic analysis of over three thousand genomes of *S. aureus* revealed that prophage-mediated inactivation of Lip2 was more common in blood and nose isolates than in skin strains, suggesting a particularly important role of Lip2 for skin colonization. Accordingly, in a mouse model of *S. aureus* skin colonization, bacteria were protected from sapienic acid (a human-specific AFA) in a cholesterol- and lipase-dependent manner. Taken together, our results suggest that *S. aureus* exoproducts target and transform environmental lipids in unforeseen ways with possible implications for the bacterium"s ability to colonize and infect its host.

OP-MP-009
Apoptosome-centered cell death restricts phagocyte entry into staphylococcal abscesses

N. Schwermann[1,2], R. Haller[1,2], S. Koch[1,2], G. A. Grassl[1,3], *V. Winstel[1,2]
[1]*Hannover Medical School, Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany*
[2]*TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany*
[3]*German Center for Infection Research, Partner site Hannover-Braunschweig, Hannover, Germany*

Manipulation of macrophage responses by the human pathogen *Staphylococcus aureus* requires the activity of two synergistically acting enzymes, secreted nuclease (Nuc) and adenosine synthase A (AdsA). Specifically, Nuc-mediated disruption of neutrophil extracellular DNA traps and subsequent bioactivity of AdsA provokes the formation of staphylococcal death-effector deoxyribonucleosides that trigger a genotoxic buildup of deoxyribonucleoside triphosphates in phagocytes that attempt to infiltrate infectious foci. However, the cellular and pathophysiological consequences that *S. aureus*-driven modulation of immune cell nucleotide metabolism may trigger remain unknown. Powered by CRISPR/Cas9 mutagenesis and in-depth analysis of cell death modalities in phagocytes, we illustrate that death-effector deoxyribonucleoside-mediated intoxication of macrophages involves destruction of mitochondria, assembly of the apoptosome, and subsequent activation of the intrinsic pathway of apoptosis. Remarkably, mice lacking caspase-9, a dominant modulator of the intrinsic pathway of apoptosis, exhibit increased resistance toward *S. aureus* invasive disease as caspase-9-deficient macrophages are resistant to death-effector deoxyribonucleosides and readily invade abscess lesions filled with staphylococci to accelerate bacterial clearance in infected host tissues. Combined with the identification of a set of candidate resistance alleles in human *CASP9* that protect macrophages from death-effector deoxyribonucleoside-mediated cytotoxicity, our data suggest that specific polymorphisms in genetic elements of the apoptotic signaling pathway may contribute to the wide array of susceptibility observed in human populations toward *S. aureus* diseases.

OP-MP-010

Chlamydia-containing spheres are the predominant egress structure of the zoonotic pathogen Chlamydia psittaci.

*J. Scholz[1], G. Holland[2], M. Laue[2], S. Banhart[1], D. Heuer[1]
[1]*Robert Koch-Institute, Unit for Sexually Transmitted Bacterial Infections (STI) and HIV, Berlin, Germany*
[2]*Robert Koch Institute, Unit of Advanced Light and Electron Microscopy, Berlin, Germany*

Egress of intracellular bacteria from host cells and cellular tissues is a critical process during the infection cycle. This process is essential for bacteria to spread inside the host and can influence the outcome of an infection. For the obligate intracellular Gram-negative zoonotic bacterium *Chlamydia psittaci* little is known about the mechanisms resulting in chlamydial egress from the infected epithelium. Using state-of-the-art imaging techniques including live cell imaging and electron microscopy, we describe and characterize *Chlamydia*-containing spheres (CCS), a novel type of egress structure of *Chlamydia*. The formation of CCS represents the predominant non-lytic egress pathway of *C. psittaci*. CCS are spherical, low phase contrast structures surrounded by a phosphatidylserine exposing membrane with specific barrier functions. They contain infectious progeny and morphologically impaired cellular organelles. CCS formation is a sequential process starting with proteolytic cleavage of a DEVD-containing substrate that can be detected inside of the chlamydial inclusions, followed by an increase in the intracellular calcium concentration of the infected cell. Subsequently, blebbing of the plasma membrane begins, the inclusion membrane destabilizes and the proteolytic cleavage of the DEVD-containing substrate increases rapidly within the whole infected cell. Finally, infected, blebbing cells detach and leave the monolayer thereby forming CCS. This sequence of events is unique for chlamydial CCS formation and fundamentally different from previously described *Chlamydia* egress pathways. Thus, CCS formation represents a new egress pathway for intracellular pathogens that could be linked to *C. psittaci* biology and might influence the outcome of the infection in organisms.

OP-MP-011

PlaD is a type IVB secreted effector phospholipase of Legionella pneumophila activated by host regulatory proteins

*C. Lang[1], M. Hiller[1], S. Wamp[2], J. Döllinger[3], A. Flieger[4]
[1]*Robert Koch Institute, Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*
[2]*Robert Koch-Institut, Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*
[3]*Robert Koch Institute, Division of Proteomics and Spectroscopy, Berlin, Germany*
[4]*Robert Koch-Institut, FG11 Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*

Introduction

Legionella pneumophila, the causative agent of a life-threatening pneumonia, intracellularly replicates in a specialized compartment in lung macrophages, the *Legionella*-containing vacuole (LCV). During infection, *L. pneumophila* secretes proteins, among others phospholipases, into the lumen of the LCV and the host cell cytoplasm via its type II (Lsp) and type IVB (Dot/Icm) secretion systems. At least 15 phospholipases A, which divide into the patatin-like proteins, the PlaB-like proteins, and the GDSL hydrolases PlaA, PlaC and PlaD, are encoded in the genome.

Goals

We here characterize the phospholipase PlaD which shows various differences to the other GDSL hydrolases PlaA and PlaC. We aim to analyze the, activity and activation mechanism, secretion path, and importance and localization in infection of PlaD.

Materials and methods

The mode of secretion of PlaD was investigated by means of Western blotting and protein translocation assays. Additionally, we determined its binding to various lipids and its interactions with eukaryotic proteins by means of lipid-protein-overlay assays, proximity ligation, and pull-down assays. Further, we analyzed the localization of PlaD during infection via immunofluorescence microscopy.

Results

We found that, during infection, PlaD is Dot/Icm-dependently injected into the host cell cytoplasm where it localizes to distinct organelles. Moreover, PlaD binds to a subset of

phosphoinositide species (PIPs) and interacts with a class of regulatory proteins of the host cell which leads to activation of phospholipase activity. Additionally, our data revealed that the C-terminal half of PlaD is essential for its secretion and phosphoinositide binding but dispensable for interaction with the regulatory proteins.

Summary

Based on its Dot/Icm dependent injection into the host cell cytoplasm, we classify PlaD as a novel type IVB secreted effector protein of *L. pneumophila*. Both its interaction with PIPs and eukaryotic proteins suggest a specific function in host cell infection.

OP-MP-012
Simkania negevensis is released from infected cells in a caspase-dependent manner
R. D. Koch[1], E. M. Hörner[1], L. Wilm[1], N. Münch[1], A. Kopfinger[1], M. L. Jekic[1], *V. Kozjak-Pavlovic[1]
[1]*University of Würzburg, Department of Microbiology, Würzburg, Germany*

Simkania negevensis (Sne) is an obligate intracellular bacterium related to Chlamydia and associated with community-acquired pneumonia and bronchiolitis in infants. Sne develops within a Sne-containing vacuole, a membranous compartment in close contact with the endoplasmic reticulum and mitochondria. During propagation, bacteria alternate between the intracellular metabolically active reticulate bodies (RB) that re-differentiate into infectious elementary bodies (EB), which are released from infected cells by an unknown mechanism. Bacteria reach a developmental plateau 3 days post-infection, and, unlike *Chlamydia*, are capable of prolonged infections, lasting up to 15 days, as well as of infecting a wide range of hosts. So far, little is known about the mechanisms that mediate the cellular release of Sne.

In this work, we show that Sne-infected epithelial HeLa, as well as macrophage-like THP-1 cells, reduce in number during infection. At the same time, the infectivity of the cell culture supernatant increases, starting at day 3 for HeLa and day 4 for THP-1 cells and reaching a maximum at day 5 post-infection. This correlates with the ability of Sne to block TNFα-, but not staurosporine-induced cell death up to 3 days post-infection, after which cell death is boosted by the presence of bacteria. Mitochondrial permeabilization through Bax and Bak is not essential for host cell lysis and release of Sne. The inhibition of caspases by Z-VAD-FMK, caspase 1 by Ac-YVAD-CMK, and proteases by an inhibitor cocktail, significantly reduces the number of released infectious particles [1]. In line with the previous results, the specific inhibition of caspase 3, 8, or 9 shows that, unlike the other two, caspase 9 is not required for the exit of Sne from infected cells. The release, however, strongly depends on caspase 8 and seems to be negatively affected by necroptosis, indicating RIPK1/RIPK3/MLKL as an alternative signaling pathway that can be used to limit bacterial propagation.

1. Koch RD, et al. Modulation of Host Cell Death and Lysis Are Required for the Release of *Simkania negevensis*. Front Cell Infect Microbiol. 2020; 10:594932.

OP-MP-013
Selective HDAC inhibition potentiates host defenses to prevent uropathogenic Escherichia coli infection
*K. Mukherjee[1], J. Saur[1], M. Becht[1], K. Emke[1], U. Dobrindt[1]
[1]*University of Münster, Institute of Hygiene, Münster, Germany*

Introduction

Uropathogenic *E. coli* (UPEC) can cause urinary tract infection (UTI) by manipulating multiple aspects of the host immune system, including epigenetic reprogramming of gene expression. Epigenetic mechanisms such as histone acetylation are a dynamically regulated process involved in many aspects of physiological and pathological immune responses. In particular, members of the histone deacetylase (HDAC) family of acetylation "erasers" are key regulators of inflammatory response during bacterial infection. Despite this association, the precise mechanistic understanding of how UPEC utilizes HDAC functions to enhance its survival and dampen host inflammatory responses remains elusive.

Goal

By delving into the functional characterization of distinct HDAC genes using complementary approaches, we aspire to uncover novel mechanisms of UPEC infection in the urinary tract.

Methods

To expedite the identification of conserved HDACs that are commonly targeted during UPEC infection, we established the surrogate insect model *Galleria mellonella* as a cost-effective, ethically acceptable system. RNA was isolated from UPEC-infected larvae and HDAC expression was analyzed by RT-PCR. Selected HDAC genes exhibiting distinct expression patterns in UPEC-infected larvae, underwent silencing using siRNAs, chemical inhibitors, and CRISPR-Cas9 based gene editing in UPEC-infected human bladder epithelial cells (RT-112). Subsequently, we systematically examined the repercussions of silencing selected HDACs on both UPEC invasion and the host immune response.

Results

We found that pronounced expression of selected HDACs during UPEC infection was associated with the increased mortality of larvae and infectivity in bladder epithelial cells. We report that loss of specific HDAC function led to reduced UPEC invasion in bladder epithelial cells. This is associated with increased expression of several immunity-related genes.

Conclusions

Our study unveils a previously unknown infection mechanism of UPEC to manipulate host responses by targeting HDACs. The results project HDACs as a promising target for developing host-directed, non-antibiotic therapies for treating UTI.

OP-MP-014
Discovery of pathogen-specific markers for novel antimicrobial targets through multi-omics investigation

*L. S. Swiatek[1], K. Surmann[2], E. Eger[1], S. E. Heiden[1], J. A. Bohnert[3], K. Becker[3], U. Völker[2], M. Schwabe[1], K. Schaufler[1,4]
[1]*Helmholtz Institute for One Health/Helmholtz Centre for Infection Research, Greifswald, Germany*
[2]*Interfaculty Institute for Genetics and Functional Genomics, University Medicine, Department of Functional Genomics, Greifswald, Germany*
[3]*Friedrich Loeffler-Institute of Medical Microbiology, University Medicine Greifswald, Greifswald, Germany*
[4]*University Medicine Greifswald, Greifswald, Germany*

Multidrug-resistant strains of *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) pose a global health threat necessitating alternative treatment approaches. Understanding key pathogenic (PAT) features and their shared regulatory mechanisms, especially when compared to commensal (COM) counterparts, holds the promise of unveiling pathogen-specific markers for anti-virulence strategies. Employing bioinformatics, we constructed pangenomes encompassing 22,267 EC and 6,740 *Klebsiella* genomes. Focused on high-risk clonal lineages, we identified genes exclusive to PAT sequence types (ST) while absent in COM ones like EC ST10, *Klebsiella variicola* (KV), and *Klebsiella quasipneumoniae*. This set, termed the patho-core genome, was investigated for intriguing markers, subject to detailed analysis and phenotypic exploration. Further investigations involved transcriptomic and proteomic analyses in a urine-like medium for select isolates. The analysis revealed 273 unique markers for KP and 24 for PAT EC including the L-sorbose phosphotransferase system. Early stationary growth phase induction in nutrient broth supplemented with sorbose prompted diauxic growth in PAT EC STs. Omics investigations revealed co-regulation of metabolic pathways (e.g., purine and tryptophan) and induction of virulence-associated pathways (e.g., flagellar and capsule production). The integrative omics analysis of KP demonstrated similar regulatory profiles for KP, while KV profiles exhibited marked differences. Enhanced growth of KP coincided with the induction of various metabolic pathways, some aligning with the patho-core genome (e.g., cellobiose), and others displaying differential expression (e.g., citrate). Our study demonstrates that a comprehensive multi-omics approach facilitates the identification of potential targets by comparing PAT with COM bacteria. This highlights the importance of distinct genetic make-ups and regulatory mechanisms that contribute to overall bacterial fitness benefits. The induction of metabolic pathways, expanding nutrient utilization, emerged as a key mechanism. Future studies are warranted to explore the targetability and *in vivo* relevance of these findings.

OP-MP-015

Intermolecular interactions in the periplasmic chaperone SurA can be linked to its activity in vitro

*F. Renschler[1,2,3], T. Kronenberger[4,5,6], J. Krusche[3], J. Zens[1], M. Schweers[1,2,3], E. Bohn[1,2,6,3], M. Schütz[1,2,3]
[1]*University Hospital Tübingen, Institut für Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*
[2]*German Center for Infection Research, Partner Site Tübingen, Tübingen, Germany*
[3]*Eberhard Karls University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*
[4]*University of Eastern Finland, School of Pharmacy, Kuopio, Finland*
[5]*Eberhard Karls University of Tübingen, Tücad2, Faculty of Science, Tübingen, Germany*
[6]*Eberhard Karls University of Tübingen, Excellence Cluster "Controlling Microbes to Fight Infections" (CMFI), Tübingen, Germany*

The periplasmic chaperone SurA shuttles outer membrane proteins (OMPs) across the periplasm in many Gram-negative bacteria. Loss or proper SurA function is linked to a reduction of virulence and outer membrane integrity (1, 2). SurA has three distinct domains, the NC-core, and the peptidyl-prolyl-isomerase domains PPI1 and PPI2, which sample various conformations in solution. NC-core and PPI1 predominantly interact with substrates whereas PPI2 and the very N-terminus can be linked to an interaction with the beta-barrel assembly machinery *in vitro* (4-9).

We established a novel SurA activity assay using a partially unfolded luciferase as a model substrate. This unfolded luciferase is catalytically inactive, upon refolding becomes active and can emit light in presence of ATP and Luciferin. Addition of SurA enhances its refolding. Molecular dynamics simulations in which we provided higher kinetic energy to overcome local minima in the SurA domain dynamics, revealed a to date unknown interaction between PPI2 and NC-core. To validate our simulations, we incubated SurA at elevated temperatures prior to the assay setup.

We observed an altered behavior of SurA after heat treatment. Instead of assisting (chaperoning) the refolding of the Luciferase, SurA inhibited refolding (holdase). Mutational analysis of the PPI2 NC-core interaction surface revealed a connection between interactions between PPI2 and NC-core and holdase activity.

In summary, we developed a novel assay to investigate the holdase activity of SurA and could show that interactions between the SurA NC-core and PPI2 domain regulate this holdase activity.

1. Klein, K et al. 2019. Front. Microbiol. 10 (FEB): 100. https://doi.org/10.3389/fmicb.2019.00100.
2. Justice, SS et al. 2006. Infect. Immun.74 (8): 4793–4800. https://doi.org/10.1128/IAI.00355-06.
3. Calabrese, AN et al. 2020 Nat. Com. 11 (1): 2155. https://doi.org/10.1038/s41467-020-15702-1.
4. Schiffrin, B et al. 2022 Com. Biol. 5 (1): 560. https://doi.org/10.1038/s42003-022-03502-w.
5. Marx, DC et al 2020 Prot. Sci. 53 (9): 1689–99. https://doi.org/10.1002/pro.3924.
6. Jia, M et al 2020 Biochem. 59 (35): 3235–46. https://doi.org/10.1021/acs.biochem.0c00507.

OP-MP-016

Antisense oligomers repress the genotoxic activity of colibactin producing E. coli

*S. Nentwich[1], L. Popella[1], J. Vogel[1,2]
[1]*Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, Germany*
[2]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*

The genotoxin colibactin is a secondary metabolite produced by several *Enterobacteriaceae* that triggers interstrand crosslinking of DNA and thereby induces DNA damage in eukaryotic cells. The presence of colibactin-producing bacteria has been correlated to the promotion of colorectal cancer and urinary tract tumors. Further, a specific mutational signature is associated with the genotoxic activity of colibactin. Colibactin is encoded by a 54-kb gene cluster, referred to as *pks* (polyketide synthase) island, which consists of 19 *clb* genes (*clbA-clbS*).

Here, we present a new strategy to repress the production of colibactin via targeted gene knockdown using antisense

oligomers. Specifically, various peptide nucleic acid (PNAs) 10mers were designed to inhibit translation of *clbA, clbP, clbQ*, and *clbR*. The efficiency of PNAs to dampen colibactin production was evaluated at different levels. First, PNA-mediated decrease of target protein level was validated *in vitro* as well as in living bacterial cells. Second, we provide evidence that PNAs can decrease the *in vitro* crosslinking capacity of colibactin-producing bacteria. Third, cell culture infection experiments revealed a reduction of the colibactin-induced mammalian DNA damage response when bacteria were pretreated with specific PNAs.

In summary, we identified three promising PNAs, two of them targeting *clbA*, involved in the activation of colibactin synthesis, and one targeting the key transcriptional activator ClbR. Further research is needed to evaluate off-target effects of the PNAs and to optimize their delivery to target infected mammalian cells and organoids.

OP-MP-017

Utilizing a novel reactive oxygen species-generating antimicrobial to potentiate antibiotics

P. Tawiah[1], G. Y. Donkor[1], L. F. Gaessler[1], K. P. Hoffmann[1,2], L. Pfirsching[1], *J. U. Dahl[1]
[1]*Illinois State University, School of Biological Sciences, Normal, IL, United States*
[2]*DAAD RISE FELLOW, Technical University Braunschweig, Brunswick, Germany*

The emerging antimicrobic resistance and decline in the discovery of new antibiotics requires drug development to consider alternative strategies. Silver-containing compounds became a promising alternative due to their multi-specific effects on inhibiting bacterial growth. We focus on the antimicrobial effects of AGXX, a novel antimicrobial composed of silver and ruthenium, which exerts its broad-spectrum effects through the production of highly cytotoxic reactive oxygen species (ROS) that inflict extensive macromolecular damage. Due to recent connections identified between ROS-stress and antibiotic activity, we hypothesized that AGXX could potentially increase the activity of conventional antibiotics. We investigated possible synergistic effects of AGXX on members of several antibiotic classes in 5 different bacterial pathogens. We discovered that combined treatment of sublethal concentrations of AGXX and aminoglycoside antibiotics exponentially enhanced their bactericidal activity and restored sensitivity in aminoglycoside resistant clinical isolates. To explore the underlying mechanism of the synergizing effects of AGXX on aminoglycosides, we utilized redox sensitive probes and found that the bactericidal effect of the combined AGXX/aminoglycosides treatment is mediated through elevated ROS production, which was attenuated with the addition of the ROS scavenger thiourea. We discovered that the increase in oxidative stress resulted in a disruption of iron homeostasis evidenced by a significant decrease in aconitase activity under combined AGXX/aminoglycoside treatment. Using spectroscopic methods and fluorescent microscopy, we further demonstrate that combined treatment of the two antimicrobials inflicted significant membrane damage, thus increasing membrane permeability. Our subsequent goal is to further delineate the mechanisms that underpin this synergistic effect by exploring how the metabolic processes and cellular targets disrupted by AGXX contributes to the synergistic effects with aminoglycosides. Our findings will potentially provide an understanding of cellular targets that could be inhibited to increase the activity of conventional antimicrobials.

OP-MP-018

Functional inhibition of the virulence factor ProA from Legionella pneumophila by novel zinc-binding compounds

*L. Scheithauer[1], A. Hirsch[2], J. Haupenthal[2], J. Selmar[1], A. Dellmann[3], R. Brouwer[4], R. Zhang[4], M. Steinert[1]
[1]*Technical University of Braunschweig, Institute of Microbiology, Brunswick, Germany*
[2]*Helmholtz Institute for Pharmaceutical Research Saarland, Drug Design and Optimisation, Saarbrücken, Germany*
[3]*Städtisches Klinikum Braunschweig, Pathology, Brunswick, Germany*
[4]*Städtisches Klinikum Braunschweig, Cardiothoracic and Vascular Surgery, Brunswick, Germany*

The M4 zinc metalloprotease ProA is an important effector protein of the human lung pathogen *Legionella pneumophila*, which is known as the causative agent of Legionnaires' disease. Due to its versatile spectrum of substrates, ProA contributes to bacterial proliferation, immune evasion, and serious tissue destruction. Successful treatment of the atypical pneumonia is based on antibiotic therapies but cannot always prevent severe symptoms and irreversible lung damage. Additional application of specific inhibitors against virulence factors like ProA might be able to reduce life-threatening complications in patients. In order to identify promising candidates, inhibitory effects of ten novel-designed zinc-binding substances were initially evaluated in azocasein assays. The three best-performing compounds were chosen for subsequent analyses regarding versatile physiological functions of ProA during infection. It was demonstrated that a 20-fold molar excess compared to the enzyme leads to successful inhibition of human collagen IV degradation, cleavage of bacterial flagellin, immune evasion from TLR5-mediated immunity, and PMN inflammation in human lung tissue explants. Interestingly, biochemical assays revealed different tendencies in terms of inhibitor efficacy than studies in a host background. The present results not only indicate great potential of the selected zinc-binding compounds for ProA research but also demonstrate methods which are suitable to test potential drug compounds on different and important organizational levels during infection.

OP-MP-019

Shiga Toxin Subunit Genes of Enterohemorrhagic Escherichia coli Are Not Transcribed in Equal Amounts

*K. Neudek[1], H. Schmidt[1]
[1]*University of Hohenheim, Institute for Food Science and Biotechnology, Department of Food Microbiology and Hygiene, Stuttgart, Germany*

Introduction and Aim: The production of Shiga toxins is the major virulence factor of enterohemorrhagic *Escherichia coli* (EHEC). Shiga toxins are AB5 protein toxins consisting of a single enzymatically active A-subunit and a pentamer of non-covalently linked B-subunits. The genes encoding the Stx2 subunits, *stxA*$_2$ and *stxB*$_2$, are located in the late-regulated phage region within the genome of lambdoid prophages, downstream of the antiterminator gene Q and upstream of the genes encoding the phage lysis cassette. The aim of this study was to quantitatively analyze the relation of the transcription of *stxA*$_2$ and *stxB*$_2$ in five EHEC wildtype strains. Due to the operon structure of the *stx* genes, we hypothesized either a 1:1 transcription ratio, or due to the AB_5 structure of the toxin, a 1:5 ratio.

Material and Methods: To perform quantitative transcriptional analysis, total RNA was isolated and purified

from *E. coli* O157:H7 strain HUSEC003, *E. coli* O157:H- strain HUSEC004, *E. coli* O103:H- strains HUSEC008, *E. coli* O26:H11 strain HUSEC018 and *E. coli* O104:H4 strain LB226692. Subsequently, qRT-PCR was performed to determine the transcript levels for $stxA_2$, $stxB_2$, and the housekeeping gene *rrsB,* which was used as an internal control.

Results and Summary: Surprisingly, $stxA_2$ was expressed approximately ~ 1.3 - 2.5 times stronger than $stxB_2$ in all investigated EHEC wildtype strains. The analyzed genes were not expressed in the expected ratios, indicating that free A-subunits might circulate in the bacterial environment which do not find a B-pentamer for holotoxin formation. Further analysis on translational and protein level should help to clarify this phenomenon.

OP-MP-020

Improved purification of the recombinantly expressed A-subunit of Shiga toxin 2a with fast protein liquid chromatography

*J. Fellendorf[1], H. Schmidt[1]
[1]*University of Hohenheim, Institute for Food Science and Biotechnology, Stuttgart, Germany*

Introduction and Aim: Shiga Toxins are the major pathogenicity factors of enterohemorrhagic *E. coli* (EHEC). Stx are AB_5 toxins, the A-subunit of which inhibits eukaryotic protein biosynthesis. The B-pentamer is responsible for binding to the receptor globotriaosylceramide. Since the A-subunit alone was already shown to be functional without its corresponding B-subunit, investigation and analysis of the mechanisms are of interest. Since mg amounts of toxin are needed for detailed analysis, current protocols of protein purification had to be optimized.

The aim of the current study was to increase the yield of the pure A-subunit to a final yield of 200-300 µg $StxA_{2a}$ per litre expression culture.

Materials and Methods: For the expression of $StxA_{2a}$, the $stxA_{2a}$ gene was cloned into the vector pET45b(+) under the control of a T7 promotor, and transformed into *E. coli* BL21 (DE3) C43. In a first step the yield of $StxA_{2a}$ was optimized under different expression conditions (expression medium, expression time, expression temperature, IPTG concentrations). In a second step, $StxA_{2a}$ was purified with a single step purification using ion exchange chromatography.

Results: The optimal expression of $StxA_{2a}$ was achieved in terrific broth after an incubation of 24 h at 30°C. The expression of $StxA_{2a}$ was induced with 1 mM IPTG. Since the His-tag present on the $StxA_{2a}$ was not accessible for purification on a metal-ion affinity column, ion exchange chromatography was used. Following this one step purification protocol, a yield of 567 µg pure $StxA_{2a}$ per litre expression culture was achieved.

Summary: The optimization of the expression and purification of $StxA_{2a}$ leaded to a 5.5 times higher yield of pure $StxA_{2a}$ to former protocols. The purified $StxA_{2a}$ will be used for further studies on the cytotoxicity, the cellular uptake and the intracellular transport of $StxA_{2a}$.

OP-MP-021

Flexibility of the PPI2 domain influences the activity of the periplasmic chaperone SurA in vivo

*J. Fischenbeck[1,2], N. Bohn[1,2], F. Renschler[1,2,3], T. Kronenberger[4,5,6], E. Bohn[1,2,3,6], M. Schütz[1,2,3]
[1]*Eberhard Karls University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*
[2]*University Hospital Tübingen, Institut für Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*
[3]*German Center for Infection Research, Partner Site Tübingen, Tübingen, Germany*
[4]*University of Eastern Finland, School of Pharmacy, Kuopio, Finland*
[5]*Eberhard Karls University of Tübingen, Tücad2, Faculty of Science, Tübingen, Germany*
[6]*Eberhard Karls University of Tübingen, Excellence Cluster "Controlling Microbes to Fight Infections" (CMFI), Tübingen, Germany*

The periplasm of Gram-negative bacteria is comprised of a complex chaperone network, facilitating the transport of unfolded proteins to the outer membrane (OM). One major chaperone is SurA, which interacts with the β-barrel assembly machinery (BAM) complex and prevents aggregation of unfolded outer membrane proteins (OMPs) during their transport across the periplasm. SurA consists of three distinct domains: the NC-core and two peptidyl-prolyl-isomerase (PPI) domains PPI1 and PPI2. Previous studies revealed flexible "closed to open" conformational dynamics of these two PPI domains (1). While the binding of substrates involves mainly interactions within the PPI1/NC-core, the PPI2 domain can be linked to BAM-complex binding *in vitro* (2). Molecular dynamics simulations suggested, that the PPI2 domain forms salt bridges with the NC-core. Destabilization of these bonds via alanine substitutions results in increased SurA activity *in vitro*. Knowledge about PPI2 and the function of its interaction with the NC-core function remained scarce *in vivo*. Hence, we wanted to find out how interference with these interactions might impact the function of SurA in whole cells.

To investigate the impact of weakened or stabilized interactions between PPI2 and the NC-core, we either created alanine substitutions or introduced disulfide bonds within the interaction surface and expressed the resulting SurA variants from the chromosome in an *E. coli* BW25113 *ΔsurA* strain. The activity of the SurA variants *in vivo* was then tested by probing OM permeability and the induction of a σE stress response using a PmicA-GFP reporter system. Thereby we aimed to quantify the cellular stress levels due to the accumulation of unfolded OMPs in the periplasm (3).

Taken together, we found distinct phenotypes regarding OM integrity and σE stress in the periplasm of *E. coli* expressing SurA variants carrying changes in the PPI2/NC core interaction interface, that were correlated with the degree of flexibility of the PPI2 domain.

1. Jia, M, et al. Biochemistry. 2020;59(35):3235-46
2. Schiffrin, B, et al. Communications Biology. 2022;5(1):560
3. Konovalova, A, et al. Proc Natl Acad Sci U S A. 2018;115(28):E6614-e21

OP-MP-022

Consequences of ClpX deficiency in Staphylococcus aureus under infection-mimicking conditions – a proteomic investigation

*L. Busch[1], C. Hentschker[1], M. Kröber[1], H. Wolfgramm[1], E. Schmidt[1], A. Ganske[1], U. Mäder[1], U. Gerth[2], S. Michalik[1], A. Reder[1], K. Surmann[1], U. Völker[1]
[1]*University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany*
[2]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*

Introduction: Pathogenic bacteria have to cope with host-mediated stressors such as iron limitation and micro aerobic conditions. To do so, bacteria have to adapt gene expression as well as proteome profile and activity. In the pathogen *S. aureus*, protein homeostasis, and especially protein degradation and folding, is largely mediated by the Clp system consisting of the ClpP peptidase and the unfoldases ClpX and ClpC. The Clp protease system is crucial for general and targeted proteolysis, which relies on unfoldases interacting with specific targets. In particular, ClpX is the most conserved Clp unfoldase and it is involved in regulation of virulence.

Goals: The majority of ClpX targets remains elusive in *S. aureus* despite the clear role of the unfoldases in virulence and fitness. We therefore aim to investigate the proteomic effect of ClpX deficiency under infection-mimicking conditions.

Methods: For investigation of the effect of ClpX deficiency the *S. aureus* mutant strain HG001 Δ*clpX*::*km* and the complemented strain HG001 Δ*clpX*::*km* pTripleTREP_clpX were constructed employing the newly developed plasmid systems pSauSE (allele exchange plasmid) and pTripleTREP (controllable expression plasmid). Mass spectrometry-based proteome profiles were then recorded for *S. aureus* HG001 and the constructed isogenic mutants under aerobic iron-rich control conditions, micro aerobic conditions and iron depleted conditions in exponential and stationary growth phase.

Results: The proteomic profiles reveal ClpX- and condition-dependent changes. Known ClpX-targets such as Spx, Sle1 and Spa were confirmed. Under infection-relevant conditions, iron limitation generally induced the Fur regulon and repressed the IsrR targetome, and under micro aerobic condition the NreBC regulon and the Rex regulon are induced, as expected. Moreover, ClpX-dependent condition-specific effects, such as a sub regulon of the known Fur regulon which is less responsive to the provoked iron limitation in the Δ*clpX* mutant, were also observed.

Summary: By presenting our proteomic study, we provide a global insight into ClpX-dependent adaptation of *S. aureus* physiology under infection-relevant conditions.

OP-MP-023
Exploring Essential Genes in Staphylococcus aureus JE2-MRSA by: Transposition-Directed Insertion Site Sequencing (TraDIS)
*S. Waheed Yousief[1], N. Abdelmalek[1], J. E. Olsen[2], B. Paglietti[1]
[1]*Sassari University, Life Science and Biotechnology, Sassari, Italy*
[2]*University of Copenhagen, Department of Veterinary and Animal Sciences, Copenhagen, Denmark*

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major public health concerns worldwide causing a variety of clinical diseases including sepsis and abscess/skin infections. Transposon Directed Insertion-site Sequencing (TraDIS) is a genome-wide mutagenesis method, which allows simultaneous assaying of large transposon mutant libraries for exploring gene significance, functionality, and inter-genetic relationships in bacteria. In the current study, our goals were i) to construct a high-density Tn-mutants library in the JE2-MRSA strain of this species, and ii) and to identify the essential genes for in growth *in vitro* and for *in vivo* infection of skin and organs in a mouse model. We transducted the Tn-5 transposon/transposase complex into the strain by phage ø11 using an improved transduction protocol to create a high-density transposon library of 10^9 mutants. After the analysis by Bio-Tradis analysis pipeline, we found that the library containing 315,400 unique insertion sites (i.e. one insertion every 9 base pairs). We identified 213 essential genes with zero transposon insertion, suggesting that these genes were essential for growth on BHI agar medium, representing 8% of the total JE2 MRSA genome. Studies to identify essential genes for *in vivo* infection is ongoing and will be reported.

Membranes and Transport

OP-MT-001
The exchange of central parts of the bacterial type III secretion system is required for assembly and function of the syste
*A. Diepold[1]
[1]*Max-Planck Institute for Terrestrial Microbiology, Ecophysiology, Marburg, Germany*

Important bacterial pathogens manipulate eukaryotic target cells by injecting effector proteins through type III secretion systems (T3SS). Recent *in situ* observations revealed that these large molecular machines, also called injectisomes, are amazingly dynamic and adaptive entities. We recently found that the cytosolic components of the T3SS form a mobile network that shuttles effector proteins from the cytosol to the export machinery (Wimmi *et al*, Nature Microbiol 2024). In contrast to these soluble components, the transmembrane rings anchoring the injectisome are stably associated – with one exception. Using live cell microscopy, single particle tracking and functional assays, we found that SctD, which forms the inner membrane ring of the T3SS, exchanges subunits in secreting injectisomes. To decipher the biological function of the unexpected mobility of this central structural component of the T3SS, we analyzed its role in assembly and function of the injectisome. Based on molecular dynamics modeling, we engineered SctD to allow to tune its exchange rate. The experiments showed that lower exchange leads to decreased secretion of effectors, revealing a direct correlation between protein exchange and the function of the T3SS. Our findings uncover a new aspect of the molecular function and regulation of the T3SS, which may be applicable to other secretion systems and molecular machines.

OP-MT-002
Functional dissection of transport processes during glycopeptide antibiotic production
*N. Gericke[1], D. Beqaj[1], T. Kronenberger[1], M. Franz-Wachtel[2], A. Gavrilidou[1], E. Stegmann[1], S. Wagner[1]
[1]*University of Tuebingen, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Tübingen, Germany*
[2]*University of Tuebingen, Proteome Center Tuebingen, Tübingen, Germany*

Glycopeptide antibiotics (GPAs) - e.g., vancomycin - are natural products that have been used for many years as a last resort and are an important group of agents to fight bacterial infections by inhibiting cell wall biosynthesis. GPAs are modified heptapeptides that are synthesized by non-ribosomal peptide synthetases (NRPS) from proteinogenic and non-proteinogenic amino acids. However, their modification results in a variety of different structures. In addition to all the biosynthetic proteins, we noticed that a transport-related protein was always co-encoded in the associated biosynthetic gene cluster (BGC). Interestingly, we found that in all 89 BGCs we analyzed, this was an ABC transporter. In *A. balhimycina*, the transporter Tba is essential for the export of the GPA balhimycin. However, a detailed functional analysis of its specificity and interactions in the cellular context is not yet available, which is the primary goal of our work.

We performed a phylogenetic analysis of GPA ABC transporters in the context of other transporters encoded by producer strains and found that they form a distinct clade. We have established a balhimycin export assay that allows the investigation of the specificity of different GPA transporters i*n vivo* by HPLC-MS and bioassays. This indicates substrate specificity of different GPA transporters, despite high similarity in sequence and structure. Furthermore, MD simulation and mutagenesis studies led us to suggest, which parts of the GPA are important for the interaction with the transporter. To confirm this *in vitro*, we are trying to purify Tba. Additionally, our data indicate a regulatory mechanism of the balhimycin production in absence of the native transporter. We have therefore used different omics approaches to investigate it further. So far, we have ruled out another regulation at the transcriptional level.
Finally, we are trying to understand the role of GPA production and export in a cellular context by using MS-based methods to identify protein-protein interactions after proximity biotinylation and immunoprecipitation.

OP-MT-003
Characterization of the membrane phosphodiesterase NbdA of Pseudomonas aeruginosa.

*S. Zehner[1], J. Rehmel[1], N. Bäuerle[1], A. Scherhag[1], M. T. Agbadaola[1], M. Schösser[1], N. Frankenberg-Dinkel[1], E. Pérez Patallo[1]
[1]University of Kaiserslautern-Landau, Microbiology, Kaiserslautern, Germany

The molecule c-di-GMP is a second messenger that controls various processes such as virulence and biofilm formation in bacteria (1). Synthesis and degradation of c-di-GMP is mediated by two types of enzymes, diguanylate cyclases (DGC) and phosphodiesterases (PDE) (1, 2). *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen and biofilm model organism, encodes more than 40 c-di-GMP modulating proteins (3). In total, 22 of these proteins are predicted to be membrane bound. Until now, the biochemical characterization of these proteins has been limited mostly to their soluble cytosolic domains (4).The phosphodiesterase NbdA comprises a membrane bound MHYT domain, predicted to be a sensor for NO, CO or O2 (5), and a cytosolic part consisting of a degenerated diguanylate cyclase and a functional phosphodiesterase domain. In vivo assays showed the phosphodiesterase activity of NbdA, that could be confirmed in vitro with the purified cytosolic part of the protein (4). We established the production and purification of the full- length protein from *E. coli* using the nanodisc forming polymer DIBMA. We obtained pure and enzymatically active NbdA and characterized the binding of heme to the full-length protein. Furthermore, to study putative interaction partners of the full-length protein NbdA, we analyzed *Pseudomonas* membranes by mass spectrometry and complexome profiling (6). Several identified interaction partners were subsequently confirmed by bacterial two hybrid analysis. With this work we shed light on another part of the complex c-di-GMP signaling network of *Pseudomonas aeruginosa*.

(1) Hengge, R. (2009) Nature Rev. Microbiol. 7: 263–273.
(2) Römling, U. et al. (2005). Mol. Microbiol. 57: 629–639.
(3) Valentini, M.& Filloux, A. (2016). J. Biol. Chem. 291: 12547–12555.
(4) Li, Y. et al. (2013) *J. Bacteriol.*, 195 (16), 3531-3542.

(5) Galperin, M. Y. et al. (2001) FEMS Microbiol. Lett 205 (1): 17–23.

(6) Scherhag, A. et al. (2023) microLife. 4:uqad028.

National Reference Centers and Consiliary Laboratories

OP-NRC-001
Carbapenemases in Germany in 2023: Report of the German National Reference Centre for Multidrug-resistant Gram-negative Bacteria

*N. Pfennigwerth[1], M. Cremanns[2], J. Eisfeld[1], J. B. Hans[1,3], A. Anders[1], S. G. Gatermann[1]
[1]Ruhr-Universität Bochum, Department of Medical Microbiology, Bochum, Germany
[2]Ruhr-University Bochum, Department of Medical Microbiology, Bochum, Germany
[3]Robert Koch Institute, Department of Infectious Disease Epidemiology, Unit 37: Healthcare-Associated Infections, Surveillance of Antibiotic Resistance and Consumption, Berlin, Germany

Question:

Multidrug-resistance in *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since hardly any innovative antimicrobial drug against gramnegative bacteria will be introduced within the next years. Among all resistance mechanisms the spread of carbapenemases is the most worrisome. However, the correct identification of carbapenemases is still challenging and molecular epidemiology of carbapenemases is required.

Methods:

The German National Reference Centre for Multidrug-Resistant Gramnegative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs. All isolates are tested by a wide array of phenotypic and molecular methods. A bioassay based on cell-free extracts and WGS methods allow the detection of still unknown β-lactamases.

Results:

A total of 8.319 isolates were investigated for carbapenemases at the National Reference Centre in 2023 until November 29th. Carbapenemases were found in 3.513 *Enterobacterales* strains, 520 of *P. aeruginosa* and 375 of *A. baumannii*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 612), NDM-1 (n = 481) and KPC-2 (n = 442), which were also found in various combinations, e.g. NDM-1/OXA-48 (n = 125). Other enzymes like NDM-4, OXA-232 or GIM-1 were found in less than 60 isolates each. In *P. aeruginosa,* VIM-2 was the most frequent carbapenemase (n = 291), followed by NDM-1 (n = 61). OXA-23 was again the most frequent carbapenemase in *A. baumannii* (n = 220), followed by OXA-72 (n = 95). NDM-1, OXA-58 and others were found in less than 10 isolates each.

Conclusions:

A large variety of different carbapenemases is detected in Germany, especially in *Enterobacterales*. The molecular epidemiology in Germany still significantly differs from observations made in other countries like Greece, Italy or the USA with a predominance of OXA-48 in *Enterobacterales*. Compared to previous years, the number of NDM-1 detections remained high. Furthermore, detection of NDM-5 and OXA-244 showed a significant increase compared to 2022.

OP-NRC-002

Genomic surveillance identifies stable C. jejuni lineages causing disease in humans

*S. Banerji[1], A. Fruth[2], A. Flieger[2]
[1]*Robert Koch-Institute, FG11/NRZ/AG Campylobacter, Wernigerode, Germany*
[2]*Robert Koch-Institute, FG11, Wernigerode, Germany*

Question: *Campylobacter* enteritis is a notifiable disease in Germany. More than 40,000 cases are reported annually. The majority of cases is caused by *C. jejuni* and is considered to be sporadic. In order to gain more insight into the population structure of clinical *C. jejuni* strains genomic surveillance was established in 2020. Here we describe major genomic lineages of *C. jejuni*, which play a central role in human disease.

Methods: *Campylobacter* spp. were cultivated on CCDA under microaerophilic conditions followed by PCR-based species determination. Whole genome sequencing was performed with Illumina NextSeq. Analysis of reads was conducted with Ridom SeqSphere+.

Results: Four years of surveillance data (2020-2024) reveal the existence of stable *C. jejuni* lineages as the cause of a significant amount of *Campylobacter* enteritis cases in Germany. To date, 14 lineages comprising approximately 850 (21%) isolates have been identified. This corresponds to approximately 8400 cases as only 4% of all notified cases are covered by the molecular surveillance program. The majority of isolates (124) belonged to complex type (CT) 2243. Case numbers peaked from February - April 2021 (36 cases) and December - February 2022 (35 cases). A smaller peak occurred in July 2023 (6 cases), indicating that this lineage is recurrent and stable over time. Another remarkable lineage is CT 2151 (ST-7355), which currently comprises 96 isolates, peaking from April – October 2022 (45 cases). This lineage has been associated with clinical cases in Denmark, Sweden and Luxembourg but has also been isolated from German poultry and poultry farms. Many of the lineages belong to CC ST-21 (6/14). Four of the six CC ST-21 lineages belong to ST-50. ST-50 has been described to occur worldwide and evolve regionally (Wallace et al. 2021). All major lineages identified so far have matching isolates from poultry origin.

Conclusion: Data from genomic surveillance points to the existence of internationally distributed recurring *C. jejuni* lineages causing the majority of *Campylobacter* enteritis cases in Germany. Therefore, an international one health approach is required for achieving reduction of cases.

OP-NRC-003

Laboratory confirmation during a large cross-border outbreak of Iatrogenic Botulism in Europe in 2023

*M. Dorner[1], C. Frank[2], M. Skiba[1], L. V. Wilk[1], M. Steinberg[1], S. Worbs[1], H. Wilking[2], K. Stark[2], B. Dorner[1]
[1]*Robert Koch-Institut, ZBS3 - Biologische Toxine, Berlin, Germany*
[2]*Robert Koch-Institut, FG35, Berlin, Germany*

Iatrogenic botulism is an adverse event after the injection of botulinum neurotoxin (BoNT) for medical or cosmetic purposes and is usually characterized by mild symptoms frequently involving the muscles adjacent to the injection site. The German national Consultant Laboratory for BoNT-producing Clostridia (Botulism, Tetanus) at the Robert Koch Institute was involved in the investigation of a highly unusual outbreak of travel-associated iatrogenic botulism: 30 out of 33 patients from Germany known to have received intra-gastric injection of BoNT/A for weight reduction in Istanbul (Turkey) developed symptoms of botulism, some of them were admitted to the ICU. The low concentration of BoNT in iatrogenic botulism usually prevents laboratory confirmation in human serum using standard assays (e.g., mouse bioassay). Indeed, state-of-the-art diagnostics of botulism is generally challenging due to the high molecular variability within the BoNT family, its limited time window for detection in serum and the ultimate sensitivity needed. We applied i) an Endopep-MS assay and ii) an Endopep-suspension immunoassay based on neoepitope-specific monoclonal antibodies recognizing the cleavage site in comparison to the classical mouse bioassay. While the mouse bioassay failed to detected any remaining BoNT/A we succeeded in the detection of BoNT/A with both the Endopep-MS and the Endopep-suspension immunoassay. The lessons-learned for botulism diagnostics in this largest outbreak of iatrogenic botulism ever noted will be discussed.

OP-NRC-004

Genomic Antibiotic Resistance Prediction in Tuberculosis: A Comprehensive Evaluation of Whole Genome Sequencing for Routine Diagnostics in Germany

*S. Andres[1,2], V. Mohr[2,3], T. Kohl[2,3], L. Bös[4], S. Kröger[4], W. Haas[4], M. Kuhns[1,2], I. Friesen[1,2], S. Niemann[1,3,5]
[1]*Research Center Borstel, Diagnostic Mycobacteriology, Borstel, Germany*
[2]*Research Center Borstel, National Tuberculosis Reference Laboratory, Borstel, Germany*
[3]*Research Center Borstel, Molecular and Experimental Mycobacteriology, Borstel, Germany*
[4]*Robert Koch-Institut, Infection epidemiology, Berlin, Germany*
[5]*German Center for Infection Research, Borstel, Germany*

Phenotypic drug susceptibility testing (DST) remains gold standard for evaluating drug resistance in Germany, despite

limitations. Since 2020, the National Reference Center (NRC) for Mycobacteria, has integrated whole genome sequencing of tuberculosis pathogens as part of the Robert Koch Institute (RKI) coordinated IMS (integrated molecular surveillance)-TB project. Accordingly, a comprehensive data set is available to test the potential of genomic resistance prediction.

This study aims to evaluate the efficacy of genome-based antibiotic resistance prediction using the expanded WHO catalog, within a low-burden setting for routine diagnostics.

Between 2020 and 2022, over 3,000 isolates underwent phenotypic DST at the NRC using the proportion method at critical concentration in the MGIT system. As part of the IMS-TB project, each of these isolates was analyzed using next generation sequencing (NGS). Genomic identification of resistance-causing variants from the extended WHO catalogue (2021) predicted resistance against first-line drugs and group A second-line drugs (Levofloxacin, Moxifloxacin, Bedaquiline and Linezolid). The results were compared with phenotypic outcome.

For 3,148 isolates, a complete phenotypic and genotypic first-line resistance profile was produced. Among these 2,279 (72.4%) were fully susceptible against first-line drugs. NGS prediction demonstrated sensitivities above 95 % for each drug except pyrazinamide (90.4%), specificities exceeding 97 % for all four first-line drugs. The negative predictive value exceeded 99% for each of all first-line antibiotics. Group A drug analysis included 341 full datasets including 66 Fluorquinolone, 11 Bedaquiline and 5 Linezolid resistant isolates. Revealing high sensitivity and specificity for the detection of Fluorquinolone resistance (95.5% and 99.6%), while Bedaquiline and Linezolid resistant isolates must be evaluated individually due to limited numbers.

NGS-based genomic DST reliably predicts first-line susceptibility. Standard sequencing of all culture-positive isolates may replace phenotypic first-line testing, given that the technical and quality management requirements can be met.

OP-NRC-005
Identification of hemolysis-related pathogenicity factors of Bartonella bacilliformis
*A. A. Dichter[1], T. G. Schultze[1], B. Averhoff[2], W. S. Ballhorn[1], D. Munteh[1], V. Kempf[1]
[1]*University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a. M., Germany*
[2]*Goethe University Frankfurt, Institute for Molecular Bio Science, Frankfurt a. M., Germany*

Question

Bartonella bacilliformis is the causative agent of Carrion's disease, a vector-borne illness restricted to the South American Andes. In the acute phase, bacteria infect erythrocytes causing severe hemolytic anemia with case-fatality rates as high as 90% in untreated patients. Erythrocyte invasion is the most important step in the pathogenesis of Carrion's disease and results in its subsequent destruction. Exact knowledge of this process is crucial for drug development.

Methods

To identify genes involved in hemolysis, a Tn5 transposon library was generated and screened for hemolytic activity. Hemolysis mutants were identified and systematically analyzed by loss of function/gain of function experiments. For this, markerless deletion was newly established for *B. bacilliformis*, and the generated deletion and complementation mutants were tested for hemolytic activity in a novel *in vitro* based hemolysis assay using human erythrocytes. To study *B. bacilliformis* hemolysis and to test potential therapeutic drugs *in vivo*, we currently establish a (humanized) murine infection model.

Results

Two hemolysis-related genes, a porin and a phospholipase, were identified. The loss of one of the two genes led to the complete inhibition of hemolysis whereas the hemolytic activity was restored by plasmid-based complementation. Although *B. bacilliformis* is believed to be a human-specific pathogen, we were still interested in whether murine erythrocytes can nevertheless be infected by *B. bacilliformis in vitro*. It turned out that murine erythrocytes undergo hemolysis upon *B. bacilliformis* infection. Murine infection experiments are ongoing to analyze the course of infection in detail.

Conclusion

Two hemolysis-related genes/proteins of *B. bacilliformis* have been identified and were functionally characterized. Ongoing work aims to establish a mouse model to study hemolysis *in vivo* and to evaluate potential anti-virulence strategies.

OP-NRC-006
Invasive meningococcal disease in Germany 2023: Requirement of an adjusted vaccination recommendation?
*H. Claus[1], K. Mohort[1], M. Krone[1], T. T. Lam[1]
[1]*Institute for Hygiene and Microbiology, University of Wuerzburg, Würzburg, Germany*

Introduction

During the COVID-19 pandemic not only a reduction of invasive meningococcal disease (IMD) cases occurred in Germany, but also changes of the affected age-groups and the serogroup distribution. At the end of 2022, the number of IMD started to increase.

Goals

To present laboratory surveillance data of IMD in Germany 2023

Materials and Methods

Data on IMD isolates and clinical samples of suspected IMD cases sent to the National Reference Center for meningococci and *Haemophilus influenzae* (NRZMHi) were analysed according to antimicrobial susceptibility, age, serogroup and whole genome sequence (WGS).

Results

In 2023, 227 *Neisseria meningitidis* (Nm) positive samples were submitted to the NRZMHi, of which 193 were viable isolates. Penicillin resistance was detected in 17 (8.8%) isolates of which one harboured a rarely occurring β-lactamase.

The age distribution of the cases was comparable to the pre-pandemic years 2018 and 2019. Most prevalent serogroups were MenB (n=107, 47,1%) and MenY (n=99, 43,6%). MenC (n=7, 3,1%) and MenW (n=10, 4,4%) cases were rare. In comparison to pre-pandemic years, MenY cases increased in the age-groups 15-19 yrs, 20-29 yrs and 80-89 yrs.

WGS data were available for 120 of the 193 meningococcal isolates. cgMLST analysis identified two genetic MenB clusters and ten genetic MenY clusters. The latter belonged to five different sequence type / finetype combinations. The genetic clusters comprised no spatio-temporally related cases.

Summary

The number of IMD cases analysed at the NRZMHi in 2023 was at a level comparable to 2018 and 2019. A major change was the increase of MenY cases from 18.4% in 2019 to 43.6% in 2023. This trend needs to be monitored and considered regarding future vaccination recommendations.

Phages and Microbial Defense Systems

OP-PMD-001
Novel sex-pilus-specific bacteriophages reduce plasmid carriage and transfer in vitro
*M. Irle[1], M. Berger[1], G. Schneider[2], U. Dobrindt[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*
[2]*University of Pecs, Department of Medical Microbiology and Immunology, Pecs, Hungary*

Antibiotic resistance genes are often encoded on conjugative plasmids, which facilitate the horizontal transfer and spreading of these genes. Bacteriophages that are targeting the sex pilus of the conjugational apparatus might therefore be useful to remove resistances from bacterial populations by (i) specifically targeting and lysing bacteria that possess a plasmid-encoded sex pilus and (ii) reducing the horizontal transfer of these plasmids.

We have identified two novel bacteriophages, ϕ4187/61 and ϕ4187/77, by a spot-assay screening of a collection of phages isolated from wastewater, that are specifically targeting plasmids belonging to Inc P, N and W. Deletion of the transfer genes from the plasmids rendered the bacteria resistant to ϕ4187/61 and ϕ4187/77, indicating that the conjugation apparatus is indeed the receptor of the phages. We show that both bacteriophages reduce the amount of plasmid-harboring bacteria and drive the selection towards plasmid-free bacterial populations in *in vitro* co-cultivation studies. Furthermore, we show that both bacteriophages also reduce the horizontal transfer of the plasmids *in vitro*. In addition to that, we have analyzed resistant bacterial isolates after phage treatment and could show that plasmid loss is one of the major phage resistance mechanisms. A second sub-population of phage-resistant bacteria still contained the plasmids. However, the vast majority of plasmid-harboring and phage-resistant bacteria had lost the capability to transfer the plasmid by conjugation, whereas a minority was still able to transfer the plasmid by conjugation, albeit at a strongly reduced frequency.

In summary, we present two novel plasmid-specific bacteriophages ϕ4187/61 and ϕ4187/77 that are specifically targeting the conjugational apparatus of plasmids. We show here that these bacteriophages can be used to drive bacterial populations towards plasmid loss and to reduce the horizontal distribution of these plasmids transfer *in vitro*. Future experiments using suitable animal models will show, if ϕ4187/61 and ϕ4187/77 are also suitable to remove plasmids from bacterial populations *in vivo*.

OP-PMD-002
PhageDive: the new data resource on prokaryotic viruses
*C. Rolland[1], J. Wittmann[1], L. C. Reimer[1], J. Sardà Carbasse[1], C. A. Dudek[1], C. Ebeling[1], B. Bunk[1], J. Overmann[1]
[1]*Leibniz Institute DSMZ, Brunswick, Germany*

Prokaryotic viruses are the most diverse and abundant biological entities on Earth. While the amount of genomic data on viruses is currently growing exponentially, mainly due to the increasing application of metagenomics techniques, these data and those generated with different experimental approaches and molecular techniques often cannot be linked to the physical virus resource. As a result, the comprehensive set of existing data for a given bacteriophage is not accessible so far and different datasets are not linked for integrative analyses. This observation led us to develop Phage*Dive* (phagedive.dsmz.de), a specific database for bacteriophages and archaeal viruses. This comprehensive database gathers all existing data on prokaryotic viruses that are dispersed across multiple sources, like scientific publications, specialized databases or internal files of culture collections. Phage*Dive* allows to link own research data to the existing information through an easy and central access. Phage*Dive* provides fields for various experimental data (lifestyle, lysis kinetics, adsorption kinetics, host range, genomic data, etc.) and all available metadata (e.g., geographical origin, isolation source). Data are standardized employing controlled vocabulary and ontologies. One important feature is to link experimental data to the culture collection number and the repository of the corresponding physical bioresource (virus and prokaryotic host strains). To date, Phage*Dive* covers 1,167 phages from three different world-renowned collections (DSMZ, Canada's Laval collection and the UK's NCTC). An advanced search using all the sections has been created and an interoperable system has been set up with other resources such as NCBI, the Institut Pasteur's Viral host range database (VHRdb) or the DSMZ's Bac*Dive* and Media*Dive*.

OP-PMD-003
Comparison of two host-phage interaction methods in Pseudomonas aeruginosa
*F. Rieper[1,2], I. H. E. Korf[2], S. Wienecke[2], D. Jahn[1], H. Ziehr[2]
[1]*Technical University of Braunschweig, Institute of microbiology, Brunswick, Germany*
[2]*Fraunhofer ITEM, Brunswick, Germany*

Goals: The aim of this work is the systematic comparison of double agar overlay plaque assay (DPA) and plankton killing assay (PKA) on a large phage-strain panel to identify strengths and weaknesses of both methods and to initially conclude which method allows for the best *in vivo* prediction.

In addition, predictions concerning a therapy regime (simultaneous / sequential) will be compared for both methods.

Introduction:During phage therapy, usually first a phagogram, similar to an antibiogram, is carried out to determine the suitability of phage(s) of interest. Various methods are described for testing phage efficacy. The two most common are DPA, which tests the host-phage interaction on solid medium, while in PKA host-phage interaction is analysed in liquid. These two methods were compared.

Materials & Methods142 *Pseudomonas aeruginosa* isolates from different clinical backgrounds (urinary tract, rectal, bronchiectasis, COPD, cystic fibrosis, wounds) were used to determine the host range of 27 phages by DPA and PKA. PKA was also performed simultaneously and sequentially on selected strains using 8 strictly lytic phages.

Results:The host spectra determined using DPA and PKA differed considerably. For *Pakpunavirus*, *Pbunavirus* and *Litunavirus*, the assessment of lytic activity demonstrated great therapeutic potential (15-47 % coverage). *Elvirus*, *Phikzvirus* (jumbophages) were underestimated with DPA (23% coverage) but lysed well in PKA (65% coverage). Many siphoviruses (mainly lysogenic) were overestimated by DPA and lysed only a few strains in PKA. *Pbunavirus, Phikzvirus* and *Bruynoghevirus* together showed an improvement in bacterial load applied sequentially and simultaneously in some strains.

Summary: Both methods have limitations. We choose PKA as standard method for phage therapy as it is automatable, independent of size/ morphology of the plaque, dependent on lifecycle of phage and comes closest to the situation in the patient. Regarding therapy regime, it is more important which phages are combined than whether it is carried out sequentially or simultaneously.

OP-PMD-004

Combining a phage cocktail with antibiotics improves recovery from Pseudomonas-induced VAP in mice

*C. V. Weißfuß[1], J. Li[1], U. Behrendt[1], M. Bürkle[1], B. Gaborieau[2,3], G. Krishnamoorthy[1], J. D. Ricard[2,3], L. Debarbieux[4], M. Witzenrath[1,5], M. Felten[1], G. Nouailles[1]
[1]*Charité - University Medicine Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany*
[2]*University Paris, INSERM, IAME, Paris, France*
[3]*APHP, Hôpital Louis Mourier, Service de Médecine Intensive Réanimation, Colombes, France*
[4]*Institut Pasteur, Université Paris Cité , CNRS UMR6047, Department of Microbiology, Bacteriophage Bacteria Host Laboratory, Paris, France*
[5]*German Center for Lung Research (DZL), Berlin, Germany*

Ventilator-associated pneumonia (VAP) poses a severe threat in hospital settings, particularly with the rise of multidrug-resistant bacteria like carbapenem-resistant *Pseudomonas aeruginosa.* Recognized as "critical priority" bacteria by the WHO, these pathogens demand innovative solutions. Our study explores the potential of bacteriophage (phage) therapy as a compelling intervention for VAP. Previously, we showed that even repetitive injections of a *P. aeruginosa* phage cocktail elicited only a marginal immune response in naïve mice.

To assess its therapeutic efficacy, we induced *P. aeruginosa*-VAP in wild-type mice through high-tidal-volume ventilation, triggering ventilator-induced lung injury, followed by intratracheal infection with *P. aeruginosa* strain PaO1. Therapy efficacy of the *P. aeruginosa*-specific phage cocktail, meropenem, or a combination of both via two intraperitoneal administrations was compared.

At 24 hpi, the combination therapy effectively reduced the bacterial load up to 3 logs in BAL and lungs, as did meropenem, but showed minimal inflammation and even faster recovery of the mice than under phage cocktail therapy. The clinical score decreased with increasing body temperatures as early as 16 hpi, whereas meropenem-treated mice did not reach normal levels until 24 hpi. Moreover, inflammatory mediators and lung permeability were reduced, but not immune cells in alveolar spaces after phage treatment.

In conclusion, our findings propose the synergistic potential of a specific phage cocktail combined with antibiotic treatment as a promising avenue for managing VAP infections caused by *P. aeruginosa*. This dual approach not only effectively combats the bacterial load, but also contributes to a swifter recovery with reduced inflammation.

OP-PMD-005

Development of a genetic system for Haloferax gibbonsii LR2-5, model host for haloarchaeal viruses

*C. Tittes[1], J. G. Nijland[1], A. M. C. Schoentag[1], T. Hackl[2], N. di Cianni[3], A. Marchfelder[3], T. E. F. Quax[1]
[1]*University of Groningen, Molecular Microbiology, Groningen, Netherlands*
[2]*University of Groningen, Eco-Evolutionary Bioinformatics, Groningen, Netherlands*
[3]*University of Ulm, Molecular Biology and Biotechnology of Prokaryotes, Ulm, Germany*

Like organisms from the other domains of life, archaea are predated upon by viruses. These viruses can have strong effects on host populations and even entire ecosystems. However, the infection mechanisms of archaeal viruses remain poorly understood. While there have been several new discoveries in viruses infecting thermoproteota (formerly crenarchaeota) in recent years, euryarchaeal viruses remain particularly understudied. To improve our understanding of these viruses, model virus-host systems are required.

The halophilic euryarchaeon *Haloferax gibbonsii* LR2-5 was isolated along with the virus HFTV1 from the hypersaline lake Retba in Senegal [1]. The strain grows well in established media used for other *Haloferax* species and is highly motile. Its genome has been fully sequenced and annotated. Additionally, it is well suited for light and electron microscopy [2]. Besides the virus it was isolated with, *Hfx. gibbonsii* LR2-5 has been shown to be infected by nine other viruses [3]. This makes it an attractive model organism for the study of virus-host interactions.

To better research the molecular mechanisms underpinning archaeal virus-host interactions, we have established a genetic system in *Hfx. gibbonsii* LR2-5 by adapting the well-established system of *Hfx. volcanii*. This system can be used for plasmid-based protein expression as well as gene deletions. Both expression- and deletion plasmids designed for *Hfx. volcanii* have been demonstrated to function in *Hfx.*

gibbonsii. This opens up a versatile genetic toolbox for the study of archaeal virus-host interactions.

References

1. Mizuno, C.M., et al., *Novel haloarchaeal viruses from Lake Retba infecting Haloferax and Halorubrum species.* Environ Microbiol, 2019. **21**(6): p. 2129-2147.
2. Tittes, C., et al., *Cellular and Genomic Properties of Haloferax gibbonsii LR2-5, the Host of Euryarchaeal Virus HFTV1.* Front Microbiol, 2021. **12**: p. 625599.
3. Aguirre Sourrouille, Z., et al., *The Viral Susceptibility of the Haloferax Species.* Viruses, 2022. **14**(6).

OP-PMD-006

Screening anti-viral compounds against a diverse set of Bacteriophages

*B. Rackow[1], N. Pozhydaieva[2], L. Kever[1], K. Höfer[2], J. Frunzke[1]

[1]*Research Center Juelich , Institute of Bio- and Geosciences, Jülich, Germany*

[2]*Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany*

Under the constant threat of predation by bacteriophages (or phages), bacteria evolved a huge arsenal of anti-phage defense mechanism. The majority of antiphage defense systems reported so far revolve around proteins or RNA complexes that function at the cellular level. Recently, the DNA-intercalating anthracyclines[1] as well as several aminoglycoside antibiotics[2], produced by *Streptomyces*, were shown to specifically block DNA replication of various dsDNA phages. Despite this discovery, the precise mechanism by which these compounds exert their effects and the specific phage characteristics that render them susceptible to these substances remain unknown.

Most antiphage molecules reported so far are produced by environmental bacteria of the genus *Streptomyces*. This study centers on antiphage compounds produced by *Streptomyces* and seeks to evaluate the specific phage characteristics that render them susceptible to chemical defense mechanisms[1,2]. For this purpose, the BASEL phage collection, comprising 69 diverse *Escherichia coli* phages, as well as several *Streptomyces* phages from our own collection were screened to investigate sensitivity determinants on the phage genome towards these anti-viral compounds[3]. This screening revealed distinct and taxonomically-related clusters of phages which are sensitive to specific compounds and emphasize an important impact of phage epigenomic modifications.

References

1 Aël Hardy, Larissa Kever, and Julia Frunzke* "Antiphage small molecules produced by bacteria – beyond protein-mediated defences" (2022), doi: 10.1016/j.tim.2022.08.001

2 Kever, L. et al. "Aminoglycoside Antibiotics Inhibit Phage Infection by Blocking an Early Step of the Infection Cycle" (2022), doi: 10.1128/mbio.00783-22

3 Maffei, E. et al. "Systematic exploration of *Escherichia coli* phage-host interactions with the BASEL phage collection" (2021), doi: 10.1371/journal.pbio.3001424

OP-PMD-007

Bacteriophages in ancient human gut metagenomes

*P. Rozwalak[1], J. Barylski[2], Y. Wijesekara[3], B. Dutilh[1], A. Zielezinski[4]

[1]*Friedrich Schiller University Jena, Microverse Cluster, Jena, Germany*

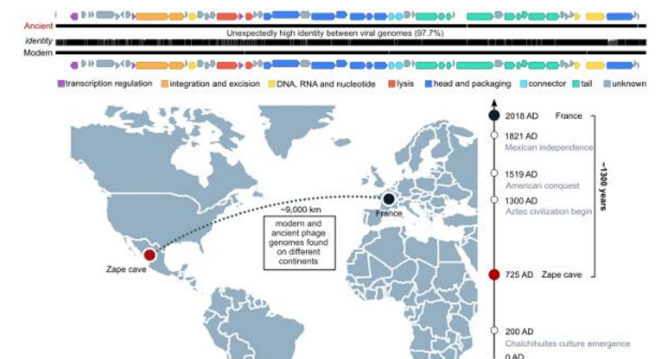
[2]*Adam Mickiewicz University, Department of Molecular Virology, Poznan, Poland*

[3]*University Medicine Greifswald, Institute of Bioinformatics, Greifswald, Germany*

[4]*Adam Mickiewicz University, Department of Computational Biology, Poznan, Poland*

Bacteriophages (phages), viruses that infect bacteria, are highly diverse and abundant everywhere on Earth. Recent advances in metagenomic sequencing and computational analysis have made it possible to explore the vast genomic diversity of phages and study their evolution and ecology. However, most studies only sample present-day phages, so they lack a long-term perspective on their evolution. In this study, we present the *de novo* assembly of 298 ancient phage genomes from publicly available ancient human gut metagenomes dated up to 5300 years before present. We authenticated their ancestral origin based on DNA deamination patterns. Next, we analyzed their relationships to modern viruses from the gastrointestinal tract and predicted hosts with cutting-edge tools. Our analysis revealed ancient representatives of known virus families and genera, as well as ancient phages that were unclassified or only distantly related to known viruses. We also discovered a genome of *Mushuvirus mushu*, a phage that infects *Faecalibacterium prausnitzii*, and other commensal bacteria in the human gut ecosystem. Despite 1300 years of evolution on different continents, modern and ancient *Mushuvirus* genomes share 97.7% nucleotide identity and suggest a long-term mutualistic relationship between the prophage and its host. Overall, our results demonstrate the feasibility of reconstructing high-quality ancient phage genomes and offer new insights into phage-bacteria interactions, expanding our understanding of the virosphere.

Fig. 1



OP-PMD-008

Wall teichoic acid substitution with glucose governs phage susceptibility of Staphylococcus epidermidis

*C. Beck[1], J. Krusche[1], A. Notaro[2], A. Walter[3], L. Kränkel[1], A. Vollert[4], R. Stemmler[1], J. Wittmann[5], M. Schaller[4], C. Slavetinsky[6], C. Mayer[3], C. De Castro[7], A. Peschel[1]

[1]*University of Tübingen, Infection Biology, Tübingen, Germany*

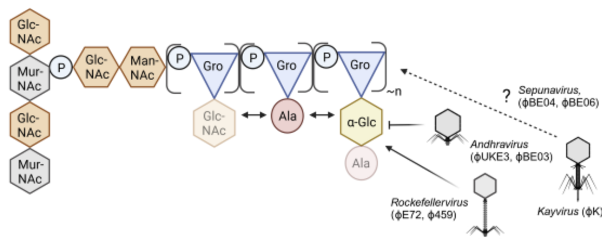
[2]*University of Naples, Department of Agricultural Sciences, Naples, Italy*

[3]*Interfaculty Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbiology/ Organismic Interactions, Tübingen, Germany*

[4]*University Hospital Tübingen, Dermatology, Tübingen, Germany*
[5]*Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures, Brunswick, Germany*
[6]*University of Tübingen, Pediatric Surgery and Urology, Tübingen, Germany*
[7]*University of Naples, Department of Chemical Sciences, Naples, Italy*

The species- and clone-specific susceptibility of *Staphylococcus* cells for bacteriophages is governed by the structures and glycosylation patterns of wall teichoic acid (WTA) glycopolymers. The glycosylation dependent phage-WTA interactions in the opportunistic pathogen *Staphylococcus epidermidis* and in other coagulase-negative staphylococci (CoNS) have remained unknown. We report a new *S. epidermidis* WTA glycosyltransferase TagE whose deletion confers resistance to siphoviruses such as ΦE72 but enables binding of otherwise unbound podoviruses. *S. epidermidis* glycerolphosphate WTA was found to be modified with glucose in a *tagE*-dependent manner. TagE is encoded together with the enzymes PgcA and GtaB providing uridine diphosphate-activated glucose. ΦE72 transduced several other CoNS species encoding TagE homologs suggesting that WTA glycosylation via TagE is a frequent trait among CoNS that permits inter-species horizontal gene transfer. Our study unravels a crucial mechanism of phage-*Staphylococcus* interaction and of horizontal gene transfer and it will help in the design of anti-staphylococcal phage therapies.

Fig. 1



OP-PMD-009
Molecular cross-talk between Sa3int phages and their Staphylococcus aureus host
*R. Dobritz[1], C. Rohmer[2], M. Bäcker[3], E. Niepoth[1], C. Wolz[1]
[1]*Interfaculty Institute for Microbiology and Infection medicine, University Tübingen, Tübingen, Germany*
[2]*Fraunhofer Institute, Stuttgart, Germany*
[3]*Friedrich Schiller University Jena, Jena, Germany*

As a major opportunistic pathogen of human and animals *Staphylococcus aureus* asymptomatically colonizes the nasal cavity, but is also a leading cause of life-threatening acute and chronic infections. More than 90% of the human nasal isolates of *S. aureus* were found to carry Sa3int phages, which integrate as prophages into the bacterial *hlb* gene disrupting the expression of an important virulence factor. The virulence factor-encoding genes carried by the Sa3-phages are all highly human-specific and probably essential for bacterial survival in the human host. However, how the *S. aureus* host modulates the life cycle of those temperate phages remains largely unknown (1). Our data suggest that this regulation is strain specific, with certain *S. aureus* strains being more prone than others to support either a lysogenic or a lytic life cycle (2). We constructed *S. aureus* single lysogens with integrated Sa3int prophages and found significant differences in phage transfer rates between different strains. Based on this finding, strains were grouped into low and high transfer strains. To get a more precise picture of the regulatory circuits we constructed replication deficient mutants, performed differential RNAseq and analysed a set of mutant strains. Comparison of different mutants in phenotypic assays revealed roles of known global regulators of *S. aureus*, in phage infection and replication regulation. By transcriptional start site prediction we identified promoter-regions that are differentially active in high and low transfer strains and identified putative transcriptional regulators on phage and host site by pull down assays. Overall, our results suggest a more prominent role of the host background regulatory processes in different growth phases and strains than previously assumed.

1. Rohmer C, Wolz C. The Role of hlb-Converting Bacteriophages in Staphylococcus aureus Host Adaption. *Microb Physiol.* 2021;31(2):109-122. doi:10.1159/000516645

2. Rohmer C, Dobritz R, Tuncbilek-Dere D, et al. Influence of *Staphylococcus aureus* Strain Background on Sa3int Phage Life Cycle Switches. *Viruses.* 2022;14(11):2471. Published 2022 Nov 8. doi:10.3390/v14112471

OP-PMD-010
Translation at the Phage Nucles in ΦKZ
*M. Gerovac[1,2], K. Chihara[2], L. Wicke[1,3], B. Böttcher[1], R. Lavigne[3], J. Vogel[1,2]
[1]*JMU Würzburg, AG Vogel, Würzburg, Germany*
[2]*HIRI, Würzburg, Germany*
[3]*KU Leuven, Leuven, Belgium*

Bacteriophages must seize control of the host gene expression machinery to promote their own protein synthesis. Since the bacterial hosts are armed with numerous anti-phage defence systems, it is essential that mechanisms of host takeover act immediately upon infection. Although individual proteins that modulate components of the bacterial gene expression apparatus have been described in several different phages, systematic approaches which capture the phage"s arsenal for immediate targeting of host transcription and translation processes have been lacking. In particular, there are no known phage factors that associate directly with host ribosomes to modulate protein synthesis. Here, we take an integrative high-throughput approach to uncover numerous new proteins encoded by the jumbo phage ΦKZ that target the gene expression machinery of the Gram-negative human pathogen *Pseudomonas aeruginosa* immediately upon infection. By integrating biochemical and structural analyses, we identify a conserved phage factor that associates with the large ribosomal subunit by binding the 5S ribosomal RNA. This highly abundant factor is amongst the earliest ΦKZ proteins expressed after infection and stays bound to ribosomes during the entire translation cycle. Our study provides a general strategy to decipher molecular components of phage-mediated host take-over and argues that phage genomes represent a large discovery space for proteins that modulate the host gene expression machinery. Gerovac et al. 2023 bioRxiv.

RNA Biology

OP-RNA-001
Regulation of the CTXϕ phage life cycle by a quorum-sensing controlled small RNA in Vibrio cholerae
*A. Lippegaus[1], M. Siemers[1,2], K. Papenfort[1,2]

[1]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany
[2]Friedrich Schiller University Jena, Microverse Cluster, Jena, Germany

Vibrio cholerae is a major human pathogen that causes the diarrheal disease cholera. A crucial aspect in the pathogenesis of the disease is cell-cell communication, a.k.a quorum sensing (QS), which coordinates community behaviours such as biofilm formation and toxin production. The cholera toxin is encoded by the filamentous bacteriophage CTXϕ which is irreversibly integrated into the host chromosome. Lysogenic induction of CTXϕ is well studied at the transcriptional level and depends on the host-encoded SOS response regulator LexA and the phage-encoded repressor RstR. However, post-transcriptional regulation of CTXϕ has not been studied and identifying potential post-transcriptional regulators of CTXϕ is challenging as it is only poorly expressed under standard laboratory conditions. To address this question, we performed transcriptomic analysis of *V. cholerae* under virulence-inducing conditions, resulting in the identification of dozens of differentially expressed sRNAs, including the well-characterized TarB and CarZ sRNAs, as well as several uncharacterized sRNAs. To investigate the global RNA-RNA network under virulence conditions, we used RIL-seq (RNA-interaction-by-ligation-and-sequencing). Our analysis captured 1,395 RNA-RNA chimeras interacting with Hfq, including a novel virulence-related sRNA that we named CisR (CTX inhibiting small RNA). We discovered that CisR base-pairs with and inhibits the expression of the *cep* mRNA, encoding a core structural element of the CTXϕ phage, and that the CisR sRNA is processed by RNAse E from the 3"UTR of the *prtV* mRNA, which encodes an M6 metalloprotease that is relevant for invasion of the mammalian host. Further, we found that two global regulators activate *cisR* transcription: the quorum-sensing regulator HapR in a cell density dependent manner, and the cyclic AMP receptor protein (CRP) in response to carbon starvation. Taken together, our data indicate that the core-genome encoded CisR sRNA controls the production of the horizontally acquired virulence gene that has a crucial role in the life cycle of the CTXϕ phage.

OP-RNA-002

RIL-seq analysis identifies the sRNA CrfA as an RNA sponge rewiring carbon starvation in *Caulobacter*

*M. Velasco Gomariz[1], L. N. Vogt[1], M. Siemers[1,2], K. Papenfort[1,2], K. Fröhlich[1,2]
[1]Friedrich Schiller University Jena, Jena, Germany
[2]Microverse Cluster, Jena, Germany

Caulobacter crescentus is an environmental bacterium living in nutrient-poor habitats. Adaptation of *Caulobacter* to changing physiological conditions has been extensively studied at the transcriptional level, but little is known about post-transcriptional control. In bacteria, small regulatory RNAs (sRNAs) contribute significantly to this layer of regulation by base-pairing mRNA targets, often with the help of RNA-binding proteins (RBPs), of which Hfq is the only one known to interact with sRNAs in *Caulobacter.*

To study the global Hfq-dependent RNA interactome in this species, we performed RIL-seq (RNA interaction by ligation and sequencing). Among the more than 1,000 new RNA-RNA interactions recovered in our analysis, we detected an abundant RNA pair involving the two sRNAs CrfA and R0014. CrfA has been previously identified in the *Caulobacter* response to carbon starvation, however, we now show that instead of acting directly on mRNA targets, CrfA functions as an RNA sponge, sequestering and inactivating the sRNA R0014. In turn, R0014 is a classical sRNA that base-pairs and represses its various target mRNAs encoding outer membrane receptors and components of the central metabolism.

Our study uncovers CrfA as an important non-coding regulator involved in rewiring the transcriptome of *Caulobacter* under low nutrient conditions, and to prime the bacterium for future outgrowth.

OP-RNA-003

Aspergillus fumigatus-infected neutrophils release extracellular RNAs bearing the capacity for host-pathogen cross-kingdom communication

*A. Bruch[1], X. Pan[1], M. G. Blango[1]
[1]Leibniz Institute for Natural Product Research and Infection Biology: Hans Knöll Institute, Junior Research Group RNA Biology of Fungal Infections, Jena, Germany

Fungal infections were spotlighted by the WHO in 2022 as being an important medical challenge of the 21st century, causing up to 3.75 million deaths among 1 billion infections annually and case-fatality rates of up to 90% in some populations. Immunocompromised individuals are especially susceptible to fungal infections, which are caused by opportunistic pathogens like *Aspergillus fumigatus*. Due to shortcomings in treatment and early diagnosis as well as an increased emergence of antifungal resistance, new diagnostic and therapeutic strategies are essential. RNA therapeutics are a potentially beneficial approach that might emerge from the expanding field of extracellular RNAs (exRNA), where a variety of RNA species were demonstrated to mediate cross-kingdom communication in fungal infection contexts of plants, insects, and mammalian immune cells. These exRNAs can be released in association with RNA binding proteins (RBPs) or extracellular vesicles (EVs), of which the latter derived from *A. fumigatus*-infected polymorphonuclear leukocytes (PMNs), were shown to bear antifungal capacity. To uncover a possible involvement of exRNAs in antifungal defense, we investigated RNA isolates from large and small EV fractions alongside small RNA-sequencing. Utilizing capillary electrophoresis, the extracellular environment was revealed to be enriched for 20-200 nt small RNAs (sRNA), which according to sRNA-sequencing displayed high abundances of miRNAs. Surprisingly, these miRNAs were enriched for specific, conserved candidates with known antimicrobial properties and potentially play a gene modulatory role in the fungus in cooperation with fungal or human RNA interference (RNAi) effectors. Subsequent investigations focused on the characterization of the identified miRNAs, involvement of key RNAi components, and definition of their role in *A. fumigatus* defense. We hope that these findings will contribute to the foundation of the exRNAs as a mediator of host-pathogen interactions and benefit development of new RNA-based diagnostics and therapeutics against this important human pathogen in the future.

Regulation & Small Proteins

OP-RSP-001

Small protein mediates inhibition of ammonium transport in Methanosarcina mazei – an ancient mechanism?

*T. Habenicht[1], K. Weidenbach[1], A. Velazquez-Campoy[2], R. Buey[3], M. Balsera[4], R. A. Schmitz[1]
[1]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for General Microbiology, Kiel, Germany*
[2]*Universidad de Zaragoza, Zaragoza, Spain*
[3]*Universidad de Salamanca, Salamanca, Spain*
[4]*Spanish National Research Council, Salamanca, Spain*

Small ORF encoded proteins, with a length less than 70 amino acids have been overlooked for long in classical bioinformatics and biochemical approaches. In the past decade however, modern genomics and transcriptomic technologies have discovered small genes containing short open reading frames in many prokaryotic genomes. By differential RNA sequencing, 1340 small open reading frames have been identified in the model organism *Methanosarcina mazei*, of which 72 have been verified by Mass Spectrometry.

While several bacterial small proteins have already been described, the number of identified and especially functionally characterized small proteins in archaea is still limited. We have discovered that the small protein 36 (sP36), which consists of only 61 aa, plays a critical role in regulating the nitrogen metabolism in *M. mazei.* The absence of sP36 significantly delays the growth of *M. mazei* when transitioning from nitrogen limitation to nitrogen sufficiency. Through *in vivo* experiments, we have observed that during nitrogen limitation, sP36 is dispersed throughout the cytoplasm; however, upon shifting the cells to nitrogen sufficiency, it relocates to the cytoplasmic membrane. Moreover, in vitro biochemical analysis clearly showed that sP36 interacts with high-affinity with the ammonium transporter AmtB1 (KD=0.26 µM) present in the cytoplasmic membrane during nitrogen limitation, as well as with the PII-like protein GlnK1 (KD=1.8 µM). We propose that in response to an ammonium up-shift, sP36 targets the ammonium transporter AmtB1 and inhibits its activity by mediating the interaction with GlnK1. This mechanism represents a fast and reversible archaeal way of AmtB regulation, in contrast to the well-studied regulation in bacteria, which depends on covalent modification of GlnK. We will in addition elaborate on the molecular sensing mechanism by sP36 and the observed correlation of sP36 presence and GlnD absence in archaea.

OP-RSP-002

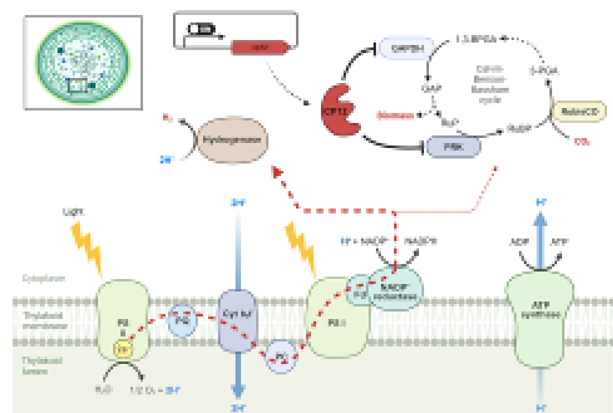
Utilization of the key regulatory protein CP12 to re-direct reductive power for production in cyanobacteria

*M. A. Itzenhäuser[1], F. Brandenburg[1], M. Theune[2], S. Grimm[1], S. Timm[3], M. Hagemann[3], K. Gutekunst[2], S. Klähn[1]
[1]*Helmholtz Center for Environmental Research – UFZ, Department of Solar Materials, Leipzig, Germany*
[2]*University of Kassel, Department of Molecular Plant Physiology, Kassel, Germany*
[3]*University of Rostock, Department of Plant Physiology, Rostock, Germany*

Cyanobacteria as phototrophic microorganisms bear great potential to produce chemicals and fuels from sustainable resources such as light, CO2, and water. The recently gained knowledge on internal electron and carbon fluxes and the regulation of cellular metabolism brings about opportunities to channel the energy towards product formation to overcome low efficiencies in product formation that hinder economical application to date. For instance, intrinsic regulators might pose interesting engineering targets to tune metabolic fluxes. In case of reductive processes like hydrogen production or redox catalysis, the partition of redox equivalents determines the efficiency. Here, the small regulatory protein CP12 controlling the major competitor for electrons – the Calvin-Benson-Bassham (CBB) cycle – was utilized to minimize electron drain in the model cyanobacterium *Synechocystis* sp. PCC 6803. In particular, we made use of phage-based CP12 variants to downregulate the CBB cycle. Unlike the native CP12 of *Synechocystis*, which only inhibits the CBB cycle in the dark and gets deactivated by a reduced cell status[1,2], CP12 homologs from cyanophages appear to be active when the infected host cell is exposed to light. This, together with other metabolic interventions, lead to an enhanced electron availability for phage reproduction[3]. To simulate this circumstance, we constructed *Synechocystis* strains that encode those CP12 homologs. In addition, we used rational protein design targeting the elimination of redox-control from the CP12 variant native to *Synechocystis.* In total, 6 different CP12 variants were inducibly expressed in *Synechocystis.* Intriguingly, impairment of growth upon induction of CP12 expression could be detected in different strengths for all variants. For promising variants, titratability of this effect could be demonstrated and their impact on the metabolism and hydrogen production was analysed. With this, we demonstrated that small proteins can be used to re-direct metabolic capacities in cyanobacteria.

1 McFarlane, et al. 2019, PNAS 116(42): 20984-20990
2 Lucius et al. 2022, Front. Plant Sci. 13: 1028794
3 Thompson et al. 2011, PNAS 108: E757-E764

Fig. 1



OP-RSP-003

Mapping prokaryotic translatomes with ribosome profiling and its derivative approaches

*E. Fiore[1], R. Gelhausen[2], L. Hadjeras[1], S. L. Svensson[1], K. Froschauer[1], F. Eggenhofer[2], R. Backofen[2], C. M. Sharma[1]
[1]*University of Würzburg, Institute of Molecular Infection Biology II, Würzburg, Germany*
[2]*University of Freiburg, Bioinformatics group, Department of Computer Science, Freiburg i. Br., Germany*

Ribosome profiling (Ribo-seq) is a powerful technique that has been widely used to study and globally annotate translatomes, including small open reading frames (sORFs). The method has enabled the detection of novel translated sORFs in the model organism *Escherichia coli* and in several other prokaryotes. However, for many bacterial and archaeal species this information is still lacking. To gain insights into the translatomes and hidden world of small proteins (≤ 50-100 amino acids), we have been adapting and optimizing Ribo-seq approaches for diverse prokaryotes. For this purpose, we have established conventional as well as optimized new Ribo-seq derivates based on specific translation inhibitors to detect translation initiation and translation termination sites (TIS and TTS). Furthermore, we have developed several computational tools to aid in the analysis of our datasets. Among these tools are the HRIBO pipeline, which processes raw data and performs metagene profiling analysis, ORF prediction, differential expression analysis, and ORFBounder, which automatically identifies peaks for TIS and TTS libraries.

We have applied such Ribo-seq profiling approaches and bioinformatics analyses to several species, including *Haloferax volcanii*, the plant symbiont *Sinorhizobium meliloti*, [CS1] and the human pathogens *Campylobacter jejuni* and *Helicobacter pylori*. The approaches, together with independent mass spectrometry and western blot validation, expanded the number of small proteins in the different organisms. Additionally, to gain insights into potential sORF functions, we have also generated Ribo-seq datasets under different growth and stress conditions. Moreover, future aims and challenges will involve applying Ribo-seq to anaerobic bacteria from the microbiota or mixtures of organisms, such as during host interaction. Additionally, we created RIBOBASE, an online repository of prokaryotic transcriptomic and translatomic datasets that facilitates the visualization, sharing of data online and direct access to the data. Altogether, our experimental and computational approaches will be beneficial for both current and future research on small proteins in prokaryotes.

OP-RSP-004
CemR atypical response regulator impacts energy conversion in Campylobacteria

*M. Noszka[1], A. Strzałka[2], J. Muraszko[1], D. Hofreuter[3], M. Abele[4], C. Ludwig[4], K. Stingl[3], A. Zawilak-Pawlik[1]
[1]*Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Laboratory of Molecular Biology of Microorganisms, Wroclaw, Poland*
[2]*University of Wroclaw, Department of Molecular Microbiology, Wroclaw, Poland*
[3]*German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany*
[4]*Technical University of Munich, Bavarian Center for Biomolecular Mass Spectrometry, Freising, Germany*

Campylobacter jejuni and *Arcobacter butzleri* are microaerophilic food-borne human gastrointestinal pathogens of the Campylobacteria class. According to WHO, campylobacteriosis is the leading cause of diarrhoeal diseases, while *A. butzleri* is an emerging pathogen associated with diarrhoea, rapidly increasing in incidence worldwide. These species face different atmospheric conditions during infection and transmission, ranging from nearly anaerobic to aerobic conditions. Thus, they need to adjust their metabolism and respiration to the changing oxygen concentrations of the colonisation sites. Interestingly, no regulators redirecting energy conversion pathways in response to oxygen as an electron acceptor have been found in Campylobacteria (doi.org/10.1016/bs.ampbs.2019.02.003).

Our previous research on gastric human pathogen *Helicobacter pylori*, relative to *C. jejuni* and *A. butzleri*, revealed that the orphan response regulator HP1021 is a redox switch protein controlling the transcription of approx. 30% of genes (doi.org/10.1093/nar/gkab440, doi.org/10.1038/s41467-023-42364-6). The HP1021 regulon includes the tricarboxylic acid cycle (TCA) genes/proteins.

Here, we studied the regulons of HP1021 homologues in *C. jejuni* and *A. butzleri*, Cj1608 and Abu0127, respectively. We used RNA-seq and LC-MS/MS to identify genes/proteins regulated by Cj1608 and Abu0127 and define the Cj1608/Abu0127 dependent pathways used by *C. jejuni* and *A. butzleri* to respond to oxidative stress. We found that these regulators are involved in the control of more than 30% of genes in each species. The genes/proteins affected most in both species are related to metabolism and energy conservation processes, mostly TCA and electron transport chain. Using phenotypic analyses, we confirmed that energy conversion processes in Cj1608/Abu0127 deletion mutants are disturbed, which causes their delayed growth. Moreover, our combined data indicate that these regulators respond to oxygen levels and redirect metabolism towards optimal energy conservation. Thus, we named the proteins Campylobacteria energy and metabolism regulators – CemR.

Secondary Metabolites and Natural Products

OP-SMNP-001
Biosynthesis of bacterial tropone natural products through enzymatic salvaging of catabolic shunt products

*R. Teufel[1]
[1]*University of Basel, Pharmaceutical Sciences, Basel, Switzerland*

Question. Various bacterial tropone natural products such as the antibiotic sulphur-containing tropodithietic acid (TDA) or (hydroxy)tropolones adopt crucial roles in symbiotic interactions with eukaryotic hosts. Their biosynthesis relies on an unusual intertwining of primary and secondary metabolism, in which the initial steps are shared for the various tropones and rely on enzymes from phenylacetic acid (paa) catabolism. In this pathway, a distinct reactive open-chain aldehyde intermediate is formed, which is usually further degraded to central metabolites. In the case of tropone biosynthesis, however, this compound is instead cyclized to the characteristic tropone scaffold. Yet, it remains largely unknown how tailoring enzymes in certain bacteria further convert the tropone scaffold into the mature natural products.

Methods. We employed *in vitro* reconstitution of diverse tropone biosynthetic pathways from Gram-negative and Gram-positive bacteria using heterologously produced enzymes from paa catabolism as well as enzymes encoded by different tropone biosynthetic gene clusters. Key enzymes were furthermore characterized mechanistically by enzyme assays and structurally by X-ray crystallography to obtain insights into their reaction mechanisms.

Results. We unravelled an unexpected mechanistic diversity for tropone functionalization involving distinct key flavoenzymes, which drastically modify the tropone scaffolds in the late-stage biosynthesis of TDA from Gram-negative *Phaeobacter* sp. and 3,7-dihydroxytropolone from *Streptomyces* sp., respectively. In both cases, these enzymes mediate several unanticipated reactions en route to the mature natural products such as a so far unique flavoenzyme-catalyzed dioxygenation in TDA biosynthesis involving oxygenolytic CoA-thioester cleavage followed by a regioselective ring epoxidation.

Conclusions. The biosynthesis of 3,7-dihydroxytropolone could be completely reconstituted and functions assigned to all involved enzymes. Similarly TDA biosynthesis was scrutinized, allowing for the discovery of a novel flavoprotein dioxygenase archetype, while final pathway steps involving the sulphur incorporation are subject to further investigation.

OP-SMNP-002
Genome sequence-based screening for novel phosphonate producers

*A. Zimmermann[1,2], J. Moschny[1,3], J. P. Gomez Escribano[2], S. Xia[1,3], J. Boldt[4,5], U. Nübel[4,5], W. Wohlleben[1,3], C. C. Hughes[1,3], Y. Mast[1,2]
[1]*German Center for Infection Research, Tübingen, Germany*
[2]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH, Bioresources for Bioeconomy and Health, Brunswick, Germany*
[3]*Eberhard Karls University of Tübingen, IMIT (Interfakultäre Institut für Mikrobiologie und Infektionsmedizin Tübingen), Tübingen, Germany*
[4]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Brunswick, Germany*
[5]*German Center for Infection Research, Hannover, Germany*

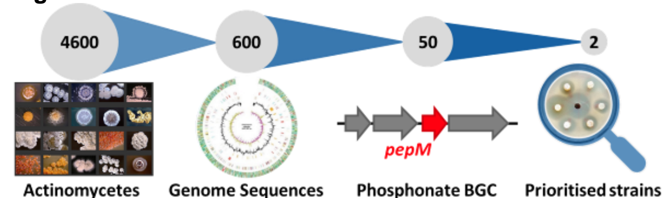
Phosphonates are a unique class of natural products with diverse chemical structures and bioactivities. Numerous phosphonate natural compounds found their way into the market as for example the herbicide bialaphos, the antimalarial agent fosmidomycin or the antibiotic fosfomycin. An initial biosynthetic isomerisation step catalysed by the enzyme phosphoenolpyruvate mutase (PepM) forms the characteristic carbon-phosphorus bond. Due to the conservation of the PepM enzymatic reaction in the vast majority of phosphonate producers, the respective biosynthetic gene *pepM* can be used as a molecular marker to screen for potential phosphonate producer strains.

The aim of the study was to screen the DSMZ *Actinomycetales* strain collection for novel phosphonate producers based on a *pepM*-guided genome mining attempt and analyse their biosynthetic products for potential novel phosphonates.

The DSMZ *Actinomycetales* strain collection harbours > 4.600 actinomycetes, many of which have already been genome-sequenced. A bioinformatic analysis of 600 genome sequences yielded 50 strains containing a *pepM* gene and thus a potential phosphonate biosynthetic gene cluster (BGC). The identified actinomycetes were cultivated on 8-10 different media following the OSMAC (one-strain-many-compounds) approach and were screened for phosphonate antibiotic production with bioassays using the phosphonate-sensitive test strain *E. coli* strain WM6242 [1], whereby 17 strains showed phosphonate-specific bioactivities. Phylogenetic analysis of the PepM amino acid sequences revealed a BGC-specific cladding. Cluster networking analysis were performed in order to prioritise strains with unique clusters for further analysis. Two strains, *Kitasatospora fiedleri* DSM 114396 [2] and *Streptomyces iranensis* DSM 41954, were prioritised for further analysis. Phosphonate production of the two strains was verified by ^{31}P-NMR analysis, whereby inactivation of the respective *pepM* genes abolished phosphonate production. Antibiotic compound isolation and characterisation is ongoing.

- [1] C. Eliot *et al.*, Chemistry & Biology. **15**, 765–770 (2008).
- [2] Zimmermann *et al.*, IJSEM. **73**, 006137 (2023).

Fig. 1



OP-SMNP-003
Discovery of a new cinnamycin analog from an Actinomadura sp. isolated from meerkat feces

*D. Iliasov[1], T. A. M. Gulder[2], T. Mascher[1]
[1]*Technical University of Dresden, Institute of Microbiology, General Microbiology, Dresden, Germany*
[2]*Technical University of Dresden, Faculty of Chemistry and Food Chemistry, Dresden, Germany*

The rapid global spread of antibiotic resistance emphasizes the need for new effective therapeutics. The isolation of strains, e.g. of the actinomycetes, from unexplored habitats could potentially provide access to new or endemic species as the new sources for novel secondary metabolites. Here, we investigate an antibiotic-producing isolate from a meerkat (*Suricata suricatta*) fecal sample with regard to its antimicrobial activity.

Diluted fecal samples were plated on MYM agar. Actinobacteria were isolated based on colony and cell morphology. The isolate was phylogenetically classified based on the 16S rDNA sequence. Mature colonies were overlayed with soft agar containing Gram-negative, Gram-positive or fungal species. Cell extracts from liquid cultures as well as colonies were analyzed regarding their antibiotic spectrum by applying a panel of whole cell biosensors, in which an antibiotic-inducible promoter is fused to the luciferase cassette. The potential antibiotic biosynthetic gene clusters (BGC) were identified by genome mining. During the plate-based biosensor screens, we identified naturally resistant *Bacillus subtilis* colonies growing in the zone of inhibition. After repeating rounds of selection, highly resistant spontaneous mutant strains were subjected to whole genome sequencing.

16S rDNA sequencing identified the isolate as *Actinomadura coerulea*. The isolate showed a strong and broad range of antibacterial activity against Gram-positive bacteria, including pathogens, as well as an antifungal effect. The secondary metabolites of the isolate potentially inhibit replication, block translation, and interfere with bacterial cell wall integrity. Amongst others, we identified loss of function mutations in *pssA*, encoding a phosphatidylserine synthase. Based on the function of PssA and genome mining, the BGC for a novel cinnamycin analog was identified. Detailed follow-up studies are currently ongoing.

Our approach demonstrates that combining the exploration of niche habitats for actinomycetes with whole-cell biosensor screening and characterization of natural resistance development provides a promising strategy for identifying novel antibiotics.

Sensing, Signaling & Communicating Microbes

OP-SSCM-001
Dynamic interplay: chemotaxis, quorum sensing and metabolism affect *Escherichia coli* interactions during gut colonization

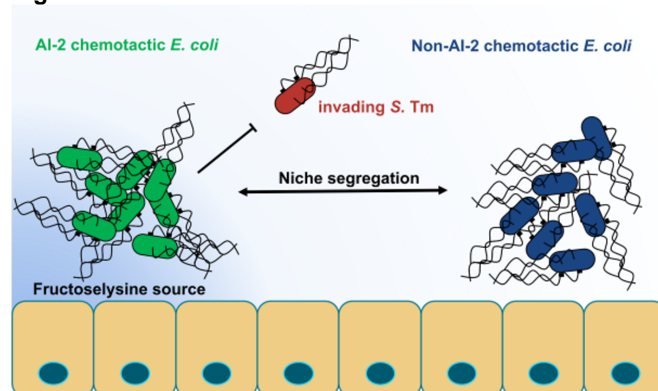
*L. Laganenka[1], J. W. Lee[2], L. Malfertheiner[3], C. Schubert[1], C. Dieterich[1], L. Fuchs[1], J. Piel[1], C. van Mering[3], V. Sourjik[2], W. D. Hardt[1]
[1]*ETH Zürich, Institute of Microbiology, Zürich, Switzerland*
[2]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[3]*University of Zürich, Department of Molecular Life Sciences and SIB Swiss Institute of Bioinformatics, Zürich, Switzerland*

Bacteria communicate and coordinate their behavior at the intra- and inter-species levels by producing and sensing extracellular small molecules called autoinducers. Autoinducer 2 (AI-2) is produced and detected by a variety of bacteria, thus playing an important role in interspecies communication. Although AI-2 is a major autoinducer molecule present in the mammalian gut, its role in bacteria-bacteria and bacteria-host interactions during gut colonization remains unclear. Through competitive infections in C57BL/6 mice, along with microscopy and bioinformatic approaches, we show that chemotaxis and AI-2 signalling promote gut colonization by *Escherichia coli*, which is in turn connected to the ability of the bacteria to utilize fructoselysine. Additionally, we show that the genomic diversity of *E. coli* strains with respect to AI-2 signaling allows ecological niche segregation and stable co-existence of different *E. coli* strains in the mammalian gut.

Furthermore, we demonstrate that *E. coli* competes with enteric pathogen *Salmonella* Typhimurium in AI-2 chemotaxis-dependent manner. Utilizing a genetically tagged library of *S.* Tm gene knockouts, we conducted high-throughput analysis to identify metabolic genes that contribute to *E. coli*-*S.* Tm competition during the murine gut colonization. Preliminary data suggests the involvement of genes related to glycerol metabolism and fumarate respiration. In summary, our results pinpoint the importance of quorum sensing- and metabolic pathways involved in both intra- and inter-species interactions of E. coli during gut colonization.

Reference: Laganenka, L. *et al.* 2022. Chemotaxis and autoinducer-2 signalling mediate colonization and co-existence of *Escherichia coli* strains in the murine gut // Nat Microbiol. 8 (2), 204-217. doi: 10.1038/s41564-022-01286-7.

Fig. 1



OP-SSCM-003
Microalgae and Bacteria Interaction—Evidence for Division of Diligence in the Alga Microbiota

Y. Astafyeva[1], M. Gurschke[1], *I. Krohn[1]
[1]*University of Hamburg, Microbiology and Biotechnology, Hamburg, Germany*

Microalgae are one of the most dominant forms of life on earth that is tightly associated with a distinct and specialized microbiota. We have previously shown that the microbiota of *Scenedesmus quadricauda* harbors less than 10 distinct microbial species. Here, we provide evidence that dominant species are affiliated with the genera of *Variovorax, Porphyrobacter, and Dyadobacter*. Experimental and transcriptome-based evidence implies that within this multispecies interaction, *Dyadobacter* is a key to alga growth and fitness and is highly adapted to live in the phycosphere. While presumably under light conditions the alga provides the energy source to the bacteria, Dyadobacter produces and releases mainly a large variety of polysaccharides modifying enzymes. This is coherent with high-level expression of the T9SS in alga cocultures. The transcriptome data further imply that quorum-quenching proteins (QQ) and biosynthesis of vitamins B_1, B_2, B_5, B_6, and B_9 are expressed by *Dyadobacter* at high levels in comparison to *Variovorax* and *Porphyrobacter*. Notably, *Dyadobacter* produces a significant number of leucine-rich repeat (LRR) proteins and enzymes involved in bacterial reactive oxygen species (ROS) tolerance. Complementary to this, Variovorax expresses the genes of the biosynthesis of vitamins B_2, B_5, B_6, B_7, B_9, and B_{12}, and *Porphyrobacter* is specialized in the production of vitamins B_2 and B_6. Thus, the shared currency between partners are vitamins, microalgae growth-promoting substances, and dissolved carbon. This work significantly enlarges our knowledge on alga-bacteria interaction and demonstrates physiological investigations of microalgae and associated bacteria, using microscopy observations, photosynthetic activity measurements, and flow cytometry.

OP-SSCM-004
Novel Inhibitors of the Two-Component System VanRS Re-Sensitize *Enterococcus Faecalis* to Vancomycin

*B. Fernandez Ciruelos[1], M. Albanese[2], A. Adhav[3], V. Solomin[4], J. Wells[1]
[1]*Wageningen University, Host-Microbes Interactomics, Wageningen, Netherlands*
[2]*Oxford Drug Design, Oxford, United Kingdom*
[3]*Instituto de Biomedicina de Valencia, Valencia, Spain*
[4]*Latvian Institute of Organic Synthesis, Riga, Latvia*

Two-component systems (TCS) are the main signalling system in bacteria and are involved in adaptation, virulence, antimicrobial resistance, etc. TCS are formed by a sensor Histidine Kinase (HK) that detects a specific stimuli and undergoes an autophosphorylation via an ATP bound to the catalytic domain (CA). The phosphate is transferred to the Response Regulator (RR) that then changes gene expression accordingly to the detected stimuli. TCS have been proposed as good targets for antibiotics due to their presence in all bacteria, each bacteria possess multiple TCS, their catalytic domains are conserved and they are absent in mammalian cells. In this research we used a fragment-based drug design approach to develop inhibitors that compete with ATP for the ATP-binding pocket on the CA domain of TCS.

Following a fragment-based drug design approach, a series of fragments were screened *in silico* for binding to the ATP-pocket of different HK. Promising fragments were synthesized and protein binding was confirmed via X-crystallography. The most promising fragment CT14 was rationally and virtually grown into a compound library. This library was synthesized, and screened for *in vitro* binding to the HKs CheA from *Thermotoga maritima* and PhoR from *Staphylococcus aureus.* Best compounds bound with Kds in the low µM range. The library was then screened for inhibition of VanRS TCS mediated vancomycin resistance in *E. faecalis* and *E. faecium.* Surprisingly, compound 752 completely re-sensitized *E. faecalis* vanA and vanB to vancomycin. Expression analysis showed a significant downregulation of *vanA* induction from vancomycin treatment when 752 was present. Even though 752 failed to re-sensitize *E. faecium* vancomycin, it led to a similar reduction in *vanA* induction.

In summary, TCS inhibitor 752 re-sensitizes *E. faecalis* to vancomycin treatment by, at least partially, inhibiting VanRS TCS. Compound 752 also reduces VanRS related pathways in *E. faecium* but this inhibition is not enough to re-sensitize it to vancomycin. However, optimization for complete inhibition of VanRS could serve to also re-sensitize *E. faecium* to vancomycin, with great implications in nosocomial infections.

Teaching Approaches & New Medical Approbation Regulations

OP-TNAR-001
Analysis of hygiene mistakes in medical training videos
*L. Behrends[1], D. Pantano[1], A. Mellmann[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*

Introduction

YouTube is a popular platform for accessing medical content, including educational videos on procedures like physical exams and blood sampling. These videos are widely watched, so it is important to check them for accurate adherence to standards. This study focused on assessing German-language videos for compliance with standard precautions for infection prevention.

Goals

The goals of this study were to assess the educational validity of the contents on this platform and whether their quality changed during and after the COVID-19 pandemic.

Materials & Methods

In November 2019, 130 German medical training videos were analyzed for hygiene errors. The search was repeated in January 2024, resulting in 61 additional videos for analysis. The search query included the German equivalent for physical examination (*körperliche Untersuchung*), intravenous line, placing venous access, peripheral venous catheter, and peripheral venous line (*venöser Zugang, Zugang legen, Peripherer Venenkatheter, Venenverweilkanül*e), along with four terms relating to blood sampling (*Blutentnahme, Blutabnahme, Blut abnehmen, Blut entnehmen*).

Results

Hygiene errors were evident in most videos from 2009 until 2019. Hand hygiene measures were rarely observed and instructions were missing. Moreover, wearing of jewelry was observed. In the more recent analyzed medical training content from 2020 until 2024, 48 of 61 of the videos displayed hygiene errors. This marks a very slight improvement in compliance with standard precautions during and after the pandemic.

Summary

The analysis of medical training videos revealed numerous hygiene errors, evident in content both from pre-pandemic times and after the advent of COVID-19. In this context, the COVID-19 pandemic did not have a significant impact on compliance with standard precautions within the observed time frame. Thus, there remains substantial room for improvement in adhering to standard precautions, with particular emphasis on the necessity of proper hand disinfection.

Poster

Archaea & Extremophiles

P-AE-001
Unraveling the distribution and diversity of methoxydotrophic archaea
*L. Hofmann[1,2,3], J. M. Kurth[1,2,3]
[1]*Philipps-University of Marburg, Chemistry, Marburg, Germany*
[2]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[3]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*

Methoxylated aromatic compounds (MACs) are a diverse group of aromatic ethers with significant relevance in biological systems. Lignin constitutes the primary source of MACs, comprising a substantial portion of organic matter in global ecosystems[1]. Conversion of MACs by bacteria and fungi has already been observed since over 40 years[2]. Yet only recently, it has been discovered that also archaea have this ability: Methoxydotrophic archaea use a bacteria-like

methyltransferase system, designated the Mto system for MAC conversion[3,4]. Given the abundance of MACs on Earth, the presence of *mto* genes in a variety of archaeal taxa[3] and the lacking knowledge about their ecology, methoxydotrophic archaea might exert an underestimated yet important influence on global climate due to CH_4 & CO_2 formation from MACs.

This project aims to elucidate the prevalence of methoxydotrophic archaea and their biogeography. For this purpose, primer pairs will be designed for amplicon sequencing and evaluated by *in silico* & wet lab experiments to specifically target archaeal *mto* gene sequences within different environmental samples based on a curated database of each gene.

In summary, the project will investigate the distribution and diversity of methoxydotrophic archaea in distinct habitats. Based on potential findings in this project, future studies will further aim to illuminate the microbial ecology of these fascinating microbes in different ecosystems.

Bibliography

[1] D. J. Burdige, *Global Biogeochem. Cycles* **2005**, *19*.
[2] R. Bache, N. Pfennig, *Arch. Microbiol.* **1981**, *130*, 255–261.
[3] C. U. Welte, R. de Graaf, P. Dalcin Martins, R. S. Jansen, M. S. M. Jetten, J. M. Kurth, *Environ. Microbiol.* **2021**, *23*, 4017–4033.
[4] J. M. Kurth, M. K. Nobu, H. Tamaki, N. de Jonge, S. Berger, M. S. M. Jetten, K. Yamamoto, D. Mayumi, S. Sakata, L. Bai, L. Cheng, J. L. Nielsen, Y. Kamagata, T. Wagner, C. U. Welte, *ISME J.* **2021**, *15*, 3549–3565.

P-AE-002

Expanding the genetic toolbox for Methanothermobacter thermautotrophicus ΔH

*L. Mühling[1], S. Farmer[1], B. Molitor[1]
[1]*University of Tübingen, Environmental Biotechnology, Tübingen, Germany*

Methanothermobacter spp. are thermophilic, chemolithotrophic methanogens with a long history in basic research as model microbes for hydrogenotrophic methanogenesis. There is also industrial interest in *Methanothermobacter* spp. as biocatalysts in the power-to-gas process in which hydrogen that is produced with electricity from renewable resources is combined with carbon dioxide to produce renewable methane. The lack of genetic tools has hindered further investigations of *Methanothermobacter* spp. and the expansion of the power-to-gas process to produce value-added products. This only changed recently with the development of a genetic system for *Methanothermobacter thermautotrophicus* ΔH in our lab. After successfully establishing the genetic system, we focus on expanding the genetic toolbox. For example, we established the formate dehydrogenase (*fdh*) operon from *M. thermautotrophicus* Z-245 as an additional selection marker. Here, an alternative transformation method to the already established conjugation protocol is presented. For this, we adapted and optimized an electroporation protocol for the use with *M. thermautotrophicus* ΔH. Furthermore, we present results on applying a thermostable Cas9 from a *Geobacillus* strain as a tool for genome editing by deleting the gene encoding uracil phosphoribosyl transferase (*upt)*, a possible counterselection marker. We discuss which adaptations to the transformation protocol and cell treatments were required to achieve a clean deletion. The presented additions to the genetic toolbox for *M. thermautotrophicus* ΔH can be used to advance industrial applications and fundamental research.

P-AE-003

Investigating the regulation of genes essential for growth of the archaeon Methermicoccus shengliensis on methoxylated aromatic compounds

*Z. Dogru[1,2,3], J. M. Kurth[1,2,3]
[1]*Microcosm Earth Center, Max Planck Institute for Terrestrial Microbiology and Philipps-Universität Marburg, Microbial Physiology, Marburg, Germany*
[2]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*
[3]*Philipps-University Marburg, Department of Chemistry, Marburg, Germany*

Lignin is one of the most prevalent organic polymers found on Earth, being a major source of methoxylated aromatics. While bacteria-mediated conversion of these compounds has been extensively documented, the archaeal degradation of methoxylated aromatics has only recently come to light. *Methermicoccus shengliensis*, a methanogenic archaeon, was identified as the pioneering archaeon capable of converting methoxylated aromatics into methane, also called methoxydotrophic methanogenesis [1]. A recent study revealed that *M. shengliensis* has an *O*-demethylase system for conversion of methoxylated aromatics that shares greater similarity with methyltransferase systems of acetogenic bacteria than the respective systems of methylotrophic archaea [2]. By using comparative transcriptomics, an operon, referred to as the *mto* operon, was found to be upregulated under methoxydotrophic growth in *M. shengliensis* [2]. This operon encodes the proteins required for the conversion of methoxylated aromatics. While the roles of most proteins encoded by this operon have been elucidated, two hypothetical proteins that have structural motifs of DNA-binding domains remain uncharacterized. Our goal is to uncover the regulation of the *mto* genes and the function of the aforesaid hypothetical proteins. To validate the DNA-binding proficiency of the putative regulators, Electrophoretic Mobility Shift Assay trials were initiated. We focused on identifying specific non-coding DNA regions within or adjacent to the *mto* operon that potentially serve as binding sites for these hypothetical proteins. In addition, we will screen the transcriptional regulators of the whole *mto* operon by using the promoter region of the *mto* gene cluster together with *M. shengliensis* cell extract and magnetic separation technology. We aim to identify the proteins that are binding to the promotor of this gene cluster. This study will help us grasp how methoxydotrophy is controlled on gene level in archaea and how specific methanogens like *M. shengliensis* regulate conversion of a variety of methylated and methoxylated compounds.

[1] Mayumi et al. (2016) Science 354:222, [2] Kurth et al. (2021) ISME J 15:3549

P-AE-004

Salt City Secrets: Astrobiological Exploration of Lüneburg's Halophilic Microorganisms

*K. Runzheimer[1], S. Leuko[1]
[1]*German Aerospace Center, Köln, Germany*

Introduction

Extreme saline habitats have emerged as captivating hotspots for astrobiological exploration. Despite being commonly perceived as inhospitable, these environments host a range of extremophiles. One such extreme habitat is the Lüneburger brine, characterized by a saturated saline solution that surpasses all other European brines in terms of salinity. Historically utilized as a source of salt, this brine offers easy accessibility for sampling campaigns. The significance of extremophiles isolated from saline environments lies in their potential to provide crucial insights into the boundaries of life on Earth and, by extension, the prospect of extraterrestrial life. The study aims to characterize the Lüneburger brine in Germany. Cultivation and cultivation independents methods will be used to further investigate the extreme microbial community and to evaluate if this brine represents an astrobiological relevant habitat.

Methods

In this study, we conducted sampling of the Lüneburger brine. Analyses encompassed the metagenome, the cultivable community, and more. Selected isolates were further tested on potential resistances to astrobiological relevant stressors like radiation.

Results

Metagenomic data unveiled the presence of halophilic Archaea, showcasing thermophilic, halophilic, and acidophilic characteristics. Several representatives were identified as candidate organisms, yet to be isolated in a pure culture. Microscopic analysis indicated variations in morphology, with the isolates displaying pleomorphism. Cultivated organisms exhibited the presence of the carotenoid bacterioruberin. Future experiments will assess the resistance of these isolates to common astrobiological stressors.

Summary

The Lüneburger brine displays an extreme habitat displaying a saturated saline solution. However, our study has shown the wide diversity of especially halophilic microorganism which thrive in this habitat. The study emphasis the potential of this easily accessible habitat for current astrobiology research and shows the presence of isolates capable of diverse resistance towards high salinity, radiation and more revealing pleomorphism and pigmentation.

P-AE-005
Important roles of Zinc Finger µ-Proteins in Haloferax volcanii

*D. Üresin[1], A. Borst[1], D. Pyper[2], L. Hadjeras[3], R. Gelhausen[4], R. Backofen[4], C. M. Sharma[3], H. Schwalbe[5], J. Soppa[1]
[1]Goethe University Frankfurt, Institute of Molecular Biosciences, AG Soppa, Frankfurt a. M., Germany
[2]Goethe University Frankfurt, Institute of Organic Chemistry, AG Schwalbe, Frankfurt a. M., Germany
[3]Julius-Maximilians University Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany
[4]University of Freiburg, Department of Computer Science, Freiburg i. Br., Germany
[5]Goethe University Frankfurt, Institute of Organic Chemistry, AG Schwalbe, Frankfurt a. M., Germany

Zinc finger proteins are known to fulfil many different roles in eukaryotes, e.g. as transcription factors, ribosomal proteins, regulators of membrane proteins, or molecular scaffolds. In archaea, they are severely understudied. In addition, most well-studied eukaryotic zinc finger proteins are large proteins containing several small zinc finger domains. In contrast, in archaea the majority of putative zinc finger proteins are very small and await experimental characterization.

Haloferax volcanii is a halophilic archaeon that can easily be cultivated, making it a frequently used model organism. Its genome encodes 282 small proteins (less than 70 amino acids), most of them having no annotated function yet. 43 of those proteins contain at least two CPXCG-like motifs, making them putative one-domain zinc finger proteins of unknown function.

For the analysis of these zinc finger µ-proteins more than 30 single gene *in frame* deletion mutants have been generated until now. They were compared to the wildtype concerning e.g. growth behaviour in different media, swarming, and biofilm formation. Most of the mutants showed a strong phenotype under at least one of the conditions tested, proving that the zinc finger -proteins fulfil various important functions in haloarchaea[1]. Co-affinity purifications have been initiated to screen for protein interaction partners. A biochemical zinc assay was performed to investigate which of the putative zinc fingers actually bind zinc ions.

The proteins HVO_0758 and HVO_2753 have been studied in detail[2,3]. RNA-Seq analyses (coll. with C. Sharma, Würzburg) revealed that movement/chemotaxis genes are downregulated in the deletion mutants, in excellent agreement with their inability to swarm. Determination of the NMR solution structures (coll. with H. Schwalbe, Frankfurt) revealed distinct structural differences between the two proteins.

Taken together, we could show that haloarchaeal zinc finger µ-proteins are important for various biological functions, e.g. glycerol metabolism, biofilm formation and swarming.

[1]Nagel et al. (2019) Genes 10:361
[2]Zahn et al. (2020) FEBS J. 288:2042
[3]Üresin et al. (2023) Front Microbiol. 14:1280972

P-AE-006
Taxonomic revision of the genus Methanobrevibacter and description of Methanomonila shimae gen. nov., sp. nov.

*E. Protasov[1], M. Shiratori[1], K. Platt[1], C. Spröer[2], S. Spring[2], M. Ohkuma[3], V. Hervé[1], A. Brune[1]
[1]Max Planck Institute for Terrestrial Microbiology, Insect gut microbiology and symbiosis, Marburg, Germany
[2]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Brunswick, Germany
[3]Japan Collection of Microorganisms, RIKEN BioResource Center, Tsukuba, Japan

Many methanogens in the digestive tract of animals fall into the radiation of the genus *Methanobrevibacter*. Recent studies revealed that the genus *Methanobrevibacter* is underclassified and comprises numerous genus-level lineages. Based on a comprehensive phylogenomic analysis, which includes all uncultured representatives with sequenced genomes and considers the relative evolutionary divergence of individual lineages, we propose seven new genera (type

species in parentheses). While *Methanacia* gen. nov. (*M. filiformis* comb. nov.), *Methanobaculum* gen. nov. (*M. cuticularis* comb. nov.), *Methanobinarius* gen. nov. (*M. arboriphilus* comb. nov.), and *Methanoflexus* gen. nov. (*M. curvatus* comb. nov.) are found almost exclusively in the digestive tract of insects and millipedes, *Methanobrevibacter* sensu stricto, *Methanarmilla* gen. nov. (*M. wolinii* comb. nov.) and *Methanocatella* gen. nov. (*M. smithii* comb. nov.) harbor all isolates from mammals and birds. Two additional lineages from termites ("Methanorudis" and "Methanovirga") are without cultured representatives. The representatives from arthropods and vertebrates form two distinct clades that show patterns of coevolution with their respective host groups. An exception is *Methanomonila* gen. nov. (*M. shimae* sp. nov.), whose members occur arthropods but are sister to the genus *Methanocatella* from vertebrates. The only isolate is strain Ec1 from the cockroach *Ergaula capucina*, a hydrogenotrophic methanogen that reduces CO2 with H2 or formate. Based on these results, we conclude that an association with animals occurred twice in the evolutionary history of the order *Methanobacteriales*, once in the ancestor of the *Methanobrevibacter* sensu lato supercomplex, and once during the radiation of the *Methanobacterium* supercomplex (i.e., in the genus *Methanosphaera*).

P-AE-007

Analysis of the Low salt transcriptome in Haloferax volcanii

*A. Borst[1], P. Brück[1], M. Hammelmann[1], J. Soppa[1]
[1]*Goethe University Frankfurt, Institut für molekulare Biowissenschaften, Frankfurt a. M., Germany*

Archaea are well known for inhabiting areas of our planet that are usually hostile for any form of life, hence they are often termed as "extremophiles". Although also found in common soil or the digestive tract of vertebrates, some species thrive on acidic or alkaline grounds, in submarine black smokers with temperatures surpassing 100°C or live in aquatic areas with a very high salinity.

Haloferax volcanii is a halophilic and mesophilic archaeon that was first discovered in the 1930s and isolated from the hypersaline environment of the Dead Sea. Its optimal growth conditions are at 42 °C in around 2 M NaCl and complex nutrient medium, however it can also grow in different synthetic media and at a wide range of temperatures and NaCl concentrations. While NaCl concentrations up to 0.7 M inhibit growth in synthetic glucose media, a concentration of 0.9 M is sufficient to sustain growth at a reduced rate.

Here we show for the first time a transcriptome-wide analysis of differential gene expression of cells grown under low salt condition. We cultivated cells at an optimal NaCl concentration of 2.1 M in glucose media and compared the expression profile of these to cells grown in 0.9 M NaCl after 26 and 68 h.

Using RNA-Seq, we identified a multitude of differentially regulated genes and gene-clusters that seem to be of importance either as a fast "stress-response" (after 26 h) to the change of ion concentrations in the environment or to enable continuous growth under these conditions (68 h). We are currently generating in-frame deletion mutants of selected genes/gene clusters and will investigate the impact of these deletions on growth under 0.9 M NaCl. To this end, two of seven tested deletion mutants (HVO_0772, annotated as a NP_1176A family transcription regulator and HVO_1863, annotated as a conserved hypothetical protein) showed a severe growth defect under low salt while having only a minor under normal salt, indicating that these genes are involved in adaption to a low salt environment.

P-AE-008

Glycerol metabolism in Sulfolobus acidocaldarius

*C. Schroeder[1], C. Schmerling[1], X. Zhou[1], J. Bost[2], B. Wassmer[2], S. V. Albers[2], C. Bräsen[1], B. Siebers[1]
[1]*University Duisburg-Essen, Essen, Germany*
[2]*University Freiburg, Freiburg i. Br., Germany*

Introduction: Glycerol is a highly abundant organic compound in nature that many organisms utilize as carbon and energy source. However, although the genetic capacity to grow on glycerol was reported for several archaea [1] the glycerol degradation has so far only been reported for the halophile *Haloferax volcanii* [2].

Objective: In this study, the glycerol degradation in the thermoacidophilic crenarchaeal model organism *Sulfolobus acidocaldarius* was analyzed.

Methods: Growth experiments, polyomics as well as biochemical and deletion mutant analyses were applied to gain comprehensive insights into the underlying mechanisms.

Results: *S. acidocaldarius* grows on glycerol as sole carbon and energy source. Glycerol degradation proceeds via glycerol kinase (GK) mediated phosphorylation to glycerol-3-phosphate followed by oxidation to dihydroxyacetone phosphate (DHAP) catalyzed by an unusual quinone reducing FAD-dependent glycerol-3-phosphate dehydrogenase (G3PDH), which shows a novel mode of membrane associtation facilitated by a small, CoxG-like protein.

The *S. acidocaldarius* genome encodes both, GK and G3PDH, in two paralogous copies which were characterized and shown to be functionally similar. However, polyomics studies indicated that only one of these GK-G3PDH couples is upregulated in presence of glycerol. Accordingly, deletion of the respective GK abolished growth on glycerol and crude extract measurements displayed an almost complete loss of GK activity. In contrast, deletion of the second GK led only to slightly diminished growth on glycerol and no significant change in GK activity in crude extracts. Thus, only one of the two GK-G3PDH couples is essential to maintain growth on glycerol.

Summary: We herein unraveled the glycerol degradation in the crenarchaeon *S. acidocaldarius* to proceed via the GK-G3PDH pathway involving a "classical" GK and an unusual G3PDH which differs in structure and membrane association from those known in bacteria and haloarchaea.

References

1. Villanueva L et al. (2017), *Environ Microbiol*, **19**(1):54-69.
2. Sherwood KEet al. (2009). *J Bacteriol*, **191**(13):4307-4315.

P-AE-009

Enzymatic synthesis of the extremolyte cyclic-2,3-diphosphoglycerate by recombinant cyclic-2,3-diphosphoglycerate synthetase from Methanothermus fervidus

*C. Stracke[1], C. Bräsen[1], B. Siebers[1]
[1]*Universtiy Duisburg-Essen, Molekulare Enzymtechnologie und Biochemie, Essen, Germany*

Introduction: Extremolytes – compatible solutes exclusively produced by extremophiles – hold great potential for applications in pharmaceuticals, healthcare, and cosmetics. Although cyclic-2,3-diphosphoglycerate (cDPG) has been detected as extremolyte in hyperthermophilic methanogenic Archaea, its potential protective properties in safeguarding membranes, proteins and DNA from damage under stressful conditions remains unused. So far, methods for protein production, stabilization and efficient synthesis of cDPG are missing as no applicable production procedure is available.

Objective: Here, we present a one-step enzymatic *in vitro* approach for the synthesis of cDPG from 2,3-diphosphoglycerate (2,3-DPG) utilizing the cyclic 2,3-diphosphoglycerate synthetase (cDPGS) from *Methanothermus fervidus*.

Methods: The heterologous production of cDPGS in *Escherichia coli* was improved via codon optimization. We implemented a streamlined two-step purification method involving heat precipitation and size exclusion chromatography. Functional enzyme characterization and conversion efficiencies were assessed and analyzed using enzyme assays and 31P-NMR.

Results: Starting from 1.7 g (wet weight) of *Escherichia coli* cells, we obtained 3.5 mg of pure cDPGS. The recombinant protein showed a *V*c of 21 U mg-1, with *K*m values of 1.4 mM and 1.1 mM for 2,3 DPG and ATP, respectively. To enhance stability for storage at -80°C, we incorporated 400 mM KCl, 5 mM DTT, 2.5 mM Mg2+ and 25% (v/v) glycerol, resulting in the retention of 95% activity even after 1.5 months. The optimized *in vitro* reaction was successfully scaled up, achieving complete conversion of 37.6 mg 2,3DPG to cDPG at 55°C within 180 minutes.

Conclusion: These results represent an important step towards a streamlined one-step *in vitro* approach to produce cDPG. The complete substrate conversion to product will simplify downstream cDPG purification, allow further up-scaling and to extend the process to a more complex enzyme cascade including e.g. an ATP recycling system or synthesis from cheaper substrates like glycerate. This paves the way for the cost-effective production of cDPG as a value-added product for application.

P-AE-010

A Drosophila Model for Infection-induced Loss of Intestinal Barrier Function and Homeostasis

*N. Broderick[1]
[1]*Johns Hopkins University, Biology, Baltimore, MD, United States*

The innate immune response is the first line of defense against infections in both *Drosophila melanogaster* and humans. In flies, the Toll and immune deficiency (Imd) pathways recognize pathogen-associated molecules, such as peptidoglycan, to activate host immune responses during infection. However, relatively few natural pathogens have been used to study mechanisms of innate immunity in *D. melanogaster*. To better understand the breadth of host responses to various microbes this study characterizes the response of *D. melanogaster* to a novel pathogen, *Chromobacterium subtsugae,* a Gram-negative environmental bacterium known for its quorum sensing production of the purple-pigment violacein. We fed *C. subtsugae* to adult *D. melanogaster* and found flies are significantly more susceptible to a purple pigmented strain that over produces violacein, compared to the wild-type non-pigmented strain. Moreover, the infectious process with this violacein producing strain follows defined events. Initially after infection, pathogen load is high and is associated with significant gut damage and the induction of immune and stress response pathways within the first 24 hours. By day 4, almost no *C. sustugae* can be cultured from flies, but death does not occur until 7-10 days post infection. This uncoupling of pathogen presence and host death, akin to sepsis, provides a unique infection course to study host responses to pathogen-induced damage. Imaging of fly guts over the course of infection indicates that after day 4 dead bacterial cells persist in the gut, which undergoes significant morphological changes as disease progresses. We will discuss our exploration of early events in the course of infection that lead to death and how host immune and stress responses contribute to pathogenesis, as well as microbial factors that elicit these impacts on the host. Altogether, this novel pathogen model provides a useful tool to study how host-microbe interactions can lead to the loss of host homeostasis.

Biotechnology & Synthetic Microbiology

P-BSM-002

Functional expression of biosynthetic magnetosome gene clusters from intractable magnetic bacteria in Magnetospirillum gryphiswaldense

*A. Knöchel[1], D. Schüler[1]
[1]*Universtiy Bayreuth, Chair of Microbiology, Bayreuth, Germany*

Magnetosomes of magnetotactic bacteria (MTB) consist of structurally perfect, nano-sized magnetite crystals enclosed within vesicles of a proteo-lipid membrane. In the well-studied model *M. gryph.* biosynthesis of its isotropic cuboctahedral-shaped crystals is controlled by more than 30 genes comprised within compact magnetosome gene clusters [1]. Similar, yet distinct gene clusters were also identified in many diverse MTB in which they likely account for the biomineralization of magnetosome crystals with a variety of more complex, genetically encoded morphologies such as anisotropic elongated shapes. However, since most of these MTB are inaccessible to genetic and biochemical approaches, their functional analysis requires the expression of magnetosome genes in a foreign host [2]. Here, we investigated whether biosynthetic multi-gene clusters from various magnetotactic Alphaproteobacteria can be functionally expressed in the tractable *M. gryph.* To this end, DNA fragments from various donors were PCR amplified and cloned by multi-fragment hot fusion assembly or yeast-based transformation-associated recombination (TAR) cloning into large expression cassettes up to 40 kb in size. Upon transfer and chromosomal integration in *M. gryph.*, gene clusters were stably maintained and restored the magnetosome

phenotypes of donor MTB to different degrees. Expression of certain parts of some of the gene clusters led to the biomineralization of magnetite particles with aberrant sizes and shapes. This suggests that the transferred genes may control the morphology of magnetosomes, which is currently explored in more detail. In conclusion, our results confirm that *M. gryph.* is a useful host for the expression and functional elucidation of morphogenic magnetosome genes from foreign MTB.

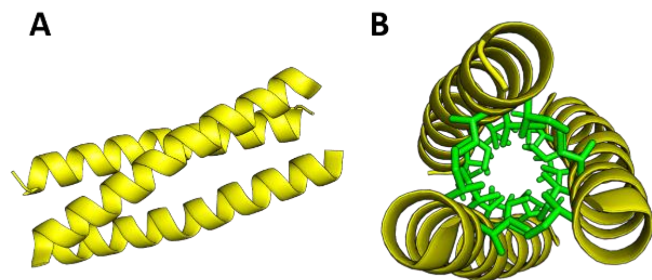
[1] R. Uebe, D. Schüler 2016. Magnetosome biogenesis in magnetotactic bacteria. Nat Rev Microbiol 14:621–637.
[2] R.P. Awal, C.T. Lefevre, D. Schüler2023, Functional expression of foreign magnetosome genes in the alphaproteobacterium Magnetospirillum gryphiswaldense., mBio 14: e0328222.

P-BSM-003
A trimeric coiled-coil motif binds bacterial lipopolysaccharides with picomolar affinity - and can be used in LPS detection and removal applications

*D. Linke[1]
[1]*University of Oslo, Biosciences, Oslo, Norway*

Modified coiled-coils sequences can be used in biotechnology, vaccine development, and biochemical research to induce protein oligomerization. A prominent model for the versatility of coiled-coil sequences is a peptide derived from the yeast transcription factor, GCN4. Unexpectedly, its trimeric variant, GCN4-pII, binds bacterial lipopolysaccharides (LPS) from different bacterial species with picomolar affinity. LPS molecules are highly immunogenic, toxic glycolipids that comprise the outer leaflet of the outer membrane of Gram-negative bacteria. Using scattering techniques and electron microscopy, we show how GCN4-pII breaks down LPS micelles in solution. In an ELISA-like assay, we find that LPS can in principle be detected in minimal concetrations, competitive to the industry gold standard for LPS detection, the LAL assay. Our findings thus suggest that the GCN4-pII peptide and derivatives thereof could be used for novel LPS detection and removal solutions with high relevance to the production and quality control of biopharmaceuticals and other biomedical products, where even minuscule amounts of residual LPS can be lethal.

Fig. 1



P-BSM-005
Parapyruvate formation during low-biomass pyruvate production with engineered Vibrio natriegens

*M. Hädrich[1], C. Schulze[1], F. Thoma[1], B. Blombach[1]
[1]*Technical University of Munich, Microbial Biotechnology, Straubing, Germany*

Most industrial production processes must operate at a high final biomass concentration to reach a high volumetric productivity (Q_P), wasting significant amounts of carbon for biocatalyst generation. Therefore, we are using *Vibrio natriegens* due to its very fast growth and associated high specific substrate uptake rate (q_S) [1] in a low-biomass processes with non-growing but metabolically highly active catalysts.

We engineered the prophage-free strain *V. natriegens* Δ*vnp12* [2] by deleting the *aceE* gene encoding the E1 subunit of the pyruvate dehydrogenase complex (PDHC). This PDHC-deficient strain is acetate auxotroph, allowing adjustment of the biomass formation by changing the amount of acetate in the process. Through this, the whole process is split into a growth and a production phase in which pyruvate is secreted into the medium.

Shaking flask experiments in minimal medium with 7.5 g glucose L^{-1} and 1 g acetate L^{-1} achieve up to 4.0 ± 0.3 g pyruvate L^{-1} in the supernatant. Therefore, batch fermentations were performed and up to 22.2 ± 0.8 g pyruvate L^{-1} were produced after 10 hours with a yield of 0.59 ± 0.04 g pyruvate g^{-1} glucose. An additional acetate feed of 8 mM h^{-1} increased the titer to 41 ± 2 g pyruvate L^{-1} with a $c_{x,max}$ of 6.6 ± 0.4 g L^{-1}. Moreover, the qs of the non-growing cells increased to 3.5 g glucose g^{-1}_{CDW} h^{-1}, close to the q_S of exponentially growing cells [1] and therefore over twice as high as the qs of growing *E. coli*.

Following gaps in the carbon balance, significant amounts of parapyruvate (4-Hydroxy-4-methyl-2-oxoglutarate, HMG) were found in the reactor samples. The ligase LigK was investigated as a possible candidate for biological parapyruvate formation using overexpression and deletion strains, showing significant phenotypes. Moreover, chemical parapyruvate formation was explored in cell-free reactor experiments.

[1] Hoffart, E., Applied and environmental microbiology, 2017. 83(22): e01614-17.

[2] Pfeifer, E., et al., Applied and environmental microbiology, 2019. 85(17): e00853-19.

P-BSM-006
Construction of a restriction deficient Clostridium kluyveri strain using the codAB counterselection system

*A. Abstreiter[1], A. Ehrenreich[1], W. Liebl[1], V. Burgmaier[2], D. Weuster-Botz[2]
[1]*Technical University of Munich, Chair of Microbiology, Freising, Germany*
[2]*Technical University of Munich, Chair of Biochemical Engineering, Garching, Germany*

Providing efficient regenerative production pathways for bulk chemicals as an alternative to the petrochemical synthesis will be inevitable for improving the carbon footprint and the transition to a circular economy. Gas-fermenting microbial co-cultures possess great potential for such recycling processes as they can utilize synthesis gas generated as an industrial off-gas or by gasification of municipal or food waste. A synthetic co-culture between *Clostridium carboxidivorans* and *Clostridium kluyveri* performing carbon chain elongation produces medium-sized alcohols from synthesis gas. The performance of *C. carboxidivorans* in this co-culture is most efficient at high

CO partial pressures, but growth of *C. kluyveri* is restricted at these CO levels. Therefore, further improvement of the process could be achieved by genetic adaption of *C. kluyveri* to high CO levels.

An *in vivo* methylation-based protocol for triparental conjugation was developed for *C. kluyveri* DSM555T, but tools for its genetic modification are still missing. To this end, the strain *C. kluyveri* was tested for its suitability for the *codAB* counter selection system and showed the necessary high 5-fluorocytosine tolerance of up to 500 µg/ml and 5-fluorouracil sensitivity below 30 µg/ml. We identified a suitable origin of replication to create a pseudo-suicide plasmid containing the *codAB* system favoring genomic integration via homologous recombination. The vector was transconjugated successfully and we were able to construct the first mutant strain of *C. kluyveri* lacking a dominant type II restriction/modification system. This 'domestication' should further increase the conjugation rates allowing to construct strains with improved CO tolerance.

P-BSM-007

Novel and efficient CRISPR/Cas systems for precise genome editing of industrially relevant microorganisms

*J. Noske[1], N. van Wyk[2], P. Scholz[1], C. Zurek[1]
[1]*Akribion Genomics AG, Zwingenberg, Germany*
[2]*BRAIN Biotech AG, Zwingenberg, Germany*

CRISPR/Cas has revolutionized genome editing and enables the precise genetic modification of various single-celled organisms in biotechnology. However, the commercial use of the well-characterized nucleases is severely hindered due to patent and licensing uncertainties.

To overcome these limitations, we have identified novel Cas nucleases with little or no homology to database Cas proteins in an elaborate metagenomics sequencing analysis of rationally selected environmental samples. Among others, several potential class 2 type V CRISPR systems suitable for genome editing resulted from this metagenome screening of over 3 tera base pairs in sequencing data. In order to evaluate the genome editing activity of our *in silico* identified sequences, we carried out experiments in *E. coli* as the classical prokaryotic model organism. We were able to show that our novel Cas nucleases, which are termed G-dase M, can be programmed to efficiently and precisely introduce a genomic DNA double-strand break. Using an additional bacterial non-homologous end joining (NHEJ) system, we then introduced gene-specific INDEL mutations with different G-dase M nucleases. Furthermore, one chosen G-dase M system in combination with the λ/Red recombinase system enabled efficient homology-directed repair (HDR) with provided DNA repair fragments in *E. coli*. We have thus proven that we were indeed able to identify functional and novel Cas proteins.

To also evaluate the activity of our G-dase M nucleases in a eukaryotic organism, we have adapted one selected system to genetically modify various *Saccharomycetes* laboratory strains and diploid commercial wine starter cultures in a targeted and efficient manner. This utilized the cell's own ability to repair introduced DNA double-strand breaks by HDR. We have even established a powerful single-plasmid system for a winemaking yeast for which no CRISPR system has been described so far. All the results of our genome editing studies have shown that our proprietary CRISPR systems are valuable tools for the genetic modification of prokaryotic and eukaryotic organisms.

P-BSM-008

Engineering of the aromatic amino acid metabolism of Corynebacterium glutamicum using biosensor-guided high-throughput screenings

*M. Krinke[1], J. Marienhagen[1]
[1]*Research Center Juelich, IBG-1, Jülich, Germany*

The engineering of microbial strains for the production of small molecules of biotechnological interest is a time-consuming, laborious and expensive process. In this context, transcription factor-based biosensors represent powerful tools for the rapid screening of large and genetically diverse strain libraries at the single cell level by translating the intracellular concentration into a fluorescence signal.

In this study, such a biosensor for the detection of intracellular chorismate concentrations in *Corynebacterium glutamicum* was constructed on the basis of the cell-own LysR-type regulator QsuR and its cognate target promoter. Subsequently, the biosensor was characterized, and combined with fluorescence-activated cell-sorting (FACS) with the aim to improve the carbon flux into the shikimate pathway.

The dynamic and operational range of the chorismate biosensor was determined by supplementation experiments using the chorismate precursor quinate, since *C. glutamicum* cannot take up chorismate. An increased fluorescence signal was detected for quinate concentrations ranging from 250 µM to 64 mM, showing a 150-fold increase in specific fluorescence at a quinate concentration of 64 mM. The ligand spectrum was determined through the addition of molecules with a structural similarity to chorismate as well as other, chemically more different intermediates of the shikimate pathway. A fluorescence signal was only detectable in response to the supplementation of quinate, which is intracellularly converted to chorismate, indicating a high specificity of the QsuR-based sensor.

For the isolation of *C. glutamicum* strains with increased carbon flux into the shikimate pathway, the biosensor was used to screen genetically diverse strain variants generated through random chemical mutagenesis of the whole genome or PCR-based targeted mutagenesis of specific genes. Here, FACS screening enabled the isolation of strain variants with increased chorismate accumulation based on their specific fluorescence signal.

P-BSM-009

Iterative in vivo "cut"n"paste" of functional genomic loci in bacteria

*S. Gude[1], A. B. Bertelsen[1], M. H. H. Nørholm[1]
[1]*DTU, Lyngby, Denmark*

Introduction:

Systematic reengineering of bacterial genomes is still in its infancy. Advances in genome engineering may enable novel applications in green biotechnology while simultaneously providing fundamental insights into the interplay between biological function and genome architecture.

Goals:

Here, we present a scalable *in vivo* method to iteratively relocate ("cut"n"paste") functional genomic loci onto artificial chromosomes in bacteria. Our method aims to facilitate the creation of auxiliary functional modules, such as the recently described iModulons[1], that can be transferred and used in a plug-and-play fashion to add specific biological functionality to a minimal cell chassis when desired.

Methods:

We combine CRISPR-Cas9 counter-selection and lambda red recombineering with standardised components and an antibiotic marker recycling scheme to drastically reduce the need for *in vitro* manipulations and locus-specific workflow optimization.

Results:

We successfully relocate multiple operons onto an artificial chromosome and assay their performance in the novel genomic context.

Summary:

In vivo "cut"n"paste" promises to overcome the stark limitations imposed by *in vitro* techniques such as PCR or restriction enzyme cloning by letting CRISPR-Cas9, homologous recombineering, and the native bacterial DNA polymerase do the heavy lifting inside the cell.

References:

1. Sastry, A.V., Gao, Y., Szubin, R. et al. The Escherichia coli transcriptome mostly consists of independently regulated modules. Nat Commun 10, 5536 (2019). https://doi.org/10.1038/s41467-019-13483-w

---

P-BSM-010

Establishing Aureobasidium as a novel, robust platform organism towards a sustainable biotechnology

*M. Driller[1], K. Stein[1], M. R. E. Dielentheis-Frenken[1], L. M. Blank[1], T. Tiso[1]
[1]*RWTH Aachen University, Institute of Applied Microbiology (iAMB), Aachen, Germany*

Aureobasidium is a genus of highly adaptable, yeast-like fungi that are widely distributed as saprophytes across various ecosystems. Demonstrating remarkable resilience, *Aureobasidium* can survive in environments characterized by extreme pH levels and high salinity. Furthermore, this fungus is notable for its production of various secondary metabolites with considerable biotechnological potential, such as polyol lipids, pullulan, and polymalate. Additionally, *Aureobasidium* can grow on a wide range of carbon sources, including renewable raw materials and waste products, positioning it as a candidate for a novel, robust platform organism.

Lignin, one of the most abundant renewable resources on earth, is highly underutilized and largely wasted. The aromatic compounds of this polymer can be a promising feedstock for a wide range of value-added chemicals. Here, we demonstrate that *Aureobasidium pullulans* can grow on a multitude of lignin-derived aromatic monomers like catechol or coumarate, even at extreme pH values. Toxicity tests revealed that the fungus can withstand high concentrations of these aromatics. Moreover, the ability to grow on mixtures of aromatic compounds found in lignin hydrolysates was also proven, while the production of the bioprivileged molecule muconic acid from the aromatics could be observed.

However, metabolic engineering tools are still rare and thus hinder the advancement of *Aureobasidium* towards a novel platform organism. To address this challenge, a genetic toolbox based on the CRISPR-Cas9 method was developed in *A. pullulans,* enabling scarless genomic modifications, integration of multiple genes, and a tuneable gene expression.

Expanding the substrate spectrum towards sustainable renewable substrates and the development of genetic tools are the first steps in establishing *A. pullulans* as a new versatile platform organism contributing to a sustainable bio-economy.

P-BSM-011

Gold nanoparticle - peptide deformylase inhibitor conjugates, a mode of action study

*A. Schultz[1], T. Zhou[2], M. John[3], P. Dietze[1], B. Menzel[4], R. Erdmann[4], N. Metzler-Nolte[3], J. Scherkenbeck[2], J. E. Bandow[1]
[1]*Ruhr University Bochum, Applied Microbiology, Bochum, Germany*
[2]*University of Wuppertal, Faculty of Mathematics and Natural Sciences, Wuppertal, Germany*
[3]*Ruhr University Bochum, Inorganic Chemistry I - Bioinorganic Chemistry, Bochum, Germany*
[4]*Ruhr University Bochum, Systems Biochemistry, Bochum, Germany*

Recent studies attributed 1.27 million deaths world-wide in 2019 to infections with multi-resistant bacteria (Murray, 2022).This emphasizes the urgency for new antibacterial agents to be developed. In this study, the utilization of small gold nanoparticles as a new drug delivery system for antibiotics was investigated. Peptide deformylase (PDF) inhibitors derived from the naturally occurring actinonin (Chen et al., 2000) were chosen as ligands for these particles (Kirschner et al., 2023). To this end, we investigated new actinonin derivates and examined the mode of action of actinonin derivative – gold nanoparticle conjugates. We screened for effective PDF inhibitors with a combination of standardized minimal inhibitory concentration assays and mass spectrometry-based analysis. For mode of action studies, gel-based proteomics was performed using the model organisms *B. subtilis* and *E. coli.* Furthermore, localization studies were performed by transmission electron microscopy.

We successfully identified derivatives with a broad activity against various gram-positive and gram-negative bacteria. The gel-based proteomics revealed that PDF is inhibited in vivo by the free actinonin derivative and the gold nanoparticle conjugate.

In localization studies it was observed that the nanoparticles did not cross the outer membrane of *E. coli.* Therefore, we conclude from the proteomic results, that the nanoparticles are able to deliver the antibiotic. Overall, we could show that gold nanoparticles have potential to deliver antibiotics without compromising the mode of action of their bound ligand.

Murray et al., 2022, Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet, 399:629-55

Chen et al., 2000, Actinonin, a naturally occurring antibacterial agent, is a potent deformylase inhibitor. Biochemistry, 15;39(6):1256-62

Kirschner et al., 2023, Structural insights into antibacterial payload release from gold nanoparticles bound to *E. coli* peptide deformylase, ChemMedChem, e202300538

P-BSM-012
Advanced workflows for the systematic identification of metabolic optimization targets in DBTL-cycles: A demonstrator for producing aromatic compounds in C. glutamicum

*N. Hollmann[1,2], S. Noack[1], J. Marienhagen[1,2]
[1]*Research Center Juelich , Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany*
[2]*RWTH Aachen University, Institute of Biotechnology, Aachen, Germany*

The establishment of rational Design - Build - Test – Learn (DBTL) cycles based on miniaturization, parallelization, automation and digitalization enables a reduction in development times and an increase in reproducibility and effectiveness of microbial strain construction for competitive biomanufacturing.

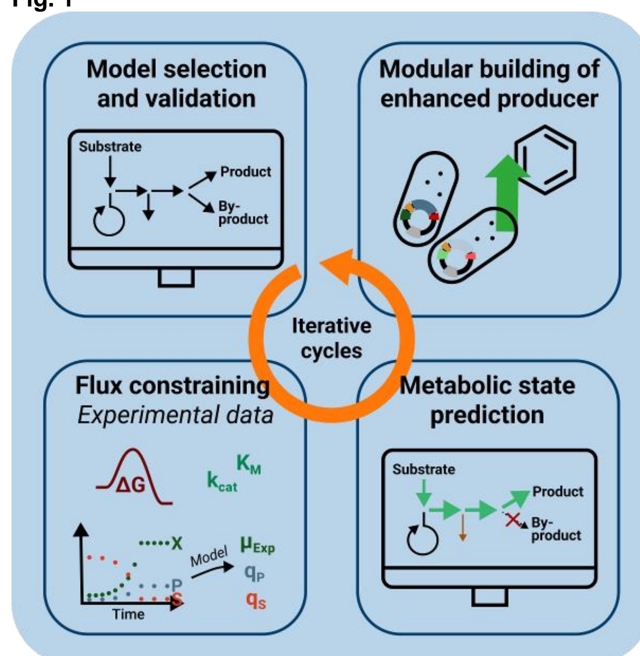
Here, we present an advanced strain design and analysis workflow to systematically improve the understanding of microbial production pathways and to enable faster identification of metabolic optimization targets (Fig. 1). As a first demonstrator, we focus on aromatic compound production in *Corynebacterium glutamicum* with the specific goal to enhance the carbon flux towards l-tyrosine through the native shikimate pathway.

The workflow harnesses the power of *in silico* metabolic modelling with parallelized strain phenotyping. A standardized library of small- to genome-scale stoichiometric network models of *C. glutamicum* in combination with thermodynamic data and available kinetic information was established. For the simulation of intracellular metabolic states, using parsimonious flux balance analysis, a python-based workflow was set up. Quantitative and fast characterization of growth and production phenotypes is realized by employing robotic-assisted micro-cultivation experiments with (un)targeted metabolomics via LC/ GC-ToF-MS. Raw data processing and modelling is performed using recently developed python tools [1]. Resulting specific rate estimates are applied to further constrain reaction fluxes for *in silico* strain design. The wider range of potential metabolic engineering targets contributing to enhanced l-tyrosine production will then be constructed using *MoClo*-based workflows on our *AutoBioTech* platform [3].

Ultimately, a standardized and validated modelling toolbox enables the design and analysis of a broad range of production strains and thus represents an essential component for the operation of biofoundries.

1. Strohmeier D et al. 2022, estim8: Parameter estimations for Dymola and FMU models. (https://pypi.org/project/estim8/)
2. https://www.biooekonomierevier.de/Innovationslabor_AutoBiotech

Fig. 1



P-BSM-013
Predictive biotechnology – A case study on 4-phenol oxidase

*D. Tischler[1], N. Weindorf[1], H. G. Weddeling[1], D. Eggerichs[1]
[1]*Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany*

Enzymes are Nature´s catalysts and have a great impact on (organic)synthesis either in their natural role or by being applied in technical processes. Certainly, the knowledge about enzymes and their functionality is steadily increasing. In addition, frequently novel enzymes and reactions are described. Herein, we present a streamlined approach of enzyme mining in order to rationally select enzymes with proposed functionalities from the ever-increasing amount of available sequence data. Hence, we want to predict enzyme functionality and applicability based on the amino acid sequence: a work related to predictive biotechnology.

In a case study on 4-phenol oxidases, to be precise on the VAO/PCMH flavoprotein family, eight enzymes were selected from about 300 sequences on basis of the properties of first shell residues of the catalytic center. Thus, a broad sequence space was covered on base of a first bioinformatic screening approach including known enzyme studies. To handle numerous sequences and easily rationalize or even predict sequence-function relations we created a computational tool, called amino acid cluster analysis (A2CA; https://doi.org/10.57760/sciencedb.09549). It allows to correlate the phylogenetic information with the data from a respective multiple sequence alignment as well as to link any other data such as activity, binding, structural data. Thereafter, selected candidates were produced and fully characterized. Correlations between important residues identified and enzyme activity yielded robust sequence-function relations, which were exploited by site-saturation mutagenesis. The application of a novel oxidase screening assay resulted in 16 active enzyme variants which were up to 90-times more active than respective wildtype enzymes.

The results were supported by kinetic experiments and structural models. The newly introduced amino acids confirmed the correlation studies which overall highlights the successful logic of the presented approach.

P-BSM-014

Reprogramming siderophore-based microbial interactions by light

*S. H. Paik[1], A. Zeisel[1], A. Krüger[2], M. Bund[2], J. Wiechert[2], D. Kohlheyer[2], J. Frunzke[2], K. E. Jaeger[1,2], T. Drepper[1]
[1]*Heinrich Heine University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Research Center Juelich, IBG-1: Biotechnology, Jülich, Germany*

Microbial communities ubiquitously inhabit diverse global ecosystems and play an important role in numerous biological processes. Social interactions such as intra- and inter-species communication have a strong influence on the composition and behaviour of these communities. In this context, the exchange of various secondary metabolites plays a central role. Notably, siderophores are an important class among these metabolites. In response to iron limitation, most bacteria secrete siderophores that exhibit high affinities to Fe^{3+}. The iron-siderophore complexes can subsequently be taken up by the microbes *via* specific receptors. Pyoverdine (PVD), a fluorescent siderophore produced by various *Pseudomonas* species, is known to be involved in several types of social interactions[1,2]. While PVD-based interactions have been extensively studied[3,4,5], the importance of the spatial and temporal distribution of these processes and their impact on community composition and development remain largely unexplored. To analyze the dynamics of PVD-mediated intra- and interspecies interactions, we aimed to develop *Pseudomonas putida* strains where PVD production can be controlled gradually and non-invasively by light. To achieve this goal, we employed the optogenetic two-component systems (TCS), Dusk[6] and REDusk[7], in *P. putida* to regulate the expression of the *pfrI* gene encoding an alternative sigma factor, which is essential for PVD production in *P. putida*. We could demonstrate that the biosynthesis of PVD can be specifically, gradually, and dynamically controlled in these strains at the single-cell level by modulating the conditions for illumination. This optogenetic approach thus enables the dynamic transition from PVD producers to non-producers and even to over-producers, thereby allowing a detailed analysis of spatial and temporal aspects of PVD-mediated intra- and interspecies communication.

[1] Kramer et al., Nat Rev Microbiol, 2020
[2]Ghssein et al., Biology, 2022
[3]Becker et al., Sci Rep, 2018
[4]Rayi et al., ACS Chemical Biology, 2024
[5]Mridha et al., Commun Biol, 2022
[6]Ohlendorf et al., J Mol Biol., 2012
[7]Multamäki et al., ACS Synthetic Biology, 2022

P-BSM-015

Exploring key enzymes of the autotrophic metabolism in Hydrogenophaga pseudoflava

*S. Grieshaber[1], P. Kehr[1], B. Blombach[1]
[1]*Technical University of Munich, Microbial Biotechnology, Straubing, Germany*

Introduction

The utilization of carbohydrate substrates for chemical production is often criticized, as it competes with food. Thus, aerobic gas fermentation to produce chemicals from C1 units is an interesting approach. One promising candidate for such processes is the carboxydotrophic bacterium *Hydrogenophaga pseudoflava* DSM 1084. This Gram-negative β-proteobacterium shows comparable high growth rates on CO, H_2 and CO_2 and mixtures thereof under aerobic conditions. Moreover, a basic genetic engineering toolbox is available which renders this bacterium an interesting host for biotechnological application. [1, 2]

Objectives

To analyze the electron transfer and its impact on energy and redox metabolism, different key enzymes of the autotrophic pathways were deleted. These mutants were cultivated with CO_2 and H_2 or CO gas mixtures. Beside the effect on growth, also the overall hydrogenase and CO dehydrogenase activity was determined.

Materials & methods

Cells were harvest in the exponential phase, stored at -80 °C and later lysed via sonication. According to Meyer and Schlegel (1980) the oxidation of CO or H_2 was measured photometrical using methylene blue as electron acceptor.

Results

The hydrogenase activity exhibited substrate-dependent behavior, with notably high activity recorded on 1 % fructose (1598 ± 201 nmol $mg^{-1}_{Protein}$ min^{-1}). Under autotrophic conditions (86 % H_2, 10 % CO_2 and 4 % O_2) the activity doubled compared to heterotrophic conditions (3021 ± 452 nmol $mg^{-1}_{Protein}$ min^{-1}). The deletion of the membrane-bound hydrogenase led to no growth under these conditions, emphasizing its crucial role. On the other hand, CO dehydrogenase 1 was identified as essential for growth on 80 % CO gas mixture.

Conclusion

Relevant enzymes for the autotrophic metabolism of *H. pseudoflava* have been identified.

[1] Grenz *et al.*, *Metab. Eng.*, Vol. 55 (2019)

[2] Siebert *et al.*, *Adv. Biochem. Eng. Biotechnol.*, Vol. 180 (2022)

[3] Meyer and Schlegel, *J. Bacteriol.*, Vol. 140 (1980)

P-BSM-016

Exploring the Diversity of Polyesterases from marine Bacteria

*T. Horbach[1], S. Thies[2], R. Molitor[1], T. Drepper[1], K. E. Jaeger[1,2]
[1]*Heinrich Heine University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Research Center Juelich, Institute of Bio- and Geosciences (IBG-1), Jülich, Germany*

In recent years, marine bacteria have proven to be a prolific source of polyester degrading enzymes. Such polymer-active enzymes can effectively break down plastic polymers into their constituent components and thus contribute to mitigate environmental pollution. With this study, we aimed to expand the repertoire of available polyesterases with a focus on enzymes showing activity at high salt concentrations, low temperatures and elevated pressure. We have isolated polyesterases from marine bacteria which inhabit plastic-polluted coasts and deep-sea environments assuming that they may have evolved an enzyme repertoire enabling the degradation of plastics. To this end, we used sediment samples for growing enrichment cultures with various synthetic polymers as sole carbon sources. More than 20 marine organisms displaying polyesterase activities were isolated, their genomes sequenced, and potentially novel polyesterase encoding genes identified by using search algorithms based on Hidden-Markov-models. Selected candidate genes were then expressed in *E. coli* and tested for polyesterase activity on indicator plates. Subsequently, 8 enzymes were purified and activity assays confirmed the hydrolysis of a broad range of synthetic substrates (eg. BHET, MHET, Impranil® DLN-SD). Some of these enzymes exhibited a high tolerance towards different organic solvents (eg. Methanol, dimethyl sulfoxide) and showed high activity also at temperatures lower than 10 °C. Our study shows that marine bacteria can be source of novel polyesterases with biochemical properties useful for biotechnological applications.

P-BSM-017
Multidisciplinary Exploration of Antimicrobial Photodynamic Therapy (aPDT) on Clinical Polymer Surfaces for Improving the Safety of Temporary Implants.

*A. Rahtz[1], S. Johannsmeier[1], T. Ripken[1], M. Weinhart[2], R. Berger[2], A. Laporte[2]
[1]*Laser Center Hannover e.V., Biomedical optics, Hannover, Germany*
[2]*Leibniz Universität Hannover, PCI, Hannover, Germany*

Abstract: Antimicrobial resistance has become a significant global issue leading to health problems and reduced treatment effectiveness, which negatively impact life expectancy. In this context, Antimicrobial Photodynamic Therapy (aPDT) emerges as a potential alternative to circumvent resistance issues. The light-triggered activation of photosensitizers (PS) induces the generation of reactive oxygen species (ROS), effectively destabilizing cell walls and instigating cellular demise. Unlike conventional antibiotics, aPDT mechanisms have evaded observable resistance.

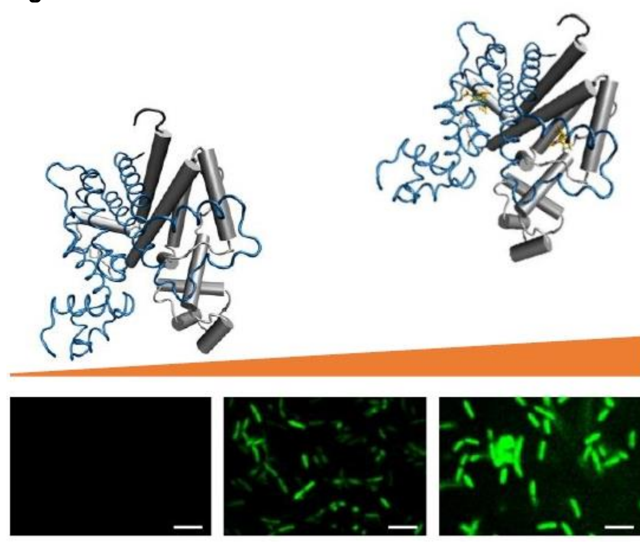
In our study, we center on the dose-response relationship, exploring the chemical aspects, including various methods for attaching PS to surfaces. We assess the efficacy of free versus bound PS in reducing bacterial counts, to gain insights into the chemical intricacies influencing the attachment strategies. The preliminary outcomes of investigation utilizing light with a wavelength of 660 nm, the PS methylene blue and *Escherichia coli* K12 bacteria reveal a reduction in bacterial count both for bound and unbound PS, depending on the concentrations and light intensity. Empirical investigation involves clinically relevant Gram-pos. and neg. bacteria. Additional parameters like PS surface loading, irradiation sequencing, potential tube material modifications, and aPDT robustness under storage, environmental, and sterilization conditions are examined. Our next step is to identify the specific ROS generated during aPDT on clinical polymer surfaces, specifically silicone and polyurethane, by combining expertise from (bio)material sciences, polymer and surface chemistry, microbiology, and optics/illumination technology. This effort aims to generate new insights into the nature and impact of ROS, establish dose-response relationships to delineate aPDT efficacy, and explore innovative strategies for covalent attachment of PS. The study will assess potential deleterious effects on surrounding tissues and critically evaluate the scalability of aPDT, contributing to a comprehensive understanding of its application in clinical settings for temporary implants like catheters, venous accesses, or dialysis shunts.

P-BSM-018
Advancement and application of tet-regulation in Stenotrophomonas maltophilia

J. Gillich[1,2], A. Reinhardt[1,2], R. Ebbert[2], J. Steinmann[1], *R. Bertram[1]
[1]*Paracelsus Medical University, Institute for Clinical Hygiene, Medical Microbiology and Clinical Infectiology, Nürnberg, Germany*
[2]*Technische Hochschule Nürnberg, Faculty of Applied Chemistry, Nürnberg, Germany*

The Gram-negative bacterium *Stenotrophomonas maltophilia* is ubiquitously present in the environment and represents a potential threat to immunocompromised patients in healthcare settings. Intrinsic resistance to different classes of antibiotics makes treatment of infections challenging. A deeper understanding of *S. maltophilia* physiology and virulence requires molecular genetic tools. We have established tetracycline-dependent gene regulation (*tet*-regulation) in *S. maltophilia* en route to further advancement and application. Our one-plasmid-based *tet*-regulation set up (doi: 10.1128/spectrum.01576-23) contains a chloramphenicol resistance marker, the *tetR* gene and three intertwined *tet*-promoters from transposon Tn*10*. Here, we replaced wt-*tetR* for a reverse *tetR* variant whose gene was codon adapted to an elevanted GC content. The dynamic range of the regulatory system was quantified using a super-folder *gfp* reporter and visualized by confocal laser scanning microscopy. Contrary to wt-TetR, cultivation in the presence of inducer anhydrotetracycline yielded reduced fluorescence compared to inducer-free conditions. Next, we addressed four potentially essential *S. maltophilia* genes using wt-*tet*-regulation by either inducible plasmid-based antisense-RNA expression, or by upstream chromosomal integration of a *tet*-regulation unit into the genome of *S. maltophilia* K279a. Growth analyses in liquid medium with or without inducer permitted an assessment of gene essentiality. Finally, the wt-*tet*-regulation unit was bracketed by 19 bp "Mosaic Elements" to allow for randomized integration into plasmid- or genomic DNA, exploiting hyperactive Tn*5* transposase. In vitro transposition of the *tet*-insertional element (1633 bp) into pUC19 yielded plasmids with insertions at different positions. We next plan to assemble ternary complexes of transposase and *tet*-insertional element (transposomes), for electroporation into *S. maltophilia*. Dependent on the yield of transformants, we can expect to identify conditional lethal mutant strains with essential genes under *tet*-control. These approaches may lead to the identification of potential targets for antiinfectives against *S. maltophilia*.

Fig. 1



P-BSM-019

Unveiling the Potential of Novel Non Haem Fe(II) Enzymes for sp^3 C-H Functionalization

*D. Calabrese[1], P. Cordero[1], L. Lauterbach[1]
[1]*RWTH Aachen University, Institute for Applied Microbiology, Aachen, Germany*

Introduction: Enzymatic functionalization of aliphatic carbons is a potent strategy in sustainable organic synthesis. Our focus is on discovering and characterizing new alpha-ketoglutarate-dependent halogenases/hydroxylases (AKGHs) capable of functionalizing sp^3 carbons in *N*-heterocycles, with wide-ranging applications in pharmaceuticals, agrochemicals, and fine chemicals.

Goals: Our objectives include discovering novel catalytic pathways by identifying and characterizing AKGHs for unexplored sp^3 carbon functionalization, thereby revealing new sustainable routes for chemical transformations. We aim to elucidate the complexities of enzyme-substrate interactions, reaction kinetics and selectivity within *N*-heterocyclic compounds. We also plan to broaden the catalytic horizons of AKGHs, expanding their substrate scope via protein engineering to enhance enzyme versatility for a variety of chemical substrates.

Methods: Our approach leveraged computational tools, such as Hidden Markov Models and AlphaFold2, for AKGH identification and structural insights. We used analytical techniques such as GC, HPLC, MS, and NMR to monitor the kinetics, identify products, and determine stereochemistry. Additionally, we implemented protein engineering methods like site saturation mutagenesis (SSM) and random mutagenesis to improve enzyme properties and expand substrate scope.

Results: Our computational screening successfully identified ten novel AKGH candidates. Through optimization of heterologous production and reaction conditions, we have achieved efficient production of these enzymes. Initial tests reveal promising catalytic potential, with these AKGHs demonstrating the ability to facilitate selective hydroxylations and halogenations in specific N-heterocyclic substrates. Current protein engineering efforts are focused on broadening their substrate scope, enhancing their applicability in diverse chemical reactions.

Summary: Our research advances AKGH understanding, revealing new catalytic pathways and enhancing substrate versatility. These enzymes offer an eco-friendly approach to chemical synthesis, aligning with green chemistry principles for a more sustainable future.

P-BSM-020

Split fluorescent protein-mediated in vivo protein immobilization

*J. Jetten[1], O. Klaus[1], T. Gensch[1,2], K. E. Jaeger[1,3], T. Drepper[1]
[1]*Heinrich Heine University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Research Center Juelich, Institute of Biological Information Processing IBI-1: Molecular and Cellular Physiology, Jülich, Germany*
[3]*Research Center Juelich, Institute of Bio- and Geosciences IBG-1: Biotechnology, Jülich, Germany*

The immobilization of enzymes on a matrix is a well-established approach to improve their stability and (re)usability for industrial applications. While many different concepts for enzyme immobilization have been developed, *in vivo* immobilization (i.e., heterologous expression of a target protein and subsequent coupling to biogenic carrier materials within living cells) is still challenging. Moreover, online monitoring of the *in vivo* immobilization process is not yet possible. Here, we present an alternative method for *in vivo* immobilization using split fluorescence proteins, such as split GFP, as a universal linker and simultaneously as a sensor for immobilization. For this, the GFP sequence is split into two non-fluorescent fragments GFP1-10 and GFP11 that have the ability to self-assemble to generate a specific green fluorescent signal. We used split GFP as a linker to immobilize target proteins on the surface of heterologous polyhydroxybutyrate (PHB) granules in *Escherichia coli*. While GFP11 is fused to the PHB synthase PhaC as an anchor protein, the target protein is linked to GFP1-10, so that successful immobilization is accompanied by a green fluorescence signal. As a proof-of-concept, the red fluorescent protein mCherry, the ester hydrolase LipD from *Alcanivorax borkumensis* and the squalene synthase from *Methylococcus capsulatus* were selected as target proteins. To verify the immobilization process in *E. coli* cells, we used fluorescence spectrometry, microscopy, and fluorescence lifetime imaging. LipD activity was analyzed *in vitro* using 4-nitrophenyl butyrate as substrate and HPLC analyses were performed to determine the amount of squalene produced *in vivo* by the squalene synthase.

Overall, we were able to show that the split GFP system can be used as a combined linker-sensor system for the *in vivo* protein immobilization on the PHB surface without affecting the function of the target proteins. Thus, we have developed a new system that enables an easy to detect decoration of the PHB surface and may therefore represent a promising approach for future applications.

P-BSM-021

Sugar-inducible promoters for "knock-down" of essential genes in a thermophilic acetogen

*A. Shakirova[1], C. Baum[1], B. Zeldes[1], M. Basen[1]
[1]*University of Rostock, Institute of Biological Sciences, Microbiology, Rostock, Germany*

Thermoanaerobacter kivui is an anaerobic thermophilic bacterium (T_{opt} 66°C) growing on sugars or H_2+CO_2. It produces acetate as a main product using the Wood-

Ljungdahl Pathway (WLP), and it is promising for biotechnological applications to produce valuable organic molecules from syngas (mixture of H_2, CO_2, and CO). However, for its application, a thorough understanding of the metabolism, as well as methods of manipulating it, are required. As some genes are essential for the cell"s survival, e.g. these encoding some enzymes of the WLP, it is difficult to study the metabolism of *T. kivui* solely by gene knockouts. For that reason, "knock-downs" of putative essential genes were performed using newly identified sugar-inducible promoters. The aim of this work was to characterise the resulting strains to better understand the redox and energy metabolism of *T. kivui*.

To generate "knock-down" strains of *T. kivui*, a Δ*pyrE* acceptor strain was transformed using PCR products containing a *pyrE* gene[1], the new sugar-inducible promoter, and flanking regions for homologous recombination to replace the native promoter. An inducible promoter was inserted in front of a gene of interest, so that it could be regulated by the presence or absence of the inducing sugar. Growth and metabolite analysis were used to compare the phenotype of cells in different conditions, and qPCR was performed to assess the expression levels of regulated genes.

Growth of *T. kivui* strains modified with inducible promoters was found to be inhibited when cells were cultured on non-inducing sugars. A "knock-down" strain with a mannitol inducible promoter (P_{Man}) in front of the major WLP gene operon accumulated formate when grown on glucose due to a metabolic bottleneck, but growth on mannitol eliminated formate accumulation. This demonstrates that sugar-inducible promoters can be used to control essential genes in *T. kivui*. Further characterization of additional strains will help to fill gaps in the current understanding of *T. kivui*"s metabolism. The new promoters also show promise for future development of metabolically engineered strains for industrial applications.

[1] Basen *et al*. 2018. *AEM*, 84, e02210-17

P-BSM-022

Towards full-length single cell RNA-seq technologies

*C. L. Chou[1], A. E. Saliba[1,2]
[1]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*
[2]*University of Würzburg, Institute of Molecular Infection Biology (IMIB), Würzburg, Germany*

Current single cell RNA sequencing (scRNA-seq) technologies rely on the capture of mRNA ends or computational inference of full-length mRNA through transcript-wide fragments (Smart-seq) to study gene expression inside a cell. While attempts are out there to overcome the challenge of full-length RNA capture major issues remain because of e.g. secondary structures or natural RNA modifications. Here, we aim to establish a new single-cell RNA-seq protocol amenable of capturing unforeseen RNA modalities.

RNA-seq approaches rely on the reverse transcription of RNAs molecules into more stable cDNA that can introduce significant biases in RNA-seq readout. The most commonly used RTs are derived from Moloney Murine Leukemia Virus (MMLV) and are typically fast in relay, do multiple cycle reactions, and thus are prone to stop at secondary structures commonly found in long transcripts. In contrast to classical retroviral RTs, Marathon RT, encoded within group II introns of *Eubacterium rectale* is ultra-processive and can reverse transcribe long transcripts (up to 30 kb) from one end to another in a single run, even in presence of highly stable structural elements. Here, we implemented a scRNA-seq method to capture full-length and structured transcripts using Marathon RT. First, we determined Marathon RTs sensitivity level using synthetic reference transcripts and adapted it to low RNA input. Once the suitability of Marathon RT for single cell level reverse transcription is validated, we incorporated structural probing into the protocol. In an infection setting, this new method would allow us to study not only pathogenic transcripts but also host RNAs in full-length on a single cell level.

P-BSM-023

Usage of lignocellulosic materials for H2 production by anaerobic gut fungi

*K. E. Schulz[1], A. Neumann[1], K. Ochsenreither[2]
[1]*Karlsruhe Institute for Technology, Institute of Bio- and Food Technology Subinstitute of Electrobiotechnology, Karlsruhe, Germany*
[2]*Technikum Laubholz GmbH, BIotechnological Conversion, Göppingen, Germany*

Introduction

Anaerobic fungi firstly classified as fungi in the 1980s by Orpin are phylogenetically confined as a whole in the phylum *Neocallimastigomycota* since 2007. These organisms had to undergo exceptional adaptation to their hosts digestive system, located in the rumen of herbivores. As primary digesters of lignocellulosic materials anaerobic fungi segregate a wide range of carbohydrate-active enzymes contained in cellulosomes. Additionally to enzymatic digestion physical disruption of plant cells through appressorium-like structures allow highly effective usage of chemically stable lignocellulosic materials. Growth is obtained through mixed acid fermentation, partially accomplished in hydrogenosomes, with the main products acetate, formate, ethanol, lactate, hydrogen, and succinate. Despite nearly 50 years of studies, the optimal cultivation conditions of *Neocallimastigomycota* remain unknown. Previously, only few physiological studies, focused on *Neocallimastix* and *Piromyces* were published. This study expands the currently known based on six genera isolated by Stabel *et al*.; 2020.

Goals

By gaining a deeper comprehension of cultivation conditions and fine-tuning the growth parameters of anaerobic fungi, we aim to enhance, the selectivity of preferred metabolites, reduce the costs and environmental problems associated with the exploitation of lignocellulosic materials for industrial use and facilitate the replacement of fossil fuels through generated hydrogen.

Materials & Methods

The used strains were cultivated in batches in serum flasks with deviating parameters. Soluble metabolites were analyzed by HPLC and H2 was analyzed by GC and pressure.

Results

Anaerobic fungi show specific tendencies in their metabolic pathway, which are able to slightly change depending on the cultivation conditions. Effects of different C-sources and concentrations, particle sizes, temperatures, batches and fed-batches reveal valuable insight.

Summary

Anaerobic gut fungi are promising organisms for H2 production from lignocellulose. The results indicate great potential for future application.

P-BSM-024

A physiological explanation for the abundance of transposon DNA in bacteria

*R. Römhild[1], C. Guet[1]

[1]*Institute of Science and Technology Austria (IST Austria), Klosterneuburg, Austria*

Introduction

Transposon DNA is surprisingly abundant in genomes, even in microbes. Typically, 1-30% of microbial genomes are active transposons. Surprisingly, the biological role of transposons within microbial genomes remains poorly understood. Transposons are disrupters of genetic information, involved in pseudogenization, the deterioration of genomes and key to the breakdown of product expression in biotechnology. On the other hand, transposons contribute to genetic change and adaptation by their importance for genetic recombination and the generation of beneficial mutations.

Goals

Here, we experimentally test for biological effects of transposons on a whole-genome level – when they are not transposing. We ask whether transposons are beneficial or deleterious to their host cells in a natural state and how they impact physiology and fitness. For this work, we focus on the group of insertion sequence elements (IS), which are the most abundant group of transposable elements in bacteria.

Materials & Methods

Using genome engineering techniques, we constructed strains with which we can look at the individual IS families (IS*1*, IS*3*, IS*5* etc.) and their mutants. The strains were validated using hybrid sequencing with Nanopore and Illumina and off-target mutations corrected. This allowed us to measure the biological impact of IS for bacterial growth and competitive fitness, by direct comparison of the strains in mono and co-culture experiments.

Results

We find that IS elements are directly beneficial to the physiology of a bacterium. They increase competitive fitness and growth rate.

Summary

We identify a beneficial physiological role of transposon DNA in bacterial cells, providing an explanation for their abundance in bacterial genomes.

P-BSM-025

Production and secretion of multimerized nanobodies exploiting unconventional secretion in Ustilago maydis

*S. Wegmann[1]

[1]*Institute for Microbiology, Heinrich-Heine Universtiy Düsseldorf, Düsseldorf, Germany*

Biotechnology has become an integral part of modern industry, especially in the realm of heterologous protein production. Various organisms serve as biotechnological production platforms, and among them, the fungus *Ustilago maydis* has gained increasing[K1] interest. Recent research has uncovered an unconventional secretion pathway that proves advantageous for exporting distinct heterologous proteins, with the chitinase Cts1 acting as a carrier. The system seems to be especially beneficial for exporting large proteins; however, the exact size limits are unknown.

This novel system has already been used to produce antibody formats like nanobodies. Nanobodies are emerging as novel and valuable products in modern industry due to their simplified structure compared to regular immunoglobulin antibodies. Simple production and the ability to easily multimerize them into large homo- or heterovalent fusion proteins offer significant advantage. The latter for example allows for the simultaneous detection of multiple targets or an increase in affinity against specific targets.

To determine the size limits of heterologous unconventional secretion cargo, we here focus on multimerization studies using nanobodies against fluorescent proteins as a proof-of-principle with an easy read-out.

As a prerequisite, production and unconventional secretion of novel nanobodies targeting mCherry and Gfp could successfully be established. In the next step, we successfully generated functional heterobivalent dimers. Now, we plan to further expand the study and multimerize the nanobodies further.

P-BSM-026

Automated engineering of Corynebacterium glutamicum using modular cloning (MoClo)-based CRISPR/Cas12a workflows

*D. Kösters[1,2], J. Marienhagen[1,2]

[1]*Research Center Juelich , IBG-1, Jülich, Germany*

[2]*RWTH Aachen University, Institute of Biotechnology, Aachen, Germany*

Automated strain construction facilities (biofoundries) employing advanced molecular cloning strategies provide the possibility of constructing a large number of genetically diverse production strains economically. Common molecular cloning tasks include synthesizing operons of varying induction strength, new-to-nature pathways, or rewired (or synthetic) genetic circuits, all encoded episomally or functionally integrated into the host genome. A new concept to meet the increasing demand for large genetic construct libraries is the standardization and modularization of genetic elements utilizing the Modular Cloning (MoClo) - principle.

In this study, a MoClo toolbox for rapid plasmid construction for episomal gene expression and CRISPR/Cas12a-based genome modifications was developed for *C. glutamicum*. A library of basic genetic parts (promoters, ribosome binding sites, enzyme encoding DNA sequences, and terminators) for transcription units was established, which facilitated the construction of plasmids pEVCg(K)_AE and pJYS3_MoClo for episomal gene expression and CRISPR/Cas12a-genome modifications, respectively.

Promoter characterization experiments (expression of fluorescent reporter protein genes) confirmed the functionality of the individual parts and resulted in a set of twelve *C. glutamicum* and three *E. coli* promoters, which can be readily used for future library constructions. To address more complex cloning needs, the parts library was expanded to enable standardized construction of synthetic operons constituted of up to four pre-assembled transcription units. The MoClo principle was then adapted for genome editing using CRISPR/Cas12a, yielding plasmid pJYS3_MoClo. Since only a limited number of genomic integration sites is known and validated for applications using *C. glutamicum*, the standardization and modularity of pJYS3_MoClo will be subsequently used to generate a data set of suitable genomic integration loci in *C. glutamicum* ATCC 13032.

Taken together, this work presents a versatile molecular toolbox for strain engineering of *C. glutamicum*, paving the way for the automated metabolic engineering of this industrial microbial workhorse.

P-BSM-027

Single-cell polymer coating to improve the drying tolerance of fungal blastospores

R. Dietsch[1], *D. Jakobs-Schönwandt[2], A. Grünberger[3], A. Patel[1]
[1]*Bielefeld Universtiy of Applied Sciences, Bielefeld Institute for Applied Materials Research, Bielefeld, Germany*
[2]*Westphalian University of Applied Sciences, Bioengineering and Sustainability, Recklinghausen, Germany*
[3]*Karlsruhe Institute of Technology, Institute of Process Engineering in Life Sciences, Karlsruhe, Germany*

Equipping cells with artificial shells or coats has been explored extensively throughout the last decade, with goals such as immuno-masking, in-vivo tracing, and imparting tolerances toward various biotic and abiotic stressors. One stressor, however, drying, has curiously been overlooked. In an industrial setting, drying of cells becomes highly relevant when a satisfactory product shelf life must be achieved at low costs. The drying of thin-walled blastospores of the entomopathogenic fungus *Metarhizium brunneum* for biocontrol in agricultural settings is a prime example of this.

Therefore, we aim to provide *M. brunneum* blastospores with an artificial biopolymer shell to protect these sensitive cells from the harsh conditions during a technical drying process. Since the de- and, in particular, the rehydration of cells is considered a time-critical process, the slowing down by an artificial shell might have a positive effect on cell viability.

We formed artificial shells by layer-by-layer coating via electrostatic interaction on the blastospore surface. The cationic polymer chitosan was coated on the negatively charged cell surface creating a dense polymer layer. This in turn, reverses the original surface charge so that another dense polymer layer with negatively charged alginate could be applied to the initial layer. By repetition of these steps, surface coats of varying thickness were created.

We were able to show that slowing down the rehydration process of blastospores increases the survival rate from 7.2 % to 48.3 % significantly. Proof of successful coating was provided by the application of fluorescence-labeled polymers and scanning electron microscopy. As the number of layers increased, the drying tolerance improved from 6.88 % to a maximum of 27.54 %. Additionally, by varying of the polymer chain length, drying tolerance was further increased up to 33.24 %.

By applying polymer layers to the surface of cells, the survival of the cells after drying could be significantly improved.

P-BSM-028

Markerless genetic modification in Clostridium cellulovorans 743B based on the CodBA counterselection system

*L. M. Mendonça de Almeida[1], A. Schöllkopf[1], H. Edelmann[1], W. Liebl[1], A. Ehrenreich[1]
[1]*Technical University of Munich, Chair of Microbiology, Freising, Germany*

Lignocellulosic biomass is an abundant renewable feedstock that can be used to produce biofuels and platform chemicals. However, its industrial valorization is a challenge due to its natural recalcitrance. *Clostridium cellulovorans* is a mesophilic cellulolytic bacterium that has received special attention from the scientific community regarding enzymatic hydrolysis of cellulose and the production of n-butanol by consolidated bioprocessing after genetic engineering. However, the lack of reliable genetic tools prevents a precise understanding of the strain's physiology and makes its biotechnological potential underexplored. Genetic modification and genomic engineering have failed due to the low transformability and the small number of characterized synthetic biology parts available for this organism. Here, we describe a genetic system comprising rationally designed deletion plasmid that allows strain engineering by the allelic-coupled exchange method. Two key features in the deletion plasmid were remarkably important. First, a suitable clostridial "pseudo-suicide" origin of replication was identified, allowing plasmid transconjugation and further integration into the chromosome. Second, an efficient counterselection method was established. The *codBA* counterselection method is based on the presence of the *codBA* cassette in the deletion plasmid and the use of the 5-fluorocytosine (5-FC) as the counterselective compound. The *codBA* cassette from *C. ljungdahlii* under the control of the P*clpB* promoter was used and determination of the minimum inhibitory concentration (MIC) of 5-FC was performed. The constructed deletion plasmids also contained flanking regions of the locus targeted for deletion. Transconjugants were cultivated on solid medium containing reduced thiamphenicol. Thereafter, potential integrants were cultivated on solid medium containing 5-FC. The confirmation of the mutation was performed via PCR. These results demonstrated the ability to obtain a scarless chromosomal mutant and contributed to expanding the molecular tool kit for *C. cellulovorans*.

Keywords: Clostridia, cellulolytic microorganisms, genetic engineering, synthetic biology tools

---

P-BSM-029

In vitro hepatotoxicity prediction in early drug discovery

*L. Chiappalupi[1]

[1]ABAC Therapeutics, Barcelona, Spain

Introduction:

One of the major causes of failure in the drug development process is related to safety concerns arising in clinical trials or drugs withdrawn during the postmarketing phase. Most frequently, hepatotoxicity represents the cause of such failure. Therefore providing a valuable tool that could predict liver toxicity in early stages of drug discovery could prevent failure in clinical trials avoiding waste of investment and time.

Goals:

In this study, we developed and validate an *in vitro* approach for hepatotoxicity prediction for its applicability in the initial phase of the drug discovery process. This approach is based on testing hepatocytes viability after acute and chronic exposure to commercial drugs.

Methods:

The cytotoxic effect induced by each compound was assessed by comparing the viability percentage of treated cells to the viability percentage of untreated cells. Cells viability was established detecting the level of ATP released by the cells, as a measure of metabolically active, thus alive cells. In every test, results were expressed as lethal concentration 50 (LC50). We tested the compounds to the human hepatocytes HepaRG, rat and pig primary hepatocytes, as rodent and non-rodent animal models, respectively. Cells were cultured in 2D configurations (monolayer and sandwich) and 3D (spheroids).

Results:

We exposed a set of commercial compounds, with different known liver toxicity profile, in three different in vitro models: monolayer, sandwich culture and 3D spheroids of human HepaRG, rat and pig primary hepatocytes. We found that spheroids were the most sensitive and responsive model to drug-induced liver injury, while monolayer and sandwich culture showed lower sensitivity. Furthermore, we observed significant differences in the hepatotoxic responses of different species, indicating that neither rodent nor non-rodent models can accurately predict human liver toxicity. Our results suggest that spheroids of human HepaRG hepatocytes are the most suitable in vitro model for screening new compounds for hepatotoxicity in the initial phase of drug discovery.

P-BSM-030

A combined metagenome and mutational approach delivers a remarkably active PET hydrolase affiliated with the genus Alkalilimnicola

*M. Gurschke[1], P. Pérez-García[1,2], E. Costanzi[3], N. Bäse[1], R. F. Dierkes[1], L. L. Nover[1], V. Applegate[3], S. Smits[3,4], J. Chow[1], W. R. Streit[1]
[1]University of Hamburg, Microbiology and Biotechnology, Hamburg, Germany
[2]Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for General Microbiology, Kiel, Germany
[3]Heinrich Heine University Düsseldorf, Düsseldorf, Germany
[4]Heinrich Heine University Düsseldorf, Institute for Biochemistry, Düsseldorf, Germany

Polyethylene terephthalate (PET) is a widely used synthetic polymer and known to contaminate marine and terrestrial ecosystems. As of today, approximately 100 PET active-enzymes (PETases) are recognized as active candidates for enzymatic degradation of PET, however, most of them only exhibit moderate activity. Therefore, we used our established metagenome-pipeline to identify novel enzymes that show high activity towards polymer degradation. Amongst others, we identified an enzyme affiliated with Alkalilimnicola ehrlichii. U-HPLC measurement of PET degradation products as well as molecular characterization implies that PET44 is a highly promiscuous esterase with the ability to effectively degrade PET, BHET and MHET down to TPA and Ethylene glycol at an optimal temperature of 40°C. It shows high sequence similarity to the IsPETase, (53.6%) and the LCC (46.4 %), two prominent and highly active PETases. Semi-rational protein engineering was used to obtain PET44 modifications similar to those of the most relevant industrial mutants of the IsPETase and the LCC (ThermoPETase, DuraPETase, HotPETase, LCC-ICCG). Our work contributes to better structure-function predictions of PETases and enhances our understanding of the essential features a true active PETase really needs in order to be efficient

---

P-BSM-031

Increasing fungal blastospore desiccation tolerance with compatible solutes

R. Dietsch[1], *D. Jakobs-Schönwandt[2], A. Grünberger[3], A. Patel[1]
[1]Bielefeld Universtiy of Applied Sciences, Bielefeld Institute for Applied Materials Research, Bielefeld, Germany
[2]Westphalian University of Applied Sciences, Bioengineering and Sustainability, Recklinghausen, Germany
[3]Karlsruhe Institute of Technology, Institute of Process Engineering in Life Sciences, Karlsruhe, Germany

In the process from fermentation, to formulation, to desiccation, and finally to storage of biocontrol agents, the low desiccation tolerance of certain active ingredients remains one of the major hurdles to overcome. A noteworthy example here are blastospores formed by the entomopathogenic fungi *M. brunneum*. Blastospores are easily produced in large quantities, but are vulnerable to desiccation. To achieve protection most desiccation-tolerant organisms are known to accumulate trehalose. Intracellularly, trehalose helps to maintain membrane flexibility by acting as water replacer, upholds protein hydration, and promotes vitrification to critically decelerate decay processes. The effectiveness is highly dependent on intracellular trehalose concentration and homogeneity.

Our aim is therefore to increase intracellular trehalose levels in *M. brunneum* blastospores through hypotonicity-induced exchange of dispensable solutes with trehalose.

Several hypotonic trehalose treatments were performed with *M. brunneum* blastospores and the influence on viability was monitored. Additionally, GC-MS was employed to reveal levels of trehalose and other solutes. Lastly, a fluorescence-based investigation of an osmotic drying process was conducted in a microfluidic environment on single-cell basis to identify critical areas in cells and stress phases.

We found a significant increase in viability of hypotonically treated blastospores after desiccation and rehydration, mirrored by significantly lower stress levels during microfluidic desiccation. Blastospore survival was increased from 7.6 % to 62.0 %. Treated blastospores exhibited significantly raised trehalose levels. The microfluidic

observation revealed that cellular stress levels were only slightly elevated during the desiccation, but strongly increased during rehydration. Newly sporulated hyphae accumulated the greatest amount of stress marker.

A hypotonic treatment led to an increased intracellular trehalose uptake which lowered the amount of stress received during de- and rehydration and increased blastospore viability. Fluorescence based single-cell monitoring gains valuable insights into critical phases during drying.

P-BSM-032

New dienelactone hydrolase from microalgae bacterial community-Antibiofilm activity against fish pathogens and potential applications for aquaculture

L. Bergmann[1], Y. Astafyeva[1], W. R. Streit[1], *I. Krohn[1]
[1]*University of Hamburg, Microbiology and Biotechnology, Hamburg, Germany*

Biofilms are resistant to many traditional antibiotics, which has led to search for new antimicrobials from different and unique sources. To harness the potential of aquatic microbial resources, we analyzed the meta-omics datasets of microalgae-bacteria communities and mined them for potential antimicrobial and quorum quenching enzymes. One of the most interesting candidates (Dlh3), a dienelactone hydrolase, is a α/β-protein with predicted eight α-helices and eight β-sheets. When it was applied to one of the major fish pathogens, *Edwardsiella anguillarum*, the biofilm development was reproducibly inhibited by up to 54.5%. The transcriptome dataset in presence of Dlh3 showed an upregulation in functions related to self-defense like active genes for export mechanisms and transport systems. The most interesting point regarding the biotechnological potential for aquaculture applications of Dlh3 are clear evidence of biofilm inhibition and that health and division of a relevant fish cell model (CHSE-214) was not impaired by the enzyme.

P-BSM-033

Tuning bacterial magnetosomes for biomedical applications by genetic display of peptide arrays

*S. Markert[1], C. Jörke[2], J. H. Clement[2], F. Mickoleit[1], D. Schüler[1]
[1]*University of Bayreuth, Microbiology, Bayreuth, Germany*
[2]*Jena University Hospital, Hematology, Jena, Germany*

Magnetic nanoparticles are of increasing importance for many applications in the (bio)medical field, for instance as agents for magnetic hyperthermia or imaging techniques. [1]. A promising alternative to chemically synthesized nanoparticles are magnetosomes biomineralized by magnetotactic bacteria. In the alphaproteobacterium *Magnetospirillum gryphiswaldense* they consist of a monocrystalline magnetite core enveloped by a biological membrane consisting of phospholipids and a set of magnetosome-specific proteins. Due to their strictly genetically regulated biosynthesis, magnetosomes are accessible to genetic engineering techniques for their functionalization, and exhibit characteristics that can hardly be achieved by chemical synthesis [2].

Utilizing abundant magnetosome proteins as anchors, we explored the display of artificial peptides on the magnetosome membrane to increase the particles" biocompatibility and to shield potentially toxic bacterial components. For this purpose, multiple arrays of the tripeptides GSA and PAS and Protein G-derived domains capable of binding Albumins and antibodies, were expressed as fusions to the magnetosome membrane protein MamC. Upon incubation with serum proteins, protein corona formation was observed, which was up to ten-fold higher compared to the wildtype as determined by ELISA and electron microscopy. The genetic introduction of a TEV protease cleavage site enabled the controlled removal of both the proteins expressed on the magnetosome surface as well as the adsorbed corona. The peptide array decorated magnetosomes were shown to be biocompatible when incubated with mammalian cells, and cell viability was kept stable upon prolonged incubation when enveloped by a protein corona. Furthermore, we expect reduced endotoxicity of the particles due to shielding effect of the corona, which is currently tested.

Thus, genetic engineering might not only provide a powerful tool to modify the magnetosome surface properties, but also to improve the biocompatibility for future *in vivo* applications.

[1] Berry C. C., Curtis A. S., *J. Phys. D: Appl. Phys.*, 2003, *36*, R198
[2] Uebe R., Schüler, D., *Nat. Rev. Microbiol.* 2016, *14*, 621

P-BSM-034

On the quest for novel CODHs: Delving into the uncultured microbial life and its hidden enzymatic potential

*S. Böhnke-Brandt[1], R. Baehrle[2], M. Perner[2]
[1]*GEOMAR, Kiel, Germany*
[2]*GEOMAR Helmholtz-Centre for Ocean Research Kie, Kiel, Germany*

Given the need to reduce global CO_2 emissions and advancing sustainable industrial approaches, microbes and their versatile biocatalysts offer enormous potential that can be used on the way to a sustainable future. However, this natural source for novel biocatalysts is currently insufficiently utilized as the vast majority of microorganisms resist cultivation. This is particularly true for deep-sea habitats, where it is estimated that 91 to 96 % of microbes cannot be cultivated. Using functional metagenomics, we gain access to this oceanic black box filled with numerous hypothetical proteins of unknown function. Here we report on a functional-screen that we have successfully developed to target carbon monoxide dehydrogenases (CODHs, EC 1.2.7.4) from the environment without relying on the cultivability of the native host.

As biocatalysts, CODHs hold great potential, as they are capable of reducing CO_2 to CO at high rates and under exclusion of any undesired carbon-containing by-products. As key enzyme of the reductive acetyl-CoA pathway CODHs can be found in physiologically versatile marine microorganisms colonizing a broad range of thermally and chemically distinct marine habitats including sediments and deep-sea hydrothermal vents. With the aim of capturing particularly active CODH enzymes from otherwise inaccessible, yet uncultured marine microbes we have developed the function-based colorimetrical CODH screening tool. We could successfully demonstrate that the activity of recombinant CODHs from phylogenetically distinct microbial species is detectable, reflecting the screen's scope. Screening of a hydrothermal deep-sea vent metagenomic library resulted only recently in the identification of two active clones. However, sequencing of the fosmid insert ends shows highest similarity to microbial species that are not yet

known to exhibit CODH activities. PacBio sequencing of the whole fosmid inserts in combination with transposon mutagenesis is now being used to identify the genes responsible for the activity.

P-BSM-035

Development of cell-permeable antimicrobial molecules (CPAMs) to treat intracellular infections

T. F. Martins[1], B. Körner[1], L. Greune[1], *C. Rüter[2]
[1]*University Münster, Institute for Infectiology, Münster, Germany*
[2]*University Münster, Institut für Infektiologie, Münster, Germany*

Infectious diseases caused by microbial pathogens, are one of the major causes of morbidity and mortality worldwide. However, they represent a great challenge for antimicrobial therapies not only due to the threat of spreading antimicrobial resistances but also due to the fact that some major bacterial pathogens can also adopt an intracellular lifestyle. Thus, intracellular pathogens are widely protected against the usual antimicrobial therapeutics and they constitute a niche for recurrence and reinfection. Commonly used antimicrobial agents are characterized by poor cellular uptake. They cannot efficiently reach the intracellular target, resulting in low antimicrobial activity against intracellular pathogens. Therefore, there is a need to develop and improve strategies for the treatment of such infectious diseases. In this regard, Cell-penetrating peptides (CPPs), also called protein transduction domains (PTDs), are small peptides that are able to cross the plasma membrane autonomously and can mediate the internalization of biologically active molecules such as antibiotics into the cytoplasm.

In this regard, we showed that CPP-gentamicin conjugates were able to penetrate different endothelial and epithelial cell lines and importantly, we confirmed that these conjugates were able to target and efficiently kill intracellular Gram-negative pathogenic bacteria in infected cells. Furthermore, we could show that Bacteria-derived peptidoglycan-degrading proteins fused to a CPP can be used to kill pathogenic Gram pos. and Gram neg. bacteria, as well. Interestingly, these fusion constructs also showed a high effectivity against biofilm formation and might represent novel therapeutic tools to treat infectious diseases.

P-BSM-036

Engineering novel metabolic pathways for the production of aromatic compounds in Pseudomonas taiwanensis VLB120

*F. Herrmann[1], B. Wynands[1], N. Wierckx[1]
[1]*Research Center Juelich , IBG-1, Jülich, Germany*

Aromatic compounds are essential components in many everyday products such as plastics, dyes, pharmaceuticals, or flavoring agents. Furthermore, aromatic hydrocarbons play an essential role as organic solvents in the industry. So far, these aromatic solvents are produced from petroleum which is associated with energy-intensive processes, ecological drawbacks, and the dependency on fossil resources. To access alternative and sustainable ways to produce such challenging chemicals, we employ microbial catalysis in highly solvent-tolerant and metabolically engineered strains of *Pseudomonas taiwanensis*. We demonstrate the production of several hydrophobic aromatics such as 4-ethylphenol and anisole. Furthermore, we are working on the production of an industrially important solvent, ethylbenzene, by employing cyanobacterial aldehyde-deformylating oxygenases, which have already been used for the biosynthesis of alkanes. We perform pathway optimization by adaptive laboratory evolution and strain and enzyme engineering to approach the solubility limit of our hydrophobic products. The final goal of our work is to achieve the formation of a second phase of the aromatic compound, which would allow one to harvest the pure product from the bacterial culture.

P-BSM-037

Biocatalytic potential of novel aminoacylases from Streptomyces griseus DSM 40236T recombinantly produced by Streptomyces lividans

G. Haeger[1], J. Probst[1], P. Siegert[1], *J. Bongaerts[1]
[1]*Aachen University of Applied Sciences, Institute of Nano- and Biotechnologies, Jülich, Germany*

Amino acid-based surfactants are skin-friendly biosurfactants with low inflammatory potential and biodegradability that are used in cosmetic formulations. Conventionally, N-acyl-L-amino acids are synthesized by the Schotten-Baumann reaction using fatty acyl chlorides. When aminoacylases are applied high conversions in biocatalytical approaches starting from free fatty acids are possible [1,2]. Bacterial aminoacylases capable of synthesis have been isolated from e.g. *Mycolicibacterium* [1], *Paraburkholderia* [2], and *Streptomyces* [3,4].

We identified and investigated two novel enzymes originating from *S. griseus* DSM 40236^T [5]. The genes were cloned and an α-aminoacylase (EC 3.5.1.14), designated SgAA, and an ε-lysine acylase (EC 3.5.1.17), designated SgELA, were recombinantly expressed in *S. lividans* TK23. A protocol for high cell density fermentation in bioreactors was established ensuring dispersed growth of the bacterial mycelium. Both aminoacylases were purified as Strep-tag II-fused proteins and biochemically characterized, focusing on its hydrolytic activity to determine temperature and pH optima and stabilities. The short-chain acyl aminoacylase SgAA hydrolyzed various acetyl amino acids at the N_α-position with a broad specificity regarding the amino acid moiety. Substrates with longer acyl chains, like lauroyl amino acids, were hydrolyzed to a lesser extent. The aminoacylase SgELA specific for the hydrolysis of N_ε-acetyl-L-lysine was unstable and lost its enzymatic activity upon storage for a longer period but could initially be characterized. While synthesis of acyl amino acids was not observed with SgELA, SgAA catalyzed the synthesis of lauroyl-methionine in aqueous buffer, which reveals the enzyme"s potential for biocatalytic applications. Future work to optimize acylation conditions is intended.

[1] Haeger, G. *et al.*, *Microb Cell Fact* 22, 77 (2023).
[2] Haeger, G. *et al.*, *App Microbiol Biotechnol* 108, 93 (2024).
[3] Koreishi, M. *et al.*, *J Am Oil Chem Soc* 82, 631-637 (2005).
[4] Koreishi, M. *et al.*, *J Biotechnol* 141, 160–165 (2009).
[5] Haeger, G. *et al.*, FEBS Open Bio, 13: 2224-2238 (2023).

P-BSM-038

Increasing electroporation efficiency in the lithoautotrophic bacterium Cupriavidus necator H16: A roadmap for non-model bacteria domestication

*M. Vajente[1,2], H. Ballerstedt[2], L. M. Blank[2], S. Schmidt[1]
[1]*University of Groningen, Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy,*

Groningen, Netherlands
[2]RWTH Aachen University, Institute of Applied Microbiology (iAMB), Aachen Biology and Biotechnology (ABBt), Aachen, Germany

Climate change is an urgent and collective challenge, and a portfolio of solutions is needed to reduce CO_2 emissions or to increase carbon capture and utilization from the atmosphere. Nature has been evolving CO_2 utilization pathways for billions of years and offers a promising repository of novel metabolisms and enzymes capable of CO_2 fixation. However, non-model bacteria are recalcitrant to genetic engineering, and the application of modern genetic tools is cumbersome. One of the main barriers is the low transformation efficiency, as most tools and technologies require the delivery of DNA molecules to tune and modify the host metabolism. This transformation barrier is a common feature of all wild-type bacteria, which employ a variety of defense systems to avoid phages, plasmids, and other mobile genetic elements in their native ecological niche. To transform them with recombinant DNA, this arsenal has to be predicted, characterized and circumvented.

In our study, we performed an in-depth analysis of *Cupriavidus necator* H16 using bioinformatic tools to study its restriction enzymes and defense systems. By using tailored plasmids, we confirmed the functional role of three systems encoded in the genome, and through a combination of plasmid mutation and demethylation, we transformed large plasmids with higher efficiency. We also succeeded in transforming suicide plasmids via electroporation, deleting the native defense systems and creating a domesticated strain.

These findings will benefit both the *C. necator* H16 community and researchers working with other non-model bacteria by providing a roadmap that can be followed to increase transformation efficiency.

P-BSM-039
Engineering of seed and scaffold regions for efficient gene regulation by synthetic small RNAs in Escherichia coli
*S. Dittmar[1], B. Berghoff[1], R. Melo Palhares[1,2], D. Schindler[2,3], B. Wiebach[1]
[1]*Justus-Liebig University Gießen, Institute for Microbiology and Molecular Biology, Giessen, Germany*
[2]*Max-Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[3]*Philipps-University Marburg, Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*

(1) Introduction

Synthetic biology is a rapidly advancing field that deals with the development and construction of novel biological systems with specific functions. One of the emerging areas within synthetic biology is the use of synthetic small RNAs. These short RNA molecules have a regulatory function. Bacteria use small regulatory RNAs (sRNAs) to regulate mRNA translation. Typical sRNAs consist of a seed and a scaffold region, which are different modules that can be designed and recombined to generate synthetic sRNAs. The seed region is a crucial component in the regulation of gene expression. The scaffold serves as a structural backbone that supports the stability of the sRNA and promotes its interaction with proteins, such as the RNA chaperone Hfq. Synthetic sRNAs offer promising potential for various applications.

(2) Goals

The aim of this study was to test different synthetic seed regions and scaffolds for efficient regulation of gene expression in *Escherichia coli*.

(3) Material & methods

Golden Gate cloning was used for assembly of seed regions and scaffolds to construct synthetic sRNA expression plasmids. Synthetic seed regions and scaffolds were predicted by computational tools. The functionality of synthetic sRNAs was tested using phenotypic screens.

(4) Results

It is assumed that the regulatory activity of synthetic sRNAs depends on the seed and scaffold regions selected during construction. Since the seed region plays an important role in binding of the target mRNA, selection of the seed region is the crucial step in synthetic sRNA design. However, we observe that some scaffolds are more effective than others in regulating certain targets. We will present computational tools for prediction and design of both seed regions and scaffolds.

(5) Summary

It may be necessary to predict and test various seed regions and sRNA scaffold variants for different mRNA targets to achieve the desired regulatory effect.

P-BSM-040
Marine fungi as a source for mycoremediation of farm manure and sewage sludge
*D. A. Stiebeling[1], A. Labes[1]
[1]*Flensburg University of Applied Sciences, Energy and Life Science, Flensburg, Germany*

The application of manure and sewage sludge on farmland is a crucial element in enhancing soil organic matter and contributing to soil carbon sequestration. However, these organic fertilizers are often contaminated with anthropogenic pollutants that can harm ecosystems and persist for extended periods. Bioremediation approaches have gained attention as a cleanup treatment in recent decades due to their cost-effectiveness and environmental friendliness. In addition to phytoremediation and bacterial bioremediation, mycoremediation has also proven successful in pollution control. While land-based fungal species, particularly white-rot fungi, have been extensively studied for mycoremediation, marine fungi are underrepresented but possess adaptive traits (e.g., halophilic, anaerobic) that could be beneficial under specific conditions, potentially expanding the degradation portfolio. In an initial step, marine filamentous fungi from the Flensburg strain collection of marine fungi were screened in a simple growth medium for their ability to tolerate and metabolize the analgesic diclofenac (DCF), a substance regularly detected in surface waters that is toxic to various organisms. The cultivation period lasted 28 days. Of the 40 isolates tested, 6 exhibited almost no metabolization, 20 showed a mediocre degradation, and 14 had a residual concentration of DCF at 15% and below. These results demonstrate the capability of some marine fungi to metabolize DCF. The second pollutant to be tested is perfluorooctanoic acid (PFOA), representing

the group of per- and polyfluoroalkyl substances (PFAS), which are also harmful and ubiquitously found in the environment. In the final step, selected fungal candidates will be employed to degrade these organic contaminants in manure and sewage sludge. The degradation rate and products will be assessed using LC-MS, with kinetics determined. This study provides an example of an applied bioremediation technique as a sustainable, eco-friendly, and inexpensive way to deal with pollutants, thereby promoting the use of organic fertilizers in agriculture to scale up soil carbon sequestration.

P-BSM-041
Maximizing phototrophic productivity - insights from computational models
*R. Steuer[1]
[1]*Humboldt University of Berlin, Fachinstitut Theoretische Biologie (ITB), Berlin, Germany*

Introduction: Cyanobacteria and other phototrophic microorganism have tremendous potential as a resource for a sustainable green economy. The economic viability of phototrophic microorganisms, however, is still limited by the low growth rates and the low areal productivities obtained in most current cultivation systems. To overcome the productivity barriers of phototrophic cultivation, there has been increasing interest in fast-growing strains. **Goals**: We discuss the main factors that determine phototrophic culture productivity. In particular, based on quantitative models, we show that high maximal growth rates are not a sufficient or necessary property for high phototrophic productivity. Rather, the light-limited specific growth rate of a phototrophic microorganism is a product of several factors, including the rate of light absorption, quantum yield and the maximal biomass yield per photon. **Materials and Methods:** Our analysis builds upon high quality computational models of cyanobacterial growth developed over the past decade. In particular, we make use of genome-scale reconstructions as well as reduced coarse-grained models of metabolism. **Results:** Computational models allow us to disentangle the prerequisites for high phototrophic productivity. Maximal productivity of a phototrophic culture is attained at high light intensities, low growth rates and high cell densities. Under such conditions, dense cultures act as an effective light dilution mechanism and alleviate the detrimental effects of photoinhibition. It is shown that antennae truncation does not result in improved culture productivities. Model results are supported by quantitative growth experiments using a high-density cultivation setup based on a membrane-mediated CO2 supply, high light intensities and thin-layer cell suspensions. **Summary:** Our results show that (i) current limits of phototrophic productivity can be overcome, that (ii) under optimal culture conditions (medium and light conditions) current model strains consistently reach high growth rates and high final cell densities, and (iii) a high maximal specific growth rate is not itself a determinant of culture productivity.

P-BSM-042
Analysis of the binding behaviour between the electron carrier cytochrome c6 and Photosystem I
*L. Mages[1], K. Christer[1], A. Kölsch[1], C. Gerischer[1], C. Schade[1], D. Nürnberg[1]
[1]*Free University of Berlin, Experimental Physics, Berlin, Germany*

Electrocatalytic- and solar-driven fuel synthesis from the greenhouse gas CO2 is a desirable approach to simultaneously produce sustainable energy carriers, and combat increasing atmospheric CO2 levels. Formate is a stable intermediate in the reduction of CO2 and is utilised in a wide range of downstream applications. Recent efforts have focused on using an all-protein, light-triggered, catalytic circuit based on photosystem I (PSI), cytochrome c6 (cyt c_6) and formate dehydrogenase (FDH), which would convert CO2 into formate. However, various challenges remained. Our research addresses the optimization of the structural basis for efficient electron transfer from cyt c_6 to a genetically engineered PSI-FDH fusion complex. Due to the transient binding of cyt c_6 to PSI it has been challenging to investigate the structural basis for this binding process and optimise binding affinities (1,2). Structural observations and models propose a specific binding site for cyt c_6 near the P700 chlorophyll pair of PSI on the lumenal side (1). Based on this model, residues likely involved in the binding mechanism were predicted. Here, heterologously expressed cyt c_6 variants from the cyanobacterium *Thermosynechococcus vestitus* BP-1 (previously known as *T. elongatus*) with a higher binding affinity to this suggested binding site were used to study the binding mechanism between cyt c_6 and PSI. These high affinity variants show increased oxygen reduction rates compared to the wildtype. Based on these results, the binding mechanism will be further specified via Isothermal Titration Calorimetry (ITC) and structural determination of the cyt c_6 -PSI complex by cryo-electron microscopy (cryo-EM).

1. Kölsch A, et al., (2020) Curr. Res. Struct. Biol. 2, 171–179.
2. Li J, et al. (2022) Commun Biol. 12;5(1):951.

P-BSM-043
Development of a screening assay for the production of functionalized sugar acids in G. oxydans and improvement of its biomass formation for industrial usage
*N. Kucher[1], L. Pütthoff[1], E. Bieringer[2], A. Zimmermann[2], D. Weuster-Botz[2], A. Ehrenreich[1], W. Liebl[1]
[1]*Technical University Munich, Molecular Life Sciences, Freising, Germany*
[2]*Technical University Munich, Energy and Process Engineering, Garching, Germany*

Gluconobacter oxydans has great biotechnological potential due to its ability to incompletely oxidize a large variety of sugars, polyols, and related compounds. These oxidations are catalyzed by membrane-bound dehydrogenases (mDHs) with the active site facing toward the periplasm, therefore avoiding the transport of substrates and products in and out of the cytoplasm. We developed a platform for the functional expression of heterologous mDHs in *G. oxydans* BP9.1, devoid of its native mDHs, thereby increasing the specific activity of the heterologous mDHs and avoiding unwanted side reactions.

This study aims to use this platform to produce cellobionic acid (CBA), galactaric acid (GA) and other relevant sugar acids. The membrane-bound glucose dehydrogenase (mGDH) from *Pseudomonas taetrolens* was expressed in *G. oxydans* BP9.1 to construct a strain that produces CBA and GA with a high space-time yield. For further optimization, random in vitro mutagenesis was used to create variants of this enzyme. An assay was established to screen for mutants with a high oxidation rate. Carbonate in the medium serves as an indicator for acidification. By oxidizing the substances

in the medium, the colonies form halos on the plates, whose size correlates with the amount of acid produced.

One common limitation of microbial processes using acetic acid bacteria compared to chemical processes is the relatively low space-time yield due to small cell concentrations, which makes industrial biomass production for oxidative biotransformation costly. Consequently, the opportunity to increase the growth yield could enhance productivity and result in improved profitability for industrial use. The non-proton pumping type II NADH dehydrogenase (NDH-2) plays a central role in the respiratory metabolism of acetic acid bacteria. It provides electrons to the electron transport chain, leading to the synthesis of ATP. Since BP9.1 seems to be limited in NADH dehydrogenase activity, its overexpression leads to a significantly increase in biomass formed. The presence of an additional NDH-2 in our platform strain enables more effective utilization of the generated NADH for energy production.

P-BSM-044

Enhancing production tolerance with growth-coupled evolution

*A. Grankin[1], B. Wynands[1], N. Wierckx[1]
[1]*Research Center Juelich, Microbial Catalysis, Jülich, Germany*

The heavy reliance of the chemical industry on petroleum for the production of fuels, solvents, and various materials is a major contributor to the alarming levels of environmental pollution caused by human activity and continued industrialization. The production of these compounds using bio-based methods offers potential solutions to environmental challenges such as climate change and pollution. However, the toxicity of these compounds to microorganisms commonly used in biotechnology is a major obstacle to effective bio-production techniques, which in turn hinders the competitiveness of biotechnological production compared to fossil-based bulk chemicals.

Addressing this challenge, the PROSPER project aims to demonstrate the bio-based synthesis of hydrophobic aromatic chemicals, with a primary focus on overcoming tolerance limitations during production. Within the scope of PROSPER, this project concentrates on enhancing production tolerance of solvents through engineering and evolution of robust producer strains. To achieve this, diverse strategies are used to establish a connection between microbial growth and solvent production.

One approach involves integrating the production pathway and rerouting the central metabolism through this pathway to stabilize the production phenotype. Another strategy utilizes solvent-sensing regulators, such as ttgVW, in conjunction with selection genes like essential genes or antibiotic-resistant markers to regulate growth based on solvent levels. In this context, we identified potential candidate genes. Once this coupling is established, adaptive laboratory evolution will be implemented to cultivate producer strains and improve their tolerance to the production of solvents. This process aims to identify key mutations responsible for enhanced tolerance against the internal production of solvents, rather than their external addition as is traditionally done. Our research will not only contributes to the development of robust *Pseudomonas taiwanensis* chassis strains but also will advances our understanding of solvent tolerance in *Pseudomonas* species.

P-BSM-045

Prospecting for novel platform organisms: Taxonomic diversity of the fungal genus Aureobasidium exploited for biotechnology

*K. Stein[1], M. Driller[1], L. M. Blank[1], T. Tiso[1]
[1]*RWTH Aachen University, Institute of Applied Microbiology, Aachen, Germany*

The genus *Aureobasidium* represents a group of highly versatile yeast-like fungi. They show great adaptability to different environments and can be found in diverse ecological niches, from cold marine habitats and temperate regions, across humid and tropical areas, to warm and dry habitats. Due to its attributes the polyextremotolerant fungus has great biotechnological potential. It not only features a large substrate and product spectrum but also tolerates cold temperatures and extreme pH values as well as high salt and sugar concentrations, thus representing an extremely robust platform organism.

To identify strains for potential biotechnological applications, we performed an extensive screening of 146 strains from the genus *Aureobasidium*. These involved 89 strains from two strain collections and 56 new wild-type strains isolated locally from diverse natural and anthropogenic environments. The tolerances of the strains towards different pH values, high salt concentrations, and different temperatures, were investigated to discover strains thriving under extreme conditions. This potentially paves the way for developing auto-sterile processes, thus eliminating the need for costly sterilization of fermenters and equipment.

In addition, we tested different low-cost and sustainable carbon sources important in the context of a circular bio-economy. The use of such carbon sources holds the potential to establish more sustainable and economically realizable processes. Most strains could grow on a variety of lignocellulose compounds, the most abundant raw material on earth. Additionally, several strains showed growth on acetate as a carbon source, which can potentially be produced from CO_2. A waste stream from industrial ethanol production was also identified as a potential carbon source.

Overall, we showcased the suitability of *Aureobasidium* as a potential microbial chassis organism for industrial biotechnology and identified strains for more detailed investigations, intending to establish economically competitive and sustainable biotechnological processes.

P-BSM-046

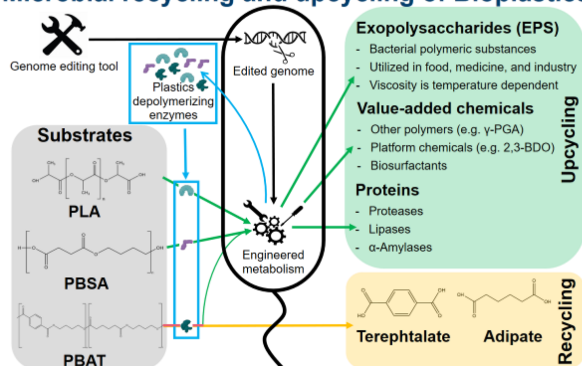
Unravelling the potential of thermophiles as *chassis* organisms for bioplastic upcycling

*T. D. Konjetzko[1], T. Becker[2], L. Müller[1], B. Wynands[1], S. Herres-Pawlis[2], N. Wierckx[1]
[1]*Research Center Juelich, Institute for Bio- and Geosciences 1 - IBG-1, Jülich, Germany*
[2]*Institute of Inorganic Chemistry, Bioanorganic Chemistry, Aachen, Germany*

Bio-based plastics are necessary to reduce the carbon footprint of everyday materials, but prominent polymers like polylactide (PLA) currently pose a challenge in traditional recycling strategies. In order to enable efficient and economically viable biorecycling of bioplastics, we envisage a consolidated bioprocess in which the depolymerizing enzymes are produced and secreted by the microbial biocatalyst that simultaneously converts the released

monomers into value-added products. Thermostable enzymes and thermophilic microbial hosts are required in order to operate closer to the glass transition temperature of the polymer and thus make it more accessible to the depolymerizing enzymes. In order to identify novel thermostable polylactide-depolymerizing enzymes, we pursued a database-assisted screening of roughly 20 000 proteins within our thermophilic strain library and additionally enriched natural producers that grow on PLA at elevated temperatures. The most promising enzymes are being characterized *in vitro* and compared to benchmark enzymes with regard to PLA degradation activity. The best performing enzymes will be overexpressed in *Parageobacillus thermoglucosidasius* while characterizing the lactic acid monomer consumption kinetics *in vivo*. Since the availability of genome editing methods for *Geobacillus* spp. is limited, such methods are being developed especially with regard to CRISPR-Cas systems. The combination of the proof of principle consolidated bioprocess and CRISPR-Cas genome editing methods will pave the way for engineering genetically stable *chassis* organisms of the genus *Geobacillus*, capable of PLA degradation and funneling the resulting carbon flux towards value-added compounds. This concept will be finally expanded towards other bioplastics such as PBAT and PBSA.

Fig. 1



P-BSM-047

Expanding the molecular toolbox for the bacterial phylum *Planctomycetota*

T. Haufschild[1], *N. Rabold[1], J. Hammer[1], *N. Kallscheuer[1], C. Jogler[1]
[1]*Friedrich Schiller University Jena, Institute of Microbiology, Microbial Interactions, Jena, Germany*

Strains belonging to the bacterial phylum *Planctomycetota* show several enigmatic cell biological features including an uncommon form of asymmetric cell division and a cell plan different from that of canonical Gram-negative model bacteria. A detailed analysis of these conspicuous traits is currently limited by the lack of sophisticated molecular biological tools for the genetic manipulation of these non-model microorganisms. Previous studies have reported the transposon-driven introduction of heterologous genes into random loci in the genome and more recently the targeted construction of single gene deletion mutants [1,2]. Still, the genetic potential remains largely untapped and the study of individual gene functions and regulatory networks is not possible without more advanced genetic engineering tools.

We here present data that considerably expands the molecular toolbox applicable for limnic and marine model planctomycetes. Extensive testing of selection marker genes yielded the first strain carrying deletions at multiple genomic loci. While testing different chromosomal locations for the introduction of heterologous genes, the expression of a set of reporter genes encoding fluorescent proteins and the multi-purpose HaloTag was achieved. The characterization of multiple novel members of the phylum in the last two years also yielded detailed information on the occurrence of plasmids. Based on bioinformatic analyses of extrachromosomal elements, we aim to construct replicative shuttle vectors as an episomal expression system based on native and foreign constitutive or inducible promoters.

The line of research will act as a platform for detailed analyses of the planctomycetal cell biology and an exploitation of their (secondary) metabolic repertoire. Genetic and metabolic engineering approaches will help to investigate the ecological role of planctomycetes in inter-species interactions and paves the way for their biotechnological applications.

References

[1] Jogler, C., et *al.* (2011). *Appl. Environ. Microbiol. 77*, 5826-5829.

[2] Santana-Molina, C., et *al.* (2022). *Proc. Natl. Acad. Sci. U.S.A.* 119, e2210081119.

P-BSM-048

Cooperative biotechnological-chemical design of active washing enzymes and sustainable surfactants

*A. Achten[1], A. Merz[1], K. Hoffmann-Jacobsen[1], *M. Wagner[1]
[1]*University of Applied Sciences Niederrhein, Faculty of Chemistry, Krefeld, Germany*

Traditionally, the development of surfactants and enzymes for laundry has been conducted in isolation due to the intricate nature of their interactions, which are only identified at advanced stages of formulation, rendering the process both time-consuming and cost-intensive. This project diverges from conventional methods by optimizing both components concurrently, thereby directly accounting for their mutual influences from the outset. This innovative approach involves the joint development of detergent enzymes and biosurfactants in order to open up new paths to more sustainable detergents.

The research focuses on the optimization of amylases derived from bacterial and fungal sources, tailored specifically for enhanced expression system compatibility through codon usage optimization and further modified with tags to facilitate visualization and purification processes. Enzyme candidates are cloned into an expression vector and subsequently expressed in a fungal system using *Aspergillus oryzae* for heterologous production. The activity of these amylases is assessed through both quantitative and qualitative assays, carried out in liquid, on culture medium or on textiles.

To navigate the biotechnological development of these enzymes, advanced data analysis techniques are employed. Spectroscopic analyses play a pivotal role in evaluating the combined cleaning efficacy of the enzyme and biosurfactant at the interface within the complex milieu of washing suds.

This project aims to refine the performance of enzyme-biosurfactant systems through enzyme engineering, enhancing their properties and ensuring their compatibility with surfactants. Finally, the synergistic biobased enzyme-biosurfactant systems are evaluated on the basis of their washing effect in washing tests.

During the project, energy, water consumption, emissions, and material use are systematically recorded, aiming to establish resource-efficient and hazard-minimized evaluation for applied research projects.

P-BSM-049
Enhancement of the arabinoxylan degradation ability of Clostridium saccharobutylicum
*H. Edelmann[1], J. Rebel[1], A. Ehrenreich[1], W. Liebl[1]
[1]*Technical University Munich, Chair of Microbiology, Freising, Germany*

In the future, chemical precursors will need to be produced from renewable rather than finite fossil resources. One novel precursor could be butanol. It can be produced biologically by ABE fermentation using solventogenic Clostridia. This process is economically viable, if low-value and abundant biomass is used as substrate for fermentation [Thieme et al. 2020]. As by-products from the milling process wheat and rye brans fulfil these criteria. The fermentable carbohydrates were mainly arabinoxylan (40%, 57%) and starch (37%, 17%) [Knudsen KEB 1997].

We found that the good solvent producer *Clostridium saccharobutylicum* DSM 13864 could only degrade 50% of pure wheat arabinoxylan. To enhance degradation, we fermented pure arabinoxylan in buffered medium and added heterologously expressed arabinofuranosidase (Axh43A), xylanase (Xyn11A, Xyn10B) and β-xylosidase (Bxl3B) from the hemicellolytic thermophile *Thermoclostridium stearcorarium* DSM8532 to support degradation [Bröcker Jannis 2019].

It was shown that the tested arabinofuranosidase (Axh43A) alone efficiently supports the native xylanolytic enzymes to increase degradation from 50% to 82%. This effect appears to be due to the side-chain cleavage of double substituted xyloses, which synergistically supports endogenous xylanolytic enzymes. A plasmid-based heterologous expression of this enzyme with a signal peptide in *C. saccharobutylicum* resulted in improved substrate degradation.

Due to the complex nature of the carbohydrates in lignocellulose, a large repertoire of glycoside hydrolases is required to effectively degrade the substrate. Extracellular saccharolytic enzymes can be exploited to improve the degradation properties of a solventogenic Clostridium. Using our genetic system for *C. sacchaobutylicum*, a stable heterologous expression of a tailored mix of enzymes produced by the solventogenic strain itself could eliminate the need for supplementation with external enzymes.

P-BSM-050
A programmable DNA ADP-ribosyltransferase for microbiota engineering.
*H. Bassett[1], C. Patinios[1], A. Del Re[1]
[1]*Helmholtz HIRI, Würzburg, Germany*

Efforts to study the role of gut microbiota on human health are hampered by the ability to manipulate specific species and strains. For this reason, CRISPR-Cas9 has become a valuable molecular tool due to its ability to be programmed to target a specific sequence. The conjugative transfer of a plasmid encoding Cas9 from E. coli to S. enterica has already been demonstrated, thus presenting an avenue for in-situ microbiota manipulation. In E. coli, a double strand break introduced by Cas9 is lethal if not repaired by the recBCD recombination pathway. An alternative recombination pathway in E. coli is via recF. Recently, a DNA ADP-ribosyltransferase (DarT) enzyme that functions as a bacterial defense toxin, has been shown to install a replication blocking lesion, that in the absence of its cognate antitoxin (DarG), leads to polymerase skipping, and the formation of a ssDNA gap. This ssDNA gap is then repaired by the recF recombination pathway. Here we demonstrate a novel gene editing system, by which a fusion of DarT to an inactivated Cas9 drives recombination in E. coli without the generation of a double strand break. Using a gene conversion assay in which a span of 8 bases are substituted, we show that our system of editing is comparable in efficiency to standalone Cas9-driven recombination, but an order of magnitude less toxic. Next, we demonstrate the ability to substitute upwards of 60 bases in either direction of the target site, in addition to insertions of 100 bases centered on the target site, and deletions of 60 bases surrounding the target site. We confirm that gene editing is dependent on the recF recombination pathway. Finally we demonstrate conjugative transfer of the system, and successive gene conversion in an E. coli host/recipient co-culture, at an efficiency that exceeds standalone Cas9 by an order of magnitude, while also an order of magnitude less toxic. DarT is the first of what we believe will become a new category of gene editors centered upon harnessing enzymes which modify DNA, in order to drive endogenous DNA repair pathways for purposes of gene editing, without generating double strand breaks.

P-BSM-051
Optimizing Microbiological Aspects of Archaeal Cultures for Power-to-Methane Trickle Bed Reactors
*D. B. Eckl[1], A. M. Hartl[1], S. Fischer[1], M. Steinmetzer[1], K. Krüger[1], A. Bellack[1]
[1]*Universtiy Regensburg, Lehrstuhl für Mikrobiologie, Regensburg, Germany*

Microbiological methane production, crucial for biogas plants and power-to-methane applications, often relies on non-defined microbial communities. While this approach offers accessibility, achieving constant, gas-grid-ready quality remains challenging. Limited understanding of these communities complicates issue identification when methane production deviates.

Addressing this, our study - within the scope of the ORBIT II project - explored defined cultures and co-cultures of methanogens for use in a trickle bed reactor. Our primary goal was to characterize and compare a methanogenic co-culture with the respective monospecies cultures. Additionally, we aimed to implement a robust RT-qPCR methodology for analyzing methanogenic co-cultures.

Laboratory experiments focused on a defined methanogenic co-culture with one representative from the order *Methanobacteriales* and one from *Methanococcales*, testing them with various gas mixtures reflecting real educt gasses at potential power-to-methane reactor sites like sewage

plants. Further, we analyzed the growth and methane production of the cultures in a temperature range from 60-70°C and with varying salts concentrations (NaCl, $MgCl_2$, $MgSO_4$). Last, a method for quantifying the microbial community, optimized for trickle-bed samples through RT-qPCR, was established.

The used archaea demonstrated efficient hydrogen utilization, a critical factor in trickle bed reactors. Even in the presence of small amounts of oxygen, most methanogens thrived and produced sufficient amounts of methane. However, the established co-culture proved more resilient to temperature, oxygen, and ion concentration fluctuations compared to monospecies cultures, therefore enhancing reactor stability. RT-qPCR emerged as a powerful tool for co-culture composition analysis when microscopic methods were impractical.

In conclusion, this study characterized a methanogenic co-culture's advantages and disadvantages, providing valuable insights for its future application in trickle-bed reactors.

P-BSM-052
A Cost-Effective Peptide Isolation Technique using a Cellulose Binding Domain

*J. Koprionik[1], M. Wagner[1]
[1]*University of Applied Sciences Niederrhein, Krefeld, Germany*

Introduction

As bioactive molecules, peptides have great application potential in various industrial sectors. However, due to the cost-intensive synthesis and purification, peptides are currently used almost exclusively in medicine. Cost-effective biotechnological production and processing would expand the market potential and fields of application for peptides.

Goals

Therefore, we want to develop a purification method for heterologously expressed peptides utilizing the cellulose binding domain CBD from *Trichoderma reesei* (*T. reesei*) in combination with an acid cleavage site with favorable cellulose particles.

Materials and methods

For the biotechnological production we are using the Ascomycete *Aspergillus oryzae* as expression host. After genomic integration of the modified expression cassette this organism expresses the heterologous peptides combined with a cellulose binding domain (CBD) into the culture medium. After cell separation the cultivation broth is incubated with microcrystalline cellulose and the CBD-tagged peptides are adsorbed from the complex nutrient medium in the presence of 1 M $(NH_4)_2SO_4$. Separation from the medium is achieved via centrifugation and washed several times with diluted $(NH_4)_2SO_4$ solution. Even washing with water did not result in CBD detachment from the cellulose indicating a very strong interaction between cellulose and the CBD. To guarantee the purification as well as to remove the peptide attached CBD we cloned an acid cleavage site in between. Finally, the detachment of the peptides from the CBD bound to the cellulose is done by chemical acid cleavage and the purified peptides are separated from the cellulose via centrifugation or filtration.

Summary

Using the CBD from *T. reesei* combined with an acid cleavage site attached to heterologously expressed peptides we developed a very cost-effective peptide isolation technique which can expand the fields of application of peptides in the future.

Diagnostic and Clinical Microbiology

P-DCM-001
Evaluation of different capsule staining methods and development of an improved capsule staining in Klebsiella pneumoniae

*E. Doğan[1], E. Eger[2], K. Schaufler[2,3], K. Becker[1], E. A. Idelevich[1,4]
[1]*University Medicine Greifswald, Friedrich Loeffler-Institute of Medical Microbiology, Greifswald, Germany*
[2]*Helmholtz Institute for One Health/Helmholtz Centre for Infection Research, Department of Epidemiology and Ecology of Antimicrobial Resistance, Greifswald, Germany*
[3]*University Medicine Greifswald, Greifswald, Germany*
[4]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

Introduction

The capsule serves as a virulence factor in various bacterial species, playing a crucial role in enabling bacteria to evade the host immune system. Although numerous methods for microscopic capsule visualization have been used for more than a century, the optimal method for capsule staining of *Klebsiella pneumoniae* has not yet been defined.

Goals

To close this gap, we compared two standard methods and a novel modified protocol to assess capsule production in *K. pneumoniae* by optical microscopy.

Materials & Methods

Fourteen *K. pneumoniae* isolates with different phenotypes as well as the reference strain *K. pneumoniae* ATCC 13883 were cultured overnight on Columbia blood agar at 35°C. Each isolate was stained using Anthony's and Maneval's methods as described by Hughes and Smith (1). Additionally, a novel modification of Maneval's method was used that incorporated an incubation in 10% skim milk broth at 35°C under aerobic conditions for 12 to 18 hours. Microscopy was performed using the Axio Imager.Z2m system equipped with the Plan-APOCHROMAT 100x/1.4 oil immersion objective and the Axiocam 305 camera (Zeiss). For comparison, the mucoid phenotype was also determined in a sedimentation assay as previously described (2).

Results

Robust and reproducible staining protocols were established to directly observe the differences in capsule by light microscopy. The most unambiguous and consistent findings were obtained with the modified Maneval's method including an overnight incubation in skim milk broth. This method effectively differentiated capsule size variations between distinct phenotypes (Figure 1). The concordance of these

findings with the results of the sedimentation assay identifying mucoid phenotypes supports the validity of this modified capsule staining technique.

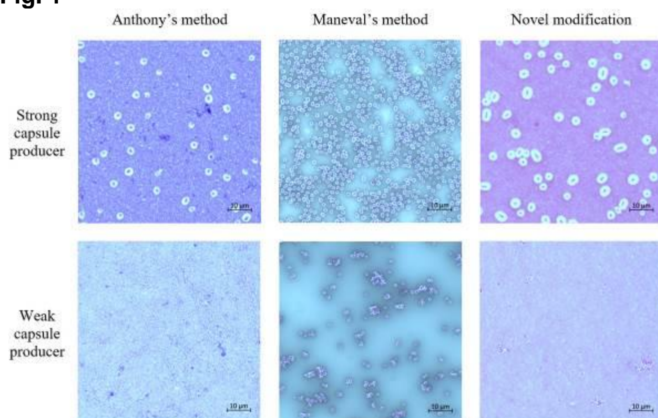
Summary

An improved staining method has been proposed, which can be used for the microscopic assessment of capsule as a virulence factor in *K. pneumoniae*.

References

1. Hughes RB, Smith AC. Capsule stain protocols. ASM. 2007.
2. Eger E et al. Hypervirulent *Klebsiella pneumoniae* sequence type 420 with a chromosomally inserted virulence plasmid. Int J Mol Sci. 2021;22.

Fig. 1



P-DCM-002

Rapid detection and real-time antibiotic susceptibility testing of Klebsiella pneumoniae and Yersinia pestis using recombinant reporter phages

*S. Kachel[1]

[1]*Fraunhofer ITMP-IIP, Penzberg, Germany*

Infections caused by the highly pathogenic bacterium *Yersinia pestis* are rare, however, the pathogen still pose a major biosecurity risk due to the potential misuse for biological warfare or bioterrorism. In contrast, the massive emergence of multi-drug-resistant (MDR) bacteria, such as *Klebsiella pneumoniae*, constitutes an enormous threat to global health as MDR-associated treatment failure causes high mortality rates in nosocomial infections. In both cases, rapid pathogen detection and antibiotic resistance screening are crucial for successful therapy and thus patient survival. Reporter phage-based diagnostics offer an avenue to expedite pathogen identification and resistance testing. Reporter phages feature integrated reporter genes that enable real-time detection of living target bacteria upon infection. Here, we developed and engineered highly specific reporter phages which produce nanoluciferase (nLuc) as a reporter enzyme upon host infection that enable rapid detection of *K. pneumoniae* or *Y. pestis* cells in clinical matrices within a few hours. At the same time, these reporter phage assays can be utilized in real-time antibiotic susceptibility testing to provide rapid identification of suitable antibiotic treatment options.

P-DCM-003

Phenotypic antibiotic susceptibility testing for last-resort antibiotics based on bacterial nanomotions provides results in two hours

*A. Sturm[1], G. Jozwiak[1], M. Pla Verge[1], G. Cathomen[1], L. Munch[1], D. Cichocka[1]

[1]*Resistell, Basel, Switzerland*

With the increasing prevalence of multi-drug resistant bacteria, last-resort antibiotics like ceftazidime-avibactam and cefiderocol have become crucial. Due to the lack of rapid diagnostic tools and their absence in most automated antibiotic susceptibility tests (AST), empirical initiation of therapy is common for critically ill patients. We propose a rapid phenotypic AST using the Resistell Phenotech, measuring bacterial nanomotions – or vibrations - within two hours. The growth-independent AST was developed and tested on clinical E. coli and K. pneumoniae isolates, recording nanomotions for ceftazidime-avibactam and cefiderocol. Machine learning algorithms achieved predictive models with 93% accuracy for spiked positive blood cultures, providing results within two hours. This rapid and accurate nanomotion AST challenges the conventional delayed gold-standard tests in hospitals, potentially transforming the assessment of antibiotic susceptibility and informing treatment decisions faster, thereby curbing the spread of antimicrobial resistance for critical drugs.

P-DCM-004

Recombinant receptor binding proteins of bacteriophages as versatile tools for pathogen detection

*P. Braun[1]

[1]*Fraunhofer ITMP-IIP, Penzberg, Germany*

For highly pathogenic bacteria, such as *Bacillus anthraci*s, *Yersina pestis*, *Burkholderia pseudomallei* or *Brucella* spp., rapid and unambiguous detection is crucial for timely antibiotic therapy of infected patients. While polymerase chain reaction (PCR) is the gold standard for diagnostics of most infectious diseases, antibody-based assays that detect specific antigens of the pathogen are commonly used as confirmatory methods. However, these antibodies often feature insufficient specificity due to the high degree of relatedness of these pathogens to their non- or less pathogenic relatives. Receptor binding proteins (RBPs) of bacteriophages, which mediate recognition and binding to host bacteria, represent a promising alternative to antibodies. Here, we identified RBPs of a variety of phages specific for *Bacillus anthraci*s, *Yersina pestis*, *Burkholderia pseudomallei* and *Brucella* spp. and utilized them to develop a set of novel tools for detection of these notorious pathogens. For this, recombinant RBPs were produced as fusions with different reporter proteins, such as fluorescent proteins or enzymes. In addition, RBPs were coupled to magnetic beads to serve as highly specific capture molecules for bacterial pathogen enrichment or isolation approaches.

P-DCM-005

Evaluation of a rapid MALDI-TOF MS-based colistin resistance test in Klebsiella pneumoniae

S. Lockwood[1], F. Nilsson[1], I. D. Nix[2], T. Maier[2], K. Sparbier[2], *B. Oberheitmann[2], S. Somajo[3], O. Ekelund[1]

[1]*Clinical Microbiology - Region Kronoberg, Växjö/Karlskrona, Sweden*

[2]*Bruker Daltonics GmbH & Co. KG, Microbiology & Infection Diagnostics, Bremen, Germany*

[3]Linnaeus University, Department of Chemistry and Biomedicine, Kalmar, Sweden

Introduction
Colistin is a last-resort antibiotic for treatment of multiple drug resistant Gram-negative bacterial infections. However, its use is hampered by the requirements of antimicrobial susceptibility testing. There is no reliable disk diffusion method, validated rapid antimicrobial susceptibility methods are scarce and only broth microdilution (BMD) is accepted by regulating bodies as EUCAST. Also, the reliability of BMD has been questioned, especially for species other than *Escherichia coli*. Here, we validated a rapid MALDI-TOF MS-based assay for lipid A characterisation in *Klebsiella pneumoniae*, using BMD and whole genome sequencing as reference.

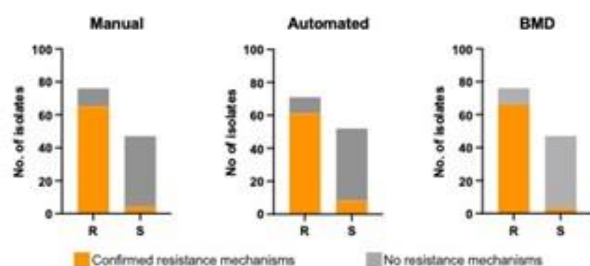
Methods
Lipid A profiling using the MBT Lipid Xtract Kit on the MALDI Biotyper Sirius System (Bruker Daltonics, Germany) was performed on 183 *K. pneumoniae* strains with previously characterised geno- and phenotype. Acquired spectra were manually examined for peaks at 1971 m/z and/or 1963 m/z, corresponding to 4-amino-4-deoxy-L-arabinose and/or phosphoethanolamine modified lipid A. If present, strains were interpreted as colistin resistant. Also, spectra were processed automated by using the prototype MBT HT LipidART Module (Bruker Daltonics). Results from both approaches were compared to BMD, and (on a subset of strains) to the presence (n=69) or absence (n=54) of confirmed mechanisms of resistance.

Results
Spectra were available for 179 isolates. Of these, 93% (manual) and 89% (automated) were S/R classified in agreement with BMD. Sensitivity for the presence of confirmed genetic resistance mechanisms was 94%, 88% and 96% for manual/automated lipid A analysis and BMD, respectively (Fig. 1).

Summary
MBT Lipid Xtract Kit is a useful and rapid screening tool for colistin resistance in *K. pneumoniae.* In the full strain collection containing 91 resistant and 92 susceptible isolates categorical agreement was 93% (automated: 88%), indicating the need for BMD confirmation. However, sensitivity for the presence of confirmed mechanisms of resistance was in line with BMD, suggesting a complex geno/pheno association behind colistin resistance in *K. pneumoniae*. This should be further investigated.

Figure 1: Colistin resistance prediction using lipid A analysis or the reference method (BMD) in K. pneumoniae with (n=69) or without (n=54) genetic markers consistent with colistin resistance.

Fig. 1



P-DCM-006
Characterisation of PVL-positive Staphylococcus aureus isolates from Saxony and Brandenburg
*E. Müller[1,2], S. Monecke[1,2,3], M. Armengol Porta[4], M. V. Narvaez Encalada[3], P. Schröttner[3,5], I. Schwede[6], H. H. Söffing[7], A. Thürmer[8], R. Ehricht[1,2,9]
[1]Leibniz-Institute of Photonic Technology (Leibniz-IPHT), Jena, Germany, member of the Leibniz Center for Photonics in Infection Research (LPI), Optical-molecular diagnostics and system technologies, Jena, Germany
[2]InfectoGnostics Research Campus, Centre for Applied Research, Jena, Germany
[3]Institute for Medical Microbiology and Virology, Dresden University Hospital, Dresden, Germany
[4]Medical Laboratory East Saxony MVZ GbR, Dresden, Germany
[5]Institute of Clinical Chemistry and Laboratory Medicine, Dresden University Hospital, Dresden, Germany
[6]IMD Labor Oderland GmbH, Frankfurt (Oder), Germany
[7]Senova Gesellschaft für Biowissenschaft und Technik mbH, Weimar, Germany
[8]MVZ Medizinische Labore Dessau Kassel GmbH, Dessau, Germany
[9]Friedrich Schiller University Jena, Institute of Physical Chemistry, Jena, Germany

Introduction: Panton-Valentine leukocidin (PVL) is a staphylococcal toxin associated with chronic/recurrent skin and soft tissue infections (SSTI) and necrotizing pneumonia. Thus, its detection warrants aggressive therapy and infection control measures. However, PVL detection is often a molecular method limiting its use.

Material and methods: In order to aid development of a lateral flow test for PVL, clinical isolates from SSTI were collected in 2020/21 at two laboratories in Dresden and at one in Frankfurt/Oder. After exclusion of duplicate and serial isolates, 83 isolates were eligible. These were characterized using DNA-microarrays that facilitated the detection of virulence and resistance markers as well as the assignment to clonal complexes and to epidemic/pandemic strains. They were also tested using an experimental lateral flow (LF) test for PVL.

Results: 39 isolates (47%) were PVL positive, and 30 isolates (36%) were mecA-positive. The MRSA rate among PVL-negatives was 20% (9 isolates), but among PVL-positives, it was as high as 54% (n=21). The most common PVL-positive MSSA lineages were CC152 (n=6), CC121 (n=4), CC5 and CC30 (each n=2). The most common PVL-MRSA strains were CC398-MRSA-VT (n=5), CC5-MRSA-IV "Sri Lanka Clone" (n=4), CC8-MRSA-[mec IV+Hg] "Latin American USA300" (n=4) and CC22-MRSA-IV (PVL+/tst+) (n=2). Other PVL-MRSA were CC1-MRSA-[V+fus+ccrAB1], CC8-MRSA-[IV+ACME] "USA300", CC30-MRSA-IV "WSPP Clone", CC88-MRSA-IV, CC152-MRSA-IV and -V, with one isolate each. LF results were in 81 cases concordant to genotyping, one false-positive and one false-negative were observed.

Discussion: Both the PVL rate as well as the MRSA rate among PVL-positives were higher than in a previous study a decade before. All PVL-MRSA strains detected as well as the most common methicillin-susceptible lineage (CC152) are known to locally predominate in other parts of the world, and thus all might be regarded as travel-associated. Therefore, patients with suspected PVL-associated disease should be asked for their travel histories, and, in case of

hospitalization, they should be treated as MRSA cases until proven otherwise.

P-DCM-007

Evaluation of different systems for identification and antibiotic susceptibility testing of bacteria directly from blood cultures

*A. Schmidt[1], J. Zens[1], P. D'Alvise[1], M. Bauer[1], L. Gouvernoy[1], J. Mack[1], S. Peter[1], M. Marschal[1]
[1]*University Hospital Tübingen, Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*

Introduction

One of the most important and timely critical procedures in microbiology diagnostics is the detection, ID and AST of microbes from blood stream infections. A delay in appropriate antibiotic therapy is detrimental to patients" outcome.

Goals

We aimed to compare two systems which promise to reduce the time to result for ID and AST directly from blood culture bottles with our routine diagnostic methods.

Materials & Methods

The study was conducted on consecutive positive blood cultures from March to May 2023. We included in total 100 monomicrobial samples with 55 gram-positive cocci and 45 gram-negative rods. For ID we compared the liquid colony from the FAST™ system (Qvella Corporation Europe) with our routine method of short-term culture analyzed by MALDI-TOF (Bruker). The AST was conducted for both by VITEK2 and compared with the AST by the dRAST (Quantamatrix).

Results

Overall, we identified 24 different bacterial species. The FAST™ system reduces the mean time to ID significantly. On a species level 95.6% of gram-negative and 85.5% of gram-positive bacteria were identified by the FAST™ system with consecutive MALDI-TOF. In 5 samples the direct ID failed, all other samples were at least identified on genus level. The mean time to AST was 9:43 h for the gram-negative and 10:48 h for the gram-positive bacteria for the FAST™ system. The dRAST takes in average 6:19 h for gram-negative and 6:45 h for the included gram-positive bacteria. 20 different antibiotics were available in all systems and were incorporated in the analyses. The rate of VME was 0.2% for the FAST™ system and 1.7% for the dRAST system.

Summary

In our study the FAST™ system reduces the time to ID considerably. It identified 95 % of the bacteria on genus level independently of restricted panel. Both systems showed satisfying and comparable performance for AST and speed up the diagnostic procedure. For the FAST™ system the usual procedure can be maintained after getting the liquid colony. The dRAST has in total a shorter hands-on time but needs a separate ID. Depending on the workflow and opening hours of the laboratory the adequate system can be chosen

P-DCM-008

Differentiation of Mycobacterium abscessus complex by Fourier-transform infrared spectroscopy

*N. Mauder[1], M. Cordovana[1], A. Curtoni[2], L. Pastrone[2], M. Genco[2], M. Sorba[2], S. Fedele[2], F. Rosati[2], M. S. Caroppo[2], A. Camaggi[3], C. Costa[2], M. Kostrzewa[1]
[1]*Bruker Daltonics GmbH & Co. KG , Bremen, Germany*
[2]*University of Turin, Microbiology and Virology Unit, Torino, Italy*
[3]*University Hospital "Maggiore della Carità" - Novara, Laboratory of Microbiology and Virology, Novara, Italy*

Background. *Mycobacterium abscessus* complex (MABSC) is one of the most frequently nontuberculous mycobacteria isolated in clinical practice, especially in immunocompromised patients. The subspecies of MABSC (*M. abscessus* ssp. *abscessus* (MAA), ssp. *massiliense* (MAM), and ssp. *bolletii* (MAB)), are associated with specific antimicrobial susceptibility profiles and different clinical outcomes. The discrimination at subspecies level is crucial for the proper patient therapeutic management, but so far it can only be achieved by genotypic techniques. In this study, we explore the potential of FT-IR spectroscopy to differentiate MABSC subspecies.

Methods. 41 patient-derived and culture collections MABSC isolates (n=18 MAA, n=13 MAM, and n=10 MAB) were analyzed by the FT-IR spectroscopy-based IR Biotyper® system (IRBT, Bruker Daltonics, Bremen). Spectra were acquired from 3 independent biological replicates of strains grown on Löwenstein-Jensen medium, incubated for 72 h at 35±2 °C. The resolution power using different regions of the IR spectrum was investigated. Predictive models were created using PCA (principal component analysis) and LDA (linear discriminant analysis), using random 67% of the acquired spectra as training set, and the remaining 33% as testing set.

Results. The IRBT analysis showed that the wavenumber range which allowed the best resolution power is the one corresponding to the absorption of carbohydrates and lipids (1300-800 cm-1, 1500-1400 cm-1 and 3000-2800 cm-1). The LDA predictive models allowed a clear differentiation of MAM from MAA and MAB, while the latter two are separable but more similar to each other. It was also observed that within each MABSC subspecies, the colony"s phenotype (rough or smooth) is responsible for a relevant spectral variance. Therefore, this feature has to be considered when defining the classes to differentiate.

Conclusions.IR Biotyper could have a future role in rapidly discriminating between MABSC subspecies. Further studies with a much larger number of samples are needed to assess the real impact of this new technology application, and to develop prediction models which are robust and generalizable to all datasets.

P-DCM-010

Antibacterial activity of monomeric and fibrillar PAP 248-286

*V. Vogel[1], R. Bauer[1], S. Burczyk[1], F. Witz[1], U. Rupp[2], C. Read[2], J. Münch[3], B. Spellerberg[1]
[1]*University Hospital Ulm, Medical Microbiology and Hygiene, Ulm, Germany*
[2]*Ulm University, Central Facility for Electron Microscopy, Ulm, Germany*
[3]*University Hospital Ulm, Institute of Molecular Virology, Ulm, Germany*

PAP248-286 is a proteolytic cleavage product of the human prostatic acid phosphatase (PAP) and is found in seminal fluid. The fibrillar form of this peptide enhances viral infections and was therefore called Semen-derived Enhancer of Virus Infection (SEVI). Like many described antimicrobial peptides, PAP248-286 is small and positively charged. Hence, we wanted to test PAP248-286 and SEVI for antibacterial activity.

Therefore, a radial diffusion assay and survival assays were performed. Since PAP248-286 is found in seminal fluid, the vaginal milieu was mimicked for our experiments. The peptide was tested against all ESKAPE pathogens as well as pathogenic bacteria of the female genital tract, like *Streptococcus agalactiae* or *Listeria monocytogenes*. Transmission electron microscopy (TEM) and membrane permeabilization assays were used to investigate putative mechanisms of action.

The survival of *Pseudomonas aeruginosa, S. agalactiae* and *L. monocytogenes* was significantly reduced after SEVI treatment. In radial diffusion assays, further activity against *Klebsiella pneumoniae, Escherichia coli* and *Acinetobacter baumannii* was detected. The antimicrobial activity of SEVI increased with lower pH. By TEM analysis, no effect of PAP248-286 or SEVI on the membrane of *P. aeruginosa* or *S. agalactiae* was visible, which was further substantiated by membrane permeabilization assays. Interestingly, TEM together with energy-dispersive X-ray (EDX) spectroscopy analysis showed that *P. aeruginosa* formed polyphosphate bodies after treatment with PAP248-286.

This study uncovers SEVI's substantial antibacterial potential against vaginal tract pathogens, suggesting a physiological role in the human body.

P-DCM-011

Evaluation of a new prototype interferon-gamma release assay for the determination of T-cell-mediated immunity in tuberculosis

M. Ibrahimová[1], K. Doležalová[2], M. Sukholytka[3], *E. Grage-Griebenow[4], D. Zapf[4], S. Saschenbrecker[4], E. Kopecká[3], M. Koziar Vašáková[3]

[1]*Laboratory of Immunology, Thomayer University Hospital, Prag, Czech Republic*
[2]*Department of Pediatrics, First Faculty of Medicine, Charles University, Thomayer University Hospital, Prag, Czech Republic*
[3]*Department of Respiratory Medicine, First Faculty of Medicine, Charles University, Thomayer University Hospital, Prag, Czech Republic*
[4]*Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany*

Objective

This study evaluates the performance of a new prototype interferon-gamma release assay (IGRA) for the determination of T-cell-mediated immunity against *Mycobacterium tuberculosis* (MTB).

Methods

Blood samples from 34 adults with active tuberculosis (TB) and from 30 children with active TB (n=1), latent tuberculosis infection (TBI, n=12) or without TB (n=17) were analyzed using the prototype Quan-T-Cell TB (EUROIMMUN) on the DSX Automated ELISA System (Dynex Technologies). The results were compared to the clinical diagnosis. Additionally, 29 of the pediatric samples were measured using the established QuantiFERON-TB Gold Plus assay (Qiagen) to determine inter-assay concordance. Both assays use stimulatory antigens that are based on the mycobacterial proteins ESAT-6 and CFP-10.

Results

The prototype Quan-T-Cell TB differentiated patients with active TB or TBI from cases without TB with a sensitivity of 91.3% at a specificity of 93.8%, while the positive and negative predictive values were 97.7% and 78.9%, respectively. Comparison between the two IGRAs showed positive, negative and overall agreement rates of 100%, 93.8% and 96.3%, respectively, with a kappa score of 0.924 indicating almost perfect agreement. Qualitative inter-assay discrepancies were found in three samples (1 TBI, 2 without TB) that were negative by QuantiFERON-TB but borderline or very weakly positive by the prototype Quan-T-Cell TB.

Conclusion

The results suggest very good performance of the prototype Quan-T-Cell TB. Unlike the QuantiFERON-TB, the Quan-T-Cell TB has a borderline range (grey zone), which is advantageous as it may help to differentiate non-specific variations near the cut-off value from true conversions or reversions. Moreover, reduction to one MTB-specific stimulation tube minimizes the required sample volume and maximizes the cost-effectiveness of laboratory testing. Using only 1.5 ml blood per analysis (QuantiFERON-TB: 4 ml) is helpful particularly in pediatric applications. Further optimization of the Quan-T-Cell TB is underway, requiring additional evaluation studies including larger, age- and gender-matched patient and control cohorts.

P-DCM-012

Comparison of prevalence of the panton-valentin leukocidin gene among clinical and community-acquired staphylococcus aureus strains

*L. J. B. Schneider[1], J. Miciulevičienė[2], M. Bratchikov[3], A. Kirkliauskienė[3]

[1]*Vilnius University, Medical Faculty, Vilnius, Lithuania*
[2]*Vilnius City Clinical Hospital, Vilnius, Lithuania*
[3]*Vilnius University, Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius, Lithuania*

Introduction *Staphylococcus aureus (S. aureus)* infections are prevalent hospital-acquired infections. Strains of *S. aureus* with the Panton-Valentin Leukocidin Toxin (PVL) acquired higher virulence. PVL gene consists of *lukS-PV* and *lukF-PV* genes.[1]

Goals To define the prevalence of PVL gene of *S. aureus* strains isolated from hospitalized patients and healthy community in Vilnius, LT.

Materials & methods A retrospective study was conducted with clinical *S. aureus* strains, which were isolated from 2018-2019 in Vilnius City Clinical Hospital, and community strains, isolated from 2017-2019 in Vilnius. All collected S. *aureus* strains were analysed for detection of the *lukF-PV* gene by our designed multiplex real-time PCR protocols. The 16S rRNA coding sequence served as an internal PCR control.

Results In total 615 hospitalized patients and 761 healthy adults from the community participated in the study. 65.7% of clinical strains were isolated from skin and soft tissues, the rest from blood, respiratory tract, urine, and other sites. From the community 329 *S. aureus* strains were isolated. Community isolation sites were 53.3% from nose, the rest - throat or throat and nose. A total of 944 S*. aureus* strains were tested for the PVL gene. PVL positive (PVL+) were 7.5% of hospital-isolated strains and 1.2% of community isolates. Among hospital PVL+ strains, the highest antibiotic resistance was found to erythromycin (32.6%), cefoxitin (30.4%) and clindamycin (8.7%). One community PVL+ strain was resistant to penicillin. 71.7% of hospital PVL+ *S. aureus* was isolated from skin and soft tissues, 13.0% blood, 10.9% respiratory tract, and 2.2% urine. All community PVL+ strains were isolated nasally.

Summary The PVL gene was more prevalent in hospital strains of *S. aureus* than in community strains, as well as the clinical strains are more likely to have antimicrobial resistance. Skin and soft tissue specimens predominated in clinical PVL+ strains, while community PVL+ strains were exclusively nasal.

References 1 Szumlanski, Tobias, et al. 2023. "Characterization of PVL-Positive MRSA Isolates in Northern Bavaria, Germany over an Eight-Year Period" Microorganisms 11, no. 1: 54.

P-DCM-013
Evaluation of microbiome enrichment and host DNA depletion in human vaginal samples using Oxford Nanopore's adaptive sequencing

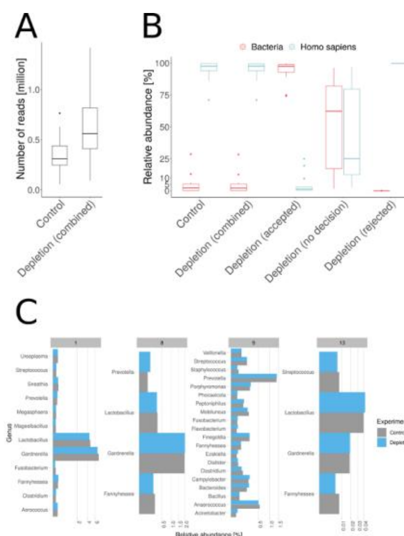
*M. Marquet[1,2], J. Zöllkau[3], J. Pastuschek[3], A. Viehweger[4], E. Schleußner[3], M. W. Pletz[2], R. Ehricht[5,6,7], C. Brandt[1,2]
[1]*University Hospital Jena, CaSe Group (Cloud computing and Sequencing), Jena, Germany*
[2]*University Hospital Jena, Institute for Infection Medicine and Hospital Hygiene, Jena, Germany*
[3]*University Hospital Jena, Department of Obstetrics, Jena, Germany*
[4]*University Hospital Leipzig, Institute of Medical Microbiology, Leipzig, Germany*
[5]*Leibniz Institute of Photonic Technology (IPHT), Jena, Germany*
[6]*Friedrich Schiller University Jena, Institute of Physical Chemistry, Jena, Germany*
[7]*InfectoGnostics Research Campus Jena, Jena, Germany*

Metagenomic sequencing is promising for clinical applications to study microbial composition concerning disease or patient outcomes. Alterations of the vaginal microbiome are associated with adverse pregnancy outcomes, like preterm premature rupture of membranes, and preterm birth. Methodologically these samples often have to deal with low relative amounts of prokaryotic DNA and high amounts of host DNA (> 90%), decreasing the overall microbial resolution. Nanopore's adaptive sampling method offers selective DNA depletion or target enrichment to directly reject or accept DNA molecules during sequencing without specialized sample preparation.

Here, we demonstrate how selective 'human host depletion' resulted in a 1.70 fold (± 0.27 fold) increase in total sequencing depth, thus providing higher taxonomic profiling sensitivity. At the same time, the microbial composition remains consistent with the control experiments. The complete removal of all human host sequences is not yet possible and should be considered as an ethical approval statement might still be necessary. Adaptive sampling increased microbial sequencing yield in all sequenced vaginal samples.

DNA of vaginal swabs was extracted via the ZymoBIOMICS DNA Miniprep kit and sequenced on the GridION (Oxford Nanopore). Adaptive sampling was enabled in the MinKNOW software. In "human depletion experiments", the human reference genome GCA_000001405.28_GRCh38.p13 was used for target depletion.

Our results demonstrated that Oxford Nanopore's unique adaptive sequencing feature has reliably increased the overall sequencing depth of bacterial sequences in clinical metagenomic samples via 'human depletion' without changing the microbial composition during sequencing. Therefore, more samples can be barcoded and sequenced simultaneously, reducing the total cost per sample in a diagnostic laboratory, making molecular monitoring of human reservoirs with low microbial concentrations and high host DNA loads more feasible. We strongly believe that adaptive sampling will prove exceptionally useful within clinical research and the individual microbiological and microbiological diagnostic approach in routine diagnostics. **Fig. 1**



P-DCM-014
Detection of *Francisella tularensis* using recombinant reporter fusion proteins

*J. K. Janssen[1], H. von Buttlar[1], G. Grass[1]
[1]*Bundeswehr Institute of Microbiology, Bacteriology, München, Germany*

Francisella tularensis causes tularemia in animals and humans. Although rare, the potential severity of human disease and the possibility of misuse as a biological agent of terrorism or warfare necessitates rapid and accurate diagnosis. Diagnostic laboratory methods commonly depend on culture, serology and molecular genetic approaches (e.g., polymerase chain reaction, PCR). All of these have limitations and require infrastructure which is frequently limited in non-traditional laboratory environments such as military field operation scenarios.

In this work we employed a *F. tularensis*-specific single-chain variable fragment antibody (scFv) to develop non-PCR identification methods for the sensitive and rapid detection

of *F. tularensis* cells. For this, synthetic genes of a *F. tularensis*-specific scFv and a green fluorescent protein were fused by PCR-amplification and cloned into the expression vector pASG-105 harboring a N-terminal twin-strep-tag-epitope. The resulting reporter fusion protein (RFP) was heterologously produced in *Escherichia coli* and purified by affinity chromatography. Binding of the RFP to a range of *Francisella spp.* and other bacteria was assessed by fluorescence microscopy. Additionally, human macrophage cell lines were infected with *F. tularensis* to determine the intracellular binding capability of the RFP. In order to assess the impact of a relevant clinical matrix on efficient labeling of *F. tularensis* bacteria with RFP, blood was used.

All three subspecies of *F. tularensis* incubated with the RFP were successfully labeled; more distantly related bacteria were not. Importantly, most close relatives of *F. tularensis* could be differentiated from *F. tularensis* with the exception of *F. hispaniensis* and certain strains of *F. novicida*. Detection of *F. tularensis* in blood posed a challenge. Labeling of intracellular *F. tularensis* was possible using the RFP. Overall, the accurate detection of *F. tularensis* by a recombinant RFP was successful and our results indicate the possibility to further develop this approach to facilitate identification of the tularemia agent in the field and laboratory.

P-DCM-015
Improvement of in vitro diagnostics for tick- and louse-borne relapsing fever used for a seroprevalence study in northern Kenya

*F. Reyer[1], M. Olesiuk[1], F. Röttgerding[1], V. Fingerle[2], A. Adamu[3], D. Waithru[4], J. Njeru[4], P. Kraiczy[1]
[1]*University Hospital of Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a. M., Germany*
[2]*National Reference Center for Borrelia, Oberschleißheim, Germany*
[3]*Usmanu Danfodiyo University, Sokoto, Nigeria*
[4]*Kenya Medical Research Institute, Nairobi, Kenya*

Introduction

Relapsing fever (RF) is a vector-borne zoonotic disease transmitted to humans by infected ticks or lice. Distinguished from all other *Borrelia* species known as causative agents of tick-borne RF, *Borrelia recurrentis* is only transmitted by the human body lice. Louse-borne RF (LBRF) is currently supposed to be endemic to the Horn of Africa, but it is conceivable, that refugees introduce the vectors into neighboring countries like northern Kenya. Unfortunately, there is no reliable *in vitro* diagnostics for RF available on the market.

Goals

The main goals of the study are (i) the improvement of our recently introduced immunoassay for LBRF by incorporating additional immunoreactive antigens and (ii) screening of serum samples collected in northern Kenya for IgG and IgM anti-*Borrelia* antibodies.

Materials & Methods

Highly immunoreactive antigens identified by mass spectrometry were used for two immunoassays, ELISA and Lineblot. Both immunoassays were incubated with confirmed *Borrelia*-positive and negative serum samples as well as, uncharacterized serum samples collected from patients with febrile symptoms in northern Kenya.

Results

Recently, GlpQ and the N-terminal fragment of ChiC (CihC-N) from *Borrelia recurrentis* were identified as reliable discriminatory antigens in combination with additional immunoreactive belonging to the Variable Major Proteins (VMP) of *B. recurrentis* to increase the sensitivity and specificity. By incorporating VMPs, in particular VlpD1, an increase in sensitivity was observed for the Line immunoblot.

Among 2,005 serum samples tested for anti-*Borrelia* IgG antibodies, 14.3 % were positive by ELISA. In addition, 152 randomly selected sera were screened for anti-*Borrelia* IgM antibodies. Unexpectedly, a high percentage of these sera tested (57.2 %) were also considered IgM positive.

Summary

The incorporation of VlpD1 leads to an increase in sensitivity of two recently introduced immunoassays. Our data on the seroprevalence of RF strongly suggest the distribution of the vector or the pathogen in the northern territories of Kenya bordered to countries where outbreaks are frequently reported.

P-DCM-016
Development of Vancomycin-resistant-enterococci rapid detection kit for routine clinical care

*L. Maus[1,2], M. Gonzalez Rodriguez[1,2], S. Mertins[1,2], P. G. Higgins[1,2], A. Klimka[1,2]
[1]*University Hospital Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*
[2]*German Center for Infection Research, Bonn, Germany*

Integrating rapid diagnostic tests into clinical management has great potential to improve, therapeutic processes, and patient outcomes. For this, we develop an antibody-based lateral flow assay (LFA) for the detection of vancomycin resistant *Enterococcus faecium* (VREfm). Resistance to vancomycin is disseminating rapidly also in other more virulent bacterial species. Hospitalized patients with gastrointestinal carriage of VREfm appear to be the major reservoir of the pathogen. Two major vancomycin resistance determinants (ligases VanA and VanB) have been described in enterococci. Expression of the ligases, VanA or VanB, results in the synthesis of altered peptidoglycan precursors to which vancomycin binds with markedly lower affinity loosing its bactericidal activity. VanA mediated resistance is characterized by high-level resistance to vancomycin, and was up to recently the most prevalent type in Germany. New epidemiological studies revealed an increasing prevalence of vanB in VREfm and particularly in sequence type ST117 during the last five years in German hospitals. VanB expressing isolates show inducible resistance to more modest levels of vancomycin rendering to false susceptible results in routine phenotypic tests.

We focused primarily on the development of an LFA to detect VanA and VanB resistance determinants with the option to expand it towards a biomarker for VREfm sequence type ST117. We cloned, expressed and purified representative VanA / VanB ligases in *E. coli* for immunization of mice. Generated monoclonal antibodies

(mAbs) by hybridoma technology were screened by ELISA and Westernblot for their binding properties.

We identified 15 mAbs that specifically react with different variants of endogenous VanA or VanB, or both of clinical isolates. In contrast to VanA, VanB expression needs to be induced by vancomycin, which will be considered in the VRE-LFA workflow. The aim is to rapidly detect VREfm in cultivated isolates from patient samples in order to simplify and expedite diagnosis and treatment options. The VRE-LFA will be integrated into a pilot study to analyze its potential impact on clinical management.

P-DCM-017

Nanopore sequencing of positive blood cultures for rapid species identification and resistance prediction

*T. Bähr[1], A. Baumhögger[1], G. Geis[1], S. G. Gatermann[1,2]
[1]IML Bochum GmbH, Bochum, Germany
[2]Ruhr-University Bochum, Department of Medical Microbiology, Bochum, Germany

Introduction

For bloodstream infections, initiation of an adequate antibiotic therapy is time critical. Whole-genome sequencing of bacterial DNA from blood cultures could provide both rapid species identification and resistance prediction, thereby reducing mortality from bloodstream infections.

We have therefore established a method for purifying bacterial DNA from blood culture bottles, which can also remove the potent enzyme inhibitor SPS from the samples. The purified DNA allows sequencing using a Nanopore sequencer, enabling both species identification and resistance prediction.

Methods

In a first step, the human DNA was depleted via gel centrifugation and selective lysis. SPS was removed by organic extraction with benzyl alcohol. The bacterial DNA was purified from the aqueous phase using a spin-column-based kit.

The purified DNA was sequenced using a shotgun sequencing workflow on a Nanopore sequencer. The generated reads were analysed in EPI2ME using the wf-Metagenomics workflow, including both species identification and a search for resistance genes.

Results

The developed protocol enabled the purification of DNA from the blood culture bottles, while at the same time efficiently removing SPS. Via the addition of the host DNA depletion steps bacterial DNA could enriched from an average of 42.3% to 79.8% of the total DNA.

In 94/96 of the blood cultures examined, all species that were detected by culture were correctly identified using sequencing. In two of these, obligate anaerobes were detected in addition to the cultured species. In the remaining two bottles, not all cultured species could be found via sequencing.

For Enterobacterales and Staphylococci, the positive and negative predictive value of the resistance prediction varies between 75% and 100%, depending on the antibiotic.

Conclusion

A protocol was successfully established that can both selectively purify bacterial DNA from blood culture bottles and remove the enzyme inhibitor SPS. By nanopore sequencing of the purified DNA, both reliable species identification and relatively accurate resistance prediction for the most relevant pathogens of bloodstream infections could be achieied.

P-DCM-018

LiquidArray® Mycobacteria direct: Detection of MTBC and NTM directly from patient specimen

L. Wolf[1], *V. Allerheiligen[1], M. Klimovich[1], K. Küspert[1]
[1]Bruker-Hain Lifescience GmbH, Research & Development, Nehren, Germany

Introduction:

Mycobacterial infections pose a major global public health challenge. Within the genus Mycobacteria, with over 200 different species, M. tuberculosis takes center stage as the main cause of tuberculosis. In addition, other species like M. avium complex (MAC) also contribute to diseases such as opportunistic infections in immunocompromised patients. The varying severity and clinical presentations of mycobacterial infections emphasize the need for tailored treatment strategies based on the specific mycobacterial species.

We are developing the LiquidArray® *Mycobacteria direct* assay as an innovative diagnostic test for the detection and differentiation of clinically significant mycobacterial species in sputum samples, such as those in the MAC, *M. xenopi*, *M. kansasii* as well as the *M. tuberculosis* complex.

Method:

The assay is powered by LiquidArray® technology, which utilizes multiplexed PCR to amplify target DNA sequences, capitalizing on the advanced capabilities of the FluoroCycler® XT for simultaneous detection across 5 fluorescence channels. The LiquidArray® technology uniquely integrates fluorescent probes that enable the detection of multiple targets in a single reaction to save time, resources and sample material. With the manual FluoroLyse® kit, as well as the automated GenoXtract® fleXT workflow for up to 96 samples, we ensure rapid DNA extraction during sample preparation.

Results:

The application of target-specific melting curve analysis enhances the assay's performance, distinguishing between closely related species. The LiquidArray® *Mycobacteria direct* can detect and distinguish between several different species from over 200 different mycobacteria in one reaction. In addition, all other mycobacteria species are detected at

the *Mycobacterium* genus level. Including DNA isolation, reliable and automatically evaluated results are obtained within 3 hours.

Summary

The LiquidArray® Mycobacteria direct will be a powerful tool for the rapid identification of clinically relevant mycobacterial species directly from patient specimen paving the way for fast specific treatment and preventive measures.

P-DCM-019

Pharmaceutical Reactive Chlorine-Oxygen Species Exhibit Antimicrobial Effects on Bacteria in Suspension and Bacterial Biofilms

*J. Sommer[1], H. Sebald[1], K. W. Stahl[2], U. Schleicher[1], C. Bogdan[1]
[1]*University Hospital Erlangen, Institute for Clinical Microbiology, Immunology and Hygiene, Erlangen, Germany*
[2]*Non-profit organization Waisenmedizin e. V., Freiburg i. Br., Germany*

Introduction:

Skin ulcers are frequently colonized with pathogens, including multi-drug resistant bacteria (MDRB). This leads to chronic, non-healing wounds. The formation of biofilms protects bacteria against antibiotics and many disinfectants. A promising treatment option is pharmaceutical NaClO2 (*sodium chlorosum*). *Sodium chlorosum* generates reactive chlorine oxygen species (RClOS) and was previously shown to promote healing of diabetic, arterial, venous and *Leishmania* ulcers and to kill *Leishmania* pro- and amastigotes *in vitro*. Based on its clinical effects, we hypothesize that *sodium chlorosum* kills antibiotic-sensitive and -resistant bacteria and disrupts bacterial biofilms.

Methods:

We used Gram-positive and Gram-negative bacterial species including antibiotic-sensitive strains and MDRB. To analyze the bacteriostatic effect, bacteria ± *sodium chlorosum* were incubated for 6 h. Optical density was measured hourly as a marker for bacterial growth. Bactericidal effects were determined by incubating bacteria for 4 h with *sodium chlorosum*. The samples were plated on agar plates and the number of colony-forming units (CFU) was determined. Biofilms were grown by incubating bacterial samples for 24 h in a 96-well plate. *Sodium chlorosum* was added, and samples were incubated again for 24 h. Biofilm viability and mass were determined with a resazurin-based stain and crystal violet, respectively.

Results:

Concentrations ≤500µM of *sodium chlorosum* inhibited the growth of most of the bacteria tested (e.g. *S. aureus*, *P. mirabilis*, *E. coli*) except for few species (e.g., *E. faecium*). Concentrations above 1000µM exhibited bactericidal effects in most bacterial species tested. While methicillin-resistant *S. aureus* (MRSA) was susceptible to low concentrations of *sodium chlorosum*, vancomycin-resistant *E. faecium* (VRE) was resistant. Biofilm viability and mass decreased in the presence of higher concentrations of *sodium chlorosum* in all tested bacterial strains.

Conclusion:

Sodium chlorosum exhibits bactericidal and bacteriostatic effects and reduces biofilm viability and mass of various bacteria. Effects on MDRB are variable and depend on the bacterial species.

P-DCM-020

***LiquidArray®* MTB-XDR VER 1.0 - a powerful molecular genetic tool for rapid diagnosis of extensively drug-resistant tuberculosis**

*V. Allerheiligen[1], K. Küspert[1], R. Spannaus[1]
[1]*Bruker-Hain Lifescience GmbH, Research & Development, Nehren, Germany*

Introduction: Extensively drug-resistant tuberculosis (XDR-TB) and pre-XDR-TB are a major challenge to TB control due to their complex diagnosis and obstacles in treatment. With LiquidArray® MTB-XDR VER 1.0 Hain Lifescience provides an assay for rapid diagnosis of XDR-TB. Besides detection of Mycobacterium tuberculosis complex (MTBC) and its mutations conferring resistance to fluoroquinolones, amikacin, and ethambutol, the assay can also detect the most significant mutations conferring resistance to the group A drug Linezolid. Due to its ability to detect linezolid resistance, LiquidArray MTB-XDR VER 1.0 is currently the only assay available that detects XDR-TB according to the revised WHO definition.

Methods: As starting material patient specimens and cultivated samples are used. DNA extraction is performed manually or automated with the GenoXtract® fleXT nucleic acid extraction system allowing high sample throughput with minimal hands-on time. Amplification, detection, and automated result interpretation is performed in the FluoroCycler® XT system within one single tube.

Results: LiquidArray MTB-XDR VER 1.0 was evaluated according to IVDR requirements. Within the analytical study an excellent performance of the assay could be demonstrated for all parameters investigated, such as analytical sensitivity, precision, analytical specificity, etc. All mutations detectable by the assay conferring resistance to specific group A and group C drugs (fluoroquinolone, amikacin, ethambutol, and linezolid) were identified 100% correctly. The excellent performance of the assay could be also demonstrated within the clinical study. In this highly complex study high sensitivities and excellent specificities for detection of MTBC could be achieved, overall and for smear negative samples. The LiquidArray MTB-XDR VER 1.0 showed excellent performance for resistance detection of the group A drugs fluoroquinolones and linezolid. Reliable detection of the most important resistance conferring mutations of group C drugs amikacin and ethambutol could also be achieved.

Summary: LiquidArray MTB-XDR VER1.0 is a powerful tool for reliable detection of resistances related to XDR-TB.

P-DCM-021

Macozinone Revealed: Nanomotion-Based Rapid Phenotypic Evaluation of New Drug Candidate for Mycobacterium tuberculosis Treatment.

*A. Vocat[1,2], A. Luraschi Eggemann[2], C. Antoni[3], V. Makarov[3], G. Cathomen[1], G. Degiacomi[4], M. Świątkowski[1], G. Wielgoszewski[1], M. R. Pasca[4], D. Cichocka[1], G. Greub[2,5], O. Opota[2], S. T. Cole[3], A. Sturm[1]
[1]*Resistell AG, Muttenz, Switzerland*
[2]*Lausanne University Hospital and University of Lausanne, Institute*

of Microbiology, Lausanne, Switzerland
[3]Innovative Medicines for Tuberculosis (iM4TB), Lausanne, Switzerland
[4]University of Pavia, Pavia, Italy
[5]Lausanne University Hospital and University of Lausanne, Service of Infectious Diseases, Lausanne, Switzerland

The surge in global *Mycobacterium tuberculosis* (MTB) infections, compounded by the post-COVID-19 landscape, underscores the urgency for swift diagnostics, especially given the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Conventional antimicrobial susceptibility testing (AST), notorious for its time-intensive nature, impedes the prompt identification of drug-resistant cases, posing a challenge in managing escalating global MTB infections. This study delves into nanomotion-based AST, lauded for its ultra-rapid phenotypical capabilities in the 2023 Tuberculosis Diagnostics Pipeline Report (2) for its high performance on established antitubercular agents (RIF, INH) (1). Our focus lies in evaluating the adaptability of this method for detecting third-line drugs, such as Macozinone (MCZ).

Employing the Phenotech AST device, we assessed its efficacy in distinguishing Macozinone profiles, utilizing MTB susceptible (H37Rv) and resistant (NTB1 with DprE1 mutations) strains (3). Changes in bacterial metabolism patterns were correlated with drug susceptibility profiles, and a machine learning approach gauged the device's accuracy, sensitivity, and specificity in predicting strain phenotypes.

Susceptible MTB strains exhibited a notable decline in cantilever oscillations, signifying reduced bacterial metabolism and eventual inactivation. Conversely, resistant strains remained unaffected by the drug in their environment. Classification models for the DprE1 inhibitor Macozinone demonstrated high training accuracy, surpassing 95%.

The nanomotion-based rapid AST protocol was effectively applied to the developmental drug MCZ. Crucially, the approach validated Macozinone's novel effects *in vitro*. The Phenotech AST device exhibits promise for direct deployment in endemic countries, facilitating timely, accurate treatment decisions for patients with results delivered in under a day (21 hours).

Fig. 1

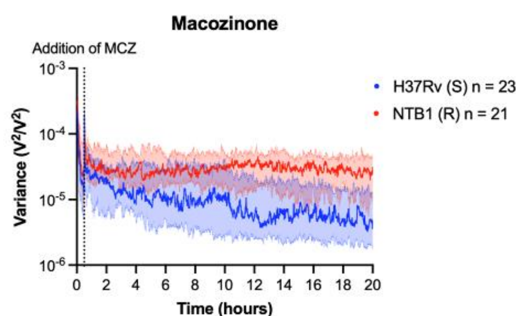


Figure 1. Nanomotion variance recordings for H37Rv (susceptible to MCZ) and NTB1 (resistant to MCZ). After the addition of MCZ, both H37Rv strains susceptible to MCZ (blue) and NTB1 strains (red) resistant to MCZ responded to the exposition of the drug, although they responded differently; towards the end of the experiment, bacterial vibrational variance was greater in the resistant strains NTB1 than in the susceptible strains H37RV. Solid lines represent the median of the variance, and the shaded background area represents the Interquartile Range of the median.

P-DCM-022
Analytical and Clinical performance evaluation of the FluoroType® MTBDR VER 2.0 with Dual Target Detection of Mycobacterium tuberculosis Complex from Native Sputum and Culture

*V. Allerheiligen[1], M. Eckart[1], L. Wolf[1], S. Fischer[1]
[1]Bruker-Hain Lifescience GmbH, Research & Development, Nehren, Germany

Introduction: The WHO-endorsed FluoroType® MTBDR VER 2.0 based on the LiquidArray® technology is an important tool in diagnosis of TB and its resistance-mediating mutations against first-line antibiotics rifampicin and isoniazid. The multi-copy target IS6110 was integrated into the FluoroType MTBDR VER 2.0 as a second target for M. tuberculosis complex (MTBC) detection. In this study we determined the analytical and clinical performance of the modified assay.

Methods: The modified FluoroType MTBDR VER 2.0 in combination the Liquefaction Set VER 1.0 allows easy and simultaneous detection of MTBC and its resistance to rifampicin and isoniazid directly from native sputum specimens. In total 8 workflows for manual and automated DNA extraction from native sputum and culture samples have been evaluated using the GenoXtract® and GenoXtract® fleXT instruments in combination with the FluoroCycler® XT. The system enables a rapid and automated processing with minimal hands-on time and fully automated evaluation. Reported mutations were adapted to the recently published WHO catalogue of resistance mediating-mutations.

Results: Integration of IS6110 leads to a high sensitivity of MTBC detection. Within the analytical performance study an excellent performance of the assay could be demonstrated. The FluoroType MTBDR VER 2.0 allows reliable detection and identification of most relevant mutations in rpoB, katG and the inhA promoter region responsible for resistance to rifampicin and isoniazid. It also identifies silent mutations within rpoB. The Liquefaction Set VER 1.0 inactivates and liquifies native sputum very effectively in one step. The excellent performance for MTBC and resistance detection was also demonstrated in a clinical study with ca. 600 patient samples.

Summary: The modified FluoroType MTBDR VER 2.0 allows a highly sensitive detection of MTBC and differentiates important mutations responsible for resistance based on the WHO catalogue of mutations. It enables sensitive detection of MTBC and identification of mutations directly from native sputum specimens. Additionally, NALC-NaOH decontaminated sputum samples and culture samples can be analyzed.

P-DCM-023
Antimicrobial activity of a nitroxoline conjugate against multidrug-resistant human pathogens

*S. R. M. Mielke[1], S. Eckert[1], D. Frank[1], D. Bachmann[2], E. Proschak[2], P. Papareddy[3], H. Herwald[3], K. Sander[4], T. A. Wichelhaus[1]
[1]Goethe University of Frankfurt, Institute for Medical Microbiology and Infection Control, Frankfurt a. M., Germany
[2]Goethe University Frankfurt, Institute for Pharmaceutical Chemistry, Frankfurt a. M., Germany
[3]Lund University, Department of Clinical Sciences, Division of Infection Medicine, Biomedical Center (BMC), Lund, Sweden
[4]University College London, Centre for Radiopharmaceutical Chemistry (CRC), London, United Kingdom

Introduction:

Nitroxoline is an approved drug with highly potent, broad-spectrum antimicrobial activity against human pathogens, including multidrug-resistant strains. The clinical use, however, is limited to uncomplicated urinary tract infections due to insufficient organ distribution and cytotoxicity at higher doses. We aim to address these limitations by developing a nitroxoline conjugate with improved pharmacokinetic properties. Here, we present data on the *in vitro* antimicrobial activity of the NC. The project was funded by JPIAMR.

Materials & Methods:

The antimicrobial activity of NC was assessed against a broad panel of multidrug-resistant as well as susceptible clinical pathogens, e.g. *Enterobacterales* (*n* = 100), *Pseudomonas aeruginosa* (*n* = 20) and *Acinetobacter baumannii* (*n* = 20) as well as tuberculous and non-tuberculous mycobacteria (*n* = 48), enterococci (*n* = 30), staphylococci (*n* = 30) and *Candida* spp. (*n* = 35). The minimum inhibitory concentrations (MIC) of the NC were determined using the broth microdilution method. The building blocks of the conjugate were used as control substances. The mode of action, i.e. bactericidal or bacteriostatic, was determined by time-kill kinetics.

Results:

The MIC data obtained confirm the broad antimicrobial activity of nitroxoline against bacteria, including mycobacteria and yeasts. The NC showed good antimicrobial activity at an equimolar concentration, comparable to that observed with nitroxoline. For example, the NC MIC50 and MIC90 in mg/L were 0.5 and 1 for *Staphylococcus aureus,* 16 and 16 for *Acinetobacter baumannii,* 32 and 32 for *Escherichia coli*, 8 and 16 for mycobacteria, and 4 and 8 for *Candida* spp.. The mode of action was determined to be bactericidal and fungicidal for *Staphylococcus aureus, Acinetobacter baumannii* and *Candida albicans*, respectively.

Summary:

Our data show good antimicrobial activity of the nitroxoline conjugate *in vitro* and may indicate a promising approach in the fight against infections caused by multidrug-resistant pathogens. Further experiments are planned to investigate the toxicity and antimicrobial activity of NC *in vivo.*

P-DCM-024

Re-emergence of Respiratory Syncytial Virus in an Adult Patient Population after the Alleviation of Non-Pharmaceutical Interventions (NPI) due to the SARS-CoV-2 Pandemic

*M. Hönemann[1], S. Thiem[1], S. Bergs[1], M. Maier[1], C. Pietsch[1]
[1]*Institute of Medical Microbiology and Virology, Virology Department, Leipzig, Germany*

Introduction: Respiratory syncytial virus represents one of the most important respiratory pathogens affecting all age groups. Following the extensive non-pharmaceutical interventions (NPIs) and societal behavioral changes of 2020 and 2021 in wake of the SARS-CoV-2 pandemic, a marked epidemiological shift was observed in 2022 and 2023.

Methods: The aim of this study was to characterize the local molecular epidemiology of RSV infections in the seasons of 2021/2022 and 2022/2023 to the three pre-pandemic seasons by sequence analysis of the complete G and F genes. Additionally, clinical data were retrieved from patient charts to determine the clinical significance of RSV infections of the same period.

Findings: In season 2021/2022 the peak of RSV detections occurred in calendar week 40 of 2021, 18 weeks before the usual peak observed in the three pre-pandemic seasons. In season 2022/2023, the peak was detected in week 1 of 2023. The sequence analysis of RSV-A and RSV-B strains revealed a close phylogenetic relatedness with assignment to the same genotype regardless of the season of origin in the G gene. The analysis of the F gene revealed a close relatedness as well, however, an amino acid drift in the antigenic regions was observed especially for RSV-B. Although the positivity rates were comparable, the cases numbers for seasons 2021/2022 and 2022/2023 were markedly different. While in season 2022/2023 as many cases as in all pre-pandemic seasons were detected, season 2021/2022 showed the lowest case number and a significantly higher amount of patients under the age of 60 (<0.001). Most of the assessed clinical parameters were similar in comparison to the three pre-pandemic seasons. High rates of comorbidities, lower respiratory tract infections, and ICU admissions prevailed in the studied adult population.

Conclusion: The NPIs in wake of the SARS-CoV-2 pandemic had a tremendous impact on the epidemiologic characteristics and seasonality of RSV. The continued effect of behavioral changes on the circulation of respiratory viruses as well as the potential implementations of new treatment strategies warrant further epidemiologic studies of this common pathogen.

P-DCM-025

Comparative Evaluation of MALDI-TOF MS-Based Assays for Carbapenemase Detection in Enterobacterales

*C. Uitz[1], K. Dichtl[1], J. Luxner[1], S. Friedl[1], E. Leitner[1], A. Grisold[1], G. Zarfel[1], I. Steinmetz[1]
[1]*Institute of Hygiene, Microbiology and Environmental Medicine, Graz, Austria*

Background: Carbapenem-resistant Enterobacterales (CRE) and carbapenemase-producing Enterobacterales (CPE) pose a global healthcare threat. Rapid, reliable assays are paramount to facilitate targeted and effective therapeutic interventions. Among various diagnostic approaches, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) holds promise for carbapenemase detection, featuring advantages over molecular and phenotypic sensitivity tests. To date, only one assay is IVD labelled and commercially available. This study aims to assess and compare its performance to a novel MALDI-TOF MS-based test.

Materials & Methods: Forty pre-characterized CRE isolates (70 % CPEs) were analysed with the established IVD CE certified MALDI-TOF MS carbapenemase detection assay (MBT STAR-Carba IVD Kit; Bruker Daltonics) on the MALDI Biotyper sirius and the newly developed Carbapenemase Activity Kit for the EXS2600 MALDI-TOF MS system (Zybio), assessing performance and handling.

Results: Valid results were obtained for 93 % and 85 % isolates using the Bruker and the Zybio assay. Sensitivities, specificities, and positive and negative predictive values were 92 %, 91 %, 96 %, and 83 % for the Bruker assay and 96 %, 64 %, 85 %, and 88 % for the Zybio assay. Considerable different assay handling aspects were noted.

Conclusion: Both assays demonstrated high sensitivities, with the Bruker assay yielding fewer false positives, and were found to be suitable for the use in a routine laboratory.

P-DCM-026

Analytical equivalence study of LiquidArray® Gastrointestinal Kit with extraction performed on GenoXtract®

*V. Allerheiligen[1], K. Dempsey[1], M. McCowan[1], J. Green[1]
[1]*Bruker-Hain Lifescience GmbH, Research & Development, Nehren, Germany*

Background: LiquidArray® Gastrointestinal VER 1.0 Kit is a rapid, sensitive, highly multiplexed PCR workflow to detect the most relevant bacterial, viral, parasitic and pathogen associated toxins which cause gastroenteritis and based on next generation LiquidArray® technology. The assay has been fully developed, having completed all design and development phases, including a comprehensive analytical and clinical study at a Public Health Wales clinical laboratory in the UK with extraction performed on GenoXtract® fleXT. An analytical equivalence study was performed using the GenoXtract® for extraction with the NA Extraction kit.

Materials/methods: Human stool samples were extracted from a Stool Stabilization Buffer using the GenoXtract®, an automated sample processor for the extraction of 12 samples per run. The PCR analysis was carried out using a FluoroCycler® XT, a thermal cycler platform specific to LiquidArray technology. The PCR is carried out over two reaction wells to achieve the high-level coverage of all pathogens. 200 samples were assessed in the study, using prospectively collected samples as well as some archived patient specimens, and enrolled under predefined inclusion and exclusion criteria. The test was compared to other CE-IVD multiplex PCR tests to enable coverage of all pathogens detected via LiquidArray Gastrointestinal.

Results: Excellent sensitivity was achieved across both PCR1 and PCR2 targets with all detected targets showing performance >95% when tested at LOD. The overall detection rates between the GenoXtract® and the GenoXtract® fleXT resulted as 97.5 % correct for the 200 clinical samples tested.

Summary: The clinical sensitivity and specificity values reported throughout the study provide evidence of clinical performance when compared to similar technologies. The assay exhibits increased pathogen coverage when compared to some current clinical diagnostics, with the ability to detect and differentiate bacterial, viral and parasitic targets. Excellent useability was demonstrated, with very low rates of invalid and indeterminate results reported. The two-well PCR workflow offers increased throughput in diagnostic capability.

P-DCM-027

A fast and economic protein-microarray based approach for serotyping of Salmonella enterica strains.

E. Müller[1,2], S. D. Braun[1,2]D. Gary[3], K. Frankenfeld[3], S. Monecke[1,2], R. Ehricht[1,2,4]
[1]*Leibniz Institute of Photonic Technology, Member of the research alliance "Leibniz Health Technolo-gies" and the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany*
[2]*InfectoGnostics Research Campus Jena, Jena, Germany*
[3]*INTER-ARRAY by fzmb GmbH, Bad Langensalza, Germany*
[4]*Friedrich Schiller University Jena, Institute of Physical Chemistry, Jena, Germany*

Salmonella enterica, a bacterium causing foodborne illnesses like salmonellosis, is prevalent in Europe and globally. It's found in food, water, and soil, leading to symptoms like diarrhea and fever. Annually, it results in about 95 million cases worldwide, with increasing antibiotic resistance posing a public health challenge. Therefore, it is necessary to detect and serotype *Salmonella* for several reasons. The identification of the serotypes of *Salmonella* isolates is crucial to detect and trace outbreaks and to implement effective control measures. Our work presents a protein-based microarray for the rapid and accurate determination of *Salmonella* serotypes. The microarray carries a set of antibodies that can detect different *Salmonella* O- and H-antigens, allowing for the identification of multiple serotypes, including Typhimurium and Enteritidis, in a single miniaturized assay. The system is fast, economical, accurate, and requires only small sample volumes. Also, it is not required to maintain an extensive collection of sera for the serotyping of *Salmonella* species and can be easily expanded and adapted to new serovars and sera. The scientific state of the art in *Salmonella* serotyping involves the comparison of traditional, molecular, and in silico methods, with a focus on economy, multiplexing, accuracy, rapidity, and adaptability to new serovars and sera. The development of protein-based microarrays, such as the one presented in our work, contributes to the ongoing advancements in this field.

Fig. 1

P-DCM-028

Molecular typing of PVL-positive Staphylococcus aureus isolates from clinical samples

*E. Müller[1,2], S. Monecke[1,2,3], A. Trimborn[4], H. H. Söffing[5], K. Frankenfeld[6], R. Ehricht[1,2,7]
[1]*Leibniz-Institute of Photonic Technology (Leibniz-IPHT), Jena, Germany, member of the Leibniz Center for Photonics in Infection Research (LPI), Optical-molecular diagnostics and system technologies, Jena, Germany*
[2]*InfectoGnostics Research Campus, Centre for Applied Research,*

Jena, Germany
[3]*Institute for Medical Microbiology and Virology, Dresden University Hospital, Dresden, Germany*
[4]*University Mannheim, Institute for Medical Microbiology and Hygiene, Mannheim, Germany*
[5]*Senova Gesellschaft für Biowissenschaft und Technik mbH, Weimar, Germany*
[6]*INTER-ARRAY, Research Center for Medical Technology and Biotechnology (fzmb GmbH), Bad Langensalza, Germany*
[7]*Friedrich Schiller University Jena, Institute of Physical Chemistry, Jena, Germany*

Introduction: Panton-Valentine leukocidin (PVL) is a staphylococcal toxin associated with recurrent skin and soft tissue infections (SSTI) and also necrotizing pneumonia. The aim of the study was to detect PVL in *S. aureus* (SA) isolates from clinical samples and to assign those isolates to clonal complexes and epidemic strains.

Material and methods: The collection of clinical SA isolates and PVL detection using RT-PCR were performed at University Hospital in Mannheim. Positive isolates were then characterized at IPHT Jena using DNA-microarrays that facilitated not only the detection of virulence genes including PVL and resistance markers, but also an assignment to CCs and strains. In addition, they were tested for PVL production using an experimental lateral flow (LF) test.

Results: Out of 556 SA isolates (MSSA and MRSA), 30 yielded PVL-positive PCR results and 27 were finally available for typing. Twelve of them were *mecA*-positive and three harboured SCC*mec*-associated *fusC* (fusidic acid resistance). For two methicillin-susceptible isolates (MSSA), PVL could not be confirmed by array and LF.

MSSA belonged to nine different lineages, with the most common one being CC30 (n=3). MRSA were assigned to seven CCs and 10 distinct strains. Strains CC152-MRSA-IV and CC22-MRSA-IV (PVL+/tst+) were found twice each, while the 8 other strains - including "USA300" - were identified only in single cases.

A concordance of PVL detection by array and LF was observed in 26/27 isolates (96%). One CC1-MRSA isolate yielded repeatedly negative LF results for PVL despite being positive in PCR and by array. Genome sequencing did not reveal significant mutations neither in its PVL genes nor in the agr locus.

Discussion: The MRSA rate among PVL-positive isolates was higher than the MRSA rate in the national average. All PVL-positive MRSA strains identified are known to be endemic in other parts of the world, thus they might be regarded as travel-associated. Providing for its frequency and clinical relevance, the implementation of screening tools for PVL – regardless whether it is PCR-based or a lateral flow assay – in routine diagnostics should be seriously considered.

P-DCM-029
From Shadows to Spotlight: Enhancing Bacterial DNA Detection in Blood Samples through Cutting-Edge Molecular Pre-Amplification
*M. Reinicke[1,2], S. D. Braun[1,2], C. Diezel[1,2], O. Lemuth[2,3], I. Engelmann[2,3], T. Liebe[2,3], R. Ehricht[1,2,4]
[1]*Leibniz Institute of Photonic Technology e.V. Jena (Leibniz-IPHT), Optical-molecular diagnostics and system technologies, Jena, Germany*
[2]*InfectoGnostics Research Campus Jena, Jena, Germany*
[3]*BLINK AG, Jena, Germany*
[4]*Friedrich Schiller University Jena, Institute of Physical Chemistry, Jena, Germany*

Introduction: Detecting target DNA at low concentrations poses a significant challenge in molecular diagnostics. Achieving high sensitivity is crucial for accurate detection, especially in cases of clinically manifest sepsis, where bacterial concentrations in the blood can be extremely low (one bacterial cell/CFU ml blood). The sample matrix, especially blood, adversely affects accuracy and sensitivity due to the presence of residual background DNA. To overcome this problem, we developed a targeted pre-amplification of marker sequences to enhance the diagnostic sensitivity down to a level of single molecules.

Material and methods: For this purpose, a sample preparation of whole blood samples directly followed by a downstream pre-amplification was developed, which amplifies species-specific and resistance markers in a multiplex procedure. A panel of 24 markers was defined. The pre-amplification was performed in a multiplex approach and quantified by qPCR. The method was tested with blood samples that were spiked with several Gram-positive and Gram-negative bacterial pathogens.

Results: When applying pre-amplification techniques, the sensitivity of the pathogen detection in whole blood samples was up to 100 times higher than in non-pre-amplified samples. By applying this method to artificially spiked blood samples, it was possible to demonstrate a sensitivity of 1 colony-forming unit (CFU) per millilitre of blood for *S. aureus* and *E. faecium*. A detection limit of 28 and 383 CFU per ml of blood was achieved for *E. coli* and *K. pneumoniae*, respectively.

Summary: In the present study, we have established a method that bridges the analytical gap between low concentrations of molecular markers and the minimum requirements for molecular testing. If the sensitivity is also confirmed for real clinical blood samples from septic patients, the novel technique can be used for pathogen detection without cultivation, which might help to accelerate diagnostics and, thus, to decrease sepsis mortality rates.

P-DCM-030
A simplified and cost-saving workflow for rapid identification and antimicrobial susceptibility testing of Gram-negative bacteria directly from positive blood cultures utilizing the lytic properties of the BD BACTEC™ anaerobic BC bottles.
J. Träger[1], *J. Esse[1], J. Held[1]
[1]*University Hospital Erlangen, Institute for Microbiology, Erlangen, Germany*

Introduction

Multiple commercial systems for rapid identification (ID) and antimicrobial susceptibility testing (AST) of bacteria directly from positive blood cultures (BC) have been developed recently. However, these systems are associated with considerable costs. In this study, we aimed to use the lytic property of the BD BACTEC™ Lytic/10 Anaerobic/F BC bottles for the simplified and cost-saving isolation of bacterial cells for downstream ID and AST by standard methods.

Methods

During seven months, all anaerobic BCs with Gram-negative rods were included in the study. 1.5 ml of blood were centrifuged for 1 min at 13.000 rpm and washed once with NaCl. The cell pellet obtained was used directly for ID using MALDI-TOF, AST using VITEK® 2 (bioMérieux) and detection of third generation cephalosporin resistance using the colorimetric β-LACTA™ test (Bio-Rad). The results were compared with the standard-of-care (MALDI-TOF and VITEK® 2 from short-term culture).

Results

104 positive anaerobic BCs, accounting for 80.7% of all BCs with Gram-negative rods, were processed. 16 (15.4%) of them were excluded because of a mixed culture (n=11) or the detection of anaerobic bacteria (n=5), leaving 88 BCs for analysis. In total, 14 different bacterial species were analyzed, mostly *Escherichia coli* (58%). ID with MALDI-TOF was correct at the species level in 93% of cases (mean score 2.18; standard deviation 0.19). Within 30 min from BC-positivity, the β-LACTA™ test identified 7 out of 8 isolates with resistance to 3rd generation cephalosporins. Complete VITEK® 2 AST results were available in the majority of cases until 8 p.m.. Most antibiotics showed an essential and categorical agreement above 96%. Few very major errors occurred with ampicillin/sulbactam (n=2) and moxifloxacin (n=2).

Conclusions

The lytic BD BACTEC™ anaerobic BC bottles enable a fast and simple workflow to accelerate ID and AST from positive BCs at minimal costs and hands-on-time.

P-DCM-031

Direct identification of bacteria from positive blood culture bottles by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry - a systematic review and meta-analysis

*J. Forster[1], T. M. Tran[1], V. Rücker[2], G. Gelbrich[2], P. U. Heuschmann[2], C. Schoen[1]

[1]*Julius-Maximilians-University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*

[2]*Julius-Maximilians-Universität, Institute for Clinical Epidemiology and Biometry, Würzburg, Germany*

Introduction

We perform a systematic review and meta-analysis of direct identification protocols of bacterial pathogens from patients with bloodstream infections (BSI) using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF).

Goals

To evaluate protocols in operation for pathogen detection directly from positive blood culture bottles.

Methods

The study was registered at PROSPERO (2022CRD42022293236). Screening and study selection followed recommendation by PRISMA. We defined correct identification rate as CIR(X) = . Meta-analysis was performed using the 'dmetar', 'meta' and 'metafor' packages of the R software. Fixed and random-effects models were used to estimate the pooled CIR of different bacterial groups and species. Upon detection of significant heterogeneity, the random-effects model was considered and influence as well outlier analysis were performed.

Results

We screened 1296 titles, 211 abstracts and 167 full texts. A total of 145 datasets from 93 studies were included. There was a significant heterogeneity in the data sets ($I^2 = 99.2\%$). The most frequent blood culture systems, mass spectrometry manufacturer, separation method and protein extraction method were BD BACTECTM (94/145, 64.8%), Bruker (117/145, 80.7%), centrifugation (119/145; 82.1%), and in-house-protocol including saponin (59/145; 40.1%). The most frequent commercial extraction protocol was the Sepsityper (37/145, 25.6%). Total CIR, CIR of gram-positive bacteria and gram-negative bacteria was 0.72 (95% CI: 0.69-0.75), 0.62 (95% CI: 0.58-0.66) and 0.85 (95% CI: 0.83-0.88), respectively. CIR of *Staphylococcus aureus, Streptococcus pneumoniae, Enterobacterales* and *Pseudomonas* spp. was 0.78 (95% CI: 0.73-0.83), 0.36 (95% CI: 0.29-0.43), 0.90 (95% CI: 0.87-0.92) and 0.86 (95% CI: 0.81-0.9). Significant differences within bacterial groups and detection methods were detected.

Summary

Direct identification protocols can detect a high rate of pathogens of BSI. However, the observed overall CIR suggests that protocols need further improvement to replace the current standard of care.

P-DCM-032

Rapid antimicrobial susceptibility testing using the RamanBioAssay platform

*M. L. Enghardt[1,2,3], R. Grohs[2,3], A. Silge[2,3], U. Glaser[2], O. Ryabchykov[2,3,4], F. Hornung[5], S. Deinhardt-Emmer[5], B. Löffler[5], J. Popp[1,2,3]

[1]*Friedrich Schiller University Jena, Institute of Physical Chemistry, Jena, Germany*

[2]*Leibniz Institute of Photonic Technology, Jena, Germany*

[3]*InfectoGnostics Research Campus Jena, Jena, Germany*

[4]*Biophotonics Diagnostics GmbH, Jena, Germany*

[5]*Jena University Hospital, Institute of Medical Microbiology, Jena, Germany*

Introduction

Antibiotic resistance has become a major public health concern, complicating infectious disease treatment and has severe medical and financial implications. To overcome these problems, there is an urgent need for faster antibiotic susceptibility testing. One approach is to use Raman spectroscopy to rapidly investigate the phenotypic response of pathogens to antibiotic treatment.

Goals

The RamanBioAssay™ (RBA) platform [Kirchhoff *et. al.* 2018] will be employed as a rapid antimicrobial susceptibility test (AST) for clinical isolates of *Escherichia coli* and *Staphylococcus aureus* strains. The information from the

Raman data obtained from both resistant and sensitive strains will be matched with the findings from microdilution tests. The results will focus on the interpretation and extrapolation of Raman-based AST results to enable reliable and growth-independent analysis for potential diagnostic use.

Methods

E. coli and *S. aureus* strains are exposed to appropriate antibiotic concentrations of respectively Ciprofloxacin and Oxacillin as part of the RBA. In a dielectrophoresis-chip, bacteria are collected in a micrometre range for high-quality Raman measurements directly from bacterial suspensions. The readout of phenotypic molecular changes during antibiotic treatment is possible after only 90 min of bacteria-antibiotic interaction, ensuring the entire test is completed in ≤ 3 hours. Finaly, chemometrics translates spectral signals into antibiograms.

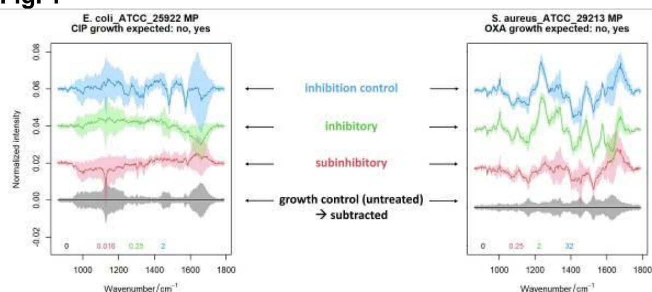
Results

A successful measurement of clinical samples within the RBA is possible. The interaction time of only 90 min between bacteria and antibiotics is enough to detect the antibiotic effects by Raman spectroscopy. The results are consistent with those of the gold standard and current routine diagnostics.

Summary

Common bacteria for bloodstream infections, *E. coli* and *S. aureus*, were successfully treated with appropriate antibiotic concentrations and tested using on-chip Raman spectroscopy. The method enables precise detection of phenotypic susceptibility to antimicrobial agents. The test therefore represents an improvement over both the gold standard and current routine diagnostics.

Fig. 1



Figure| Raman spectra of *E. coli* treated with Ciprofloxacin (left) and *S. aureus* treated with Oxacillin (right) at different concentrations both below and above the MIC, wherein the growth control was subtracted. These spectra showing distinct differences compared to the untreated control. The difference spectra of sufficiently treated (blue and green) and insufficiently treated (red and black) samples contrast, indicating molecular changes induced by the antibiotics within 90 minutes of interaction.

P-DCM-033

Differentiation of a novel, non-core Brucella Isolate from a White`s Tree frog

*C. M. Ufermann[1], D. Hofreuter[1], A. Gadicherla[1,2], R. Oehme[3], F. Cantet[4], S. Köhler[4], S. Al Dahouk[1,5]

[1]*German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany*
[2]*University of Wisconsin, Center for Quantitative Cell Imaging, Madison, WI, United States*
[3]*Ministry of Social Affairs, Health and Integration Baden-Wüttemberg, State Health Department, Stuttgart, Germany*
[4]*University Montpellier, Institut de Recherche en Infectiologie de Montpellier (IRIM), CNRS, Montpellier, France*
[5]*German Environment Agency, Department of Environmental Hygiene, Berlin, Germany*

The genus *Brucella* has gained several species and numerous yet unclassified isolates with atypical phenotypes and genome sequences that differ significantly from the classical, highly pathogenic species such as *B. melitensis*. Despite the increasing number of these novel, atypical *Brucella* spp., major information about their reservoirs, pathogenicity, and zoonotic potential are vague. Moreover, monitoring of atypical *Brucella* remains challenging, since misidentification with environmental *Ochrobactrum* spp. or *B. melitensis* occurs frequently. Microbiologists, clinicians and veterinarians are further confused by the recent inclusion of *Ochrobactrum* into the genus *Brucella*.

Here, we report a case of two diseased exotic frogs presenting with dermal abnormalities. Tissue samples were tested positive by a *Brucella*-specific PCR. We also characterized one isolate by standard microbiological methods, species-specific multiplex PCR and WGS for phylogenetic differentiation. Its pathogenic potential was analyzed in an *in cellulo* infection assay.

The suspected *Brucella* isolate displayed non-fastidious, rapid growth and showed microbiological characteristics similar to novel, atypical *Brucella* strains, such as *B. inopinata* or *Brucella* sp. from African bullfrogs. Differential metabolic phenotyping with a specific set of growth substrates enabled its discrimination from classical *Brucella* as well as *Ochrobactrum*. By genomic and phylogenetic characterization, this atypical *Brucella* isolate was positioned within the novel, non-core clade including various amphibian and human *Brucella* isolates distant from *Ochrobactrum*. Similar to other atypical *Brucella*, this new isolate rapidly replicated in macrophages.

Here we describe another example of amphibian brucellosis, caused by a new member of the non-core *Brucella* clade. In addition, we present a phenotyping approach that facilitates the differentiation of *Ochrobactrum*, atypical and classical *Brucella* by a cost effective and simple microbiological method. Our and previous reports suggest that exotic frogs are reservoirs for potentially pathogenic *Brucella* spp., and therefore might pose an underestimated zoonotic hazard.

P-DCM-034

Analyzing Antibiotic Prescribing Patterns Following Blood Culture Diagnostics in an Emergency Department Setting: A Monocentric Retrospective Study

*M. Misailovski[1,2], N. Srivastava[1], S. Blaschke[3], A. Fuchs[3], H. Kaba[1], M. Kaase[1], A. Dudakova[4], A. Beste[1], M. Alrifai[1], J. Gerlach[1], S. Scheithauer[1], M. H. Schulze[1]

[1]*University Medical Center Göttingen, Department of Infection control and Infection Diseases, Göttingen, Germany*
[2]*University Medical Center Göttingen, Department of Geriatrics, Göttingen, Germany*
[3]*University Medical Center Göttingen, Emergency Department, Göttingen, Germany*
[4]*University Medical Center Göttingen, Institute for Medical Microbiology and Virology, Göttingen, Germany*

Introduction: Blood culture (BC) results serve as a determinant in shaping both diagnostic and therapeutic strategies for the effective management of (systemic bloodstream) infections. Despite its crucial role, there exists a limited body of literature scrutinizing the BC diagnostics

and antibiotic prescriptions, particularly within the Emergency Departments (ED).

Goals: In this study, we explore the landscape of BC diagnostics and antibiotic prescribing in the ED.

Materials & Methods: Adult patients admitted to the ED at the University Medical Center Goettingen between June and July 2023 and with at least one pair of BCs within the first 48 hours were included. The outcome was defined as blood culture positivity (BCP) in at least one BC.

Results: In total 413 patients were included. Approximately 80% of patients had ≥2 BCs collected and 20% had only one BC. The BCP rate was 21% (n=88). The three most common pathogens were *Escherichia coli* (20%), *Staphylococcus aureus* (7%) and *Klebsiella oxytoca* (7%). The most prevalent potential contaminants were *Staphylococcus hominis*, *Staphylococcus epidermidis,* and *Cutibacterium acnes* (35%). Overall, 69% of patients (n=286) in whom BC was made underwent antibiotic treatment in the ED (28% received ≥2 antibiotics). Of these, 25% had a positive BC (n=74). The top 5 antibiotics prescribed were: piperacillin/tazobactam, ceftriaxone, ampicillin/sulbactam, azithromycin, and metronidazole.

Summary: The results of our study aim to contribute valuable insights into optimizing BC diagnostics and antibiotic stewardship protocols, enhancing rational prescriptions and antibiotic interventions for bloodstream infections in the EDs.

P-DCM-035

Performance characteristics of the RADI COVID-19 IgG rapid test for SARS-CoV-2 Anti-S IgG detection compared to an ELISA assay

*H. Nyawale[1,2], I. Wagenhäuser[3,4], J. Reusch[3,4], J. Mees[3,5], S. Masoud[6], D. Kamori[6], N. Moremi[7], S. Mshana[2], M. Krone[3,1], M. Mirambo[2]

[1]*Univesity of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*

[2]*Catholic University of Health and Allied Sciences, Department of Microbiology and Immunology, Weill Bugando School of Medicine, Mwanza, Tanzania, United Republic Of*

[3]*University Hospital Würzburg, Infection Control and Antimicrobial Stewardship Unit, Würzburg, Germany*

[4]*University Hospital Würzburg, Department of Internal Medicine I, Würzburg, Germany*

[5]*University Hospital Würzburg, Pediatric Rheumatology/Special Immunology, Würzburg, Germany*

[6]*Muhimbili University of Health and Allied Sciences, Department of Microbiology and Immunology, Dar es Salaam, Tanzania, United Republic Of*

[7]*National Public Health Laboratory, Dar es Salaam, Tanzania, United Republic Of*

Question: The infection with severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) and the development of coronavirus disease 2019 (COVID-19) represents a global health care challenge. World Health Organization (WHO) insists on the use of diagnostic tools which are affordable, and easy to perform with high sensitivity and specificity. WHO suggests the use of SARS-CoV-2 antibody-rapid diagnostic tests in low and middle income countries (LMIC) for sero-epidemiological studies that meet the least performance prerequisites of ≥ 80% sensitivity and ≥ 97% specificity.

We, therefore, validated a SARS-CoV-2 antibody rapid detection test against a reference standard for antibody diagnostics, the Enzyme-linked Immunosorbent Assay (ELISA) performance characteristics

Methods: We collected blood specimens from consented participants during regional wide serological survey in three districts in Mwanza; Ukerewe, and Magu/Tanzania. The blood samples were tested directly after sample asservation for SARS-CoV-2 IgG on site using RADI COVID-19 IgG rapid test kits (KH Medics Limited). The results were compared to aquantitative ELISA (SERION ELISA *agile* SARS-CoV-2 IgG) performed from frozen samples. Data management and analysis was done using STATA version 12.

Results: A total of 710 samples were evaluated for performance characteristics of the test. Out of the 368 samples which were positive in the RADI COVID-19 IgG rapid test kits, 312 were concordant positive for both RADI rapid test and Serion IgG ELISA, thus, giving a sensitivity of 84.7% (95 CI, 81.2-88.3%). Out of 342 samples which tested negative for the RADI rapid test, 333 were concordant negative, giving a specificity of 97.4%. The positive predictive values and negative predictive values of the tests were 97.2% and 85.6% respectively.

Previous history of fever was significantly associated with false positive RADI COVID-19 IgG test results (p=0.012).

Conclusions: The performance characteristics of the RADI COVID-19 IgG rapid test kits compared to the Serion IgG ELISA are within recommended WHO performance characteristics which can still be used for serological surveys in African settings.

P-DCM-036

Characterisation of carbapenem-resistance in carbapenemase-negative Klebsiella pneumoniae for development of an antibody-based rapid detection kit

*L. Maus[1,2], M. Gonzalez Rodriguez[1,2], S. Mertins[1,2], M. Martoccia[1,2], P. G. Higgins[1,2], M. Krönke[1,2,3], A. Klimka[1,2]

[1]*University Hospital Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*

[2]*German Center for Infection Research, Bonn, Germany*

[3]*Cologne Cluster of Excellence on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Köln, Germany*

Background

Carbapenem-resistant *Klebsiella pneumoniae* (Kp) is of serious concern in hospitals. Carbapenem-resistance is mediated by carbapenemases, however, for carbapenemase-negative carbapenem-resistant Kp (CNCRKp), a combination of ESBL and porin loss is an alternative resistance mechanism. Detection of carbapenemases by lateral flow assays is well-established, yet for CNCRKp no such test exists. We therefore sought to develop a rapid diagnostic test to identify CNCRKp.

Materials

Thirty-two CNCRKp clinical isolates from Germany were sequenced (MiSeq) and resistance to ertapenem, meropenem and imipenem was confirmed by agar dilution. For gene expression analysis, total RNA was extracted and qRT-PCR was performed with primers targeting ESBLs (SHV, CTX-M, TEM, DHA, OXA-1 and OXA-9) and porins (OmpK35 and OmpK36).

Results

All isolates were resistant to ertapenem, 25 (78 %) were resistant to meropenem and 17 (53 %) to imipenem according to EUCAST guidelines. Sequence analysis revealed deleterious mutations in the *ompK*35 and *ompK*36 genes in 14 and 26 isolates, respectively. All remaining isolates with intact gene sequences showed decreased OmpK35 and OmpK36 expression on mRNA level compared to the reference carbapenem-susceptible strain ATCC13883. Porin loss/reduced expression combined with SHV expression was associated only with ertapenem resistance. qRT-PCR data demonstrate that isolates expressing either DHA or an increased expression of CTX-M, OXA-1 or OXA-9 were resistant to all tested carbapenems. ESBL and porin genes were cloned into *E. coli* to retrieve recombinant proteins used for the generation of monoclonal antibodies (mAbs) by hybridoma technology. Expression of endogenous ESBLs and porins in lysates of Kp clinical isolates was detected in Western blot using our antigen-specific mAbs.

Conclusion

These data support the hypothesis that porin loss (deleted/mutated gene) or decreased expression in combination with overexpression of ESBLs is associated with carbapenem resistance in CNCRKp. Our results have direct impact on the combination of ESBL-/porin-specific mAbs in an immunochromatographic lateral flow assay to rapidly detect CNCRKp.

P-DCM-037
Assessment of three MALDI-TOF MS Systems for High-Throughput Microbial Identification in the Setting of Routine Diagnostics
*I. Klugherz[1], H. Greimel[1], S. Friedl[1], D. Siebenhofer[1], I. Steinmetz[1], K. Dichtl[1]
[1]*Medical University Graz, Graz, Austria*

Background: In clinical microbiology, MALDI-TOF MS based identification of cultivated microorganisms is an integral part of routine diagnostics. The selection of a system for routine laboratory use should consider factors beyond result accuracy, such as the time to (valid) results, hand-on time, and consumable costs. Literature on these aspects is limited, and to our knowledge there is no such study comparing three devices. Our study aims to compare the suitability of three MALDI-TOF systems, namely bioMérieux"s MS Prime, Bruker"s Biotyper sirius and Zybio"s EXS2600, for use in a high-throughput setting in a diagnostic laboratory.

Methods: During the study period, all isolates that were identified using MALDI-TOF in routine diagnostics were prospectively collected and measured in parallel on the three devices. All days of the week are represented twice. Times for sample preparation and analysis were documented. Samples without valid results were subjected to repeated testing. Validity and agreement between all instruments were assessed.

Results: A total of 2,433 samples was tested. The initial runs yielded valid results for 92 % (Biotyper sirius), 88 % (EXS2600) and 86 % (MS Prime) of the samples. In the repeat measurements carried out with formic acid, valid results were obtained in 49 %, 59 % and 38 % respectively. Mean measuring times per sample varied largely between the test systems (4 – 30 seconds). Average hands-on time varied between 22 and 24 seconds per sample. For 1,862 samples all three systems yielded identical species results. 14 samples were measured as "no ID" by all devices. For 114 samples no overlaps of results at species level were observed for any of the three devices in the initial runs. Of those 58 displayed an overlap at genus level for at least two devices and 13 for all three.

Conclusion: This study provides the first comprehensive overview comparing performance and handling of these three MALDI-TOF systems in a high-throughput setting as it is encountered in routine microbiology laboratories. Depending on the laboratory's specific needs, the selection of a device may prioritize rapid measurement time or cost-effective consumables.

P-DCM-038
Efficacy of CorA Against S. aureus and Clinical Strains of CNS
*J. Karacic[1], K. Pfarr[1], A. Schiefer[1], T. Schneider[2], M. Grosse[3], M. Stadler[3], A. Hoerauf[1], G. Bierbaum[1]
[1]*University Hospital Bonn, Institute of Medical Microbiology, Immunology and Parasitology, Bonn, Germany*
[2]*University of Bonn, Institute for Pharmaceutical Biology, Bonn, Germany*
[3]*Department of Microbial Drugs, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany., Brunswick, Germany*

Corallopyronin A (CorA) is an antibiotic produced by C*orallococcus coralloides*. CorA effectively targets bacterial RNA polymerase and has shown activity against Gram-positive bacteria, including MRSA. Since CorA has a different mode of action from the rifamycin class, it is effective against rifampicin-resistant *S. aureus*. Because *S. aureus* is a major contributor to periprosthetic joint infections (~60%) (PJIs), CorA has the potential to be a promising therapeutic option for treating these infections.

The study aims to further understand the antimicrobial potential of CorA against *S. aureus* and coagulase-negative staphylococci (CNS), including strains from prosthesis infections and stable Small Colony Variants (SCV). The minimum inhibitory concentrations (MICs) of CorA were determined for 45 *S. aureus* and 15 coagulase-negative staphylococci (CNS), showing promising results with MICs ranging from 0.125 mg/l to 1 mg/l. In addition, time-kill curves in the presence of 4 x MIC performed with 5 different *S. aureus* strains showed a significant decrease of 1.5-3 Log10 cfu mL-1 of viable cells within 25h. Minimum bactericidal concentration (MBC) tests were conducted for 29 strains *S. aureus* by checking the survival rates in the MIC determination. MBC results demonstrated that CorA was bactericidal against 80% of tested CNS strains and 37% *S. aureus* strains. The results of this study provide robust evidence of CorA efficiency against *S. aureus* lab strains, clinical isolates, SCVs and CNS.

P-DCM-039
Ability of two rapid phenotypic AST methods to detect carbapenem-resistance in Gram-negative bacteria directly from positive blood cultures
*N. Degel-Broßmann[1], B. Berinson[1], L. Reibenspies[2], J. Pärssinen[2], A. Åman[2], E. Davies[2], J. Fernberg[2], J. Torpnér[2], H. Öhrn[2], C. Johansson[2], J. Ångström[2], M. Christner[1], H. Seifert[3], P. G. Higgins[3], M. Aepfelbacher[1], C. Malmberg[2,4], H. Rohde[1]
[1]*University Hospital Hamburg-Eppendorf, Institute for Medical Microbiology, Virology and Hygiene, Hamburg, Germany*
[2]*Gradientech AB, Uppsala, Sweden*

[3]Faculty of Medicine and University Hospital Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany
[4]Uppsala University, Departement of Medical Sciences, Uppsala, Sweden

Background: Bacteremia and sepsis are life-threatening diseases associated with high mortality. Ineffectiveness of empiric treatments underscore the urgent need for accurate and timely detection of resistant organisms from blood cultures. The EUCAST Rapid antimicrobial susceptibility testing (RAST) provides phenotypic susceptibility data from positive blood cultures in 4-8 h. Recently, the QuickMIC system was launched, which provides MICs in 2-4 h, while reducing hands-on time. While rapid AST may improve patient management, clinical usefulness is determined by their sensitivity to detect carbapenem-resistant organisms (CRO). This study evaluates the ability of RAST and QuickMIC to detect carbapenem-resistance in Gram-negatives directly from positive blood cultures using Broth microdilution (BMD) as reference.

Methods: 101 carbapenem-resistant or carbapenemase-producing Gram-negative isolates, (E. coli n=24, K. pneumoniae n=23, A. baumannii-complex n=29, P. aeruginosa n=25), were spiked into blood culture bottles and underwent testing using QuickMIC and EUCAST RAST and BMD as reference method. Genomes of all isolates were sequenced and analysed.

Results: QuickMIC compared to BMD resulted in an overall categorical agreement (CA) of 87.7 % for meropenem and third-generation cephalosporins. RAST CA ranged between 84.2-86.3% with 4, 6, 8 and 20 h readout (Figure1). The CA for meropenem alone was 77.3% with QuickMIC, and ranged from 59.4-71.6% with RAST at 4, 6, 8 and 20 h. QuickMIC displays higher major discrepancies (MD) for ceftazidime-avibactam compared to RAST at any assay endpoint, whereas RAST hat significantly higher very major discrepancies (VMD) for meropenem. The VMD for meropenem in RAST and QuickMIC was mostly associated with *blaOXA* and *blaNDM* gene groups.

Conclusions: QuickMIC and RAST perform similarly, but QuickMIC has a lower rate of VMD compared to RAST and provides actionable results at 2-4 h while best RAST performance is at 8 h. Genotype influences the rapid AST result quality, especially in meropenem and other beta-lactam antibiotics, which is important to think of when implementing rapid AST methods in clinical practice.

Fig. 1

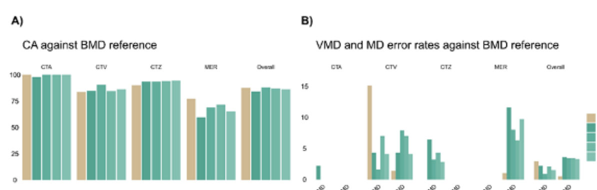


Figure 1 | A) Categorical agreement for QuickMIC and EUCAST RAST at 4, 6, 8 and 20h readout for third-generation cephalosporins cefotaxime (CTA), ceftazidim-avibactam (CTV), ceftazidime (CTZ) and the carbapenem meropenem, as well as overall CA. **B)** Major Discrepancy (MD) and Very Major Discrepancy (VMD) error rates for the methods.

P-DCM-040
Diagnostic performance of two lateral flow assays for measurement of mannan antigen and anti-mannan IgG antibodies in patients with candidemia: a retrospective case-control study

J. Träger[1], S. Mihai[2], N. Rakova[2], J. Esse[1], *J. Held[1]
[1]*University Hospital Erlangen, Institute for Clinical Microbiology, Immunology and Hygiene erlanegn, Erlangen, Germany*
[2]*University Hospital Erlangen, Central laboratory, Erlangen, Germany*

Introduction: Candidemia is the forth most common blood stream infection on intensive care units and is associated with a high mortality. Rapid diagnosis is essential for timely initiation of antifungal therapy. We aimed to analyse the performance of two lateral flow assays (LFA) for the measurement of mannan antigen and anti-mannan antibodies in sera of patients with candidemia.

Methods: Serum samples of 250 patients with blood culture-proven candidemia and 200 controls were tested with the TECO® Fast *Candida* mannan antigen LFA and the TECO® Fast *Candida* IgG antibody LFA, respectively. Both assays have a turn-around-time of approximately 40 min. The control sera were taken from hospitalized patients with negative blood cultures (n=140) and bacteremia (n=60), respectively.

Results: The mean age of the study patients was 64.6 years and 63.5% were male. Candidemia was caused by *Candida* (C.) *albicans* (46.6%), *C. glabrata* (27.7%), *C. parapsilosis* complex (9.9%), *C. tropicalis* (5.1%), *C. krusei* (4.7%), *C. lusitaniae* (1.6%) and other *Candida* spp (1.2%). A mixed candidemia occurred in 3.2% of patients. The sensitivity, specificity and area under the ROC-curve (AUC) was 46.2% (95%-CI: 40.0-52.6), 87.1% (95%-CI: 81.6-91.4) and 0.667 (95%-CI: 0.621-0.710) for TECO mannan antigen; 45.1% (95%-CI: 38.9-51.4), 75.6% (95%-CI: 69.1-81.4) and 0.604 (95%-CI:0.557-0.649) for TECO anti-mannan-antibodies and 65.9% (95%-CI: 59.7-71.7), 65.7% (95%-CI: 59.7-71.7) and 0.658 (95%-CI:0.612-0.701) for the combination of both assays.

Conclusion: The diagnostic performance of the TECO® Fast Candida Ag & IgG LFAs is comparable to the widely used Platelia *Candida* Ag and Ab Plus enzyme immunoassays (EIA). However, the LFA format is much more practical, especially for small sample numbers, and the time-to-result compared to the EIA is significantly reduced.

Epidemiology and Antimicrobial Resistance of Zoonotic Pathogens

P-EAZP-001
Prevalence, phenotypic and genotypic characteristics of Staphylococcus aureus isolated from small ruminants with mastitis in Sardinia, Italy

*A. Moawad[1], S. Dore[2], H. Tomaso[3]
[1]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, IBIZ, Jena, Germany*
[2]*National Reference Center for Sheep and Goat Mastitis-Experimental Zooprophylactic Institute of Sardinia, Sassari, Italy, Sassari, Italy*
[3]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, IBIZ, Jena, Germany*

Background: Mastitis is a common disease of small ruminants causing major economic losses due to reduced yield and poor quality of milk worldwide. The problem is even

more relevant in the rural areas of the Mediterranean region, where almost two thirds of the global sheep and a quarter of the global goat milk are produced. The current investigation aimed to gain insight into the genetic diversity, antimicrobial resistance profiles and virulence associated factors of *Staphylococcus* (*S.*) *aureus* isolated from animals with clinical mastitis in dairy farms in Sardinia, Italy.

Methods: Thirty-four *Staphylococcus aureus* isolates collected from clinical mastitis cases from sheep and goat from 26 dairy farms were characterized using whole genome sequencing and antibiotic resistance testing.

Results: All isolates proved to be phenotypically methicillin sensitive *S. aureus* (MSSA). Only few isolates showed resistance against tetracycline (12.5%) and erythromycin (3.12%). The isolates were assigned to seven different sequence types: ST133 ($n = 15$), ST700 ($n = 9$) were the main two sequence types. Resistance genes *bla*Z, *dfr*G and *mec*A were found in one isolate, each (3.12%). All tetracycline resistant isolates harbored either *tet*M or *tet*K.

Conclusion: ST133 had the highest potential to cause disease and was found frequently in sheep mastitis cases. Seven different sequence types were identified among a limited number of isolates. The circulation of some clusters in different regions shows the persistence of cluster-associated infection. Although all isolates were from clinical mastitis cases, they have showed almost no resistances to the tested antimicrobials. This indicates the highly developed hygienic measures in the surveyed farms, and the routine antimicrobial resistance testing in combination with prudent use of antimicrobials to avoid the emergence of resistance. This is in line with the current agricultural regulations, i.e., implementation of EU legislation, increases the farmers´ income through avoiding losses due to reduced milk yield and decreases the veterinary costs. Most importantly, the burden of disease is reduced and animal welfare improved.

P-EAZP-002

Novel aminoglycoside resistance mechanism in Campylobacter coli from turkey caecal content isolated in Germany

*M. Zarske[1], C. Werckenthin[2], J. Golz[1], K. Stingl[1]
[1]*German Federal Institute for Risk Assessment, Biologische Sicherheit, Berlin, Germany*
[2]*Lower Saxony State Office for Consumer Protection and Food Safety, Food and Veterinary Institute, Oldenburg, Germany*

Introduction:

Pheno- and genotypic analysis of thermotolerant *Campylobacter* spp. identified a *C. coli* isolate from turkey caecal content to exhibit resistance to gentamicin (GEN) without harbouring known resistance determinants.

Goals:

The study aimed to identify the molecular determinant conferring resistance to GEN in the *C. coli* isolate. Also, cross-resistance to other aminoglycosides, transferability of the resistance among *Campylobacter* spp. and its persistence upon *in vitro* passaging were characterized.

Materials & Methods:

Antimicrobial susceptibility was tested using microdilution. Whole genome sequencing (WGS) was performed with Illumina NextSeq and Oxford Nanopore Technology. Genomic DNA (gDNA) or a PCR fragment from the resistant donor, harbouring the 16S rRNA gene, was transferred from the resistant isolate to sensitive recipient *C.* spp. isolates using natural transformation. Transformants were selected on 8-16 mg/L tobramycin (TOB) and reanalysed by WGS. Sanger sequencing was performed to assess transitions in 16S rRNA copies in the transformants upon passaging.

Results:

C. coli BfR-CA-15687 had been isolated during the zoonosis monitoring from turkey caecal content in 2018. Antimicrobial susceptibility tests showed resistance to apramycin (APR), GEN, kanamycin (KAN) and TOB, while the isolate was sensitive to streptomycin (STR). The resistance was naturally transformable to sensitive recipient *C. jejuni* and *C. coli* using BfR-CA-15687 gDNA. The transformation rate was ~2 log10 lower compared to the control *rpsL*-A128G point mutation, conferring STR resistance. SNP analysis of WGS data revealed a novel A1387G mutation in the 16S rRNA gene associated with APR^R-GEN^R-KAN^R-TOB^R resistance. Transformation of this mutation within a 16S rRNA PCR fragment showed causal relationship between 16S rRNA_A1387G and resistance. Sanger sequencing revealed A1387G transition in all three or less copies of the 16S rRNA genes in transformants, impacting resistance stability.

Summary:

We unveiled a novel, rare APR^R-GEN^R-KAN^R-TOB^R resistance mechanism in *C. coli*, mediated by the point mutation 16S rRNA_ A1387G, which was transferable via natural transformation.

P-EAZP-003

INNOTARGETS: An EU funded doctoral training school dedicated to innovative approaches to find novel antimicrobials

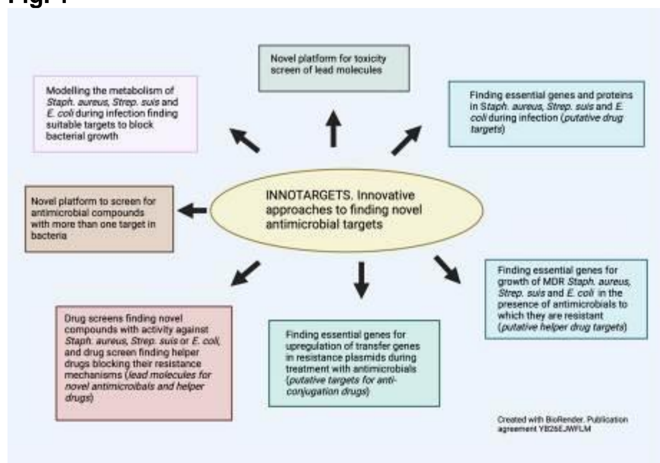
*J. E. Olsen[1], L. E. Thomsen[1]
[1]*University of Copenhagen, Veterinary and Animal Sciences, Frederiksberg, Denmark*

INNOTARGETS (https://innotargets.ku.dk/about/) is a Marie Skłodowska-Curie training network, in which five universities in Europe (Copenhagen, DK; Wageningen, NL; Barcelona, ES; Sassari, IT and Duesseldorf, GE) have joined forces with two research organizations (CRESA, ES and Porto Conte Ricerche, IT) and two antimicrobial discovery companies (Naicons, IT and ABAC, ES) to train 12 doctoral students in aspects related to identification of novel antimicrobials. Topics span from basic research into molecular host pathogen interaction, in situ modelling of the metabolism of pathogenic bacteria during infection, understanding responses in antimicrobial resistant bacteria to treatment with the antimicrobials to which they are resistant, understanding mechanisms behind increases in transfer frequency of some resistance-plasmids due to treatment with antimicrobials, novel screening platforms for antimicrobials and antimicrobial helper-drugs, and faster ways to perform toxicity screens of drug-candidates (Figure). Doctoral students in the network are trained with a unique combination of microbiology and mathematical modelling

skills, as well as soft skills related to science communication, data management, drug development, IPR-rights, and general industry perspectives on research – making them putative key persons in society"s fight to keep up with the everlasting resistance development in pathogenic bacteria.

This project has received funding from the European Union"s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 956154

Fig. 1



P-EAZP-004

Unravelling the mechanisms of albicidin resistance in Acinetobacter baumannii IMT51508

*S. Khan[1], J. Kupke[1], R. Süßmuth[1], M. Fulde[1]
[1]*Microbiology and Epizootics, Veternary medicin, Berlin, Germany*

Introduction:

Antimicrobial resistance has emerged as a major clinical and public health challenge worldwide. According to one recent report, antimicrobial resistance caused 5 million human deaths in 2019 and estimated up to 10 million deaths by 2050 [1].

Albicidin is a promising antibacterial peptide that inhibits the bacterial DNA gyrase's activity [2]. It is extensively studied in *Salmonella*Typhimurium and *Escherichia coli*. The reported resistance mechanisms included degradation by the endopeptidase AlbD, and binding of albicidin through MerR-like transcriptional regulator AlbA [3]. The goal of our study is to identify the mechanism of albicidin resistance to ESKAPE pathogens that are leading cause of nosocomial infections. The study is particularly focused to understand the resistance mechanism in a nosocomial pathogen *Acinetobacter baumannii* IMT51508.

Methodology:

The study is designed to measure the Minimum Inhibitory Concentration (MIC) of albicidin in corresponding bacterium, leading to laboratory evolution and Next generation sequencing (NGS) of evolved eight independent mutants. We also measured the cross-resistance of bacteria to other antibiotics and effect of antimicrobial on bacterial growth through resistogram and time kill assay respectively.

Results:

The experiments deduced the MIC of IMT51508 0.06250 µg/mL followed by evolution of bacteria to 64xMIC (4mg/mL) of albicidin. However, considerate genetic analysis demonstrated that eight independent mutants have developed the resistance in DUF445 domain containing protein (*YjiN*) and additionally, three out of eight mutants have also mutated the DNA gyrase subunit A (*gyrA*). However, mutagenesis experiments are in process to investigate the explicit mechanism of resistance. Interestingly, the resistogram analysis predicted the elevated level of resistance to ciprpfloxacin in all independently evolved mutants.

Conclusion:

In conclusion, the perspective study has potential to understand the diversified strategies of bacterial resistance mechanisms to a potential peptide antibiotic. The better understanding will ultimately lead to design specified and precise resistance coping approach.

P-EAZP-005

Antimicrobial resistances and microbiome of red deer and sympatric cattle

*N. Lechleiter[1], J. Wedemeyer[1], A. Schütz[1], J. Sehl-Ewert[1], T. Homeier-Bachmann[1]
[1]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Institute of Epidemiology, Greifswald, Germany*

When habitats of wild animals and livestock intersect, a possibility for the transmission of microbiota and pathogens is created. This can be the case when cows are kept on pastures close to natural areas for reasons of animal welfare or landscape conservation. Since red deer (*Cervus elaphus*) originally are inhabitors of a structured open landscape, pastures of cows are occasionally used for grazing. Even if the contact arising from this is indirect, faecal-oral transmission routes can develop, that have so far been passed over by most research.

Faecal samples from sympatric red deer and cattle will be analysed through shotgun metagenomics to investigate the resistome and the microbiome, as well as potential spillover between the species. In addition to the sequencing, swabs of the sampling material will be cultivated on selective media to isolate ESBL *E. coli*, if present. The samples are taken from the colon of slaughtered cows and hunted red deer. In addition, we will compare the results with those obtained from fresh droppings to assess whether a non-invasive sampling strategy could be used. Since some bacteria are more prone to carry specific resistance genes, the resistome and microbiome of the investigated animals will be tested for correlations.

One of the 70 red deer investigated so far was positive for ESBL *E. coli* and an isolate of the strain was obtained. Due to the informative character of the microbiome composition, some deductions based on factors like the Firmicutes-to-Bacteroidetes ratio, bacterial diversity and prevalence of pathogens can be made as to the general state of health of the animal. The use of faecal metagenomics is expected to allow for the non-invasive monitoring and investigation of antimicrobial resistances and gastrointestinal microbiome in sympatric animals.

P-EAZP-006

The effect of different antimicrobial peptides on biofilm formation of Klebsiella pneumoniae in vitro

*S. Hanstein[1], T. Grochow[2], S. Fietz[2], M. Mötzing[3], R. Hoffmann[3], C. G. Baums[1], S. Kähl[1]
[1]*Leipzig University, Institute of Bacteriology and Mycology, Faculty of Veterinary Medicine, Leipzig, Germany*
[2]*Leipzig University, Institute of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Leipzig, Germany*
[3]*Leipzig University, Bioanalytics, Centre for Biotechnology and Biomedicine, Leipzig, Germany*

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is an important opportunistic pathogen in humans and animals that causes pneumonia and urinary tract infections and is capable of biofilm formation. Antimicrobial Peptides (AMPs) are small cationic amphipatic peptides and considered to be a promising alternative for the development of new therapeutic options.The aim of this study is to investigate the effect of different AMPs on the biofilm formation of *K. pneumoniae.*

Materials & Methods

First, the ability of biofilm formation *in vitro* of various human and veterinary strains of *K. pneumoniae* was investigated using a crystal violet assay. The formed biofilms and the kinetics of biofilm formation were visualized using scanning electron microscopy. In the next step, the effect of eight AMPs on biofilm formation of three different strains of *K. pneumoniae* was analyzed using a serial dilution test followed by a crystal violet assay.

The viability of the formed biofilms was investigated using a resazurin assay. Further the effect of AMPs on mature biofilms was analyzed using the crystal violet assay and scanning electron microscopy.

Results

Biofilm formation of three *K. pneumoniae* strains was confirmed *in vitro* in accordance with a calculated cut-off value, which seperates biofilm formers from non-biofilm formers.

Scanning electron micrographs showed that *K. pneumoniae* is forming a pronounced 3D structure of bacteria adhering to the surface in contrast to the negative control *Muribacter muris*.

The AMPs human β-defensin 3 and LL-37 showed the greatest effect with a reduction of biofilm formation by more than 75% while planctonic growth was not affected under these conditions. A reduction of biofilm formation by more than 50% for only one of the three tested strains was demonstrated for Onc72 and Onc112. No reduction in biofilm mass was shown for Api137.

Summary

Distinct AMPs such as human β-defensin 3 and LL-37 reduce biofilm formation of *K. pneumoniae in vitro*, while other AMPs with a described bactericidal effect against planctonic klebsiellae, such as Api137, do not suppress biofilm formation under the chosen experimental conditions.

P-EAZP-007

Antibiotic Resistance in the Equine Microbiome

*J. Wedemeyer[1], N. Lechleiter[1], A. Schütz[1], T. Homeier-Bachmann[1]
[1]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Institute of Epidemiology, Greifswald, Germany*

Antimicrobial resistance (AMR) is a critical challenge in both human and veterinary medicine. The microbiome of animals such as horses can act as a reservoir for genes conferring resistance. Primarily kept as companion animals in close contact to humans, horses are an essential part of the One Health approach to targeting antibiotic resistance. However, the microbiome and resistome of horses remain understudied.

The goal of this study was therefore to gain further insight into the microbiome and resistome of horses. Twelve fecal samples were obtained from healthy adult horses in six different locations in Western Pomerania. Shotgun metagenomic sequencing was performed to analyze antimicrobial resistance genes and to gain insight into the microbiome composition. Additionally, ESBL*E. coli* isolates could be obtained from two fecal samples and were further analyzed by antimicrobial susceptibility testing and whole genome sequencing.

In the metagenomic datasets genes conferring resistance to the antimicrobial class of Macrolides, Lincosamides and Streptogramines (MLS) could be identified in all samples. Next to MLS the highest relative abundance occurred in the classes of Tetracyclines, and Sulfonamides. Overall, the normalized abundance of AMR gene counts was 0.0041 on average. The ESBL*E. coli* isolates carried a blaCTX-M-15 gene and displayed phenotypical resistance against Gentamicin and Trimethoprim in addition to Cephalosporins. The predominant phyla in the microbiome of all samples were Pseudomonadota, Bacteroidota, Actinomycetota, and Bacillota.

These findings provide valuable insights into the burden of AMR genes in the microbiome of horses in the Western Pomeranian region, which was generally lower than in farm animals like cattle in the same area. It may be useful to conduct further studies on horses in the future, ideally involving different species on the premises of horse stables and their owners, to identify possible transmission routes in this environment. This study also offers a unique opportunity to compare and evaluate the respective advantages of metagenomic and cultural approaches in studying the resistome and microbiome of animals.

P-EAZP-008

Extended-Spectrum β-Lactamase-producing Escherichia coli and Methicillin-resistant Staphylococcus aureus in Laying hen and broiler farm.

*J. Junker[1], T. Homeier-Bachmann[1]
[1]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Institute of Epidemiology, Greifswald, Germany*

One of the biggest global problem is the increasing amount of antimicrobial resistances (AMR) leading to challenges in treatment of bacterial infections. The AMR problem is not limited to human medicine, but is also present in livestock. For the monitoring of ESBL-E. coli and MRSA in two different poultry farms we are conducting a longitudinal study that started December 2023. These two pathogens are characterized by a high zoonotic potential. With this study we

want to gain insights into the on-farm dynamics of ESBL E. coli and MRSA in poultry farms. To achieve this, animals are sampled weekly (broiler farm) and every two weeks (laying hen farm) and in addition farm related data are collected monthly. Fecal samples are then examined for the presence of ESBL E. coli and MRSA using chromogenic media. Obtained isolates are subjected to antibiotic susceptibility testing using a VITEK 2 apparatus. Additionally, the isolates will be whole genome sequenced and analyzed.

So far, no MRSA were detected. ESBL-E. coli was present on the broiler farm, only sporadically at first, two weeks after stabling in all samples. In the laying hen farm, ESBL-E. coli was only detected sporadically from the 62nd week of life (two months after start of sampling), both in floor-and free-range farming. Additionally, to the investigation of the further samples, we evaluate the information regarding potential risk factors for the prevalence. The Study will take a total of two years.

Environmental Microbiology & Processes

P-EMP-001

Oxygen respiration and polysaccharide degradation by a sulfate-reducing Acidobacterium

*S. Dyksma[1], M. Pester[1]
[1]*Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Microorganisms, Brunswick, Germany*

Microorganisms can derive energy from dissimilatory sulfate reduction, a process that drives the biogeochemical sulfur cycle and is tightly linked to carbon, nitrogen and metal cycling. Recent metagenomic surveys greatly expanded the diversity of microorganisms that possess the genomic potential for sulfate reduction, highlighting our incomplete understanding of this functional group. In the wake of these studies, an energy metabolism based on the dissimilation of sulfur compounds was proposed for members of the globally distributed and abundant phylum *Acidobacteriota*. However, fundamental aspects of their ecophysiology are still unknown.

To disentangle the physiology of sulfur compound-dissimilating *Acidobacteriota*, we followed sulfur-cycling in a long-term continuous culture that was consecutively exposed to oxic and anoxic conditions over a period of more than 200 days. Genome-centric metatranscriptomics embedded into controlled bioreactor operation revealed the unique metabolic flexibility of a representative acidobacterium to switch from sulfate reduction under anoxic conditions to aerobic respiration when oxygen was available as electron acceptor, providing experimental evidence that facultatively anaerobic sulfate-reducing bacteria exist within the *Acidobacteriota.* This versatile acidobacterium utilized pectin polysaccharides during both sulfate reduction and aerobic respiration. The combination of facultative anaerobiosis and polysaccharide degradation suggested an unprecedented metabolic versatility among sulfate reducers.

These results break three central dogmas in microbiology: sulfate reduction and aerobic respiration are not mutually exclusive in the same organism, sulfate reducers can mineralize organic polymers, and the anaerobic mineralization of complex organic matter is not necessarily a multi-step process involving different microbial guilds but can be accomplished by one microorganism.

P-EMP-002

Investigating the diversity of viruses infecting Nitrospira across different ecosystems

*J. Starke[1], C. Moraru[1], T. L. Stach[1], S. Eßer[1], K. Sures[1], J. Plewka[1], T. L. V. Bornemann[1], A. R. Soares[1], A. J. Probst[1]
[1]*University of Duisburg-Essen, Chemistry, Essen, Germany*

Nitrogen serves as a vital element in all living organisms and acts as the primary nutrient that restricts life on Earth in all known ecosystems. Its biological availability is determined by a variety of microbial-driven transformations, known as the nitrogen cycle. Various microorganisms participate in the nitrogen cycle, making them key to global biogechemistry and making viral infection of nitrogen cyclers an ecological process of global relevance. One phylum of these microorganisms is *Nitrospira*, which can be found in various ecosystems where they are often involved in of nitrification. Here, we investigated the unexplored diversity of *Nitrospira*-infecting viruses and their ecological impact on nitrification in diverse ecosystems. We conducted genomic analyses on 20 publicly available metagenomes from a range of environments, including activated sludge, bioreactors, lakes, and groundwater, where *Nitrospira* presence was detected. Additionally, we incorporated metagenomes from a mesocosm experiment simulating streams and from a sedimentation basin. We identified CRISPR-Cas systems in different Nitrospira MAGs that were matching unrecognized viral particles, indicating either past or current viral infections. This allowed for the reconstruction of viral infection histories of *Nitrospira* across various ecosystems. We further explore potential differences between viruses infecting *Nitrospira* engaged in complete ammonia oxidation (COMAMMOX) and those involved solely in nitrite oxidation. We hypothesize that viruses significantly influence impactthe ecological functions of *Nitrospira* within these ecosystems, thereby exerting a direct impact on the global nitrogen cycle.

P-EMP-003

Merging two worlds: Synergy between a photobioreactor and a chicken coop from the microbiome point of view

*M. Velaz Martin[1], L. Meisch[1], T. Glockow[2], A. K. Kaster[3], K. S. Rabe[1], C. M. Niemeyer[1]
[1]*Karlsruhe Institute of Technology (KIT), IBG-1, Karlsruhe, Germany*
[2]*Acheron, Bremen, Germany*
[3]*Karlsruhe Institute for Technology (KIT), IBG-5, Karlsruhe, Germany*

The growing global demand for food, coupled with diminishing arable land and environmental concerns, requires the development of sustainable agricultural practices. While urban controlled environment agriculture is effective for plant agriculture, sustainable approaches in circular farming for animals are underdeveloped. Photosynthetic microorganisms (PMO's), such as microalgae and *Cyanobacteriota (Spirulina)*, in mixed populations can be harnessed in photobioreactors within conventional animal houses to utilize exhaust components for growth.

We investigated the capacity of the PMOs to transform animal housing emissions into valuable biomass as well as potential community shifts in the mixed population.[1] To this end, a suspended, innovative cone-shaped, helical tubular photobioreactor was introduced into a chicken coop and demonstrated its efficiency in purifying exhaust air while producing *Spirulina*.

Metagenomics analysis revealed the impact of the chicken house exhaust on the algal culture and provided insights into the NH3 metabolism. The taxonomic classification of the microbial community in the photobioreactor revealed a predominance of bacterial organisms before aeration, with a notable shift to a more diverse community, including eukaryotic species, after exposure to exhaust air from the chicken coop. The emergence of *Viridiplantae*, and *Stramenopiles*, particularly *Chlorella* and diatoms, suggests a significant impact on the microbial composition influenced by the exhaust air.

References:

[1] T. Glockow, M. Velaz Martín, L. Meisch, D. Kapieske, K. Meissner, M. Correa Cassal, A.-K. Kaster, K. S. Rabe, C. M. Niemeyer, A photobioreactor for production of algae biomass from gaseous emissions of an animal house, *Appl. Microbiol. and Biotechnol.,* **2023**.

P-EMP-004

Biofilm formation and persistence of various anthrax causing bacteria on leaves

*L. Borst[1], S. Klee[1]
[1]*Robert Koch Institute, Berlin, Germany*

Introduction

Bacillus cereus biovar *anthracis* (*Bc*bv*a*) is a novel anthrax causing agent infecting animals in African rainforest areas. Human infections were not yet confirmed, but exposure is likely due to hunting and consumption of "bush meat" and was also evidenced by seroprevalence studies in humans living in affected regions.

It is still largely unknown how animals get infected with *Bc*bv*a*. One route of infection might be similar to that of *Bacillus anthracis* (*BA*) via spores released into the soil by dead animals. However, also monkey species living only in trees get regularly infected by *Bc*bv*a*. It was shown that carrion flies which had fed on *BA*-infected carcasses can contaminate leaves with their excretions and thereby transmit the disease. Indeed, also *Bc*bv*a* has been confirmed in carrion flies. These flies could potentially spread *Bc*bv*a* and its spores to leaves and fruits which in turn get consumed by various monkey species.

We hypothesize that *Bc*bv*a* is capable of persisting and even replicating on these leaves as biofilm and thereby considerably increasing the chance of infecting animals.

Methods

In this study, we use an *in vivo* model with *Chlorophytum comosum* (Spider Plant) to reconstruct biofilm formation and persistence of *Bc*bv*a* and *BA* on the leaf surface.

To gain insight into biofilm formation, we use confocal laser scanning and electron microscopy. Multiplication and spore formation of *Bc*bv*a* are confirmed by quantification methods such as colony forming unit determination and quantitative PCR (qPCR). At last, we aim to characterize and quantify the biofilm matrix composition by labeling with specific fluorescent markers and photometric measurement.

Results

First results of confocal and electron microscopy showed biofilm formation of *Bc*bv*a* and *BA* on leaves. Quantification with qPCR even points to *Bc*bv*a* being able to multiply on the leaf surface under our model conditions.

Conclusion

Even though the project is still ongoing, first results support our hypothesis on *Bc*bv*a* and *BA* producing biofilm on leaves. Further insight on the biofilm formation could lead to a better understanding on how this deadly disease is spreading in wildlife.

P-EMP-005

DNAse-insensitive transfer of a linear megaplasmid within Micrococcus species

J. R. Dib[1,2,3], M. Übelacker[4], M. F. Perez[3], M. E. Farías[3], *M. Baudrexl[4], W. Liebl[4]
[1]*Georg-August University Göttingen, Institut für Mikrobiologie und Genetik, Göttingen, Germany*
[2]*Universidad Nacional de Tucumán, Tucumán, Argentina*
[3]*PROIMI - CONICET, Tucumán, Argentina*
[4]*Technical Univeristy of Munich, Chair of Microbiology, Freising, Germany*

Micrococcus is an ubiquitous genus from the phylum *Actinomycetota* which can be found in harsh environments exposed to extreme conditions. Megaplasmids have been identified in *Micrococcus* strains and thought to play a role in mediating resistance against, e.g., UV irradiation, dryness, hypersalinity, high concentrations of heavy metals and different macrolid antibiotics.

Horizontal gene transfer may be important to disseminate these survival factors. Natural transformation, i.e. the uptake of naked DNA from the environment is a known trait for *Micrococcus* strains, while conjugation of linear plasmids has not been shown for this genus before.

This study presents compelling evidence for the transfer of a linear invertron-type megaplasmid from the original host to a recipient *Micrococcus* strain and further transfer to another *Micrococcus* strain in a DNAse-insensitive and competence genes-independent way.

Mating experiments were performed after growth in full medium on minimal medium plates for 48 h with and without DNAse I. The donor strain harbored the linear plasmid which contains an erythromycin resistance gene, whereas the kanamycin resistant recipient strain was incapable of DNA uptake via natural transformation due to a mutation of an essential competence gene. In addition, control experiments were performed with isolated plasmids to show that cells on selective plates were not transformed by natural transformation.

Transconjugants were selected on full medium plates containing both antibiotics, and were extensively checked via PCR, while the presence of the megaplasmid was also verified using PFGE. To determine the conjugation frequency, the CFU of recipient cells were determined on plates containing only kanamycin. Notably, the addition of DNAse I did not impede the transfer of the linear plasmid to recipient cells.

This study uncovered a novel mechanism for horizontal gene transfer of linear plasmids between *Micrococcus* strains, which awaits further investigation in the future.

P-EMP-006
Evaluation of the ddPCR assay to identify and quantify Alexandrium pseudogonyaulax through eDNA

*G. Koc[1,2], A. Kremp[2], P. Retzlaff[2], U. Tillman[3], B. Krock[3], M. Labrenz[1,2]
[1]*Klaipeda University, Klaipeda, Lithuania*
[2]*Leibniz Institute for Baltic Sea Research Warnemuende, Rostock, Germany*
[3]*Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany*

Harmful algal blooms (HABs) are pervasive worldwide, posing significant threats to marine ecosystems as well as fisheries and aquaculture. In Northern Europe, HAB occurrences are frequent, notably in the Baltic Sea and eastern North Sea. *Alexandrium pseudogonyaulax*, a dinoflagellate species, stands out from other species groups due to its distinct nutritional mode, phylogenetic relatedness, and toxigenic capacities. Populations can be toxic, producing the cytotoxic macrolide polyether goniodomin A (GDA), with potential impacts on fish populations. Its increasing prevalence in coastal waters of central and northern Europe raises concerns. The feeding mechanism of *A. pseudogonyaulax*, trapping and immobilizing prey with toxic mucus, enhances its growth and competitive advantage, likely benefiting from environmental changes such as increased river discharge and favourable temperatures attributed to climate change. This may also explain the recent increased biomass of the dinoflagellate in phytoplankton in summer in the western and southern Baltic Sea. This study aimed to explore the potential of using digital droplet PCR (ddPCR) for *A. pseudogonyaulax* detection and enumeration in Northern European water bodies, offering a rapid and reliable alternative to traditional microscopy methods. In 2020 an expedition was carried out in Northern European sea areas, covering 35 stations along a salinity gradient from the North Sea and Baltic Sea, where plankton net tow samples were collected from the research vessel Uthörn to detect and quantify *A. pseudogonyaulax*. Additionally, water samples collected from the coastal German long-term monitoring station Heiligendamm over four months in 2021 were evaluated. For each sample, ddPCR was conducted using a species-specific 28S rDNA primer. To our knowledge the ddPCR approach was not applied to this species before. Results were compared with microscopic cell counts, validating the assay's efficacy. More detailed results will be presented at the conference, but overall, it seems that the ddPCR approach is as reliable as traditional microscopy, crucial for mitigating the impacts of HABs on marine ecosystems and associated industries.

P-EMP-007
Nitrous oxide respiration of Neobacillus vireti in pure culture and soil microcosms

*L. Geisenhof[1], J. Simon[1], K. Zeiter[1], J. Fischer[1]
[1]*Technical University of Darmstadt, Microbiology and energy conversion, Darmstadt, Germany*

Biologically catalysed reduction of nitrous oxide (N_2O, laughing gas) to dinitrogen gas is a desirable process in view of the increasing atmospheric concentration of this important greenhouse gas and ozone-depleting substance [1]. A diverse range of bacterial species produce the copper cluster-containing enzyme N_2O reductase (NosZ). Based on phylogenetic analyses, NosZ enzymes have been classified as clade I or clade II, and the corresponding organisms are expected to differ significantly in their electron transport processes to NosZ as well as in the maintenance and repair of the NosZ active site. In some organisms, N_2O reduction contributes to a distinct mode of anaerobic respiration, termed N_2O respiration [2].

In the present study, the N_2O-respiring and nitrate-ammonifying soil organism *Neobacillus vireti* (phylum Bacillota) [3] was investigated for its ability to reduce N_2O in pure cultures and in soil microcosms when introduced as vegetative cells or spores. The abundance of added *N. vireti* cells in soil microcosms was determined by qPCR using appropriate primer pairs.

Specific N_2O turnover rates, apparent N_2O affinities and survival of *N. vireti* cells in soil will be presented.

The data suggest that *N. vireti* cells are a potential candidate for use in mitigating nitrous oxide emissions from agricultural soils, the largest anthropogenic source of N2O [1].

References

1 Tian H, Xu R, Canadell JG, Thompson, RL, Winiwarter W *et al.* (2020) A comprehensive quantification of global nitrous oxide sources and sinks. Nature **586**, 248–256
2 Hein S & Simon J (2019) Bacterial nitrous oxide respiration: electron transport chains and copper transfer reactions. Adv. Microb. Physiol. **75**, 137–175
3 Mania D, Heylen K, van Spanning RJM & Frostegård Å (2014) The nitrate-ammonifying and *nosZ*-carrying bacterium *Bacillus vireti* is a potent source and sink for nitric and nitrous oxide under high nitrate conditions. Environ. Microbiol. **16**, 3196–3210

P-EMP-008
A methylamine-driven long term groundwater enrichment containing members of the Candidate Phyla Radiation

*M. Taubert[1], V. Reilly-Schott[2], M. Sprenger[3], K. Papenfort[3], E. Gabashvili[1]
[1]*Friedrich Schiller University Jena, Aquatic Geomicrobiology, Jena, Germany*
[2]*Helmholtz Centre for Environmental Research (UFZ), Biophotovoltaics, Leipzig, Germany*
[3]*Friedrich Schiller University Jena, General Microbiology, Jena, Germany*

Bacteria of the recently discovered and widespread Candidate Phyla Radiation (CPR) metabolically depend on host species. As a consequence, CPR are typically lost once removed from their natural environment for enrichment and cultivation approaches. Only few CPR are currently available as isolates, and even initially successful enrichments showed a tendency to crash for unknown reasons after prolonged incubations.

Here, we report on an aerobic groundwater-derived enrichment containing CPR that has been continuously maintained for more than two years, and has been monitored using 16S rRNA gene amplicon sequencing, quantitative PCR, BONCAT and metagenomics. Fed with methylamine (MA) as carbon and energy source, the activity of methylotrophs affiliated with *Methylobacterium* and *Aminobacter* fueled a community of 500 to 700 bacteria

based on ASV analysis, including 28 ASVs of the CPR. In total, CPR contributed up to 15% of the community based on relative abundance of 16S rRNA genes. As CPR usually have less 16S rRNA gene copies than other bacteria, their actual abundance might be even higher. From a total of 132 high quality metagenome-assembled genomes (MAGs), 18 MAGs of CPR were recovered, affiliated with *Cand.* Magasanikbacteria of the ABY1 group and *Cand.* Kaiserbacteria of the *Cand.* Paceibacteria. Both BONCAT-activated cell sorting and 16S rRNA gene copy numbers suggested proliferation of the CPR. Following passaging of the enrichment, CPR typically increased after 4 to 6 weeks, when nutrients were depleted and oxygen consumption ceased, suggesting their growth to benefit from conditions where other microbes are starving. Apart from CPR and MA degraders, also MAGs implicated in nitrogen cycling like *Nitrosomonas* and *Nitrospira* were recovered, housing ammonia monooxygenase and nitrite oxidoreductase genes. This indicated that MA degradation fueled a network of nitrogen based metabolic interactions and thus supported the complex community observed, potentially also benefitting CPR. The enrichment culture represents a valuable model system to investigate the role of CPR for the rest of the community and the potential functional interplay among these organisms.

P-EMP-009

Metaproteomic approach to discovering novel biocatalysts from environmental sources

*F. Dicks[1], L. I. Leichert[1], J. E. Bandow[2], S. Schäkermann[2], D. Sander[1], S. Heinrich[3], P. Sukul[1]

[1]*Ruhr-University Bochum, Biochemistry, Bochum, Germany*

[2]*Ruhr-University Bochum, Applied Microbiology, Bochum, Germany*

[3]*Ruhr University Bochum, Applied Microbiology, Bochum, Germany*

Our group has developed a functional metaproteomic method, in which we combine cultivation-independent -omics methodologies with the immediacy of activity screening. It has the potential to discover all enzymes with a given activity in an environmental community and does not require prior assumptions about the biocatalyst"s structure. It combines metaproteomic extraction of samples with direct, zymography based, activity screening. The type of zymogram can be easily adapted, to find a variety of desired enzyme functions. In this project we will apply this method to discover novel glycosidases, specifically amylases, cellulases and hemicellulases, as these enzymes are key to a sustainable utilization of renewable plant-based resources. We established two dye-based methods for activity detection. One based on the reaction of iodine with starch (for amylases), the other based on congo red, which can form a complex with cellulose and hemicellulose. For esterases, we used 4-Methylumbelliferone (MU) as a fluorescent substrate. MU can also be tailored to form ester bonds with a desired substrate. We used enantiomeric forms of amino acids to find enantioselective esterases. We performed assays to determine characteristics of this enzyme, like kinetics and E-value. For these assays, we will utilize derivates of methylumbelliferone, with which we already have experience. We found four candidates for different amino acids and varying degrees of enantioselectivity. The highest E-value was with around 1000 a remarkable find.

Taken together, we can screen multiple enzyme classes in environments that naturally select for desired enzyme properties, making them accessible for industrial or medical applications. Our method is versatile, as it can be adapted quickly to not only screen for different properties, but different enzyme classes entirely.

P-EMP-010

Bacterial selection for enhanced biofiltration and control of fish pathogens in Recirculating Aquaculture Systems

*J. Clols-Fuentes[1], J. García Márquez[2], H. W. Palm[1], S. Arijo Andrade[2]

[1]*University of Rostock, Aquaculture and sea ranching, Rostock, Germany*

[2]*University of Malaga, Microbiology, Malaga, Spain*

Introduction: The biofilter of a RAS is a key element for the water treatment since it removes byproducts originated during the fish production. A proper balance of the microbial community maintains safe levels of these residual compounds. An imbalance in the biofilter's microbiome reduces the water quality, which causes stress to the fish physiological system. This situation could result in a disease outbreak and great economic losses.

Aim: The aim of the present work is the selection of microorganisms to enhance the biofiltration system in a RAS, and investigate its capacity to reduce the abundance of pathogens in the system.

Methods: Samples were obtained from the biofilter of a freshwater RAS with reared *Clarias gariepinus*. Different bacterial colonies growing on Tryptic Soy Agar (TSA) plates were isolated and biochemically characterized. The phytase, lipase, amylase, caseinase, gelatinase, cellulase, nitrate reduction and nitrite reduction activities were determined with differential mediums. Isolates with interesting metabolic profiles were selected and tested for biofilm formation ability, quorum sensing activity and antagonistic effect against seven potentially pathogenic bacteria.

Results: Two strains identified as *Bacillus* sp. and *Lysinibacillus* sp. were selected according to their overall metabolic profile. *Bacillus* sp. showed a strong inhibition of the growth from *Staphylococcus aureus* and *Pseudomonas veronii*. It was able to catalyze phosphate, glucose, casein, gelatin, cellulose and reduce nitrate. *Lysinibacillus* sp. had an antagonistic effect on the growth of *Vibrio anguilarum* and *P. veronii,* and could reduce nitrite and catalyze gelatin. The two Gram-positive spore-forming bacterial strains have the capacity to recover after a lyophilization treatment and were able to cohabit together on the same medium.

Conclusions: The two candidates had essential functional traits with the potential to improve the water biofiltration processes and control the overgrowth of pathogenic bacteria. They also had advantageous biotechnological and functional characteristics for the future development of probiotics which could be used at the production sector.

P-EMP-011

The compositional nature of microbial sequencing datasets hides significant community changes in wetlands

*J. Schwarzer[1,2], P. Mueller[1], G. Noyce[3], A. Bartholomäus[2], S. Liebner[2,4]

[1]*Universtiy Münster, Institute for Landscape Ecology, Münster, Germany*

[2]*German Research Centre for Geosciences, Potsdam, Germany*

[3]*Smithsonian Environmental Research Center, Edgewater, Germany*

[4]*University of Potsdam, Potsdam, Germany*

Wetland research today is concerned about the biochemical responses to climate and land-use change. These changes are substantially mediated by microbial activity. But often enough, these changes are not reflected in microbial community compositions.
In the SMARTX experiment in a coastal wetland on the Chesapeake Bay (USA) biogeochemical responses to several years of simulated elevated temperatures and atmospheric CO_2 were observed. However, similar to comparable studies on microbial compositional changes, no significant change in microbial community composition was observed. We propose that the discrepancy between measurable biogeochemical changes and the lack of an apparent microbial response stems to a large part from our data analysis approach, which does not account for the compositional nature of microbial sequencing datasets. There are two reasons why sequencing datasets should be analysed as compositional. Firstly, to determine mean microbial community compositions, the overall count quantity, so the sequencing depth, is not relevant. However, it is relevant when comparing compositions. Additionally, the statistical error strongly depends on the total number of counts. This also matters when investigating the variability of community compositions. Secondly, the bias induced through nucleic acid extraction, amplification and sequencing cannot be expected to cancel out when comparing community compositions, even if the bias is constant for each amplicon sequence variant (ASV, a proxy for species).Approaching our data as compositional solves both of the above described problems. Firstly, the total sum of counts becomes irrelevant for our statistical analysis (Subcompositional Coherence). Secondly, the analysis is invariant to the constant extraction bias (Perturbation Invariance). By adapting our data analysis approach, we expect to see changes of other measurable parameters reflected in microbial community compositions. In a first attempt to reanalyse ASV reads from the SMARTX experiment, a temperature effect on community composition, which was not apparent when using rarefaction and Bray-Curtis distance measures, could be identified.

P-EMP-012
Towards exploring the diversity of magnetotactic bacteria – one single cell at a time
*L. Raupach[1], S. P. E. Kreiss[1], C. E. Wurzbacher[1], J. Hammer[1], N. Kallscheuer[1], M. C. F. van Teeseling[1], *T. Haufschild[1], C. Jogler[1]
[1]*Friedrich Schiller University Jena, Institute of Microbiology, Microbial Interactions, Jena, Germany*

Magnetotactic bacteria (MTB) are a phylogenetically heterogenous group of prokaryotes united by one special trait: their ability to sense magnetic fields. It is mediated by nano-sized iron mineral crystals, forming organelles known as magnetosomes. Allowing the bacteria to use earth"s magnetic field for orientation, magnetotaxis in particular facilitates MTB"s navigation through habitats characterized by a complex system of gradients. To this day, less than 30 species of these remarkable bacteria are validly published and a similar number has candidate status. However, it is possible to observe MTB in the sediment of the majority of water bodies, ranging from small rivers over lakes to the coastal areas of the oceans. Therefore, a tremendous amount of hidden biology awaits discovery in the realm of MTB.

To get a hold onto the diversity of MTB, we hosted a broad-range citizen science project in 2022 resulting in 225 sediment samples of Germany-wide origin. Using light microscopy, we screened each sampled habitat for MTB presence and collected information about their number and morphology. Next to many single-celled coccoid, rod-, oval- and spirilla-shaped MTB, we identified two spherical multicellular magnetotactic prokaryotes (MMP), one from a sampling site previously not known to host such an organism.

Combining micromanipulation with a single/oligo-cell approach, we investigated some of the most promising MTB in our samples in-depth. Isolated single/oligo cells were subjected to light microscopy, whole genome sequencing, and subsequent whole genome analysis. The results shed light on the selected MTB"s phylogenetic positions in the tree of life and the distribution/organization of their magnetosome gene clusters.

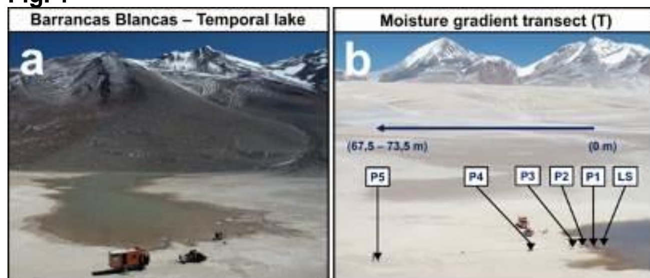
By increasing the number of available genomes, the metabolic capabilities of MTB could be identified to facilitate cultivation of novel species in the future while enabling the comparison of magnetosome genes from species of various habitats. Thereby, the project contributes to unveiling the diversity of magnetotactic bacteria and creates the base to broadscale research with these highly interesting specialists.

P-EMP-013
Hotspot for microbial life in the Puna de Atacama (Dry Andes): Effect of a fresh-water high-altitude temporary lake on the andesite microbial communities in the Barrancas Blancas Plain (Chile)
*D. Medina[1], A. García[2], R. Oses[3], D. Wagner[1,4], A. Schleicher[1,4]
[1]*GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Geomicrobiology, Potsdam, Germany*
[2]*Universidad de Atacama, Laboratorio de Investigación de la Criósfera de los Andes (LICA), Copiapó, Chile*
[3]*Universidad de Atacama, Centro Regional de Investigación y Desarrollo Sustentable de Atacama (CRIDESAT), Copiapó, Chile*
[4]*University of Potsdam, Geosciences, Potsdam, Germany*

The Central Andes region, encompassing the Central Volcanic Zone and the Altiplano–Puna High Plateau, one of the most extreme mountain environments globally. Here we found the Barrancas Blancas Plain (68°39' W, 27°02' S, 5000 m.a.l.s), a unique ecological niche hosting a temporary lake. This area serves as a natural laboratory, facilitating the study of extremophiles and water's impact on microbial life in a desert environment. A 70-meter long moisture transect, from the lake to a dry reference site, uncovered a diverse microbial community using a novel intracellular DNA extraction method. Depth-related clustering was evident, with surface samples dominated by *Proteobacteria* and *Bacteroides*, while *Gemmatimonadota* increased with depth. As other Andean Lake environments, the prevalence of common primary producers such as *Cyanobacteria* was low in all samples. Gemmatimonadota exhibited higher abundance compared to similar environments, potentially playing a role in phototrophy and the nitrogen cycle. Additionally, surface samples at 8-23 meters away from the lake exhibited heightened ATP content and enzymatic activity, mirroring levels observed in forests and other water-favourable extreme environments. This heightened activity correlated with the prevalence of *Proteobacteria* and *Bacteroidota*. Despite being recognized as opportunistic microbes capable of degrading complex organic matter, studies in desert environments have revealed their adaptability in using alternative energy pathways, aligning with metabolic prediction analyses. In summary, this location emerges as a life hotspot amid the harsh mountain desert

landscape, showcasing the significant influence of available water on microbial communities and their activities in the development of the ecosystem.

Fig. 1



P-EMP-014
Community structure and abundance of prokaryotic communities in arctic-alpine soils are influenced by topography and time

*M. Reiser[1], K. Frindte[2], J. Löffler[3], C. Knief[2]
[1]*INRES/ GIUB Universität Bonn, Bonn, Germany*
[2]*Universtiy Bonn, Institute of Crop Science and Resource Conservation, Bonn, Germany*
[3]*Universtiy Bonn, GIUB, Bonn, Germany*

Several studies have shown that microbial communities in alpine soils change with elevation. For arctic-alpine soils an additional effect of microtopographic exposition has recently been shown. However, it is less known how annual weather fluctuations influence microbial communities and modulate such differences and whether this is dependent on continentality. To investigate the prokaryotic community in response to continentality, elevation and microtopography over time, we collected soil samples over three years (2020, 2021, 2022) from two mountains in the Norwegian Scandes in late summer before the first snowfall. We covered seven elevational levels above the treeline with four microtopographic positions at each level (ridges, depressions, southern and northern exposed slopes). All study sites are equipped with permanent data loggers to document temperature and soil moisture. Prokaryotic community structure was analysed by 16 S rRNA gene amplicon sequencing and abundance by quantitative PCR of 16S rRNA. The most abundant phyla across all study sites were *Acidobacteriota*, *Proteobacteriota*, *Chloroflexi* and *Actinobacteriota.* Strong differences in the abundance of phyla were observed between the years. Differences in community composition were assessed by Permanova and revealed that the origin of the samples from different microtopographic positions and different elevations contributed significantly to the observed variation in community composition, therewith confirming previous results. Beyond, we observed strong year-to year variation and a slight effect of continentality. Further, substantial variation was explained by combinations of these factors. We currently evaluate the role of study-site specific climatic variation on the observed differences in community structure.

P-EMP-015
The importance of root-soil contact in the soil-rhizosphere-plant continuum

*A. S. Wendel[1], S. Bauke[1], W. Amelung[1], C. Knief[1]
[1]*University Bonn, INRES, Bonn, Germany*

At the root-soil interface several important processes take place like nutrient and water uptake and the interaction with microorganisms. Rhizosphere establishment at this interface is hypothesized to be affected by root-soil contact level and being modulated by physicochemical properties of the soil interface, with implications for plant performance.

To investigate the importance of root-soil contact, we modified root-soil contact levels and properties. We grew maize plants in the presence of artificial pores (abiogenic pores), artificial pores incubated with earthworms (biogenic pores) or without pores under greenhouse and field conditions. Plant performance and rhizosphere traits were analyzed, including bacterial abundance and community composition, with a special focus on nitrogen cycling bacteria as indicators for rhizosphere nutrient cycling.

Using endoscopy, we observed that roots in pores grew in part without any contact to soil. Fluorescence microscopy and quantitative PCR (qPCR) revealed that root surface colonization was strongly impaired without root-soil contact. Amplicon sequencing (16S rRNA, ITS1) showed that reduced contact had also consequences for the composition of the rhizosphere microbiota, especially the bacterial community. It was furthermore essential for nitrogen cycling in the rhizosphere, as nitrifying bacteria were more abundant in the rhizosphere with full contact to the soil according to qPCR (*amoA*). However, root-soil contact had no effects on early plant performance in the greenhouse experiments. By comparison, in the field experiment covering also later growth stages, reduced root -soil contact due to the presence of abiogenic pores resulted in growth deficits compared to the control, which were fully compensated when the interface between root and soil was enriched in nutrients due to the activity of the soil fauna in biogenic pores.

Our findings underline the importance of root-soil contact for rhizosphere processes and plant performance.

P-EMP-016
Hexavalent chromium-reducing bacteria isolated from Korean fermented food

*H. Park[1], J. I. Won[1], Y. Lee[1], H. S. Lee[1], J. H. Lee[1], B. S. Jeon[1]
[1]*Korea Institute of Ceramic Engineering and Technology, Biomaterials & Processing Center, Cheongju-si, South Korea*

Introduction

Chromium exists mainly in trivalent and hexavalent forms, of which hexavalent chromium has carcinogenic and mutagenic properties. Hexavalent chromium, widely used in industry, quickly enters soil and groundwater, and pollutes the environment. Using microorganisms is one of the safest and most effective alternatives for regenerating soil and groundwater contaminated with hexavalent chromium. Among them, the use of Generally Recognized as Safe (GRAS) strains and edible strains can be a safer alternative. We sought to isolate bacteria that remove hexavalent chromium from Korean fermented food as an alternative.

Goals

- Isolate of bacteria capable of removing hexavalent chromium from fermented foods
- Identification of hexavalent chromium removal mechanism in isolated bacteria

Materials & Methods

A bacteria with chromium-reducing ability was newly isolated from doenjang, a traditional Korean fermented food. A comparative evaluation of the ability of several bacteria to hexavalent chromium removal was conducted, and resistance to various concentrations of hexavalent chromium was tested.

Results

The isolate strain was designated *Bacillus* sp. KICET-4. The reduction performance for hexavalent chromium of KICET-4, which evaluated several bacteria's characteristics, was observed. Although growth was inhibited at a hexavalent chromium concentration of 200 mg Cr/L, KICET-4 still played a role in removing hexavalent chromium. Also, when the medium containing hexavalent chromium was treated with KICET-4, it was confirmed that no cytotoxicity was observed compared to the untreated control group.

Summary

We isolated several bacteria capable of removing hexavalent chromium from Korean fermented foods. Among these, KICET-4 showed the most excellent hexavalent chromium removal ability. In addition, it was confirmed through cytotoxicity experiments that the high concentration of hexavalent chromium present in the medium was removed to a level below the toxic concentration. It is expected that KICET-4 can be applied as a sustainable purification technology to environments polluted with hexavalent chromium.

Key words: Hexavalent chromium, KICET-4, GRAS strains

P-EMP-017
Novel Cultivation Technique for Enriching and Decoding Microbial Dark Matter in Environmental Microbiomes
*L. Meisch[1], M. Velaz Martin[1], A. K. Kaster[2], K. S. Rabe[1], C. M. Niemeyer[1]
[1]*Karlsruhe Institute for Technology, Institute for Biological Interfaces (IBG-1), Eggenstein-Leopoldshafen, Germany*
[2]*Karlsruhe Institute for Technology, Institute for Biological Interfaces (IBG-5), Eggenstein-Leopoldshafen, Germany*

Introduction: Microorganisms occur in an immense variety on earth, that inhabit a wide range of diverse habitats. A significant portion of these microorganisms, collectively termed microbial dark matter (MDM), are still unknown. Since most of these microorganisms do not follow an isolated planktonic lifestyle, cultivating these microorganisms in laboratory settings is challenging. Consequently, there is a great need for matrices that facilitate the cultivation of complex microbial communities.

Goals: Development of a macroporous elastomeric silicon foam (MESIF) as a matrix integrated into a chip with a media reservoir to enable the growth of complex microbial communities.

Methods: MESIF materials with varying pore sizes and chemically modified surfaces were prepared. Integration of MESIF into a chip was followed by a characterization of its colonization by the model organism *Escherichia coli*. The impact of MESIF on the enrichment of environmental microbiomes was assessed through genomic DNA sequencing and bioinformatics.

Results: The model organism *Escherichia coli* was used to validate the rapid colonization of the modifiable MESIF in the chip. Environmental microbiomes were enriched in the MESIF chip within a few days. Especially organisms belonging to the *Candidate Phyla Radiation* could be enriched in chips placed in a moving bed biofilter of a fish tank. From dry air from a chicken coop a variety of biotechnologically interesting *Actinobacteriota* could be enriched and detected in the chip. Sampling with the herbicide glyphosate as the sole carbon source enriched *Desulfobacteria*, which are known to include species metabolizing glyphosate.

Summary: The MESIF materials serving as macroporous enrichment matrix, can be easily prepared with reproducible properties. The chip design provides a platform for enriching uncultured microorganisms, facilitating the systematic exploration of microbial dark matter.

Literature:Zoheir AE, Meisch L, Velaz Martín M, Bickmann C, Kiselev A, Lenk F, Kaster A-K, Rabe KS and Niemeyer CM.: Macroporous Silicone Chips for Decoding Microbial Dark Matter in Environmental Microbiomes. *ACS Appl. Mater. Interfaces* (2022) *14* (44):49592

P-EMP-018
Microbially induced hypoxic and sulfidic zones in times of global warming – a threat to the Baltic Sea?
*N. Adam-Beyer[1], F. Scholz[2], M. Perner[1]
[1]*GEOMAR Helmholtz-Centre for Ocean Research Kie, Geomicrobiology, Kiel, Germany*
[2]*Universtiy Hamburg, Biogeochemistry in the Earth System, Hamburg, Germany*

In recent decades rising temperatures have led to the continuous global expansion of severely reduced oxygen levels and hypoxia (<63 µM) in aquatic habitats. These effects predominantly result from increasing nutrient inputs and reduced oxygen solubility due to increasing water temperatures. Microbially driven organic matter degradation represents the main driver of oxygen consumption in seafloor habitats. In oxygen-depleted sediments sulfate reduction and methanogenesis are two of the most important metabolic pathways, which result in the formation of toxic hydrogen sulfide and the greenhouse gas methane. These compounds may be released from the sediments, posing a threat to marine life, affecting global warming and resulting in potentially severe socio-economic impacts.

In order to gain a deeper understanding of the mechanisms involved in the expansion of hypoxic and sulfidic zones in coastal regions of the Baltic Sea, we analyzed geochemical parameters and microbial communities of sediments underneath a seasonally hypoxic water column at the time series station Boknis Eck (Eckernförde Bay). First results of the porewater geochemistry, modeled turnover rates and 16S rRNA gene assessments suggest an intensification and upwards shift of sulfate reduction and methanogenesis in warmer periods and after hypoxic events compared to the winter period. This trend is also reflected in the respective microbial community compositions, especially the abundances of typical sulfate reducers, sulfide oxidizers and methanogens. In comparison to prior campaigns over the last 15 years, our data also indicate an overall increase of

reducing processes in sediments of the Eckernförde Bay. Our findings will be further evaluated during future sampling campaigns to Boknis Eck and other areas in the southwestern Baltic Sea. Our ultimate goal is to develop a sediment biogeochemical model, which can be used to spatially predict the onset of anoxic-sulfidic bottom waters and the associated risk to ecosystems and local stakeholders.

P-EMP-019

Effect of temperature and oxygen on nitrous oxide reducing bacteria from biogas and wastewater treatment plants

*T. Laader[1], S. Mielke[1], J. Simon[1]
[1]*Technical University of Darmstadt, Microbiology, Darmstadt, Germany*

Nitrous oxide (N2O; laughing gas) is a potent greenhouse gas and one of the main causes of ozone layer depletion [1]. Over the last century, human activities have greatly increased N2O emissions, mainly from agriculture, livestock farming and wastewater treatment. N2O is produced by nitrifiers, denitrifiers and microorganisms that catalyse dissimilatory nitrate/nitrite reduction to ammonia (DNRA) [1-3]. However, only one type of nitrous oxide reductase (NosZ) has been described. This enzyme is found in bacterial and archaeal species from several phyla, including Proteobacteria and Firmicutes [2]. Many of these organisms are able to grow by N2O respiration.

Several N2O-reducing strains of the genera *Pseudomonas*, *Alcaligenes* and *Comamonas* were recently isolated from an anaerobic digester and a wastewater treatment plant. Here, the effects of temperature and oxygen availability on these cells were investigated. Growth curves were determined in the range of 15 °C to 37 °C using an acetate-based medium and supplying an atmosphere of pure N2O. Microrespirometry was used to measure specific N2O reduction rates. In all isolates, the presence of oxygen either inhibited or abolished N2O reduction. However, upon oxygen depletion, nitrous oxide reduction rapidly resumed, albeit at a slightly reduced rate. Hypotheses to explain this phenotype at the molecular level will be discussed.

References

1 Stein LY (2020). The long-term relationship between microbial metabolism and greenhouse gases. Trends Microbiol. 28, 500–511

2 Torres MJ, Simon J, Rowley G, Bedmar EJ, Richardson DJ, Gates AJ & Delgado MJ (2016). Nitrous oxide metabolism in nitrate-reducing bacteria: physiology and regulatory mechanisms. Adv. Microb. Physiol 68, 353–432

3 Hein S & Simon J (2019) Bacterial nitrous oxide respiration: electron transport chains and copper transfer reactions. Adv. Microb. Physiol. **75**, 137–175

P-EMP-020

Occurence of multi-drug resistant pathogens in bathing waters in Schleswig-Holstein: are hospital wastewaters a problem?

*M. Decius[1], U. Möbius[1], B. Christiansen[1], G. Petzold[2], S. Simon[2], A. Marcic[3]
[1]*Institute for Hygiene and Environmental Medicine, Kiel, Germany*
[2]*Ministry of Justice and Health, Environmental Health Protection Unit, Kiel, Germany*
[3]*Office for Health, Infection protection, Kiel, Germany*

Introduction: The natural ability of bacteria to develop resistance has been accelerated by the selective pressure exerted by the improper and increased use of antibiotics in human therapy. Wastewater systems, especially those receiving hospital or livestock wastewater, were demonstrated to be significant sources of significant epidemic pathogens such as Klebsiella pneumoniae or Pseudomonas aeruginosa.

Goals: The aim of the study was to examine if clinically relevant multi-drug resistant pathogens occur in bathing waters in Schleswig-Holstein and if these are associated with the discharge of hospital wastewater. If necessary, updated recommendations for action and measures should be developped.

Methods: The following monitoring sites were included: hospital wastewater (collection chamber), influents and effluents from sewage treatment plants and bathing waters with discharge of treated wastewater. Focus was placed on multi-drug resistant gram-negative rods with carbapeneme resistance (4MRGN), which are spread into the environment through discharge into water/hospital wastewater: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Klebsiella oxytoca.* cultivation was carried out on selective culture media with species identification. Confirmed isolates were further examined using a Vitec antibiogram and, if necessary, an E-test and/or carbapenemase test. The results were interpreted according to EUCAST criteria.

Results: We did not detectany multi-drug resistant gram-negative rods with carbapeneme resistance in the bathing water samples tested. Only one isolate of multi-drug resistant Klebsiella pneumoniae (3MRGN) was detected in a bathing water sample.

Conclusions: In our analysis we did not detect multi-drug resistant gram negative rods with carbapeneme resistance in bathing waters. Thus, lacking an acute health risk for the general population there is no need to change routine testing and parameters for bathing waters in Schleswig-Holstein.

P-EMP-021

Immobilization of nitrous oxide respiring bacteria

*J. Polat[1], S. Lackner[2], J. Simon[1]
[1]*Technical University of Darmstadt, Department of Biology, Microbial Energy Conversion and Biotechnology, Darmstadt, Germany*
[2]*Technical University of Darmstadt, Department of Civil and Environmental Engineering, Water and Environmental Biotechnology, Darmstadt, Germany*

Atmospheric nitrous oxide (N_2O), known for its much higher greenhouse gas potential than CO_2, plays a crucial role in climate change [1]. N_2O emissions from wastewater treatment plants (WWTPs), resulting from biological processes within these facilities, are emerging as a relevant concern [2]. This study aims to investigate the use of immobilised N_2O-respiring microorganisms [3] as a potential method to mitigate N_2O emissions from WWTPs.

Pure cultures of several N_2O-respiring microorganisms (isolates belonging to the genera *Pseudomonas*, *Comamonas* or *Alcaligenes* as well as *Wolinella succinogenes*) were successfully immobilised in Ca^{2+}-alginate beads (2 or 4 %, w/v, alginate). Cell proliferation and colonies formation were observed under appropriate anaerobic N_2O respiration conditions with 10 or 100 % N_2O in the culture headspace. At high cell densities, gas bubbles, presumably consisting of N_2 gas, appeared and occasionally even disrupted the integrity of the alginate beads. Microrespirometry was used to investigate the N_2O consumption rates of alginate-immobilised cells as a function of the number of alginate beads.

This study demonstrates the potential of alginate-immobilised bacteria as a method for reducing N_2O emissions in wastewater treatment plants, which holds promise for climate change mitigation strategies.

References

1 Griffis, T.J., Chen, Z., Baker, J.M., Wood, J.D., Millet, D.B., Lee, X., Venterea, R.T. and Turner, P.A. (2017) Nitrous oxide emissions are enhanced in a warmer and wetter world. Proc. Natl. Acad. Sci. USA **114**, 12081-12085.

2 Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S.M. and van Loosdrecht, M.C.M. (2009) Nitrous oxide emission during wastewater treatment. Water Res. **43**, 4093-4103.

3 Hein, S. and Simon, J. (2019) Bacterial nitrous oxide respiration: electron transport chains and copper transfer reactions. Adv. Microb. Physiol. **75**, 137-175.

P-EMP-022

Enhanced dissolved oxygen levels in sediment: Investigating the impact of calcium peroxide application for eutrophicated lake restoration

*T. Kaupper[1], F. Kreher[2], J. Ruecker[3], D. M. Gampe[4], T. Lueders[1]

[1]*University of Bayreuth, Ecological Microbiology, Bayreuth Center of Ecology and Environmental Research (BayCEER), Bayreuth, Germany*

[2]*Wertec GmbH, Chemnitz, Germany*

[3]*Brandenburg University of Technology (BTU) Cottbus-Senftenberg, Aquatic Ecology, Bad Saarow, Germany*

[4]*Söll GmbH, Hof, Germany*

Restoring eutrophicated lakes is vital for improving water quality in aquatic ecosystems often highly valued by local communities. Nutrient influx, mainly from changing land-use and agricultural runoff, exacerbates the trophic status, especially in smaller lakes. Traditional dredging, though prevalent, is expensive and environmentally harmful. An alternative approach uses water-insoluble calcium peroxide (CaO_2) for lake restoration, offering a cost-effective and less labor-intensive solution. CaO_2 slowly releases oxygen, increasing dissolved oxygen levels (eDO), promoting aerobic microbial degradation of organic matter in eutrophicated sediments. It may also mitigate harmful algal blooms through phosphorus elimination.

In this project funded within the "Water4All" ZIM network, several industry and university partners collaborate to analyze factors influencing organic matter degradation and sediment reduction in small lakes (2 amendment. Despite previous successful applications, uncertainties prevail about the specific biotic and abiotic factors affecting its efficacy. Several small lakes in Northern Bavaria and Saxonia were treated with CaO_2, with intensive monitoring of biotic and abiotic parameters. Concurrently, laboratory microcosm incubations are investigated for fine-scale assessment of CaO_2-induced effects through microsensor measurements and 16S rRNA gene amplicon sequencing.

Preliminary tests show increased levels of bioavailable O_2 in the sediment surface, showing a short-term increase *in situ*. Ongoing field data analysis will unveil further effects. In laboratory microcosms, intensive bioturbation of sediment columns observed even for unrealistically high CaO_2 dosage complicates biogeochemical data interpretation but alleviates concerns regarding ecotoxicological assessment. Amplicons for 16S rRNA gene sequencing are currently analyzed, to further examine the influence of CaO_2 amendment on sediment microbial community development. Taken together, the project aims to provide a comprehensive mechanistic and also freshwater ecology perspective of CaO_2-based treatment strategies for eutrophicated small lakes.

P-EMP-023

Microbial transcriptome patterns highlight increased pedogenesis-related activity in arid compared to semiarid soils under humid conditions.

*V. Rodriguez[1], A. Bartholomäus[1], R. Oses[2], S. Liebner[1,3], D. Wagner[1,4]

[1]*GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Section Geomicrobiology, Potsdam, Germany*

[2]*University of Atacama, Centro Regional de Investigación y Desarrollo Sustentable de Atacama, Copiapó, Chile*

[3]*University of Potsdam, Institute of Biochemistry and Biology, Potsdam, Germany*

[4]*University of Potsdam, Geosciences, Potsdam, Germany*

In arid and semiarid environments, microbial activity is restricted by limited water and high evapotranspiration, impacting soil formation. Recognizing that this limitation can be overcome under humid conditions, accelerating soil formation by influencing microbial processes, our study aims to investigate which microorganisms transform initial arid and semiarid soils into resilient ecosystems. Additionally, we characterized essential microbial processes and how their gene expression differs between arid and semiarid soils under simulated humid climate conditions. We assessed changes in bacterial and archaeal communities using advanced metagenomic and metatranscriptomic approaches through a sixteen-week climate simulation experiment. The results revealed the triggering of an intense functional response in arid soils compared to semiarid soils under humid climate conditions. *Proteobacteria* and *Actinobacteriota* dominated the overall transcriptional profile and specific functions associated with the early stages of soil formation. Arid soils undergo higher transcription of genes linked to soil formation, particularly those associated with soil aggregation, phosphate metabolism, and weathering, potentially promoting the succession of arid soils. Our findings demonstrate the rapid activation of soil formation-related pathways under humid climate conditions, particularly in arid soils, occurring within short timeframes of weeks.

P-EMP-024

Biological nitrogen fixation, diversity and community structure of diazotrophs in two mosses in 25 temperate forests

C. Groß[1], S. Hossen[2], S. Dittrich[3], K. H. Knorr[4], W. Borken[1], *M. Noll[2]

[1]*Univerity of Bayreuth, Center of Ecology and Environmental Research (BayCEER), Bayreuth, Germany*

[2]*University of Applied Science and Arts Coburg, Institute for Bioanalysis, Coburg, Germany*

[3]Technical Univeristy of Dresden, Biodiversity and Conservation, Tharandt, Germany
[4]University of Münster, Institute of Landscape Ecology, Münster, Germany

Many moss species are associated with nitrogen (N)-fixing bacteria (diazotrophs) that support the N supply of mosses. Our knowledge relates primarily to pristine ecosystems with low atmospheric N input, but knowledge of biological N fixation (BNF) and diazotrophic communities in mosses in temperate forests with high N deposition is limited. We measured BNF rates using the direct stable isotope method and studied the total and potentially active diazotrophic communities in two abundant mosses, *Brachythecium rutabulum* and *Hypnum cupressiforme*, both growing on lying deadwood trunks in 25 temperate forest sites. BNF rates in both mosses were similar to those observed in moss species of pristine ecosystems. *H. cupressiforme* fixed three times more N_2 and exhibited lower diazotrophic richness than *B. rutabulum*. *Frankia* was the most prominent diazotroph followed by cyanobacteria *Nostoc*. Manganese, iron, and molybdenum contents in mosses were positively correlated with BNF and diazotrophic communities. *Frankia* maintained high BNF rates in *H. cupressiforme* and *B. rutabulum* even under high chronic N deposition in Central European forests. Moss N concentration and ^{15}N abundance indicate a rather minor contribution of BNF to the N nutrition of these mosses.

P-EMP-025

The bicarbonate transporter (MpsABC) is essential for growth in *Staphylococcus aureus*

*E. Liberini[1], S. H. Fan[2], I. Koch[3], B. Sailer[3], K. Hipp[3], J. Biboy[4], D. Vollmer[4], J. Gray[4], A. S. Bayer[2], W. Vollmer[4,5], P. Francois[6], F. Götz[1]
[1]University Tübingen, Microbial genetics, Tübingen, Germany
[2]The Lundquist Institute, Torrance, CA, United States
[3]Max-Planck-Institute for Biology, Tübingen, Germany
[4]Biosciences Institute, Newcastle upon Tyne, United Kingdom
[5]Institute for Molecular Bioscience, Brisbane, Australia
[6]Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland

Introduction. Bicarbonate and CO_2 are important substrates for carboxylation reactions in bacterial central metabolism. In the opportunistic pathogen *Staphylococcus aureus,* the bicarbonate transporter, MpsABC (membrane potential-generating system), is the only uptake system of the membrane-impermeant bicarbonate, which is crucial for growth in ambient air. The *S. aureus* Δ*mpsABC* mutant's growth deficit in ambient air, could be rescued by 5% CO_2. **Goals.** We aimed to investigate the impact of bicarbonate depletion on the phenotypic and genotypic changes that enable the Δ*mpsABC* mutant to counteract CO2/bicarbonate deficiency. **Material & Methods.** We compared WT and mutant by electron microscopy (EM), analyzed the cell wall (CW) composition by HPLC and mass spectrometry (MS), investigated the susceptibility to cell wall lytic enzymes, and carried out comparative RNAseq analysis for gene expression. **Results.** EM revealed that the CW of Δ*mpsABC* was twice as thick compared to the parent strain. The mutant was largely resistant to lysostaphin due to the incorporation of L-alanine in the interpeptide bridge. The RNAseq profiling revealed that particularly genes encoding CW-hydrolases were highly upregulated; while genes encoding CW-anchored proteins, wall teichoic acid biosynthesis, secreted proteins, transporters and toxins were downregulated. **Conclusion.** Bicarbonate depletion in *S. aureus* induces major changes in cell wall composition and global gene expression. The changes may indicate general survival strategies adopted by bacteria which face CO_2/bicarbonate limitation.

P-EMP-026

The diversity of non-rhizobial endophytes and abundance of AMF in response to P fertilizers in Pisum sativum

*S. K. Thaqi[1,2], N. Hensel[3], N. Vitow[4], K. Panten[5], L. M. Streb[1], S. Kublik[1], C. Baum[4], P. Leinweber[4], M. Schloter[1,3], S. Schulz[1]
[1]Helmholtz Center Munich, Comparative Microbiome Analysis, Neuherberg, Germany
[2]Technical University of Munich, Crop Physiology, Freising, Germany
[3]Technical University of Munich, Environmental Biology, Freising, Germany
[4]University of Rostock, Soil Science, Rostock, Germany
[5]Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Crop and Soil Science, Brunswick, Germany

Introduction

Non-rhizobial endophytes (NRE) enhance plant growth via functions like nutrient mobilization and pathogen suppression. NRE are strongly influenced by several abiotic and biotic factors. In legumes, for example, phosphorus (P) availability and concurrent arbuscular mycorrhizal fungi (AMF) root colonization may affect NRE diversity and nodulation. Recently, NRE have been detected in the root interior and as an integral part of root nodules.

Goals

Here, we studied how different P fertilizers and AMF colonization rates affect NRE communities in nodules. It was hypothesized that increased P availability would increase the number of nodules for stochiometric reasons while reducing the abundance of AMF and the diversity of NRE in the nodules.

Materials & Methods

In a field trial, four fertilizer treatments with increasing solubility were tested, namely no P fertilizer (P0), bone char (BC) and bone char+ (BC+) as alternative fertilizer and triplesuperphosphate (TSP) (n=3). Bulk soil, rhizosphere soil, surface sterilized nodules and roots of Pisum sativum were sampled, followed by DNA extraction, quantitative PCR (16S rRNA, AMF, nifH, gcd), and amplicon sequencing for 16S rRNA.

Results

Contrary to our expectations, TSP resulted in the highest NRE diversity, number of nodules and abundance of AMF. Further, the overlap between nodules, roots and rhizosphere soil was most pronounced in this treatment, indicating a less strict filtering effect. In contrast, the BC+ treatment had the highest number of vital nodules, abundance of Rhizobiaceae, levels of nifH and gcd. The pronounced overlap between the roots and nodules suggested a significant influence of root endophytes on NRE. The BC and P0 treatments showed the lowest diversity and minimal overlap between compartments but had increased levels of AMF.

Summary

This study provides insights into the impacts of different P availability on symbiosis and NRE. While TSP shows a tripartite symbiosis, the recruitment of NREs is less targeted. BC+ enhances nodulation with focused NRE recruitment, possibly linked to P turnover. Conversely, BC and P0 promote symbiosis with AMF and show fewer nodules to conserve energy.

P-EMP-027

Microbial interactions in nitrogen cycling in oligotrophic groundwater

*M. A. Rahman[1], R. Nafeh[1], A. Sharma[1], M. Krüger[1], B. Thamdrup[2], M. Kündgen[3], C. Jogler[3], K. Küsel[1], M. Herrmann[1]
[1]*Friedrich Schiller University Jena, Institute of Biodiversity, Jena, Germany*
[2]*University of Southern Denmark, Department of Biology, Odense, Denmark*
[3]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*

Understanding the links between anaerobic and aerobic processes in the nitrogen cycle is crucial for studying nitrogen dynamics in natural environments. Coupling between anaerobic and aerobic nitrogen transformation processes was previously shown to support nitrogen loss in the marine water column. However, the importance of such interactions for N dynamics in groundwater environments is poorly understood. Previous findings demonstrated a high relevance of anaerobic ammonia oxidation (anammox) for nitrogen loss from carbonate-rock aquifers in the Hainich Critical Zone Exploratory (Germany). Here, we hypothesize that (i) anammox in oligotrophic groundwater is supported by tight coupling with dissimilatory nitrate reduction to ammonium (DNRA) and aerobic ammonia oxidation, providing ammonium and nitrite, respectively, for the anammox reaction, and (ii) that this coupling is reflected by spatial co-localization of the respective microbial groups in the aquifer system. Comparative investigations of two suboxic groundwater wells (0.3 to 2.3 µmol L^{-1} dissolved oxygen) pointed to overall favorable conditions for anammox (12-25 µmol L^{-1} NO_3^-, 29-40 µmol L^{-1} NH_4^+) along with anammox activity (H52: 0.9 nmol N_2 L^{-1} d^{-1}, H53: 2.5-9.7 nmol N_2 L^{-1} d^{-1}). Metagenomic analysis indicated the genetic potential for DNRA by members of the class Thermodesulfovibrionales (Nitrospirota), which were found to consistently co-occur with anammox bacteria in those two wells, together accounting for 13-25% of the bacterial community. Although oxygen is limited, aerobic nitrifiers, especially the genus *Nitrospira* (Nitrospirota) constituted 1-15% across both wells which may support anammox activity. Ongoing experiments utilize CARD-FISH to detect anammox bacteria along with members of the Nitrospirota in attached aquifer communities. Potential coupling between anammox, DNRA, and aerobic ammonia oxidation is being tested using ^{15}N-based approaches. Our findings suggest that differences in anammox activity in the studied aquifer system could primarily be linked to the presence of oxygen and the intensity of coupling with aerobic N cycling processes.

P-EMP-028

Microbial composition of aerosols collected during Saharan dust storm events in Cape Verde

M. Frentrup[1], *J. Degenhardt[2], S. Gómez Maqueo Anaya[3], K. Schepanski[4], E. José dos Santos Souza[3], H. Herrmann[3], K. W. Fomba[3], U. Nübel[2]
[1]*Leibniz-Institute DSMZ–German Collection of Microorganisms and Cell Cultures, Bioinformatics and databases, Brunswick, Germany*
[2]*Leibniz-Institute DSMZ–German Collection of Microorganisms and Cell Cultures, Microbial Genome research, Brunswick, Germany*
[3]*Leibniz-Institute for Tropospheric Research (TROPOS), Leipzig, Germany*
[4]*Free University of Berlin, Institute of meteorology, Germany, Germany*

Mineral dust is an air pollutant that has been linked to respiratory and cardiovascular diseases. During the Harmattan annual season, large regions in Western Africa, including the islands of Cape Verde, are exposed to high concentrations of mineral dust that gets emitted from areas in the Sahara and transported over long distances. Both the chemical composition and the microbiological properties of dust may influence its detrimental health impact, but their specific effects have rarely been disentangled. We investigated the microbiome associated with aerosol particles collected at the Cape Verde island Sao Vicente, comparing reference periods with low dust concentrations to periods with pronounced Saharan dust storm events. Samples were collected simultaneously on the island's east coast where the dust arrived ('inflow' site) and on the northwest coast close to the island's major city ('outflow' site). By doing so, we wanted to elucidate which microorganisms had been transported with the dust from the African continent and which had originated from the island itself. 16S rDNA amplicon sequencing revealed that Saharan dust carried enormous microbial diversity. The most abundant bacterial taxa were also represented among strains successfully cultivated. The microbial composition of collected aerosols changed between dust storm events and reference periods, but also between in- and outflow sites on the island. Dust events were characterized by increased relative abundances of the genera *Domibacillus* sp., *Acinetobacter* sp. and *Cytophaga* sp. which contain several opportunistic pathogens. Further analysis showed that samples taken at the outflow site contained higher relative abundances of *Nocardioides* sp., *Acinetobacter* sp. and *Pseudomonas* sp., which presumably had been mobilized from sources on the island. Inflow samples were distinguished by lower relative abundances of certain bacteria. This may be caused by the large continental area from which the dust had been mobilized, resulting in a diverse microbiome with few dominating organisms. Lastly, the method of sampling clearly had an effect on the observed results, which should be considered in future studies.

P-EMP-029

Combined TCBS and CHROMagar analyses allow basic identification of Vibrio vulnificus within a 48h incubation period in the coastal Baltic Sea

*C. Glackin[1], M. Labrenz[1], D. Riedinger[1], S. Thota[1], S. Dupke[2]
[1]*Leibniz Institute for Baltic Sea Research, Rostock, Germany*
[2]*Robert-Koch Institute, Berlin, Germany*

With rising infection rates in recent years *Vibrio vulnificus* pose an increasing threat to public safety in the coastal brackish Baltic Sea. It is therefore important to be able to monitor this organism and assess its risk potential for the society on a more regular basis. However, as the coastline of the Baltic Sea is 8,000 km long and shared by nine nations, a convenient, fast, inexpensive, yet efficient *V. vulnificus* identification method is essential. We evaluated the effectiveness of a two-step agar-based approach for *V. vulnificus* in comparison with *V. cholerae*, *V. parahaemolyticus*, and *V. alginolyticus* consisting of successive *Vibrio* isolation and cultivation on thiosulphate-

citrate-bile salt sucrose (TCBS) and CHROMagar, respectively. Our study contains isolates from water and sediment across a broad expanse of the Baltic Sea including 13 locations and 2 different summers, the time of year during which *Vibrio* infections are usually much more frequent. Confirmation of isolate species was carried out using molecular analyses. The two-step agar plating method performed well across different locations and timeframes in identifying *V. vulnificus* by more than 80%, but sensitivity in other *Vibrio* species varied. Thus, our approach yielded promising results as a potential tool for early *V. vulnificus* detection across a broad timeframe and transect of the Baltic Sea and potentially other brackish environments.

P-EMP-030

Insights from a four year in-situ incubation experiment at the Indian Ridge: How microbes impact seafloor massive sulfide weathering

*A. Tecza-Wiezel[1], K. Laufer-Meiser[1], C. H. Solterbeck[2], J. Schloesser[2], S. G. Sander[3], M. Perner[1]

[1]GEOMAR Helmholtz-Centre for Ocean Research Kie, Department of Geomicrobiology, Kiel, Germany

[2]University of Applied Sciences Kiel, Institute for Materials and Surfaces, Kiel, Germany

[3]GEOMAR Helmholtz-Centre for Ocean Research Kie, Marine Mineral Resources, Kiel, Germany

Seafloor massive sulfide (SMS) deposits form on the modern ocean seafloor at active hydrothermal vent systems through mixing of mineral-rich, hydrothermal fluids with ambient oxygenated seawater. SMS deposits are primarily comprised of pyrite and chalcopyrite, and serve as source for valuable metals such as copper. Once hydrothermal activity ceases these SMS deposits undergo abiotic oxidative weathering when penetrated by oxygenated seawater. Microbial activity considerably accelerates this process transforming sulfide minerals, enhancing metal transport and mineral dissolution. Under certain circumstances the formation of iron-rich silica caps can create low oxygen conditions below the caprock, shielding SMS deposits from both abiotic and biotic oxidative weathering likely extending the lifetime of these deposits. Here we will examine the impact of microbial activity for SMS transformation and dissolution of minerals under oxic and low oxygen conditions. For this we incubated sulfide minerals for four years on the seafloor at an active and an inactive venting side on the Indian Ridge and used hydrothermal vent material for laboratory experiments. We employed a combination of molecular biological techniques, microbial enrichment experiments, physiological studies, microscopy, and geochemistry, to identify key microbial actors responsible for mineral transformation and metal mobilization. SEM images reveal diverse mineral structures which are indicative of the presence of various microorganisms involved in Fe-cycling, including twisted stalks and nanowires. By determining turnover rates and mineral modifications, we aim to predict how microbial activity influences the lifetime of SMS deposits under different oxygen conditions.

P-EMP-032

Community composition of soil bacteria and soil respiration under different land use patterns in the Fichtelgebirge

*S. Anthony[1], N. Meyer[2], D. Thomas[1]

[1]University of Bayreuth, Center of Ecology and Environmental Research BayCEER, Ecological Microbiology, Bayreuth, Germany

[2]Goethe University Frankfurt, Soil Geography and Ecosystem Research Institute of Physical Geography, Frankfurt a. M., Germany

Aboveground–belowground interactions exert critical controls over many parameters of terrestrial ecosystems, i.e., community composition, primary production, and nutrient cycling. They are expected to continuously change regarding vegetation and basic soil properties, that is, C: N ratio, soil pH, and microbial community.

This project aimed to evaluate microbial community composition and the land use effect on the soil respiration rate of three different land types in a selected Fichtel Mountains temperate zone (Germany) region. Further, it aimed to identify the alpha and beta diversity variations in each land type and the genus-level microbial communities.

The project selected one hundred and twenty sample sites from forests, grasslands, and arable lands in the Fichtel mountains for soil DNA extraction, followed by 16S rRNA gene amplicon sequencing. In parallel, soil basal respiration was measured by a respirometer (respicond VI), and microbial biomass carbon was determined by substrate-induced respiration.

Using these methods and statistical models, the representative results demonstrated significant differences in bacterial community composition among the different land types. Bacterial communities in forest soils were clearly differentiated from those in grassland and arable land. Further, a general linear model analysis showed a strong correlation between forest land type and microbial biomass carbon. *Candidatus Udaeobacter* was found to be the most abundant genus in sample sites, which showed the highest soil respiration.

Results from this project will contribute to a better understanding of how alpha and beta diversity of microbial communities vary with land types and how microbial biomass affects the soil respiration amounts in temperate regions.

P-EMP-033

Environmental degradation of the natural colorant curcumin processes through chemical autoxidation and microbial degradation of the respective products

*J. Wessel[1], F. Gorecki[1], N. Theiß[1], C. L. Krämer[1], B. Philipp[1]

[1]University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany

While the environmental fate of non-degradable and fossil-based plastics is of high societal interest, the fate of plastic additives, such as colourants, is often overlooked. Some plastic additives are toxic and often poorly biodegradable, and could, thus, be considered as chemicals of emerging concern. The CurCol (curcumin-based sustainable colourants) project aimed to develop biodegradable colourants from natural and chemically modified curcumin. It was investigated whether these bio-based colourants are biodegradable and what happens to them when they are used as additives in bio-based plastics and exposed to the environment.

Various approaches were used to enrich and isolate bacteria from different environmental samples and the rhizosphere of curcuma plants using natural curcumin and structurally similar aromatic metabolites (ferulic acid, cinnamic acid, eugenol or vanillin). Neither the enrichment cultures nor the bacteria isolated with aromatic compounds were able to utilise curcumin as the sole carbon source. There was also no indication of co-metabolic conversion of curcumin.

Studies on the chemical stability curcumin and various of its yellow and red colour derivatives indicated pH-dependent autoxidation of curcumin to a bicyclopentadione (BCP). At elevated temperatures curcumin was chemically degraded to vanillin. Also, purified BCP was decomposed to vanillin and feruloylmethane at temperatures from 60 °C on. Curcumin, BCP and vanillin were also released from coloured polymers in a temperature-dependent way. No toxic effects of the colourant and its degradation products were detected, and vanillin was readily degraded by most of the isolated bacteria as well as by model organisms such as *Pseudomonas putida* KT2440.

These experiments showed that curcumin and its derivatives can be chemically degraded under environmental conditions occurring during composting and that the respective degradation products can serve as carbon and energy sources for environmental bacteria. Thus, curcumin-based colourants could constitute a bio-based and environmentally friendly additive for polymers.

P-EMP-034

Raw vegetables as a vehicle for pathogens and antimicrobial resistances in the Indian food-chain

*P. Kalpana[1], S. Yasobant[2], D. B. Saxena[2], C. Schreiber[3]
[1]*Center For Development Research (ZEF), ZEF-C, Bonn, Germany*
[2]*Indian Institute of Public Health Gandhinagar, Department of Public Health Sciences, Gandhinagar, India*
[3]*University Hospital Bonn, Institute for Hygiene & Public Health, Bonn, Germany*

Introduction: Antimicrobial resistance (AMR) has arisen as one of the fronting threats to public health. It possesses a multidimensional (social, economic, and environmental) challenge encompassing the food production system, influencing human and animal health. Antibiotics and antibiotic-resistant pathogenic bacteria are present in the environment naturally more important, anthropogenically influenced. Resistances can disseminate via raw vegetables consumption to human. But studies integrating the environment into health risks assessment by AMR transmission are rare, esp. in the south Asian region.

Goals: The study aims to investigate the role of vegetables in AMR spread by an agroecosystem exploration from a One Health perspective in Ahmedabad, India. This abstract focus on (resistant) human pathogenic bacteria in soil and raw vegetable.

Materials & Methods: A total of 312 samples were collected from peri-urban agricultural farms from 08/2022-05/2023. It comprises most frequently produced and raw consumed vegetables (spinach, spring onion, green garlic, coriander, radish) and the respective soils. Analysis of indicator bacteria in crops for human consumption as recommended by WHO (*E. coli, Salmonella, Klebsiella, Pseudomonas*) followed cultural standard procedures. AST pattern of the isolates were done by Kirby-Bauer disk diffusion technique using Muller Hinton Agar and CLSI guidelines. Microbiome and resistance genes analysis are recently ongoing.

Results: The microbiological assessment recorded that both soil and vegetable samples had considerable levels of microbial contamination, with highest loads of *Salmonella* (86.5% positive vegetable samples), and lowest of *E. coli* (6.4%). The corresponding soil samples were positive from 96.15%, and 14.7%.

AMR patterns of isolated shows multidrug resistance to at least 4 antibiotics in 36.4% of *E. coli* and 81% of *Salmonella.* 32.6% of *Klebsiella* but no *Pseudomonas* showed multidrug resistances.

Summary: The study shows the importance of microbiological assessment in agroecosystems in a country like India, one of the largest producers of fresh vegetables. Resistant pathogens found pose a certain health risk for consumers.

P-EMP-035

Closer to reality? Soil-free microbial cell extracts as a novel approach to understand microbial carbon use efficiency in soils.

*M. Varsadiya[1], F. Dehghani[2], E. Blagodatskaya[2], T. Lueders[1]
[1]*University of Bayreuth, Microbiology Ecology, Bayreuth, Germany*
[2]*UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Halle (Saale), Germany*

Microbial carbon use efficiency (CUE), the ratio of C retained in biomass to C assimilated by microbes, is central to our understanding of organic C turnover in soil. To unravel the factors that control CUE and to decipher patterns in C utilization and metabolic activity, the use of soil-free microbial cell extract (SFCE) seems promising. We propose that by isolating active microbial cells from soil, the amount of C taken up by specific populations and their efficiency of C utilization can be more precisely quantified in short-term incubations, than while facing the complex background of the soil matrix.

Therefore, we have revisited and optimized established protocols to extract microbial cells from agricultural soil via Nycodenz density gradients. The extracted cells were counted via fluorescent live-dead staining and accounted for up to ~25% of the original soil biomass. We then used calorespirometric measurements (metabolic heat and respiration) to compare CUE values of SFCE and intact soil under-provisioning of different substrates (glycerol, glucose, glutamine, and citric acid). Respiration data was collected for 24 and 48h, whereas metabolic heat was continuously measured.

A substantial fraction of viable microbial cells were extracted from the soil using Nycodenz, with numbers ranging from 10^7 to 10^8 per gram of soil. The CUE values calculated from calorespirometric ratios suggested that SFCE had a relatively higher per-cell CUE than intact soil during the initial 24h of incubation. Substrate-specific distinctions in heat production between both approaches were clearly apparent. Prokaryotic communities in soil and SFCE before and after incubation were analyzed via amplicon sequencing, to identify the taxa most responsive to substrate addition. The number of significantly enriched taxa in soil and SFCE compared to control samples were glycerol (0, 16), glucose (6, 41), glutamine (2, 46), and citric acid (1, 38), respectively.

The data generated from studies using SFCE provides a valuable new handle to refine models which are needed to predict how changes in environmental conditions, or climate scenarios may impact microbial CUE and C cycling in soils.

P-EMP-036

Impact of untreated and treated wastewater irrigation on antibiotic resistance genes and mobile genetic elements in three fifferent soil types

*L. Soufi[1], S. Gallego[2], K. Werner[1], B. J. Heyde[3], I. Kampouris[2], J. Siemens[3], E. Grohmann[1], K. Smalla[2]
[1]*Berliner Hochschule für Technik, Berlin, Germany*
[2]*Julius Kuehn Institute, Brunswick, Germany*
[3]*Justus-Liebig-University Giessen, Institute of Soil Science and Soil Conservation, Giessen, Germany*

Introduction: Wastewater containing pathogens, antibiotics, and antibiotic resistance determinants raises concerns about the risk of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) spreading to the environment, being mobilized from the resistome and transferred to potentially pathogenic bacteria.

Goals: This study investigates the impact of the change from untreated to treated wastewater for irrigation on the abundance and diversity of ARGs, MGEs, and the associated soil microbiome. The objective is to improve our understanding of the impact of plasmids and pollutants present in wastewater on the soil microbiome and the dissemination of ARGs.

Materials & Methods: In a soil incubation experiment, three different soil types were subjected to irrigation with treated or untreated wastewater, either spiked or not with antibiotics and biocides. Samples were collected at both four days and four weeks post-irrigation. DNA-based analysis was conducted by qPCR and amplicon sequencing of 16S rRNA gene.

Results: Soils irrigated with spiked wastewater containing antibiotics showed a higher relative abundance of sulfonamide resistance (*sul1*) gene, class 1 integron-integrase (*intI1*) gene, and plasmids belonging to the IncP-1 and pSK1 group. In soils irrigated with unspiked wastewater, trimethoprim and erythromycin resistance genes as well as *Staphylococcus* plasmids of the pI258 family were detected. Principal component analysis revealed a distinct separation in the distribution of ARGs and MGEs depending on the spiking level, sampling date and soil type. Conversely, the analysis of the soil microbial community by NMDS showed that only the soil type and sampling date had an impact on the composition of the soil microbial community.

Conclusions: Antibiotics and disinfectants applied to irrigation water increased the relative abundance of several ARGs in soils irrigated with wastewater. Antibiotics and disinfectants may have enhanced the occurrence of ARGs and the spread of MGEs, but they had little impact on the soil microbial composition.

P-EMP-037

Tree species specific rhizosphere microbiomes in the forest floor of temperate forests

*S. Bibinger[1], M. Wannenmacher[2], S. Kublik[1], C. Werner[2], F. Lang[3], M. Schloter[1,4], S. Schulz[1]
[1]*Helmholtz Center Munich, Institute of Comparative Microbiome Analysis, München, Germany*
[2]*University of Freiburg, Chair of Ecosystem Physiology, Freiburg i. Br., Germany*
[3]*University of Freiburg, Chair of Soil Ecology, Freiburg i. Br., Germany*
[4]*Technical University Munich, Environmental microbiology, München, Germany*

In forest ecosystems, the forest floor represents the boundary between mineral soil and atmosphere and as such acts as a hub for microbial nutrient turnover and transport. Likewise, the rhizosphere plays a pivotal role in nutrient turnover and is crucial for both plant nutrition and health. However, how plant-specific rhizosphere selection takes place in the forest floor, a hotspot for fine root biomass, remains insufficiently understood. The goal of this study was to elucidate, how different tree species (F. sylvatica, A. pseudoplatanus, P. abies) shape their bacterial rhizosphere microbiome in the forest floor as compared to the mineral soil. One the one hand, tree-specific effects are generally thought to dominantly impact the associated microbiome composition, especially based on their differential mycorrhization profiles and nutrient requirements. On the other hand the rhizosphere selection in the forest floor is generally expected to impact the community composition less than in the mineral topsoil, especially with regards to the increase of copiotrophic bacteria. Rhizosphere samples were collected from the three tree species in two horizons (forest floor and mineral topsoil) at four sites of contrasting climate and at two timepoints (May and September). Bulk soil samples were collected alongside and 16S rRNA gene amplicon sequencing was carried out with all samples. Microbial community composition but not alpha diversity was impacted by all analysed factors. Surprisingly, the soil compartment had the overall smallest impact on the microbiome composition while the tree species had a strong impact on its associated microbiome irrespective of compartment. The tree-specific selection effect was more pronounced in the mineral soil than in the forest floor as revealed by ordination. 16S rRNA gene copy number prediction hinted at species-specific enrichment of copiotrophic bacteria in the rhizosphere of either the mineral soil or the forest floor. Findings from this study enhance our understanding of how different tree species influence the composition of their associated microbiome in the mineral soil and in the forest floor.

P-EMP-038

Cultivation-based quantification of antimicrobial-resistant heterotrophic bacteria upon changing irrigation from untreated wastewater to treated wastewater in soils with different clay contents

*D. Pulami[1], D. Aryal[1], P. Isack[1], S. P. Glaeser[1], P. Kämpfer[1], B. Heyde[2], J. Siemens[2], C. Siebe[3]
[1]*Justus Liebig University Giessen, Institut für Angewandte Mikrobiologie, Giessen, Germany*
[2]*Justus Liebig University Giessen, Institute for Soil Science and Soil Conservation, Giessen, Germany*
[3]*Universidad Nacional Autónoma de México, Instituto de Geología, Mexico City, Mexico*

Reclaimed wastewater (WW) is often used for irrigation. After changing irrigation with untreated to treated WW it is supposed, that there could be short-term increase in antimicrobial resistant bacteria (ARB) following release of adsorbed pollutants. We hypothesize heterotrophic ARB abundance increase after changing irrigation (untreated to treated) in soils.

Here, an incubation experiment was performed with three soil types, Phaeozem, Vertisol and Leptosol with historically extended irrigation with untreated WW. Soils were incubated with untreated-unspiked, treated-unspiked, untreated-spiked and treated-spiked WW. Soil samples before incubation with WW were used as controls. A spot-assay technique was established to determine ARB abundance on MH (37°C/24h/potential pathogens) and R2A

(25°C/48h/environmental bacteria) both without and with CIP, TRI/SUL, ERY/CLI and BAC-C12. Samples were taken 4 weeks after irrigation.

WW treatment and spiking effected the abundance of ARBs (on MH, R2A) in irrigation water. Especially *Pseudomonas* and *E. coli* were present in treated and spiked WW. Independent of the soil type the irrigation shift from untreated to treated WW showed no significant effects on the relative abundance of ARBs (on MH, R2A). However, the relative abundance of potential pathogens (MH) was significantly higher ($p<0.05$) in the presence of TRI/SUL and ERY/CLI upon irrigation (all WW types) compared to the control for phaeozem. Presence of BAC-C12 caused significantly higher relative abundance of potential pathogens after irrigation (exception: untreated-unspiked) for phaeozem. Similarly, potential pathogen relative abundance was significantly higher after irrigation (all WW types) in presence of TRI/SUL (exception, treated-unspiked vs control) and BAC-C12 for leptosol. No interactions were observed between soil-types, treatments and spiking considering abundance of ARB (on MH, R2A).

In phaeozem and leptosol irrespective of the WW types, the fraction of TRI/SUL and BAC-C12 resistant potential pathogens increased compared to non-irrigated soil. Resistance screening will provide insights into the WW treatment based modification of the ARBs resistome.

P-EMP-039

Promoting autotrophic denitrification in shallow porous aquifers for drinking water production

*F. Pfaff[1], A. Seeholzer[2], A. Wunderlich[2], F. Einsiedl[2], T. Lueders[1]
[1]University of Bayreuth, Ecological Microbiology, Bayreuth, Germany
[2]Technical University, Hydrogeology, München, Germany

Global nitrate pollution in groundwater poses a great challenge. In Europe, groundwater is crucial for drinking water, necessitating sustainable management for shallow aquifers with high recharge. However, their attenuation potential against pollutants like nitrate is often limited. High nitrate levels persist in many aquifers even long after reducing nitrogen inputs. Persistent high nitrate levels in oligotrophic shallow groundwater systems call for urgent development of innovative and cost-effective technologies to eliminate nitrate loading and ensure a sustainable water supply, meeting the growing demand.

In this BMBF-funded project under the "LURCH – Nachhaltige Grundwasserbewirtschaftung" initiative, we aim to implement a novel approach for nitrate removal from shallow groundwater. Via the injection of reduced gaseous electron donors (H_2/CH_4), we aim to stimulate autotrophic denitrification directly upstream of burdened drinking water wells. In lab scale microcosms, we are currently simulating *in situ* conditions for a range of aquifer materials and amendments, while enrichment cultures of autotrophic denitrifying communities are established for potential stimulation within natural aquifers. A mesoscale flume experiment tests gas injection principles and monitors stimulation effects. The final aim is to implement this approach at a field-scale pilot plant, eliminating nitrate in an actual drinking water production system.

Preliminary data is suggesting that injecting H_2/CH_4 can effectively eliminate nitrate from groundwater aquifers without adversely impacting water quality or hydraulic characteristics through secondary reactions, like mineral dissolution or biofilm clogging. Current techniques for nitrate elimination mostly involve post-treatment of pumped groundwater with methods like osmosis, anion exchange, electrodialysis, or water treatment in bioreactors. Here, we want to harness autotrophic microbial potentials intrinsic to groundwater systems for a new and sustainable solution to a notorious problem. A more precise understanding of the microbiology involved in autotrophic denitrification in drinking water aquifers will support these developments.

P-EMP-040

Experimental biofilm-mediated mineralization of leaves

*S. Karacic[1], B. Palmer[2], C. Gee[2], G. Bierbaum[1]
[1]University Hospital Bonn, Institute of Medical Microbiology, Immunology and Parasitology, Bonn, Germany
[2]Institute of Geosciences, Division of Palaeontology, University of Bonn, 53115 Bonn, Germany, Bonn, Germany

Microorganisms can colonize and form biofilms on leaves and soft tissues. The mineralization of soft tissues is influenced by biofilm development and complex microbial communities. This study aimed to develop leaf biofilms in vitro on *Hedera* leaves, explore the microbial community composition, and investigate conditions under which the leaf biofilms will form a mineralized surface layer.

We examined the temporal dynamics of microbial communities over three weeks under aerobic and anaerobic conditions and isolated single bacterial strains from decaying leaves to reconstitute biofilm microbial communities in the laboratory. Biofilm-covered leaf samples were collected weekly and investigated by scanning electron microscopy. We used 16S rRNA to measure microbial diversity and temporal changes in microbial communities of *Hedera* leaves. To characterize the conditions that lead to mineralization, the biofilm-coated leaves were exposed to different compounds that might enhance mineralization. We observed a compositional shift in bacterial communities over three weeks, with oxygen availability and incubation time influencing microbial diversity. The genera *Vogesella*, *Tolumonas*, *Rhodoferax and Aeromonas* were initially abundant and decreased over time. We isolated 83 bacterial strains from biofilm-coated decaying leaves in vitro. The addition of a minimum of 80mg/ml of $Ca2+$ concentration was found essential for mineralization, and the addition of the *Shewanella sp.* strain enhanced FeSx mineral formation under slightly acidic pH. These findings provide valuable insights for further investigations of leaf biofilms in the context of biomineralization and preservation.

P-EMP-041

Bacterial degradation of polymer plasticizers on the example of diethyl phthalate (DEP)

*S. Bertoldi[1], Z. Chauhan[1], N. Sharma[1], D. Schlosser[2], N. Marcel[2], H. J. Heipieper[1], C. Eberlein[1]
[1]Helmholtz Centre for Environmental Research (UFZ), Molecular environmental biotechnology, Leipzig, Germany
[2]Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany

The continuing reports of plastic pollution in various ecosystems highlight the threat posed by the ever-increasing consumption of synthetic polymers. Plastics are frequently associated with additives such as phthalic acid esters. Such compounds are used as plasticizers to provide flexibility to plastic products and as common additives in various

consumer products, and suspect to cause endocrine disruption in animals.

The bacterial degradation of diethyl phthalate (DEP) as a model compound for plasticizers was studied. In order to isolate bacterial DEP degraders, samples from biofilm on a polyurethane tubing were taken. A bacterial isolate from this biofilm was able to grow in mineral medium with DEP as sole carbon and energy source. The complete degradation of up to 4 mM DEP was confirmed by UPLC analysis. Furthermore, the substrate spectrum of the bacterial culture was assessed by testing different carbon source like trihydroxybenzene, phthalic acid and other aromatics. The bacterial isolate was taxonomically characterized and probably contains at least two bacterial species. In depth 16S rRNA gene sequencing and genome sequencing were conducted to identify the bacterial composition of the DEP degrading culture.

P-EMP-042

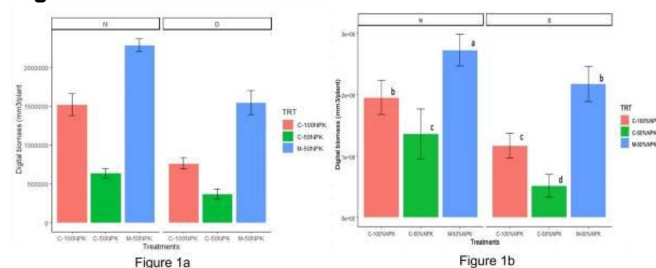
Plant Growth Promoting Bacteria from Mangrove Ecosystems Enhance Tomato (Solanum lycopersicum) seedling growth under induced Salt, Drought Stress and reduced chemical fertilization

*M. A. Khan[1], R. S. Alhemeiri[1], S. Tounsi-Hammami[1]
[1]*Zayed University, College of Natural and Health Sciences, Dubai, United Arab Emirates*

Abiotic stress conditions like salinity, drought, and climate change threaten agriculture and food security worldwide. Drought and salt stress alleviation in plants might be substantially facilitated by plant growth-promoting bacteria (PGPB). The main research questions addressed were: Do the selected native PGPB strains tolerate abiotic stress such as pH variation, salt, and drought stress under *in vitro* conditions? Do native PGPB strains and a reduced rate of NPK chemical fertilization improve tomato seedling growth parameters compared to seedlings given 100%NPK? Two endophytic bacteria isolated from the roots of mangroves in the United Arab Emirates, *Bacillus wiedmannii* (S1) and *Bacillus licheniformis* (S2), were used to inoculate the plants. The strains and their mixture underwent tolerance testing to abiotic stresses, including pH, salinity, and drought stress. To induce salt stress, 100 mM NaCl solution was applied to the tomato plants after germination in the pot experiment. During the last two weeks of the study, plants were not irrigated to initiate drought stress.

A fully randomised design with three replicates demonstrated that the PGPB strains could improve the digital biomass, leaf area, plant height, greenness average, and the normalized digital vegetation index under induced saline and drought conditions. Inoculated plants that were exposed to drought stress exhibited enhanced levels of digital biomass (Figure 1a), leaf area, plant height, average greenness, and normalized digital vegetation index by 102%, 75%, 15%, 20%, and 2%, respectively compared to plants that received the complete dose of NPK fertilizer during drought stress. Digital biomass (Figure 1b), leaf area, plant height, and normalized digital vegetation index were higher in PGPB-inoculated plants when subjected to salt stress by 87%, 37%, 39%, and 21%, respectively, compared to plants receiving 100% NPK. This study concluded that mangrove-derived PGPB could encourage tomato seedlings' growth and resilience to salt and drought stress under minimum chemical fertilizer application.

Fig. 1



P-EMP-043

Enrichment of CO_2 utilizing microorganisms from the Eger Rift subsurface ecosystem

*D. Lipus[1], Z. Jia[1], A. Bartholomäus[1], D. Wagner[1,2], J. Kallmeyer[1]
[1]*GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Geomicrobiology, Potsdam, Germany*
[2]*University of Potsdam, Geosciences, Potsdam, Germany*

Introduction:

The Eger Rift in Western Bohemia (CZ) stands out as a unique subsurface ecosystem due to frequent tectonic activity, high CO_2 fluxes, and periodic seismic release of H_2. This environment offers a scientifically relevant opportunity to study microbial subsurface processes, particularly carbon cycling. Genomic analyses of drill core and formation water samples reveal a surprisingly diverse microbial community, including methanogenic archaea, acetogens, sulfate reducers, and cyanobacteria, suggesting Eger Rift microorganisms to employ a range of metabolic approaches to utilize CO_2.

Goal:

Our study aims to investigate native Eger Rift organisms by employing diverse culturing methods to enrich, isolate, and characterize potential CO_2 utilizers. The overarching objective is to identify and describe native microbial carboxylation reactions.

Methods:

We conducted anaerobic enrichments over six months under a high H_2/CO_2 atmosphere to enrich methanogenic archaea, and six-week aerobic enrichments focused on the growth of cyanobacteria under atmospheric conditions. Characterization involved amplicon and metagenomic ONT sequencing. Metagenome assembled genomes (MAGs) were reconstructed and functionally annotated. Ongoing isolation efforts using agar shakes (anaerobic) and streak plates (aerobic) are in progress.

Results:

Anaerobic enrichments yielded a circular *Methanobacterium* genome and a MAG of a novel species of hydrogenotrophic *Methanosphaerula* from 60 m deep Eger sediments. Cyanobacteria only grew in cultures inoculated with 118 m and 194 m deep sediments, while other aerobic

microorganisms, including *Arthrobacter*, *Polaromonas*, and *Rhizobium*, could be enriched from different depth. Genomic and functional characterization revealed the metabolic potential of anaerobic (Wood Ljungdahl) and aerobic (CBB) CO_2 fixation, as well as H2 utilization pathways.

Summary:

Our ongoing cultivation work supports previous findings, suggesting that, in addition to methanogenic archaea, several aerobic microorganisms, including cyanobacteria, likely inhabit the Eger Rift subsurface and utilize naturally occurring CO_2.

P-EMP-044
Identification of phage receptors in the fire blight pathogen Erwinia amylovora

L. E. Knecht[1,2], S. Gayder[1], C. Pelludat[3], M. J. Loessner[2], *L. Fieseler[1]
[1]*Zurich University of Applied Sciences, Institute of Food and Beverage Innovation, Wädenswil, Switzerland*
[2]*ETH Zurich, Institute of Food; Nutrition and Health, Zürich, Switzerland*
[3]*Agroscope, Phytopathology of Fruits and Vegetable Crops, Nyon, Switzerland*

Erwinia amylovora is a member of the *Erwiniaceae* and the causative agent of fire blight, a severe disease of *Rosaceae* plants. *E. amylovora* usually infects host blossoms via the stigma and invades the ovary. It spreads through the xylem of an infected plant. In the xylem *E. amylovora* produces exopolysaccharides (EPS), e.g., a capsule, which leads to ooze formation and canker development. The capsule is composed of amylovoran, levan, and cellulose, respectively.

Transposon mutagenesis of *E. amylovora* revealed that adsorption of the T7-like phage L1 and the SP6-like phage S2 (both *Autographiviridae*) is dependent on the amylovoran synthesis (*ams*) operon. Accordingly, both phages exhibit a Depolymerase (Dpo) with 59 % amino acid identity. The enzyme is a structural component of the virion. DpoL1 binds specifically to amylovoran and cuts the galactose backbone. In addition, adsorption of phage S6 (*Schitoviridae*) and M7 (*Myoviridae*) depends on the bacterial cellulose synthesis (*bcs*) operon. Deletion of the *bcs* operon or associated genes (*bcsA*, *bcsC,* and *bcsZ*) verified the crucial role of bacterial cellulose for S6 and M7 infection. Application of the cellulose binding dye Congo Red blocked infection by both phages. In addition, we demonstrate that infective S6 virions degraded cellulose to glucose molecules and that Gp95, a phage encoded cellulase, is involved to catalyze the reaction. *In vitro* treatment of a growing *E. amylovora* culture with DpoL1 or GP95 did not inhibit growth. However, application of DpoL1 together with the capsule-independent phage Y2 (*Chaseviridae*) revealed a strong synergistic inhibitory effect and caused a 4 log reduction of viable cell counts. Y2 is highly specific to *E. amylovora* and solely relies on LPS for adsorption. Transposon mutagenesis revealed four so far unrecognized genes in *E. amylovora*, which play a central role in both, LPS and/or EPS synthesis. Deletion of each gene affected either LPS or EPS composition and resulted in highly reduced adsorption and efficacy of plating not only for Y2, but also for other phages that infect *E. amylovora*. Most of the mutants were highly attenuated in virulence on detached blossoms.

Eukaryotic Pathogens

P-EP-001
The effect of lung colonisation with Candida albicans on Staphylococcus aureus infection

*T. Köhler[1], N. E. Nieuwenhuizen[1], I. D. Jacobsen[2], E. S. Ibrahim[3], K. Ohlsen[3], O. Kurzai[1]
[1]*Institute for Hygiene and Microbiology, Institute for Hygiene and Microbiology, Würzburg, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute, Microbial Immunology, Jena, Germany*
[3]*Institute of Molecular Infection Biology, AG Ohlsen, Würzburg, Germany*

Introduction: *C. albicans* is a commensal fungus found on mucosal surfaces, such as the gastrointestinal tract. The gram-positive bacterium *S. aureus* is also part of the human microbiome. In immunocompromised patients, both pathogens can cause severe systemic infections by disseminating via the gut or skin into the bloodstream. However, whereas *S. aureus* regularly causes pneumonia and disseminates from the lung into other parts of the body, *C. albicans* almost never causes invasive lung infections, despite massively colonising the lungs of mechanically ventilated patients.

Goals: We aim to determine the mechanism behind the different behaviour of *C. albicans* and *S. aureus* in the lung, and the effect of *C. albicans* on *S. aureus* infection.

Materials & Methods: We established a novel murine lung colonisation/infection model using 8-10-week-old Balb/c mice. Mice were administered $5x10^6$ colony forming units (CFUs) of *C. albicans* intranasally at day 0 and infected intranasally with 10^8 CFUs *S. aureus* on day 1. At 24 and 48 hours post *S. aureus* infection, lung, liver and kidney bacterial/fungal burdens as well as lung and spleen immune responses were analysed.

Results: After intranasal administration, *C. albicans* numbers decreased while *S. aureus* numbers increased. *S. aureus* disseminated into the liver and kidney, whereas *C. albicans* did not. This mimics the clinical situation where *C. albicans* does not cause invasive infection via the lungs, but *S. aureus* does. *S. aureus* did not affect lung *C. albicans* loads, but *C. albicans* had a slight effect on *S. aureus* dissemination. Short-term *C. albicans* colonisation led to increased numbers of neutrophils, eosinophils and CD11b+ dendritic cells in the lung. Single cell RNA sequencing allowed us to analyse a wide range of lung myeloid cells including neutrophils, monocytes, inflammatory macrophages, natural killer cells, alveolar macrophages and dendritic cells.

Summary: We successfully established a mouse model of *C. albicans* and *S. aureus* colonisation/infection in the lungs. The effects of *C. albicans* on *S. aureus* infection will be further investigated, and mutant strains will be used to explore host-pathogen interactions.

P-EP-002
Towards the establishment of functional epidemiology for human cryptosporidiosis

*C. Klotz[1], R. Ignatius[2,3], S. Navaratnam[1], M. Grensemann[1], M. Laue[4], T. Aebischer[1]

[1]Robert Koch-Institute, Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany
[2]MVZ Labor 28, Berlin, Germany
[3]Charité-University Medicine, Institute of Microbiology, Infectious Diseases and Immunology, Berlin, Germany
[4]Robert Koch-Institute, Unit for Advanced Light and Electron Microscopy, Berlin, Germany

1. **Introduction**

Tools for functional assessment of human cryptosporidiosis are still inadequate. Advances in stem cell-based organoid technologies resembling the primary intestinal epithelium of humans supports full life cycle progression of the parasite and allows new applications toward the comparison of the pathogenicity of different species or genotypes.

2. **Objectives**

The overall aim of the project is to compare the pathogenicity of major Cryptosporidium genotypes circulating in Germany in stem cell derived organoid systems. In a pilot study, we evaluated the most common Cryptosporidium types in humans in Germany. We furthermore established an experimental infection model based on a previously described human stem cell derived intestinal organoid system.

3. **Materials & Methods**

Cryptosporidium positive samples were characterized by PCR for species determination and genotyping. Experimental infection of human small intestinal organoid derived monolayer (ODM) were performed with C. parvum gp60 genotype IIaA15G2R1 and evaluated by qPCR, immunofluorescence analysis (IFA) and by electron microscopy.

4. **Results**

Of 125 Cryptosporidium positive human cases, 28% were determined as C. hominis and 65% as C. parvum. Gp60 typing revealed a variety of genotypes for both C. hominis (dominant type Ib10G2) and C. parvum (dominant type IIaA15G2R1). Evaluation of experimental infection of ODMs with sporozoites by qPCR showed approximately 10-fold increase of genome equivalents after 6 days post infection. The IFA analysis confirmed parasite infection and proliferation within the brush border of enterocytes. In the EM analysis trophozoites and meront stages were detected after 24 hours whereas sexual stages could be observed after 72 hours.

5. **Conclusion**

Our pilot study provides a first overview of dominant C. hominis and C. parvum genotypes circulating in Germany. We confirmed the suitability of our well-established ODM model for Cryptosporidium infection. Next steps will include more systematic sampling of human cryptosporidiosis cases in Germany and we will further pursue the ODM infection model to unravel the molecular basis of host-parasite interaction.

P-EP-003

The evolution of Candida albicans in the modern human host – Analysis and comparison of strains from unusual sources

*P. M. Jansen[1], S. Brunke[1], B. Hube[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*

In modern Western societies, the yeast *Candida albicans* is frequently found as a commensal member of the gut microbiota, which serves as a reservoir for severe infections. The human gut microbiota has changed significantly due to industrialization. Interestingly, a microbiome study of a mostly isolated, non-Western human population found little indications of *C. albicans* colonization. Furthermore, *C. albicans* strains from potential environmental reservoirs are still rarely found and little researched.

We aim to understand the evolution of *C. albicans* as it adapted to the human host and what effect the changes in human lifestyle had during industrialization and the advent of modern medicine on this member of the human microbiome.

Over 30 strains from the environment, isolated human populations, and animals were examined to elucidate the changes *C. albicans* has undergone during its co-evolution with modern humans. This included metabolic, genomic, antifungal sensitivity, and virulence tests. Laboratory evolutionary experiments explored how *C. albicans* adapted to conditions in the modern Western gut.

We found differences between human-derived and environmental strains, with the former showing greater virulence potential as measured in vitro damage to epithelial cells. Some of these human commensal isolates were even more damaging than clinical isolates. In general, the metabolic spectrum of environmental strains was narrower than that of human-derived strains, especially for sugars. Importantly, several non-clinical strains showed resistance to typical antifungal agents. We continuously cultured non-human adapted strains in media containing different dietary sugars for months. The resulting human-adapted strains were specialized to grow better on the dietary sugar provided, but worse on other sugars. A genome-level comparison of the metabolic pathways of the adapted strains, their environmental progenitors, and human isolates is underway.

Our findings highlight substantial differences in metabolism and virulence between human-derived and environmental *C. albicans* strains. An evolutionary trade-off in adapting to sugars typical of the human diet seems evident.

P-EP-004

The role of putative mTORC-related kinases in translational regulation during Plasmodium falciparum transmission

*S. Bennink[1], F. Müller[1], A. Rnjbal[1], J. Müller[1], M. Wesselbaum[1], C. Kühne[1], G. Pradel[1]
[1]*RWTH Aachen University, Division of Cellular and Applied Infection Biology, Aachen, Germany*

After transmission from the human to the mosquito, the malaria parasite *Plasmodium falciparum* encounters a new environment to which it quickly needs to adapt. Among

others, stage specific proteins must be synthesized, e.g. for host cell egress and sexual reproduction. Transcripts coding for some of these proteins are already synthesized in the human host, while translation is initiated only after transmission. We have identified the protein 7-Helix-1 as a crucial component in the process of translational re-initiation. 7-Helix-1 was shown to localize to stress granules where it interacts with known ribonucleoproteins and binds repressed mRNAs. In silico analyses demonstrated that 7-Helix-1 is homologous to the human stress regulator hLanCL2, which is involved in the mTORC-pathway. Although *P. falciparum* has lost most of the mTORC-components, we hypothesize that 7-Helix-1 regulates translation via an mTORC-like signaling cascade.

In order to decipher the potential link between 7-Helix-1, mTORC-related components and translation, we will characterize the downstream kinases of the mTORC-pathway, KIN, PI3K and S6K, which are present in *P. falciparum*. To investigate the role of the three kinases during translational regulation, knockout (KO) parasite lines are generated and characterized. Further, the KO studies will be complemented with chemical inhibition/activation studies using commercial inhibitors and activators.

RT-PCRs revealed transcript expression for all three kinases in mature and activated gametocytes. Chemical inhibition of KIN by dorsomorphin lead to impaired parasite growth and developmentally arrested schizonts. Further, dorsomorphin-treated parasites were shown to be impaired in stress-induced regulation of translation activity.

The mTORC pathway is one of the most important signaling cascades in mammalian cells integrating nutrient availability and regulating protein translation. In this project, we will investigate the potential role of mTORC-related kinases in translational regulation. Data gained in this project will help us understand how the parasite is able to specifically regulate protein synthesis depending on the environment.

P-EP-005

A novel pangenome reference for capturing the interstrain diversity of Aspergillus fumigatus in 'omics approaches

*M. Perrier[1,2], A. Barber[1,2]
[1]*Friedrich Schiller University Jena, Institute for Microbiology, Jena, Germany*
[2]*Friedrich Schiller University Jena, Cluster of Excellence Balance of the Microverse, Jena, Germany*

Aspergillus fumigatus is a ubiquitous pathogenic mould found in the environment. This fungus is the species most frequently responsible for invasive aspergillosis disease, a serious infection with a high mortality rate responsible for over 1.5 million deaths annually. *A. fumigatus* possesses phenotypic variations among its strains, including in the degree of virulence. Pangenomic studies on *A. fumigatus* have hypothesised that these differences could come from the wide variation in gene content among strains. This includes a large number of accessory genes that are specific to subpopulations of *A. fumigatus* and represent 30% of the pangenome. Due to their absence in the main reference strains, most of these genes are not yet functionally characterised.

To better understand the functional role of these uncharacterised genes, we have created a pangenome reference of *A. fumigatus* that encodes the entire gene collection of the species and their variation. This novel tool can be used as a reference for genomic and transcriptomic analyses. To construct the pangenome reference, we used a representative collection of 15 isolates' long-read sequence data that we de novo assembled into near chromosomal assemblies. Individual genomes were combined into a pangenome graph, and the gene annotations were imposed onto the graph. This reference pangenome encapsulates the diversity of *A. fumigatus* by including genes absent from the linear reference Af293 and opens new perspectives to describe these genes functionally. By using this new reference with transcriptomic data, we aim to understand the role of the accessory genome and intraspecies diversity in the virulence and phenotypic differences of *A. fumigatus*.

P-EP-006

Phosphoproteomic analysis of A. fumigatus reveals autophagy regulation during hypoxic growth

L. Ivanova[1], T. Krüger[1], A. Bigalke[1], T. Heinekamp[1], A. A. Brakhage[1,2], *O. Kniemeyer[1]
[1]*Leibniz-Institut für Naturstoffforschung und Infektionsbiologie Hans-Knöll-Institut, Molecular and Applied Microbiology, Jena, Germany*
[2]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*

Introduction

The mould *Aspergillus fumigatus* is an opportunistic human pathogen, which can cause multiple diseases ranging from life-threatening invasive pulmonary aspergillosis in immunocompromised patients to chronic, noninvasive forms of infection and allergies. The ability of the fungus to sense and adapt to low environmental oxygen concentrations is an important virulence trait. In order to study the adaptation response on the protein and posttranslational level, we performed quantitative proteomics and phosphoproteomics analyses by comparing fungal mycelium grown under either normoxic or hypoxic (0.2% O2) conditions in an oxygen-controlled fermenter. The goal was to find novel regulatory circuits in hypoxic adaptation.

Methods

Proteins were extracted from *A. fumigatus* mycelium and digested in solution with a trypsin/LysC mixture. Subsequently, phosphopeptides were enriched using a TiO_2/ZrO_2 solid phase extraction protocol. All samples were analyzed in triplicates by nanoLC-MS/MS (Thermo QExactive HF). The pTmRS algorithm was used to calculate phosphosite probabilities and the Minora algorithm for label-free quantification.

Results

We identified in total 5136 proteins, of which 318 proteins and 1674 phosphopeptides showed significantly different abundance upon hypoxia (fold change >4, ratio-adjusted p-value <0.05). In particular proteins involved in mitochondrion organization, amino acid metabolism, and lipid metabolic processes increased in abundance under hypoxia. The phosphoproteomic data indicated that the mitotic cell cycle and autophagy processes are differentially regulated under hypoxic growth. Indeed, phosphopeptides derived from proteins of the Atg1/Atg13 complex, which is known to initiate autophagosome formation, showed drastic changes in phosphorylation under hypoxia.

Conclusions

Phosphoproteomics of *A. fumigatus* revealed an involvement of autophagy under hypoxic growth conditions. Initial experiments have started to investigate the impact of autophagy on hypoxic growth in *A. fumigatus*

P-EP-007

The Candida Albicans quorum-sensing molecule farnesol modulates lipid metabolism in human monocyte-derived dendritic cells

*M. Batliner[1], F. Schumacher[2], D. Wigger[2], W. Vivas[3,4,5], I. Fohmann[1], A. Prell[2], T. Köhler[1], A. Riedel[6], M. Vaeth[7], B. Kleuser[2], O. Kurzai[1,8,9], N. E. Nieuwenhuizen[1]
[1]*Julius-Maximilians-University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[2]*Free University of Berlin, Institute of Pharmacy, Berlin, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute, Jena, Germany*
[4]*Jena University Hospital- Friedrich Schiller University, Institute for Infectious Diseases and Infection Control, Jena, Germany*
[5]*Jena University Hospital- Friedrich Schiller University, Department of Anesthesiology and Intensive Care, Jena, Germany*
[6]*University Hospital of Würzburg, Mildred Scheel Early Career Center (MSNZ), Würzburg, Germany*
[7]*Julius-Maximilians-University of Würzburg, Institute of Systems Immunology, Max Planck Research Group, Würzburg, Germany*
[8]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute, Research Group Fungal Septomics, Jena, Germany*
[9]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute, National Reference Center for Invasive Fungal Infections, Jena, Germany*

Introduction: Lipids play a regulatory role in immune responses and inflammation. Sphingolipids can influence the expression of surface markers on dendritic cells (DCs) and affect their capacity to phagocytose the fungal pathogen *Candida albicans*. Farnesol, a quorum-sensing molecule produced by *C. albicans*, alters the expression of antigen-presentation markers on human monocyte-derived DCs (moDCs) resulting in less potent T cell priming. Therefore, the aim of this study is to investigate the effects of farnesol on the sphingolipid metabolism in human moDCs and explore the underlying molecular mechanism and functional consequences of farnesol-induced changes in sphingolipid composition.

Methods: Primary human monocytes were differentiated to moDCs in the presence of farnesol or solvent control. Quantification of sphingolipid metabolites was achieved by HPLC-MS/MS. The activity of specific enzymes involved in the *de novo* synthesis of sphingolipids was detected using a cell-free sphingolipid *de novo* synthesis assay and deuterated metabolites. The molecular mechanism underlying the changes in lipid composition was investigated by detecting reactive oxygen species (ROS) using DCF and MitoSox dyes. Mitochondrial respiration was measured using an Extracellular Flux Seahorse Analyzer.

Results: We found that farnesol increases the intracellular content of dihydrosphingolipid species in moDcs. The activity of the first enzyme in the sphingolipid *de novo* synthesis pathway is significantly enhanced upon treatment with farnesol, causing the accumulation of downstream metabolites. In addition, farnesol inhibits dihydroceramide desaturase (Des) activity, the last enzyme in the *de novo* synthesis pathway. We could link the indirect inhibition of Des by farnesol to the generation of ROS originating from the mitochondria. Further, the associated dihydroceramide accumulation may most likely account for the deficient mitochondrial function observed in our model.

Summary: In summary, our data reveals novel effects of farnesol on sphingolipid metabolism and mitochondrial function in DCs, indicating that *C. albicans* can manipulate host cell metabolism via farnesol secretion.

P-EP-008

Identification of host immune mediators promoting Candida albicans adaptation to inflammation

*C. Fernández Fernández[1], S. Dinçer[1], M. Himmel[1], T. Krüger[2], O. Kniemeyer[2], A. A. Brakhage[2,3], A. Dietschmann[1], M. S. Gresnigt[1]
[1]*Leibniz-HKI, Junior Research Group Adaptive Pathogenicity Strategies, Jena, Germany*
[2]*Leibniz-HKI, Department of Molecular and Applied Microbiology, Jena, Germany*
[3]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*

During microbial infections, there is a dynamic interplay between the host and the pathogen. An appropriate adaptation to the inflammatory environment of the host during infection is crucial for pathogen success and persistence within the host. *Candida albicans* is an opportunistic pathogen that can cause invasive life-threatening infections in immunocompromised individuals. Yet, its existence as a common commensal yeast on human mucosal surfaces fostered the evolution of different adaptation strategies that allow it to evade or escape from the host immune response. In line with this, we have investigated how *C. albicans* uses soluble signals from activated human macrophages to increase its stress resistance and, consequently, its ability to escape macrophages.

To understand how *C. albicans* senses and adapts to the inflammatory environment during its pathogenic stage, an inflammation-adaptation model has been established. In this model, monocyte-derived macrophages (MDMs) are stimulated with inactivated *C. albicans* morphotypes, which elicit inflammatory responses. Macrophage activation results in the release of a complex mixture of immune mediators into the supernatant, which is then used for *C. albicans* culture *in vitro* and assessment of fungal adaptations. Supernatant composition was analyzed by fractionation and proteomics to identify potential immune mediators inducing fungal adaptation.

Several immune mediators that could potentially be sensed by the fungus to trigger fungal adaptations were identified. Among the molecules present in the supernatants, the macrophage cytoplasmic enzyme lactate dehydrogenase (LDH) has been identified as a potential promoter of fungal stress resistance. Specific effects on *C. albicans* virulence, growth, and metabolism are currently being assessed.

Collectively we observe that changes in the host inflammatory environment can induce *C. albicans* stress resistance. Understanding how specific immune mediators influence this adaptation and the host-pathogen interplay can foster the understanding of the persistence of pathogens like *C. albicans* and might be relevant to identify new therapeutic avenues.

P-EP-009

Exploring the role of Candida albicans Ece1 peptides in promoting fungal gut commensalis

*T. B. Schille[1,2], S. H. Liang[3], S. Sircaik[3], M. Hänel[1], A. Starick[4], S. Mogavero[1], S. Allert[1], K. Papenfort[4,2], R. Bennett[3], B. Hube[1,4,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*
[2]*Friedrich Schiller University Jena, Cluster of Excellence Balance of the Microverse, Jena, Germany*
[3]*Brown University, Department of Molecular Microbiology and Immunology, Providence, RI, United States*
[4]*Friedrich Schiller University Jena, Institute of Microbiology, Faculty of Biological Sciences, Jena, Germany*

Candida albicans, an opportunistic fungal pathogen, poses a significant risk to human health by causing very frequently superficial infections, but also severe systemic infections under certain predisposing conditions. The current dogma of *C. albicans* commensalism is that the yeast morphology is the preferred growth form during gut colonization, while hyphae are detrimental for commensal growth in the gut and rather required and indispensable for pathogenesis. Hypha formation is associated with a strong induction of the gene *ECE1,* encoding candidalysin (CaL) - the first (ribosomal) peptide toxin identified in a human pathogenic fungus – and seven additional non-candidalysin Ece1 peptides (NCEPs). While CaL directly inflicts damage to human cells, we propose that Ece1 may also act on bacteria of the human microbiota during commensalism.

Using *in vivo* competition models, we confirmed that the yeast morphology in fact favors murine gut colonization in models using antibiotics to remove antagonistic bacteria. However, hypha formation plays a crucial role to facilitate *C. albicans* colonization in hosts with either an undisturbed gut microbiota or carrying specific bacterial populations. In these niches, hyphal competent *C. albicans* wildtype cells exhibited a fitness advantage over yeast-locked mutant cells, promoting successful colonization. This attribute is primarily due to the expression of *ECE1*.

We explored the effects of Ece1 on co-colonizing bacteria by assessing the susceptibility of selected bacteria from different body sites to Ece1 peptides. Our screening revealed that CaL influences the growth of several members of the microbiota and modulates bacterial properties. We are currently analyzing the transcriptional response of gut bacteria to CaL stimulation to understand the mechanisms of *Candida*-bacteria cross-kingdom interactions.

Our study provides evidence that CaL has evolved to improve fungal fitness during competition with bacterial members of the human microbiota, shaping microbial communities during commensalism or polymicrobial infections in the human gut through inter-kingdom competition.

P-EP-010

Employing various human Macrophage in vitro models to determine early key factors in successful defense upon Aspergillus fumigatus encounter

*J. Söhnlein[1], Z. Abboud[2], M. Seif[1], D. Sheta[2], H. Einsele[3], A. Beilhack[2], J. Löffler[1]
[1]*University Hospital Würzburg, Medicine II - AG Löffler, Würzburg, Germany*
[2]*University Hospital Würzburg, ZEMM - AG Beilhack, Würzburg, Germany*
[3]*University Hospital Würzburg, Würzburg, Germany*

Aspergillus fumigatus, a saprotrophic mold commonly found in soil on decaying matter, propagates via spore formation and aerial distribution. Inhalation of these spores by immunocompromised patients can cause life-threatening invasive aspergillosis. Healthy individuals also inhale hundreds to thousands of spores daily but still maintain lung homeostasis. The mechanisms determining whether this airborne pathogen can be contained or will invade the alveolar epithelia to cause infection remains elusive.

We aim to investigate early decision points in alveolar macrophages upon initial encounter with *A. fumigatus* by deploying various human macrophage (MΦ) populations.

We utilized the recently published AML cell model [1] and screened it for primary alveolar MΦ markers. Likewise, we stimulated these cells with *A. fumigatus*, employing GM-CSF MΦs as controls and ELISA as well as qPCR as readouts. For further comparison, AML cells and GM-CSF MΦs were challenged with *A. fumigatus* conidia at different time intervals, followed by dual RNA-sequencing. Transcriptomic profiles were evaluated comparing shared and characteristic expression patterns of MΦs specific to defined *A. fumigatus* morphotypes. Likewise, gene expression of *A. fumigatus* confronted with different MΦ populations was analyzed. In addition, we compare these MΦ profiles with pre-existing gene expression profiles of other myeloid immune cells challenged with *A. fumigatus* based on archived GEO data sets. This will reveal shared but also population-specific profiles as well as characteristic *A. fumigatus* counter defense strategies. Furthermore, we employ the FLARE conidia model [2] in flow cytometry and Single-Cell analysis to decipher molecular differences in phagocytic and killing ability of the MΦ types.

We evaluate the AML model for infection with the fungal pathogen *A. fumigatus* and compare it with GM-CSF and primary MΦs. Through this, we will be able to identify initial key factors of MΦs to prevent lung infection by *A. fumigatus.*

[1] Pahari et al. *mBio* vol. 14,4 (2023): e0083423. doi:10.1128/mbio.00834-23

[2] Jhingran et al. *Cell Reports* vol. 2,6 (2012): 1762-73. doi:10.1016/j.celrep.2012.10.026

P-EP-011

Identification of Candida albicans protein kinase genes which regulate filamentation-independent cytotoxicity

*A. Möslinger[1], B. Ramírez-Zavala[2], R. Alonso-Román[1], S. U. J. Hitzler[3], S. Allert[1], J. Morschhäuser[2], L. Kasper[4], M. S. Gresnigt[3], B. Hube[4,5]
[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Department of Microbial Pathogenicity Mechanisms, Jena, Germany*
[2]*Julius Maximilians University of Würzburg, Institute of Molecular Infection Biology, Würzburg, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute, Junior Research Group Adaptive Pathogenicity Strategies, Jena, Germany*
[4]*Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute, Department of Microbial Pathogenicity Mechanisms, Jena, Germany*
[5]*Friedrich Schiller University Jena, Faculty of Biological Sciences, Institute of Microbiology, Jena, Germany*

Microbial signal transduction pathways regulate adaptation to various environmental conditions and enable pathogens to cause infections. Most of these pathways are regulated by protein kinases. The opportunistic fungal pathogen *Candida albicans* exists as a commensal of the intestinal mycobiota and can cause disseminated candidiasis upon certain circumstances. Both commensalism and pathogenicity require a complex network of signalling pathways. The *C. albicans* genome was predicted to encode 108 protein kinases yet nearly 50% remain uncharacterised. We aim to dissect the role of *C. albicans* protein kinases during commensalism and infection. To investigate the pathogenicity of *C. albicans*, an *in vitro* intestinal epithelial cell (IEC) infection model was used. A library containing individual mutants lacking each of the 108 protein kinase genes identified in *C. albicans* was screened for their ability to damage the host tissue by measuring the activity of IEC cytoplasmatic lactate dehydrogenase in the supernatant. Mutants exhibiting an increased or decreased cytotoxicity were validated and their morphology on IECs was assessed.Our kinase mutant screen revealed that deletion of around 30% of all kinases resulted in altered cytotoxicity compared to the wild-type (18 increased and 19 decreased damage). As filamentous growth is a major virulence factor of *C. albicans*, the morphology of these mutants on IECs was investigated. We identified five mutants (lacking *CRK1*, *HOG1*, *PBS2*, *SOK1*, or *SSK2*) which showed increased IEC damage but reduced or wild-type-like filamentation. So far, members of the HOG pathway and *CRK1* were described to be indispensable during commensalism and/or systemic infection but seem to negatively impact pathogenicity during epithelial infection. To fully determine the role of these protein kinases during epithelial infection, specific virulence attributes such as adhesion, invasion and translocation as well as metabolic adaptation are assessed for the corresponding mutants.Collectively, we identified a crucial role for several protein kinases during infection of IECs including kinases that negatively and filamentation-independently impact pathogenicity.

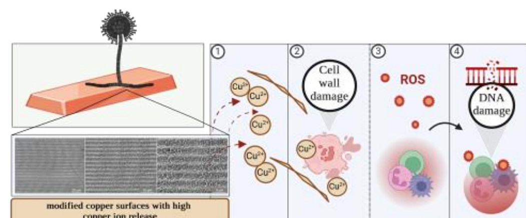
P-EP-012

Copper stress response of aspergillus niger spores and the implications for antifungal surface functionalization

*S. M. Koch[1], K. Siems[1], A. Schiele[1], D. W. Müller[2], A. Ahmed[2], K. Brix[3], F. Mücklich[2], R. Kautenburger[3], R. Möller[1]
[1]*German Aerospace Center, Institute for Aerospace Medicine, Radiation Biology, Köln, Germany*
[2]*Material Engineering Center Saarland (MECS), Department of Material Science of Engineering, Saarland, Germany*
[3]*Saarland University, Department of Inorganic Solid-State Chemistry, Saarbrücken, Germany*

Microbial life has been an enduring presence in various environments, prompting research into understanding the interactions between microorganisms and antimicrobial agents. Not only in terrestrial settings but even in human spaceflight related environments, *Aspergillus niger* has been identified alongside *Penicillium ssp*. as predominant species in HEPA filters and dust within the international space station. As especially fungal contaminations could have detrimental effects on built materials over an extended amount of time and negatively affect not only the structural integrity of a confined built environment, but cause negative effects on human health, it underscores the need to understand fungal responses to stress. Within this study we researched the fungal spore stress response from novel functionalized copper surfaces. The undertaken research focuses on the interaction between *A. niger* spores and innovative antimicrobial surfaces, that are made from copper and copper-alloys (brass) which have been micro-topographied through Ultrashort pulses Direct Laser Interference Patterning (USP-DLIP). The pulsed patterns show patterns of 3 µm or 9 µm depths which enhances the release of copper ions up on contact with microbial cells. The preliminary findings of contact – killing assays using spores from a wildtype and melanin deficient mutant strain of *A.niger*, reveal an unexpected heterogeneity in *A. niger* spore germination responses to copper stress, shedding light on the sophisticated regulation of copper homeostasis by this fungus. The results of those assays were further investigated using varied microscopy based methods like Fluorescence staining, SEM and Live - microscopic imaging of germinating spores under copper stress. For further analysis a detailed RNA profile analyses to discern the molecular mechanisms underlying any observed damage is planned, to gain deeper insights into the intricacies of *A. niger's* response to copper-induced stress. Understanding how *A. niger* reacts to copper stress is important for refining effective antimicrobial strategies with broader applications, addressing fungal contamination challenges across diverse settings.

Fig. 1



Food Microbiology and -hygiene

P-FMH-001

Different methods of employing the CRISPR-Cas9 toolbox for mutagenesis of starter culture strains of Lactococcus lactis

*M. Piesch[1], A. Weiß[1]
[1]*Universtiy Hamburg, Food Microbiology, Hamburg, Germany*

Clustered, Regularly-Interspaced Short Palindromic Repeats (CRISPR) and their associated enzyme (Cas9) are a widely used tools for mutagenesis like insertion, deletion and point mutations. In recent years the implementation of CRISPR-Cas9 systems in procaryotes has increased. It is an excellent tool for genome editing of known strains of *Lactococcus lactis* as NZ9000, but little research focuses on the editing of starter culture strains.

In this study, CRISPR-Cas9 will be established in *L. lactis* strains isolated from cheese manufacture. The aim is to create a CRISPR-Cas9 system that can achieve site specific mutagenesis of a cell-enveloped proteinase gene to investigate the effects on the bitter peptide content in dairy products.

Two strains of *L. lactis* isolated from cheese production were used. The plasmid systems for CRISPR-Cas9 mediated Gene mutagenesis were developed by combining bioinformatic methods, CRISPR-Cas9 programs and the results of whole genome sequencing.

The comparison of the different tools shows that not all systems already developed in *L. lactis* NZ9000 can be transferred to *L. lactis* starter cultures without problems. The nisin-induced systems can only be used by introducing the necessary genes *via* another vector. The applied *L. lactis* strains from starter cultures contain native plasmids. Therefore, a comparison of the origin of replication of the plasmids is necessary. Furthermore, it must be investigated whether the strains' own recombinase activity is sufficient to effectively utilize single plasmid systems without foreign recombinase. In addition, it must be investigated whether the already published single plasmid systems, which are mainly used for deletion, are suitable for a single base pair exchange if the repair template is integrated on the CRISPR-Cas9 vector.

In summary, plasmid systems designed in *L. lactis* NZ9000 cannot be easily transferred to *L. lactis* strains present in starter cultures. Further studies are needed to show whether a single plasmid system consisting of only one CRISPR-Cas9 plasmid is sufficient to perform site specific mutagenesis in *L. lactis* isolates.

P-FMH-002

Heat inactivation of foodborne pathogens in whey concentrate

*C. Franz[1], G. Fiedler[1], S. Nöbel[1], S. Matzen[1], M. Samtlebe[1]
[1]*wheyco, Hamburg, Germany*

Introduction: Pasteurization aims to achieve at least a 5 log10 reduction of heat-resistant microorganisms in raw whole milk. In Europe it is done by high temperature short time at 72°C for at least 15 s, according to regulation 853/2002. While heat inactivation of pathogens has been well studied in raw milk, their inactivation in whey concentrate has not received attention.

Goals: The study aimed to determine the inactivation of heat-resistant strains of the pathogens *Escherichia coli* and *Salmonella enterica* in whey concentrate with 30% dry matter by applying of HTST-treatment comparable to industrial practice.

Methods: Whey concentrate was inoculated with *E. coli* AW1.7 or *S. enterica* serovar Senftenberg 775W at an initial concentration of ca. 5 x 105 CFU/ml and then inactivated in a pilot plant pasteurizer. All experiments were set to a constant product flow rate of approx. 25 L/h and nominal heating temperatures were set to 72.0, 68.0, 64.0, 60.0, and 56.0°C. Heating was done at each temperature for an average of 17.5 s. Survivors were enumerated by plating aliquots of suitable dilutions on agar media.

Results: At the lower heating temperatures of 56°C and 60°C only *S.* Senftenberg showed a minor reduction in counts of 0.4 ± 0.4 log10 CFU/mL and 0.6 ± 0.7 log10 CFU/mL, respectively. At 64°C, the numbers of S. Senftenberg 775W were slightly reduced by 1.1 ± 0.6 log10 CFU/mL, while *E. coli* AW1.7 showed a minor reduction of 0.7 ± 0.5 log10 CFU/mL at this temperature. Starting from 68°C, the strains *S.* Senftenberg 775W and *E. coli* AW1.7 showed a reduction in cell counts of 3.1 – 4.2 log10 CFU/mL. At 72°C, viable cells of both strains tested were reduced by over 5 log10 CFU/mL.

Summary: Results showed that a 5 log10 reduction of both the highly heat-tolerant strains of *Salmonella* and *E. coli* in whey concentrate can be achieved when heating at 72°C for an average of 17.5 s in a continuous pasteurization unit close to industrial practice. Thus, products made from whey concentrate, when used as an ingredient in foods, should not lead to contamination with these bacterial pathogens, provided that the whey concentrate was pasteurized at 72°C for 17.5 s.

P-FMH-003

Influence of environmental conditions on the thermal resistance of a surrogate for Salmonella spp.

A. Gedas[1,2], H. Schmidt[2], *A. Weiß[1]
[1]*University of Hamburg, Food Microbiology, Hamburg, Germany*
[2]*University of Hohenheim, Institute of Food Science and Biotechnology, Stuttgart, Germany*

Question: *Salmonella enterica* is an important prominent foodborne pathogen. Its ability to survive in acidic conditions raises safety concerns within the fruit juice industry. Additionally, the continuous exposure to stress conditions facilitates the development of tolerance rather than immediate bacterial death. The organism's adaptive response to environmental changes is vital to survive the challenges. Nonetheless, the precise timeline for such adaptations in the microorganism is still not completely understood. The objective of this study was to determine the thermal resistance of *Escherichia coli* ATCC 11229 as a surrogate for *S. enterica*. after pre-incubation under different environmental conditions.

Methods: The heat resistance of *E. coli* ATCC 11229 was determined, after 15 min and 3 h pre-incubation at 20°C. Three sugar content levels, namely 7° Brix, 12° Brix, 17° Brix, as well as three pH-ranges, from 3.1-3.3, 6.7-7.0, and 9.3-9.6, were investigated. To exclude other environmental factors, pre-incubation was performed in strawberry nectar (12° Brix, pH 3.5) and artificial nectar (12° Brix, pH 3.5). After the incubation time, thermal inactivation at 60°C in the strawberry nectar in biological triplicates and technical duplicates was performed.

Results: The heat sensitivity of the investigated strain remained consistent after 15 min pre-incubation in both strawberry nectar and artificial nectar with identical sugar content and pH levels. Therefore, the 3 h pre-incubation was performed only in strawberry nectar. Almost in all cases the determined D60-value was higher after the longer pre-incubation period. However, only in the 12°Brix strawberry nectar sample with a pH range of 9.3-9.6, a statistically significant higher D60-value was obtained.

Conclusions: This study showed clearly the impact of environmental stress, such as increased sugar content and extreme pH values, on thermal resistance of *E. coli* ATCC 11229. Nevertheless, more research is needed to verify the influence of incubation stress conditions and duration on bacterial thermal resistance.

P-FMH-004

Compound structure and hydrophobicity contribute to antimicrobial activity of short-chain carboxylic acids

*K. S. Ng[1], T. Busche[2], C. Rückert-Reed[2], M. F. Bambace[1], C. Schwab[1]
[1]*Aarhus University, Biological and Chemical Engineering, Aarhus, Denmark*
[2]*Bielefeld University, Center for Biotechnology (CeBiTec), Bielefeld, Germany*

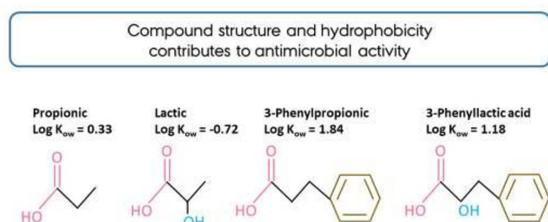
Utilization of short-chain carboxylic acids (SCCA) is a feasible approach to reduce food waste by preventing microbial spoilage. However, the antimicrobial mode of action of SCCA has not been fully uncovered. This study aimed to evaluate the effects of structurally different SCCA with 3 carbons in the backbone on the antimicrobial activity against *Salmonella enterica,* including hydrophobicity (log K_{ow}) as a factor of compound structure that is often ignored.

The minimum inhibitory concentrations (MIC) of propionic (log K_{ow} = 0.33), lactic (log K_{ow} = –0.72), 3-phenylpropionic (log K_{ow} = 1.84) and 3-phenyllactic acid (log K_{ow} = 1.18) were tested using a high-throughput broth dilution assay at pH 4.5, and the OD_{600} was recorded after 24 h incubation. To determine microbial response, *Salmonella* cells were harvested at log phase (OD_{600} 0.2~0.3), RNA was extracted, reverse transcribed and sequenced. DeSeq2 was used to compare the transcriptional profiles of treated cells and controls that grew without subinhibitory levels of SCCA.

The antimicrobial activity of SCCA increased with increasing compound hydrophobicity: 3-PP > 3PL > propionic > lactic acid. 3-PP inhibited the growth of *S. enterica* the most and reduced the final cell density to 50% (MIC_{50}) at 1.8 mM, followed by 3PL (5.0 mM), propionic acid (5.2 mM), and lactic acid (28.2 mM). RNA-seq indicated higher expression of *lldPRD* and *nap* operon encoding proteins related to lactate uptake and nitrate reductase, which has been linked to acid stress responses. When subjected to propionic acid and 3PP, *eut* and *pdu* genes that are responsible for ethanolamine and glycerol/1,2-propanediol utilization were upregulated, respectively.

In conclusion, we identified 3-PP as a potential biopreservative to inhibit *Salmonella* with hydrophobicity as a determining factor. Gene expression responses differed between compounds suggesting that compound structure is an important contributor to the inhibitory activity of SCCA.

Fig. 1



P-FMH-005
Use of bacterial surrogates for the microbial safety evaluation of fermented pea protein
*J. Hollmann[1], H. Schmidt[1]
[1]*University of Hohenheim, Institute of Food Science and Biotechnology, Stuttgart, Germany*

Microbial Fermentation is a traditional method for food preservation, extending shelf life, and ensuring microbial stability. This study aimed to assess the microbial safety of a fermented pea protein-based product using a mixed starter culture of *Lactococcus lactis* LTH 7123 and *Yarrowia lipolytica* LTH 6056. Safety evaluation was conducted through challenge tests with surrogates of common foodborne pathogens to determine a potential inhibition by the starter cultures.

Experiments were performed in fermentation mixtures consisting of commercial pea protein isolate and glucose. The fermentation samples were first inoculated with *L. lactis* or the mixed starter culture and additionally inoculated with either *Escherichia coli* ATCC 11229, *Listeria innocua* ATCC 33090, *Salmonella enterica* subsp. *enterica* serovar Senftenberg LTH 5703 and *Bacillus cereus* DSM 31T. As a growth control, pea protein suspensions were inoculated only with the tested surrogates. Viable counts and pH-values were determined after 16 hours and subsequently every 24 h throughout a 96-hour fermentation period.

All surrogates exhibited an initial increase in their viable counts by about 3 log units within 16 h of fermentation. While the bacterial counts of the surrogates in the mixed culture fermentation were comparable to their growth control, their bacterial count after 24 h decreased in presence of *L. lactis* alone after 24 h. However, no complete elimination was achieved after 96 h, except for *S.* Senftenberg. *B. cereus* counts in both fermentations remained constant throughout the fermentation time with about 10^6 cfu/ml after 24 h. The weaker growth of *L. lactis* and the slightly higher final pH-value in the mixed culture may explain variations in pathogen inhibition.

The results indicated insufficient inhibition of surrogates using *L. lactis* and *Y. lipolytica* as a mixed culture. However, growth inhibition of *E. coli*, *L. innocua* and *S.* Senftenberg could be achieved with *L. lactis* as a single strain. *Y. lipolytica* probably limited the inhibition of the surrogates during the fermentation of pea protein. To ensure process safety, further hurdles should be implemented.

P-FMH-006
Establishment of a bacteriophage catalogue: Phage isolation, characterization, and catalogue compilation
*C. Franz[1], S. Sprotte[1], N. Biere[1], F. Hille[1]
[1]*Max Rubner-Institut, Microbiology and Biotechnology, Kiel, Germany*

Introduction: Bacteriophages are recognized for their potential contribution towards enhancing food safety through their use as biocontrol agents in the food industry. Moreover, they are being explored as alternative human therapeutics, particularly in response to the growing need for antibiotic alternatives. Recognizing their significance as potential biocontrol and therapy tools, the Bacteriophage Competence Centre of the Max Rubner-Institut (MRI) aims to create a Bacteriophage Catalogue containing phages that can be provided for research.

Goals: The objectives includes isolating phages from various environmental sources, characterizing their morphological and genomic features, and presenting the compiled information as standardized profiles in a catalogue that will be available in a public repository and from which the phages can be ordered by researchers.

Materials & Methods: Phages were isolated from environmental samples using established protocols, followed by purification and amplification. Characterization involved morphological and genomic analysis. Standardized data, including host range and key genomic features are recorded.

The compiled information is formatted into concise profiles for inclusion in the catalogue.

Results: A collection of phages for *E. coli*, *Enterococcus*, *Salmonella*, *Pseudomonas* and other bacterial strains that are successively listed in the catalogue will be made available. Morphological analysis revealed a spectrum of phage shapes and sizes, while genomic sequencing provided insights into their genetic diversity. The host range data demonstrated specificity, further highlighting the uniqueness of each phage.

Summary: In summary, our institute has successfully isolated, characterized, and compiled a diverse array of phages to establish a comprehensive catalogue. This catalogue, intended for publication in an open data repository, serves as a valuable resource for the scientific community. By providing detailed information on phage morphology, genomics, and host range, we aim to facilitate research in this field and promote the sustainable use of phages in food industry and clinics, subject of course to local governmental regulations.

P-FMH-007

Diversity of psychrotrophic pseudomonads from raw milk - a method for rapid identification using MALDI-TOF-MS

S. Gieschler-Lübbehüsen[1], C. Grimmler[2], E. Brinks[1], C. Franz[1], *C. Böhnlein[1]
[1]*Max-Rubner Institute, Department of Microbiology and Biotechnology, Kiel, Germany*
[2]*Max Rubner-Institute, Department of Safety and Quality of Meat, Kulmbach, Germany*

Introduction: Refrigerated storage of raw milk is a fundamental hygienic measure in the dairy industry. However, under these conditions psychrotrophic bacteria can proliferate in raw milk and secrete enzymes that contribute to product spoilage. Heat-resistant peptidases, which are mainly produced by bacteria of the genus *Pseudomonas*, can cause spoilage effects of ultrahigh temperature (UHT) treated and sterilized milk which is manifested by symptoms such as bitterness, increased viscosity and gelling. Rapid and reliable identification of pseudomonads in raw milk and tracing their sources on farms and in processing plants is therefore of great importance in order to avoid economic losses and food waste in the milk value chain.

Goals: Our study aimed to rapidly identify spoilage bacteria of the genus *Pseudomonas* isolated from raw milk by the means of their protein fingerprints using the mass spectrometric technique MALDI-TOF-MS.

Methods: For this purpose, a custom database with more than 260 reference spectra of 104 different food-relevant *Pseudomonas* species was created, as the Bruker MALDI Biotyper® system could only identify a subset of the raw milk associated pseudomonads. A total of 1,200 isolates from 356 raw milk samples were picked from *Pseudomonas* CFC selective agar and subsequently identified using the MALDI biotyper.

Results: Overall, 80% of the isolates from CFC agar could be assigned to the genus *Pseudomonas* (*P.*), while growth of other genera, such as *Stenotrophomonas* and *Hafnia*, was also observed. Twice as many isolates from organic milk could not be identified when compared to isolated from conventional milk. Based on the number of genera and species identified, the diversity in organic milk was higher than in conventionally produced milk. At the species level, *P. lundensis* was the most frequently detected species present in the raw milk samples investigated.

Summary: The analyses for rapid identification of raw milk isolates using MALDI-TOF indicated a high diversity with a large number of unknown species which may exhibit spoilage characteristics and impact the shelf life of ultrahigh temperature (UHT) treated and sterilized milk.

P-FMH-008

Using 3D-Laserscanning Microscopy for the absolute quantification of viable Campylobacter spp. in chicken rinse matrix

*T. Nicola[1], S. Lick[1], D. A. Brüggemann[1], J. F. Evers[2], *S. Knorr[1]
[1]*Max Rubner-Institute, Kulmbach, Germany*
[2]*Cairn Research GmbH, Heidelberg, Germany*

Introduction

Campylobacteriosis is the most common food-borne disease caused by bacteria. During slaughtering, chicken skin can be contaminated by *Campylobacter* spp. due to the evisceration process. Although the number of *Campylobacter* spp. on broilers is controlled by process hygiene criteria that are constantly being improved, the number of human infections still remains high. Under stress conditions, *Campylobacter* spp. can enter a viable but non-culturable (VBNC) state, which is poorly understood. VBNC *Campylobacter* spp. cannot be detected with the DIN EN ISO 10272-2:2017 method and therefore an underestimation of the number of live *Campylobacter* spp. on chicken might be the result.

Goals

The aim was to develop a quantification method for live *Campylobacter* spp. cells in chicken meat samples with fluorescence microscopy, combining live-dead and species-specific antibody staining.

Methods

Campylobacter jejuni cells were embedded in agarose pads prepared either with Bolton broth or unsterile chicken rinse. They were either stained directly or incubated at 42 °C for 22 h under microaerobic conditions until microcolonies (µCFU) were formed. For live cell staining, tetrazolium salt CTC, an indicator for metabolic activity, was used. In addition, a specific fluorophore-tagged antibody was applied. Using CLSM, 3D-scans of a defined volume were taken. *Campylobacter* live numbers were automatically counted where CTC and antibody signals were co-localized.

Results

Live-dead discrimination could be achieved quantitatively in serial tenfold dilutions of dead and living *C. jejuni* cells. Absolute quantification was linear for µCFU formation within a range of 3x 10^1-10^5 µCFU/mL and for single cells in a range of 10^3-10^6 cells/mL, both in Bolton broth and chicken rinse.

Summary

A new 3D-scanning microscopy method has been developed for quantitative detection of low *Campylobacter* spp. numbers in a complex agarose-based growth matrix, using fluorophore-tagged *Campylobacter*-specific antibodies and CTC for live-dead discrimination. The method requires little time and sample preparation and allows simultaneous detection of reproducing cells (µCFU), VBNCs and dead cells.

Fig. 1

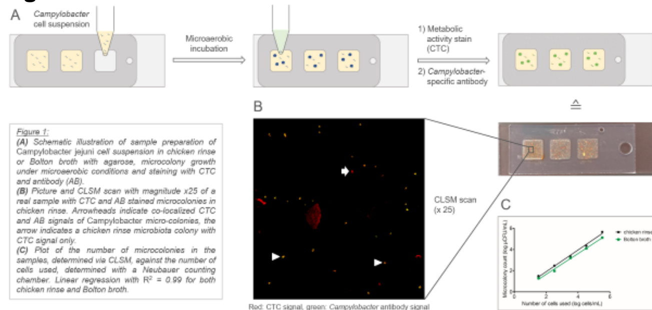


Figure 1:
(A) Schematic illustration of sample preparation of Campylobacter jejuni cell suspension in chicken rinse or Bolton broth with agarose, microcolony growth under microaerobic conditions and staining with CTC and antibody (AB).
(B) Picture and CLSM scan with magnitude x25 of a real sample with CTC and AB stained microcolonies in chicken rinse. Arrowheads indicate co-localized CTC and AB signals of Campylobacter micro-colonies, the arrow indicates a chicken rinse microbiota colony with CTC signal only.
(C) Plot of the number of microcolonies in the samples, determined via CLSM, against the number of cells used, determined with a Neubauer counting chamber. Linear regression with $R^2 = 0.99$ for both chicken rinse and Bolton broth.

P-FMH-009

Investigation of β-Glucan-Producing lactic acid bacteria strains for the stabilization of fruit preparations

*D. Zipori[1], S. Festini[2], S. Neidhart[2], M. Jekle[2], H. Schmidt[1]
[1]*University of Hohenheim, Food Microbiology and Hygiene, Stuttgart, Germany*
[2]*University of Hohenheim, Plant-based Foods, Stuttgart, Germany*

Introduction: Fruit preparations are used as intermediate products in many applications in the food industry. Hydrocolloids, predominantly polysaccharides, are usually added as thickeners and stabilizers to prevent sedimentation or floating of fruit pieces in a fruit puree matrix.

Goal: The reported study aimed at the development of a screening procedure enabling the selection of β-D-glucan-producing lactic acid bacteria (LAB) strains and their application *in situ* for the stabilization of fruit preparations as additive replacement.

Materials & Methods: Potential LAB strains were isolated from environmental sources and investigated together with over 200 strains from our strain collection. The screening procedure included the confirmation of phenotypic slime formation on solid media, PCR-based detection of the gene-encoding β-D-glucan-producing glycosyltransferase (*gtf*-PCR), detection of β-D-glucan polysaccharide capsule by specific antibodies, and an *in situ* qualitative assay for the thickening of fruit, vegetable or mixed fruit-vegetable purees. Two strains were selected for a controlled fermentation process involving peach, strawberry, carrot and beetroot purees. The fermentation products were freeze-dried and then incorporated into fruit preparations, with subsequent measurement of the viscosity.

Results & Summary: The screening resulted in 50 strains exhibiting slimy phenotype, 11 of which were carrying the *gtf* gene. Six strains exhibited slime production in strawberry and peach purees combined with beetroot or carrot puree, respectively. These strains were identified as belonging to the species *Pediococcus parvulus*, *Lacticaseibacillus paracasei*, *Levilactobacillus brevis*, *Pediococcus claussenii*, and *Furfurilactobacillus rossiae*. Pasteurized model strawberry fruit preparations containing fermentates of the two tested strains *L. brevis* TMW 1.2112 and *P. parvulus* LTH 1110 showed no significant increase in viscosity compared to the control products.The chemical composition of the occurring polysaccharides is being studied. Further approaches are included in the ongoing research into the applicability of β-D-glucan-producing LAB in fruit preparations.

P-FMH-011

A Caenorhabditis elegans life-dead-assay for the estimation of the pathogenic potential of pathogenic Escherichia coli from food samples

*M. Projahn[1], N. Baschinski[1], E. Dukadjinac[1], K. Detert[2], H. Schmidt[2], E. Schuh[1], M. Fischer[1]
[1]*German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany*
[2]*University of Hohenheim, Department of Food Microbiology and Hygiene, Stuttgart, Germany*

Shiga toxin-producing *Escherichia coli* (STEC) and diarrheagenic *E. coli* (DEC) are important food-borne pathogens. The severity of clinical symptoms can vary from diarrhea to haemolytic-uremic syndrome and death. In STEC, the Shiga toxin subtype and the occurrence of additional virulence factors have an impact on the clinical manifestation. STEC from food can vary in serotype and virulence associated genes (VAG) from those usually connected to severe clinical cases. Therefore, an assessment of the pathogenic potential of food isolates for humans would be helpful. Here, we established a *Caenorhabditis (C.) elegans* life-dead-assay to estimate the pathogenic potential of STEC from food samples. A collection of non-pathogenic *E. coli*, DEC, STEC and respective *stx* deletion mutants were tested concerning their impact on the lifespan of *C. elegans* SS104. For the life-dead-assay, worms were synchronized to the same L4 larval stadium. Fifteen worms were seeded on fresh nematode growth medium plates with either the non-pathogenic *E. coli* OP50 control strain or a challenge strain. Numbers of life and dead worms were counted each day. Kaplan-Meier survival curves were calculated using R and the median survival times (MSTs) of three technical and biological replicates were determined. For all tested strains, MSTs of the worm populations were reduced compared to the control strain OP50. Worms fed on OP50 had an MST of 11 days. MSTs for non-pathogenic *E. coli* strains were reduced to 10 to 7 days, respectively while DEC and STEC strains led to a further reduction of the MST to 6 days. Deletion of the *stx* gene led to an increase of the MST compared to the wild type strain. A *C. elegans* life-dead-assay for *in vivo* pathogenicity determination experiments for DEC and STEC from food samples was successfully established. Comparable reductions in the MSTs for DEC and STEC were determined. The shape of the Kaplan-Meier survival curves differed between DEC and STEC strains and the investigated deletion mutants, respectively. Furthermore, one tested STEC strain harbour the Stx2g Shiga toxin subtype, which is assumed to be less toxic, therefore STEC with other Stx-subtypes shall be tested.

P-FMH-012

Identification and characterisation of thuricin 17-like bacteriocins produced by Bacillus thuringiensis and Bacillus cereus isolates

*S. G. Batman[1], N. Jessberger[1], M. Plötz[1], S. Kittler[1]
[1]*Institute for Food Quality and Food Safety, University of Veterinary Medicine Hannover, Hannover, Germany*

Introduction

Bacillus species are bacteria with a broad application profile in food production. They are used as starter cultures in modern biotechnological processes and in traditionally fermented products. They are capable of producing bacteriocins, ribosomally synthesised peptides with high antibacterial activity. Bacteriocins do not affect the sensory properties of foods or the composition of the human gut microbiota, as they are degraded by proteases in the gastrointestinal tract. The *Bacillus cereus* group consists of several genetically closely related species, such as *B. cereus sensu stricto, B. anthracis*, or *B. thuringiensis*. The latter has recently come to the fore for its ability to produce bacteriocins, in particular thuricins (thuricin 17 and thurincin H). However, their properties have not yet been fully characterised.

Goals

The aim of the present study was the identification and characterization of bacteriocins from different *B. cereus* and *B. thuringiensis* isolates.

Materials & Methods

Supernatants of various *Bacillus* strains isolated from foods were tested by the agar well diffusion method for their antimicrobial activity towards *Listeria monocytogenes*. To determine the molecular weight of the bacteriocins, SDS-PAGE and silver staining were performed. Finally, the sensitivity of the bacteriocins to temperature and enzymes such as proteinase K, pronase E, trypsin, lipase and amylase was evaluated.

Results

Six *Bacillus* isolates showed antibacterial activity towards *L. monocytogenes*. The molecular weight of the corresponding bacteriocins was approx. 6 kDa. Antimicrobial activity was eliminated by proteinase K and pronase E. No decrease in activity was observed when samples were treated with trypsin, lipase, amylase, or temperatures up to 65 °C. Examination of the putative gene clusters in the genomes of the tested *Bacillus* isolates revealed high similarity of the peptide sequences to thurincin H and thuricin 17 as well as a conserved motif specific to these bacteriocins.

Summary

We report 6 bacteriocins from *B. cereus* and *B. thuringiensis* isolates with high similarity to thuricin 17. Confirmation via MALDI-ToF-MS is ongoing.

P-FMH-013
Systematic evaluation of the suitability of a phage product for biocontrol of Listeria monocytogenes
*C. Brieske[1], H. Z. Low[1], C. Böhnlein[1], C. Franz[1]
[1]*Max Rubner Institute, Federal Research Institute of Nutrition and Food, Microbiology and biotechnology, Kiel, Germany*

Introduction: Foodborne bacterial pathogens can cause significant health and economic impacts worldwide. *Listeria* (*L.*) *monocytogenes* frequently leads to outbreaks and food recalls due to contamination. Bacteriophage (phage) -based products claiming to have broad host ranges against *Listeria* are commercially available. Although these products are already approved for use in countries like Israel, Canada, China, Switzerland, Australia and New Zealand, they are still awaiting approval in Germany. **Goals**: The aim of this study is to systematically investigate the efficacy of these phage-based products against sequenced and genotypically distinct clinical (n=20 most common genotypes) and food-associated (n=30) *L. monocytogenes* strains currently circulating in Germany. We also compared various experimental techniques for the determination of phage susceptibility and evaluated their strengths and weaknesses. **Methods**: A commercially available phage product against *L. monocytogenes* was used. The determination of susceptibility of clinical and food-associated *L. monocytogenes* strains was investigated by spot assays, plaque assays, colony reduction, flow cytometry and optical density measurements (OD_{600}). **Results**: Out of 50 *Listeria monocytogenes* isolates, 70% and 76% of the strains were found to be susceptible to the phage product in spot and plaque assays, respectively. When using the colony reduction method (n=20), flow cytometry (n=50) and OD_{600} (n=50), phage incubation substantially reduced live cell counts after 24 hours for all isolates compared to the control without phage. With flow cytometry, a substantial reduction could even be seen after 3 hours incubation (Fig. 1). **Summary**: Preliminary results demonstrate the effectiveness of the phage-based product on currently circulating strains of *L. monocytogenes* and indicate the biocontrol potential for the reduction of listeriosis should it be approved. It might also be prudent to rethink the use of plaque and spot assays, which are currently considered to be the gold standard for phage susceptibility testing in favor of methods that directly address bacteria reduction, e.g. colony reduction, flow cytometry and OD_{600}.

Fig. 1

	Spot/Plaque Assay	Colony Reduction	Flow Cytometry	OD600
			Live / Dead Staining	
Time to Results	24 hours	48 hours	3 hours	24 hours
Susceptibility according to assay	70/76% (n=50)	100% (n=20)	100% (n=50)	100% (n=50)

created in BioRender.com

P-FMH-014
From science to market: Potential use of novel weissella strains as protective cultures
*S. Fischer[1], A. Euler[1], N. Mariani Corea[1], F. Titgemeyer[1]
[1]*University of Applied Sciences Münster, FB Oecotrophologie, Münster, Germany*

Introduction: Lactic acid bacteria (LAB), of which over 260 species have been described to date, are widely used in food fermentation to produce a variety of tasty foods. They can also have numerous health-promoting effects and effectively combat pathogens in food. A less studied group are the 21 species of the genus Weissella, although they are frequently found in fermented foods.

Objective: We aimed to isolate novel Weissella strains in order to evaluate their potential as food protecting cultures.

Results: In recent years, we have compiled a strain collection of more than one thousand LAB that were isolated from milk samples and teat canal biofilms of milk cattle. In this environment, we found 22 *Weissella* strains comprising six species. Binary matrices obtained from Random Amplified Polymorphic DNA (RAPD) experiments revealed that most of them could be assigned to different subspecies. However, two isolates of *W. paramesenteroides* and two isolates of *W. thailandensis* showed identical banding patterns. Eight food pathogens were selected to test how well the *Weissella* isolates inhibit them. The data from the spot-on-lawn tests showed that 14 of the 22 isolates had strong activity, seven had medium activity and one isolate had low activity. Among those, isolates of *W. paramesenteroides* were the most effective. They were also the only species that were able to produce hydrogen peroxide as an inhibiting agent. No correlation was found in relation to acid production. In summary, *Streptococcus dysgalactiae*, various *Escherichia coli* strains and *Staphylococcus aureus* were efficiently inhibited by almost all *Weissellae*, while *Klebsiella pneumoniae*, *Streptococcus uberis*, and *Enterococcus faecalis* were efficiently inhibited by four or less isolates.

Conclusions: The Weissella wild-type strains isolated here show a high potential for combating pathogenic bacteria in food. They will therefore be subjected to further investigations with regard to their use as starter, probiotic or protective cultures.

Gastrointestinal Infections

P-GI-001

Cytokine secretion and DNA damage in H. pylori infection relies on sub-lethal signals in the mitochondrial apoptosis apparatus

*P. Neubert[1], B. Dörflinger[1], S. Kirschnek[1], G. Häcker[1]
[1]*University Hospital and Medical Center Freiburg, Institute of Medical Microbiology and Hygiene, Freiburg i. Br., Germany*

Introduction: *Helicobacter pylori* (*Hp*) colonizes the gastric mucus layer of around half of the world"s population. Although the infection is mostly asymptomatic, it is a major risk factor for gastritis, gastric and duodenal ulcers and gastric adenocarcinoma. *Hp* is recognized by epithelial cells and activates various signaling pathways through for instance pattern recognition receptors, resulting in inflammation. *Hp* can also induce DNA damage including double-strand breaks. The apoptosis system can be activated by infection, and pathogens have pro- or antiapoptotic effects. We have recently shown that various intracellular pathogens (bacteria and viruses) trigger sub-lethal signals in the mitochondrial apoptotic pathway.

Methods: We infected human gastric carcinoma cells (AGS) with *Hp* and tested for sub-lethal signals in the mitochondrial apoptosis pathway. We determined cytokine/chemokine release and DNA damage as a readout. We further investigated the release of mitochondrial cytochrome *c* and SMAC by Western Blot and confocal microscopy.

Results: Conditions could be established where infection with *Hp* did not induce cell death, but where sub-lethal signals in the mitochondrial apoptosis pathway could be identified. The pathway required the pattern recognition receptor NOD1 and specifically the BCL-2-family member BAK. We found that *Hp*-infection caused a pulse of release of the mitochondrial intermembrane-space protein SMAC apparently from the entire mitochondrial network into the cytosol. SMAC activated alternative NF-kB and was required for the normal secretion of chemokines upon infection. The activity of the mitochondrial apoptosis-pathway also caused DNA-ds-breaks, and in biopsies from *Hp*-patients we observed a correlation of evidence of sub-lethal signaling and the DNA-damage response.

Summary: The results identify sub-lethal signals in the mitochondrial apoptosis pathway during *Hp*-infection. With the release of mitochondrial proteins from the mitochondrial network, the results suggest a new model of mitochondrial signal. The evidence suggests that these signals contribute to inflammation and genome stress including mutations during chronic *Hp*-infection

---

P-GI-002

Development of primary cell-based infection models to study pathogenesis of Campylobacter jejuni

*T. Bruchhardt[1], M. Alzheimer[1], M. Neyazi[1], S. Bartfeld[2], C. M. Sharma[1]
[1]*Julius-Maximilians-Universität Würzburg, Würzburg, Germany*
[2]*Technische Universität Berlin, Berlin, Germany*

Campylobacter jejuni is currently the leading cause of bacterial foodborne gastroenteritis in humans. However, only a few *C. jejuni* virulence-determining factors involved in host cell interactions *in-vivo* have been identified, in part due to the lack of appropriate infection models that accurately reflect the human host environment. So far, infection studies have mainly employed animal or *in-vitro* cell culture models based on immortalised cell lines (e.g., Caco-2 cells), which are limited in their ability to model the complexity of intact three-dimensional (3D) tissue in the human host. To add physical characteristics of host intestinal tissue, like the structure of crypts and villi, we previously developed a 3D infection model based on tissue-engineering that incorporates a biological scaffold, the small intestinal submucosa, seeded with Caco-2 cells. This 3D infection model allowed for monitoring pathogenesis related phenotypes of *C. jejuni* mutant strains analogous to phenotypes previously observed in animal models, which could not be reflected in 2D monolayers. Nevertheless, the use of immortalized cell lines in an infection model limits the resemblance of specific host tissue characteristic, such as cell type diversity. These shortcomings hinder the identification of host cell types targeted by *C. jejuni*, as well as the determination of bacterial survival and replication within host cells. Therefore, we are now adapting primary cell-based *in-vitro* infection models of the small intestine by employing human intestinal organoids for *C. jejuni* infections. So far, we have successfully set up 3D and 2D monolayer cultures of human jejunum and ileum organoids in our lab. First infection experiments of organoid-derived monolayers demonstrate that independent of the multiplicity of infection (MOI) and patient origin of organoids, *C. jejuni* can infect primary jejunum cells and in several of these, clusters of multiple bacterial cells can be observed. Using these humanized infection models, we aim to get a deeper understanding of *C. jejuni* pathogenesis, including host target cell identification as well as epithelial and pathogen factors mediating infections.

P-GI-003

High density genomic surveillance and risk profiling of clinical Listeria monocytogenes subtypes in Germany, 2018-2021

*S. Halbedel[1], S. Wamp[2], R. Lachmann[3], A. Holzer[3], A. Pietzka[4], W. Ruppitsch[5], H. Wilking[3], A. Flieger[1]
[1]*Robert Koch Institute, FG 11 Division of Enteropathogenic Bacteria and Legionella, Consultant Laboratory for Listeria, Wernigerode, Germany*
[2]*Robert Koch Institute, FG11 - Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany*
[3]*Robert Koch-Institut, FG35 Division of Gastrointestinal Infections, Berlin, Germany*
[4]*Austrian Agency for Health and Food Safety, Graz, Austria*
[5]*Austrian Agency for Health and Food Safety, Wien, Austria*

Introduction: Invasive listeriosis is a notifiable foodborne disease caused by the bacterium *Listeria monocytogenes* and characterized by high fatality rates. Systematic collection of pathogen isolates from listeriosis cases, whole genome sequencing (WGS) and phylogenetic analyses allow recognition and termination of outbreaks after source identification as well as risk profiling of abundant lineages. For surveillance of listeriosis in Germany, clinical *L. monocytogenes* isolates are collected and their genomes sequenced at the Consultant Laboratory for *Listeria* located at the RKI (1).

Methods: 1802 clinical *L. monocytogenes* isolates from Germany collected in 2018-2021 were subjected to WGS for subtyping, cluster recognition and virulome/resistome analyses. Clinical information on the different disease manifestations was used for the calculation of the risk for developing materno-fetal and brain infections for the most abundant phylogenetic sublineages.

Results: The collected isolates covered 62% of all notified cases and belonged to 188 infection clusters, partially reflecting large outbreaks (2-4). 42% of the clusters were protracted, 60% generated cases cross-regionally, including 11 multinational clusters. Strain risk profiling for development of materno-fetal and brain infections allowed discrimination of hypervirulent (ST1) and hypovirulent phylogenetic sublineages (ST8, ST14, ST29 and ST155), confirmed by *in vitro* infection experiments. In hypovirulent sublineages, naturally occurring loss of function mutations were found in several virulence and house-keeping genes.

Summary: Our work provides a comprehensive insight into the population structure of clinical *L. monocytogenes* isolates in Germany and the genetic and clinical characteristics of the most abundant sublineages. The assignment of hyper- and hypovirulent lineages might lead to the discovery of relevant virulence factors outside of reference strains.

1 – Halbedel *et al.* ***J Clin Microbiol***. 2018; 56(6):e00119-18
2 – Halbedel *et al.* ***Emerg Infect Dis.*** 2020; 26(7):1456-1464
3 – Lachmann *et al.* ***Clin Microbiol Infect.*** 2021; 27(7):1035.e1-1035.e5
4 – Halbedel et al. ***Microbiol Spectr.*** 2023; 10:e0352022

P-GI-004

The Ig-like glycoprotein CD101 amplifies anti-bacterial properties of phagocytes

M. Wrage[1], J. Kaltwasser[1], P. Seeberger[2], K. Dettmer[3], M. Rauh[1], *J. Mattner[1]
[1]*Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany*
[2]*Max Planck Institute, Potsdam, Germany*
[3]*Institute for Functional Genomics, University of Regensburg, Regensburg, Germany*

Introduction

T lymphocytes and myeloid cells express CD101, an Ig-like glycoprotein, predominantly in the gut. While CD101-expressing T lymphocytes ameliorate the severity of DSS- and T cell transfer-induced colitis and sustain the function of regulatory T cells, the biology of CD101 on myeloid cell subsets is less well understood.

Goals

Therefore we investigated the myeloid cell-specific functions of CD101 in oral and systemic *Salmonella* infection models.

Materials & Methods

We assessed the regulation of CD101 expression, the composition of intestinal microbiota, the intraluminal metabolome, *Salmonella* replication and the severity of intestinal inflammation in mouse models of acute and chronic *Salmonella*-induced colitis using conditional CD101-knockout mice crossed to CD11c Cre, LyzM Cre and Cx3Cr1 Cre mice as well as in patients suffering from inflammatory bowel disease (IBD).

Results

Neutrophil specific conditional CD101 KO mice harbored less neutrophils in the bone marrow, like conventional CD101 KO mice and exhibited distinct variations in the composition of intestinal microbiota following *Salmonella* infection. CD101-expressing neutrophils, but not other CD101-expressing myeloid cell subsets restrained *Salmonella* infection *in vitro* and *in vivo*. CD101-intrinsic and -extrinsic mechanisms contributed to the control of bacterial replication and systemic spread, and the CD101-dependent containment of *Salmonella* infection depended on the concomitant expression of *Irg-1* and *Nox2* along with an increased accumulation of itaconate and reactive oxygen species in neutrophils. An increased circulation of intestinal microbial antigens in the sera of IBD patients correlated inversely with the distribution of CD101 expression on myeloid cells, mirroring the suppression of CD101 in mice following *Salmonella* infection.

Summary

When expressed on neutrophils, CD101 restrains infection due to the control of metabolic, immune-regulatory and anti-microbial processes. To what extent CD101 shapes the composition of intestinal microbiota and how CD101-dependent microbiota promotes colonization resistance to *Salmonella* infection, is subject of further investigation.

P-GI-005

Title: Impact of hypoxia on epithelial function upon exposure to Clostridioides difficile toxins

*I. Ben Brahim[1], A. Mosig[2], K. Huber[1]
[1]*Paul Ehrlich Institute, Microbiology, Langen, Germany*
[2]*Jena Klinikum, Research Building F2, Jena, Germany*

Introduction and goals: Hypoxia, regulates cell proliferation, differentiation and effector function, which is mainly driven by hypoxia inducible factors (HIFs). In pathological conditions hypoxia facilitates tissue damage and increases disease severity. The aim of our project is to investigae whether *C. difficile* toxins A (TcdA) and B (TcdB) influence hypoxic factors (HIF-1/2 α) and tissue damage in *C. difficile* infection.

Materials and methods: The C2BBe1 colonic epithelial cell line was exposed to *C. difficile* TcdA and TcdB under hypoxic and normoxic conditions. Hypoxia was induced by atmospheric oxygen reduction (1% O2) or chemically with CoCl2 or deferoxamine (DFO). We assessed production of proinflammatory cytokines, cell cytotoxicity, HIF1/2α levels and epithelial barrier permeability.

Results: While C2BBe1 cells challenged with TcdA and TcdB produced IL-8, exposure to 1% O2 - but not CoCl2 - reduced the amount of IL-8 secreted by C2BBe1 cells upon TcdA stimulation. We further observed an increase in cell cytotoxicity and epithelial permeability under 1% O2 with TcdA and TcdB when compared to normoxia and CoCl2 treatment. Furthermore, we saw that toxins did not affect HIF1α and HIF-2α stabilized protein levels. To understand the contribution of HIF factors on toxin-mediated permeability increase, we next assessed activation of either HIF-1α by CoCl2 or HIF-1α and HIF-2α by DFO. Interestingly, highest permeability increase was observed in 1% O2 hypoxia, whereas CoCl2 and DFO exhibited a similar level of damage as under normoxia.

Conclusion: TcdA and TcdB-mediated effects on the cytokine response and epithelial barrier function are altered by hypoxia. The effects observed with chemically-induced hypoxia differ from those obtained at 1% O2. Thus, these effects are most likely not due to direct engagement of HIF factors, but rather to increased susceptibility of epithelial cells to toxins under hypoxic conditions. Future work aims to investigate the underlying molecular mechanisms.

Funding: Innovative Medicines Initiative 2 Joint Undertaking (grant agreement No 101007799 (Inno4Vac)).

Key words *C. difficile*, gastrointestinal mucosa, hypoxia, HIF

P-GI-006

Using spatial transcriptomics to resolve the host-pathogen interaction in vivo.

*L. Chiggiato[1], P. Dersch[2], M. Hornef[3], A. E. Saliba[1]
[1]Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany
[2]University of Münster, Center for Molecular Biology of Inflammation, Münster, Germany
[3]RWTH Aachen University, Institut für Medizinische Mikrobiologie, Aachen, Germany

Single-cell transcriptomics is revolutionizing our understanding of host-pathogen interactions by providing a cellular-level overview of infection trajectories. However, because cells needs to be dissociated, the technology falls short in providing *in vivo* spatial information crucial for tissue analysis. Furthermore, while some commercial solutions are emerging, they cannot provide the sufficient resolution to detect and localize pathogens accurately. To overcome these limitations, we are establishing a novel methodology combining near-single-cell resolution spatial transcriptomic approach with pathogen-targeting probes. We will validated through fluorescence microscopy and H&E staining, offering visual confirmation of bacterial presence in intestinal tissues. This integrated approach enables the simultaneous profiling of host-transcriptomes and precise spatial mapping of bacterial activity, facilitating a more comprehensive understanding of host-pathogen interactions during infection.

P-GI-007

Towards real-time imaging of Salmonella enterica Serovar Typhimurium infection and modulation using metabolic engineering and in vivo bioorthogonal click chemistry

*E. Laing[1,2,3], L. Maier[2,3], N. Beziere[1,2,4], J. Cotton[1,4]
[1]Werner Siemens Imaging Center, Universitätklinikum of Tübingen, Preclinical imaging and radiopharmacy, Tübingen, Germany
[2]Cluster of Excellence CMFI (EXC 2124) "Controlling Microbes to Fight Infections", Eberhard Karls University, Tübingen, Germany
[3]University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany
[4]Cluster of Excellence iFIT (EXC 2180) "Image Guided and Functionally Instructed Tumour Therapies", Eberhard Karls University, Tübingen, Germany

Understanding of the gut microbiome's protective mechanism is limited. Fecal sampling and *ex vivo* strategies offer little real-time information and need many animals for longitudinal studies. Metabolic engineering permitted species-specific labeling and fluorescence imaging of *Bacteroides fragilis* (*Bf*) *in vivo* (1), albeit with shallow depth and low resolution. Further work using positron emission tomography (PET) showed promise but was limited by time constraints due to *in vitro* labeling with short-living radioisotopes (2). We aim to optimize the conditions of metabolic engineering-based protocols formerly established, "clicking" a radiotracer *in vivo*, to monitor modulation of *Salmonella* Typhimurium (*S*Tm) infection with *Escherichia coli* Nissle (*Ec*N). Bacterial membranes were thus decorated with azide-modified sugars. Verification of labeling efficiency was performed by clicking alkyne conjugated fluorescent agents (AlexaFluor488) before fluorescence-activated cell sorting (FACS) analysis and *in vitro* fluorescent imaging. The bound fluorescent-agent was localized by fluorescent microscopy. Mean fluorescence intensity significantly differed from the negative control (fluorophore unconjugated cells) for *ST*m and *Ec*N. Yet, this was non-significant compared to control samples with fluorescent agent added without azidosugar pre-incubation. This indicates nonspecific intercalation of the fluorophore, with labeling not resulting from the desired click chemistry reaction. Compared to percent labeled cells for the published positive control (*Bf*) (1,2) neither *Ec*N or *ST*m were labeled significantly. Microscopy results supported this, where bound-fluorescent agent was localized to the membrane in *Bf*, but dispersed randomly for *Ec*N and *ST*m. Here, we have shown that metabolic engineering-click chemistry labeling is not presently translatable to all gram-negative species and success is dependent on fine-tuning conditions based on lipopolysaccharide structure and sugar metabolism of each. Further adaptation is vital before use in PET imaging of *ST*m infection and modulation.

1. Geva-Zatorsky. Nat Med. 2015;21(9):1091-1100.
2.Wang. Eur J Nucl Med Mol Imaging. 2020;47(4):991-1002.

Fig. 1

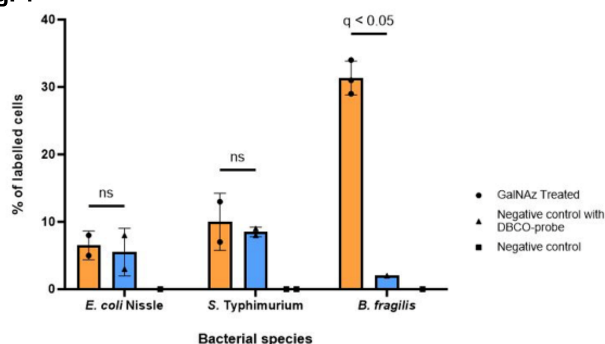


Figure 1. Fluorescence-activated Flow Cytometry results depicting percentage of labelled bacterial cells (*Escherichia coli* Nissle, *Salmonella enterica* Serovar Typhimurium, and *Bacteroides fragilis*) following pre-incubation with *N*-Azidoacetylgalactosamine-tetraacylated (GalNAz) and a 2-hour conjugation with DBCO-AlexaFluor488.

P-GI-008

The role of the ATTAAT-specific methyltransferase M.HpyAVII in transcriptional regulation of Helicobacter pylor*i*

*W. Gottschall[1], F. Ailloud[1,2], C. Josenhans[1,2], S. Suerbaum[1,2]

[1]*Ludwig-Maximilians University, Max von Pettenkofer-Institute / Medical Microbiology and Hospital Epidemiology, München, Germany*

[2]*German Center for Infection Research, München, Germany*

Background: *Helicobacter pylori* (Hp) is a bacterial carcinogenic pathogen infecting about 50% of the human population. Hp is genetically highly diverse and harbors one of the largest portfolios of restriction-modification (RM) systems. Several RM systems have been related to gene regulation and we have shown previously that this effect is partially mediated by direct methylation of promoter regions.

Aims and Methods: Here, we analyzed M.HpyAVII, an ATTAAT-specific methyltransferase, aiming to understand its role in transcriptional regulation of Hp and underlying mechanisms. The M.HpyAVII gene is highly conserved in Hp, and also its only strictly orphan MTase. We performed bioinformatic analyses, transcriptomic assays and phenotypic characterization of Hp wild-type strains and M.HpyAVII mutants.

Results and Conclusions: RNA-Seq analyses showed a pronounced effect of ATTAAT methylation on the Hp transcriptome. Yet, only part of these changes could be explained as direct methylation effects by the presence of an ATTAAT motif in promoter regions. However, pathways related to acid stress and iron metabolism can be observed among differentially regulated genes lacking a motif in their promoters, suggesting that the MTase might influence transcription indirectly through regulation cascades. Following this hypothesis, we could show that the targeted disruption of a methylated motif within a promoter could lead to a significant change in expression not only on the downstream gene but on other distant, functionally related genes as well. Furthermore, we also demonstrate that this type of methylation-dependent regulation cascade can be associated with phenotypic changes, such as copper, nickel and iron sensitivity.

In summary, we combined genome-wide and targeted analysis of gene expression to provide new insights into the regulatory effects of methyltransferases in *H. pylori.*

References:

Malfertheiner P, … & Suerbaum S. *Nat Rev Dis Primers* 2023

Krebes J, … & Suerbaum S. *Nucl. Acids Res.* 2014

Estibariz I, …, Josenhans C & Suerbaum S. *Nucl. Acids Res.* 2019

Ailloud F, Gottschall W & Suerbaum S. *Commun Biol.* 2023

P-GI-009

EVREA-Phage: management of intestinal vancomycin resistant Enterococcus faecium through phage therapy

*A. F. Moreira Martins[1], C. Rohde[1], J. Wittmann[1], A. Gatzmeier[1], B. Henze[1], S. Peter[1]

[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Phage genomics and application Team Department Bioeconomy and Health Research, Brunswick, Germany*

Enterococcus faecium is a versatile Gram-positive bacterium adaptable to a wide variety of environments. It is considered a commensal bacterium of the human gastrointestinal tract although it can become an opportunistic pathogen with potentially fatal outcomes. Vancomycin-resistant *E. faecium* (VREfm) are of great concern, within health care settings with infections rising steadily over the last decade and strains carrying additional resistance to last-resort antibiotics such as linezolid, daptomycin and tigecycline. Colonization of immunosuppressed patients with VREfm is linked to high mortality when compared to non-colonized patients. Currently no effective treatment strategy exists to eradicate VRE colonization and in this context, bacteriophages show great potential as therapeutic tools without known severe[RC1] [AFMM2] adverse events once the phages are sufficiently purified. The aim of the EVREA-Phage project, publicly funded under DZIF support, is the isolation and characterization of potential phage candidates, determining their potential for therapeutic application towards eradicating intestinal VREfm without disrupting the normal gut microbiota. Phages were isolated from multiple waste water and fecal samples, purified and characterized regarding morphology, *in vitro* lytic potential and host range against an extensive panel of clade A1 *E. faecium* strains, mainly represented by internationally relevant clones such as ST80, ST117 and ST203. Through EVREA-Phage a broad VREfm host range phage cocktail has been selected, promising candidates were selected for kinetics, sequencing, phage synergism, phage-antibiotic activity, eliciting of refractory behavior over time and the effect on other gut commensal bacteria. This translational project will include subsequent preclinical investigation in an *in vitro* intestinal model assay taking place at the University Hospital Bonn and an *in vivo* mouse model at the Helmholtz Centre for Infection Research in Braunschweig allowing for a thorough corroboration of the best candidates to finally produce an efficient cocktail against VRE strains and for pharmaceutical purification before later human application.

P-GI-010

Role of host glycosylation in susceptibility to C. difficile infection

*A. Suwandi[1], A. Galeev[2,3], S. Sharma[2,3], A. Kotlyarov[4], J. F. Baines[5,6], M. Gaestel[4], G. A. Grassl[2,3]

[1]*Hannover Medical School, Institute of Cell Biochemistry, Hannover, Germany*

[2]Hannover Medical School, Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany
[3]German Center for Infection Research, Hannover, Germany
[4]Hannover Medical School, Institute of Cell Biochemistry, Hannover, Germany
[5]Institute of Experimental Medicine, CAU Kiel, Kiel, Germany
[6]Max Planck Institute for Evolutionary Biology, Evolutionary Genomics, Plön, Germany

Clostridioides difficile (CD) is a gram-positive anaerobic and spore-forming bacterium that causes diarrhea and colitis. It is the leading cause of nosocomial antibiotic-associated diarrhea in adults that have high levels of recurrence and mortality. Toxin A and B (TcdA/TcdB) are the main CD virulence factors that cause cytotoxic effects and inflammation. Gastrointestinal tract expresses substantial amount of glycan structures and glyconconjugates which is an important factor contributing to the composition and physiology of intestinal microbiota. These glycoconjugates were utilized by intestinal microbiota and pathogens for microbial attachment site and as nutrients. Therefore, variation in host glycosylation can mediate susceptibility to intestinal inflammation such as to enteric pathogens. The glycosyltransferase gene b4galnt2 encodes a beta-1,4-N-acetylgalactosaminyltransferase known to catalyze the last step in the biosynthesis of the Sd(a) and Cad blood group antigens and is expressed in the GI tract of most mammals, including humans. Loss of B4galnt2 expression is associated with altered intestinal microbiota composition. In our previous study, we found B4galnt2 intestinal expression was strongly associated with increased susceptibility to Salmonella infection which is microbiota dependent. However, in our current study, B4galnt2-deficient mice showed increased susceptibility to CD infection. B4galnt2-deficient mice showed significantly higher bacterial loads and increased level of lipocalin-2 as well as inflammation score. *Ex vivo* experiments using intestinal crypt organoids derived from wild type and B4galnt2 deficient mice showed higher cell death in organoid from B4galnt2-/- after CD culture filtrate and CD toxin TcdA treatment. Our preliminary experiments showed that glycosylation can influence the signal activation such as ERK 1/2, p38/MK2 and JNK1 after CD toxins treatment.

Thus, glycosylation can influence to the regulation of signal transduction particularly upon bacterial infection.

P-GI-011

Target identification of cell penetrating Shigella effector protein IpaH7.8

T. Karagöz[1], I. Ngueya Yango[1], Y. Teschke[1], P. Dersch[2], *C. Rüter[1]
[1]University Münster, Institute for Infectiology, Münster, Germany
[2]University Münster, Institute for Infectiology, ZMBE, Münster, Germany

Modulation of host cell processes by bacterial effector proteins is often referred to as a key virulence factor upon infection. By injecting these effector proteins directly into the cytoplasm of the host cell via the type 3 secretion (T3SS) Gram-negative bacteria can alter induced immue responses to their benefit.

Interestingly, previous reports could identify some, usually T3SS delivered proteins, to have the ability to translocate autonomously into eukaryotic cells such as the Yersinia outer protein M (YopM), being able to inhibit caspase 1 and thus promote *Yersinia* infection. Moreover, sequence analysis revealed a species-spanning family of effector proteins bearing strong homologies in protein structure and function including candidates from *Yersinia*, *Salmonella* and *Shigella*.

Another LPX-effector-family member is the *Shigella* effector protein IpaH7.8. By recombinantly expressing and purifying the protein we could show a T3SS-independent, endosomal uptake of IpaH7.8, mediated by two highly-conserved N-terminal α-helices. The latter could also be shown to facilitate an endosomal escape and thus, access to the cytoplasm. To eventually modulate the host's immune response, the well-conserved C-terminal domain of IpaH7.8, comprising an E3 ubiquitin ligase activity, allows hijacking the ubiquitination system of the host. To asses affected target proteins in human macrophages, we conducted a comparative signalotome and ubiquitome analysis upon treatment of THP-1 derived macrophages, revealing potential interaction partners of IpaH7.8. With regard to the anti-inflammatory properties of other LPX effector proteins, the impact of the ubiquitination of target proteins is currently investigated and may allow a therapeutic application of IpaH7.8 to combat inflammatory diseases.

Healthcare-Associated Infections and Pathogens: Prevention, Surveillance, Outbreaks and Antibiotic Stewardship

P-HAIP-001

EC-COMPASS: Long-term, Multi-Centre Surveillance of Enterobacter cloacae complex – an Interpretive Clinical Viewpoint

*M. Mauritz[1], B. Claus[2], *J. Forster[3], M. Petzold[4], S. Schneitler[5], A. Halfmann[5], S. Hauswaldt[6], D. Nurjadi[6], N. Toepfner[4]
[1]Vestische Kinder- und Jugendklinik Datteln, Pediatrics, Datteln, Germany
[2]Pedscience, Datteln, Germany
[3]Institute for Hygiene and Microbiology, Würzburg, Germany
[4]Institute for Medical Microbiology and Virology, Dresden, Germany
[5]Institute of Medical Microbiology and Hygiene, Homburg, Germany
[6]Department of Infectious Diseases and Microbiology, Lübeck, Germany

Introduction: *Enterobacter cloacae* complex (ECCO) comprises closely related Enterobacteriaceae, causing various infections ranging from mild urinary tract infections to severe bloodstream infections [1]. In particular, ECCO has emerged as a significant cause of healthcare-associated infections, particularly in neonatal and adult intensive care [2]. Increasing broad-spectrum antibiotic resistance in ECCO has become an important public health concern [3].

Goals: The aim of this "Enterobacter Cloacae COMplex PASsive Surveillance" (EC-COMPASS) is to provide a detailed overview of the epidemiology and resistance patterns of ECCO isolates detected in routine microbiological diagnostics in four German tertiary care hospitals.

Materials & Methods: Routine microbiological diagnostic data was extracted from the laboratory information systems via Hybase® software of four German tertiary care hospitals.

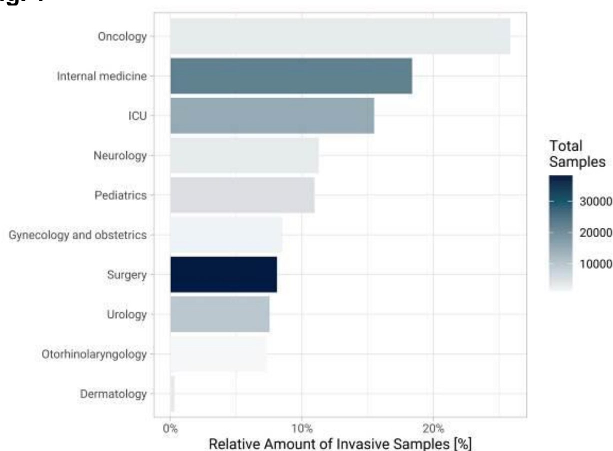
Results: Analysis of datasets from 14,311 patients revealed a higher incidence in male patients. Sources of ECCO were swabs, urine, respiratory secretions, blood cultures, and tissue samples. The highest proportion of invasive ECCO isolates relative to the number of total detections was seen in

the oncology, medical ICU, and surgical ICU departments (Figure 1). Carbapenem resistance was 0.6%, with no major temporal variation between the hospitals.

Summary: The EC-COMPASS provides a comprehensive overview of ECCO infections and colonization in tertiary medical care patients. Clinical specimens most likely came from the Internal Medicine, General Surgery, and Neonatology departments. Detection in urine samples and respiratory specimens was most frequent. Bloodstream infections primarily affect older men treated in the oncology or ICU.

[1] Mezzatesta ML et al. Enterobacter cloacae complex: clinical impact and emerging antibiotic resistance. Future Microbiol 2012;7:887–902.
[2] Dalben M et al. Investigation of an outbreak of Enterobacter cloacae in a neonatal unit and review of the literature. J Hosp Infect 2008;70:7–14.
[3] Annavajhala MK et al. Multidrug-Resistant Enterobacter cloacae Complex Emerging as a Global, Diversifying Threat. Front Microbiol 2019;10:44.

Fig. 1



P-HAIP-002
High hydrostatic pressure as a means to eliminate bacteria from bone tissue

*H. Loeffler[1], J. Waletzko-Hellwig[1], R. J. Fischer[2], M. Basen[2,3], M. Frank[4,5], A. Jonitz-Heincke[1,2], R. Bader[1], A. Klinder[1]
[1]*Rostock University Medical Center, Department of Orthopaedics, Biomechanics and Implant Technology Research Laboratory, Rostock, Germany*
[2]*University of Rostock, Institute of Biological Sciences, Microbiology, Rostock, Germany*
[3]*University of Rostock, Department Maritime Systems, Rostock, Germany*
[4]*Rostock University Medical Center, Medical Biology and Electron Microscopy Center, Rostock, Germany*
[5]*University of Rostock, Department Life, Light and Matter, Rostock, Germany*

Bone tumors, severe fractures, infections, or revision arthroplasties require surgical intervention of the musculoskeletal system, that often results in defects exceeding the self-healing capabilities of bone. Allografts, mostly taken from deceased donors, are crucial in the reconstruction of such defect areas. However, allogeneic bone grafts need preoperative processing to reduce the immunogenic burden and to eliminate a potential microbial load.

This study investigates the microbiological aspects of high hydrostatic pressure (HHP) treatment as a processing method for bone tissue. In order to reduce contaminations while preserving its osteoinductive features and structural integrity, HHP treatment parameters were systematically optimized.

Two model organisms, *Escherichia coli* and *Micrococcus luteus*, underwent HHP treatment of up to 600 MPa, with variations in pressure level, treatment duration, temperature, and pressurization medium. Colony forming units were determined for the calculation of logarithmic reduction factors. Bovine cancellous bone blocks, artificially contaminated with *E. coli* and *M. luteus*, were subjected to a tailored HHP protocol to assess the protective effect of the surrounding tissue.

Results indicate a correlation between bacterial load reduction and applied pressure level. Lowering treatment temperature from 20 °C to 10 °C significantly increased treatment efficiency. Furthermore, the pressurization medium had a significant influence, with 0.9% sodium chloride solution supplemented with low concentrations of surfactants like SDS and EDTA proving most effective in inactivation. Despite being less effective than in bacterial suspensions, a reduction in bioburden was also observed in the artificially contaminated bone blocks.

This study demonstrates the efficiency of HHP treatment in inactivating bacteria in suspension and when associated with bone tissue. Further investigation of HHP treatment protocols that balance microbiological safety, biocompatibility and tissue integrity and analysis of underlying mechanisms may enable improvement of clinical outcome after allogeneic bone grafting.

P-HAIP-003
The influence of established and novel antibiotics on the activity of the WalRK and the LiaRS two-component systems of Bacillus subtilis

*M. Karcher[1], G. Bierbaum[1], M. Gajdiss[1], S. Kirsch-Dahmen[2], R. Müller[2], V. Franz[1]
[1]*UKB IMMIP, AG Bierbaum, Bonn, Germany*
[2]*The Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany*

Due to the increasing number of antibiotic-resistant bacteria, the demand for new antibiotics and new antibiotic targets is growing. Two-component systems (TCS) are attractive targets for antimicrobials. They consist of a histidine kinase (HK) that detects a signal and a response regulator (RR) that conveys adaption of gene expression in response to environmental changes. Mainly, HK and RR genes are encoded on the bacterial genome, a few are found in lower eukaryotes. TCS genes were not found in mammalian genomes. Therefore, a TCS is an optimal new target for old and new antibiotics without affecting human cells. WalRK represents the only essential TCS in Gram-positive bacteria like the pathogen *Staphylococcus aureus*. It controls cell wall metabolism and the expression of autolysins, which are involved in cell wall modeling during cell growth and division. WalRK activates the expression of *ssaA* and ihnhibits *iseA*. The upstream sequences of *liaI*, *iseA* and *ssaA* were fused to the luminescence gene cluster of *Photorhabdus luminescens* and integrated into the *B. subtilis* genome. With these reporter clones, luminescence assays were performed using a TECAN plate reader in the presence of different antibiotics. The activity of the reporter strains was evaluated

and showed typical expression patterns for all three clones in the presence of different antibiotic classes. The expression of *ssaA* was always downregulated. Lipid II binders enhanced the expression of *liaI* and *iseA*. A low activation of *liaI* and a strong upregulation of *iseA* were observed with β-lactams. The expression of all three promoters was downregulated in the presence of protein, RNA and DNA biosynthesis inhibitors. Published WalRK inhibitors resulted in a downregulation of *iseA* and *ssaA* at concentrations below the minimal inhibitory concentration and an upregulation of *liaI* at higher concentrations. WalRK is a target for new antibiotics in bacteria. In this study, reporter systems were created in *B. subtilis*, to test compounds for an inhibitory effect against WalRK. The system showed not the expected upregulation of *iseA* in the presence of published WalRK inhibitors. The expression pattern was not comparable with the observed profiles in presence of established antibiotics. Therefore, an inhibition of the phosphorylation and dephosphorylation activity of WalK is discussed.

P-HAIP-004

First real-world application of Ambient Artificial Intelligence Sensors for automated and real-time early detection of Respiratory Infections in a group of European outpatient clinics based on ambient symptom load to enable better infection prevention policies and hygiene compliance.

*M. Groh[1], P. Tinschert[1], D. Cleres[1], R. Naidoo[2]
[1]*Resmonics AG, Zürich, Switzerland*
[2]*University of Oxford, Nuffield Department of Medicine, Oxford, United Kingdom*

Question

Acute respiratory infections (ARIs) cause 1.3M cases and 20-30% of HAI mortality in Europe.[1,2] Sick days and lost shifts caused by ARIs have a heavy toll on the stretched resources of hospitals, costing millions. Despite this, implementation of respiratory hygiene measures is inconsistent and can be as low as 25%.[3,4]

Methods

We present a novel AI-powered sensor technology that aims to address the medical and economic impact of respiratory infections in healthcare facilities. It uses CE-certified acoustic AI[5] to monitor rooms for symptoms of respiratory infections. Detected symptoms are classified and risk scores provided in real-time, incorporating additional ambient sensorial data. The solution provides on-device warnings or automatic reports received before weekly or daily shift planning meetings to review and adjust infection prevention policies. Here we present results from a 4-month trial in three European outpatient clinics during the recent influenza/COVID-19 season.

Results

Preliminary results suggest that the technology can serve as an early indicator of evolving infection risk. Increased risk scores preceded both publicly available regional data on infections and occurrences of staff illness and absenteeism. The data allowed real-time risk stratification of different rooms within the same facilities and the facilities themselves. In the intervention phase of the study, the integration of the solution in the daily clinical workflow was assessed.

Conclusions

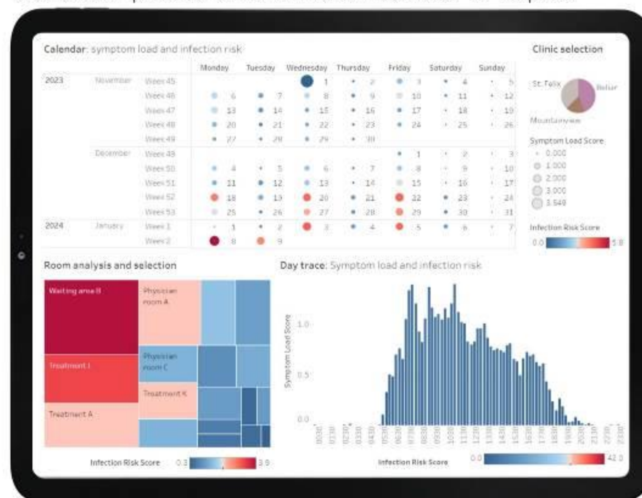
As the solution can be implemented with minimal burden to the clinical workforce, the awareness for following and implementing hygiene measures could be increased, leading to a material increase in employees taking infection prevention actions to mitigate preventable and nosocomial respiratory diseases in hospital.[6] On a larger scale, the solution could contribute to national pandemic early warning and preparedness systems.[7]

References

1. European CDC, PPS. 2023
2. Zacher B. et al., Euro Surv. 2019
3. Kuster S.P. et al., Inf Ctrl & Hosp Epid 2021.
4. Glöckner A., Interview, Hygiene Hub 2018
5. Tinschert et al., J Ast All 2020
6. Resmonics AG, unpublished dat.
7. Husain et al., OJEMB 2022

Fig. 1

Groh et al. AI-powered real-time infection risk tracker for hospitals



P-HAIP-005

Behaviour of Candida auris and Candida parapsilosis in comparison to other Candida species in laundry hygiene tests according to EN 17658

*B. Brands[1], D. Bockmühl[1,2]
[1]*Dr. Brill + Prof. Bockmühl GmbH Institute for Applied Hygiene, Kleve, Germany*
[2]*University of Applied Sciences Rhein-Waal, Faculty of Life Sciences, Kleve, Germany*

In 2022, the standard EN 17658 for laundry disinfection was released which uses *Candida albicans* as a test organism for yeasts.

Recently, a rise in infections by different *Candida* species, such as *C. auris* and *C. parapsilosis,* have been reported [1–3], which could result in a higher relevance of these species for laundry hygiene, not only in the hospital, but also in domestic health-care settings.

This study focuses on the reduction of four different *Candida* strains in standardized laundry processes according to EN 17658 to detect possible differences between the original test strain and emerging pathogenic *Candida* species.

A commercially available powder colour detergent, colour detergent combined with addition of a bleach releasing additive and a heavy-duty detergent were compared to the IEC A standard detergent with and without added activated

oxygen bleach. Test were performed at 40 °C, which on the one hand is the highest allowed temperature for the test method but on the other hand ensures sufficient bleach activation. The experimental setup allows for an assessment of the hygiene efficacy based on domestic washing procedures, which are important for people being cared for at home but could also be used to estimate the efficacy of professional hospital laundering.

Our results show a differential behavior of the investigated strains, thus suggesting a need for monitoring the hygienic efficacy of laundry processes on emerging *Candida* species.

References:

1. Branco, J., Miranda, I. M. & Rodrigues, A. G. Candida parapsilosis Virulence and Antifungal Resistance Mechanisms: A Comprehensive Review of Key Determinants. *J. Fungi* **9**, 80 (2023).
2. Sun, M. *et al.* Increase in Candida parapsilosis candidemia in cancer patients. *Mediterr. J. Hematol. Infect. Dis.* **11**, 1–7 (2019).
3. Cristina, M. L. *et al.* An Overview on Candida auris in Healthcare Settings. *J. Fungi* **9**, 913 (2023).

P-HAIP-006

Application of Fourier-transform infrared spectroscopy for typing in a neonatal Kluyvera ascorbata clinical outbreak

*M. Cordovana[1], J. B. Hans[2], A. B. Pranada[3]

[1]*Bruker Daltonics GmbH & Co. KG, Bremen, Germany*

[2]*National Reference Center for multidrug resistant Gram-negative bacteria, Department for Medical Microbiology Ruhr-University Bochum, Dortmund, Germany*

[3]*MVZ Dr. Eberhard & Partner Dortmund, Department of Medical Microbiology, Dortmund, Germany*

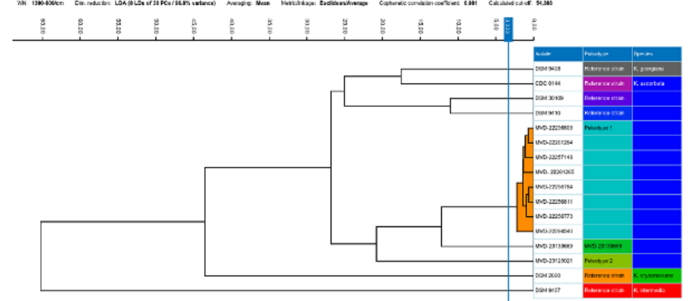
Introduction. Despite not commonly found in clinical samples, *Kluyvera ascorbata* has the potential to cause clinically relevant infections, especially in healthcare settings. Typing methods have to be applied to recognize and track the transmissions and outbreaks. Fourier transform infrared spectroscopy (FT-IRS) is a recently introduced method for microbial typing on different intraspecies levels with the potential of a near-time monitoring due to its speed. In this study, we evaluate the applicability of FT-IRS methodology for the typing of a less common pathogen.

Methods. Sixteen strains of *Kluyvera* spp. were included in this study. Among them, n=10 *K. ascorbata* isolates collected during the routine screening of a neonatal intensive care unit (NICU) in September 2022, n=3 *K. ascorbata* reference strains from culture collections and the type strains of *K. cryocrescens, K. georgiana* and *K. intermedia* (total n=3). The strains collected during the screening underwent typing by PFGE, performed by the National Reference Center in Bochum, Germany. Eight out of ten screening *K. ascorbata* strains were typed by PFGE as belonging to the same pulsotype. FT-IRS analysis was performed with the IR Biotyper® system (IRBT; Bruker Daltonics, Germany). Spectra were acquired from 3 independent biological replicates, cultivated on Columbia blood agar (Becton Dickinson) at 37 °C for 24±2 h. Exploratory analysis for clustering was performed by Hierarchical Cluster Analysis (HCA) applying Euclidean average linkage.

Results. IR Biotyper HCA shows that the eight *K. ascorbata* outbreak isolates cluster together, in complete agreement with PFGE results (Figure 1). Further, the outbreak isolates resulted clearly differentiated from the two non-outbreak isolates, as well as from the three culture collection strains. Also, the type strains of the other *Kluyvera* species resulted well differentiated from *K. ascorbata*.

Conclusions. FT-IRS could successfully reveal the clonality among *K. ascorbata* outbreak isolates in this study. Due to its speed and ease-of-use the method has the potential to be applied in a routine setting for near-time monitoring in hospital hygiene.

Fig. 1



P-HAIP-007

Tackling the mechanism of species-specific drug combinations in bacteria

*M. Airoldi[1], A. R. Brochado[1]

[1]*University of Tuebingen, IMIT, Tübingen, Germany*

Antibiotic combinations have been previously shown to be highly species-specific (Brochado et al. Nature, 2018). While a mechanistic basis for such specificity is yet to be understood, the potential to use combinations for narrow-spectra treatment, where the pathogen is primarily targeted and the commensal bacterial population is protected, is there. Here we investigate species-specific synergistic combinations against *Pseudomonas aeruginosa,* a particularly problematic Gram-negative pathogen, given its intrinsic resistance to major classes of antibiotics. Here we focus on understanding the mechanism of action underlying synergy between aminoglycosides and β-lactams, because of it clinical relevance – it is used to treat infections by several Gram-negative pathogens.

We started by assessing synergy across different bacterial strains beyond *P. aeruginosa*, including *Escherichia coli, Salmonella Typhimurium, Klebsiella pneumoniae, Acinetobacter baumannii, Burkholderia pseudomallei.* Our data shows that synergy potency widely varies across all species. It is particularly potent against *P. aeruginosa*, while the weakest effect is observed for *E. coli* strains.

Plotz and Davis (Science, 1962) previously showed that *Escherichia coli* pre-treatment with the β-lactam penicillin, quicken the subsequent killing by the aminoglycoside streptomycin, due to increased uptake. However, it is not understood why the activity of the combination is stronger against *P. aeruginosa.* In trying to understand the reasons for such species specificity, we next evaluated the activity of the individual antibiotics against *E.coli* and *P.aeruginosa* in relation to growth inhibition, killing kinetics and morphology changes. While aminoglycoside activity is very similar between the two species, greater differences can be

observed on the β-lactam side, thus suggesting that β-lactam activity is determinant for the synergy. We are currently investigating how beta-lactam specificity impacts synergy outcome. This study will help understanding and improving antibiotic action against *P. aeruginosa*, one of the most-difficult-to-treat bacterial pathogens of today.

P-HAIP-008

Impact of gender, medical qualifications and area population on the heads of municipal public health departments during the SARS-CoV-2/COVID-19 pandemic

D. Mitic[1,2], J. Franke[3], H. Henke-Möller[4], N. Wischnewski[5], N. L. Schwerdtner[6], A. Jurke[7], R. Brosow[8], V. Kinne[1,2], F. Kipp[1,2], *S. Trommer[1,2]
[1]*Institute of Infectious Diseases and Infection Control, Jena, Germany*
[2]*University Hospital Jena, Institute of Infectious Diseases and Infection Control, Jena, Germany*
[3]*Thuringian Ministry of Labor, Social Affairs, Health, Family and Women's Affairs, Specialist matters ÖGD, Erfurt, Germany*
[4]*Thüringer Landesverwaltungsamt, Gesundheitswesen, Weimar, Germany*
[5]*Charlotteburg-Wilmersdorf Health Department, Health Department, Berlin, Germany*
[6]*Saxon State Ministry for Social Affairs and Social Cohesion, Public health service, infection protection, Dresden, Germany*
[7]*State Center for Health NRW, Healthy living environments, Bonn, Germany*
[8]*Bavarian State Ministry of Health, Care and Prevention, Fundamental matters of the ÖGD, München, Germany*

Introduction

The SARS-CoV-2/COVID-19 pandemic exposed deficiencies in the management of German municipal public health authorities. The BMBF joint project SARSCoV2Dx aims to identify the needs of the heads of these authorities who were involved in pandemic management and propose measures for future crises. This study assessed the impact of gender, medical qualifications, and population size on the resilience and psychosocial workload of heads during the pandemic.

Materials and Methods

In June 2023, an online questionnaire surveyed heads in Thuringia, Saxony, North Rhine-Westphalia, Bavaria, and Berlin. Analysis included gender, medical qualifications, and population size influences on workload, assessed through the ERI questionnaire and RS-13 scale.

Results

Results showed 54.1% female, 44.6% male, and 1.4% diverse heads. Medical qualifications included 71.9% public health, 67.2% other medical, and 17.2% public health degree, with an average of 1.5 specializations. No significant gender-medical qualification correlation was found ($\chi^2(6) = 6.32$, $p = 0.38$). Mean RS-13 score was $M = 72.66$ ($SD = 12.42$), indicating high stress resilience. Male heads had slightly higher scores ($M = 74.37$; $SD = 10.55$) than females ($M = 73.14$; $SD = 8.48$). Psychosocial stress, indicated by ER-ratio ($M = 1.54$; $SD = 0.50$), was higher for females ($M = 1.61$; $SD = 0.63$) than males ($M = 1.47$; $SD = 1.47$). Most heads managed populations > 50,000 ≤ 150,000 (40.5%), with a moderate correlation between population and resilience ($\rho = .341$, $p = .010$).

Summary

In summary, the study found a majority of female heads, no significant gender-medical qualification correlation, and high stress resilience among heads with medical specializations. Regardless of gender, heads exhibited high resilience. Larger responsibilities due to heads managed regions with higher population correlated with higher resilience, but psychosocial distress at work was prevalent. Further research is required to explore additional factors influencing pandemic management.

P-HAIP-009

Evaluation of the Vivalytic one Analyser for detecting uropathogenic bacteria and antimicrobial resistances in urine samples of urological patients

*J. Hartmann[1], F. Wagenlehner[1], C. Imirzalioglu[2], M. Fritzenwanker[2], T. Hain[2]
[1]*Clinic for Urology, Pediatric Urology and Andrology, Justus Liebig University, Giessen, Germany*
[2]*Institute of Medical Microbiology, Justus Liebig University, Giessen, Germany*

Question:

Does the transport of urine samples of urological patients have influence on uropathogen detection rate and antibiotic susceptibility testing of the Vivalytic Urinary Tract Infections (UTI) analyser, a qualitative microarray-based point-of-care (POC) test.

Methods:

During a period of four weeks, we used the Vivalytic test to analyze 126 consecutive urine samples of urological patients (51 women, 75 men) with a mean age of 62,9 years. Samples processed with the Vivalytic UTI test were preselected for bacteriuria by screening with urine flow cytometry (cut-off ≥ 70 cells/µl). To evaluate the transport effect on the uropathogens within the urine samples, we performed the Vivalytic UTI test first at the point-of-care (urological department; before transport) and secondly at the microbiological laboratory (after transport) and compared the results to standard urine culture and antibiotic sensitivity testing according to EUCAST.

Results:

As described in **Table 1**, the species identification of the nineteen uropathogens reached a diagnostic accuracy of ≥ 90.27% with a negative predictive value (NPV) ≥ 93.67%, except for *Staphylococcus epidermidis. Klebsiella aerogenes*, *Providencia stuartii* which were not detected in the tested urine specimens. *Staphylococcus saprophyticus* was detected solely by Vivalytic UTI before the transport. The results displayed a higher degree of concordance when comparing the standard test with Vivalytic UTI tested after the specimen transport (p = 0.0336). The antibiotic resistance gene detection has a higher diagnostic accuracy after the transport (≥ 84.15) compared to POC-testing (≥ 81.7%), except for Vancomycin resistance genes, that were solely found with the Vivalytic UTI test.

Conclusion:

In this study, the Vivalytic UTI test displayed high sensitivity and specificity in identifying uropathogenic organisms and antibiotic resistance markers directly from clinical urine samples in a point-of-care setting within 146 min. According to our results, the transport had an influence on pathogen detection rate and antibiotic susceptibility testing of the Vivalytic UTI analyser.

Fig. 1

Table 1. N-no.of uropathogens found by POC with Vivalytic UTI; SE-Sensitivity; SP-Specificity; ACC-Accuracy

Comparison Vivalytic UTI to standard urine culture and antibiotic sensitivity test **before** transport					Comparison Vivalytic UTI to standard urine culture and antibiotic sensitivity test **after** transport				
POC detected uropathogens before transport	**N**	**SE (%)**	**SP (%)**	**ACC**	**POC detected uropathogens after transport**	**N**	**SE (%)**	**SP (%)**	**ACC**
A.baumani	**2**	100.00	99.15	99.15	*A.baumani*	**2**	100.00	99.11	99.12
C.freundii	**3**	100.00	99.14	99.15	*C.freundii*	**3**	100.00	99.10	99.12
C.koseri	**1**	0.00	99.15	98.13	*C.koseri*	**0**	0.00	100.00	99.12
E.cloacae	**10**	100.00	97.30	97.46	*E.cloacae*	**7**	83.33	98.13	97.35
E.coli	**39**	94.29	92.77	93.22	*E.coli*	**36**	90.62	91.36	91.15
E.faecalis	**39**	87.18	93.67	91.35	*E.faecalis*	**41**	88.10	94.37	92.04
E.faecium	**7**	80.00	97.35	96.61	*E.faecium*	**7**	100.00	98.15	98.23
K.aerogenes	**0**	/	100.00	/	*K.aerogenes*	**0**	/	100.00	/
K.oxytoca	**3**	66.67	99.13	98.31	*K.oxytoca*	**1**	50.00	100.00	99.12
K.pneumoniae	**11**	90.00	98.15	97.46	*K.pneumoniae*	**18**	88.89	90.38	90.27
M.morganii	**3**	33.33	98.26	96.61	*M.morganii*	**3**	66.67	99.09	98.23
P.aeruginosa	**12**	90.91	98.13	97.46	*P.aeruginosa*	**9**	72.73	99.02	96.46
P.rettgeri	**4**	100.00	98.28	98.31	*P.rettgeri*	**2**	100.00	99.11	99.12
P.stuartii	**0**	/	100.00	/	*P.stuartii*	**0**	/	100.00	/
Proteus	**12**	100.00	96.36	96.61	*Proteus*	**13**	100.00	94.83	95.12
S.agalactiae	**6**	100.00	97.39	97.47	*S.agalactiae*	**1**	50.00	100.00	99.12
S.aureus	**4**	0.00	96.55	94.92	*S.aureus*	**3**	0.00	97.30	95.58
S.epidermidis	**26**	62.50	80.91	79.66	*S.epidermidis*	**23**	62.50	83.81	82.30
S.marcescens	**17**	0.00	85.59	/	*S.marcescens*	**11**	100.00	91.89	92.04
S.saprophyticus	**1**	/	99.15	/	*S.saprophyticus*	**0**	/	100.00	/
C.albicans	**5**	50.00	98.17	96.46	*C.albicans*	**4**	80.00	99.12	98.31
Antibiotic resistance genes **before** transport					Antibotic resistance genes **after** transport				
Trimethoprim		62.50	93.1	84.15	Trimethoprim		68.18	95.00	87.80
Methicillin		50.00	83.33	81.71	Methicillin		100.00	83.54	84.15
Vancomycin		/	98.78	/	Vancomycin		/	98.78	/

P-HAIP-010
Development of the consumption of reserve-antibiotics in German acute care hospitals from 2018 to 2022

*B. Schweickert[1], M. Feig[1], M. Schneider[1], M. Behnke[2], L. A. Peña Diaz[2], C. Geffers[2], K. Gröschner[1], D. Richter[1], T. Eckmanns[1], M. Abu-Sin[1]
[1]Robert Koch Institute, Department of Infection Epidemiology, Berlin, Germany
[2]Charité - University Medicine Berlin, Institute for Hygiene and Environmental Medicine, Belin, Germany

Background: The SARS-CoV-2-pandemic has affected the framework conditions in which hospital antimicrobial prescribing occurred.

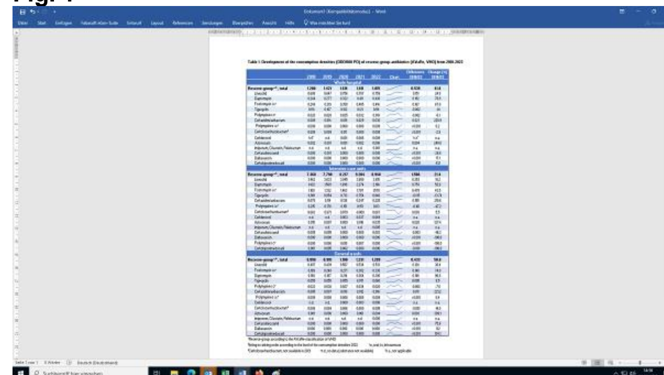
Goals: Data on the development of the consumption of reserve-antibiotics in the hospital sector from 2018 to 2022 are presented.

Materials and Methods: Data from 180 hospitals, continuously participating from 2018 to 2022 in the national surveillance system of hospital antimicrobial consumption (AMC, Robert Koch Institute), were analyzed according to the ATC (Anatomical Therapeutic Chemical)/DDD (Defined Daily Dose)-method of WHO. AMC was quantified by consumption density (CD) expressed in DDD/100 patient days (PD) and DDD/100 admissions (AD). Data on antibiotics belonging to the reserve-group according to the AWaRe-classification of WHO have been analyzed for the whole hospital and stratified by ward type (intensive care unit (ICU), general ward).

Results: From 2018 to 2022 the CDs of reserve-antibiotics increased from 1.3 to 1.8 DDD/100 PD by 42% and accounted for 2.4% and 3.4% of total consumption in 2018 and 2022, respectively (Table 1). The strongest increase occurred in 2020 and 2021 and slowed down in 2022. Stratification by ward type revealed that on ICUs the CDs showed the steepest increase in 2021 followed by a decrease in 2022. On general wards the CDs presented with a linear increase from 2018 to 2021 that slowed down in 2022. Except for polymyxines i.v and tigecycline, a rise of varying degrees could be observed for linezolid, daptomycin, fosfomycin i.v., ceftazidim/avibactam, cefiderocol and aztreonam. The most prominent increases could be observed in antibiotics assumed to be effective against carbapenem-resistant gram-negative pathogens. On ICUs, CDs expressed in DDD/100 AD showed relatively higher increases during the two pandemic years compared to those expressed in DDD/100 PD, which went along with a rise of the length of stay.

Summary: The rise of most reserve-antibiotics showed the strongest increase during the pandemic years 2020 and 2021 and no return to pre-pandemic values in 2022. Close monitoring of these agents and assurance of their prudent use constitute important targets of antibiotic stewardship activities.

Fig. 1



P-HAIP-011
Phenotypic within-host evolution of vancomycin-resistant Enterococcus faecium

*V. Blaschke[1], V. Rauschenberger[1], S. Kampmeier[1]
[1]University Würzburg, Institute of Hygiene and Microbiology, Würzburg, Germany

Introduction

Vancomycin-resistant *Enterococcus faecium* (VREfm) are emerging multidrug-resistant pathogens. The number of nosocomial infections caused by VREfm is increasing, posing a major threat worldwide. VREfm colonization of the gastrointestinal tract often precedes the development of invasive infection. The underlying mechanism by which a colonizing strain becomes invasive in terms of phenotypic changes is still not clear. The aim of this study was to investigate the phenotypic differences between colonizing and infection isolates of VREfm.

Materials & Methods

In total 27 VREfm isolate pairs were collected from patients who were initially colonized with VREfm and subsequently developed a bloodstream infection (BSI). Each isolate pair consisted of one colonizing isolate (rectal swab) and one subsequent BSI isolate. First, the ability of VREfm isolates to form biofilm was compared. For this, overnight cultures were grown in tryptic soy broth (TSB) and diluted in a 96-well plate. After incubation, the biofilms were stained with crystal

violet and OD_{595} was measured. A spot-killing assay was performed to assess the competition between the paired isolates. Each colonizing isolate was spotted on Brain Heart Infusion (BHI) agar containing the corresponding infection strain and vice versa.

Results

The mean OD_{595} measured of all isolates indicated an overall limited ability to form robust biofilms. In total, four isolates (three invasive and one colonizing isolate) significantly exceeded this mean by 2.1–3.1 times (p=0.002–0.03). In the spot killing assay four isolates were identified which lysed the corresponding strain. Of these, three were colonizing, while one was an invasive isolate. Thus, no statistically significant increase in lysis of either the colonizing or the infection strain was observed.

Summary

Our preliminary data showed no significant phenotypic differences between colonizing and infection isolates with respect to biofilm formation and competition. However, some isolates showed an increased biofilm or lysis by others. Further investigation is necessary to address the underlying mechanisms and to correlate phenotypic with genotypic data.

P-HAIP-012
Characterising a cluster of NDM-5-positive Escherichia coli clinical isolates

R. Arazo del Pino[1,2], T. Burgwinkel[1,2], L. Casselman[1], S. Chorlton[3], V. Persy[1,2], J. Zweigner[2,4], P. G. Higgins[1,2], *K. Xanthopoulou[1,2]
[1]*Institute for Medical Microbiology, Immunology and Hygiene, Faculty of Medicine and University Hospital Cologne, Köln, Germany*
[2]*German Center for Infection Research, Partner site Bonn-Cologne, Köln, Germany*
[3]*Bugseq Bioinformatics, Vancouver, Canada*
[4]*Department of Infection Control and Hospital Hygiene, Faculty of Medicine and University Hospital Cologne, Köln, Germany*

Introduction

Based on data from the National Reference Center for Gram-negative bacteria, the prevalence of NDM-5 carbapenemase in Germany has increased in recent years. In the present study, we investigated the clonality and genetic background of NDM-5 positive *Escherichia coli* isolates within a German hospital.

Materials

Four isolates obtained from four patients between February and September 2023 were subjected to whole-genome sequencing (WGS) using the MiSeq (Ilumina) and MinION (Nanopore). Species identification, core-genome MLST (cgMLST), 7-loci MLST, resistome, and plasmidome analysis relied on assemblies generated using Unicycler (Ubuntu) and BugSeq (https://bugseq.com/), an automated bioinformatics platform. Furthermore, plasmids were annotated using BAKTA and compared by BRIG.

Results

The four *E. coli* isolates (AML1585, AML1672, AML1679, and AML1698) were tested carbapenem-resistant using VITEK (bioMérieux) by the routine diagnostic laboratory. The isolates differed by up to 2302 alleles based on cgMLST and were therefore considered unrelated. However, all isolates shared antimicrobial resistance genes, i.e., bla_{NDM-5}, *aadA2*, *dfrA12*, and *sul1,* which were encoded on multireplicon plasmids, containing the IncFII, IncFIB and IncFIA replicons (Table 1). The BugSeq platform grouped pAML1585 and pAML1679 as closely related (plasmid cluster A). Plasmid cluster A was confirmed using BRIG. Furthermore, plasmid analysis confirmed that pAML1585 and pAML1679 shared not only the plasmid backbone but also antimicrobial resistance genes including the NDM-5 suggesting a mobile genetic element transfer between the two isolates. The NDM-5 encoding plasmids pAML1672 and pAML1698 were unrelated to plasmid cluster A and were considered as unique.

Summary

Our findings revealed the presence of the carbapenemase NDM-5 encoded on a plasmid in four unrelated *E. coli* isolates. Two NDM-5-encoding plasmids formed a cluster confirmed by the BugSeq platform and our in-house workflow. These data underline the crucial contribution of WGS in tracking plasmid spread as well as a user-friendly application of bioinformatics pipelines for analysing bacterial genomes and plasmids.

Fig. 1

Table 1. Summary of sequence types (STs), antimicrobial resistance genes, replicon types, plasmid size and plasmid cluster for the four unrelated *E. coli* isolates.

Isolate	Sequence Type	NDM-5-encoding plasmid			
		Acquired Antimicrobial Resistance Gene	Replicon Type	Plasmid Size (bp)	Plasmid Cluster
AML1585	648	*aadA2; $bla_{CTX-M-15}$; bla_{TEM-1B}; bla_{OXA-1}; bla_{NDM-5}; catB3; sul1; dfrA12*	*IncFIA; IncFIB; IncFII*	137,747	A
AML1679	46	*aadA2; $bla_{CTX-M-15}$; bla_{NDM-5}; erm(B), sul1, dfrA12*	*IncFIA; IncFIB; IncFII*	110,516	A
AML1672	405	*aac(3)-lia; aadA; aadA5; aac(6')-Ib-cr; $bla_{CTX-M-15}$ bla_{OXA-1}; bla_{NDM-5}; mph(A); catB3; sul1; tet(B); dfrA12; dfrA17*	*IncFIA; IncFIB; IncFII, p0111*	170,631	unique
AML1698	410	*aadA5, $bla_{CTX-M-15}$, bla_{NDM-5}; mph(A), sul1, tet(A); dfrA17*	*IncFIA; IncFIB; IncFII*	136,349	unique

---

P-HAIP-013
The Flavin Transferase ApbE as a key drug target - structural revelations for antibiotic discovery

*M. Zeno[1], J. Vering[1], G. Fritz[1], J. Steuber[1]
[1]*Universtiy Hohenheim, Cellular Microbiology, Stuttgart, Germany*

The flavin transferase ApbE is a lipoprotein located in the periplasm of numerous bacterial species. ApbE binds to FAD noncovalently and facilitates the exclusive insertion of the FMN cofactor via a phosphoester bond to a threonine residue within the target protein [1]. Through this posttranslational modification (FMNylation), ApbE matures several bacterial proteins involved in energy metabolism, including subunits of unique multienzyme complexes: the Na+-translocating NADH: ubiquinone oxidoreductase (C and B) [2] and the Rhodobacter nitrogen fixation (RNF) complex (G and D) [3], along with other proteins like fumarate reductase and urocanate reductase [4]. As a critical assembly factor, ApbE emerges as an excellent target for

drug discovery. Inhibiting the active site of ApbE could impede the maturation of Na+ -NQR and other enzymes vital for microbial cellular respiration. Our pursuit of this target in multidrug-resistant pathogens utilizes the structure-based drug discovery approach: Crystallography-based fragment screening. For this, the ApbE enzyme from *E. coli* was produced recombinantly and purified by affinity and size exclusion chromatographic steps under retention of catalytic activity. Crystals of ApbE, obtained through automated screening and optimized for X-ray diffraction up to 1.8 Å, revealed the FAD binding pocket. Identification of unique interactions and key residues within the 3D structure of ApbE sets the stage for the next step. Using ApbE crystals, we aim to implement a fast fragment and compound screening pipeline, providing a potential starting point for developing a new class of antibiotics to combat antimicrobial resistance, especially in multidrug-resistant gram-negative bacteria

[1] Bogachev, Baykov,and Bertsova(2018). Biochemical Society transactions 46, 5, 1161–1169.
[2] Méheust, Raphaël; Huang, Shuo; Rivera-Lugo, Rafael; Banfield, Jillian; Light, Samuel (2021) eLife 10. DOI: 10.7554/eLife.66878
[3] Hau, Kaltwasser, Muras, Casutt, Vohl, Claußen, Steffen, Leitner, Bill, Cutsail, DeBeer, Vonck, Steuber, Fritz(2023). Nat. Struct. Mol. Biol. 2023
[4] Vitt, Prinz, Eisinger, Ermler, and Buckel. (2022). Nature Communications 13,6315

P-HAIP-014

Microbiological evaluation of an anti-infective biopolymer implant coating with two modes of operation

*M. Nonhoff[1], J. Pützler[1], M. Fobker[2], S. Niemann[3], J. Hasselmann[1,4], G. Gosheger[1], M. Schulze[1]
[1]*University Hospital Münster, Clinic for General Orthopaedics and Tumor Orthopaedics, Münster, Germany*
[2]*University Hospital Münster, Central laboratory, Münster, Germany*
[3]*University Hospital Münster, Institute for Medical Microbiology, Münster, Germany*
[4]*University of Applied Sciences Münster, Labor für Werkstofftechnik, Steinfurt, Germany*

Question

Periprosthetic joint infections (PJI) are more likely to occur with increasing implant volume, which can lead to clinical challenges due to the need for multiple revision surgeries. Currently, only a few anti-infective implant coatings are in clinical use with the aim to reduce this risk [1]. Furthermore, biofilm growth on the implant surface is not adequately inhibited by systemic antibiotic treatments.

A biocompatible implant coating with an anti-infective concentration of homogeneously dissolved silver ions has been developed to prevent biofilm formation on the implant surface. Additionally, extracorporeal shock waves (ESW) can be used to non-invasively activate the targeted burst-release of silver ions into the vicinity of the implant at any time.

Methods

The study analyzed the two modes of operation. Biofilm inhibition assays were conducted using *Staphylococcus epidermidis* RP62A. The silver released by activation with ESW was analyzed using graphite furnace atomic absorption spectrometry (GF-AAS), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), confocal laser scanning microscopy (CLSM) and growth curve analysis. Additionally, a combined biofilm and activation assay was also performed.

Results

The biofilm of *S. epidermidis* was inhibited by 1000-fold with a 6% silver coating. Activation resulted in the release of 57.8 ± 0.04738 mg/L of silver. SEM, EDX and CLSM analyses indicate complete local release of Ag by disrupting small areas of the coating. The analysis of the growth curve revealed a delay of 12 hours in growth. The combination of both modes resulted in complete eradication after 24 hours.

Conclusions

This implant coating has the potential to protect against PJI and enables non-invasive activation to release silver in a targeted and controlled manner. The coating could be a complementary treatment option to systemic antibiotics for PJI.

References

[1] Wildemann B, Jandt KD (2021) Infections @ Trauma/Orthopedic Implants: Recent Advances on Materials, Methods, and Microbes-A Mini-Review. Materials (Basel) 14. https://doi.org/10.3390/ma14195834

P-HAIP-015

Genome-oriented outbreak identification reveals a gross overestimation of outbreak frequency for vancomycin-resistant Enterococcus faecium

*A. Rath[1], B. Kieninger[1], N. Mirzaliyeva[1], A. Caplunik-Pratsch[1], J. Fritsch[1], S. Schmid[2], P. Mester[2], W. Schneider-Brachert[1]
[1]*University Hospital Regensburg, Department of Infection Prevention and Infectious Diseases, Regensburg, Germany*
[2]*University Hospital Regensburg, Department for Internal Medicine I, Regensburg, Germany*

Introduction

Precise outbreak detection is crucial for efficient outbreak management. The German legal outbreak definition implies the nosocomial acquisition of the same bacteria by minimum two epidemiologically linked patients. For vancomycin-resistant enterococci (VRE) the "same bacteria" is typically understood as identical species and *van*-genotype. In this study, we evaluated how the additional use of whole-genome sequencing (WGS) affects outbreaks identification at an intensive care unit of a tertiary care hospital during January 2020 – February 2021.

Methods

All patients with VRE (*E. faecium* only) were identified retrospectively. Admission period and time of VRE detection were documented. VRE detection >48h after admission was considered nosocomial. Two outbreak definitions were used: 1. the legal definition as stated above, and 2. the legal definition combined with WGS data. Hereby outbreak confirmation was achieved, if a difference in ≤3 alleles in pairwise comparison by core-genome (cg) multilocus sequence typing (MLST) was detected. WGS (Illumina MiSeq) was performed on the first VRE isolates per patient, and analyzed using SeqSphere+.

Results

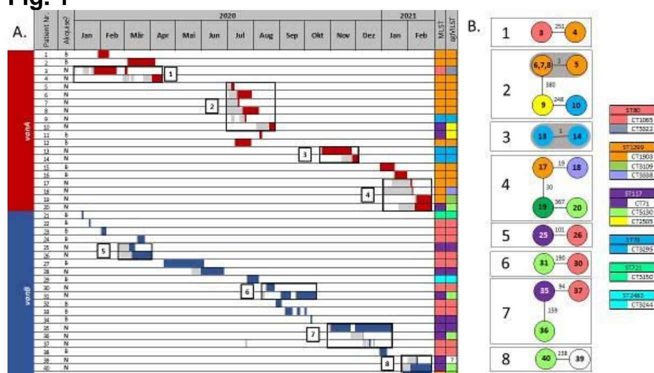
41 VRE-positive patients were identified, including 22 nosocomial cases. The median length of stay was 20 days (2-113 days), whereas nosocomial VRE detection occurred after a median of 17 days (3-52 days). According to the legal definition, eight VRE outbreaks involving 2-6 patients were suspected during the study period.

WGS revealed the presence of six different VRE sequence types (ST) - predominantly ST1299, ST80, and ST117 - and 11 complex types (CT). Four of the suspected outbreaks proved to be false as isolates differed by MLST. Moreover, cgMLST only confirmed two outbreaks of the remaining four clusters. These involved two and four (of initially six) patients, respectively.

Conclusion

Only two of eight legally suspected VRE outbreaks could be confirmed using WGS. This proves both a gross overestimation of the frequency of VRE outbreaks, and a clear deficit in precision of outbreak identification on a daily basis without the use of WGS, which lead to wasteful use of outbreak management resources.

Fig. 1



P-HAIP-016
The effect of serum origin on the anti-infective properties of a biopolymer implant coating containing silver Ions

*M. Nonhoff[1], J. Pützler[1], M. Fobker[2], S. Niemann[3], J. Hasselmann[1,4], G. Gosheger[1], M. Schulze[1]
[1]*University Hospital Münster, Clinic for General Orthopaedics and Tumor Orthopaedics, Münster, Germany*
[2]*University Hospital Münster, Central laboratory, Münster, Germany*
[3]*University Hospital Münster, Institute for Medical Microbiology, Münster, Germany*
[4]*University of Applied Sciences Münster, Labor für Werkstofftechnik, Steinfurt, Germany*

Question

During the development of an activatable anti-infective coating based on poly-L-lactic acid and silver nitrate, concerns arose regarding the potential inhibition of the silver's effect by physiological fluids. The literature indicates that silver ions can bind to thiol groups, which can shift the minimum inhibitory concentration (MIC) upwards [1]. Therefore, the glutathione in serum may play a role in the effectiveness of the coating in clinical applications and *in vivo* studies. To evaluate the potential inhibition or shift of the MIC of the activated coating *in situ* and in various *in vivo* or *in vitro* models, growth curves were applied using the average measured value of silver release from a 6% silver nitrate coating (57.8 mg/L).

Methods

Test solutions were prepared using a 1280 mg/L stock solution of silver nitrate and either human, fetal bovine (FBS), mouse, and rabbit serum, or tryptic soy broth (TSB). The concentrations used were 50, 100, 150, and 200 mg/L for the sera and 50 and 100 mg/L for the TSB. The solutions (100 µL) were incubated with a *Staphylococcus epidermidis* RP62A culture in TSB (100 µL) containing a bacterial load of 105 CFU/mL. The incubation was carried out in technical duplicates and biological triplicates for 24 hours at 37°C. The OD was measured every 30 minutes at 578 nm.

Results

Concentrations of 50 mg/L silver nitrate and higher inhibited the growth of *S. epidermidis* in human serum, FBS, mouse serum, and rabbit serum for 24 hours. No differences in growth were observed between TSB and any of the serum samples.

Conclusions

The MIC of silver nitrate is below 50 mg/L for all sera, indicating that the implant coating's silver ion release is at a therapeutically effective level. It is important to note that the MIC may have increased due to the binding of serum components, but this is not relevant to the coating's effectiveness as long as the previous values of silver ion release can be guaranteed in all models.

References

[1] Mulley G et al. (2014) Inactivation of the antibacterial and cytotoxic properties of silver ions by biologically relevant compounds. PLoS One 9:e94409. https://doi.org/10.1371/journal.pone.0094409

P-HAIP-018
Reduction and inactivation of microorganisms with tolerance to quaternary ammonium compounds by chlorine dioxide

S. Herzog[1], L. Hackel[1], J. Diehr[1], E. Suermann[1], A. Mellmann[1], *T. Kuczius[1]
[1]*Institute of Hygiene, University Hospital Münster, Münster, Germany*

Introduction: Quaternary ammonium compounds (QACs) are globally used as active substances in bactericides. Since QACs are known to cause bacterial tolerances associated with increased tolerances to antibiotics, concerns about human health risks by QAC-tolerant bacteria are increasing. Once adapted to QAC, tolerant microorganisms can persist in tap water despite ongoing disinfection with QACs. For the inactivation of potential problematic QAC-tolerant microorganisms from tap water, the impact of chlorine dioxide as an alternative biocide was tested.

Goal: The aim of this study was to evaluate the susceptibility of QAC-tolerant gram-positive and gram-negative microorganisms to the oxidative disinfectant chlorine dioxide.

Material & Methods: The gram-negatives *Escherichia coli*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* as well as the gram-positives *Staphylococcus aureus* und *S. epidermidis* were successively adapted to tolerance to benzalkonium chloride (BAC) representative for QACs by repeated inoculation and regrowth. Analyzing the susceptibility to oxidative disinfection, BAC tolerant bacteria were treated with chlorine dioxide at low (0.4 mg/L) and increased concentrations (1 mg/L) for 25 min followed by plating to determine the inactivation rates.

Results: The adaption to BAC resulted in a fast occurring tolerance, which was stable over weeks. The phenotype of BAC tolerance in tested bacteria remained even without BAC supplementation. The bacterial biofilm formation increased with the adaption to BAC. An exposure to 1 mg/L chlorine dioxide was adequate to remove densities of 10^7 cells of *E. coli*, *S. maltophilia*, *S. aureus* and *S. epidermidis.* In contrast, treatment with 0.4 ml/L chlorine dioxide limited the bacterial inactivation.

Conclusion: BAC-tolerant bacteria demonstrated challenges towards a successful disinfection. Regarding a high bacterial load of BAC-tolerant bacteria, an increased concentration of chlorine dioxide inactivated the microorganisms successfully. A regular change of disinfectants with different modes of action is recommended to avoid formation of tolerances and resistances.

P-HAIP-019

The Risk of Bloodstream Infections in Patients with Central Venous Access Port in Non-ICUs in Germany

*B. Weikert[1], S. Saydan[1], F. Schwab[1], C. Geffers[1]
[1]*Charité - University Medicine Berlin, Institute of Hygiene and Environmental Medicine, Berlin, Germany*

Background / Question

Catheter-related bloodstream infections (CR-BSI) are one of the most common complications of central venous access ports. There is a lack of evidence on CR-BSI rates in patients with such ports in hospital and outpatient settings. We analyzed national surveillance data and reported incidence densities of CR-BSI in patients with central venous access ports and central venous catheters in non-intensive care units (Non-ICUs) in Germany.

Methods

Surveillance of hospital-acquired infections in Non-ICUs is performed in the STATIONS-KISS module, www.nrz-hygiene.de. Non-ICUs that had collected data on patients with central venous access ports and central venous catheters for at least one month were included in the present analysis. We calculated incidence densities of port-related BSI from 2011 to 2021 and compared these rates with CVC-related bloodstream infection rates.

Results

A total of 268 port-related BSI were documented from 2011 to 2021 in 118 participating Non-ICUs. The overall incidence density for port-related BSI was 0.67 per 1000 device-days in patients with central venous access port. The utilisation rate of central venous access ports has remained relatively stable over the years and is around 18.79 per 100 patient-days. Facility-based BSI rates differed only slightly between internal medicine, oncohaematology, interdisziplinary, surgical and neurological facilities. Compared to patients with central venous access port, patients with CVC had a 3-fold higher risk of bloodstream infection in our cohort (0.67 versus 2.16 per 1000 device-days). The most commonly isolated pathogens were coagulase-negative staphylococci and staphylococcus aureus, however, gram-negative microorganisms, including Klebsiella pneumonia, were also prevalent.

Conclusions

Our results are consistent with previous international studies, which showed CR-BSI rates between 0.11 and 0.76 per 1000 device-days in patients with central venous access ports. Given the increasing use of such vascular access devices in hospital and outpatient settings, infection prevention bundles are needed to reduce the risk of bloodstream infections.

P-HAIP-020

Demonstrating shedding of vancomycin-resistant Enterococcus faecium (VREfm) into the hospital environment by colonized patients based on typing by whole genome sequencing

*N. Kucheryava[1], F. Pankok[1], E. Nikiforov[1], L. Stieg[1], M. Hoch[1], G. Herold-Schulze[1], A. Bludau[1], G. Hernandez-Mejia[2], A. Bartz[2], B. Lange[3], A. Kuhlmann[4], A. Karch[2], W. Sunder[5], M. Kaase[1], S. Scheithauer[1]
[1]*University Medical Center, Georg-August University Göttingen, Department of Infection Control and Infectious Diseases, Göttingen, Germany*
[2]*University of Münster, Institute of Epidemiology and Social Medicine, Münster, Germany*
[3]*Helmholtz Center for Infection Research, Department of Epidemiology, Brunswick, Germany*
[4]*Martin Luther University Halle-Wittenberg, Faculty of Medicine, Halle (Saale), Germany*
[5]*Technical University of Braunschweig, Institute of Construction Design, Industrial and Health Care Building, Brunswick, Germany*

Introduction: Colonized or infected patients and contaminated hospital contact surfaces serve as reservoir of nosocomial pathogens and risk for transmission.

Goals: Contamination of patient contact surfaces in hospital environment was examined regarding the persistence and transmission of vancomycin-resistant *Enterococcus faecium* (VREfm).

Materials & Methods: Environmental samplings by swabbing the patient contact sites were performed on 20 hospital wards in 56 isolation rooms occupied with VREfm colonized or infected patients detected on admission. Some samplings were repeated over multiple follow-ups during a patient's stay in the room and after discharge to assess VREfm persistence on hospital fomites. Collected environmental samples were evaluated for contamination with *E. faecium.* VREfm isolates recovered from

environmental sites and strains derived from positive surveillance cultures of inpatients were examined for molecular epidemiological associations by clonal relatedness using whole genome sequencing (WGS) and core-genome multi-locus sequence typing.

Results: Of 550 surface samples, 122 sites (21.8%) were contaminated with *E. faecium*, 8.2% with VREfm and 12.3% with vancomycin-susceptible *E. faecium* (VSEfm). The VRE and VSEfm isolates were recovered in 36 (64.3%) isolation rooms occupied with VREfm or VSE colonized patients. In total, 57 environmental isolates and 45 VREfm strains derived from patient clinical or screening cultures were subjected to WGS. Molecular WGS-based typing revealed 7 genotypically distinct clusters of VREfm of diverse sequence type (ST117, ST80, ST2528, ST1299) with *vanA*, *vanB* genotypes including indistinguishable and genetically closely related (≤3 alleles differing) patient and environmental isolates.

Summary: WGS-based typing identified indistinguishable environmental VREfm contaminants on hospital fomites and strains from inpatients with colonization suggesting VREfm shedding from patient to environment as potential reservoirs of nosocomial pathogens and vectors for cross-transmission in hospitals highlighting the importance of disinfection, surveillance cultures, contact precautions and isolation of VREfm-positive patients.

P-HAIP-021

Systematic screening of 42 major VRE Enterococcus faecium strains towards changes in the antibiotic resistance, biofilm formation and desiccation tolerance after exposure to simulated microgravity

*F. Arndt[1,2], K. Siems[1], S. Leuko[1], S. V. Walker[3], R. Möller[1], A. L. Boschert[2]
[1]*German Aerospace Center, Institute of Aerospace Medicine, Radiation Biology, Köln, Germany*
[2]*University Hospital Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*
[3]*Regional Clinics Holding GmbH RKH, Institute for Clinical Microbiology and Hospital Hygiene, Ludwigsburg, Germany*

Vancomycin resistant *Enterococcus* (*E.*) *faecium* (VRE) is a healthcare-associated pathogen causing severe diseases in immunocompromised patients. The dysregulation of immune functions in astronauts during spaceflight, as previously shown, elevates the risk of infections from both endogenous and exogenous pathogens. Compounding this concern, treatment options are restricted during long-term missions. Consequently, we seek to understand how opportunistic gastrointestinal bacteria, such as *E. faecium*, may alter their antimicrobial susceptibility, desiccation tolerance, and biofilm formation in response to the absence of gravity. Understanding these dynamics has implications for healthcare on Earth and in space.

We tested the changes in antibiotic susceptibility of Vancomycin susceptible (VSE), Vancomycin resistant (VRE) and Vancomycin-variable (VVE-B) *E. faecium* isolates to 22 different antibiotics, after 7 days of incubation in simulated microgravity (sim. µ*g*) and at normal gravity (1 *g*). All isolates were also characterized regarding their biofilm formation and tolerance to desiccation after sim. µ*g*. We observed in- and decrease in MIC (Minimum Inhibitory Concentration) for seven isolates to Ceftarolin, Gentamycin, Moxifloxacin, Oxacillin, Rifampicin, Synercid and Vancomycin after sim. µ*g*. Moreover, 55 % (23/42 isolates) showed an increase in biofilm formation and 59 % (25/42) increase in desiccation tolerance after sim. µ*g*.

This study provides first insights on how *E. faecium* adapts its resistance behavior under spaceflight analog conditions like sim. µ*g*. The found alterations distinctly demonstrate the adaptability of *E. faecium* while also showing a great variability to sim. µ*g*. To prevent VRE infections and guarantee health of the crewmembers for future long-term space missions, it is essential to improve the understanding about the tenacity of VRE and gain knowledge about the influence of microgravity on the resistance behavior to different stressors. Understanding these dynamics has implications for healthcare on Earth and in space.

P-HAIP-022

Determination of cgMLST Thresholds for MRSA, MDR E. coli, and VRE by correlating molecular typing data with epidemiological information

*H. Tönnies[1], M. Fechner[2], A. Mellmann[1]
[1]*University Münster, Institute of Hygiene, Münster, Germany*
[2]*University Münster, Institute of Medical Informatics, Münster, Germany*

Question

In outbreak investigations, two primary Whole Genome Sequencing (WGS)-based methods, namely cgMLST and SNP, quantify typing differences through differing alleles or nucleotides, respectively. Currently, there is no universally accepted threshold for these measures to assess the likelihood of transmission. Thresholds in the literature are frequently determined through retrospective analysis of outbreaks within their epidemiological context. This retrospective approach results in the adoption of diverse thresholds, undermining the potential standardization achievable with the cgMLST method. Our aim is to establish universally applicable threshold values for MRSA, MDR E. coli, and VRE through a novel correlation method.

Methods

We conducted routine WGS-based cgMLST typing on inpatient-derived multidrug-resistant organisms (MDROs). Allelic profiles of eligible MDROs were exported, and cgMLST distances were computed. For each observed cgMLST distance, we calculated patient pairs with a successful identification of an epidemiological link. Considering different levels of spatial and temporal interactions, various epidemiological cut points were examined. The threshold was defined as the cgMLST distance beyond which the percentage of epidemiological contacts found no longer changed significantly.

Results

Threshold values were determined as 4 for MRSA and 3 for both *E. coli* and VRE. A notable finding was that even with identically typed isolates, no epidemiological link could be identified in half of the cases. For VRE, the value was even below 40%.

Discussion

We present our correlation method as an innovative approach for establishing universally applicable threshold

values in molecular typing. However, it's important to note that the thresholds presented were derived exclusively from our dataset and may not hold general validity. We encourage other university hospitals to employ this method with their datasets to validate and confirm the findings.

P-HAIP-023
Strengthening Molecular Surveillance of ARIs in Bavaria: Expansion of the Bavarian Influenza + Corona Sentinel (BIS+C)

*J. Flechsler[1], H. Angermeier[1], S. Lacroix[1], S. Schmidt[1], J. Weber[1], N. Paravinja[1], J. Lutmayr[1], U. Eberle[1], S. Heinzinger[1], R. Konrad[1], N. Ackermann[1], A. Sing[1]
[1]*Bavarian Health and Food Safety Authority, Public Health Mikrobiologie, Oberschleißheim, Germany*

Introduction

In 2022, the Bavarian Ministry of Health decided to strengthen the Bavarian Influenza + Corona Sentinel (BIS+C).

Goals

For molecular surveillance of acute respiratory infections (ARIs), sentinel practices (general practitioners and paediatricians) send weekly nasopharyngeal swab samples from patients with ARIs to the LGL for diagnosis of influenza, SARS-CoV-2, and respiratory syncytial virus (RSV).

Materials and methods

The BIS+C has initially focused on the diagnosis of influenza cases, as well as RSV cases in children <5 years of age. Due to the pandemic, SARS-CoV-2 diagnostics has been included in the sentinel. Since calendar week (CW) 40/2021, all samples were additionally tested for RSV regardless of patient age. Furthermore, the ARI swab sampling period has been extended from seasonal to year-round. To achieve comprehensive coverage of BIS+C sentinel surveillance for ARI pathogens throughout Bavaria, additional BIS+C sentinel practices were recruited per county or urban municipality with respect to population density through an extensive marketing campaign.

Results

Participation in BIS+C has more than doubled, from 92 practices in 2021/2022 to 196 in 2023/2024. So far, the 2023/2024 season in Bavaria has shown an RSV epidemic according to RKI definitions since CW 45/2023, mainly affecting children <4, followed by the age group 5-14 years, and an influenza A(H1N1)pdm09 epidemic beginning in CW 51/2023, primarily impacting children and middle-aged individuals. Notably, influenza's onset was later compared to the previous season (CW 44/2022). SARS-CoV-2 viruses have predominantly affected adults and the elderly > 60 years of age.

Summary

The BIS+C sentinel network represents a molecular early warning system that monitors influenza, SARS-CoV-2, and RSV in Bavaria. Together with the Bay-VOC - and wastewater monitoring project, BIS+C is one of the three pillars of molecular surveillance of viral respiratory infections in Bavaria.

P-HAIP-024
EU targets for 2030 from the European action plan to combat antimicrobial resistance: how do German federal states compare in the outpatient sector? Germany, 2019-2022.

*S. B. Schink[1], A. Mlaouhi-Müller[2], W. Wittmüß[2], M. Schneider[3], M. Feig[3], T. Eckmanns[1], J. Schleeff[2], M. Abu-Sin[1], J. Hermes[1]
[1]*Robert Koch-Institut, Nosocomial infections, surveillance of antibiotic resistance and consumption, Berlin, Germany*
[2]*GKV-Spitzenverband, National Association of Statutory Health Insurance Funds, Berlin, Germany*
[3]*Robert Koch-Institute, Methods Development, Research Infrastructure and Information Technology, Berlin, Germany*

INTRODUCTION

On June 6th, 2023, the European Union published a recommendation on stepping up EU actions to combat antimicrobial resistance and set as target to increase the proportion of antibiotics from the access group as defined in the AWaRe (access, watch, reserve) classification of the WHO to 65% by 2030.

GOALS

We analyzed overall antibiotic prescription volume and access prescription use from 2019 to 2022 in the light of new EU targets for 2030.

MATERIALS & METHODS

We applied the WHO AWaRe classification to all prescriptions issued to outpatients and reimbursed by statutory health insurance by federal state in Germany in 2019-2022. Using Stata 17, we analysed overall prescription numbers and proportion of access prescriptions by federal state and year, and mapped the results with RegioGraph.

RESULTS

Overall, 87% of the population in Germany is covered by statutory health insurance. We could classify 99% of all systemic antibiotic prescriptions to outpatients according to AWaRe criteria.

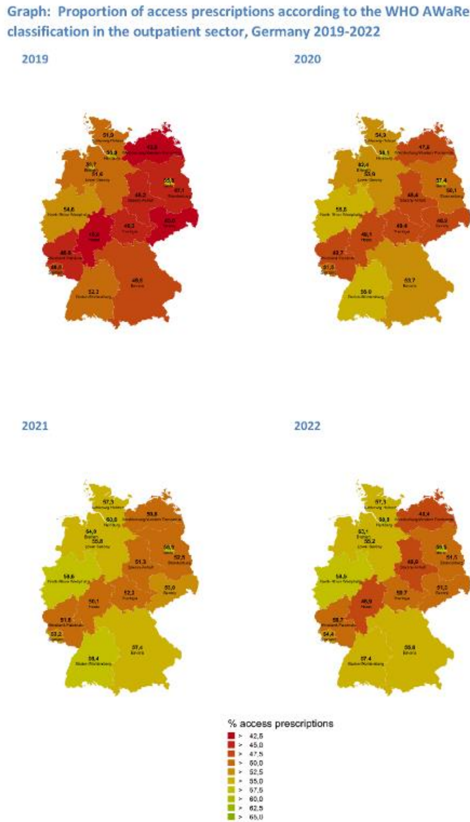
Prescribing decreased significantly with the COVID-19 pandemic in Germany from 26.6 million (mil.) prescriptions in 2019 to 19.2 mil. in 2020, stayed low throughout 2021 (17.2 mil.) and in the first three quarters of 2022, but exceeded pre-pandemic times in the last quarter of 2022 (total 2022: 22.3 mil.). By federal state, decrease ranged from -12.4% in Berlin and -13.8% in Saxony to -20.3% in Rhineland-Palatinate and -22.2% in both Saarland and Schleswig-Holstein.

Overall access prescriptions in Germany increased from 51% in 2019 to 55% in 2022 [graph]. Geographical differences point to a smaller proportion of access use in mainly eastern regions compared to western regions, with lowest proportion in Mecklenburg-Western Pomerania (48.4%), Hessen (48.9%) and Saxony-Anhalt (48.9%) and highest in Bremen (63.1%) in 2022.

SUMMARY

While the overall number of antibiotic prescriptions decreased, the proportion of access prescriptions grew. Both changes vary regionally across Germany. This points to nuanced challenges to further improve antibiotic stewardship measures in the outpatient sector to meet EU 2030 targets.

Fig. 1



P-HAIP-025

Microbiological epidemiology in patients undergoing allogeneic hematopoietic stem cell transplantation (2015-2020) – first results of a retrospective cohort study

L. Knegendorf[1], *C. Baier[1], D. Schlüter[1], N. Brüder[2], F. H. Heidel[2], M. Eder[2], E. Ebadi[1], G. Beutel[2]

[1]*Hannover Medical School, Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany*

[2]*Hannover Medical School, Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover, Germany*

Background

Infections, especially bloodstream infections (BSI), are a common complication in patients undergoing allogeneic stem cell transplantation (allo-SCT). Beyond the detection of infections, microbiological screening performed on patients with hematologic neoplasms frequently detects bacterial isolates that colonize patients.

Methods

We retrospectively identified all adult patients who underwent allo-SCT at Hannover Medical School (Germany) between 2015 and 2020. Microbiological data was extracted from the laboratory information system. Screening results (from for example stool samples/rectal swabs) were included from specimens collected up to 100 days prior to and 20 days after allo-SCT. The assessment of blood culture specimens was limited to a period of 10 days prior to and 20 days after allo-SCT.

Results

During the study period, 503 patients underwent allo-SCT, resulting in 516 cases. Among the collected specimens, blood cultures from 216 cases showed at least one positive result. Gram-negative bacteria (GNB) and enterococci were found in 104/216 (48.1%) of these cases (Figure 1A). In 57/216 (26.4%) of the cases the isolates in the blood were also found in intestinal screening specimens (stool/rectal swab), as determined by species comparison. All matching isolates were either GNB or enterococci (57/104; 54.8%).

Of the 516 transplantation cases, 6 had MRSA colonization, 173 had VRE, 7 had Carbapenem-resistant (CR) Enterobacterales or *Acinetobacter sp.* and 65 had ESBL-phenotype GNB in combination with fluroquinolone resistance (Figure 1B).

Discussion

In this analysis, we evaluated bacterial colonization and BSI of allo-SCT patients. Our findings reveal a high prevalence of VRE and a low occurrence of CR GNB. Both have important implications for IPC strategies, as these organisms can precipitate nosocomial infections. Furthermore, over half of the BSI cases with GNB and enterococci showed a species match with intestinal screening samples. In the context of oral decontamination strategies, the identification of ESBL-producing bacteria resistant to fluoroquinolones is particularly remarkable.

Fig. 1

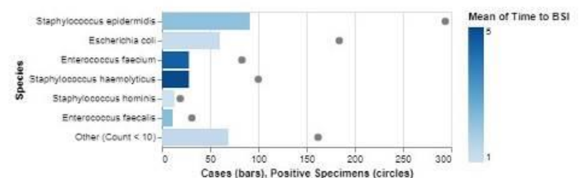


Figure 1A. Distribution of positive blood culture specimens

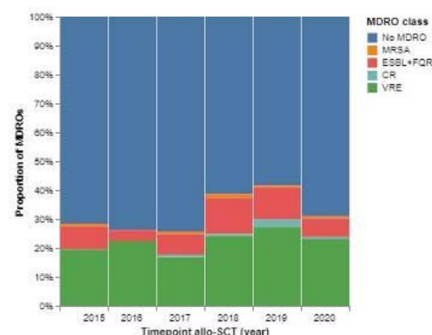


Figure 1B. Proportion of MDROs in all allo-SCT cases

P-HAIP-026

Zapnometinib as a new antibacterial compound targeting cell division of Streptococcus pneumoniae

*J. Jacob[1], M. Wert[1], F. Herrmann[2], A. L. Linard Matos[3], O. Soehnlein[3], S. Ludwig[1], Y. Boergeling[1]
[1]*University of Münster, Institute of Virology, Münster, Germany*
[2]*University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Münster, Germany*
[3]*University of Münster, Institute of Experimental Pathology, Münster, Germany*

Introduction: *Streptococcus pneumoniae* is known to be the causative agent of pneumonia, otitis media and even systemic infections. Increasing resistance of *S. pneumoniae* towards commonly used antibiotics makes the treatment of these infections very challenging. Thus, the WHO identified *S. pneumoniae* as a priority pathogen highlighting the urgent need for new antibiotic agents against *S. pneumoniae*. Our lab previously showed that the human MEK inhibitor Zapnometinib reduces growth of several gram-positive bacteria.

Goals: In the current project, we investigate the potential of Zapnometinib as a new antibiotic agent against *S. pneumoniae*. We characterized the effects of Zapnometinib on *S. pneumoniae* growth behavior and morphology with the ultimate goal to identify the mechanism of action.

Methods: Atomic force microscopy, Fluorescence microscopy, growth reduction studies, growth kinetics, EC_{50} determination

Results: Treatment of *S. pneumoniae* with Zapnometinib resulted in a dose-dependent growth reduction, with the EC_{50} being in the low micromolar range. Growth kinetic experiments revealed a bacteriostatic mode of action of Zapnometinib in *S. pneumoniae.* Further characterization of the morphological changes induced by the treatment showed that Zapnometinb interfered with the process of pneumococcal cell division which resulted in significantly elongated cell chains. Atomic force microscopy confirmed that the formation of septa was impaired upon treatment with Zapnometinib. Fluorescence microscopy showed that this was accompanied by a dysregulated incorporation of peptidoglycan and defective cell pole maturation.

Summary: Our data confirm that Zapnometinib harbors antibacterial properties against *S. pneumoniae*. Zapnometinib treatment resulted in defective cell division, which was characterized by elongated cell chains, impaired septum formation, dysregulated peptidoglycan incorporation as well as cell pole maturation defects.

P-HAIP-027

Connecting actual user data from SARS-CoV-2 test centers with a user survey in a mid-sized German city

*M. Grohmann[1], B. Conrady[2,3], F. Wolschendorf[4], S. Lindner[4], L. Schomakers[4], A. K. Witte[4]
[1]*HTK Hygiene Technologie Kompetenzzentrum GmbH, Bamberg, Germany*
[2]*University of Copenhagen, Department of Veterinary and Animal Sciences, Frederiksberg, Denmark*
[3]*Complexity Science Hub Vienna, Wien, Austria*
[4]*HTK Hygiene Technologie Kompetenzzentrum GmbH, Bamberg, Germany*

Introduction

Since March 2021, Germany has been offering cost-free tests for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and negative test results have become a prerequisite for various day-to-day activities following the lockdowns.

Goals

We aimed to understand the usage patterns of tests and explore any connections with mitigation measures. Additionally, we sought to correlate this data with the results of a user survey.

Materials & Methods

We analyzed over 50,000 anonymized records from eight test centers in the typical medium-sized city of Bamberg with one of them remaining open continuously from March until December 2021. We gathered data about the motivations of 1,347 participants through a user survey conducted over a ten-week period in summer 2021. Participants were recruited via the online booking system, regional and social media, and requests in the test centers.

Results

The actual user data revealed that distinct patterns of visitor types were tested in the different test centers and the majority tested only once. Individuals who underwent repeated testing tended to favor the same location. A preference for spontaneous testing grew in proportion to the availability of spare tests. Compared to the local demographic visitors aged 18 to 30 years were distinctly overrepresented. A negative binominal model showed that implemented mitigation measures had an impact on the number of tests conducted. In the survey, most frequently cited motivations were visits with family and friends, access to gastronomy, shopping & culture, and tourism. In contrast to the actual individuals tested, most participants of the survey used the tests more than once, although not regularly.

Summary

On the one hand, actual user data demonstrate that test centers were rarely used for regular testing but rather for meeting requirements of certified tests. On the other hand, most participants of the survey have been tested several times and indicated as one main motivation for testing meetings with family and friends. This emphasizes the strength and bias of surveys and the importance of analysis of actual data.

P-HAIP-028

Zapnometinib: An antiviral and antibacterial agent for combatting Streptococcus pneumoniae infections with synergistic effects with penicillin

*M. Wert[1], J. Jacob[1], S. Ludwig[1], Y. Boergeling[1]
[1]*University of Münster, Institute of Virology, Münster, Germany*

Introduction: *Streptococcus pneumoniae* (*S. pneumoniae*) is a commensal pathogen of the nasopharynx and one of the leading causes of bacterial death in children under the age of

five. Especially prior infection with respiratory viruses like influenza A viruses is known to pave the way for secondary bacterial infection with severe outcome. *S. pneumoniae* displays high genetic plasticity allowing fast adaptation to antibacterial compounds and spread of resistances within and among species. The worldwide emergence of antibiotic resistance rates further dramatizes the clinical situation and presents a major threat to the global health system underscoring the pressing need for innovative solutions to replace or at least reduce the use of currently available antibiotics.

Goals: Zapnometinib, a compound with antiviral and antibacterial properties, is a promising candidate to combat both viral and bacterial infections. Here, we investigate the antibacterial activity of Zapnometinib against *S. pneumoniae* and analyze its interaction with commonly used antibiotics.

Materials & Methods: Besides EC50 determination of zapnometinib against S. pneumoniae, standard MIC assays were performed with different antibiotics. To further analyze drug interactions, we tested a diagonal synergy method that is derived from the checkerboard assay and allows a fast and easy screening for additivity or synergism.

Results: Zapnometinib exhibits remarkable antibacterial activity against *S. pneumoniae*, with an effective concentration 50% of 5 µM *in vitro.* Moreover, pretreatment of *S. pneumoniae* with zapnometinib reduces the minimal inhibitory concentration of various antibiotics potentially sensitizing the bacteria for antibiotic treatment. Interestingly, combination therapy with Penicillin-G resulted in a drug interaction score of 0.8, which corresponds to a synergistic effect.

Summary: Our initial findings on the promising interaction of zapnometinib with common antibiotics highlight its potential future importance for the treatment of co-infections and the fight against increasing antimicrobial resistance.

P-HAIP-029

Identification of risk factors for long-term persistence of linezolid resistant enterococci - experiences from a tertiary care centre

*V. Rauschenberger[1], H. Claus[1], S. Kampmeier[1,2]
[1]*University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[2]*University Hospital Würzburg, Infection Control and Antimicrobial Stewardship Unit, Würzburg, Germany*

Introduction

Linezolid-resistant enterococci (LRE) have raised concern worldwide due to their increased detection in healthcare settings. Infections are often preceded by colonisations and intensified infection prevention and control strategies are established to minimize spreading. As specific eradication strategies are lacking, these precautions are often continued until spontaneous clearance occurs. However, LRE may persist over long periods of time. Here we aim at identifying factors determining a prolonged colonisation.

Materials & Methods

Patients that were admitted on an intensive care unit, oncological or infectious disease ward at a tertiary care hospital were screened for LRE via rectal swab during one year study period. Patients colonized for more than ten weeks were defined as long-term carriers. LRE-isolates were subjected to whole genome sequencing and compared using a core genome Multilocus Sequencing Typing approach. Demographic and clinical risk factors of admitted patients were recorded.

Results

In the study period, 77 patients colonized with LRE (59 LR *E. faecium*, 18 LR *E. faecalis*) were identified. Of these, 30 (39%) were female. The median age was 65 years (range 23-90 years). Of all patients, 9 (12%) were LRE long-term carriers according to above mentioned classification. Spontaneous LRE clearance was observed in 40 patients (52%). Comparison of risk factors in the LRE persistence vs. clearance group resulted in liver dysfunction being significantly more often associated with LRE persistence (p=0.03). Other clinical or demographic factors did not differ significantly between both groups. Assessment of genetic relatedness of detected LRE revealed 7 clusters comprising 1-12 genotypes of LR *E. faecium* and 4 clusters comprising 2 genotypes of LR *E. faecalis.*

Summary

First results of our study suggest that persistence of LRE colonisation is potentially overestimated. Long-term-colonisation is favoured by patient associated risk factors, whereby liver dysfunction plays a relevant role. Genetically, the LRE isolates of this study show a high diversity, revealing that LRE are rather acquired due to antibiotic treatment than by transmission.

P-HAIP-030

From airport to school to hospital - An Outbreak of SARS-CoV-2 Infections Among German Schoolchildren Caused by A Hitherto Rare Viral Variant

*S. Sachse[1], *I. Kraiselburd[1], O. Anastasiou[2], C. Elsner[2], S. Roß[2], S. Goretzki[3], A. Thomas[1], S. Goer[4], R. Nagel[4], M. Koldehoff[4], F. Meyer[1], U. Dittmer[2], *R. Schmithausen[1,4]
[1]*Institute for Artificial Intelligence in Medicine, Data Science, Essen, Germany*
[2]*University Hospital Essen, Institute of Virology, Essen, Germany*
[3]*Pediatric Hematology and Oncology, Department of Pediatrics III, University Hospital Essen, University of Duisburg-Essen, Essen, Germany*
[4]*University Hospital Essen, Department of Hygiene and Environmental Medicine, Essen, Germany*

In December 2021, we investigated a COVID-19 outbreak in a German high school, marking the first case of the previously rare viral variant B.1.640.2, potentially originating from Cameroon. The full-length SARS-CoV-2 sequences from infected individuals were classified within the B.1.640.1 and B.1.640.2 pangolin lineages (IHU variant). The Robert Koch Institute confirmed that this variant had been identified only once before in Germany, without an associated outbreak.

The index case, an adult returning from a three-week development aid mission in the Republic of the Congo, introduced the atypical variant to the school via his children. This led to further transmission to a vulnerable hospital ward and subsequently several other communities. A unique biphasic pattern in CT-value courses over 3-4 weeks was observed in all cases, coinciding with the period immediately

following the mandatory 10-14 day quarantine, underscoring the distinctive features of the B.1.640.2 variant.

Additionally, it was established that the index case and another known case likely infected each other on an airplane, highlighting the variant's transmission dynamics. These findings underscore the unpredictability of SARS-CoV-2 variant emergence and their potential introduction into new geographical areas and vulnerable settings from abroad.

Despite these challenges, early management strategies and research into the clinical signs associated with self-quarantine proved effective in controlling the outbreak. This instance further illustrates the critical importance of continuous vigilance and preparedness in the global response to emerging SARS-CoV-2 variants.

P-HAIP-031

A new antibiotic: Evaluation of a Photoactivatable Ciprofloxacin and the role of Bile Acids to diminish Biofilms on Bile Duct Catheters

*A. Busch[1,2], N. Eberhard[3], B. Gonzalez Santamarina[2], M. L. Enghardt[1], O. Rohland[4], I. Hussain[2], I. Rubio[2], L. Thieme[5], J. Rödel[6], B. Löffler[6], H. D. Arndt[3], M. Bauer[2]
[1]*Friedrich Schiller University Jena, Theoretical Microbal Ecology, Jena, Germany*
[2]*Jena University Hospital, Department of Anesthesiology and Intensive Care Medicine, Jena, Germany*
[3]*Friedrich Schiller University Jena, Institute for Organic and Macromolecular Chemistry, Jena, Germany*
[4]*Jena University Hospital, Department of General, Visceral and Vascular Surgery, Jena, Germany*
[5]*Jena University Hospital, Institute of Infectious Diseases and Infection Control, Jena, Germany*
[6]*Jena University Hospital, Department of Medical Microbiology, Jena, Germany*

We would like to present the effectiveness of a newly developed photoactivatable ciprofloxacin on pathogenic bacteria and biofilms derived infections associated with biliary catheters. The research addresses the challenge of elusive bile microbiomes linked to hepatobiliary diseases, particularly those resulting from biofilms on internal-external biliary drainage catheters WE could demonstrate that the photo-activated antibiotic effectively prevents biofilm formation and reduces bacterial viability without harming eukaryotic cells. Additionally, the combined impact of bile acids in combination with antibiotics was investigated. A combinational treatment involving antibiotics and bile acids, such as ursodesoxycholic acid (UDCA), shows promise in reducing bacterial survival within biofilms. The findings provide evidence for mode of action of the treatment of UDCA in biliary infections, like cholangitis.

P-HAIP-032

Exploring the pathogen-killing potential of AGXX® surfaces: A game-changer in antimicrobial technology for healthcare settings

*D. Wischer[1], R. Hadersbeck[1], L. Morad[1], R. Haag[2], E. Grohmann[1]
[1]*Berliner Hochschule für Technik, Faculty of Life Sciences and Technology, Department of Microbiology, Berlin, Germany*
[2]*Free University of Berlin, Institute of Chemistry and Biochemistry, Berlin, Germany*

The escalating threat of antibiotic resistance has led to the exploration of alternative antimicrobial strategies. Antimicrobial surfaces have emerged as a promising means of infection-control in healthcare settings by preventing bacterial surface colonisation and transfer of germs. AGXX®, utilising contact-catalytic antimicrobial technology efficiently eliminates pathogenic bacteria, including multiresistant organisms, filamentous fungi, yeasts, and viruses by release of ROS. While AGXX®"s broad-range antimicrobial potential has been well demonstrated, quantitative data on its bactericidal efficacy are lacking.

Our study employed a modified ISO 22196 assay to quantitatively assess the bactericidal efficacy of AGXX®-surfaces. Testing against pathogenic *Escherichia coli* and *Staphylococcus aureus* (MRSA) strains, AGXX®-sheets (Ru-coated silver) demonstrated a remarkable 5 log reduction in viable bacteria within 3 hours, outperforming uncoated silver sheets, which achieved a 1 log reduction in the same timeframe. Ongoing assessments include live/dead stains and microscopic evaluations as well as testing of further pathogenic bacteria and fungi under the same test protocol.

Standardized ISO tests provide an initial assessment for comparing and generating reproducible data to evaluate antimicrobial materials. Surface performance must be validated under application-specific conditions. Our preliminary data underscore AGXX®'s substantial pathogen-killing activity, positioning it favourably against plain silver surfaces. Future studies will explore specific healthcare applications, addressing implementation challenges and limitations. Expanding assessments to real-life conditions will allow for the definition of the most effective applications of AGXX® in healthcare settings. AGXX® stands out as a promising antimicrobial technology, deserving further exploration in the pursuit of robust infection control solutions.

P-HAIP-033

Medical tourists – a source of imported pathogens? – A review of Methicillin-Resistant Staphylococcus aureus (MRSA)-, Multiresistant Gram-negative bacteria (MRGN)-admission-screenings in Arab and Russian medical tourists at University Hospital Düsseldorf (UKD) from 2017 to 2021

*A. Eyking-Singer[1], S. Geis[2], D. Kalhoefer[1], A. Jurke[1]
[1]*Landeszentrum Gesundheit NRW, Bochum, Germany*
[2]*University Hospital Düsseldorf, Hospital hygiene staff unit, Düsseldorf, Germany*

Introduction

Many foreign patients travel to Germany solely for medical treatment (medical tourism), potentially carrying various pathogens. Saudi Arabia and Russia exhibit higher prevalence of multi-resistant bacteria compared to Germany. The Commission for Hospital Hygiene and Infection Prevention (KRINKO) recommends MRSA-admission-screenings for patients from such risk regions and MRGN-admission-screenings for patients with recent healthcare system exposure in regions with high MRGN-prevalence.

Goals

We aimed to investigate whether medical tourists from the Arabian Peninsula and Russia were routinely screened for MRSA and MRGN upon admission to UKD and whether the results comply with current screening recommendations.

Materials & Methods

We analysed MRSA- and MRGN-screening results of Arab and Russian patients admitted to UKD from 2017 to 2021.

Results

239 Arab and 135 Russian patients were admitted to UKD from 2017 to 2021, including 52% Arab and 21% Russian minors. While the gender distribution was similar, men were screened more frequently. MRSA-screening rates were higher (Arab 80%, Russia 70%) than MRGN-screenings rates (Arab 12%, Russia 24%). More Arab patients were positive for MRSA or MRGN (MRSA: Arab 7%, Russia 2%; MRGN: Arab 21%, Russia 6%). Notably, 72% of Arab MRSA- and 100% of Arab MRGN-positive patients were male. The proportion of MRSA- und MRGN-positive patients was comparable between Arab minors (MRSA 8%; MRGN 21%) and Arab adults (MRSA 7%; MRGN 20%). No Russian minors tested positive for MRSA or MRGN. Arab patients carried 11 (3x 4MRGN) and Russian 6 (1x 4MRGN) distinct MRGN species. Five MRGN species were detected in Arab minors and one MRGN in a Russian minor.

Summary

UKD implemented MRSA- and MRGN-screening recommendations. The respective KRINKO recommendations lead to variations in the proportions of MRSA- and MRGN-screened medical tourists. Arab patients showed higher rates of MRSA- and MRGN-positive screenings in adults and minors and a greater variety of MRGN species compared to Russian patients. Thus, our results underscore the importance of tailored screenings for multidrug-resistant bacteria based on the specific patient population.

P-HAIP-034
Localisation of indication-based hand desinfections.

*D. Sons[1], F. Mattner[1], S. Herbrandt[2], M. Stolte[2], A. Hamid[1], R. Otchwemah[1]
[1]*Cologne Merheim Medical Centre, Institute of Hygiene, Köln, Germany*
[2]*Technische Universität, Dortmund, Germany*

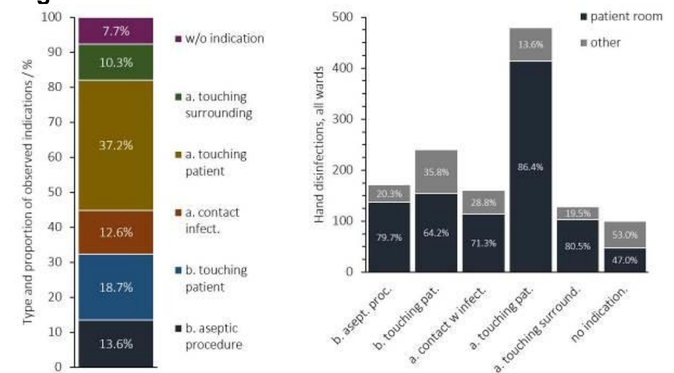
Questions: Does the location of disinfectant dispensers (DD) influence its usage for hand disinfection? Is the type of medical indication decisive for using a certain DD in respect to its location? Is it possible to define favourable and unfavourable positions of DD in a patient room by an electronic monitoring system (EMS)?

Methods: Study was from Feb.2022-Dez.2023, with direct observations (dO) by staff on 3 wards (ICU, IMC, Normal). dO included the localisation of indication-based HD, distinguishing between patient room and outside. We also applied an EMS (NosoEx® GWA Hygiene, Paul Hartmann AG) permanently recording HD. EMS data were sorted as dO data but further specified adressing DD types and locations in patient room. DD types: wall mounted, freestanding, hanging. Raw data were processed by TU Dortmund.

Results: We analysed 1324 dO seeing most HD executed in patient room with 72,2%, 67,9% and 84,9% for Normal, IMC and ICU, respectively. The most indications observed are after and before touching a patient and before aseptic procedures with 37.2%, 18.7% and 13.6%. Regarding the location of HD the indication before touching a patient has the lowest and after touching a patient the highest values for HD in patient room (64.2% and 86.4%). EMS recorded 985740 HD. Normal ward and ICU prefer HD in patient room with 77% and 71%, while IMC executes less HD inside (47%). Hanging DD in direct proximity to patient beds are preferred for HD on Normal ward (70%). On IMC and ICU hanging DD placed at the window side were not used frequently (16% and 5 %). Both, IMC and ICU execute most HD with wall mounted DD (56% and 79%).

Conclusions: Here, the type of medical indication does not obviously influence the location of HD. However, the positions of DD have great impact on frequency of its use. Striking are here the two analysed locations of hanging DD. Further, is the position of a DD more crucial than its type. Nevertheless, when it comes to the integration in the health care workflow and acceptance by the medical staff, type and handling might be mandatory. We want to point out, that the conditions on each ward are unique and must be assessed individually.

Fig. 1



P-HAIP-035
Touching surfaces- antibacterial surfaces for spaceflight and clinical applications

*C. L. Krämer[1,2], K. Siems[2], D. W. Müller[3], A. L. Boschert[4], A. Schiele[1,2], S. Leuko[2], F. Mücklich[3], R. Moeller[1,5]
[1]*University of Applied Sciences Bonn-Rhein-Sieg, Natural Sciences, Rheinbach, Germany*
[2]*German Aerospace Center, Department of Radiation Biology, Köln, Germany*
[3]*University of Saarland, Department of Materials Science and Engineering, Saarbrücken, Germany*
[4]*University Hospital Cologne, Institute of Medical Microbiology, Immunology and Hygiene, Köln, Germany*
[5]*German Aerospace Center, Institute of Aerospace Medicine, Köln, Germany*

The International Space Station (ISS) is a unique environment with its microbiome originating mostly from the inhabiting crew. Due to its remoteness and the extreme conditions, such as increased radiation and microgravity, the ISS represents an ideal testing ground for studying microbial adaptation in confined habitats. On the ISS, but similarly also in other restricted areas such as intensive care units, fomites can be niches for opportunistic pathogens, which are then further spread via contact. To reduce bacterial contamination on surfaces, antibacterial surfaces can be implemented.

In the Cosmic Kiss project "Touching Surfaces" novel copper- based antibacterial surfaces were tested under real spaceflight conditions, in schools, and in clinical settings. The surfaces combine chemically antibacterial properties through the use of copper and brass as a copper-alloy as

well as topographic properties to enhance antibacterial efficacy. Three different metals were implemented: Stainless steel as inert reference surface and copper as well as brass as antibacterial metals. Using Ultrashort Pulsed Direct Laser Interference Patterning (USP- DLIP) micro- and nanometer structures were created in each metal. The micro-structures were supposed to enhance the contact between surface and bacteria, thereby increasing the antibacterial activity of copper. Whereas the nanostructures were employed to hamper adhesion of bacteria to the surface. The surfaces were inserted into spaceflight hardware, so-called "Touch Arrays". These were then installed in schools in Germany as part of a citizen science project, a university hospital, and on the ISS, where they were touched frequently over a defined timeframe.

After the experiment duration, surfaces were tested for their robustness against frequent touching using electron microscopy. Results showed that structured surfaces remained intact despite frequent contact and organic contamination. Additionally, the microbial community was analyzed using culture-dependent and independent approaches. All copper surfaces and nanostructured brass surfaces in particular showed reduced microbial contamination.

P-HAIP-036

Detection of SARS-CoV-2 RNA in stool samples from patients with acute gastroenteritis during a low prevalence endemic period in summer 2023

*F. Mattner[1,2], A. F. Wendel[1,2], M. Malecki[1,2], I. Winterfeld[1,2]
[1]*University Witten/Herdecke, Köln, Germany*
[2]*Witten-Herdecke, Infection Control and hospital epidemiology, Köln, Germany*

Introduction

Following its emergence in 2020, we are currently undergoing a transition from a pandemic to an endemic state for SARS-CoV-2. In order to comprehend the endemic nature of the virus, it is feasible to derive epidemiological insights not only from surveillance but also from diagnostic screening tests.

To examine whether persistent shedding of SARS-CoV-2 virus RNA in stool samples occurs in patients with gastroenteritic symptoms during a low prevalence period following the declared end of the SARS-CoV-2 pandemic.

Material and Methods

Stool samples, collected for gastroenteritis screening between June 12, 2023, and July 20, 2023, and initially tested negatively for Adenovirus, Norovirus, Rotavirus, *Campylobacter spp., Salmonella spp., Yersinia enterocolitica, Clostridioides difficile*, enterohemorrhagic *E. coli*, enteropathogenic *E. coli*, and enteroinvasive *E. coli*, were subjected to testing for SARS-CoV-2 and influenza A/B RNA at our diagnostic laboratory in a tertiary care center in Cologne. Automated nucleic acid extraction using the Maxwell®RSC Blood DNA Kit (Promega), followed by the RIDA®GENE Flu & SARS-CoV-2 PCR assay (R-Biopharm) conducted on a CFX96 real-time cycler (Bio-Rad) was performed.

Routine surveillance data of SARS-CoV-2 positive cases were used.

Results

After the substantial Omicron waves, SARS-CoV-2 RNA and influenza RNA was found in one each out of 103 (0.97%; CI95% 0; 0.029) consecutive stool samples from patients presenting gastroenteritic symptoms during a period of low prevalence.The weekly prevalence of admissions was less than 0.5 per 100 admissions, and there were no occurrences of nosocomial cases throughout the investigation period.

Summary

Given that a substantial portion of the population had been infected by the time the extensive Omicron wave ended, there was a possibility that persistent SARS-CoV-2 in the gastrointestinal tract could be identified in symptomatic patients. However, this expectation did not align with the actual findings.

P-HAIP-037

Prevalence of co-colonisation of multidrug-resistant organisms in intensive care.

*B. Schlosser[1], E. Lemke[1], C. Geffers[1], R. Leistner[1,2]
[1]*Charité - University Medicine Berlin, Institut für Hygiene und Umweltmedizin, Berlin, Germany*
[2]*Charité - University Medicine Berlin, Division of Gastroenterology, Infectious Diseases and Rheumatology, Medical Department, Berlin, Germany*

Background: Multidrug resistant organisms (MDROs) including Methicillin resistant S. aureus (MRSA), vancomycin resistant Enterococcus (VRE) and MDR-GNB - mainly distinguished by susceptibility (MDR-GNB-CS) or resistance (MDR-GNB-CR) to carbapenems - are of global concern. There is a paucity of evidence on the prevalence of MDRO co-colonisation, which can pose a challenge to clinical management and affect patient's outcomes. In addition, the risk factors for co-colonisation with multiple MDROs are not well defined. Methods: We performed a retrospective analysis using electronic data of 70,479 patients admitted to one of our 16 intensive care units at a university hospital in Berlin between 2018 and 2023. Only patients who had at least one nasal or rectal screening or whose clinical samples tested positive for MDROs were included in the analysis. Associations between the detection of multiple MDROs and clinical findings were assessed in multivariable analyses. Results: 47% (n=33,088) of patients admitted to our ICUs were screened for MDROs and included in our analysis. 20.5% (n=6,781) of screened patients tested positive for MDRO. A further 834 patients who were not screened had MDROs detected only in clinical specimens, giving a total of 7,705 MDRO-positive patients. 4.9% of MDRO patients were co-colonised with more than one MDRO, most commonly with VRE and MDR-CS-GNB (44% of co-colonised patients). Analysis of potential risk factors for co-colonisation showed increased likelihoods for patients with cystic fibrosis (CF, OR: 5.7), chronic kidney disease (CKN; OR: 1.3), pulmonary disease (CPD; OR: 1.5) and younger age (OR: 1.2). Conclusion: Co-colonisation occurred in almost 5 % of the screened patients in our intensive care unit. MDR-CS-GNB and VRE were most frequently detected in co-colonised patients (44%). We showed that conditions with a potentially high rate of antibiotic prescriptions such as CF or CPD were associated with a higher probability of co-colonisation but further investigations are required.

Host-Associated Microbiomes and Microbe-Host Interactions

P-HAMI-001
Snail gastrointestinal tract bacterial communities show a different biogeographical pattern than sediment and water habitats
*D. Herlemann[1,2], H. Tammert[3], C. Kivistik[3], K. Käiro[3], V. Kisand[3,4]
[1]*Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Section Biological Oceanography, Rostock, Germany*
[2]*Estonian University of Life Sciences, Chair of Hydrobiology and Fishery, Tartu, Estonia*
[3]*Estonian University of Life Sciences, Centre for Limnology, Vehendi küla, Estonia*
[4]*University of Tartu, Institute of Technology, Tartu, Estonia*

Host associated bacteria face a different set of dispersal challenges since they often depend on their hosts providing specific environments compared to the surrounding. Here we investigate the impact of biogeographic distances on the bacterial diversity and composition of *Ampullaceana balthica*-gastrointestinal tract bacteria. Effects on the host-associated bacterial community are compared to water and sediment communities using 16S rRNA gene sequencing. The bacterial communities sampled in Estonia, Denmark and Northern Germany differed between water, sediment and gastrointestinal tract and also between countries within each substrate indicating that each substrate has specific communities. The turnover rate over increasing geographic distances were lowest for gastrointestinal tract and highest for water bacterial communities. Also, the overlap of 16S rRNA gene amplicon sequencing variants (ASVs) between countries was lowest for gastrointestinal tract bacteria. The low turnover rate of the gastrointestinal community combined with little ASV overlap between counties suggests unspecific ASVs colonization from the local bacterial communities. It also supports that the host applies little phylogenetic selection on its gastrointestinal bacteria. The resulting country specific gastrointestinal bacterial community that is independent from the geographic distance supports that the gastrointestinal tract can act as habitat islands despite being strongly influenced by local environmental factors.

P-HAMI-002
Selectively targeting microbes associated with childhood stunting
*N. Akduman[1]
[1]*University of Tübingen, Tübingen, Germany*

Stunted growth is a condition in which children have a short height for their age as a result of chronic or recurrent malnutrition. Affecting millions of children worldwide and often associated with environmental enteric dysfunction (EED), stunted growth can lead to increased morbidity and mortality, loss of physical growth potential, impaired neurodevelopmental and cognitive function, and increased risk of chronic disease in adulthood.

Recent studies of the microbiome of stunted children have found that small intestinal bacterial overgrowth (SIBO) is extremely common, affecting more than 80% of stunted cases. SIBO is often characterized by an overgrowth of oral bacteria, leading to increased permeability and inflammation, and the replacement of classical small intestinal strains, causing a microbial contribution to stunting through an imbalance in the microbiome.

Our goal is to selectively inhibit strains associated with stunting using compounds while preserving the commensal gut microbiota. To achieve this, a systematic in vitro high-throughput screening method was used to identify compounds that selectively inhibit enteric pathogens and oral bacteria associated with stunting while sparing gut commensals. Clinical isolates from stunted children were identified using a MALDI-TOF MS library method, which allows rapid and cost-effective identification of human commensal gut bacteria. The most promising compound candidates were then tested for selective inhibition in clinical isolates from stunted children, as well as in synthetic and patient stool bacterial communities. We are currently testing the most promising drug candidates in vivo in gnotobiotic mice models colonized with either a synthetic community associated with stunting or stool-derived communities from stunted children.

Restoring a healthy microbiome balance is expected to have anti-inflammatory effects, which in turn could alleviate the symptoms of stunting and positively impact children's health and development.

P-HAMI-003
Systematically Investigating the Interactions between the Gut Microbiome and Psychotropic Drug
*L. Berg[1], C. Obermüller[1], P. Müller[1], L. Maier[1]
[1]*University Hospital Tübingen, M3 Reseach Center for Malignome, Metabolme and Microbiome, Tübingen, Germany*

Introduction: Mental illnesses continue to be a global health burden and only recently has the intimate physiological connection between the gut microbiome and brain become clear. Gut microbes not only affect host physiology, but also drug metabolism, and it has been demonstrated that the gut microbiome is particularly affected by antidepressant and antipsychotic drugs. As the gut microbiome plays a pivotal role in signaling across the gut-brain axis, it is plausible that the therapeutic and adverse effects of psychotropic drugs may be influenced by the microbiome. Consequently, interpersonal differences in treatment outcome (intensity, duration, onset, and side effects) may be explained by compositional variation of the microbiome from person to person.

Methods: To decipher the extent of these complex drug-microbiome interactions, a representative group of human gut bacterial species were tested in a high-throughput *in vitro* anerobic screening of over 1000 marketed drugs, which revealed a remarkably strong and direct antibacterial effect of antipsychotics on gut microbes. Following up on this therapeutic drug class, 1641 agents targeting neuronal signaling were tested on six phylogenetically diverse gut microbes, including commensals, pathobionts and pathogens.

Results and outlook: 14% of the 1614 compounds exhibited antibacterial potential against at least one of the tested strains. Strains that play a crucial role in keeping the human gut healthy, such as butyrate and propionate producers, were significantly more inhibited by these compounds. 40 marketed antidepressants and antipsychotics have been chosen to be screened in both a synthetic model bacterial community and *ex vivo* (patient

stool samples) complex microbiome communities. A detail understanding of the underlying mechanisms may create new opportunities to enhance treatment methods and foster the development of innovative microbial treatment strategies for mental illnesses.

P-HAMI-004

Investigating molecular decision points that determine colorectal cancer colonization by Fusobacterium nucleatum

*V. Cosi[1], F. Ponath[1], J. Vogel[1]
[1]*Helmholtz Institute for RNA-based Infection Research, RABI, Würzburg, Germany*

The anaerobic oral microbe *Fusobacterium nucleatum* has recently gained attention for its ability to colonize tumors in distal sites from its primary niche. Its presence in the tumor tissue is linked to tumor growth, metastasis, and resistance to chemotherapy. While bacterial removal reduces tumor burden, sustained systemic antibiotic treatment has severe side effects. To interfere with tumor colonization in a more selective manner, it is essential to understand the molecular host factors that enable fusobacterial colonization of the tumor environment.

In order to address this question, we established a hypoxic colonization protocol using three different colon cancer cell lines (Caco-2, HT-29, and HCT116) and GFP-expressing fusobacteria in physiological 1 % O_2 conditions. Interestingly, we observed different colonization rates for each cell line. This might be due to distinct cellular properties such as observed differences in cytokine profiles that could affect the colonization of fusobacteria. To further dissect host and bacterial gene expression changes upon colonization, we separated bystander from colonized cells using fluorescence-activated cell sorting and performed dual RNA-seq. This method allows monitoring gene expression changes of both organisms in parallel as the separation of host and bacterial transcripts is done *in silico*, which increases the sensitivity for detecting bacterial-induced responses in colonized cells. We will be focusing further analysis on differentially expressed genes to determine key factors that facilitate successful colonization by *F. nucleatum*, with the ultimate goal of defining new targets for specific intervention.

P-HAMI-005

Investigating the mode of action of non-antibiotic drugs on human gut microbes

*L. Boldt[1,2,3], L. A. Esquembre[2,3], H. Brötz-Oesterhelt[2,3], L. Maier[1,2,3]
[1]*University Hospital, M3 Research Center, Tübingen, Germany*
[2]*University of Tuebingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*
[3]*University of Tuebingen, Cluster of Excellence "Controlling Microbes to Fight Infections", Tübingen, Germany*

Consumption of non-antibiotic human-targeted drugs (HTDs) was shown to have a major impact on the composition of the human gut microbiome. To date, however, little is known about the exact mechanisms of how HTDs directly influence the physiology and growth of gut bacteria.

Fluorescence microscopy is routinely employed in antibiotic research providing quantitative insights into cellular and molecular responses of bacteria to drugs. To establish fluorescence microscopy assays for anaerobic bacteria, we selected 16 abundant obligate anaerobic bacterial species of the human gut microbiome and optimised high-throughput imaging methods for DNA, membrane and Live/Dead staining. As proof of concept, we selected six HTDs from different therapeutic classes that have been shown to inhibit the growth of the species subset. In the next step, we aim to optimise peptidoglycan staining, implement time-resolved data acquisition and detailed image-quantification, and perform cytological profiling to systematically extend our analysis. Collectively, the readouts from these fluorescence microscopy assays will allow mechanistic investigation of the physiological consequences of HTDs on specific cellular processes in anaerobic bacteria. The immediate response of different gut microbes to HTD exposure will be further investigated using transcriptomic analyses and compared to other stress responses, such as exposure to antibiotics.

In the long term, the combination of fluorescence microscopy assays with transcriptomic analyses will allow us to better understand the consequences of drug therapy on the gut microbiome. This understanding will pave the way for minimising the collateral damage of drugs on the gut microbiome, thereby reducing gastrointestinal side effects.

P-HAMI-006

Design and characterization of synthetic model communities for the human neonatal gut microbiota

*E. Niedermeier[1], T. Hitch[2], T. Clavel[2], L. Hall[3], A. Flemmer[4], B. Stecher[1]
[1]*Ludwig-Maximilians University Munich, Medical Faculty Max von Pettenkofer-Institute, München, Germany*
[2]*RWTH Aachen University, Aachen, Germany*
[3]*Quadram Institute, University of Birmingham, Birmingham, United Kingdom*
[4]*Haunersches Kinderspital & Perinatalzentrum, LMU Klinikum, München, Germany*

The mammalian gut is colonized by diverse microbial communities that provide its host with different functions, e.g. nutrition or protection against invading pathogens. These microbiota functions are dependent on the microbial community composition (*who is there?)* and inter- and intraspecies interaction networks (*what do they do?*).

Profound understanding of individual species interactions and their consequences for the mammalian host is often hindered by the microbiome´s diversity and difficulty in conducting experimental manipulations. Here, synthetic communities (Syncoms) can serve as tool to analyze bacterial ecology in detail by reducing complexity and increasing experimental manipulability. We previously used Syncoms to gain a mechanistic understanding of microbiome-mediated colonization resistance against pathogens (Eberl et al. 2021), bacterial interactions networks (Weiss et al. 2022) and the systematic investigation of bacterial keystone species (Weiss et al. 2023).

Building on our expertise, we now aim to generate a synthetic community to model the human neonatal gut microbiota (NeoSyn). The microbiota plays a crucial role in various aspects of early development and health. NeoSyn is intended to mimic the highly variable neonatal gut microbiome both functionally and taxonomically.

The variations in the neonatal gut microbiome are driven by many different factors, such as the mode of delivery, the type of feeding or the use of medication (especially antibiotics).

Using MiMiC (Kumar et al. 2021), a tool which aids the design of minimal microbial consortia based on the functional potential identified in a given metagenomic sample, we designed different variants of NeoSyn consisting of 12 members to mimic an infant's microbiota of vaginally-born (NeoVaSyn) and caesarean-born (NeoCeSyn) infants. This core composition can be complemented with additional species of interest to perform research specific experiments. Using NeoSyns, we plan to investigate and understand e.g. differences in microbial ecology and microbiome functions or apply them for studying different antimicrobial compounds.

P-HAMI-007

Age matters: Exploring differential effects of antimicrobial treatment on Gut Microbiota of adult and Juvenile Brown Trouts

*L. M. Streb[1], P. Cholewinska[2], S. Gschwendtner[1], S. Kublik[1], N. Geveke[3], J. Geist[3], M. Schloter[1,4]

[1]*Helmholtz Center Munich, Research Unit Comparative Microbiome Analysis, Neuherberg, Germany*

[2]*Ludwig-Maximilian-University, Chair for Fish Diseases and Fisheries Biology, München, Germany*

[3]*Technical University Munich, Chair of Aquatic Systems Biology, München, Germany*

[4]*Technical University Munich, Chair of Environmental Microbiology, München, Germany*

Introduction

Amid the growing demand for sustainable protein-rich nutrition, particularly in regions disproportionally affected by the ongoing food crisis, aquaculture has become a pivotal resource. Antibiotics and Antiparasitics, extensively used in hatcheries to maximize production yields and as surrogates for inadequate hygiene, have been linked to increased abundance of antibiotic resistance genes and persistent shifts in the intestinal microbiome of various farmed fish species. Given that the gut microbiome of juvenile fish is still maturing, it likely exhibits greater susceptibility to external perturbations compared to the more stable microbiome of adult fish.

Goals

Using a metabarcoding approach, this study focuses on the distinct impact of antimicrobial treatment on the gut microbiome of juvenile and adult brown trouts (*Salmo trutta fario*), exploring the co-selective pressure of combined florfenicol (FF) and peracetic acid (PAA) application as well as the influence of age.

Material & Methods

In a freshwater RAS, one and two summer-old brown trouts underwent three treatments (10 mg/kg FF via medicated feed, FF+PAA, PAA) compared to an untreated control group. Fecal samples were collected prior to administration, on the last day of administration (day 10) and four times during the post-treatment phase, followed by 16S rRNA gene based amplicon sequencing on the Illumina MiSeq platform.

Results

Results show distinct changes in the gut microbiome composition of juvenile trout following both FF and PAA treatment, marked by decreased abundance of several beneficial core taxa (*Shewanella, Lactococcus, Deefgea*) and an increase of putative pathogens (*Streptococcus, Acinetobacter*). Notably, these effects were absent in adult fish.

Summary

Addressing a critical knowledge gap regarding the extent to which the response to antimicrobial treatment is dependent on the developmental stage of the fish, this study emphasizes the importance of understanding differential effects between developmental stages and highlights the potential long-term consequences of combined application of antibiotics and antiparasitics in aquaculture.

P-HAMI-008

Revolutionizing Arbuscular Mycorrhizal Fungi (AMF) production: A Plant-Free Cultivation Approach

*N. Neetu[1], K. Burow[1], P. Franken[1,2]

[1]*Friedrich Schiller University Jena, Microbiology, Jena, Germany*

[2]*Erfurt university of applied sciences, Research Centre for Horticultural Crops (FGK), Erfurt, Germany*

AMF play a pivotal role in enhancing plant nutrient uptake and fostering sustainable agriculture. However, their widespread application has been hindered by the reliance on host plants for propagation, limiting their availability. The OPT4AMF (Optimization of microbial production biostimulants with innovative additives and mycorrhizal helper bacteria) research project, in collaboration with Inoq GmbH, aims to revolutionize AMF production by pioneering plant-free cultivation techniques, thus ensuring year-round availability of AMF inoculum to support sustainable agriculture worldwide.

The primary strategy involves harnessing mycorrhizal helper bacteria (MHB) from the plant growth-promoting rhizobacteria (PGPR) genera, Azotobacter and Azospirillum, isolated from grass rhizosphere (Jena Experiment) and roots using selective media. The Azotobacter and Azospirillum genera are selected as PGPR for this study due to their ability to fix atmospheric nitrogen, enhance nutrient uptake, and stimulate plant growth through hormone production and root colonization. MHB's ability to form biofilms on AMF surfaces protects them from mycophagous bacteria and enhances their vitality. MHB strains capable of biofilm production will be evaluated for their impact on AMF spore production by testing the bacterial-fungal interaction on root organ culture (ROC) and in the absence of a plant partner.

Plant-free AMF production could revolutionize sustainable agriculture, enabling scalable inoculum production and year-round availability of these beneficial fungi. This approach supports environmentally-friendly practices by reducing reliance on synthetic fertilizers and in consequence enhancing global food security.

P-HAMI-009

Exploring the gut microbiome and mycobiome in Glioblastoma patients

*M. Herz[1], K. Schmid[2], A. Cattaneo[2], A. Keßler[2], R. Nickl[2], R. I. Ernestus[2], C. Hagemann[2], M. Löhr[2], O. Kurzai[1], V. Nickl[1]

[1]*University Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*

[2]*University Hospital Würzburg, Neurosurgery, Würzburg, Germany*

Objective:

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults and remains associated with a poor prognosis despite aggressive therapeutic interventions. This study investigates potential differences in the gut microbiome and mycobiome compositions between primary GBM patients and healthy control subjects.

Methods:

We enrolled 23 GBM patients treated at the University Hospital in Wuerzburg and 23 control participants in a prospective case-control study. Rectal swabs from GBM patients were collected before surgery. DNA was extracted from rectal swabs for 16S and ITS sequencing. We also performed FISH analysis using universal eukaryotic and fungal probes on GBM sections

Results:

Preliminary results indicated differences in the composition of the microbiome and mycobiome between GBM patients and controls. Bacteroidaceae and Lachnospiraceae were lower abundant in GBM patients compared to healthy controls. In contrast, Candida and Cryptococcus were found in higher abundance in GBM patients. FISH analysis did not detect any bacteria or fungi in GBM sections.

Conclusions:

Our preliminary findings provide insights into the microbiome in GBM patients. These results will contribute to our understanding of the gut microbiome in GBM patients and potentially might lead to therapeutic avenues in the pursuit of improved outcomes.

P-HAMI-010

Single-cell analyses to dissect morphological heterogeneity in the gut commensal Bacteroides thetaiotaomicron

*E. Bornet[1]

[1]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*

Genetically identical bacteria within the same micro-environment may not behave alike. This is exemplified by the abundant gut microbiota member *Bacteroides thetaiotaomicron*, whose name derives from the three morphotypes (dubbed "*Theta*", "*Iota*" and "*Omicron*") that these bacteria can adopt. However, the molecular basis of this morphological heterogeneity and its functional consequences are unknown. Here, we map the transcriptome of *Bacteroides* morphotypes using sensitive "mini-bulk" RNA-seq. We observed that across four biological replicates, the different morphotypes upregulate specific sets of marker genes, encoding proteins involved in primary metabolism and membrane-associated processes. A subset of morphotype-specific markers are validated using single-molecule fluorescence *in-situ* hybridization and by single-bacterium RNA-seq. Lastly, reanalysis of existing high-throughput imaging data derived from an ordered transposon insertion library of *B.thetaiotaomicron* in combination with confocal microscopy of clean deletion mutants, reveals the influence of individual genes on bacterial cell shape. Together, our work lays the ground to dissect the extent and impact of cell-to-cell variability in this dominant bacterial species of the human microbiota.

P-HAMI-011

The murine lung microbiome and its disbalance by the fungal lung pathogen Aspergillus fumigatus

*L. Nikitashina[1,2], X. Chen[2,3], L. Radosa[1], K. Li[2,3], M. Straßburger[4], B. Seelbinder[3], W. Krüger[2,5], S. Vielreicher[2,5], T. Heinekamp[1], I. D. Jacobsen[2,5], G. Panagiotou[2,3], A. A. Brakhage[1,2]

[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department of Molecular and Applied Microbiology, Jena, Germany*

[2]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*

[3]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department of Microbiome Dynamics, Jena, Germany*

[4]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Transfer Group Anti-infectives, Jena, Germany*

[5]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Research Group Microbial Immunology, Jena, Germany*

The presence of a stable residential microbiota in the lungs and their potential function for health and disease remain a matter of debate. Moreover, little is known about interconnections between the lung microbiota and lung diseases e.g. caused by pathogenic microorganisms. *Aspergillus fumigatus* is a clinically relevant fungus causing life-threatening lung infections in immunocompromised patients. Here, we aimed to analyze the lung microbiota of mice both by DNA sequencing and cultivation approaches. This way, we aimed at the identification of the species characteristic of the murine lung microbiome. To bridge the gap between the lung microbiome and invasive aspergillosis, we investigated the lung microbiome upon its disbalance by an *A. fumigatus* infection and a possible rebalancing by antifungal treatment. Finally, we aimed to gain the information on the mechanistic basis of potential interactions between lung bacteria and the pathogen.

We could isolate bacteria belonging to 11 species from the lower airways of mice. One of the most prominently isolated bacteria was *Ligilactobacillus murinus*. We have developed a genetic transformation system for *L. murinus* to label the bacteria with GFP. We noticed that GFP-labelled *L. murinus* cells were internalized by murine alveolar epithelial cells. This finding suggests that lung bacteria can be recognized by alveolar epithelial cells, found intracellularly and expected to modulate the immune system of the lung.

Further, we analyzed changes in the lung microbiome upon immunosuppression, infection with *A. fumigatus*, and antifungal treatment in a mouse model of invasive aspergillosis. Sequencing analysis showed that the composition of the microbiome changed dramatically under all tested conditions. Interestingly, *L. murinus* was detected in the lungs under all treatments and increased in abundance in the lungs infected with *A. fumigatus.*To gain insight into potential cause-effect relationships between lung microbiota and a pathogen, isolated bacteria were co-cultivated with *A. fumigatus*. We found that *A. fumigatus* promotes growth of *L. murinus* indicating a direct influence of the pathogen on resident bacteria.

P-HAMI-012

Staphylococcal lipases generate wax esters to detoxify host-derived antimicrobial fatty acids.

*J. Camus[1], C. D. Freeman[2], K. M. Hines[2], A. Kengmo Tchoupa[1]
[1]*Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, Tübingen, Germany*
[2]*University of Georgia, Department of Chemistry, Athens, GA, Germany*

Host-derived antimicrobial fatty acids (AFAs) are essential to restrict the proliferation of opportunistic microorganisms like *Staphylococcus aureus*. This bacterium has evolved several adaptation strategies to resist AFAs. Recently, we have discovered that *S. aureus* utilizes its lipase Lip2 to esterify AFAs with cholesterol, leading to AFA detoxification. However, the full extent of staphylococcal lipase-mediated changes of the host lipid landscape remains elusive. For instance, fatty acids esterified with long-chain fatty alcohols (wax esters), that play important roles for skin structure and function, could represent products or substrates of staphylococcal lipases. Wax ester production from AFAs and long-chain fatty alcohols as a strategy to escape the AFA toxicity is unexplored. Here, we demonstrate that Lip2 detoxifies AFAs with various fatty alcohols via esterification. This enabled Lip2-expressing *S. aureus* to grow and form biofilms in the presence of fatty alcohols and otherwise toxic concentrations of AFAs. Moreover, we investigated lipases from coagulase-negative staphylococci (CoNS), which share similarities with Lip2, for esterification capacities. To do so, we heterologously expressed CoNS lipases in a lipase-deficient *S. aureus* mutant. The *Staphylococcus simulans* SsL and *Staphylococcus epidermidis* GehD lipases could protect the *S. aureus* lipase-deficient mutant from AFA toxicity in the presence of either cholesterol or fatty alcohols. Our current lipidomics analyses are revealing the ability of Lip2 and CoNS lipases in shaping the lipid landscape of the bacterium and its environment. Collectively, these results suggest that (i) lipase-mediated AFA detoxification is common in CoNS, (ii) different hydroxylated substrates found on the skin surface can be used to alleviate the toxicity of AFAs and (iii) lipases exquisitely manipulate environmental lipids to promote bacterial growth while changing the host lipid landscape. Considering their ability to both degrade lipids and detoxify AFAs, staphylococcal lipases may play an underappreciated role at the host-microbe interface with potential implications for the microbiome composition.

P-HAMI-013
Functional bacterial diversity of the digestive tract of chicken

*B. Rios-Galicia[1], J. S. Saenz[1], A. Camarinha-Silva[1], J. Seifert[1]
[1]*Universtiy Hohenheim, Animal Science, Stuttgart, Germany*

The study of intestinal microbial communities complements the knowledge gained through metagenomic studies with cultivation approaches. Although cultivation methods carry a selection bias to microorganisms that adapt better to the culture conditions, sequenced genomes obtained from cultivation play an important role in providing reference material to deeply characterise metagenomic observations. The objective of this work is to estimate the functional diversity along the gastrointestinal tract of chicken considering the prevalence, abundance and functional annotation of the cultured fraction reported so far. To address this, genome assemblies generated from cultivation studies obtained from crop, jejunum, ileum, caeca, and faeces were collected, quality filtered and compared against metagenomic sequences derived from studies on the chicken gastrointestinal tract (GIT). Genes obtained from genome annotation were mapped to estimate abundance of functions along the different GIT regions. Members of *Lactobacillaceae* dominated the upper regions and this was reflected on the redundancy of isolation. *Limosilactobacillus* species were found to be more abundant in the crop, while *Ligilactobacillus* species dominated the small intestine. Species from upper regions encode a high number of glycosidases specialised in complex polysaccharides compared to species found in lower regions (caeca and faeces). Along all regions, genes that encode collagenases and hyaluronidases are equally found, indicating a common adaptation to along the gastrointestinal tract. These results enlighten the microbial ecology of the GIT of chicken through the observation of species and functions distribution.

Fig. 1

P-HAMI-014
Establishing a molecular toolkit for ectomycorrhizal symbiosis

V. Kreszies[1], H. Reichelt[1], J. Bock[1], *I. Teichert[1]
[1]*Georg-August University Göttingen, Forest Botany and Tree Physiology, Göttingen, Germany*

Mycorrhizal symbiosis is a mutualistic interaction between plants and fungi. Most trees in temperate forests show colonization of fine roots by ectomycorrhizal fungi (ECM). The trees benefit from this symbiosis by easier access to water and mineral nutrients, and the fungi acquire carbohydrates from the trees. Many studies have focused on ectomycorrhizal interaction, and for example transcriptomic analysis has identified a huge number of genes as differentially regulated during the onset and / or maintenance of symbiosis. Yet, molecular analysis of mycorrhizal interaction partners in laboratory model systems as well as functional analysis of genes remains scarce.

To establish such models, we morphologically and genetically analyze ECM from fine roots in temperate forests. Microscopic analysis is complemented by DNA isolation and sequencing of different morphotypes. Further, we work on transformation systems for ECM that are able to grow in the lab and are able to mycorrhize poplar trees, a genetically tractable system. This will enable us to analyze the basis for initiation and maintenance of the ECM symbiosis on a molecular level.

P-HAMI-015
A rosy future for caries patients? - Effects of roseoflavin on the human oral microbiota

*N. Schwendenmann[1], L. Steiner[1], S. Burger[1], L. Seufert[2], M. Mack[2], M. Egert[1]
[1]*Furtwangen University of Applied Sciences, Institute of Precision Medicine, Villingen-Schwenningen, Germany*
[2]*Mannheim University of Applied Sciences, Institute of Technical Microbiology, Mannheim, Germany*

A recent study [1] suggested that the bacterium *Streptococcus mutans* is auxotrophic for riboflavin (RF, vitamin B_2) and thus depends on uptake. In soil, *Streptomyces davaonensis* produces the antivitamin roseoflavin (RoF), a toxic RF analogue, to reduce growth of competing microorganisms [2]. We hypothesized that RoF might be useful to reduce the cariogenic potential of the human oral microbiota. However, little is known about the antimicrobial efficacy of RoF against members of the human oral microbiota.

In the cultivation-based arm of our study, more than 300 microbial isolates were obtained from saliva samples of 41 healthy human volunteers and identified by MALDI-TOF-MS. Securely identified species were tested with regard to RF auxotrophy and susceptibility to RoF in RF-free media supplemented with RF and/or RoF, respectively. None of the isolated Gram-negative species were RF auxotrophic, while 7 out of 19 Gram-positive species grew significantly better when media were supplemented with RF (100 µM). Similarly, growth of 11 out of 12 tested Gram-negative species was not negatively affected by RoF (100 µM), whereas 11 out of 19 Gram-positives showed growth reduction, including *S. mutans.* These data suggest that RoF-treatment might shift the human oral microbiota to the Gram-negative fraction.

To obtain a cultivation-independent view on the effects of RF and RoF on the oral microbiota, fractions of pooled saliva from 15 patients were incubated for up to 24 h at 37 °C in the presence or absence of different concentrations of RF and RoF, respectively. Total DNA and RNA were extracted from actively growing cells and used as (RT-)PCR-templates for 16S rRNA gene and 16S rRNA-based community analyses. The results are supposed to unravel the effect of RF and RoF on the "present" (rDNA-based community composition) and "active" (rRNA-based community composition) fractions of the oral microbiota. Future research aims at a deeper understanding of the inhibition mechanisms of RoF on selected species as well as the effects of RoF on human epithelial cells.

[1] Turner et al. (2020). J Bacteriol 203(2):e00293-20 ; [2] Pedrolli et al. (2013). Curr Pharm Des 19(14):2552-60.

P-HAMI-016
Serratia sp. profiles in bronchoalveolar lavage specimens from patients with tuberculosis and non-tuberculous mycobacterial lung diseases

*M. Belheouane[1], B. Kalsdorf[1], J. Heyckendorf[1,2], S. Niemann[1,2,3], K. I. Gaede[1,2,3,4,5], M. Merker[1,2,3,4,5]
[1]*Research Center Borstel Leibniz Lung Center, Evolution of the Resistome, Borstel, Germany*
[2]*University Medical Center Schleswig-Holstein, Department of Internal Medicine I, Kiel, Germany*
[3]*German Center for Infection Research, Brunswick, Germany*
[4]*Airway Research Centre North (ARCN), German Centre for Lung Research (DZL), Großhansdorf, Germany*
[5]*University Hospital Schleswig-Holstein, PopGen 2.0 Biobanking Network (P2N), Kiel, Germany*

Introduction

Mycobacterium tuberculosis complex (Mtbc) bacteria causing tuberculosis (TB) are among the leading infectious killers worldwide. Additionally, opportunistic non-tuberculous mycobacteria (NTMs) can cause severe respiratory infections in patients with pre-existing lung conditions. Yet, it is unclear if the lung microbiome is a risk factor to develop active TB and NTM lung disease or influences treatment outcomes.

Goals

We sought to contrast the lung microbiome of TB, and NTM patients, and identify key taxa associated with disease states.

Materials & Methods

We profiled the lung microbiomes of 23 TB, 19 NTM, and 4 non-infectious lung disease patients prior their main therapy by analyzing bronchoalveolar lavage fluid (BALFs) collected at the Research Center Borstel, Germany over 14 years. We depleted human cells and extracellular DNA, and profiled the microbiome using 16S rRNA amplicon- and exploratory whole metagenome sequencing following state-of-the-art laboratory, and analytical protocols designed for low biomass specimens.

Results

We found that the genus *Serratia* strongly dominates the lung microbiome of TB, and NTM patients. At the sub-genus level, we find that i) TB patients harbor higher community diversity, ii) two distinct *Serratia* traits are significantly associated to TB patients, one of which belongs to *Serrartia grimesii* as disclosed by exploratory metagenomic analysis, iii) disease state significantly influences microbiome community structure.

Summary

Serratia sp. plays a pivotal role for our understanding of microbial interactions in the lung microbiome of patients infected with Mtbc and NTMs. Future studies investigating the mechanisms of *Serratia-Mycobacterium* interactions and their impact on disease progression and therapy outcome are warranted.

P-HAMI-017
A headful of germs - Metagenomic analysis of the scalp microbiota in young and elderly women

*S. Jacksch[1], S. Gruedl[2], R. Simmering[3], T. Welss[2], M. Egert[1]
[1]*Furtwangen University, Institute of Precision Medicine, Villingen-Schwenningen, Germany*
[2]*Henkel AG & Co KGaA, Global Category Hair, Düsseldorf, Germany*
[3]*Henkel AG & Co KGaA, Department of Microbiology, Corporate Scientific Solutions, Düsseldorf, Germany*

The human scalp carries a moderately diverse microbiota [1]. Ageing changes skin and scalp physiology which might impact the resident microbiota [2,3] and call for adapted care

strategies. Our previous, PCR-based research suggested that age might increase scalp species richness [1]. This prompted us to investigate age-related changes in the scalp microbiota in more detail with a PCR-independent, metagenomic approach to also address the functional potential of the scalp microbes. We collected a total of 46 scalp swab samples from healthy, preconditioned women aged 21-36 years ("young") and 64-87 years ("old"), respectively. After DNA isolation and Illumina MiSeq-based shotgun sequencing, the samples underwent bioinformatic analyses. Taxonomic profiling of quality-filtered sequences (average sequencing depth: 131150 reads/sample) against the RefSeq database, limited to microbial sequences, revealed that bacterial (36 %) and eukaryotic (34 %) sequences were relatively most abundant. Viral and archaeal sequences accounted for less than 1 % of all sequences. Consistent with other studies, the most common bacterial genera were *Cutibacterium* and *Staphylococcus*, while the primary fungal genus was *Malassezia* [1,3]. 30 % of the sequences could not be taxonomically annotated using this database. To gain insight into potential functional properties, sequences were summarised using GO terms, based on their SwissProt match. However, neither taxonomic nor functional analyses revealed significant differences between younger and older subjects, even after increasing the average sequencing depth for selected samples to 285543 reads. So far, our data do not indicate a significant effect of age on the structure and function of the scalp microbiota. However, future studies might include a greater sequencing depth to better account for rare sequence types and more function-oriented approaches, such as metatranscriptomics. In addition, male test persons might be included and the pre-conditioning treatment adapted.

[1] Jacksch et al. (2023) *Int J Cosmet Sci*, 10.1111/ics.12895; [2] Shibagaki et al. (2017) *Sci Rep* 7(1):10567; [3] Saxena et al. (2018) *Front Cell Infect Microbiol* 8:346

P-HAMI-018

Bacterial aggregation in the imbalanced intestinal microbiota

*S. Wagner[1,2], A. Busch[1,2,3], M. Bauer[2,3], R. Allen[1,2]
[1]*Friedrich Schiller University Jena, Institute of Microbiology/Theor. Microb. Ecology, Jena, Germany*
[2]*Friedrich Schiller University Jena, Cluster of Excellence Balance of the Microverse, Jena, Germany*
[3]*University Hospital Jena, Dept of Anesthesiology and Intensive Care Medicine, Jena, Germany*

Bacterial aggregates, comprising single or multiple species, are prevalent across diverse ecological niches. Such cellular clustering is regarded as a shield against environmental adversities and suspected to be the initial phase in biofilm formation. This ubiquitous phenomenon can be observed among both environmental and pathogenic bacterial populations. Thus, raising interest of aggregation within human microbiomes. Particularly in highly competitive environments like the human intestine, the protective characteristic of multicellular aggregates is thought to lead to a selective advantage for pathogenic species. Perturbations in the equilibrium of the intestinal microbiota may promote the proliferation of unfavorable bacteria. Moreover, dysbiosis is frequently observed during or after sepsis. However, the specific role of bacterial aggregation in this context remains elusive.

To investigate the impact of aggregation on a disbalanced human gut microbiota, *Enterobacteriaceae* were isolated from fecal samples of intensive care patients (from study MS-ICU) who were treated with the antibiotics meropenem, piperacillin/tazobactam, or with no antibiotics. Isolates were screened for their ability to aggregate and form biofilms. As bile acids accumulate in patients" blood during sepsis, we also assessed the influence of this substances on cell size, shape and aggregation via cell counting and microscopy.

Our study demonstrates the presence of several aggregate- and biofilm-forming *Enterobacteriaceae* among the surviving strains isolated across all patient treatment cohorts. Additionally, bile acids significantly influence those processes. We suggest that bacterial aggregation and biofilm formation may promote the survival of specific strains within the gut. Subsequent experiments will consider the impact of host-pathogen interactions and also of antibiotics. Integrate our results, we aim to understand the role of bacterial aggregation in the maintenance of a balanced human intestinal microbiota.

P-HAMI-019

Airway microbial metagenomics from premature birth to end-stage lung disease

*I. Rosenboom[1], A. Thavarasa[1], H. Richardson[2], C. F. Davenport[3], L. Wiehlmann[3], D. Viemann[1], J. D. Chalmers[2], B. Tümmler[1]
[1]*Hannover Medical School, Clinic for Paediatric Pulmonology, Allergy and Neonatology, Hannover, Germany*
[2]*University of Dundee, School of Medicine, Dundee, United Kingdom*
[3]*Hannover Medical School, Research Core Unit Genomics, Hannover, Germany*

Background: Progressive respiratory diseases are characterised by chronic infections. Shotgun metagenomics identifies bacteria, fungi and DNA viruses at higher resolution than amplicon sequencing commonly applied to study this habitat.

Question: The airway metagenome of preterm infants (n=24), healthy adults (n=88) and people with bronchiectasis (n=101) was examined to identify microbial community members that distinguish disease from lung health.

Methods: Shotgun metagenomics sequencing was performed on an Illumina NextSeq system. The generated short read data sets were then processed by our in-house developed sequencing alignment pipeline Wochenende.

Results: During their stay at the neonatal intensive care unit, preterm neonates acquired individual non-maternal airway metagenome signatures from the hospital environment. After hospital discharge, airway metagenomes developed towards a common taxonomic structure, but did not achieve the stable bacterial community structures seen in healthy full-term infants. In case of the people with bronchiectasis, the individual metagenomes clustered by the absence or presence of *H. influenzae* and *P. aeruginosa*. Severe bronchiectasis was characterised by low diversity metagenomes. Comparison with the sputum metagenome of healthy non-smokers revealed a gradient of depletion of commensal taxa in bronchiectasis, even in the absence of any respiratory pathogen.

Conclusions: As signs of microbial dysbiosis, commensal species become gradually suppressed in people with

bronchiectasis and the bacterial metagenome of preterm infants is still immature by 15 months of age.

P-HAMI-020
Gene Disruption of a Novel ABC Transporter in Staphylococcus epidermidis 1457 Sensitizes the strain to Staphylococcal bacteriocin Epifadin
T. Türkoglu[1], *L. Schulze[1], B. Krismer[1], A. Peschel[1]
[1]*University of Tuebingen, IMIT - Infectionbiology, Tübingen, Germany*

Staphylococcus aureus (S. aureus) is a prevalent opportunistic pathogen that can cause superficial infections as well as a range of severe diseases, possibly leading to death. Around 30 % of the population is colonized by S. aureus, preferably in the anterior nares. Overuse of antibiotics in healthcare and livestock accelerates the development of resistances in bacteria, which can lead to the emergence of multidrug-resistant species, such as methicillin-resistant S. aureus (MRSA). An alternative, promising control strategy is based on microbiome-mediated colonization resistance. Colonization resistance may occur via various mechanisms of complex microbial interactions, including the production of antimicrobials, referred to as bacteriocins. Staphylococcus epidermidis (S. epidermidis) is an important member of the nasal microbiota due to its production of diverse bacteriocins, which is associated with a high capacity for colonization resistance, especially against S. aureus. Nasal S. epidermidis strain IVK83 produces a highly potent bacteriocin, called epifadin, which effectively inhibits S. aureus. However, the phenotypic response to epifadin varies among Staphylococci, and our understanding of how resistance is mediated remains limited. We aimed to identify genetic features of Staphylococci that confer resistance to epifadin. We investigated the ABC transporters EfiF and EfiG, encoded in the biosynthetic gene cluster of epifadin, as potential resistance factors. To this end, we performed spot assays on susceptible S. aureus strains provided with a heterologous expression of the respective transporters, which did not change epifadin susceptibility in S. aureus. However, we demonstrated that S. epidermidis IVK83, deficient in both efiFG expression, shows decreased antimicrobial activity on S. aureus suggesting an impaired secretion of the bacteriocin. Independently, transposon mutagenesis of the epifadin-resistant S. epidermidis 1457 revealed an epifadin-susceptible clone, disrupted in the expression of a putative ABC transporter, referred to as ergB. We therefore propose that the erg-encoded transport system contributes to epifadin resistance in Staphylococci.

P-HAMI-021
Tillandsia landbeckii lomas provide refugia for microbial life in the hyper-arid Atacama Desert
*S. Velte[1], A. Hakobyan[1], C. Knief[1]
[1]*University of Bonn, Institute of Crop Science and Resource Conservation, Bonn, Germany*

The Atacama Desert, one of the oldest and driest non-polar deserts on Earth, displays extreme conditions for microbial life due to water scarcity and low soil organic carbon availability. We hypothesized that well-adapted desert plants support microbial life and provide different habitats for bacteria. We investigated the habitat function of *Tillandsia landbeckii* lomas. *T. landbeckii* has developed strategies to survive even under the most arid conditions and is found in isolated populations in the Atacama Desert. We collected plant and soil samples from different *T. landbeckii* lomas and analyzed the bacterial communities that were associated with the plants and present in the nearby soil based on 16S rRNA gene amplicon sequencing. Our data revealed a clear compartment-specific colonization of *T. landbeckii* plants, i.e. the above-ground part (phyllosphere) hosted a different community than the below-ground part (laimosphere). Further, plant-associated microbial communities were distinct from those of barren surface soil. Besides well-known phyllosphere colonizers (*Alphaproteobacteria, Gammaproteobacteria* or *Actinobacteria*), we detected rather unusual taxa in the phyllosphere, especially "*Candidatus* Uzinura", an insect symbiont. The microbial community of the laimosphere was comprised of soil and rhizosphere dwelling bacteria as well as phyllosphere colonizers (*Gammaproteobacteria, Actinobacteria* or *Bacteroidia*). In case the plants are grown on dunes with relic *T. landbeckii*, the habitat function is further extended into the deeper soil. We observed an increased bacterial abundance, richness and diversity, especially in dune layers with elevated amounts of plant material. Taken together our findings indicate that *T. landbeckii* lomas provide different habitats for microorganisms. Adaptation strategies of these microorganisms in the different habitats need to be studied in more detail in the future.

P-HAMI-022
Oral biofilms in axial spondyloarthritis: High correlation of NGS results and spatial analysis of the oral microbiome
M. Gühmann[1], Z. Xiong[2,1], M. Muchova[1], J. Schmidt[2,1], J. Rademacher[3], K. A. Schildhauer[4], D. Poddubnyy[3], H. Dommisch[4], A. Moter[1,5], *J. Kikhney[2,1]
[1]*Charité - University Medicine Berlin, Biofilmcenter, Institute for Microbiology, Infectious Diseases and Immunology, Berlin, Germany*
[2]*MoKi Analytics GmbH, Berlin, Germany*
[3]*Charité - University Medicine Berlin, Medizinische Klinik für Gastroenterologie, Infektiologie und Rheumatologie, Berlin, Germany*
[4]*Charité - University Medicine Berlin, Charité Center for Dental, Oral and Maxillofacial Medicine, Berlin, Germany*
[5]*Moter Diagnostics, Berlin, Germany*

Question

Axial spondyloarthritis (axSpA) is a complex rheumatic disease, mainly manifested at the spinal column and symptomatic by pain in the lower back. The exact pathogenesis is still not fully understood. It is thought to be caused by a combination of genetic as well as environmental factors, with microbial influences currently discussed as relevant.

Microbiome analysis using NGS alone does not provide information about abundance or spatial distribution of bacterial species and thus does not allow identifying the key microorganisms in mixed biofilms. Novel techniques such as fluorescence in situ hybridization (FISH) allow a more detailed analysis of the architecture of subgingival bacterial plaque.

To gain validated and multidimensional insights into the microbiomes, the combination of two complementary analysis techniques was developed.

Aim was to shed light on the oral microbiota in patients with axSpA and possible association with disease activity / severity.

Methods

23 patients and 4 healthy controls were included. Oral biofilms were sampled using a carrier system that was placed in a periodontal pocket for 5 days. Carriers were embedded and consecutive sections were submitted to NGS analysis. Based on those results, FISH was performed using specific probes for two bacterial species identified as potentially interesting to the disease, to gain further insight into the spatial structures of the microbiomes and to confirm the results obtained by NGS.

Results

Distinct differences between four groups of axSpA patients and between patients and healthy controls were detected. It could be shown that the results of the NGS analysis agree with those of FISH, with a very high degree of agreement.

Conclusions

Using the two different molecular techniques, a comprehensive and detailed "landscape" of the oral microbiome could be provided and we succeeded in giving microbiome data by NGS a spatial resolution.

The microbial differences that were be detected, are to be linked to clinical data. Information on the spatial distribution of the bacteria visualised by FISH could be used to find key species and investigate interactions between oral bacteria

---

P-HAMI-023

Microbiome disturbance by antibiotic treatment affects the metabolic responses of the host (Crassostrea gigas) to abiotic stressors

*M. Gaikwad[1,2], T. Bruhns[2], C. Hassenrück[1], I. Sokolova[2], M. Wegner[3], M. Labrenz[1]
[1]*Leibniz-Institut für Ostseeforschung Warnemünde, Biological Oceanography, Rostock, Germany*
[2]*University of Rostock, Institute of Biological Sciences, Rostock, Germany*
[3]*Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, List/Sylt, Germany*

Intertidal species, including many invaders, thrive in stressful and highly fluctuating environments. A prominent invasion hotspot, the Wadden Sea, witnessed the dramatic dispersal of the Pacific oyster *Crassostrea gigas* from aquaculture into the wild. Recent studies delving into the molecular mechanisms behind *C. gigas* broad stress tolerance found evidence of amplification of the genes involved in cellular stress protection and immunity. However, the potential role of host-microbiome interactions in the exceptional stress tolerance of *C. gigas* and the significance of the native microbiome structure for host performance in fluctuating environments remain unexplored. To understand the link between microbiome integrity and the host"s physiological performance under stress, we investigated the impact of antibiotic-induced microbiome disturbance on oysters" responses to abiotic stress including intermittent hypoxia, temperature, and salinity fluctuations. We analyzed microbiome dynamics in gills, digestive glands, and haemolymph of 300 *C. gigas* using high-throughput sequencing of the 16S rRNA gene with the DADA2 algorithm pipeline. Statistical analyses determined temporal shifts, alpha and beta diversity, and taxonomic composition of oyster microbiome under different conditions. We found pronounced within-individual heterogeneity in microbial composition across tissues, with *Proteobacteria, Bacteroidota, Spirochaetia,* and *Firmicutes* consistently dominating. Antibiotic treatment induced significant shifts in alpha diversity, indicating temporal changes in depletion effects. Moreover, the microbiome responded deferentially to stressors when combined with antibiotic disruption, with outcomes varying across tissues. We observed marked taxonomic alterations in antibiotic-depleted oysters, rendering the native microbiome more susceptible to a shift toward a community dominated by opportunistic pathogens, thereby increasing disease risk. These observed shifts in microbial composition and diversity offer valuable insights into the resilience of oyster-associated bacteria and their potential implications for host health and survival in the face of environmental challenges.

P-HAMI-024

Uncovering host-pathogen interaction dynamics in human intestinal epithelial cells

*A. Binder[1,2], M. Neyazi[1], L. Knodler[3], S. Bartfeld[1,4], A. E. Saliba[1,2]
[1]*University of Würzburg, Institute for Molecular Biology (IMIB), Würzburg, Germany*
[2]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*
[3]*University of Vermont, Burlington, Germany*
[4]*Institute for Biotechnology, Berlin, Germany*

Salmonella enterica serovar Typhimurium (henceforth *Salmonella*) is a common cause of gastroenteritis in humans. *Salmonella* bacteria infect the intestine and actively invade intestinal epithelial cells (IECs), where they either remain in the *Salmonella*-containing vacuole (SCV) or escape into the cytosol. The determinants of these different life-style remain poorly understood. Research on those two distinct populations of *Salmonella* inside epithelial cells has mostly been done on cancer cell lines, which are known to lack components of the *in vivo* tissue that might be crucial to infection with *Salmonella*. The growing field of organoid research provides new models, such as organoid-derived monolayers, that bridge the practicability of cell lines with a higher level of complexity, by providing different cell types of a certain tissue, in a 2D monolayer structure. Here we describe and characterize a human small intestinal epithelial organoid-derived monolayer model for infection with *Salmonella*, using single-cell RNA sequencing (scRNA-seq). We furthermore infect the organoid-derived monolayer model with *Salmonella* and investigate the existence of vacuolar and cytosolic *Salmonella*respectively, using a staining for the SCV surface marker LAMP-1. To study the dynamics of the infection, we have implemented a high-throughputmethod, combining single-cell RNA-seq and RNA metabolic labeling. Altogether, we expect our multi-dimensional single-cell analysis to reveal the host factors that underliethe fate decision of bacterial divergent lifestyles.

P-HAMI-025

Sphingosine kinase 1/S1P receptor signaling axis is essential for cellular uptake of Neisseria meningitidis into brain endothelial cells

*I. Fohmann[1], A. Weinmann[1], F. Schumacher[2], S. Peters[1], A. Prell[2], C. Weigel[3], S. Spiegel[3], B. Kleuser[2], *A. Schubert-Unkmeir[1]
[1]*University of Wuerzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[2]*Free University of Berlin, Institute of Pharmacy, Berlin, Germany*
[3]*Virginia Commonwealth University School of Medicine, Department of Biochemistry and Molecular Biology and the Massey Cancer Center, Richmond, VA, United States*

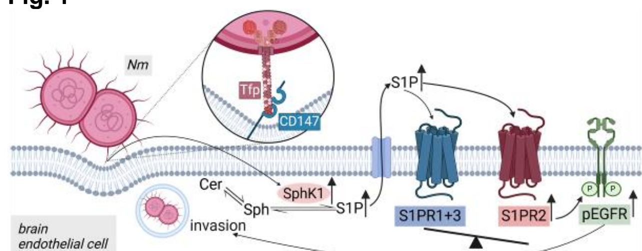
Neisseria meningitidis (*Nm*) can cause life-threatening meningitis after crossing the blood-brain barrier (BBB). The small lyso-phospholipid sphingosine 1-phosphate (S1P) is an important regulator of BBB integrity which is formed by sphingosine kinases (SphKs). The barrier function of the BBB is mainly exerted by brain endothelial cells (BECs). BECs express 3 types of S1P receptors (S1PR1-3), which, upon binding to S1P, can positively or negatively influence BBB permeability. We hypothesize that *Nm* manipulates S1P-S1PR signaling to favor uptake into BECs as a first step to cross the BBB.

We used LC-MS/MS to generate a time-resolved picture of sphingolipid metabolism and secretion after infecting BEC cell line hCMEC/D3 with *Nm* serogroup B strain MC58. qPCR in combination with phospho-specific western blots of BEC subcellular fractions was applied to determine transcriptional and (post-)translational regulation of S1P-metabolizing enzymes and receptors. SphK enzymatic activity assays were used to determine bacterial virulence factors as well as cellular interactors leading to an increase of S1P after *Nm* infection. Antibiotic killing assays and differential fluorescent staining detected with confocal microscopy were conducted to identify the role of S1P and S1P signaling during bacterial adherence and invasion. ELISA and qPCR experiments shed light on the role of S1P signaling during BEC inflammation.

We found that *Nm* infection of BECs induced continuous production and secretion of S1P. S1P production was facilitated by increased SphK enzymatic activity, which could be ascribed to neisserial type IV pilus' (Tfp) interaction with cellular surface receptor CD147. Activation appeared due to transcriptional upregulation and phospho-activation of isozyme SphK1. Phospho-activation of EGFR, a pre-requisite for *Nm* invasion, was dependent S1P on secretion. Moreover, we found that inhibiting S1P production or signaling over S1PR2 prevented uptake of *Nm* into BECs and reduced inflammatory cytokine expression and release.

Taken together, our results highlight the SphK-S1P-S1PR signaling axis as a potential target for adjuvant therapy during *Nm* meningitis.

Fig. 1



P-HAMI-026
The intricate relationship between Drosophila and Caenorhabditis in their natural habitat
*J. Johnke[1], C. Petersen[1], M. Knop[2], T. Roeder[2], H. Schulenburg[1,3]
[1]*CAU Kiel, Evolutionary Ecology and Genetics, Kiel, Germany*
[2]*CAU Kiel, Molecular Physiology, Kiel, Germany*
[3]*MPI for Evolutionary Biology, Plön, Germany*

Model organisms play a crucial role in advancing our current understanding of biology. However, we often overlook the importance of considering their ecology to comprehend their biology fully. In this context, we examine the microbiomes of two common model organisms— the nematode *Caenorhabditis* and the larvae of the fruit fly *Drosophila*—which share the same natural habitat and thereby potentially the same resource, i.e. bacteria.

Taking samples from a compost heap from September to December reveals that *Caenorhabditis* and *Drosophila* larvae coexist on rotten apples, sharing most and specifically abundant members of theirmicrobiome. However, certain ASVs are enriched in the respective sample types. Additionally, microbiomes from both hosts cluster separately in a PCoA. Laboratory compost experiments corroborate the observations and reveal that the presence of *Drosophila* negatively impacts worm population size. Digging deeper, a complex interaction network emerges: *Drosophila* larvae can ingest eggs and larval stages of the worm, sometimes releasing live worms after gut passage. Conversely, the presence of L4 and adult worms accelerates the fly's development, indicating a stress response likely triggered by resource competition. Notably, dead fly larvae become a food source for the nematode. Worms seemingly enter fly larvae corpses through the mouth opening, proliferating both inside and outside the deceased body.

In summary, our studies reveal a complex relationship of dual nature between *Drosophila* and *Caenorhabditis* in their shared habitat. *Drosophila* feeds on specific worm stages, while smaller worms survive gut passage, dispersing across the habitat, potentially finding new resources, including dead fly larvae. Competition for resources might result in fly larvae entering pupation earlier for survival, creating an opportunity for the worm population to increase.

P-HAMI-027
Antibiotic-induced changes in the microbiota affect susceptibility and immune responses to systemic candidiasis
K. Merga[1], S. Chakraborti[1], *I. D. Jacobsen[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Microbial Immunology, Jena, Germany*
[2]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*

Prolonged treatment with antibiotics is a risk factor for systemic candidiasis. By affecting overall bacterial burden in the gut and depletion of antagonistic bacteria, antibiotic treatment reduces colonization resistance and thereby promotes fungal overgrowth. As the gut is an important source for translocation of *Candida* into the blood stream, high intestinal fungal burden increases the likelihood of translocation and subsequent dissemination. We hypothesized that antibiotic treatment might additionally affect host susceptibility by impacting tissue physiology or immune responses to infection.

To test this hypothesis, we systemically infected mice by intravenous injection of *C. albicans*. Pretreatment with a combination of antibiotics led to significantly reduced survival in specific-pathogen free (SPF) mice, indicating that antibiotic treatment indeed affects host susceptibility. This effect was reproducible across SPF mice that differed in their microbiome composition prior to treatment. However, antibiotics had no effect on the susceptibility of germ free mice, suggesting that changes in the microbiota, rather than a direct impact on host cell physiology, are responsible for the negative impact of antibiotics on host resistance.

Immunophenotyping revealed a profound impact of antibiotic treatment on immune cell numbers in different compartments, cytokine production after infection, and the antifungal efficacy of innate immune cells. The later is likely to explain the significantly higher renal fungal burden observed in antibiotic-treated mice two days after infection.

Importantly, intestinal colonization with *C. albicans* prior to infection, resembling the situation in patients, abrogates antibiotic-induces susceptibility differences, but antibiotic treatment of colonized mice leads to stronger Th17 responses and increased amounts of *C. albicans*-specific IgG. This could lead to increased immunopathology in hosts that are not able to clear the infection.

P-HAMI-028

Sterol-accumulating bacteria function as a cholesterol source for sterol-auxotrophic bacterivorous nematodes.

D. Schmidt[1,2], D. Leusder[2], C. Kaiser[2], B. Philipp[1,3], E. Liebau[2], *J. Holert[1]

[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

[2]*University of Münster, Institute of Integrative Cell Biology and Physiology, Münster, Germany*

[3]*Fraunhofer Institute for Molecular Biology and Applied Ecology, Environmental Microbiology, Schmallenberg, Germany*

During evolution, some *Ecdysozoa* such as insects, nematodes, and crustaceans have lost the ability to synthesize steroids *de novo*, making them dependent on exogenous sterols. In *Caenorhabditis elegans*, dietary sterols are primarily required as hormone precursors to regulate reproduction and life stage development. Strikingly, *C. elegans* are filter-feeding bacterivores relying on bacteria as their main nutrient source. Since most bacteria do not synthesize steroids, it remains an open question how these nematodes acquire sterols to maintain their life cycle. We recently found that sterol-degrading Actinobacteria from the *Rhodococcus* and *Mycobacterium* genera accumulate significant amounts of sterols in intracellular lipid droplets under stress conditions such as nitrogen limitation. We hypothesized that these sterol-accumulating bacteria may constitute an important sterol source for *C. elegans* in oligotrophic habitats.

To test this hypothesis, we measured *C. elegans* reproduction rates by comparing brood sizes with different sterol-accumulating and sterol-free bacteria as food sources on sterol-free medium. While *C. elegans* was not able to produce offspring with sterol-free *E. coli*, *M. smegmatis,* or *M. aurum,* around 250 offspring were produced per worm when *M. smegmatis* and *M. aurum* had accumulated intracellular sterols. Similar numbers were produced with sterol-free bacteria on cholesterol-supplemented medium. To track bacteria- and medium-derived sterols in *C. elegans*, we performed feeding assays with fluorescently labelled cholesterol. Confocal microscopy showed that bacteria-derived cholesterol was taken up via the pharynx into the gut and across the gut wall of *C. elegans*. In contrast, medium-derived cholesterol localized primarily in the nerve ring and sensory dendrites but was almost absent from the pharynx and the intestine. Experiments with *C. elegans* sterol uptake mutants are currently underway.

Our results show that *C. elegans* can use intracellular bacterial sterol storage compounds to maintain its reproductive cycle and suggest that different uptake mechanisms for sterols derived from bacterial lipid droplets and from the medium exist in *C. elegans*.

P-HAMI-029

Nasal commensals reduce Staphylococcus aureus proliferation by restricting siderophore availability

Y. Zhao[1], A. Bitzer[1], J. J. Power[1], D. Belikova[1], B. Torres-Salazar[1], L. Adolf[1], D. Gerlach[2], B. Krismer[1], *S. Heilbronner[2]

[1]*University of Tübingen, Infectionbiology, Tübingen, Germany*

[2]*Ludwig-Maximilians University Munich, Microbiology, Martinsried, Germany*

Introduction: The human microbiome is critically associated with human health and disease. One aspect of this is that antibiotic-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* can reside within the nasal microbiota which increases the risk of infections. Epidemiological studies of the nasal microbiome have revealed positive and negative correlations between non-pathogenic species and *S. aureus*, but the underlying molecular mechanisms remain poorly understood. The nasal cavity is iron-limited and bacteria are known to produce iron-scavenging siderophores to proliferate in such environments. Siderophores are public goods that can be consumed by all members of a bacterial community. Accordingly, siderophores are known to mediate bacterial competition and collaboration but their role in the nasal microbiome is unknown**.**

Goals: We sought to assess the nature und physiological relevance of siderophore-mediated interaction between *S. aureus* nasal commensals.

Methods: We screened 94 nasal bacterial strains from seven genera for their capacity to produce siderophores as well as to consume the siderophores produced by *S. aureus.* We assessed the effects of siderophore provision and piracy on the fitness of *S. aureus* and use cotton rat models of nasal colonization to assess the relevance of siderophores during colonization.

Results: We found that 80% of the strains engaged in siderophore-mediated interactions with *S. aureus* including collaborative and competitive interactions. Non-pathogenic corynebacterial species were found to be prominent consumers of *S. aureus* siderophores. In co-culture experiments, consumption of siderophores by competitors reduced *S. aureus* growth in an iron-dependent fashion. Additionally, we show that siderophore acquisition is crucial for *S. aureus* nasal colonization *in vivo.*

Summary: Our data show a wide network of siderophore mediated interactions between the species of the human nasal microbiome and provide mechanistic evidence for inter-species competition and collaboration impacting pathogen proliferation. This opens avenues for designing nasal probiotics to displace *S. aureus* from the nasal cavity of humans.

P-HAMI-030

Resilience of chicken towards *Salmonella*: using surrogate infection models to define a protective microbiome (ChiSaRe)

*B. Becker[1], A. Berndt[1], M. Weber[1], E. M. Liebler-Tenorio[1], T. M. Fuchs[1]

[1]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Institut für molekulare Pathogenese, Jena, Germany*

In 2021 the European food safety authority (efsa) reported 60,050 cases of Salmonellosis. This makes *S. enterica* the second most prevalent bacterium for zoonotic infections in humans.[1] These infections are often associated with ingestion of chicken (G*allus domesticus*) produce. Besides its ability to infect humans, *S. enterica* is a threat for its avian host as well. E.g. Serovars *S. enterica pullorum and S. enterica galliarum* are the causing agents of diseases, associated with mortality rates approaching 100 % in young chicken.[2]

We currently establish a protective chicken microbiome, that confers resilience *S. enterica*. The first step was the identification of 10 dominant and representative microbes of the gastro-intestinal tract (GIT) of *G. domesticus* from literature that define a synthetic oligo-chicken microbiota (OCM). These candidates belong to six common orders of bacteria, including Lactobacillales, Bacteriodales and Bifidobacteriales. A commercially available, complex microbiota was shown to inhibit the growth of *S. enterica*, and this microbiota is used as a positive control to compare the efficiency of the defined OCM. The OCM is subjected to competitive growth assays (CGA) with different strains and mutants of *S. enterica* to identify promising phenotypes. The OCM will be applied to surrogate infection models, namely larvae of *Galleria mellonella* and organ-on-chip based on cells of the avian GIT. The models are infected with *S. enterica,* to investigate possible protective functions of the OCM. The underlying molecular factors will be characterised using omics approaches with a focus on (meta-)transcriptomics and proteomics especially.

[1]The European Union One Health 2021 Zoonoses Report; European Food Safety Authority, European Centre for Disease Prevention and Control; Report Approved: 11 November 2022; doi: 10.2903/j.efsa.2022.7666

[2]SHIVAPRASAD H.L.. Fowl typhoid and pullorum disease. Scientific & Technical Review. 2000 08 1; 19 (2): pp. 405-424. doi: https://doi.org/10.20506/rst.19.2.1222

P-HAMI-031

Mycobiome analysis of wild rodents in Thuringia

*S. Müller[1], I. D. Jacobsen[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Microbial Immunology, Jena, Germany*
[2]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*

The significance of the gastrointestinal microbiota composition towards the balance between human health or disease has been demonstrated in numerous studies over the last decades. Most research focused on bacteria as the most numerous components of the intestinal microbiota. More recently, however, fungal colonization has been shown to have a specific and profound impact on development and function of the immune system.

As functional studies in humans are often not possible due to ethical and practical constraints, laboratory mice are commonly used to investigate the impact of the microbiota and fungal colonization on the host. However, laboratory mice are usually maintained in a highly standardized environment with strict hygiene regimens. Compared to wild mice, they exhibit less immune activation and lower microbial diversity due to the lack of environmental exposure to a wider range of microbes, including fungi.

In order to identify fungi that can colonize the gut of wild rodents, and to determine the possible impact of host-specific factors and the environment, we analyse over 300 gut samples from different mouse species captured in different habitats in Thuringia. Overall abundance of fungal DNA is determined by qPCR; ITS-sequencing will be used for phylogenetic analysis. This provides not only additional information on the microbiome of wild rodents, but might also identify fungi that colonize a range of host species in different environments, or as specialists are associated with specific host species or habitats.

P-HAMI-032

Oral microbiome composition in adolescent smokers and non-smokers

*P. Schaefer-Dreyer[1,2], W. Behrens[1,2], A. Winkel[1,2], P. C. Pott[2], M. Paulsen[3], F. Tanisik-Damrah[2], A. Melk[3], I. Yang[1,2], M. Stiesch[1,2]
[1]*Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Hannover, Germany, Hannover, Germany*
[2]*Hannover Medical School, Dental prosthetics and biomedical materials science, Hannover, Germany*
[3]*Hannover Medical School, Klinik für Pädiatrische Nieren-, Leber- & Stoffwechselerkrankungen, Hannover, Germany*

Objectives

Smoking is associated with shifts in the composition of the oral microbiome that can contribute to the development and progression of periodontitis in adults [1]. While most smokers start smoking before the age of 18 [2], data on the effects of smoking on the oral microbiome in adolescents is very limited. The current study investigates the effects of smoking on the composition of the oral microbiome in a local cohort of secondary-school pupils.

Materials and Methods

The adolescent cohort of this study comprised 196 secondary-school pupils aged 14 to 21, who were sampled between June and December 2020. We collected a swab of the buccal mucosa as well as information on smoking behavior from every participant. 98 adolescent smokers fulfilled the inclusion criteria. Their oral microbiome composition was compared to that of 98 non-smokers matched for age, gender, BMI and medication intake. The oral microbiome composition was analysed using high-throughput sequencing of the full-length 16S rRNA gene. Analyses of bacterial diversity, abundance comparisons of individual bacterial taxa in smokers and non-smokers, and other statistical analyses were performed.

Results

Smoking pupils were distributed over five categories of smoking frequencies from "less than once/month" to "every day". Thirteen of 98 smokers smoked daily. None of the pupils met the definition for heavy smokers (more than 20 cigarettes per day). Overall microbiota composition as measured by weighted UniFrac distances differed significantly between smokers and non-smokers. Smoking exerted a discernible influence on the abundance of several key microbial families, indicating an early onset in smoking-related changes in the oral microbiome.

Conclusion

This study shows the early onset of smoking-related alterations in the oral microbiome.

References

[1] Shchipkova, A. Y., Nagaraja, H. N., & Kumar, P. S. (2010). Subgingival microbial profiles of smokers with periodontitis. *Journal of dental research*, 89(11), 1247–1253. https://doi.org/10.1177/0022034510377203
[2] DEBRA: https://www.debra-study.info/wp-content/uploads/2022/12/Factsheet-09-v3.pdf

---

P-HAMI-033
Neisseria meningitidis disrupts P-glycoprotein but not BCRP functional activity in induced pluripotent stem cell-derived brain-like endothelial cells

*F. Noratabadi[1], L. Enders[1], B. J. Kim[2], A. Schubert-Unkmeir[1]
[1]*Julius Maximilians University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[2]*University of Alabama, Department of Biological Sciences, Tuscaloosa, AL, United States*

The blood-brain barrier and the meningeal blood-cerebrospinal fluid barrier (mBCSFB) are formed by brain endothelial cells (BECs), protecting the brain from harmful substances. BECs express efflux transporters, P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP), which actively pump endogenous and exogenous substances from the brain to the blood. Despite the crucial role of efflux transporters in the brain, there is still a considerable gap in our understanding of their activity in response to environmental factors such as pathogens.

Neisseria meningitidis (Nm), a commensal bacterium of the respiratory tract, can cause meningitis through systemic spread and transmigration across mBCSFB. Little is known about the impact of *Nm* on efflux transporters in the brain. This study aims to decipher the impact of *Nm* on P-gp and BCRP activity in BECs.

iPSCs were differentiated into BECs as previously described (Stebbins et al., 2016). The impact of *Nm* on P-gp and BCRP was analyzed by RT-qPCR and immunoblotting. P-gp and BCRP activity was assessed by measuring intracellular accumulation of Rhodamine 123 (R123) and Chlorin e6 (Ce6), respectively.

The uptake of R123 and Ce6 by P-gp and BCRP under exposure to their inhibitors, PSC833 and Ko143, respectively confirmed their activity in iBECs. Following *Nm* infection, a notable decrease in P-gp activity was observed despite changes in expression and protein level. In contrast to P-gp, BCRP activity remained unaffected. Our data suggested an essential role for the *Nm* capsule in P-gp dysfunction since non-capsulated *Nm* did not impact the R123 accumulation. Moreover, we observed that live *Nm* is required for P-gp inhibition.

The decline in P-gp activity during *Nm* infection suggests a dynamic response to *Nm* at the level of activity. Consistent with earlier research, unaltered BCRP activity suggests a potential compensatory relationship with P-gp (Lye et al., 2023). Our data identified a crucial role for the *Nm* capsule in P-gp inhibition, presenting a potential therapeutic target for drug delivery. Our observation emphasizes the requirement of live *Nm* for P-gp inhibition, highlighting the host-pathogen interaction.

P-HAMI-034
E. faecalis reduces the pathogen abundance in a C. muridarum mouse model and impacts on fusion of chlamydial inclusions

*S. Graspeuntner[1], N. Loeper[1], C. Scholz[1], L. Semmler[1], N. Sempf[2], I. Laumonnier[2], P. König[2], J. F. Baines[3], J. Rupp[1]
[1]*University of Lübeck, Department of Infectious Diseases and Microbiology, Lübeck, Germany*
[2]*University of Lübeck, Lübeck, Germany*
[3]*Max Planck Institute of Evolutionary Biology, Plön, Germany*

Asymptomatic and hence untreated genital chlamydial infection can cause ascension of the pathogen, leading to severe sequels such as pelvic inflammatory disease and infertility. However, the complex interplay between the infection, the immune response, and particular the role of the vaginal microbiota is not well understood yet. We have shown that in a progesterone-dependent mouse model, genital tract abundance of *C. muridarum* is being reduced in an *E. faecalis*-associated manner. While this goes along with further changes in the urogenital environment (e.g. tissue structure, immune cell abundance), we have experimentally proven that *E. faecalis* is providing partial reduction of the pathogen when modulating the vaginal microbiota of the mouse. We were wondering by which mechanisms *E. faecalis* may provide this protective role in our model and analyzed its impact on *C. muridarum* in cell culture. We identified that transient co-incubation of *E. faecalis* with *C. muridarum*-infected cells delays the fusion of multiple inclusion which form at chlamydial cell entry. This homotypic fusion of multiple inclusion into one inclusion is an important mechanism for *Chlamydia* pathogenicity. While it is yet unknown, how *E. faecalis* impairs this fusion process, it may be central to how E. faecalis reduces chlamydial infection in our mouse model. Our model further underlines that experimental modulation of the vaginal microbiota may become a key in understanding bacteria-bacteria and bacteria-host interactions in prevention of sexually transmitted chlamydial infections.

P-HAMI-035
Neisseria meningitidis leads to an increase in dihydroceramide levels in brain endothelial cells

*A. Weinmann[1], I. Fohmann[1], A. Prell[2], F. Schumacher[2], D. Wigger[2], D. Brenner[3], J. Eilts[4], M. Batliner[1], O. Kurzai[1], B. Kleuser[2], J. Seibel[3], A. Schubert-Unkmeir[1]
[1]*Julius-Maximilians-Universität Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[2]*Free University of Berlin, Institute of Pharmacy, Berlin, Germany*
[3]*Julius-Maximilans-Universität Würzburg, Institute for Organic Chemistry, Würzburg, Germany*
[4]*Julius-Maximilans-Universität Würzburg, Chair of Bio Technology and Bio Physics, Würzburg, Germany*

Introduction

Neisseria meningitidis (*Nm*) is a human-specific pathogen that can interact with and penetrate brain endothelial cells (BECs) of the blood-brain barrier, and cause meningitis. *Nm* can modulate the sphingolipid metabolism in BECs and hijack the sphingolipid balance in the host to promote cellular invasion. We found that dihydroceramide (dhCer) levels are significantly increased during infection of BECs with *Nm* and

therefore aimed to investigate their role in the interaction of *Nm* with BECs.

Methods

BECs (hCMEC/D3s) were infected with *Nm* and transcriptional regulation of enzymes of the *de novo* synthesis pathway was assessed by qRT-PCR. Protein levels of dihydroceramide desaturase (DEGS) of infected hCMEC/D3s were investigated by western blotting. DEGS activity was determined by addition of deuterated dhCer during infection and measurement of deuterated dhCer and Cer with LC-MS/MS. Furthermore, subcellular localization of dhCer production after infection was examined by live-cell confocal microscopy using clickable ω-azido-dihydrosphingosine in combination with wheat germ agglutinin-AF Plus 405 for plasma membrane staining in mApple-Sec61b-C1 transfected hCMEC/D3s to detect ER localization. Biotin/streptavidin-AF 647 staining was used to localize *Nm*.

Results

We found that the increase in dhCer after *Nm* infection is not caused by transcriptional regulation of enzymes of the *de novo* synthesis pathway (including orosomucoid-like protein, serine palmitoyltransferase, 3-ketodihydrosphingosine reductase, ceramide synthase, ceramidase and DEGS) as well as by DEGS protein levels. DEGS enzymatic activity was, however, reduced after 8 h of *Nm* infection. Interestingly, we detected ER localization of clicked sphingolipid species and translocation to the plasma membrane after *Nm* infection.

Summary

Taken together, we showed that *Nm* leads to an increase in dhCer levels in BECs. This increase was not caused by the transcriptional regulation of enzymes of the *de novo* synthesis pathway but is due to reduced DEGS activity. Moreover, clickable ω-azido-dihydrosphingosine can be used to investigate the localization of dhCer within the infected host cells by confocal microscopy.

P-HAMI-036
Systemic control of symbiotic competence through mobile miRNAs

*K. Markmann[1,2,3], D. Tsikou[3], Z. Yan[3], M. Sexauer[1,2], C. Fröschel[1], H. Bhasin[2], E. Roitsch[2], J. Stougaard[3]
[1]*JMU Würzburg, Julius-von-Sachs-Institute, Würzburg, Germany*
[2]*Tübingen University, Tübingen, Germany*
[3]*Aarhus University, Aarhus, Denmark*

Legume plants balance symbiotic interactions with nitrogen-fixing rhizobial bacteria via a systemic feedback system tightly controlling rates of bacterial infection and nodulation events. This host regulatory system, termed autoregulation of nodulation (AON), prevents nutritional misbalances and is key to maintaining the association at a mutualistic state. We identified a riboregulator, micro RNA miR2111, that undergoes shoot-to-root translocation to control infection (1) through specific post-transcriptional regulation of the Kelch-repeat F-box gene *TOO MUCH LOVE* (*TML*), a root-acting repressor of infection and nodulation. Our results reveal miR2111 as a key systemic activator of symbiosis that maintains a susceptible default status in noninfected hosts. Looking beyond symbiotic legumes, we found this regulon to be involved in root system architectural control across plant lineages (2). This suggests that bacterial endosymbiosis recruited functional elements from core developmental processes (2). Excitingly, we identified another miRNA, miRSDI1, as a second mediator of systemic symbiosis control, counteracting the miR2111-*TML* node and balancing shoot control of root symbiosis to optimize host plant benefits (Fröschel et al., unpublished).

(1) Tsikou et al. (2018) Science 362: 233-236
(2) Sexauer et al. (2023) Nature Comm 14:8083

P-HAMI-037
Biofilm development of the prevalent gut bacterium Segatella copri

*S. E. Kurrer[1], R. Fischer[1], D. R. Momo Kenfack[1], M. Basen[1]
[1]*University of Rostock, Institute of Biological Sciences, Microbiology, Rostock, Germany*

Bacteria of the phylum *Bacteroidota* are important representatives of the gut microbiome, where they break down dietary fibers and contribute to a healthy gut [1]. Because they naturally produce organic acids which may be used as commodity chemicals, our objective was to study *Bacteroidota* as platform organisms for bioconversions. Furthermore, *Bacteroidota* have also been described to form biofilms, but studies that do not focus on pathogenic strains are scarce [2]. For us, this would be of interest, since we may utilize them in bioreactors as catalytic biofilms.

Initially, we screened twelve anaerobic *Bacteroidota* strains for their ability to form biofilm *in vitro*. For that purpose, strains were incubated in 24-well plates in PYG medium for 24 – 72 h. Biofilm formation was observed after crystal violet staining by measuring absorption at 540 nm (A540) and protein content and conversion of glucose to organic acids were quantified as well. Of all tested strains, *Segatella copri* (former *Prevotella*) showed the best biofilm formation.

We studied the time course of its biofilm development: After 4 h the protein content started to increase in the biofilm, reaching a maximum after 12 h. Within this time, glucose was completely consumed, and succinate and acetate were produced in amounts similar to batch culture. Interestingly, the A540 still increased between 12 and 16 h, although biomass formation already stagnated, raising the question if the higher A540 results from changes in biomass composition. Additionally, we performed a live-dead staining and monitored CFUs: Here, the first sample showing a biofilm was at 8 h. Up to 36 h the biofilm mainly consisted of living cells, while at later time points the proportion of dead cells increased, and the CFU decreased.

Our results indicate that the most important phase during biofilm development is within the first 12 to 16 h. Currently, we study the influence of different culture conditions on biofilm formation and organic acid production, and also focus on the matrix composition in more detail.

[1] Wexler 2007, *Clin Microbiol Rev*
[2] Béchon and Ghigo 2022, *FEMS Microbiol Rev*

P-HAMI-038
Implementing thick-section spatial transcriptomics to investigate host-pathogen interactions

*T. Beste[1], A. E. Saliba[1,2]

[1]Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany
[2]University of Würzburg, Institut for Molecular Infection Biology (IMIB), Würzburg, Germany

Single-cell transcriptomics has proven to be an extremely powerful tool to investigate host-pathogen interactions heterogeneity. Most popular single-cell genomics techniques require the cells to be dissociated, leading to the loss of the spatial context, which could give more insight into the identity or function of involved cells. Yet, the emergence of spatial transcriptomics methods aims to preserve cell-to-cell interactions and keep the tissue intact. However, current commercially available methods (e.g., Visium) lack the ability to resolve infected cells in whole tissues and therefore are not amenable to detect rare events like in chronic infections where only a few infection foci are present in a whole organ.

Here, we aim to develop thick-section spatial transcriptomics amenable to capture host and pathogen interactions to reveal infection foci in 3D. We have implemented expansion microscopy and single-molecule FISH to identify rare events during infection.

We have achieved successful expansion of tissue sections from the lung, liver, and intestine, achieving a thickness of up to 300 µm. The expansion process is isotropic, with samples expanding by a factor of 3 in the x/y direction and 1.5 in the z direction. Notably, even after undergoing rigorous treatment, the samples retain their original structure post-embedding and expansion.

Leveraging existing single-cell data from mice, we have discerned marker genes associated with specific cell types. Subsequently, we have designed probes to precisely target these marker genes within our samples. Our efforts have yielded promising results, enabling us to identify distinct cell types such as alveolar macrophages and ciliated cells.

Looking ahead, our focus will shift towards extending this methodology to investigate infected tissues. Our initial experiments will concentrate on exploring the dynamics of Staphylococcus aureus infection in the lung.

The identification of single cells inside the tissue can be achieved by training neural networks like Cellpose or Stardist on organ-specific data.

P-HAMI-039

Insights into the culturable community and symbiosis-related traits of bacteria from long-term aquarium and Red Sea tropical octocorals

M. Marques[1], R. Militão[1], E. Santos[2], N. Baylina[2], R. Peixoto[3], *T. Keller-Costa[1], R. Costa[1]
[1]Instituto Superior Técnico, University of Lisbon, Institute for Bioengineering and Biosciences, Department of Bioengineering, Lisbon, Portugal
[2]Oceanário de Lisboa, Lisbon, Portugal
[3]King Abdullah University of Science and Technology, Red Sea Research Center, Thuwal, Saudi Arabia

Corals are under unparalleled pressure due to climate change, with octocorals (*Octocorallia, Cnidaria*) being affected by rising temperatures, pathogens, and anthropogenic disturbances too. Host-microbe interactions play key roles in octocoral health, but our ability to benefit from such interactions to preserve octocorals remains underexplored. This project examines the role of aquarium facilities in octocoral microbiome conservation and aims to develop a "probiotic cocktail" to mitigate climate change-induced stress in octocorals. Three tropical octocoral species, *Litophyton* sp., *Lobophytum* sp. and *Sclerophytum* sp., from a long-term aquarium mesocosm (Oceanário de Lisboa), and two octocoral species, *Litophyton* sp. and *Sclerophytum* sp., from the Red Sea, were sampled. A total of 152 bacterial strains were isolated in our culture-dependent approach with strains grouping into six bacterial classes, 14 orders and 27 genera. Additionally, six unclassified isolates were obtained, likely representing new bacterial families in the *Alteromonadales* and *Cellvibrionales* orders. The collection comprised multiple, "hard-to-cultivate" genera such as *Endozoicomonas*, *Fictibacillus*, and *Flammeovirga*. The *Endozoicomonas* genus has been frequently suggested as a coral health indicator. Based on genus-level diversity, phenotypic screenings for host-beneficial properties of 25 bacterial isolates were performed. Twenty-two isolates presented antioxidant properties, eighteen cellulose- and nine chitin-degradation capabilities, with *Actinobacteria* frequently degrading both polysaccharides. Antimicrobial activity was tested against coral bacterial and fungal pathogens under control (26ºC) and stress (34ºC) temperatures, and 19 isolates displayed antagonism towards pathogens under heat stress conditions. The preservation of core symbionts of corals in captivity highlights the possibility of using sustainable, man-made ecosystems as repositories of stable and healthy coral microbiomes. The observed antimicrobial and enzymatic activities indicate potential beneficial traits among our isolates. Mesocosm experiments are now underway to determine their probiotic effects in octocorals.

P-HAMI-040

Infection of human respiratory tissue models with Staphylococcus aureus and influenza A

*C. Popp[1], H. Oberwinkler[2], N. Pallmann[2], T. Weigel[1], T. Ehret Kasemo[3], A. Scherzad[3], J. Bodem[4], S. Hackenberg[3], M. Steinke[1,3]
[1]Fraunhofer Institute for Silicate Research ISC, Translational Center Regenerative Therapies, Würzburg, Germany
[2]University Hospital of Würzburg, Chair of Tissue Engineering and Regenerative Medicine, Würzburg, Germany
[3]University Hospital of Würzburg, Department of Oto-Rhino-Laryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery, Würzburg, Germany
[4]University of Würzburg, Institute for Virology and Immunobiology, Würzburg, Germany

Human airway tissue models (hATMs) have emerged as valuable tools for studying host-pathogen interactions involving airborne bacteria and viruses. Our human 3D tissue models of the conducting airways reflect the architecture of the conducting airways in humans. These hATMs consist of fibroblast-loaded connective tissue and a respiratory epithelial layer. The tissue models show the mucociliary phenotype and, thus, facilitate the mucus transport that is necessary to remove trapped pathogens.

Until recently, we built the respiratory tissue models on a 3D scaffold derived from decellularized porcine small intestine. In order to replace the associated animal experiments, the objective of this study was to substitute the biological scaffold with a synthetic polyamide 6 (PA6) matrix. Additionally, we evaluated the suitability of these models for investigating viral and bacterial infections.

To address this objective, both scaffold types were seeded with human respiratory fibroblasts and epithelial cells. The morphology of PA6- and aECM-based models was analyzed

using histological methods. Furthermore, we measured the epithelial barrier and conducted RT-qPCR and immunofluorescent staining to study tissue model infection with influenza A virus (IAV). In addition, we investigated the infection of PA6-based hATMs with *S. aureus* with CFU assays and histological methods.

Comparing respiratory tissue models based on the biological and synthetic scaffold, respectively, no obvious differences concerning tissue model morphology, cell type composition, epithelial barrier properties and susceptibility to IAV infection were observed. Furthermore, PA6-based hATMs were successfully infected with S. aureus.

Our data show that PA6 fibers are suitable scaffolds for building human respiratory tissue models and can be used to replace biological scaffolds and associated animal experiments. The advanced features enable us to simulate the dynamics of infections and study the interactions between pathogens and human tissue *in vitro*. With their high *in vitro-in vivo* correlation, our models provide a promising platform for preclinical research and the investigation of infection mechanisms.

P-HAMI-041

Comparative genome analysis of strain Priestia megaterium B1 reveals conserved potential for adaptation to endophytism and plant growth promotion

*F. M. Mahmoud[1], K. Pritsch[2], V. Radl[3], R. Siani[1], S. Benning[1], S. Kublik[1], B. Bunk[4], C. Spröer[4], M. Schloter[1,5]
[1]*Helmholtz Center Munich, Research Unit for Comparative Microbiome Analysis, Neuherberg, Germany*
[2]*Helmholtz Center Munich, Research Unit for Environmental Simulations, Neuherberg, Germany*
[3]*Helmholtz Center Munich, Neuherberg, Germany*
[4]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Brunswick, Germany*
[5]*TUM School of Life Sciences, Chair for Environmental Microbiology, Freising, Germany*

Introduction: *Priestia megaterium* is noted as soil and plant microbe, with antimicrobial (Cui et al., 2023) and plant growth promotion (Bhatt et al., 2020) activities. Genomic analysis of endophytes, along with comparative genomics revealed potential genes linked to endophytism (Pinski et al., 2019). **Goals:** We aimed to assess potential genetic and physiological features employed by *P. megaterium* B1, isolated from apple roots, to adapt to the plant niche and enhance its growth. Also, we intended to explore genetic markers distinguishing plant-derived *P. megaterium* strains from soil ones, to deepen our understanding of possible traits influencing B1's plant association. **Methods:** We extensively analysed strain B1's genome, emphasizing genes tied to endophytism, and plant growth promotion. Validation through physiological tests was performed. Our genomic comparison included pan-genome and sparse partial least square discriminant (sPLS-DA) analyses, based on high quality genomes of 27 plant strains and 31 soil strains, beside strain B1. Functional enrichment of Pfam domains in both groups was done. Genes involved in plant-microbe interaction and plant growth promotion were explored for each strain. **Results:** Genomic analysis of B1 revealed putative genes related to motility and biofilm formation, confirmed by physiological tests. B1 showed genetic and physiological potential to produce indole-3-acetic acid and siderophores, and solubilize phosphate and zinc. The pan-genome analysis of 59 strains uncovered a close pan-genome. Strains from soil and plants displayed comparable genome sizes. SPLS-DA showed discriminative clustering, but functional enrichment analysis did not reveal significant enrichment of Pfam domains in either habitats. Also genetic elements associated with endophytism and plant growth promotion were present in strains from both habitats. **Summary:** B1 possesses physiological and genomic potential to adapt to plant niche and promote its growth. Comparative genomic analysis implied a conserved genetic structure for potential endophytism among strains from both habitats, suggesting a possible transition between free-living and host-associated lifestyles.

P-HAMI-043

Discrimination between MSSA and MRSA strains in neutrophils and monocytes by Raman spectroscopy

*A. Pistiki[1], E. Ibukun Osadare[1], S. Monecke[1], A. Ramoji[1], O. Ryabchykov[1], T. W. Bocklitz[1], P. Rösch[1], R. Ehricht[1], J. Popp[1]
[1]*Leibniz-IPHT, Jena, Germany*

Introduction: Current microbiological methods can only partly cover the requirements in speed and precision that are needed for timely and effective treatment of patients with infections. Improvement of infectious disease diagnostics is needed using fast, precise, inexpensive and culture-independent technologies. Raman spectroscopy is a fast, label free analytical technique that can also provide information on the biochemical composition of immune cells [1, 2].

Goal: We investigated whether Raman spectroscopy enables the differentiation of blood phagocytes that have engulfed methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococcus aureus* strains. To limit genome-related variabilities, an isogenic strain pair was used.

Materials & Methods: Blood phagocytes were isolated from peripheral blood of 8 healthy donors and were incubated in the presence/absence of MRSA and MSSA at multiplicity of infection (MOI) 3. Neutrophils were incubated for 1 and 3 h and monocytes for 8 and 24 h. Raman imaging was applied onto 15 cells/treatment and data were analysed using Partial Least-Squares Discriminant Analysis **(**PLS-DA).

Results: Staphylococcus infected neutrophils could be discriminated form their naïve controls with 80% balanced accuracy (BA) after 1 h and 83.3% after 3 h, whereas monocytes with BA of 66.7% after 8 h that increased to 80% after 24 h. MRSA and MSSA infected neutrophils could not be discriminated in both time points (50% BA). In monocytes however, the BA of 50% obtained after 8h increased to 70% after 24 h.

Summary: The results of this first, prove-of-concept study show that Raman spectroscopy enables the discrimination of *ex-vivo* MRSA and MSSA phagocytosis by blood monocytes. This first step points towards a direction that is worth considering for further investigation and development, leading to better management of infectious diseases.

Financial support of the MCSA-COFUND Multiply Project (H2020 GA 713694) and the research campus InfectoGnostics (FKZ 13GW0096F) is gratefully acknowledged.

References

[1] Pistiki et al., *Int. J. Mol. Sci.* **2021**, *22*(19), 10481
[2] Ramoji A, et.al. Crit Care Explor. 2021;3(5): e0394-e

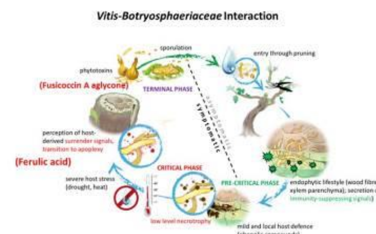
P-HAMI-044
Grapevine Trunk Diseases: a case of hijacked chemical communication between fungal endophytes and plants and the potential of soil microbiome on promoting plant defence against trunk diseases
*I. Khattb[1], P. Nick[1], A. K. Kaster[1]
[1]*Karlsruhe Institute of Technology, Joseph Gottlieb Kölreuter Institute for Plant Sciences, Karlsruhe, Germany*

With the ongoing climate change, grapevine trunk diseases became a threatening challenge for viticulture worldwide. European wild grapevines from the last viable population in Germany along the Rhine river showed variable degrees of resistance against *Neofusicoccum parvum* Bt-67, a fungus causing Botryosphaeria dieback. Resistant genotypes showed robust production of Non-glycosylated stilbenes, viniferin trimers. By contrast, the susceptible genotypes accumulated less stilbenes with a significantly higher proportion of glycosylated piceid (**Khattab**, et al., *New Phytologist,* 2021).

In addition, the disease outbreak under dry hot summers seems to be controlled by chemical communication between the host and colonizing fungus. We introduce the new concept of a "plant surrender signal" accumulating in host plants under stress and facilitate the fungal transition from latent phase to aggressive necrotrophic behaviour causing apoplectic breakdown in grapevines. Using a cell-based experimental system (Vitis cells) and bioactivity-guided fractionation, trans-ferulic acid, a monolignol precursor, was identified as a "surrender signal". This signal specifically activates the secretion of the fungal phytotoxin fusicoccin A aglycone, which targets 14-3-3 proteins, manipulating molecular players that regulate programmed cell death, e.g. ROS, actin filaments and metacaspases. Therefore, the channelling of phenylpropanoid pathway from ferulic acid to the trans-resveratrol phytoalexin could be a target for future therapy (**Khattab**, et al., *Plant Cell and Environment,* 2022). Interestingly, in the absence of ferulic acid, the fungus secretes 4- hydroxyphenylacetic acid, mimicking auxins on grapevine defence and facilitating fungal spread (Flubacher et al., *Plant Cell and Environment,* 2023).

As an immediate strategy, we target the soil microbiota that promote viticulture resilience against trunk diseases. Using Shotgun metagenomics as well as molecular and cellular aspects, we identified that enriching the soil with compost and biochar significantly promoted the soil microbiome diversity, and function and it limited the fungal spread in the infected vines.

Fig. 1



Host-Pathogen Interactions and Clinics of Zoonotic Infections

P-HPIZ-001
The impact of malaria on lipids and glucocorticoids in children under ten years at the Korle-Bu Teaching Hospita
*B. Armah[1]
[1]*St. Adelaide International School, Biochemistry, Accra, Ghana*

Background: Malaria is a common and life-threatening disease in Ghana. Malaria infection has been implicated in lipid and glucocorticoid imbalances among children. Cortisol-induced stresses and parasitaemia, may affect the brain development and risk of cardiovascular disorders among children.

Aim: To investigate the impact malaria has on lipids and glucocorticoids.

Method: A comparative cross-sectional study using random sampling method between February and May, 2019 was used. A sample size of 77 participants comprising 46 cases and 31 controls were involved in the study. Thick and thin blood smears were made for each participant, stained with Giemsa and examined under microscope. Plasma total cholesterol, triglycerides, HDL and LDL were estimated using a chemistry analyzer. Cortisol levels of participants were measured by Enzyme-linked immunosorbent assay.

Result: *Plasmodium falciparum* was responsible for all identified cases of malaria infection in this study. Ages 1-5years (n=11) had a prevalence of 23.9% whiles 6-9years (n=35) had a prevalence of 76.1%. Children aged 6-9 years were more likely to get malaria than those in the 1-5 years group (OR=1.966, $p<0.001$). HDL-Chol associated negatively with level of parasitaemia (rho=-0.538, $p<0.0001$). Triglycerides correlated weakly but positively with malaria count (rho=0.296, $p<0.05$). No association were observed for LDL-Chol, VLDL-Chol and Total-Chol versus malaria count ($p >0.05$). Cortisol was not associated with level of parasitaemia in this study ($p>0.05$).

Conclusion: This study showed no association between cortisol and malaria in affected patients.

HDL-Chol impacted negatively with level of parasitaemia. The implications of malaria on glucocorticoids however merit further research.

P-HPIZ-002

Identifying novel virulence factors in canine infective endocarditis caused by Streptococcus canis using transposon directed insertion sequencing

*M. Katsburg[1], A. Kopenhagen[2], E. Aubry[1], S. Bergmann[2], M. Fulde[1]
[1]*Free University of Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*
[2]*Technical University of Braunschweig, Institute of Microbiology, Brunswick, Germany*

Question

Infective endocarditis (IE) is a bacterial infection of the endocardium. *Streptococcus canis* (SC) was found to be involved in almost a quarter of canine IE cases. SC colonizes the skin and mucosae of asymptomatic cats and dogs, and is associated with both superficial and severe infections. Cases of SC causing severe infection or IE in humans have been described after bite or scratch injuries from dogs or cats. The overall prognosis of dogs with IE is poor. The goals of the study are identification and verification of novel virulence factors in SC. This will give new options for vaccine development or treatment of infective endocarditis by for example antibody-antibiotic conjugates where it is essential to have a known target.

Methods

In this study, we use a random transposon mutant library (TraDIS) to discover virulence factors in the adhesion and invasion of SC to endothelial cells. This input library was made by transposon directed insertion-site sequencing, starting from a clinical endocarditis SC strain. After infection of HUVEC cells in vitro, the mutants that did not adhere or invade were sequenced, and this output pool was compared to the input library. This infection was done both statically and under flow conditions to mimic the behaviour of endothelial cells on different locations of the heart valve. By comparing output and input pool, potential virulence factors could be identified. As a selection assay for biofilm formation, a biofilm will be formed on a fibrin matrix under flow conditions to create a pool with mutants that are unable or able to participate in this formation. After sequence analysis we will confirm these factors by mutagenesis.

Results

Genes that are relevant for the pathogenesis of SC in IE could be identified using the TraDIS method. Within these potential virulence factors, some have homologues in other streptococcal species, but for many others the function is unknown. This method tells us which of these are worth investigating.

Conclusions

TraDIS is a great sequencing technique for screening the whole SC genome on a specific function, like in this case infection of endothelial cells and biofilm formation.

P-HPIZ-003

Streptococcus canis interacts with smooth muscle cells in a 3D-co-cultivation system with endothelial cells under microfluidic conditions

*D. Simmert[1], A. Kopenhagen[1], M. Müsken[2], M. Steinert[1], S. Bergmann[1]
[1]*Technical University of Braunschweig, Institute for Microbiology, Brunswick, Germany*
[2]*Helmholtz Center for Infection Research, Zentrale Einheit für Mikroskopie, Brunswick, Germany*

Introduction: The opportunistic zoonotic bacterium *Streptococcus canis* (*S. canis*) is known as a bacterial pathogen of companion animals and of domesticated live stock. The range of infection severity extends from minor urinary tract infections to systemic diseases such as necrotizing fasciitis, and even sepsis. Consequently, *S. canis* can enter the blood circulation and adheres to the blood vessel endothelium. The inner lining of the vasculature is formed by endothelial cells (EC) embedded in a collagen-rich extracellular matrix supported by smooth muscle cells (SMC).

Goals: So far, the pathophysiology of the transcellular interaction between *S. canis* and each single component forming the blood vessel wall remains elusive. We aim to decipher bacterial and cellular factors mediating *S. canis* adherence, cell internalization and transmigration of the complex blood vessel wall throughout systemic bloodstream infection.

Materials & Methods: Cell culture-based reconstruction of vessel wall was achieved by co-cultivation of human primary EC and SMC within a 3D-collagen matrix. To mimick the physiological bloodstream, the co-cultivation was introduced to a microfluidic system. After optimization of cell-type-specific immunofluorescent staining procedure, bacterial infection with *S. canis* was conducted. Electron microscopic (EM) imaging visualized morphological response to bacterial infection.

Results: A 3D-co-cultivation system based on EC and SMC embedded within a collagen matrix was successfully established in a microfluidic system. Microscopic and EM visualization showed that *S. canis* adheres to EC and is also invasive. Furthermore, *S. canis* traverses even under high shear stress conditions the EC layer and the collagen matrix and enters deeper layers of the co-cultivation system, thereby attaching to underlaying SMC.

Summary: The established 3D-co-cultivation system provides a suitable infection model for the elucidation of pathomechanisms determining bacterial bloodstream infections.

P-HPIZ-004

Investigating the link between the Stx2 phage carriage dependent SOS response and the metabolism of Escherichia coli

*I. Hastor[1], M. Berger[1], U. Dobrindt[1], A. Mellmann[1], P. Berger[1]
[1]*University Hosptial Münster, Institute of Hygiene, Münster, Germany*

Introduction

Shiga toxin (Stx) 2, which is encoded on a phage, is the main virulence factor of the 2011 enterohemorrhagic *Escherichia coli* O104:H4 outbreak strain. Stx2-encoding phages can infect and lysogenize other bacterial strains. Our former investigation showed a drastic decrease in the metabolic potential and upregulated SOS response genes due to the presence of ϕO104 in *E. coli* K-12 MG1655. In contrast, we observed no drastic decrease in the respiration potential

after transducing ϕO104 Δ*stx2* in the *E.coli* O104:H4 strains C227-11ϕcu and 55989.

Goals

The goal is to elucidate whether the degree of metabolic alterations in strains carrying ϕO104 is linked to differences in their SOS response.

Materials & Methods

E. coli strains MG1655, C227-11ϕcu and 55989 were lysogenized with ϕO104 Δ*stx2* from the *E. coli* O104:H4 isolate LB226692 Δ*stx2*. Bacterial strains were transformed with the SOS reporter plasmid pMBM25 carrying a *recA*P-*cfp* fusion. A Gam-expressing plasmid was conjugated in MG1655::ϕO104 Δ*stx2* to block the SOS response. CFP production and bacterial growth kinetics were analyzed during growth in LB medium for 24 hours at 37 °C.

Results

We analyzed the effects of ϕO104 Δ*stx2* carriage in *E. coli* strains MG1655, C227-11ϕcu and 55989 on the SOS response and bacterial growth kinetics. Interestingly, we observed an induction of the SOS response during exponential growth phase followed by a drop in the optical density (OD) at the onset of stationary phase in MG1655::ϕO104 Δ*stx2* but not in *E. coli* O104:H4 backgrounds (C227-11ϕcu and 55989). We did not detected a drop in the OD in MG1655::ϕO104 Δ*stx2* carrying the Gam-expressing plasmid, confirming that the observed changes in growth kinetics of this Stx2 lysogen were indeed SOS response-dependent.

Summary

Stx2 phage carriage leads to a strong induction of the SOS response in *E. coli* K-12 MG1655 but not in the analyzed *E. coli* O104:H4 backgrounds. Ongoing BIOLOG experiments will assess the changes in metabolism of strain MG1655::ϕO104 Δ*stx2* upon blocking its SOS response and will clarify, if there is a direct correlation between the elevated SOS response and the drastic decrease in metabolic respiration in *E. coli* MG1655.

P-HPIZ-006

Sublethal systemic LPS exposure promotes intestinal opportunistic pathogen blooms

*S. Kroon[1], D. Malcic[1], L. Weidert[2], L. Bircher[2], L. Boldt[3], P. Christen[1], P. Kiefer[1], A. Sintsova[1], B. Nguyen[1], M. Barthel[1], Y. Steiger[1], M. Clerc[1], M. K. Herzog[1], C. Chen[4], B. Guery[4], E. Slack[2], S. Sunagawa[1], J. A. Vorholt[1], L. Maier[3], C. Lacroix[2], A. Hausmann[1,5], W. D. Hardt[1]
[1]*Institute of Microbiology, ETH Zürich, Zürich, Switzerland*
[2]*Institute of Food Science and Nutrition, ETH Zürich, Zürich, Switzerland*
[3]*University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*
[4]*Infectious Diseases Service, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland*
[5]*reNEW - Novo Nordisk Foundation Center for Stem Cell Medicine, University of Copenhagen, Copenhagen, Denmark*

The cause of opportunistic pathogen infections in patients with systemic immune activation often remains unresolved. The intestinal microbiota serves as a gut-luminal reservoir for opportunistic pathogens, from where they can translocate to systemic sites. Systemic immune activation is associated with perturbations in the intestinal microbiota and increased risk of systemic infection with opportunistic pathogens. However, the causal link between these phenomena remains unclear. To address this, we employ a mouse model of sublethal systemic lipopolysaccharide (LPS) exposure, quantify intestinal growth of clinically relevant opportunistic pathogens, and use a related gut pathogen, *Salmonella* Typhimurium, to dissect the underlying mechanisms. We show that systemic LPS exposure opens an intestinal niche for *Enterococcus*, *Klebsiella*, *Escherichia coli* and *Salmonella* blooms. Knockout mice, microbiota growth assays, transcriptomics, metabolomics, and oxygen species measurements indicate that systemic immune activation elevates intestinal oxygen species levels in a Toll-like receptor 4 (TLR4)-dependent manner. This acutely halts microbiota fermentation and enables oxidative respiration by facultative anaerobic bacteria both in mouse and human microbiota, fuelling luminal opportunistic pathogen blooms and increasing the likelihood of systemic spread. LPS-triggered release of oxygen species, its growth-halting effect on the microbiota and the associated blooms of opportunistic pathogens provide a mechanism underlying the onset of systemic infections in sepsis patients.

P-HPIZ-007

Unveiling the mechanisms of NK cell defense against fungal infections: exploring the crucial role of conserved surface proteins in the NK cell-fungus interaction

*F. Natasha[1], L. Heilig[2], J. Springer[2], M. Sauer[1], J. Löffler[2], U. Terpitz[1]
[1]*University Würzburg, Biotechnology and Biophysics, Würzburg, Germany*
[2]*University Hospital, Internal Medicine II, Würzburg, Germany*

Every year, a total of around 2 million people worldwide develop Invasive pulmonary aspergillosis (IPA), with a crude annual mortality rate of 85.2% as published in 2024 (D. Denning)[1]. *Aspergillus fumigatus*, the causative agent itself usually stays in its air-borne, spore-forming form and transforms into a life-threatening pathogen, particularly in immuno-compromised patients. On the other hand, natural killer (NK) cells serve as the initial line of defense in the human body. The function of NK cells against *A. fumigatus* in the human body remains a puzzle. We have previously reported that NK cells establish a stable immunological synapse with *A. fumigatus*, and CD56 is a pathogen recognition receptor on human NK cells[2]. CD226 (DNAM-1), a cell-surface glycoprotein, plays vital roles in several diseases ranging from autoimmune diseases to cancer to viral infections. Additionally, it has been published that CD226 serves as an activating receptor for NK cells and is involved in the immunological synapse (IS) formation. However, it is not clear yet if this is only true for NK-cell-cancer-cell interaction or also in the NK-cell-fungus interaction. In our research, we are working to unlock the mechanism by visualizing the interface of CD226- *A. fumigatus* interaction. Our findings have already indicated that, unlike CD56, CD226 does not significantly relocate at the interaction site. Furthermore, our results suggest that the interaction between CD226 and *A. fumigatus* occurs through a direct mechanism. A CRISPR-Cas-engineered knock-out CD226 NK cells is assisting us to envisage the significance of CD226 in fungal infections.

References:

1. Denning, W. D., Global incidence and mortality of severe fungal disease. Lancet Infect Dis, (2024).

https://doi.org/10.1016/S1473-3099(23)00692-8
2. Ziegler, S., Weiss, E., Schmitt, AL. et al. CD56 Is a Pathogen Recognition Receptor on Human Natural Killer Cells. Sci Rep 7, 6138 (2017). https://doi.org/10.1038/s41598-017-06238-4

P-HPIZ-008

Sec pathway-dependent bacterial signal peptides are a major source for formyl peptide receptor 1 activation in mice and humans

C. Intzen[1,2,3], H. Heilmann[3], M. Abdrabou[1,3], F. Zufall[2], *M. Bischoff[1], B. Bufe[3]
[1]*Saarland University, Center for Integrative Physiology and Molecular Medicine, Homburg, Germany*
[2]*Kaiserslautern University of Applied Sciences, Faculty of Computer Sciences and Microsystems Technology, Zweibrücken, Germany*
[3]*Saarland University, Institute for Medical Microbiology and Hygiene, Homburg, Germany*

Recruitment of immune cells to the site of infection is a major component of the innate immune response. Formyl peptide receptors (FPRs) promote this process by recognizing N-terminally formylated peptides that act as chemotactic stimuli on neutrophils. Formyl peptides are very characteristic for bacterial infections, because N-terminally formylated methionine residues are required in bacterial protein biosynthesis to initiate translation, whereas the majority of eukaryotic peptides are, with the exception of mitochondrial proteins, unformylated. Recent work suggests that bacterial signal peptides, which serve as signal sequences to translocate proteins across cellular membranes, may be a major source of formyl peptides.

Here we report that batch-cultured *Escherichia coli* BL21 (DE3) cells secrete ligands into the growth medium, which activate the FPR1 homologs of humans (hFPR1) and mice (mFpr1) in HEK293T transfected reporter systems, respectively. Notably, no relevant activation of these two FPRs was observed when the FPR1-expressing reporter cells were challenged with *E. coli* cell lysates. Activation of the FPR1 homologs by cell culture supernatants was growth phase-dependent and strongest with supernatants harvested from exponential growth phase *E. coli* cultures. Size exclusion filtration and protease treatment of the *E. coli* culture supernatants revealed that the FPR1-activating ligands are of proteinaceous nature and smaller than 3 kDa in size. Treatment of the *E. coli* cultures with the general secretory (Sec) pathway inhibitor erythrosine B almost completely abolished the secretion of the FPR1-activating ligands into the extracellular milieu, while the Sec ATPase inhibitor only marginally affected the viability of *E. coli* and HEK293T cell cultures, respectively.

Our results indicate that the release of formyl peptides from *E. coli* occurs post-translationally through the Sec pathway, supporting the hypothesis that signal peptides are a major source of bacterial N-formylated peptides.

P-HPIZ-009

Characterization of hypoxia-induced dormancy in Coxiella burnetii

*F. Asghar[1], I. Hayek[1], A. Lührmann[1]
[1]*University Hospital Erlangen, Microbiology Institute - Clinical Microbiology, Immunology and Hygiene, Erlangen, Germany*

Coxiella burnetii is an obligate intracellular bacterium that causes Q fever in both humans and domestic ruminants. Humans get infected by inhalation of contaminated aerosols from infected ruminants. Almost 95% of cases are either asymptomatic or present as mild flu-like illness, pneumonia or hepatitis. However, 3-5% cases can develop chronic Q fever months or years after primary infection. The clinical picture of chronic Q fever suggests that *C. burnetii* establishes a persistent state. Yet, information about the induction of persistence is rare. STAT3 is important for host immunity and controls the expression of citrate transporter and citrate synthase. Under hypoxic conditions (0.5% O2), stabilization of HIF1a impairs the STAT3 activity, resulting in reduction of the TCA cycle intermediate citrate. Citrate limitation results in inhibition of *C. burnetii* replication without interfering with the viability of the pathogen. Here, we aim to characterize hypoxia-induced persistence of *C. burnetii,* to clarify whether *C. burnetii* might undergo stringent response or enters the metabolically inactive small cell-variant form (SCV) to survive this environmental stress condition. Thus, we infected primary murine macrophages with *C. burnetii* under normoxic (21% O2) and hypoxic (0.5% O2) conditions and analysed the expression of stringent response and SCV genes. Our data suggests that *C. burnetii* does not undergo stringent response, but instead enters the SCV as non-replicating persistent form. Further research is required to validate this assumption. For this bacterial morphology, bacterial ability to invade new target cells and to withstand adverse conditions, e.g. antibiotic treatment will be analysed.

P-HPIZ-010

The analysis of 'Candidatus Phytoplasma fraxini' illustrates the small core genome of phytoplasmas

*J. W. Böhm[1], B. Hüttel[2], B. Schneider[3], M. Kube[1]
[1]*University of Hohenheim, Integrative Infection Biology Crops-Livestock, Stuttgart, Germany*
[2]*Max Planck Genome-centre Cologne, Max Planck Institute for Plant Breeding, Köln, Germany*
[3]*Julius Kühn-Institut, Federal Research Centre for Cultivated Plants,, Dossenheim, Germany*

The taxon '*Candidatus* Phytoplasma' (Mycoplasmatota) is characterised by intracellular bacteria that colonise the plant phloem and are transmitted by sap-sucking insect vectors. Infection of these nutrient-rich environments is reflected in reduced genome sizes <1 Mb, but they are blown up by mobile elements. The obligate parasitic lifestyle suggests even smaller genomes. However, some phylogenetic branches have not yet been covered by complete genome sequences, raising the question whether the conserved gene core may be even smaller than expected or, as recently shown [1], encoding additional metabolic functions.

We analysed the complete genome of '*Candidatus* Phytoplasma fraxini', the first member of the ash yellows group, and performed a pangenome analysis.

The genome was determined by SMRT sequencing and assembled by Canu [2]. Consistency in annotation was ensured by re-annotating all genomes using RAST [3]. Orthologs were determined and used as previously described [1].

Themodel organisms such as genome of '*Ca*. P. fraxini' is 598 kb in size, encoding 552 CDS. It lacks lactate metabolism, separating it from the closely related elm yellows group but shares a gene set of 242 genes limited to the basic functions of replication, protein biosynthesis, membrane transport, lipid and carbohydrate metabolism. Effector proteins involved in disease pathogenesis are not

part of the shared genome. The phytoplasma gene set is not covered by *Mycoplasma genitalium* and *Bacillus subtilis*.

The analysis supports the additional evolutionary pathway of phytoplasmas, characterised by simplified parasitism and adapted pathogen-host interactions.

Böhm, J.W.; Duckeck, D.; …. Kube, M. Genome Comparison of "*Candidatus* Phytoplasma rubi" with Genomes of Other 16SrV Phytoplasmas Highlights Special Group Features. Applied Microbiology 2023, 3, 1083–1100.

Koren, S.; Walenz, B.P.; ... Phillippy A.M. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Research 2017.

Aziz, R.K.; Bartels, D; … Disz, T.; Edwards, R.A.; Formsma, K.; et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008, 9, 75.

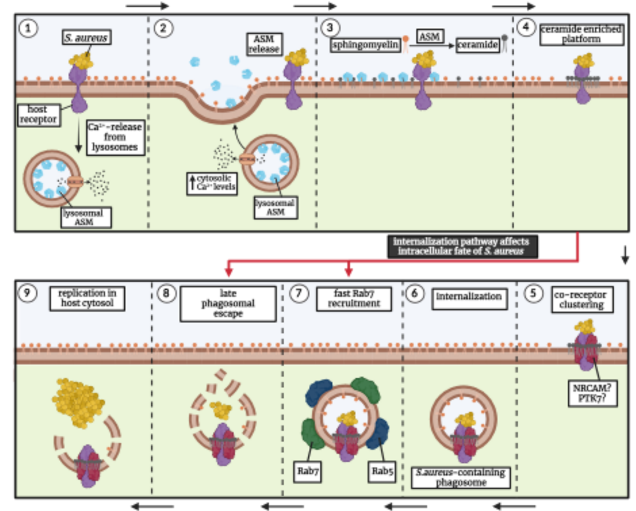
P-HPIZ-011

Decision at the Plasma Membrane: Pathway of Invasion Affects Intracellular Fate of Staphylococcus aureus

*M. Rühling[1], F. Schmelz[1], K. Ulbrich[1], N. Knoch[1], C. Kappe[2], A. Iwanowitsch[1], K. Paprotka[1], A. Kempf[1], C. Arenz[2], M. Fraunholz[1]
[1]*University of Würzburg, Chair of Microbiology, Würzburg, Germany*
[2]*Humboldt University of Berlin, Institute of Chemistry, Berlin, Germany*

Staphylococcus aureus is a Gram-positive opportunistic human pathogen that can cause severe infection such as endocarditis, sepsis, and toxic shock syndrome. Due to rising antibiotic resistance, as seen for example in Methicillin-resistant *S. aureus* stains (MRSA), treatment of infection becomes more and more challenging, making *S. aureus* to one of the most important pathogens in healthcare. In the late 1990s, it was discovered that *S. aureus* can reside within non-professional phagocytic cells (NPPC), such as endothelial and epithelial cells, a feature that often was associated with antibiotic resistance as well as chronic infection. Thus, we try to understand how *S. aureus* is able to enter its host cells and which strategies are used by the pathogen to replicate within the intracellular niche and moreover, how these processes could be targeted for treating *S. aureus* infection.Here, we provide evidence that the uptake of the MRSA strain JE2 by host cells requires liberation of Ca2+ from the endolysosomal compartment and activity of acid sphingomyelinase (ASM), a lysosomal enzyme involved in the degradation of the sphingolipid sphingomyelin. ASM likely remodels plasma membrane during pathogen internalization resulting in a rapid uptake, whereas other invasion pathways that are ASM-independent possess slower kinetics. Furthermore, we established an APEX2-based proximity labeling approach that enables identification of host molecules that interact with *S. aureus*. Thereby, we found novel receptor candidates that likely are enrolled in the ASM-dependent internalization pathway.In NPPCs, *S. aureus* lyses the phagosomal membrane and invades the cytosol of host cell, a process that we call phagosomal escape. We found that phagosomal escape rates of bacteria entered their host cell via the ASM-dependent pathway are lower than for bacteria that used other internalization pathways, suggesting that the outcome of an intracellular *S. aureus* infection is at least partially decided at the host"s plasma membrane. Moreover, we found that pharmacological inhibition of ASM protected host cells from infection, suggesting ASM as a promising target for treatment of *S. aureus* infection.

Fig. 1



P-HPIZ-012

rpoS deletion profoundly alters the carbon source utilization and fitness of E. coli O104:H4

*P. Berger[1], D. Loewe[1], K. Bosse-Plois[1], M. Berger[1], U. Dobrindt[1], A. Mellmann[1]
[1]*University of Münster, Institute of Hygiene, Münster, Germany*

Introduction

Escherichia coli (*E. coli*) O104:H4 caused in 2011 in Germany the enterohemorrhagic *E. coli* (EHEC) outbreak with the highest incidence rate of hemolytic uremic syndrome worldwide. We recently described an *E. coli* O104:H4 isolate that had acquired a single nucleotide polymorphism in the start codon (ATG>ATA) of *rpoS,* coding for the alternative RNA polymerase sigma factor S (RpoS), which resulted in enhanced virulence gene expression and drastically altered metabolism.

Goal

The goal of this work was to monitor the effect of *rpoS* deletion (Δ*rpoS*) on *E. coli* O104:H4 carbon source utilization and fitness.

Material and Methods

The *rpoS* gene was deleted by homologous recombineering in *E. coli* O104:H4 Δ*stx2*. The effect of Δ*rpoS* on carbon source utilization was analysed by phenotype microarrays using the BIOLOG PM1 plate. *E. coli* O104:H4 Δ*stx2* and Δ*stx2* Δ*rpoS* were co-cultured for 24 hours in minimal M9 medium supplemented with 0.2% single carbon source and the bacterial cell numbers were determined by counting colony forming units.

Results

Biolog PM1 phenotype microarrays revealed a strong correlation between the effect of *rpoS* ATG>ATA and Δ*rpoS* on *E. coli* O104:H4 carbon source utilization. Namely, the

strains carrying an intact *rpoS* allele assimilated more efficiently sugars, e.g. arabinose, galactose and fucose, whereas both *rpoS ATG>ATA* allele and Δ*rpoS* resulted in stronger metabolic activity with amino acids, carboxylic and dicarboxylic acids (TCA cycle substrates). Interestingly, competition experiments with *E. coli* O104:H4 Δ*stx2* and Δ*stx2* Δ*rpoS* in M9 supplemented with single carbon sources revealed a dramatic competitive advantage of the Δ*rpoS* strain with the tested amino acids, i.e. L-alanine, L-glutamine and L-aspartic acid, however, no and only moderate advantage of the *rpoS* wild type strain with galactose and arabinose, respectively.

Summary

We could show that *rpoS* deletion profoundly alters the carbon source utilization and fitness of *E. coli* O104:H4. Thus, this work provides additional evidence that RpoS acts as a global regulator of *E. coli* O104:H4 metabolism and further emphasizes its importance in EHEC colonization and virulence.

P-HPIZ-013

Impaired functionality of alveolar type 2 cells in TERC ko/ko mice results in severe influenza A virus infection

*A. Häder[1], Y. Reißer[1], F. Hornung[1], B. Löffler[1], S. Deinhardt-Emmer[1]
[1]*University Hospital Jena, Medical Microbiology, Jena, Germany*

INTRODUCTION: Aging is correlated with increased mortality during influenza A virus pneumonia. A model to investigate the impact of aging is the use of TERC ko/ko mice, characterized by accelerated telomere shortening and telomerase independent impact on cell cycle and inflammation. Within lung tissue, alveolar type 2 (AT2) cells serve as progenitor cells, crucial for maintaining lung homeostasis. Aged AT2 cells display indications of cellular senescence and heightened inflammatory signaling. Therefore, these cells are of special interest for analyzing the pathomechanisms during influenza virus infection.

GOALS: Investigation of the impact of telomerase dysfunction and the telomerase independent role of TERC on the immune response and disease manifestation during respiratory virus infection.

MATERIALS & METHODS: TERC-ko/ko and WT mice were intranasal infected with influenza A virus for 2 and 21 days. Viral load was quantified via plaque assay, cytokine secretion was measured by using flow cytometry and surfactant A protein was determined via ELISA. To further determine the infection event, a murine *ex vivo* infection model of lung slices was used. mRNA-sequencing of infected AT2 cells from TERC ko/ko mice was performed.

RESULTS: We observed a severe disease progression in TERC ko/ko mice during the first 10 days of influenza A virus infection. Throughout the acute phase, TERC ko/ko mice showed lower viral load in the lungs with a significantly higher pro-inflammatory cytokine response and surfactant protein A production. These results were confirmed using a murine *ex vivo* lung model. To further analyze the infection process, global transcriptome response of infected primary AT2 cells from TERC ko/ko and WT mice was performed. We identified a dysregulation in the inflammatory host response and cell cycle, as evidenced by the PI3/Akt pathway.

SUMMARY: TERC ko/ko mice exhibited an increased susceptibility to influenza A virus infection. Specifically, evidence from AT2 cells suggests that the knockout of TERC contributes to restricted proliferation, elevated inflammatory response, and impaired surfactant function in the lung.

P-HPIZ-014

Quantification of Mycoplasma hyopneumoniae in cell culture systems and host tissues by a new duplex qPCR-based method.

N. Mierswa[1], R. Spriewald[1], D. Höltig[2], *J. Meens[1]
[1]*University of Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany*
[2]*University of Veterinary Medicine Hannover, Clinic for small cloven-hoofed animals and forensic medicine and outpatient clinic, Hannover, Germany*

Introduction

Mycoplasma hyopneumoniae is the causative agent of Enzootic pneumonia (EP) in pigs, a highly prevalent, chronic respiratory disease, which causes considerable economic losses in the swine industry. The infection leads to damage of the ciliated swine respiratory epithelium, either by direct cytotoxic processes or indirectly, by the induction of a strong, damaging inflammatory response. Despite the medical and economical importance of EP, most pathomechanisms are not yet fully understood.

We describe here a duplex qPCR-based method to quantify the number of *Mycoplasma hyopneumoniae* cells interacting with the host, normalized to the number of host cells. This method allows a better comparison of pathogen burden in 3D organotypic tissue models, like tissue explants or Precision-cut lung slices (PCLS), with samples obtained from in-vivo infections.

Objective

Establishing a duplex qPCR to quantify *M.°hyopneumoniae* cells, normalized to the number of porcine host cells.

Materials & methods

Lungs were obtained from healthy slaughtered pigs. Accessory and cranial lobes were used for the preparation of PCLS. Slices were infected with freshly grown *M.°hyopneumoniae* for 4 h or 24 h. Tissue samples from experimentally infected pigs were obtained during in-house animal experiments. DNA was isolated from PCLS and tissue samples by standard methods. PCR methods were developed on Biometra Tone and Stratagene™ Mx3005P cyclers.

Results

We compared different porcine target genes to determine host cell numbers. Finally, we have chosen the porcine Carcinoembryonic antigen-related cell adhesion molecule 18 (CEACAM18) gene and combined it with the already established p102 gene from *M.°hyopneumoniae* in a duplex TaqMan-based assay system. Absolute copy numbers of the genes were calculated according to plasmid standards containing the cloned target sequences.

Conclusion

The new duplex qPCR is well suited quantify the pathogen burden normalized to host cell numbers. It allows a better comparison of 3D infection models with in-vivo samples.

P-HPIZ-015
Population dynamics reveal bottlenecks during neonatal infections with non-typhoidal Salmonella
*C. Lemke[1], K. Tedin[1], M. Hensel[2], M. Hornef[3], M. Fulde[1], K. van Vorst[1]
[1]*Free University Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*
[2]*University Osnabrück, Biology / Chemistry, Osnabrück, Germany*
[3]*RWTH Aachen University, Medical Microbiology, Aachen, Germany*

1. Introduction

Non-typhoidal *Salmonella* (NTS) are a common global health concern in human and veterinary medicine. Whereas healthy adults usually suffer from self-limiting gastroenteritis, children younger than 5 years are at increased risk for systemic distribution, often leading to severe and potentially life-threatening conditions. After oral uptake, NTS secrete a variety of effector proteins encoded by *Salmonella* Pathogenicity Islands (SPIs) to overcome the epithelial barrier in the intestine. In previous studies, we identified SPI2 effectors as essential for epithelial evasion and subsequent systemic dissemination in the neonate host.

2. Goals

Our objective is to attain a deeper understanding of egress mechanisms, selection processes as well as potential bottleneck identification during NTS pathogenesis.

3. Materials and Methods

Wild-type isogenic tagged strains (WITS), either in a wt NTS or SPI2-deficient background, are orally applied to newborn mice in accordance with our previously established neonatal infection model. Tissue was harvested at 2 or 4 days post infection (dpi) and sequence abundance comparison of populations isolated from distinct organ compartments within the same host allow us to draw conclusions of disseminative pathways.

4. Results

Intestinal inflammation imposed a bottleneck in wt-infected animals in the small intestine. A few founder bacteria reach systemic compartments after oral uptake independent of SPI2. However, wt bacteria successfully transmigrate the intestinal epithelium and disseminate to systemic sites. Consequently, bacterial loads in liver tissue of wt-infected neonates increased between 2 and 4 dpi, whereas SPI2-deficient bacterial loads remained similar. Sequencing revealed an assimilation of subpopulations at systemic sites compared to bacteria isolated from the intestine within 4 days in the wt background. In contrast, subpopulations in systemic organs and gut of the same host infected with SPI2-deficient WITS display low similarities.

5. Summary

Epithelial transmigration is the source of the majority of bacteria isolated from systemic site dependent on SPI2 effector proteins.

P-HPIZ-016
Host-pathogen interplay revealed by the analysis of human enteropathogenic Escherichia coli (EPEC) isolates using a murine neonatal infection model
A. Z. Bachmann[1], D. Friske[1], N. M. K. Gubbi[1], U. Repnik[2], T. Hitch[1], M. Berger[3], U. Dobrindt[3], D. Rasko[4], M. Hornef[1], *A. Dupont[1]
[1]*RWTH Aachen University, Institute of Medical Microbiology, Aachen, Germany*
[2]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Faculty of Mathematics and Natural Sciences, Kiel, Germany*
[3]*Münster University, Institute for Hygiene, Münster, Germany*
[4]*University of Maryland School of Medicine, Baltimore, MD, Germany*

Typical enteropathogenic *Escherichia coli* (tEPEC), a causative agent of watery diarrhoea in infants, has been scarcely studied *in vivo* due to the lack of a suitable small animal model. Taking advantage of our recently established murine neonatal infection model [1], we analysed the formation of A/E lesions and deciphered the intestinal epithelial host response upon infection with different clinical isolates as well as with the type strain E2348/69. Interestingly, despite a similar degree of gut colonization, the number of attaching and effacing (A/E) lesions and the intensity of the epithelial host response varied greatly between the different tEPEC strains. As our mouse model controls for variations in host genetics and environmental factors, our data suggest that EPEC interaction with the host mucosa is strongly modulated by yet unidentified bacterial factors *in vivo*. In addition, our study highlights the heterogeneity of clinical EPEC isolates and illustrates the importance to study many representative EPEC strains to better understand EPEC pathogenesis and develop appropriate therapeutics.

References

[1] Dupont *et al.*, PLoS Pathog., 2016, 12(5):e1005616

Infection Immunology

P-II-001
Regulation of low local tissue oxygenation in cutaneous leishmaniasis
*B. Holoborodko[1], V. Schatz[1], F. Weber[2], K. Dettmer[3], A. Grüneboom[2], J. Jantsch[4]
[1]*University Hospital Regensburg, Institute of Clinical Microbiology and Hygiene, Regensburg, Germany*
[2]*Leibniz-Institut für Analytische Wissenschaften, – ISAS – e.V., Dortmund, Germany*
[3]*University of Regensburg, Institute of Functional Genomics, Regensburg, Germany*
[4]*University Hospital Cologne, Institute for Medical Microbiology, Immunology, and Hygiene, Köln, Germany*

In infected or inflamed tissues, low oxygen (O_2) levels prevail, affecting the O_2-dependent antimicrobial effectors of professional phagocytes, thereby promoting the survival of the intracellular parasite *Leishmania (L.) major*. However, the

mechanism causing the local O_2 deficiency in the infected lesion is unknown. The focus of this study is to determine the microenvironmental factors associated with low tissue O_2 levels in the *L. major* lesions as well as to assess the contribution of these factors on lesional tissue O_2 and host defense.

Using non-invasive O_2 and perfusion measurement in a C57BL/6 (healer) / BALB/c (non-healer) mouse model, we demonstrate that the course of infection has a bi-phasic discrimination. The first phase is characterized by formation of the lesion. While the size of the lesion as well as oxygen levels remains indistinguishable between the strains, C57BL/6 animals exhibited elevated perfusion during this phase. In the second phase, the healer strain displays normalization of tissue oxygenation with increased tissue perfusion levels. In contrast, non-healer strain present a non-resolving course of infection without normalization of oxygen levels and only minimal changes in tissue perfusion.

Furthermore, by infecting T and B lymphocyte deficient (Rag2-KO) C57BL/6 mice, we investigated the contribution of the adaptive immune system in this context. These showed a similar non-healing course with a minimal increase in perfusion as BALB/c mice, but with a time delay in terms of lesion formation and decrease in oxygen levels.

Our data suggest that the adaptive immune response is involved in early oxygen depletion in infected tissues. We will use this experimental setup to uncover immunovascular crosstalk and its impact on host defense in the future.

P-II-002

Staphylococcus epidermidis prosthetic joint infections are associated with an expansion of distinct immune cell populations

*P. Bartsch[1], Z. I. Junginger[2], R. Hackbusch[2], M. Arndt[3], T. Gehrke[3], M. Aepfelbacher[4], E. Tolosa[2], H. Rohde[4]

[1]*University Hospital Hamburg-Eppendorf, Institute of medical microbiology, virology and hygiene, Hamburg, Germany*

[2]*University Hospital Hamburg-Eppendorf, Institute of Immunology, Hamburg, Germany*

[3]*Helios Endo-Clinic Hamburg, Hamburg, Germany*

[4]*University Hospital Hamburg-Eppendorf, Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany*

Staphylococcus epidermidis is a leading pathogen in prosthetic joint infections (PJI). Biofilm formation plays a key role in *S. epidermidis* immune evasion and is essential for the development of chronic, therapy-resistant infections. In PJI, the host mounts a significant immune response, mainly characterized by infiltration of the joint and synovial fluid (SF) by neutrophils. In addition, myeloid-derived suppressor cells (MDSCs) are increased in the SF of infected prosthetic joints and are important in dampening the proliferation and activation of other leukocytes such as NK or T cells. Own evidence from in vitro studies supports the idea that in *S. epidermidis* implant infections, biofilm formation induces the development of anti-inflammatory macrophages. While this mechanism might be relevant for chronic infections, at present the importance in vivo is unknown. Therefore, the major aim of this study was provide a high resolution population analysis of immune cells in the SF from *S. epidermidis* PJI.

To study immune cell distribution SF and whole blood were obtained from seven patients (two female, five male) undergoing revision arthroplasty of the knee (n=4) or hip (n=3) for late chronic PJI by *S. epidermidis*. Flow cytometric analysis of SF revealed a consistent increase in monocytes (especially $CD14^{dim}$), conventional dendritic cells (type 1 and 2) and chronically activated $CD4^+$ T cells as compared to the corresponding blood sample. Plasmacytoid dendritic cells did not change significantly between SF and blood samples.

The higher levels of non-classical monocytes and $CD127^{neg}$ $CD4^+$ T cells may represent a state of chronic infection that cannot be cleared by immune cells due to biofilm formation of the pathogen. In order to investigate the interaction between *S. epidermidis* and immune cells in the infected joint, we are currently analyzing the differentiation profile and cytokine production of the infiltrating monocytes in relation to virulence factors of the patient-isolated *S. epidermidis* strains.

P-II-003

Decoding Persistent Salmonella Infections: High Throughput Exploration of Molecular Decision Points

*A. Tietze[1], D. Gerlach[2], A. R. Brochado[3]

[1]*Julius-Maximilians-Universität Würzburg, Microbiology, Würzburg, Germany*

[2]*Ludwig Maximilians University Munich, Microbiology, München, Germany*

[3]*Eberhard Karls University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*

Bacterial pathogens exhibit resistance to antibiotic treatments through either antimicrobial resistance mechanisms or the adoption of bet-hedging strategies, where a slow-growing subpopulation becomes tolerant to antibiotics (persisters). Such treatment-resistant pathogens not only contribute to the spread of antibiotic resistance but are also linked to chronic and recurring infections. While extensive research is conducted to decipher the mechanisms of antibiotic resistance, it is largely unknow how the existence of persister subpopulations is triggered and sustained during infections, hindering the development of efficient treatment strategies. To address this research gap, we have designed a high-throughput approach to uncover key molecular decisions for sustaining or aborting a persistent state during Salmonella infections within macrophages. Our approach involves the simultaneous targeting of the bacterium and the macrophage using a combination of antibiotics and immunomodulatory drugs to identify conditions that modify Salmonella's intracellular survival. Subsequent in-depth exploration of the most promising conditions will unveil the genetic landscape of bacterial and host pathways facilitating intracellular pathogen survival and or exit from the persistence state. In the long term, we aim to investigate how pathogen- or host cell-specific our findings are, which will hint towards the need for broad-spectrum or targeted therapies.

P-II-004

Deciphering the complex interplay between liver Kupffer cells and Staphylococcus aureus during bloodstream infection

*O. V. Nwofor[1], Q. Chen[1], O. Goldmann[1], *J. Wolf[2], M. Fraunholz[2], E. Medina[1]

[1]*Helmholtz Center for Infection Research, Infection Immunology, Brunswick, Germany*

[2]*Julius-Maximilians-Universität Würzburg, Microbiology, Würzburg, Germany*

Staphylococcus aureus is an opportunistic human pathogen and a leading cause of severe and life-threatening systemic

infections, which occur when the bacteria disseminate from an initial site of infection to distant organs via the bloodstream. *S. aureus* bacteremia is associated with high mortality rates. Understanding the mechanisms of host defense against *S. aureus* systemic infections is therefore crucial for developing effective strategies for prevention and treatment of these infections. Kupffer cells (KC), specialized liver-resident macrophages, are key players in the removal of bacteria in the bloodstream. However, *S. aureus* has developed strategies to resist elimination by KC that contribute to its ability to persist and colonize additional infection sites such as the kidneys.

Here we sought to decipher the complex interplay between *S. aureus* virulence factors and the anti-microbial mechanisms of KC *in vitro* and *in vitro*. KC were isolated from the livers of C57BL/6 mice and were infected with *S. aureus in vitro*, followed by monitoring of bacterial intracellular killing and phagocytosis rates. In the *in vivo* experiments, mice were infected intravenously with GFP-expressing *S. aureus*, and the number of viable bacteria in the liver was determined 4 and 24 hours after infection. By flow cytometry we determined that monocytes and neutrophils were also recruited to the liver 4 h after infection and thus may contribute to bacterial killing.

Our results demonstrate that *in vitro*-infected KC efficiently phagocytosed and eradicated *S. aureus* 2 h after infection. *In vivo,* we observed a substantial reduction of *S. aureus* in the liver 4 h after inoculation. However, a bacterial subpopulation survived and disseminated to other organs at later times of infection. We will investigate by Tn-seq, if this subpopulation of *S. aureus* requires specific virulence determinants to escape KC. Furthermore, only a subpopulation of KC harbored *S. aureus*, suggesting heterogeneity in the bactericidal capacity of the phagocytes. Single cell RNA sequencing will be used to investigate if this KC heterogeneity influences their antibacterial capacity.

P-II-005

How FHR-1 deficiency affects the infection with the malaria pathogen Plasmodium falciparum

*T. Reiß[1], A. Gonzalez-Delgado[2], J. Venrath[1], I. Bruchhaus[3], A. Bachmann[3], R. Fendel[4], C. Skerka[2], G. Pradel[1]
[1]RWTH Aachen University, Cellular and Applied Infection Biology, Aachen, Germany
[2]Leibniz Institute for Natural Product Research and Infection Biology, Department of Infection Biology, Jena, Germany
[3]Bernhard Nocht Institute for Tropical Medicine, Cellular Parasitology Department, Hamburg, Germany
[4]University of Tübingen, Institute for Tropical Medicine, Tübingen, Germany

The obligate intracellular parasite *Plasmodium falciparum* causes malaria tropica, the most severe form of malaria, which is particularly acute in the WHO African region. With more than 247 million cases and 619,000 deaths each year, malaria is still one of the deadliest infectious diseases in the world. Malaria infection is characterized by multiple symptoms, like fever, head- and body aches, and diarrhea. Severe complications further include anemia, shock, cerebral malaria, multiple organ failure, and ultimately death. During evolution, *P. falciparum* has developed various mechanisms to evade attack by human complement, such as binding of the human complement regulator Factor H. In previous studies, we have shown that the Factor H-Related Protein 1 (FHR-1) competes with Factor H for binding sites and thereby prevents Factor H-mediated complement evasion. Furthermore, FHR-1, when binding to malaria-lysed erythrocytes, activates neutrophils and thus contributes to inflammation. Remarkably, up to one third of the African population harbors a chromosomal deletion of the FHR-1 gene. To investigate a potential link between FHR-1 deficiencies and malaria severity, we investigated two cohorts of malaria tropica-infected African patients. All individuals gave informed consent to participate in a clinical trial. From the study participants, serum was collected and used to detect the FHR-1 protein by Western blot analysis. In addition, other protein concentrations, such as inflammation markers, were measured using in-house and commercial ELISA. We show that FHR-1 deficient patients are less likely to suffer from severe malaria. In addition, these patients have less signs of anemia compared to FHR-1 expressing individuals. Further, we measured inflammation markers. Both in FHR-1-deficient patients and in patients with a mild course of malaria, we were able to demonstrate a significant decrease in the concentration of inflammation markers. Our data underline the potent role of FHR-1 in inflammation, which likely enhances the immune response in malaria infections and the risk of severe malaria anemia.

P-II-006

Toll-like receptor 4 improves replication of Chlamydia trachomatis serovar D in the uroepithelial T24/83 cell line

*S. Kuhn[1], S. Albrecht[1], L. Kellner[1], X. Rait[1], H. Griffiths[1], C. De La Torre[2], N. Gretz[2], T. Miethke[1]
[1]University Mannheim, Institute of Medical Microbiology and Hygiene Medical Faculty Mannheim, Mannheim, Germany
[2]Center of Experimental Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Chlamydia trachomatis infection remains mostly symptomless but can cause tissue modulating processes like hydrosalpinx, pelvic inflammatory disorder or lead to infertility. Uroepithelium is the point of entry for the chlamydial infection. In order to identify the immune signaling cascades activated by *Chlamydia*, compared to polyI:C or LPS, we infected T24/83 human uroepithelial cell line with *C. trachomatis* serovar D. Here we used microarray analysis and quantitative Real Time PCR, and observed an induction in the mRNA transcription of inflammatory genes such as il-1β, il-6 and tnf-α. However, the ELISA showed, that the corresponding cytokines were not secreted upon infection. T24/83 cells secrete the mentioned cytokines after a stimulation with polyI:C or LPS which are ligands for Toll-like receptor (TLR) 3 and 4, respectively. Thus, the pattern of the gene induction is different compared to infection with *C. trachomatis.* Additionally, we identified a set of genes like matrix metallopeptidase 1 and 3 and histatin 1, which were most induced upon *C. trachomati*s infection. It was reported, that TLR3 and TLR4 play an important role in the host pathogen interaction. Therefore we wanted to identify the role of TLR3 and TLR4 in T24/83 cells using CRISPR/Cas9 knock-outs. Unexpectedly we could show that in TLR4KO cells *C. trachomatis* serovar D replicate less well than in the wildtype. Whereas TLR3KO cells showed no difference in chlamydial replication compared to the wild type host cells. Hence, we assume that chlamydia can use TLR4 as a supporter for its own growth.

P-II-007

Co-stimulation of formyl-peptide receptor 1 and 2 leads to synergistic activation of neutrophils

*J. Becker[1], E. Weiß[1], M. Lebtig[1], A. Peschel[1], D. Kretschmer[1]
[1]Eberhard Karls University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, Tübingen, Germany

As innate immune cells, neutrophils play a critical role in defending the human body against invading pathogens. To detect foreign organisms and initiate an immune response, neutrophils use a set of pattern-recognition receptors (PRRs) that are activated by pathogen-associated molecular patterns (PAMPs). These PRRs include the formyl-peptide receptors (FPRs). While FPR1 senses short formylated peptides of bacteria in general, FPR2 is activated by specific bacterial molecules including phenol-soluble modulins (PSMs). PSMs are small toxic peptides produced in high amounts by the pathogenic bacterium *Staphylococcus aureus*. Both FPR1 and FPR2 ligands stimulate neutrophils to release cytokines like IL-8 and produce reactive oxygen species (ROS) or guide neutrophil chemotaxis.

Since both FPR1 ligands and PSMs as FPR2 ligands are present at the site of infection with *S. aureus*, the aim of this work is to analyse the impact of co-stimulation of FPR1 and FPR2 on neutrophils. Preliminary data indicate that the activation of both receptors synergistically enhances the release of the chemokine IL-8 and the antimicrobial peptide LL-37 by neutrophils. Likewise, cell migration and ROS production are increased, resulting in an overall higher inflammatory immune response.

Activation of FPRs plays an important role during an immune response. Therefore, deciphering the interaction between FPR1 and FPR2 promises to shed light on the regulation of inflammation and pave the way for the development of new therapeutic approaches.

P-II-008

The role of extracellular vesicles in escape of Candida albicans from phagocytosis by neutrophils

*J. J. Patitz[1], N. E. Nieuwenhuizen[1], A. K. Zimmermann[2], M. G. Blango[3], T. Krüger[2], O. Kniemeyer[2], A. A. Brakhage[4,2], O. Kurzai[1,5], K. Hünniger[5]
[1]Institute for Hygiene and Microbiology, Medical Mycology, Würzburg, Germany
[2]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department of Molecular and Applied Microbiology, Jena, Germany
[3]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Junior Research Group RNA Biology of Fungal Infections, Jena, Germany
[4]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany
[5]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Fungal Septomics, Jena, Germany

Introduction: Polymorphonuclear granulocytes (PMN) provide a rapid innate immune response against fungal infections. Using an ex vivo human whole blood model, we confirmed their central role in immune responses against *Candida albicans*. In this model, a fraction of *C. albicans* cells escaped phagocytosis and extracellular killing.

Goals: We aimed to identify the mechanisms by which *C. albicans* cells escape from phagocytosis.

Materials & Methods: PMNs were isolated from whole blood with the MACSxpress Whole Blood Neutrophil Isolation Kit (Miltenyi) and confronted with opsonized *C. albicans*. Using flow cytometry, we investigated remodelling of the surface of *C. albicans* after incubation with PMNs or extracellular vesicles (EVs) released by PMNs. EVs were isolated by high-speed centrifugation. EVs released by PMNs after contact with *C. albicans* (CaEVs) were compared with those released from mock-infected PMNs (sponEVs) using nanoparticle tracking analysis and mass spectrometry.

Results: After 1 h of confrontation, 90 % of *C. albicans* cells had been phagocytosed by neutrophils. Extracellular *C. albicans* acquired PMN markers (e.g. CD66b) and Annexin V, suggesting that EVs may be involved in surface remodelling of the extracellular fungi. Incubation of *C. albicans* with isolated EVs confirmed transfer of host cell markers by EVs. Both sponEVs and CaEVs transferred host cell markers. The size and protein content of sponEVs and CaEVs were similar, but confrontation of PMNs with *C. albicans* increased the amount of EVs released. Pre-incubating *C. albicans* with EVs decreased the percentage of fungal cells phagocytosed by neutrophils.

Summary: EVs released by PMNs may contribute to the escape of *C. albicans* from phagocytosis by transfer of host cell proteins. These results increase our understanding of host-cell interactions with *C. albicans* in the blood.

P-II-009

Elucidating the immunopathogenesis of vulvovaginal candidiasis using an organ-on-chip model

*K. O. Cheng[1], Ö. Kırav[1], M. Valentine[1], D. Montaño[1], A. Mosig[2], A. Dietschmann[1], M. S. Gresnigt[1]
[1]Leibniz-HKI, Jena, Germany
[2]Jena University Hospital, Jena, Germany

Vulvovaginal candidiasis (VVC) is one of the principal infectious causes of vaginal and vulvar inflammation. Approximately 75% of women suffer from this infection at least once during their reproductive years, and a subgroup suffers from recurrent infections that dramatically impact quality of life. A hyperinflammatory innate immune response associated with excessive neutrophil recruitment drives symptoms of VVC. Current models such as classical *in vitro* cell culture and mice models cannot completely mimic human VVC for the absence of a physiological tissue environment and intrinsic pathophysiological differences. Conversely, organ-on-chip models provide a closer look at human physiology with higher capacity to control microenvironment as well as tissue-specific functions due to their design. The recent technological advances in microfluidics and organ-on-chip systems paved the way to establish a VVC-on-chip model. We therefore established a novel VVC-on-chip model consisting of an epithelial and a vascular compartment to dissect the complex host-pathogen interplay of VVC in a more human context. A-431 vaginal epithelial cells co-cultured with human monocyte-derived macrophages, and human umbilical vein vascular cells were contained in the two cavities, respectively. Primary human neutrophils were perfused in the vascular compartment to study the immunopathology during *Candida* infections. The flexibility of this model enables *real-time* monitoring and easy manipulations, for instance, immunofluorescence staining can be performed to visualize infection of the vaginal epithelial cells *in situ* while immunopathogenesis can be evaluated through lactate dehydrogenase release and quantification of neutrophil activation markers. Consistent with what is known about the immunopathogenesis of VVC, our model demonstrated that perfused neutrophils from the vascular cavity migrated to the infected epithelium, promoting higher vaginal epithelial cell damage and IL-8 release during *C. albicans* infection. Collectively, this VVC-on-chip model possesses potential to illuminate molecular mechanisms underlying the over-aggressive immune responses to the vaginal *Candida* infection.

P-II-010

Spontaneous activity of the mitochondrial apoptotic apparatus controls mitochondrial antiviral signaling protein (MAVS) and cyclic GMP-AMP synthase (cGAS) aggregation and activity

*S. Gradzka-Boberda[1], I. Parui[1], I. Gentle[1], G. Häcker[1]
[1]*University of Freiburg, Institute of Medical Microbiology and Hygiene, Medical Center, Freiburg i. Br., Germany*

Introduction

MAVS is the adaptor protein of cytosolic helicases. Upon activation, MAVS monomers aggregate to serve as a platform for recruitment of effector proteins, leading to IRF3 and NF-κB activation. cGAS recognizes dsDNA and synthesizes cGAMP, leading to an IRF3 and NF-kB response. cGAS also aggregates when activated. Spontaneous sub-lethal activation of the mitochondrial apoptotic apparatus has been observed in proliferating epithelial cells, generating low-level caspase activity. Caspases play various roles in inflammation. One of the anti-inflammatory functions of caspases is the proteolytic degradation of signaling proteins during apoptosis. MAVS, cGAS and IRF3 are substrates of caspase-3 and their cleavage limits the IFN response. We propose a novel role of sub-lethal activity of the mitochondrial apoptotic apparatus that tunes MAVS/cGAS activity.

Goals

To provide an exact measurement of steady-state sub-lethal activity of cell death caspases and to understand how the mitochondrial apoptotic apparatus controls MAVS/cGAS signaling.

Materials & Methods

We analyzed MAVS aggregation and activity in cells deficient in mitochondrial apoptosis apparatus components. To detect MAVS aggregation, we performed SDD-AGE. To answer the question of MAVS-sensitivity to low-level caspase-3 activity, we exposed mitochondria to recombinant caspase-3. We also assessed IFN type I response and IL-6 secretion.

Results

Spontaneous activity and substrate cleavage by caspase-9 and -3 but not -7 were observed in non-apoptotic HeLa cells. Loss of caspase-activity in the mitochondrial apoptosis pathway increased MAVS aggregation at steady-state and upon experimental stimulation of MAVS. We observed that MAVS complexes have a greater sensitivity to low-level caspase-3 activity than monomers. In the absence of caspase activity, MAVS-signals were enhanced. cGAS activity was also regulated by steady-state caspase-activity.

Summary

Spontaneous activation of the mitochondrial apoptotic apparatus generates low-level caspase-3 activity that specifically cleaves MAVS- and probably cGAS-complexes but not the monomers. We propose that this is a way to tune their basal activity.

P-II-011

Serological Testing for Assessing the Seroprevalence of Acute Respiratory Infections: a systematic review

*H. Müller[1,2], *M. Krone[1,3], I. Wagenhäuser[1,2]
[1]*University Hospital Würzburg, Infection Control and Antimicrobial Stewardship Unit, Würzburg, Germany*
[2]*University Hospital Würzburg, Department of Internal Medicine I, Würzburg, Germany*
[3]*University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*

Question

In recent times, serological testing has been an important and well-established method to detect and track acute respiratory infections (ARI) in the infection epidemiological population monitoring above all as an important addition to the reporting figures and symptom-based surveillance.

This systematic review aims a comprehensive overview, systematically examining the current evidence on the suitability and applicability of seroprevalence assessments for the ARI pathogens RSV, Influenza, and SARS-CoV-2 to evaluate real infection rates and on the definition of qualitative and quantitative serological changes that can detect an infection. In addition, the review asseses the extend to which serological testing can identify breakthrough infections after a vaccination or a previous infection and considering the respective seasonal dynamics of an ARI pathogen.

Methods

The search was conducted on PubMed® platform, with the following pre-determined search terms: RSV, respiratory syncytial virus, Influenza, SARS-CoV-2, seroconversion, seroprevalence, seroepidemiology, and infection rate. Publications that met the following criteria were included: English-speaking, human species as the main study collective. After screening the titles regarding suitability, the abstracts and the full texts of the publications deemed relevant were screened accordingly. The selected studies were then evaluated, and the results comparatively assembled.

Results

The initial search for seroprevalance assessments on RSV yielded 107 results. Respectively, the search for SARS-CoV-2 led to 2,889, the search for Influenza to 1,638 results. The complete results of the evidence screening process and the consequent evaluation will be presented at the conference.

Conclusions

The ongoing screening process has so far resulted in the inclusion of a systematic review covering seroprevalence studies regarding RSV infections. Therefore, the results presented and discussed at the conference will focus on the evidence on seroprevalence concerning Influenza and SARS-CoV-2.

P-II-012

Role of cmv-IL-10 in an HCMV & Aspergillus fumigatus co-infection model

L. Bussemer[1], *L. Heilig[1], S. Schäuble[2], L. Strobel[1], O. Kurzai[3,4], A. Grothey[5], L. Dölken[5,6], K. Laib Sampaio[7], C. Sinzger[7], H. Einsele[1], S. Wurster[8], J. Löffler[1]
[1]*University Hospital Wuerzburg,, Medical and Polyclinic II, Würzburg, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute (HKI), Department of Microbiome Dynamic, Jena, Germany*
[3]*University of Würzburg, Institute for Hygiene und Microbiology, Würzburg, Germany*
[4]*Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute, National Reference Center for Invasive Fungal Infections, Jena, Germany*
[5]*University of Wuerzburg, Institute for Virology and Immunobiology, Würzburg, Germany*
[6]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*
[7]*University Medical Center, Institute for Virology, Ulm, Germany*
[8]*The University of Texas MD Anderson Cancer Center, Department of Infectious Diseases, Infection Control and Employee Health, Houston, TX, United States*

Introduction: Human cytomegalovirus (HCMV) causes severe infections in immunocompromised patients and can also predispose them to fungal co-infections. For instance, we previously found that HCMV co-infection attenuated proinflammatory response of human monocyte-derived dendritic cells (moDCs) to the opportunistic mold *Aspergillus fumigatus*. Of note, HCMV employs several immune evasion strategies, including its interleukin-10 homolog (cmv-IL-10), to counteract host immunosurveillance and establish life-long latency.

Goals: We studied the role of HCMV in the interplay between *A. fumigatus* and human moDCs, focusing on the role of cmv-IL-10 in immune cell-mediated cross-kingdom interactions.

Materials & Methods: We exposed moDCs to wildtype HCMV TB40E or *UL111A* (HCMV lacking cmv-IL-10) and/or *A. fumigatus*, either individually (single infection) or in combination (co-infection). In addition, moDCs were stimulated with recombinant cmv-IL-10, with and without subsequent *A. fumigatus* challenge. We used a multifaced approach (RNA-seq, flow cytometry, ELISA) to study moDC activation and various effector responses.

Results: In contrast to single *A. fumigatus* infection, co-infection with *A. fumigatus* and TB40E but not *UL111A* resulted in reduced expression of various genes associated with DC activation (e.g., *IRF5* and *TLR4*). Moreover, exposure of moDCs to cmv-IL-10 led to diminished expression of genes involved in the initiation of anti-*Aspergillus* defense and tissue repair (e.g., *CXCL8* and *VEGFA*). Additionally, simultaneous confrontation with rcmv-IL-10 dampened several DC effector responses to *A. fumigatus*, including IL-1β and CXCL10 release.

Conclusion: We provide inaugural evidence that cmv-IL-10 contributes to attenuated antifungal host response during HCMV-associated aspergillosis.

P-II-014
Analyzing and Differentiating Immune Dysregulation in COVID-19 and Influenza Patients Predisposing to Mold Infections

*B. Tappe[1], L. Kimmes[1], C. D. Lauruschkat[1], J. Schoppa[1], H. Einsele[1], S. Wurster[2], J. Löffler[1]
[1]*University Hospital Würzburg, Medical Clinic and Polyclinic II, Würzburg, Germany*
[2]*The university of Texas MD Anderson Cancer Center, Department of Infectious Diseases, Infection Control and Employee Health, Houston, TX, United States*

Patients suffering from respiratory viral infections are susceptible to developing secondary fungal infections. During the SARS-CoV-2 pandemic, COVID-19-associated aspergillosis (CAPA) has been diagnosed in up to 39% of COVID-19 patients admitted to the ICU, resulting in a significant concern for the healthcare system. Therapeutic approaches for CAPA have been mainly influenced by the treatment strategies for influenza-associated aspergillosis (IAPA), although there may be distinct features caused by the underlying viral infection. To enhance patient therapy, it is crucial to evaluate the infection-specific or shared immune alterations that contribute to fungal susceptibility.

Utilizing our previously published whole blood (WB) stimulation assays, we examine the immune response of influenza, COVID-19, or non-infected control patients to the human pathogenic mold *Aspergillus fumigatus*. Additionally, we identify underlying mechanistic impairments in COVID-19 and influenza patients by stimulating WB with various TLR agonists.

For innate immune cell stimulation, Hirudin anticoagulated WB is incubated with stimuli for 6 hours under constant rotation. T-cell immune responses are evaluated using Lithium-Heparinized whole WB incubated for 24 hours. Plasma from stimulated WB is collected for multiplex cytokine analysis (e.g., IFN-γ, TNF-α, IL-17A, G-CSF), and cells are lysed for subsequent RNA isolation. Gene expression analysis is performed using the NanoString nCounter Host Response Panel with 785 genes. Furthermore, we are performing flow cytometry to phenotype immune cells and to evaluate *Aspergillus* stimulated T cells, monocytes, and granulocytes. Additionally, we quantify the direct fungicidal activity of PMNs by measuring hyphal length using live-cell imaging.

This enables us to identify generic impairments in host immunity against *A. fumigatus* among patients suffering from viral infections. This analysis include discerning infection-specific or shared motifs and improved understanding of signaling pathways involved in the mold susceptibility of patients with an underlying SARS-CoV-2 or Influenza infection.

P-II-015
Myeloid cells in a mouse model of chlamydial infection

*L. Peng[1], T. Zortel[1], S. Barth[1], G. Häcker[1], S. Kirschnek[1]
[1]*University Hospital and Medical Center Freiburg, Institute of Medical Microbiology and Hygiene, Freiburg i. Br., Germany*

Objectives

Chlamydia (C.) trachomatis infects the female genital tract and may cause permanent tissue damage.The exact mechanism of bacterial clearance and development of tissue damage is still unknown. Our previous work has focused on the role of neutrophils on bacterial clearance and tissue destruction. Little is known about other myeloid cells in the female genital tract at steady state and during chlamydial infection. In this project, we characterize the recruitment of myeloid subpopulations and their impact on chlamydial infection and tissue damage.

Methods

In a *C. muridarum* infection mouse model, we investigated the infiltration of myeloid cells, bacterial load and tissue damage in the genital tract. The contribution of neutrophils was characterized in mice deficient in mature neutrophils (Mcl-1 mice). The effect of monocytes/inflammatory macrophages was investigated in CCR2-KO mice deficient in the recruitment of inflammatory monocytes.

Results

Myeloid cells infiltrated the genital tract early in infection, with a maximum for neutrophils and macrophages at 7 dpi, and for dendritic cells at 14 dpi. Chlamydial DNA was detected early in the upper genital tract. Chlamydia was efficently cleared at 31- 35 dpi from all parts of the genital tract. Mcl-1 mice lacking neutrophils showed substantial alterations of the inflammatory infiltrate and had higher chlamydial burden and reduced tissue damage. In wt mice, upon entry into the genital tract, inflammatory monocytes differentiated into macrophages and gave rise to different subpopulations. In CCR2-KO mice, the accumulation of macrophages was reduced, which suggests that the increase in macrophages in infected tissue is caused by recruitment and differentiation of inflammatory monocytes. CCR2-deficiency did not affect infiltration of neutrophils, DCs or T cells but reduced cytokine production by T cells.

Conclusions and Outlook

Neutrophils play a substantial role in chlamydial clearance and tissue destruction. Our data indicate the differential presence of individual subgroups of monocytes/macrophages during the course of infection, which may play different roles in immune reaction and tissue destruction.

P-II-016
Exploring the immune response of humanized mice to Staphylococcus aureus infection

*T. Hertlein[1], S. Hung[1,2,3], L. Dreher[1], A. Kasperkowitz[1], F. Kurz[4], J. Diessner[5], E. S. Ibrahim[1,6], S. Schwarz[2,3], K. Ohlsen[1]
[1]*Julius Maximilians University of Würzburg, Institute of Molecular Infection Biology, Würzburg, Germany*
[2]*Free University of Berlin, Department of Veterinary Medicine, Berlin, Germany*
[3]*Free University of Berlin, Veterinary Centre for Resistance Research (TZR), Berlin, Germany*
[4]*Julius Maximilians University of Würzburg, Institute of Pathology, Würzburg, Germany*
[5]*Julius Maximilians University of Würzburg, Department of Obstetrics and Gynecology, Würzburg, Germany*
[6]*Cairo University, Faculty of Pharmacy, Cairo, Egypt*

Humanized hemato-lymphoid system mice, or humanized mice, became in recent years a valuable tool to study the course of infection of pathogens with human host tropism. These immunodeficient mice, which are engrafted with human immune cells, showed remarkably increased susceptibility against *Staphylococcus aureus* infection.

Most of the early studies employed humanized NSG (huNSG, NOD-*scid* IL2Rgnull) mice which are widely used in the scientific community, but show poor human myeloid cell reconstitution. Since the innate immune response plays a decisive role in the defense against *S. aureus*, we conducted infection experiments in next-generation humanized NSG-SGM3 mice (huSGM3, NOD-scid IL2Rgnull-3/GM/SF), which have an improved myeloid cell compartment. We could demonstrate that huSGM3 showed an even higher susceptibility than huNSG although having higher numbers of human immune cells. Importantly, when looking at individual mouse data, it became clear, that the higher the number of human immune cells the more severe the infection in these mice. This was furthermore accompanied by increased levels of human cytokines and chemokines.

Currently, we are expanding our portfolio of infection models in humanized mice in terms of infection types and pathogens with the aim to learn more about the chances and limitations of this model. Additionally, we have started to investigate the human immune cell repertoire by single-cell RNA sequencing and preliminary data indicates a wide variety of human immune cell lineages and subtypes.

Knut Ohlsen and Tobias Hertlein contributed equally to this work

Relevant publications:

Hung S *et al.* 2022. MRSA Infection in the Thigh Muscle Leads to Systemic Disease, Strong Inflammation, and Loss of Human Monocytes in Humanized Mice. Front Immunol. 13:892053.
Hung S *et al.* 2023. Next-generation humanized NSG-SGM3 mice are highly susceptible to *Staphylococcus aureus* infection. Front Immunol. 14:1127709.

P-II-017
The antibacterial activity of Human Histone Peptides against ESKAPE Pathogens

*N. Jaber[1], A. Rodriguez[2,3], L. Ständker[2], S. Wiese[3], B. Spellerberg[1]
[1]*Institute of Medical Microbiology and Hygiene/ Ulm Univeristy Clinic, Faculty of Medicine, Ulm, Germany*
[2]*Core Facility for Functional Peptidomics/ Ulm University, Ulm, Germany*
[3]*Core Unit of Mass Spectrometry and Proteomics/ Ulm University, Ulm, Germany*

Antimicrobial resistance (AMR) represents a global health crisis associated with 1.27 million deaths in 2019 [1], [2]. New therapeutic approaches include antimicrobial peptides and proteins (AMPs), which are part of the innate immune defense, as alternatives to antibiotics [3]. Histones represent a family of small basic proteins that associate with DNA, whose antibacterial activity was first reported in 1942. These proteins and their fragments can be found in the cytoplasm, cellular membrane, extracellular fluid, and neutrophil extracellular traps (NETs) [4]. However, the antibacterial activity of human histones or their fragments has never been investigated in detail. We used a hemofiltrate peptide database for antimicrobial peptide prediction to identify novel human AMPs. Thirteen histone H1 sequences were identified as putative AMPs, synthesized, and tested against bacterial ESKAPE pathogens in a radial diffusion assay. A prominent dose-dependent antibacterial activity was detected for twelve fragments of H1 against *P. aeruginosa*. These histone H1 fragments inhibited the growth of *P. aeruginosa* without disrupting the bacterial membrane, as shown by a Sytox assay. H1 fragments incubated with THP-1 cells were not cytotoxic in LDH and Alamar assays. The characterized antimicrobial activity points to a role for human histone H1 fragments in innate immunity and may represent a promising approach for the development of novel antibacterial therapies.

P-II-018

Stromal cells shape the immune response to mycobacteria

*A. K. Lösslein[1,2,3], P. Henneke[1,2]
[1]*Medical Center and Faculty of Medicine, Institute for Infection Prevention and Control, Freiburg i. Br., Germany*
[2]*Medical Center and Faculty of Medicine, Institute for Immunodeficiency, Center for Chronic Immunodeficiency (CCI), Freiburg i. Br., Germany*
[3]*Medical Center and Faculty of Medicine, Institute of Medical Microbiology and Hygiene, Freiburg i. Br., Germany*

Introduction & Goals

Mycobacterial tissue infections lead to the formation of granulomas, which are complex immune cell structures. Key player of the granuloma is the macrophage (MΦ). Nevertheless, a mature granuloma with a necrotic core not only induces the accumulation of immune cells but also leads to a remodelling of the tissue with an impact on fibroblasts and stromal cells (SC). In this project, we aim to characterize the role of SC for granuloma formation and control of mycobacteria, as well as the interaction of MΦ and non-immune cells in mycobacterial infections.

Methods

To address these questions, we used murine *M. bovis* BCG infection models and *in vitro* co-cultures of SC and MΦ. We further characterized SC in mycobacterial infections by flow cytometry analysis, confocal and live cell imaging as well as transcriptomic analysis.

Results

Mycobacterial infections lead to the accumulation of bone-marrow derived monocytes at the site of infection, which then further contribute to granuloma formation. We found high expression levels of CCL2 and CCL7 in SC for up to 16 weeks post infection, indicating an involvement in the recruitment of monocytes. Although in infection mostly macrophages take up mycobacteria, flow cytometry and microscopy revealed that BCG could infect SC *in vitro*. With the help of adoptive cell transfers, we were able to prove that infections of SC with mycobacteria also occur *in vivo*. Additionally, killing assays suggested that the bacteria could survive within SC over several days. Of interest, SC accumulated lipids and showed changes in lipid metabolism during the infection, which might be relevant for the intracellular survival of mycobacteria as those are highly lipid-dependent.

Currently, we are performing transcriptome analyses to elucidate mechanisms of bacterial uptake in SC as well as MΦ-SC interactions.

Summary

Our data indicate that SC play a crucial role in mycobacterial infections and that they influence the MΦ immune response. It is highly relevant to identify potential host cells of mycobacteria as this is of importance for the duration of antibiotic treatment and the pathogenesis of latency in mycobacterial infections.

P-II-019

The deubiquitinating enzyme Cylindromatosis (CYLD) inhibits the control of Staphylococcus aureus in murine and human macrophages

C. Schmidt[1], M. Raptaki[1], F. Stavrou[2], L. Rösner[2], T. Werfel[2], *N. Gopala Krishna[1], D. Schlüter[1]
[1]*Institute of Medical Microbiology and Hospital Hygiene, Hannover Medical School, Hannover, Germany*
[2]*Department of Dermatology and Allergy, Hannover Medical School, Hannover, Germany*

Objectives: *Staphylococcus aureus is a major cause of* skin, soft tissue and severe systemic infections. The emergence of methicillin-resistant *Staphylococcus aureus (MRSA)* has further increased the risk of mortality. Therefore, development of adjunct therapies targeting the host immune response becomes important. The innate immune response against *S. aureus* is substantially mediated by macrophages and involves the NF-κB pathway. The deubiquitinating enzyme CYLD negatively regulates the NF-κB pathway by cleaving K-63 linked polyubiquitin chains from several signaling molecules. This project aims to investigate how CYLD regulates the outcome of *S. aureus* infection in murine and human macrophages.

Methods: Wild-type (WT) and Cyld -/- mice were intravenously infected with *S. aureus* and the weight loss was monitored daily until day 49 p.i.. The bacterial loads in liver, kidneys and spleen were quantified. To delineate the role of CYLD in macrophages, bone marrow-derived macrophages (BMDM) from WT and Cyld-/- mice were stimulated *in vitro* with IFN-g and LPS for 24 hours followed by infection with *S. aureus*. The bacterial load in the macrophages was enumerated 24 hours p.i. To identify the underlying CYLD-regulated signaling pathways, the activation of NF-κB, ERK1/2, and p38MAPK was analyzed by WB in *S. aureus* infected BMDM and MRSA-infected THP-1 macrophages. Furthermore, CYLD was deleted in human THP-1 cells by CRISPR-Cas9 and differentiated into M0, M1 and M2 macrophages. The bacterial load in WT and Cyld-/- THP-1 macrophages was enumerated after infection with MRSA.

Results: CYLD-deficiency reduced bacterial burden in *S. aureus* infection both in vivo and in vitro. Additional in vitro experiments using murine BMDM and human THP-1 derived macrophages showed enhanced activation of NF-κB, p38MAPK and ERK1/2 pathways, and increased production of anti-bacterial antibacterial ROS in CYLD deficient macrophages leading to better control of *S. aureus*.

Conclusions: Our study *identifies CYLD as an inhibitor of anti-staphylococcus immune responses and a potential therapeutic target to treat S. aureus* infections.

P-II-020

Non-selective Janus-Kinase-Inhibitors (ns-JAKi) significantly impair signal-transducer-and-activator-of-transcription (STAT) type 1 (STAT1) signaling of varicella-zoster-virus-(VZV)-specific cellular memory responses

*G. Almanzar[1], F. Baum[1], M. Steimer[1], L. Jahn[1], S. Hick[1], A. Ess[1], C. Rack[1], I. Turin[1], A. Schäfer[2], P. Arampatzi[3], T. Bischler[3], T. Gräfenhan[3], F. Erhard[4], T. Hennig[4], L. Dölken[4], M. Feuchtenberger[5], M. Schmalzing[2], M. Prelog[1]
[1]*University Hospital Würzburg, Department of Pediatrics, Würzburg, Germany*
[2]*University Hospital Würzburg, Department of Internal Medicine,*

Medicine II, Würzburg, Germany
[3]*University Hospital Würzburg, Core Unit Systems Medicine, Würzburg, Germany*
[4]*University of Würzburg, Department of Virology and Immunology, Würzburg, Germany*
[5]*Bayern Ost Medical Center Altötting Burghausen, Department of Rheumatology, Burghausen, Germany*

Background: Janus-Kinase-inhibitors (JAKi) have been approved for several indications in autoimmune diseases. Clinical studies showed that non-selective JAKi (ns-JAKi) are associated with an increased risk of herpes zoster (HZ) by reactivation of varicella-zoster-virus (VZV), whereas selective JAK-1 inhibitors (JAK-1i) have a lower risk for HZ.

Goals: In order to understand the molecular mechanisms behind the different HZ risk between ns-JAK und JAK-1i, the study aims to analyze transcriptome patterns and VZV-specific cellular responses induced by administration of JAKi in vitro on lymphocytes obtained from patients with rheumatoid arthritis (RA) and healthy controls (HC).

Methods: Lymphocytes were stimulated by VZV-antigens in vitro under different concentrations with JAK-1i or ns-JAKi. ELISpot and immune-phenotyping by flow-cytometry were performed to screen for cellular reactivity. mRNA sequencing techniques were used for transcriptome analysis. Western-blot-analysis was performed to determine the phosphorylated signal-transducer-and-activator-of-transcription-(STAT) proteins to estimate functional JAK activity.

Results: JAKi-treated lymphocytes showed significantly lower spot-forming-units (SFU) in a concentration-dependent manner compared to untreated controls and a reduced expression of activation markers (CD69). Relative intensity of phosphorylated STAT1 (pSTAT1) was reduced by 81% by JAK-1i and by 94% by ns-JAKi using plasma-concentration-equivalent doses. JAKi-untreated VZV-stimulated conditions induced expression of STAT1 2.4-fold, STAT2 2.8-fold, STAT3 1.2-fold and STAT4 1.7-fold. Interferon-response-factor-7 (IRF7) was induced 3.6-fold. After treatment with JAK-1i, STAT1 was still induced 1.5-fold, but significantly reduced by 82% by ns-JAKi.

Summary: The data demonstrated that the impact on VZV-specific cellular responses was significantly higher in ns-JAKi-treated than in JAK-1i-treated lymphocytes. This may result in less severe impairment of VZV-induced STAT-signaling by selective JAK-1i compared to ns-JAKi and may explain the lower HZ incidence in JAK-1i compared to ns-JAKi-treated patients.

P-II-021

Ubiquitin E3 ligases mediating the intracellular recognition of S. aureus

*O. Plöhn[1], A. K. Singh[1], K. Becker[1], C. Cammann[1], U. Seifert[1]
[1]*University Medicine Greifswald, Friedrich Loeffler-Institute of Medical Microbiology, Greifswald, Germany*

Introduction

Ubiquitin E3 ligases catalyse the transfer of ubiquitin moieties to protein substrates, affecting protein function, stability and localisation. During infection, ubiquitination is critical for regulating host cellular signalling pathways to recognise and eliminate pathogens. Intracellular pathogens are ubiquitinated by specific E3 ligases that target e.g. bacteria for autophagosomal degradation. However, knowledge of the E3 ligases involved is very limited, especially for Gram-positive bacteria such as *Staphylococcus aureus*.

Method

Lung epithelial cells are the first line of defence against infections of the respiratory tract. Therefore, we analysed A549 lung epithelial cells during *S. aureus* infection, focusing on the role of the E3 ligase LRSAM1. We used the CRISPR-Cas9 system to generate LRSAM1-deficient A549 cells and compared their cellular response to that of LRSAM1-expressing cells during *S. aureus* infection by monitoring the activation of cellular signalling pathways and the subsequent cytokine production, host cell death and the intracellular survival of *S. aureus*.

Results

The analysis of the LRSAM1-deficient A549 cells revealed a strong impact on the intracellular replication of *S. aureus* during infection which was accompanied by an increased host cell death compared to control cells. Moreover, by monitoring IκBα degradation an altered activation of the NFκB-pathway could be observed which resulted in elevated secretion of the pro-inflammatory cytokines IL-6 and IL-8. Currently LRSAM1 deficient cells are analysed for their capability to ubiquitinate intracellular *S. aureus* as well as the impact of LRSAM1 on autophagy.

Discussion

In conclusion, our results indicate a prominent role for LRSAM1 during *S. aureus* infection. Further experiments are needed to clarify its exact substrates and thus elucidate the mechanism by which this E3 ligase affects intracellular bacteria recognition and thus directly or indirectly impacts intracellular bacteria survival and host cell death. Understanding the molecular mechanisms underlying the role of LRSAM1 will help to develop new therapeutic strategies against host cell persistence of e.g. *S. aureus*.

P-II-022

Changes in the host ubiquitination machinery as response to S. aureus infection

*A. K. Singh[1], O. Plöhn[1], S. Holtfreter[2], K. Becker[1], B. M. Bröker[2], C. Cammann[1], U. Seifert[1]
[1]*University Medicine Greifswald, Friedrich Loeffler-Institute of Medical Microbiology, Greifswald, Germany*
[2]*University Medicine Greifswald, Institute of Immunology, Greifswald, Germany*

Introduction

S. aureus is an important bacterial pathogen that causes a wide range of clinical manifestations. Post-translational modifications such as ubiquitination regulate host cell recognition of *S. aureus* by pattern recognition receptors and their respective downstream signalling pathways. Ubiquitination affects protein function, stability and localization, and is facilitated by ubiquitinating enzymes known as E3 ligases. Our aim was to analyse a selection of E3 ligases thought to be involved in regulating host cell responses to *S. aureus* infection.

Method

Macrophages are the frontline defenders of the innate immune response and are crucial in combating infections caused by *S. aureus*. To study the host cellular response, we infected THP-1 macrophage-like cells with different *S. aureus* strains and analysed E3 ligase expression at protein level via immunoblotting and RNA using qPCR. The role of selected E3 ubiquitin ligases was further investigated in the context of bacterial infection using the CRISPR-Cas9 knockout technique.

Results

This study revealed changes in the expression levels of selected E3 ligases in response to *S. aureus* infection in THP-1 cells. Furthermore, these changes could be confirmed by exposure of the cells to specific virulence factors such as staphylococcal protein A. In addition, an altered cytokine production was observed, suggesting a dynamic interplay between the virulence factors of the pathogen and the host immune response. Currently, the function of specific E3 ligases is analysed with respect to their impact on cellular signalling pathways, cell survival and cytokine production by comparing CRISR-CAS9-mediated knockout cells with their wild-type counterparts.

Discussion

In conclusion, our findings highlight the intricate molecular mechanisms involved in the ubiquitin-mediated host-pathogen interaction and provide potential approaches for targeted therapeutic intervention. Based on our results it will be of interest to deepen the knowledge on the function of selected E3 ligases. Furthermore, the observed changes in E3 ligase activity and cytokine profiles provide valuable insights into *S. aureus* pathogenicity.

P-II-023

Production and characterisation of monoclonal antibodies generated against pneumococcal proteins

F. Weber[1], S. Weber[2], B. Köllner[2], *S. Hammerschmidt[1]
[1]*Universtiy Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany*
[2]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Institute of Immunolgy, Greifswald, Germany*

Introduction Characterization of host-pathogen interactions of *Streptococcus pneumoniae* is important as this pathobiont poses still a danger to human health (1) Monoclonal antibodies (mAb) specific for bacterial proteins are very suitable for precise and reliable microbial and infection-biological studies (2)

Goals. The aim was to establish monoclonal antibodies specific for mainly surface-associated pneumococcal proteins to investigate the interplay between the pathogen and host in detail.

Material and Methods. Mice were immunised with 73 purified recombinant pneumococcal proteins and the obtained hybridomas were screened stepwise in an indirect ELISA using three different antigen preparations: (A) whole pneumococci and pneumococcal lysate; (B) the purified recombinant pneumococcal proteins divided into groups and (C) single proteins according to the positive reaction in step B to enlighten the antibodies' specificity.

Results. So far, mAb specific for >20 different pneumococcal proteins were identified. A high number of them recognise specifically surface-associated proteins of which 10 are specific for lipoproteins, 3 for sortase-anchored proteins, 2 each for non-clasical surface proteins (NCSP) and choline-binding proteins (CBPs) as well as for 4 non-classified proteins. Two mAb are directed against intracellular proteins. A large proportion of the hybridomas created have to be screened in follow up studies.

Summary. The established stepwise screening strategy enables a quick and precise determination of mAb specificity in a targeted and reliable manner. The established mAb will contribute to elucidate the functions of pneumococcal proteins in metabolism and host-pathogen-interactions.

References:

1. Mehr, S. & Wood, N. *Streptococcus pneumoniae* – a review of carriage, infection, serotype replacement and vaccination. *Paediatr. Respir. Rev.* **13**, 258–264 (2012)
2. Nelson, P. N. Demystified ...: Monoclonal antibodies. *Mol. Pathol.* **53**, 111–117 (2000)

P-II-024

Chlamydia-like Bacterium *Simkania negevensis* exploits host sphingolipids for infection and progeny formation

J. D. Weinrich[1], F. Schumacher[2], M. Rühling[1], B. Kleuser[2], *A. Mohanty[1], V. Kozjak-Pavlovic[1]
[1]*Julius-Maximilians-Universität Würzburg (JMU), Chair of Microbiology, Würzburg, Germany*
[2]*Free University of Berlin, Berlin, Germany*

The obligate intracellular pathogen *Simkania negevensis* (Sne) resides within a Simkania Containing Vacuole (SnCV), closely interacting with the host endoplasmic reticulum and mitochondria [1]. It is dependent on host metabolites, like sphingolipids, due to limited metabolic capacity. To unravel the role of sphingolipids in both Sne infection and its life cycle, our aim was to investigate the involvement of mitochondrial sphingolipids in Sne infection and examine the trafficking of ceramide among host cell organelles, plasma membrane, and SnCV.

To check the mitochondrial status, Mitochondria-ceramide transport protein expression (Mito-CERT), altering CERT to target mitochondria, was performed. Despite cell survival, this modification had no effect on Sne infection. Subsequently, we screened various inhibitors of sphingolipid metabolism and employed knockout cell lines to identify key factors for Sne survival. We showed that, unlike *Chlamydia trachomatis*, Sne does not depend on *de novo* ceramide synthesis or CERT. However, the synthesis of sphingomyelin from ceramide by sphingomyelin synthases 1/-2 is necessary for successful infection. Acid sphingomyelinase and acid ceramidase are crucial as well, indicating the role of lysosomes and the salvage pathway. Sphingolipidome analyses in two cell lines showed a significant change in the ratio between sphingomyelin and ceramide in favor of ceramide, as well as the increase of sphingosine-1-phosphate in infected cells.

Currently, our focus is on whether Sne, similar to Chlamydia, serves as a platform for synthesizing sphingomyelin or ceramide within the host cell. Additionally, we aim to explore the role of lysosomal sphingolipid and cholesterol trafficking, as well as cholesterol modification, in Sne infection.

1.Kunz TC, Kozjak-Pavlovic V. Diverce Facets of Sphingolipid Involvement in Bacterial Infections, Front Cell Dev Biol. 2019; 7:203. doi: 10.3389/fcell.2019.00203
2.Derré I, Swiss R, Agaisse H. The Lipid Transfer Protein CERT Interacts with the *Chlamydia* Inclusion Protein IncD and Participates to ER-*Chlamydia* Inclusion Membrane Contact Sites, PLoS Pathog. 2011; 7(6): e1002092 doi:10.1371/journal.ppat.1002092

P-II-025

Streptococcus pneumoniae **and** ***Staphylococcus aureus*** **toxins can trigger alteration in oxidation metabolism and induce procoagulant state in platelets**

*A. Ozhiganova[1], K. Jahn[2], M. Chatterjee[3], S. Hammerschmidt[1]
[1]*Universtiy Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany*
[2]*University of Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany*
[3]*University of Tübingen, Department of Pharmacology, Experimental Therapy and Toxicology, Tübingen, Germany*

Introduction. Platelets actively fight infections by producing antibacterial substances, enhancing immune activity, and form clots to prevent bacterial dissemination. Previous studies have observed platelet activation following incubation with various bacterial toxins (1,2) that might potentially result in transition to a procoagulant state promoting thrombus formation.

Goals. We hypothesized that under sublytic conditions *S. pneumoniae* proteins pneumolysin (Ply) and *S. aureus* proteins a-hemolysin (Hla), EapD3D4, CHIPS, FlipR, and AtlA trigger reactive oxygen species (ROS) generation in platelets, leading to increased lipid oxidation to favour procoagulant activation.

Methods. To test this hypothesis, toxins were incubated with washed platelets. ROS (DCFH2-DA), and mitochondrial superoxide (MitoSOX) generation, intraplatelet oxidized low-density lipoprotein-(anti-oxLDL-FITC) levels, platelet activation (degranulation-CD62P), and phosphatidylserine (PS; Annexin V) exposure were measured via flow cytometry.

Results. While all proteins increased platelet activation, toxins Ply and Hla preferably induced PS exposure substantiating a procoagulant state, along with increased ROS generation. Superoxide production also increased in the presence of toxins and *S. aureus* EapD3D4. Intraplatelet oxLDL levels were enhanced following incubation with CHIPS.

Summary. Our data suggest that Ply and Hla toxins turn platelets procoagulant without implying nonenzymatic lipid oxidation. Staphylococcal proteins activated platelets but did not induce a procoagulant state. Notably, CHIPS and EapD3D4 induced intraplatelet lipid oxidation without inducing a procoagulant phenotype. Such differential impact of bacteria and bacterial toxins may prevent bacterial dissemination, but could also contribute to increased organ damage through platelet derived oxidized (phospho)lipids and procoagulant platelet-assisted thrombosis, necessitating further research in this direction.

References:

1. Li, C., Li, J., & Ni, H. (2020). Frontiers in Immunology, 11, 1962.
2. Jahn, K., T.P. Kohler, L.S. Swiatek, S. Wiebe, and S. Hammerschmidt. 2022. Cells 11(7):1121.

P-II-026

Neutralising Antibodies and Avidity to SARS-CoV-2 spike protein in patients undergoing haemodialysis

*R. Götte[1], M. Fladerer[1], L. Huth[1], L. Schäfer[1], G. Almanzar[1], T. Stövesand[2], C. Drechsler[2], M. Prelog[1]
[1]*University Hospital Würzburg, Pediatric Clinic, Würzburg, Germany*
[2]*Kuratorium für Heimdialyse, Würzburg, Germany*

Introduction:
Patients undergoing haemodialysis (HD) have been recommended to receive booster doses of mRNA vaccines against SARS-CoV-2 as part of the group of persons at risk for severe sequelae of coronavirus disease (Covid-19).

Question:
The aim of this study was to longitudinally analyse the humoral immune response to spike protein and the impact breakthrough infections (BTI) in HD.

Materials & Methods:
82 HD and 38 healthy controls (HC) had received a total of 3 to 5 mRNA vaccinations. Blood samples were taken before and at 2 and 4 weeks and at 3, 6 and 9 months after vaccination. The concentration anti-spike-IgG (binding antibody units, BAU/ml) and the relative avidity index (%) were assessed by adaption of a commercially available ELISA assay using thiocyanate as chaotropic reagent.

Results:
HD who had received a 5th vaccination 6 months after the 4th vaccination showed a higher anti-spike-IgG (mean 3335,35 BAU/ml) after 6 months compared to HD who had received only 4 vaccinations and were evaluated at time point 12 months after the 4th vaccination (2344,83 BAU/ml). The RAI remained similar between HD who were vaccinated five times and those who had 4 vaccinations (76,64% versus 79,33%) with no significant difference compared to HC (78,08%). HD with BTI in the last 6 months showed 1,57-fold higher anti-spike-IgG compared to those without BTI.

Conclusion:
The results revealed an immunological benefit of a fifth vaccination for HD patients by higher anti-spike-IgG concentrations. Hybrid immunity by BTI was able to further induce anti-spike-IgG. The findings corroborate the recommendation for repeated COVID19 mRNA vaccinations in HD patients.

P-II-027

Pre-existing SARS-CoV-2 spike-specific T-helper cells and spike-induced cytokine secretion after BNT162b2 booster vaccination determine sustained humoral and cellular immune responses

*L. Page[1], K. Dennehy[1], K. Müller[2], P. Girl[2], H. Buijze[1], E. Löll[1], J. M. Classen[3], H. Messmann[3], C. Römmele[3], R. Hoffmann[1], A. Fuchs[3], S. Wurster[4]

[1]University Hospital Augsburg, Institute for Laboratory Medicine and Microbiology, Augsburg, Germany
[2]Bundeswehr Institute of Microbiology, München, Germany
[3]University Hospital Augsburg, Internal Medicine III - Gastroenterology and Infectious Diseases, Augsburg, Germany
[4]The University of Texas MD Anderson Cancer Center, Department of Infectious Diseases, Infection Control, and Employee Health, Houston, TX, United States

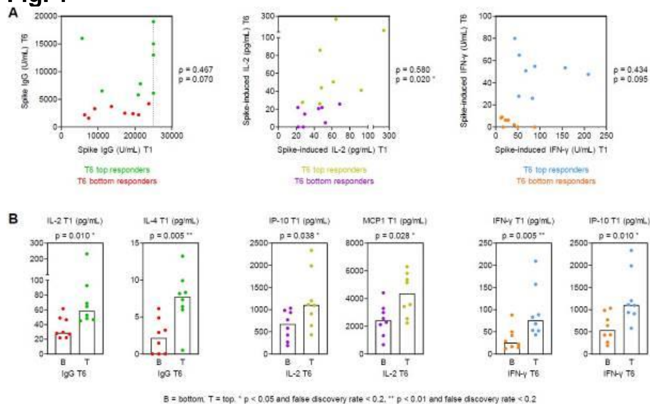
Question: SARS-CoV-2 spike (S) specific T-cell and antibody (AB) responses after primary BNT162b2 vaccination partially depend on the phenotypic composition of pre-existing T-helper (Th) cell populations (Saggau et al., 2022). However, adaptive cellular responses and their impact on sustained immune protection after booster vaccination are incompletely understood. We monitored cellular and humoral immune responses before and up to 6 months after the 3rd BNT162b2 vaccination to identify early determinants of sustained responses.

Methods: Blood of 30 healthy subjects was collected before (T0), and 1 and 6 months after (T1, T6) their 3rd BNT162b2 vaccination. Serum samples were tested for nucleocapsid (N), S, and neutralizing AB responses. Subjects developing N IgG or those with incomplete follow-up were excluded. Whole blood was stimulated with S peptides (0.6 nMol/peptide) as previously described (Weis et al., 2020, Lauruschkat et al., 2021) and subsequently analyzed by flow cytometry (CCR7, CD3, CD4, CD45RO, CD69, CD154, IFN-γ) and 13-plex multiplex cytokine assays. Significant associations between readouts were identified by multiple testing-adjusted rank correlation analysis.

Results: We found moderate correlation between S IgG (r=0.47), S-induced IL-2 (r=0.58) and IFN-γ (r=0.43) at T1 and T6 (Fig. 1A). When comparing the top and bottom half of S IgG responders at T6, sustained robust IgG responses were significantly associated with S-specific ($CD69^+ \pm CD154^+ \pm IFN\text{-}\gamma^+$) Th-cell frequencies at T0 ($p=0.038$), especially multifunctional type-1 Th cells. Furthermore, S IgG, S-induced IL-2, and S-induced IFN-γ at T6 were significantly associated with increased IL-2 & IL-4, IP-10 & MCP1, and IFN-γ & IP-10 levels at T1, respectively (Fig. 1B). Using support vector machine models, pre-booster S-specific T-cell frequencies and cytokine responses at T1 predicted T6 responses with F1 scores of 0.82-1.00.

Conclusions: Pre-existing S-specific Th cells and T-cellular cytokine signatures shape sustained adaptive cellular and humoral responses after BNT162b2 booster vaccination. Functional T-cell assays might facilitate early identification of potential non-responders.

Fig. 1



P-II-028

Physiological significance of bacterial RNA recognition by the innate immune system

*L. Findeis[1], L. S. Chen[2], A. Dalpke[2]
[1]Technical University of Dresden, Institute for Medical Microbiology and Hygiene, Dresden, Germany
[2]Heidelberg University Hospital, Medical Microbiology and Hygiene, Heidelberg, Germany

Introduction: Bacteria express various pathogen-associated molecular patterns which are recognized by pattern recognition receptors (PRR). PRRs are expressed throughout all immune cells and divided into different groups such as Toll-like receptors (TLR). TLR8 is a sensor of single-stranded RNA, sensing RNA degradation products from viruses or bacteria. It is highly expressed by monocytes, yet its contribution to recognition of bacteria is poorly understood. Goals:The aim of this study was to examine the role of TLR8 for the recognition of whole, viable bacteria in human cell line, thus analysing the importance of bacterial RNA/TLR8 recognition when, during infection, a plethora of different PAMPs is present. The study explored the question of non-redundancy of bacterial RNA mediated TLR8 stimulation. Method:Experiments were conducted with WT and TLR8-defecient BLaER1 cells, which can be transdifferentiated into monocytes. WT and knock-out (KO) cells were infected with different living bacteria. After 1 hour of infection, bacteria were killed by gentamycin and cells were cultivated for another 15 hours. Secreted cytokines in supernatants were quantified. In parallel, we conducted live infections in primary human PBMC pre-treated with the TLR8 inhibitor CU-CPT9a. Results:We identified several bacteria that showed reduced cytokine secretion when TLR8 was missing (BLaER1 KO) or inhibited (PBMCs). Most of them were Gram-positive bacteria including *Bacillus cereus.* Further, we found *Mycoplasma pneumoniae* to be dependent on TLR8, indicating that bacterial RNA is a significant PAMP. Reduction in cytokine production in KO cells showed a wide range with some bacteria showing almost no cytokine induction at low MOIs. Gram-negative bacteria, usually recognized via TLR4, surprisingly showed dependence on TLR8 in cases like *Haemophilus influenzae.* Conclusion: Altogether this highlights the importance of TLR8 for recognition of whole bacteria regardless of the Gram strains and its importance in innate immune response. The study identifies good candidates for further research on bacterial RNA recognition during infection to unravel the complexities of host-pathogen interaction.

P-II-029

Cross-sectional assessment of T cellular SARS-CoV-2 immunity following a fourth COVID-19 vaccination using a whole blood-based IFNg release assay

*J. Mees[1,2], J. Rätz[3], I. Wagenhäuser[1,4], J. Reusch[1,4], A. Gabel[1], N. Petri[4], B. Weißbrich[3], L. Dölken[3], K. Knies[3], N. Beyersdorf[3], M. Krone[1,5]
[1]University Hospital Würzburg, Infection Control and Antimicrobial Stewardship, Würzburg, Germany
[2]University Hospital Würzburg, Pediatric Rheumatology/Special Immunobiology, Würzburg, Germany
[3]University of Würzburg, Institute for Virology and Immunobiology, Würzburg, Germany
[4]University of Würzburg, Department of Internal Medicine I, Würzburg, Germany
[5]University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany

Question

In addition to innate immunity, adaptive immunity, i.e. T and B cells substantially contribute to the control of viral infections with e.g. SARS-CoV-2. Interferon-gamma (IFNγ) serves a central function in T cell-mediated immunity. To date, a limited number of studies have investigated the factors influencing T cell reactivity to SARS-CoV-2 in exposed, COVID-19-vaccinated, healthcare workers (HCWs).

Methods

For this cross-sectional analysis, as part of the longitudinal CoVacSer study, blood samples with a paired study questionnaire were collected in September 2023 from 113 HCWs who had already received at least four COVID-19 vaccinations. T cell reactivity was assessed using the Elecsys® IFNγ Release Assay (IGRA), for which a SARS-CoV-2 peptide mix was used as Antigen (Ag) to stimulate T cells. A negative control (NC) without Ag was used to determine background release of IFNγ. The T cell reactivity was assessed by calculating Ag-NC in IU/ml. Additionally, anti-SARS-CoV-2-Spike IgG levels were measured using an Enzyme-linked Immunosorbent Assay (ELISA).

Results

The T-cell reactivity analysed in the study showed a median of 0.75 Ag-NC (IU/ml) [IQR: 0.32-1.28 Ag-NC (IU/ml)]. Univariate analyses showed that T-cell reactivity was significantly higher in individuals who had been infected at least once (p=0.049), had no regular patient contact (p=0.005), or were non-smokers (p=0.026). No significant difference was found for gender (p=0.645). IgG levels (r=0.152, p=0.110), age (r=-0.174, p=0.855), and BMI (r=0.110, p=0.248) were nor significantly correlated with T-cell reactivity. Infection was identified as the only significant influencing factor in a multivariable regression including the variables age, BMI, smoking, infection and patient contact (p=0.044).

Conclusion

As expected, past infection with SARS-CoV-2 was associated with increased T-cell reactivity. HCWs without regular patient contact showed a higher T-cell reactivity because the number of infections was higher in this group. Lower T-cell activity in smokers was probably caused by a combination of slightly lower T-cell responses after vaccination and the lower SARS-CoV-2 infection rate.

P-II-030
The influence of postnatal infectious challenge on enteric tissue maturation

S. Schlößer[1], A. L. Jürgens[1], N. Modares[1], I. Richter[1], M. Schmitz[1], K. Zhang[1], U. Repnik[2], U. Rolle-Kampczyk[3], M. von Bergen[3], *A. Dupont[1], M. Hornef[1]
[1]*RWTH Uniklinik Aachen, Institute of Medical Microbiology, Aachen, Germany*
[2]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Faculty of Mathematics and Natural Sciences, Kiel, Germany*
[3]*Helmholtz Centre for Environmental Research (UFZ), Department of Molecular Systems Biology, Leipzig, Germany*

Gastrointestinal infections caused by *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) remain a major health problem for neonates and young infants worldwide. A better understanding of the mechanisms responsible for disease development and progression during the postnatal period is crucial to improve therapeutic options. Here, using our murine neonatal *Salmonella* infection model, we analysed the gene, protein and metabolic expression profiles of *S.* Typhimurium-infected and healthy age-matched wild type and gene-deficient newborn mice. Those analyses revealed alterations of the proliferative and metabolic activities as well as of the antimicrobial peptide repertoire of epithelial cells in infected animals. Several target genes exhibited expression levels comparable to those usually observed in more mature animals. Those alterations were not observed in infected newborn mice lacking MyD88 signalling in their hematopoietic cell compartment. Stimulation of 3D intestinal organoids with various cytokines produced by intestinal immune cells could in part recapitulate the phenotype. Together, our results suggest that *S.* Typhimurium infection induces an accelerated maturation of the newborn small intestinal epithelium *via* an immune cell-mediated mechanism. Our work is expected to gain further insight into the short- and long-term consequences of neonatal enteric infections on intestinal homeostasis and disease susceptibility. Additionally, our results might identify therapeutic targets to improve the clinical outcome of infants upon gastrointestinal infection.

Microbial Cell Biology

P-MCB-001
CRISPR-Cas Spacer Integration in a type III-A system

*M. Seimel[1], S. Willkomm[1], D. Grohmann[1]
[1]*University Regensburg, Microbiology and Archaea Center, Regensburg, Germany*

Adaptive immunity is an important asset to compete in the universal arms race of evolution. Prokaryotes possess an adaptive immune system in the form of clustered regularly interspaced short palindromic repeats (CRISPR) and their CRISPR-associated (Cas) proteins. New immunological memories are saved in the form of spacers in between repeats of the CRISPR array. The key components to acquire new spacers include a Cas1-2 complex, a CRISPR array comprising a leader sequence with at least one repeat and often auxiliar proteins like IHF. In the type I-E system of *Escherichia coli*, IHF binds within the leader junction and bends the CRISPR array in a sharp U-Turn. This interaction specifies correct spacer integration. *Thermotoga profunda* encodes a III-A Cas1-2 complex and HU, a protein similar to IHF. Both proteins belong to the type II DNA-binding protein family and have a highly conserved structure. IHF and HU-like proteins are typically 10 kDa in size, share an α helical core with two positively charged β-ribbon arms and form a dimer. We demonstrate specific integration of new spacers only in the presence of the complex and TpHU. Additionally, TpCas1-2 has low intrinsic specificity and TpHU is necessary to avoid ectopic integration. Our results enhance the current state of knowledge as HU proteins were previously not associated with CRISPR-Cas spacer integration.

P-MCB-002
Illuminating the two oscillators of Synechocystis sp. PCC 6803 using backscatter measurements

*F. P. Stirba[1], A. Wiegard[1], N. M. Schmelling[1], A. Pawlowski[1], K. N. Sebastian[2], L. C. Berwanger[1], N. Thumm[1], A. Wilde[2], I. M. Axmann[1]
[1]*Heinrich Heine University Düsseldorf, Institute for Synthetic Microbiology, Düsseldorf, Germany*
[2]*University of Freiburg, Institute of Biology III, Freiburg i. Br., Germany*

Circadian clocks are a mechanism that evolved in diverse groups of species. Although their details differ, their purpose is always the same. Through a central oscillator with a period of ~24 hours, daily changes in the environment due to the diurnal cycle can be anticipated. This 24-hour rhythm is robust, even in varying temperature conditions, and can be reset by external stimuli. In the cyanobacterial clock model *Synechococcus elongatus* PCC 7942 the central oscillator consists of the proteins KaiA, KaiB and KaiC. Through rhythmic phosphorylation and dephosphorylation of KaiC, this oscillator regulates a multitude of cellular processes. *Synechocystis* sp. PCC 6803 possesses orthologs of this core oscillator but additionally exhibits two paralogs of KaiB and KaiC. Our recent findings have uncovered a novel chimeric KaiA-like protein (KaiA3) which completes the KaiB3-KaiC3 oscillator[1]. In a previous study, our group could show that backscatter measurements can detect oscillations in the growth signal of *Synechocystis* that follow the circadian rhythm[2]. To investigate the newly discovered KaiA3-KaiB3-KaiC3 oscillator and its influence on the circadian rhythm of *Synechocystis*, we used online backscatter measurements of several knockout mutants of the *kai* genes. Our results show that knockouts of *kaiC3* and *kaiA3* lead to reduced amplitude and shifted phase whereas knockout of *kaiB3* almost completely abolishes oscillation. These results suggest a connection between the two oscillators of *Synechocystis* which indicates that bacterial circadian clocks may be more complex than previously thought. Since *kai* homologs were detected in several prokaryotes including archaea, the impact of these results may reach beyond cyanobacteria.

1. Köbler, C. *et al.* Two circadian oscillators in one cyanobacterium. *bioRxiv preprint* (2023) doi:10.1101/2021.07.20.453058.
2. Berwanger, L. C. *et al.* Self-sustained rhythmic behavior of Synechocystis PCC 6803 under continuous light conditions in the absence of light-dark entrainment. *bioRxiv preprint* (2023) doi:10.1101/2023.09.26.559469.

P-MCB-003
Vibrios with fused chromosomes

*L. Abendroth[1], M. Runge[1], B. Bunk[2], R. L. Hahnke[2], C. Spröer[2], J. Overmann[2], T. Waldminghaus[1]
[1]*TU Darmstadt, Molecular Microbiology, Darmstadt, Germany*
[2]*German Collection of Microorganisms and Cell Cultures GmbH, Brunswick, Germany*

The genetic content of most bacteria is inherited in a single chromosome. However, bacteria of the Vibrionaceae family encode their genetic information on two separate chromosomes. While studying the two-chromosome system in *Vibrio*, three natural single-chromosome *Vibrio* strains (NSCV) were discovered. In these strains chromosomes are stably fused and do not revert into the two-chromosomal state. The genetic mechanism underlying the unidirectional chromosomal fusion events and how the fusion is stably maintained remain unknown.

Recombination between homologous sequences in the chromosome of NSCV strains can potentially lead to the separation of the fused chromosomes. To test whether NSCV strains are still able to perform homologous recombination, we treated NSCVs with UV-irradiation and Mitomycin C. These agents cause DNA double strand breaks, which are repaired via homologous recombination. Our results show that NSCV strains overcome this treatment indicating recombination proficiency. Although proven to be capable of homologous recombination, pulsed-field gel electrophoresis detected no division of the fused chromosome in NSCV clones irradiated with elevated UV doses.

To find more natural occurring examples of fused chromosomes to establish general "fusion rules", we screened 180 strains of the Vibrionaceae via PacBio sequencing. The analysis of new NSCV genomes could reveal specific genetic characteristics which might explain the locked single-chromosomal state.

P-MCB-004
Inhibitors targeting LPS biosynthesis have diverse antimicrobial effects

*L. J. Tilg[1], A. M. Möller[1], F. A. Kraft[1], B. Kutscher[1], F. Narberhaus[1]
[1]*Ruhr-University Bochum, Biology of microorganisms, Bochum, Germany*

The balance between phospholipids and lipopolysaccharides (LPS) in the outer membrane (OM) of Gram-negative bacteria is crucial for viability. Since an imbalance in OM homeostasis can lead to cell death, the LPS biosynthesis pathway is a promising antibiotic target for difficult-to-treat Gram-negative bacteria. The first enzyme in the LPS pathway is LpxA, which utilizes acyl-ACP and UDP-N-acetylglucosamine. Since this reaction is bidirectional, LpxC, the second enzyme in the pathway constitutes the first committed step. LPS levels are adjusted by tightly controlled FtsH-mediated proteolysis of LpxC. While LpxC leads to a deacetylation of UDP-N-acetylglucosamine, LpxD is responsible for adding the second fatty acid chain. The next synthesis step involves LpxH, which is the first membrane-bound protein. After the assembly of Kdo2-Lipid A by five other membrane-bound proteins and the attachment of the core oligosaccharides, the LPS precursor molecule is transported into the periplasmic space by the ABC transporter MsbA. Finally, the O-antigen is attached to the molecule and LPS is translocated into the outer layer of the outer membrane by the Lpt-complex.

To gain insights into the mode of action of inhibitors targeting five different enzymes in the LPS pathway, we conduct a comparative phenotypic analysis. Treatment of *E. coli* with these compounds changed the cell shape and the viability in different ways. Whereas inhibitors of early steps of LPS synthesis and the Lpt-complex led to cell lysis, the LpxH inhibitor and the MsbA inhibitor did not. Interestingly, LpxC, LpxA and MsbA inhibition sensitized *E. coli* to cell wall antibiotics, which typically do not cross the outer membrane, indicating an imbalance of the LPS to phospholipid ratio.

Our study shows that in addition to well-studied LpxC inhibitors, compounds targeting earlier or later steps of LPS synthesis deserve further attention for the development of antibiotics.

P-MCB-005

A hexameric ribonuclease - the decision maker of survival?

*M. Meiser[1]
[1]*Philipps Universität Marburg, Chemistry, Marburg, Germany*

All organisms, including bacteria, need to be able to adapt quickly to changes in their environment in order to ensure their survival. An important step of regulation takes place at the level of RNA, what requires key players like Ribonucleases and RNA-binding proteins.

Ribonucleases (RNases) and RNA-binding proteins (RBPs) play a crucial role in all kingdoms of life and provide a level of regulation by catalyzing or influencing the degradation and maturation of RNA. RNases are mainly divided into two different groups, meaning that they catalyze the hydrolysis of RNA from the inside of the molecules (endoribonucleases) or the outside (exoribonucleases). Currently 21 ribonucleases in the well-studied soil bacterium *Bacillus subtilis* are known.

Here we present a hexameric nucleolytic enzyme which might hve a global role in cell differentiation in *B. subtilis*. Our protein of interest is described as ribonuclease and is characterized by its unique domain structure, containing a C-terminal OB domain (oligonucleotide binding) and a N-terminal HD (metal dependend phosphohydrolase) domain. The specific function of this RNase in *B. subtilis* is currently unknown.

Our preliminary *in vivo* and *in vitro* analysis of this hexameric enzyme indicate a regulatory role in cell differentiation in *B. subtilis*.

P-MCB-006

Infection of cornea models with different Neisseria gonorrhoeae derivatives to study the importance of the Type IV pilus for bacterial adherence and infection outcome

*L. M. Kafuri Cifuentes[1], N. Knetzger[2], C. Lotz[2], T. Rudel[1], V. Kozjak-Pavlovic[1]
[1]*Julius-Maximilians-University of Würzburg, Microbiology, Würzburg, Germany*
[2]*Fraunhofer, Translational Center Regenerative Therapies, Würzburg, Germany*

Neisseria gonorrhoeae (Ngo) is a human obligate pathogen and is the main cause of *ophthalmia neonatorum*, a type of conjunctivitis in newborns of women infected with Ngo, which untreated can lead to blindness (1). One of the most important virulence factors of Ngo is the Type IV pilus, filamentous protein structures that facilitate the adhesion of the bacteria to the target tissue. In this project, we studied the importance of the Type IV pilus for Ngo adherence, infection and its outcome using cornea tissue models (2). We infected the models with MS11 Ngo derivatives: MS11 F3 (Pili+, RecA+), MS11 N159 (Pili+, RecA-, Adh+cornea) and MS11 N191 (Pili+, RecA-, Adh-cornea). We observed no significant difference in the tissue integrity between the non-infected and infected models. Measurements of the cell death by LDH method showed that the highest cytotoxicity was present after 72 h of infection in models infected with MS11 N159 Ngo. The bacterial adherence was also the highest for N159, which could be confirmed using fluorescence microscopy and SEM. Lastly, to analyze the outcome of the infection, the concentration of different important cytokines for infection was measured. IL-8 was the most secreted cytokine and was the highest in the samples infected with MS11 N159 for 72 h, suggesting a specific response that could be possibly receptor-dependent. These results show the importance of the functional Type IV pilus for the infection of the cornea tissue by Ngo, and its role not only for bacterial adherence but also on the outcome of the infection.

1. Tjia, K., van Putten, J., Pels, E., & Zanen, H. (1988). The interaction between Neisseria gonorrhoeae and the human cornea in organ culture. *Ophtalmology*, 341-345.
2. Lotz, C., et al. (2016). Alternative methods for the replacement of eye irritation testing. *ALTEX - Alternatives to animal experimentation*. 55-67.

P-MCB-007

Influence of an SPFH protein on developmental programs in Bacillus subtilis

*S. Baur[1], U. Repnik[2], L. Rau[1], A. Mondry[1], A. Savietto-Scholz[1], M. Bramkamp[1]
[1]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for General Microbiology, Kiel, Germany*
[2]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Central microscopy, Kiel, Germany*

As a soil dwelling bacterium *Bacillus subtilis* is exposed to constant, often life-threatening changes in conditions. To manage these challenges, the bacterium employs alternative σ-factors, activating a response that enables cellular adaptation to environmental stressors. Membrane-associated proteins play a crucial role in perceiving these environmental changes, with one notable complex being PspA-YdjGHI, housing the SPFH (Stomatin/Prohibitin/Flotillin/HflK-C) protein, YdjI (1). Members of this widely conserved family are associated with regulation of membrane fluidity (2,3) and are known for their impact on a variety of membrane bound processes, including developmental processes in *B. subtilis* (4,5). Here, we show that YdjI is required for efficient motility and development of social communities. Deleting *ydjI* impairs biofilm formation and prevents the formation of the BlsA hydrophobic biofilm coat. We further show that the deletion of *ydjI* leads to an upregulation of the σ^B answer and a downregulation of the *ytrABCDEF* operon. This operon encodes for an ABC transporter of unknown function, its own regulator, and the FtsX-like membrane associated protein YtrF (6). Deletion of *ytrF* phenocopies the biofilm defects observed for the *ydjI* deletion. Interestingly RNA Seq of biofilms of *ydjI* and *ytrF* mutants revealed an induction of gene clusters associated with σ^B activation. Together, our data strongly suggest that the biofilm related phenotypes are caused by a defect during the initiation of differentiation that likely originates from the general stress response due to energy starvation.

(1) Savietto Scholz, A., et al. (2021) Front. Microbiol. 12: 754924
(2) Bach, J.N. et al. (2013) Mol. Micro. 88:1205-1217
(3) Zielińska, A., et al. (2020) Elife 9:e57179
(4) Yepes A, et al. (2012) Mol Microbiol. 86(2):457-471
(5) Dempwolff F, et al. (2012) J Bacteriol. 194(17):4652-61
(6) Benda M, et al. (2021) Front Microbiol. 12:587035

P-MCB-008

Activities of ADEP derivatives on prokaryotic and eukaryotic ClpP

*Y. Thoma[1], H. Brötz-Oesterhelt[1]

[1]Universtiy Tübingen, Mikrobielle Wirkstoffe, Tübingen, Germany

With the increase and spread of bacterial resistance, the importance of discovering and developing new antimicrobial compounds has reached a new level. One of the more recent antibacterial targets is the caseinolytic serine protease, which emerged as the target structure of a new class of antibiotics named acyldepsipeptides (ADEP). ADEP has a unique dual mode of action, activating the proteolytic core ClpP for non-regulated proteolysis and disrupting the natural interaction of ClpP with its associated ATPases, abolishing all natural functions of the protease. In its natural function, Clp protease maintains cellular homeostasis and plays a key role in various stress responses. In the presence of ADEP this balance is severely disturbed. Natural substrates accumulate while other crucial proteins are degraded in an ATP-independent, unregulated fashion. When bound to ADEP, ClpP wreaks havoc in the cell, overwhelming its coping mechanisms and ultimately leading to cell death. ClpP is conserved not only in prokaryotes but also in eukaryotes, where it is found in chloroplasts and mitochondria but not in the eukaryotic cytoplasm. Like its bacterial homolog, mitochondrial ClpP has important functions in eukaryotic cell homeostasis, such as protein quality control and regulation of several stress responses, including the mitochondrial unfolded protein response (UPRmt). Recent studies have shown that ClpP is upregulated and essential for proliferation and metastasis in several cancer cell types, making ClpP not only interesting as an antimicrobial target but also as a target for anti-cancer therapy. Here, we report on the effects of ADEP on mitochondrial ClpP compared to bacterial ClpP. ADEP can dysregulate mitochondrial ClpP in vitro and enter the eukaryotic cell, but its impact on the physiology of eukaryotic cells has not been studied at the molecular level. Different ADEP derivatives are compared, to determine the structure-activity relationship, and assessed side-by-side with a ClpP activator from the structurally unrelated imipridone class. We aim at increasing our understanding about the physiological role of mitochondrial ClpP and how ADEP affects its function.

P-MCB-009
Characterization of the trans-translation inhibitors KKL-35 and KKL-588

*F. Demirbas[1], M. Vázquez-Hernádez[1], J. E. Bandow[1], K. Keiler[2]
[1]*Ruhr University Bochum, Department of Applied Microbiology, Bochum, Germany*
[2]*Pennsylvania State University, Department of Biochemistry and Molecular Biology, Pennsylvania, PA, United States*

The worldwide rise in multidrug resistant pathogens and the lack of new antibiotics for clinical use with novel targets or mechanisms constitutes a major medical crisis. *trans*-Translation, the main ribosome rescue mechanism in all bacteria, is a promising target (Keiler, 2008). Inhibitors of this process include synthetic compounds, that show a broad spectrum of activity against gram-positive and gram-negative bacteria, including *Bacillus anthracis* or *Escherichia coli* Δ*tolC (*Ram*adoss* et al., 2013*).* We found that complexation of these *trans*-translation inhibitors with Cu(II) can lead to potentiation of the antibacterial effects in *Bacillus subtilis* and *Escherichia coli* Δ*tolC.* Here, we report the antibacterial activity of KKL-35 and KKL-588 against *E. coli* Δ*tolC* as determined by minimal inhibitory concentration (MIC) assays and growth experiments. Complex formation of KKL-35 and KKL-588 with Cu(II) was characterized using absorption spectra, Jobs plots, hydrophobicity and binding affinity assays. Both compounds have high binding affinities for Cu(II) of 3.01·10-14(KKL-588) and 7.17·10-25 (KKL-35), with KKL:Cu(II) stoichiometries of 4:1 for KKL-35 and 1:1 for KKL-588. Furthermore, for both compounds hydrophobicity increases when complexed with Cu(II). Treatment of *E. coli*Δ*tolC* with KKL-588 resulted in a significant inhibition of growth. This effect was abolished when simultaneously treating the cells with Cu(II). The mechanism underlying this antagonistic effect of Cu(II) on the anti-*E.coli* activity of KKL-588 remains to be investigated.

Keiler, K.C., 2008. Biology of *trans*-Translation. Annu. Rev. Microbiol. 62, 133–151.

Ramadoss, N.S., Alumasa, J.N., Cheng, L., Wang, Y., Li, S., Chambers, B.S., Chang, H., Chatterjee, A.K., Brinker, A., Engels, I.H., Keiler, K.C., 2013. Small molecule inhibitors of *trans*-translation have broad-spectrum antibiotic activity. Proc. Natl. Acad. Sci. U.S.A. 110, 10282–10287. https://doi.org/10.1073/pnas.1302816110

P-MCB-010
Effects of cell shape changes on Listeria monocytogenes in vitro virulence

*S. Wamp[1], J. Rismondo[1,2], J. Döhling[1], S. Halbedel[1,3]
[1]*Robert Koch-Institute, FG11 - Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany*
[2]*Georg-August University Göttingen, Department of General Microbiology, Göttingen, Germany*
[3]*Otto von Guericke University Magdeburg, Institute for Medical Microbiology and Hospital Hygiene, Magdeburg, Germany*

Introduction: *Listeria monocytogenes* is a foodborne pathogen characterized by its capability to invade and replicate in multiple human cell types and to spread from cell to cell. In addition to known virulence factors, also the absence of the late cell division protein DivIVA leads to severe virulence defects (1, 2). Based on the characteristic cell-chaining phenotype of the *L. monocytogenes divIVA* mutant, the question arose how the cell shape effects *in vitro* virulence. Therefore, we here compare the virulence properties of coccoid and filamentous *mreB* (involved in lateral cell growth) and *ezrA* (involved in Z-ring formation) mutants to the cell-chaining phenotype of the *divIVA* mutant.

Material and Methods: IPTG-dependent *mreB* and *ezrA* depletion strains were constructed and the presence of the expected coccoid and filamentous phenotypes was verified during growth under standard laboratory conditions and in *in vitro* infection experiments. All cell morphotypes were tested for replication, invasion and cell-to-cell spread in various eukaryotic cell lines. Additionally, the expression of actin tails, required for movement and spreading during infection, was analysed by phalloidin staining.

Results: The coccoid and filamentous growth of the *mreB* and *ezrA* depletion strains did neither impair the multiplication in infected macrophages nor did it influence the invasion in human hepatocytes, as opposed to the replication and invasion deficient *divIVA* mutant. On the other hand, filamentous growth of the *ezrA* depletion strain led to reduced plaque sizes compared to the wild type. No cell-to-cell spread could be observed for the coccoid growing *mreB* depletion strain as well as the *divIVA* mutant, which coincided with a reduced abundance or absence of actin filaments.

Summary: Our findings indicate that the cell shape plays a minor role for efficient *in vitro* infection. The severe virulence

defect of the *divIVA* mutant therefore seems to be associated with a DivIVA-dependent influence on other virulence factors or their release via the SecA2 secretion route (1).

1 – Halbedel *et al.* (2012), ***Mol Micro***, 83: 821-839.
2 – Kaval *et al.* (2014), ***Mol Micro***, 94: 637-654.

P-MCB-011
A novel putative DNA damage-repair system Dip in Corynebacterium glutamicum
*E. Karnaukhova[1], L. Griem[1], J. Grund[1], B. Rackow[1], G. Giacomelli[1], M. Bramkamp[1,2]
[1]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for General Microbiology, Kiel, Germany*
[2]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Central Microscopy Facility, Kiel, Germany*

In bacterial species, most types of environmental stresses ultimately result in varying degrees of DNA damage. The bacterial SOS response is a generalized response aimed not only at repairing DNA damage, but also at pausing cell division to avoid segregation of damaged chromosomes. This response is controlled by LexA which, under physiological conditions, represses the expression of SOS genes. In *Corynebacterium glutamicum*, a model organism for the cell biology of *Corynebacterineae* and a biotechnological workhorse, the LexA regulon consists of 48 genes. Apart from genes encoding homologous recombination systems, HNH endonucleases and others, nearly half of the LexA-regulated genes have an unknown function (1). In this project, we identified a new putative SOS-regulated operon in *C. glutamicum* containing the DNA damage-induced proteins (*dipABCD*). Having proven its induction upon exposure to the DNA-damaging compound mitomycin C, we aimed to elucidate the role of Dips in the SOS response and, more specifically, DNA repair. Using a variety of *in vitro* and *in vivo* approaches, we could show that the system comprises a divalent ion-dependent nickase and GTPase, DipA, and an ATP-dependent helicase which binds in proximity to DNA damage, DipD. Moreover, based on the single-molecule localization microscopy, we hypothesized that DipD and DipA are recruited to the DNA damage site sequentially, e.g. the DNA nicking activity of DipA precedes the unwinding activity of DipD. The Dip system as a whole might interact with DivIVA, a polar scaffold for cell elongation (RodA) and chromosome segregation (ParB-*parS*) machineries (2), as evidenced by pull-down assay and SMLM. In summary, our results demonstrate a putative role of the Dip system in DNA damage repair and contribute to the studies of non-canonical DNA repair systems.

1. Jochmann et al., *Microbiology* **2009**, 155.
2. Giacomelli et al., *Genes* **2022**, 13, 278.

P-MCB-012
Biofilm viability assessment with Calcein-AM and TMA-DPH assay in comparison to SYTO9 and PI staining
T. Tchatchiashvili[1], L. Thieme[1], *O. Makarewicz[1], M. W. Pletz[1]
[1]*Jena University Hospital, Institute of Infectious Diseases and Infection Control, Jena, Germany*

Background: Accurate assessment of bacterial cell viability within biofilms is vital for evaluating the efficacy of anti-biofilm agents. A common staining approach for this involves SYTO9 and propidium iodide (SYTO9/PI), however, the application of these probes often leads to an underestimation of biofilm cell viability, attributed to the presence of extracellular DNA within the biofilm, and introduces other notable limitations.

Aims: This study explores whether biofilm viability staining with the dye combination of Calcein acetyl methyl ester (Calcein-AM) plus 1-(4-Trimethylammoniumphenyl)-6-Phenyl-1,3,5-Hexatriene *p*-Toluenesulfonate (TMA-DPH) in comparison to the conventionally used SYTO9/PI combination shows a superior correlation with the Colony forming units (CFU) determination method. Calcein-AM specifically stains metabolically active cells, providing a direct indication of viable cells, while TMA-DPH penetrates all cells and also provides insights into membrane fluidity patterns.

Methods: Biofilms of Gram-positive and Gram-negative bacterial species, treated with antibiotics, were stained with Calcein-AM/TMA-DPH and SYTO9/PI, then imaged using Confocal laser scanning microscopy (CLSM). CFU determination, the gold standard in microbiological viability assessments, was performed on the same biofilms. CLSM images were analyzed using an ImageJ-based biofilm analysis tool, comparing data to respective CFU numbers of the treated biofilms.

Results: CLSM micrographs showed divergent patterns between Calcein-AM/TMA-DPH and SYTO9/PI stainings in all bacterial species. Quantitative analysis in Gram-positive species revealed a positive correlation between Calcein-AM image analysis data and CFU, while no correlation was observed with SYTO9/PI. Work on Gram-negative bacteria is ongoing.

Conclusion: Accurate biofilm viability assessment is crucial for evaluating anti-biofilm agents. Preliminary results suggest Calcein-AM and TMA-DPH staining may provide a more precise method, emphasizing the superior efficacy of physiological state probes over DNA-binding fluorophores.

P-MCB-013
Hypochlorous acid (HOCl)-derived lipid N-chloramines in host-microbe interactions
*L. R. Knoke[1], S. U. Abad Herrera[2], S. Heinrich[3], N. Lupilov[1], J. E. Bandow[3], T. Günther-Pomorski[2], L. I. Leichert[1]
[1]*Ruhr University Bochum, Microbial Biochemistry, Bochum, Germany*
[2]*Ruhr-University Bochum, Molecular Biochemistry, Bochum, Germany*
[3]*Ruhr University Bochum, Applied Microbiology, Bochum, Germany*

When bacteria encounter neutrophils, the first line of defence against pathogens in humans, they are phagocytised and killed by a cocktail of oxidants, including the highly toxic hypochlorous acid (HOCl), produced by the neutrophils in the phagolysosome. *In vitro* studies have shown that, like in proteins, HOCl results in the chlorination of the membrane lipids" amino residues found in the head groups of some lipids. However, reliable methods for the *in vivo* detection of lipid N-chloramines are lacking and hence their physiological importance is still under investigation.

Here, we used the dansyl derivative dansyl sulfinic acid ($DANSO_2H$) to investigate N-chlorination of membrane lipids in living bacteria and mammalian cells exposed to HOCl and *in vitro* studies with model membranes to identify target lipids and activity of N-chloramines towards biomolecules.

First, we showed that exposure of living mammalian (THP1) or bacterial cells (*E. coli*) to HOCl results in the formation of lipid N-chloramines in cell membranes using $DANSO_2H$-derivatization followed by thin layer chromatography (TLC). Furthermore, HOCl treatment of living cells also results in protein N-chlorination. *In vitro* studies of model membranes in large unilamellar vesicles (LUV) single out phosphatidylethanolamine (PE), a major phospholipid in *E. coli*, also present in the mammalian cytoplasmic membrane, as a main target of HOCl. These N-chloramines were reversed by treatment of chlorinated LUV with the cellular antioxidant glutathione, indicating a role of this cellular reduction system in counteracting N-chloramines. To investigate the activity of chlorinated LUVs towards biomolecules, we used the redox active protein roGFP2. Exclusively PE-containing, HOCl-treated LUVs oxidize the probe, suggesting that lipid N-chloramines exhibit oxidative capacity. Taken together, our experiments suggest that lipid N-chloramines have a biologically relevant oxidative activity, and hence potentially accelerate the host immune response similar to protein N-chloramines. Furthermore, this work lays the foundation for future experiments aiming at their occurrence and biological role(s) in host pathogen interactions.

P-MCB-014

Unveiling the dynamics of chromosome segregation in Rhodomicrobium vannielii DSM166

*V. Bieberstein[1], F. D. Müller[1]
[1]*University of Bayreuth, Microbiology, Bayreuth, Germany*

The segregation of bacterial chromosomes is typically controlled by the conserved tripartite ParABS system. This system orchestrates the segregation of the origin of replication early in the cell cycle, concomitantly with DNA replication and is essential for the faithful distribution of the paired chromosomes. The ParABS system comprises the ATPase ParA, the ori-proximal sequence *parS*, and the *parS* binding CTPase ParB. Upon ATP binding and dimerization, ParA is believed to interact with DNA in a non-specific manner. ParB proteins bound to *parS* interact with each other to form a spreading nucleoprotein complex that interacts with DNA-bound ParA dimers. This tripartite chromosome partitioning system is hypothesized to mediate the net movement of the replicated chromosomal origin to the opposite cell pole. However, research on chromosome segregation has predominantly focused on bacteria with simple rod-like or coccoid morphology. As a result, the corresponding processes in prosthecate budding bacteria are largely unknown. These bacteria must move the chromosome through a narrow hypha to reach distant progeny. Current models cannot explain this process suggesting that our understanding of chromosome segregation in bacteria is incomplete. Nonetheless, the absence of suitable model organisms impedes the study of this process. We have successfully genetically accessed *Rhodomicrobium vannielii*, a notable multipolar Alphaproteobacterium, known for its pleomorphic cells that reproduce through bud formation at the tips of extended hyphae. We traced the dynamic localization patterns of both ParA and ParB proteins by fluorescent labelling and fluorescence time-laps microscopy. Statistical analysis of the fluorescence signals indicates that chromosome translocation begins only after the bud has reached a certain size, suggesting the existence of an unidentified checkpoint. Furthermore, translocation occurs rapidly, indicating an active DNA transport mechanism of unknown identity.

P-MCB-015

Septal protein FraI is a new player in cell-cell communication and cell differentiation in multicellular cyanobacteria

A. K. Kieninger[1], A. Janović[1], T. Müller[1], D. Risser[2], *I. Maldener[1]
[1]*Universtiy Tübingen, IMIT Organismic Interactions, Tübingen, Germany*
[2]*University of Colorado, Department of Biology, Colorado Springs, CO, United States*

Filamentous cyanobacteria are prominent examples of bacterial multicellularity. Hundreds of photosynthetic cells are interconnected in a homeostatic network and are capable of cell differentiation in adaptation to changing environment [1]. Depending on species, dormant spore-like akinetes, N_2 fixing heterocysts and motile hormogonia are formed[1]. To coordinate the physiological processes, these cyanobacteria exhibit a sophisticated cell-cell communication system [2,3]. The cellular basis of molecule exchange between the cells are septal junctions (**SJ**) [4], bridging the septum thru 20-nm wide nanopores in the peptidoglycan [5,6]. The SJ consist of 3 modules: the septum crossing tube, the plug residing in the cytoplasmic membrane and a cap on the cytoplasmic side [7]. A mutant of *Nostoc punctiforme* ATCC 29133 lacking the cell wall amidase AmiC2 was not able to form the nanopores and to undergo cell differentiation [4,5]. Similarly, the homologues proteins AmiC1 and AmiC2 of *Anabaena* PCC 7120 have functions in nanopore and heterocyst formation [8].

So far, we identified two proteins of the SJ, FraD and SepN [7,9]. FraD mutants lack cap and plug; SepN mutants just the plug. Consequently, SJ gating, which allows closure of SJs in stress situations[7,9], is impaired and the filaments exhibit severe phenotypes, like fragmentation and aberrant heterocyst differentiation.

Here we present FraI, encoded by *alr4714* (*Anabaena* PCC 1720) and NpF4142 (*Nostoc punctiforme* ATCC 29133), respectively. Mutants of both strains show defects in cell differentiation and diazotrophic growth, and almost no cell-cell communication. We show that this is due to the lack of nanopores and SJ, proving the important role of the nanopore array for the multicellular lifestyle of these cyanobacteria.

1 Maldener *et al.* 2014 in *The Cell Biology of Cyanobacteria* 239–304
2 Flores *et al.* 2019 *Life* 9
3 Nieves-Morión *et al.* 2017 *mBio* 8, e01756-16
4 Kieninger *et al.* 2021 *Curr Opin Microbiol* 61, 35–41
5 Lehner *et al.* 2013 *FASEB Journal* 27, 2293–2300.
6 Nürnberg *et al.* 2015 *mBio* 6
7 Weiss *et al.* 2019 *Cell* 178, 374-384.e15
8 Bornikoel *et al.* 2017 *Front Cell Infect Microbiol* 7
9 Kieninger *et al.* 2022 *Nat Commun* 13

P-MCB-016

Antibiotic tolerance of biofilms emerging from multicellular effects of antibiotic efflux

*V. Jaut[1], F. Schreiber[1], R. Allen[1], S. Vareschi[1]
[1]*Federal Institute for Materials Research and Testing (BAM), Department of Materials and the Environment, Berlin, Germany*

Introduction: Biofilms are multicellular assemblages of bacteria living in a self-produced extracellular matrix. One characteristic of biofilms is that they are difficult to kill.

Different mechanisms, like the development of highly tolerant persister cells or increased expression of efflux pumps, which pump certain antimicrobials out of the cell, make them tolerant.
Goals: The overall project goal is to develop a predictive model for efflux-mediated antimicrobial multicellular assemblies.Our central hypothesis is that efflux pump activity causes emergent antimicrobial tolerance of multicellular bacterial populations, through the interplay of efflux-mediated spatial interactions and efflux-linked persistence.
Methods & Results: To test the hypothesis, we are combining computational modelling with information gained from 3 types of multicellular assemblages, i.e. colonies on agar, multicellular populations grown in a monolayer microfluidic device, and 3D biofilms grown in flow chambers. We are currently generating strains that differ in their levels of efflux activity. In parallel the strains are characterized in terms of growth, minimum inhibitory concentration of different antimicrobial substances, colony morphology, and biofilm formation ability. The strains will be mixed and then cultivated together in the 3 model systems. In colonies, the link between colony structure and spatial patterns of gene expression will be characterized. Experiments in microfluidic devices will be used to determine the interactions range of cells with high efflux activity on neighboring susceptible cells, as well as to analyze the correlation between efflux and persister formation. The results will be used to construct individual-based models that predict the effect of efflux on biofilm structure and antimicrobial tolerance. Flow chamber 3D biofilms will be generated to test the model predictions.
Summary: Using combination of computational modelling and lab experiments with multicellular assemblages, we want to shed light on the interplay between spatial biofilm organization, multidrug efflux and antimicrobial tolerance.

P-MCB-017
Functional analysis of a bipartite aerotaxis sensosr in M. gryphiswaldense
*C. Weigel[1], J. Herz[1], D. Pfeiffer[1]
[1]*University of Bayreuth, Department of Microbiology, Bayreuth, Germany*

- Magnetotactic bacteria (MTB) employ a unique navigation strategy known as magneto-aerotaxis, guiding them towards preferred micro- or anoxic zones.
- The genetically tractable Alphaproteobacterium *Magnetospirillum gryphiswaldense* has ~56 putative genes encoding MCPs, whereby the bipartite CetBA2-like receptor system was previously identified to play a superior role during aerotaxis
- Deletion of CetBA2-like system genes resulted in the formation of aberrant aerotactic swim halos, and impaired aerotactic band formation towards higher oxygen concentrations Three-dimensional structured illumination microscopy (3D-SIM) revealed a polar-lateral cellular organization of the CetBA systems and presumably no crosstalk between the paralogous systems. To characterize the interaction between CetB2 and CetA2, truncated CetB2 variants were generated and tested against CetA2 by Bacterial Adenylate Cyclase-Based Two-Hybrid (BACTH) assay

---

P-MCB-018
Assessing the O2 tolerance of M. oralis and M. smithii
*S. D. Akinci[1], B. Molitor[1,2]
[1]*Eberhard Karls University of Tübingen, Dept. of Geosciences, Environmental Biotechnology Group, Tübingen, Germany*
[2]*Cluster of Excellence, "Controlling Microbes to Fight Infections", Tübingen, Germany*

Anaerobes possess oxidative defenses found in their aerobic counterparts, as they have evolved tactics to minimize the metabolic disruption caused by O_2 or restore function after oxidative stress dissipates. Notably, Methanobrevibacter species have shown substantial tolerance to O_2 along with catalase activity. Recent studies revealed that Methanobrevibacter reduces O_2 to H_2O via $F_{420}H_2$ oxidase while remaining metabolically active. For example, *Methanobrevibacter smithii* NADH-oxidase activity, an essential enzyme in O_2 detoxification with the capacity to reduce O_2 to H_2O. Collectively, cellular oxidative damage is multi-layered, given the diradical and oxidative nature of O_2.

Despite recent insights, the enzymatic repertoire involved in protection against oxidative stress in Methanobrevibacter species associated with the human microbiome remains poorly understood. The unique microenvironment within human periodontal pockets supports the growth of anaerobes. However, given the predominant aerobic nature of the oral cavity, O_2 exposure occurs during the early stages of dental plaque development, and even in already established periodontal pockets. A similar situation unfolds in the gut, where primarily anoxic conditions prevail, yet microbes are challenged with varying O_2 gradients.

Shedding light on the O_2 detoxifying properties of *Methanobrevibacter oralis* and *M. smithii* concerning their respective ecological niches might reveal novel adaptation strategies from an evolutionary point of view. To assess the O_2 tolerance of *M. oralis* and *M. smithii*, batch bottle experiments are carried out to determine an appropriate O_2 concentration for subsequent bioreactor and proteomics studies. The aim is to identify conditions in which cells face O_2 stress while still exhibiting growth and metabolic activity. Preliminary findings suggest that the growth and metabolism of *M. smithii* are influenced by various factors, including the quantity of added O_2 and the specific time point at which O_2 is introduced. To substantiate these observations, the integration of proteomics data is crucial to elucidate potential adaptations of these methanogens in response to O_2-induced stress.

P-MCB-019
Genetic characterization of the Vibrio cholerae chromosome 2 replication triggering site
*A. Steinhoff[1], J. Seidlitz[1]
[1]*TU Darmstadt, Moleculare Microbiology, Darmstadt, Germany*

Most bacterial species carry their genetic information on a single chromosome. However, members of the Vibrionaceae family (e.g. *V. cholerae, V. natriegens*) have two chromosomes of uneven size. The two chromosomes are each replicated from a single replication origin (*ori*). Interestingly, the replication of chromosome 2 (chr2) starts delayed ensuring that both chromosomes terminate replication in synchrony. It was found that the replication of the so called *crtS* (chr2 replication triggering site) on chr1 provides the signal for the replication initiation at *ori2*. Integration of a single *crtS* into the *E. coli* chromosome stabilizes the maintenance of an *ori2*- based plasmid (synVicII). We found that additional *crtS*-sites increase the copy number of the synVicII gradually. To identify the minimal spacing of two *crtS* to remain functionality, we

integrated *crtS* pairs separated by 100 bps up to 8 kbps in *E. coli* strains harboring a synVicII. Indirect copy number measurements suggested a minimum spacing to maintain the functionality of two adjacent *crtS*- sites. In addition, we designed an approach to identify the variable and the interchangeable bases of roughly 80 bp long *crtS* by systematically introducing up to four N throughout the whole sequence making use of DNA oligo libraries. A genetic selection system would then separate respective mutant libraries to more or less functional *crtS*-sequences. As a first step, we were able to show that a differentiation of functional and non-functional *crtS*-sequences is possible in a competitive setting using *E. coli* harboring a synVicII carrying an ampicillin resistance marker. We found that at increasing ampicillin concentrations, an *E. coli* harboring a functional *crtS* sequence can overgrow an *E. coli* with a non- functional *crtS*- sequence. Application of this screen to *crtS* mutant libraries combined with next-generation-sequencing readout should allow complete dissection of the functional *crtS* sequence space as next step to uncover its mechanism of action.

P-MCB-020
Some noteworthy updates on the planctomycetal cell biology

*C. E. Wurzbacher[1], J. Hammer[1], T. Haufschild[1], N. Kallscheuer[1], C. Jogler[1,2]
[1]*Friedrich Schiller University Jena, Department for Microbial Interactions, Jena, Germany*
[2]*Friedrich Schiller University Jena, Cluster of Excellence Balance of the Microverse, Jena, Germany*

Since their discovery, members of the phylum *Planctomycetota* have surprised scientists with their unique features. Although the molecular mechanism is yet to be elucidated, bacteria from the class *Planctomycetia* are well known for their budding cell division. Recently, several environmental isolates belonging to the phylum *Planctomycetota* were discovered, which challenge commonly accepted principles of bacterial cell biology yet again. One of these exceptional strains was reported by Shiratori et al. (2019): In addition to its eukaryotic-like locomotion and division, it is capable of engulfing prey bacteria in an endocytosis-like mechanism. As many scientists were rather sceptical about the bacterial identity of this organism, we investigated its characteristics in more detail and confirmed the findings of Shiratori et al. (2019). Furthermore, we isolated several similarly behaving bacteria from environmental samples.

References:

Shiratori, T., Suzuki, S., Kakizawa, Y., and Ishida, K. I. 2019. 'Phagocytosis-like cell engulfment by a planctomycete bacterium', *Nature Communications*, 10: 5529.

P-MCB-021
Mechanism behind antibiotic enhanced plasmid transfer and identification of compounds which inhibit this mechanism

*J. Moussa[1], D. Gargallo Viola[2], L. E. Thomsen[1]
[1]*University of Copenhagen, Veterinary and Animal Sciences, Copenhagen, Denmark*
[2]*ABAC Therapeutics, Barcelona, Spain*

Background

Horizontal gene transfer plays a major role in transferring genetic material between bacteria. The most common type is conjugation, which is considered a significant contributor in the emergence of new antimicrobial-resistant pathogens. This process is highly efficient and depends on the presence of conjugative plasmids. These plasmids provide the necessary genes for their transmission including the transfer (*tra*) genes involved in the type IV secretion system. Previous work has shown that transfer frequency of a cefotaxime (CTX) resistance plasmid was significantly enhanced when the donor was pre-grown in CTX (1). However, it is still unknown how CTX has this effect.

Objectives

In this study, we aim at identifying genes and regulatory pathways involved in the conjugative spread of resistance plasmids in *E. coli* exposed to CTX. Furthermore, we intend to identify compounds which prevent plasmid conjugation.

Methods and results

The screening of a transposon mutant library led to the identification of eight genes, five more genes were selected based on their suspected effect on antibiotic-induced conjugation. All genes were deleted using the lambda-red recombinase system. Eleven of these mutants led to reduced antibiotic-induced conjugation frequency compared to the wild-type, while two had the opposite effect. Five mutants, which significantly reduced antibiotic induced conjugation, were complemented to confirm their effect.

A novel protocol was designed to screen a large compound-library for compounds that can inhibit or reduce conjugation. The method provides a rapid and easy way for identifying potential plasmid conjugation inhibitors from a large library.

Summary

These data identify chromosomally encoded genes that contribute to the CTX induced up-regulation of conjugation. By further understanding the regulatory pathway we will be able to identify targets for future new antimicrobial agents.

(1) Liu G, Olsen JE and Thomsen LE (2019) Identification of Genes Essential for Antibiotic-Induced Up-Regulation of Plasmid-Transfer-Genes in Cephalosporin Resistant *Escherichia coli*. Front. Microbiol. 10:2203. doi: 10.3389/fmicb.2019.02203

P-MCB-022
Fis-mediated transcriptional dynamics in Yersinia pseudotuberculosis

*S. Javadi[1], D. Meggers[1], S. Pienkoß[1], A. Krüger[2], J. Frunzke[2], F. Narberhaus[1]
[1]*Ruhr-University Bochum, Microbial Biology, Bochum, Germany*
[2]*Research Center Juelich, Jülich, Germany*

Yersinia pseudotuberculosis is a facultative intracellular pathogen that migrates between two dissimilar habitats: the external environment, particularly in soil and water, and the human host, where it causes an intestinal infection known as yersiniosis. The transition between these habitats is tightly regulated by complex regulatory networks. Changes in the

DNA topology of specific chromosomal regions (e.g. gene promoters) play an important role as well as global changes in DNA architecture mediated by nucleoid-associated proteins. The histone-like protein Fis is known as a transcriptional regulator found in many bacteria that regulates virulence factors and optimizes bacterial adaptation to different environments. Although the role of Fis has been described in various organisms, the regulatory networks specific for temperature fluctuations in *Y. pseudotuberculosis* have not yet been elucidated.

To gain a comprehensive insight into the global effects of Fis in *Y. pseudotuberculosis* at ambient and host body temperature, we conducted comparative RNA sequencing in the wild-type and a Δ*fis* mutant at 25 and 37 °C. Remarkably, the results uncovered more than 300 misregulated genes in Δ*fis* that were distributed among 14 functional gene categories. Furthermore, to identify specific DNA-binding sites of Fis, we performed chromatin affinity purification and sequencing (ChAP-Seq). These data in combination with phenotypic analyses show that the absence of Fis has significant physiological consequences in *Yersinia*. Most notably, the absence of Fis resulted in the upregulation of numerous virulence genes, including the main virulence regulator LcrF, and the loss of motility at 25 °C.

Overall, our data highlight the critical role of Fis in modulating *Yersinia*´s lifestyle by regulating genes involved in various cellular processes.

P-MCB-023
Polar localization of the chemotaxis system in Pseudomonas putida

*L. M. Schmidt[1], L. Zehner[1], T. S. Bentele[1], K. Thormann[1]
[1]*Justus-Liebig University Gießen, Institute for Microbiology and Molecular Biology, Giessen, Germany*

It is important for bacteria to be able to perceive and react to changes in their environment. This includes positive changes in the nutrient gradient, i.e. an attractant that would trigger movement towards the substance, as well as low concentrations and deterrents that trigger movement away from it. The bacterial chemotaxis system can sense these gradient changes and transmit them via a signaling cascade within the cell to the designated flagellar motors. In the lophotrichous flagellated bacterium *Pseudomonas putida*, the chemotaxis system, like the flagella, is localized to the cell pole. To investigate the factors contributing to the polar localization of the chemotaxis system, deletion mutants of various proteins (e.g. FlhF, FimV and PocB) were generated that have previously been shown to be involved in the recruitment and correct localization of motility structures in Pseudomonads. Using a genomic translational fusion of CheA-mCherry, the localization of the chemotaxis system in the deletion strains was detected via fluorescence microscopy. In addition, the spreading behavior of the strains was examined. Our results indicate that deletions of a CheW domain protein and a ParA-family protein lead to a strong delocalization of the chemotaxis system, whereas deletions of FlhF and PocB have only a minor effect. Remarkably, the deletion of FimV does not result in delocalization of the chemotaxis system. In addition, a deletion of FlhF significantly reduces the number of cells with a chemotaxis system. The spreading behavior of *P. putida* is substantially influenced by all deletions. The study shows that the mechanism underlying polar localization of the *P. putida* chemotaxis system differs from that of other monopolarly flagellated gammaproteobacteria and potentially also from that of other *Pseudomonas* species.

P-MCB-024
Escherichia coli susceptibility to fosfomycin revisited: deciphering cellular perturbations beyond target inhibition

*M. Bianchi[1], M. Borisova-Mayer[1], T. Harbig[2], J. Rapp[1], J. Bornikoel[1], K. Nieselt[2], H. Link[1], H. Brötz-Oesterhelt[1], C. Mayer[1]
[1]*Eberhard Karls University of Tübingen, IMIT, Tübingen, Germany*
[2]*Eberhard Karls University of Tübingen, IBMI, Tübingen, Germany*

Introduction:

Antimicrobial resistance worsens every year due to the growing gap between MDR bacteria proliferation and the slow rate of new antibiotics" discovery. However, many old antimicrobial drugs, like fosfomycin (FOS) first discovered in 1969, are still active against MDR pathogens. FOS blocks the MurA enzyme, and this unique mechanism of action makes it ideal for combinatorial therapies. Despite routine FOS prescription for urinary tract infections caused by *Escherichia coli*, relapse rates remain high. FOS-treated *E. coli* rapidly develops resistance to FOS through genetic mutations, but little is known about possible metabolic adaptations that also enable its survival. Moreover, it is still obscure how FOS globally impacts the cell, to carry on its bactericidal effect.

Goal:

Investigate FOS effects on *E. coli*, beyond its action on MurA, and elucidate metabolic pathways crucial for the drug bactericidal effect or for bacterial survival.

Material and methods:

Experiments were performed on *E. coli* BW25113 and on KO-mutants of the genes of interest. We obtained omics data of FOS-treated *E. coli* through Illumina RNA Sequencing and time-resolved metabolomics (HPLC-MS). Time-lapse microscopy of FOS-treated cells showed morphological alterations that allow bacterial survival. The evaluation of the metabolic pathways of interest was performed through antibiotic susceptibility testing.

Results:

We showed that *E. coli* BW25113 responds to FOS by deep alterations of its metabolomic and transcriptional state. Moreover, treated bacteria transform into L-form like spheroplasts, survive for a long time in such state and eventually revert to a bacillar form and grow exponentially again. We then focused our investigation particularly on a CreBC-related response and evaluated its role in the bacterial response to the drug.

Summary:

FOS is still active against MDR bacteria, but how it globally affects the cells is still not understood, even if the antimicrobial mode of action should be investigated beyond the drug-target mere interaction. We showed global alterations in FOS-treated *E. coli* and further studied the role of a FOS-induced CreBC-related response.

Microbiology in the Digital Era

P-MDE-001
BakRep – A searchable web repository for bacterial genomes and standardized characterizations
*L. Fenske[1], L. Jelonek[1], A. Goesmann[1], O. Schwengers[1]
[1]*Justus Liebig University Giessen, Bioinformatics and Systems Biology, Giessen, Germany*

The abundance of bacterial genomic data in public genome databases is crucial for research in various fields. However, most data has been processed differently, making accurate comparisons challenging. *Blackwell et al.* used an uniform approach to assemble and characterize 661,405 bacterial genomes retrieved from the European Nucleotide Archive in November 2018. First, this revealed a highly uneven taxonomic composition, with just 20 of the 2,336 species making up 90% of the genomes. Secondly, new genomes of 311,006 isolates which had not been assembled before, were added. This data resource has been published by *Blackwell et al.*, with the intention to be used as a comprehensive basis for further analysis.

Based on this we further analyzed the assembled genomes in a standardized way. A taxonomic classification was achieved using the Genome Taxonomy Database and all eligible genomes were further typed via multilocus-sequence typing. In addition we annotated all genomes assigning functional categories and database cross references to public databases. Here, we present a searchable web repository for bacterial genomes, to make this resource accessible to scientists through an interactive website. This repository provides researchers with a flexible search engine to query the data and search for specific subsets of genomes based on various features. This platform allows for customized searches integrating taxonomic, genomic, and meta information.

To handle this challenging amount of data and addressing upcoming data influx, the BakRep web repository is build on a scalable and reliable backend comprising a REST API server, an Elasticsearch cluster and S3 cloud storage. This setup is deployed in a Kubernetes cluster hosted within the deNBI cloud computing infrastructure. 751 GB of genome assemblies were imported and processed resulting in 6.15 TB of generated results. Out of the 661,405 input assemblies, 640,090 were effectively characterized. The BakRep project conducts comprehensive and standardized characterization of one of the largest collections of bacterial genomes worldwide. We envision it as a high-quality open resource for microbial researchers wordwide.

P-MDE-002
Speaking the same language: Changing the way to share microbial strain data
*J. F. Witte[1], L. C. Reimer[1], A. Yurkov[1], B. Bunk[1], J. M. López-Coronado[2], S. Alokozay[2], A. Zuzuarregui[2], R. Aznar[2], R. P. de Vries[3], V. Robert[3], J. Overmann[1]
[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Brunswick, Germany*
[2]*Spanish Type Culture Collection-University of Valencia (CECT-UVEG), Paterna, Spain*
[3]*Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands*

Researchers worldwide are working with millions of microbial strains, with about 530.000 strains registered in the Global Catalogue of Microorganisms (gcm.wdcm.org). The data on these microorganisms is exchanged in different, largely unstructured ways. This hinders comparative analyses and synthesis of data. To facilitate data sharing and contribute to more open and FAIR data, we developed and applied a new common data format and established a technical infrastructure for exchanging microbial strain data in a systematic approach.

While there have been several attempts in the past to establish standards suitable for the exchange of microbial data, these were limited to just basic strain data for identification and growth conditions and also lacked a technical concept for making this standard accessible and effectively usable for the wider microbial research community. Here we present a new approach, that aims to establish a modular standard gradually integrating various data. The data that now can be accommodated by this standard include basic growth conditions and detailed information about the metabolism and even kinetic growth parameters of microbial strains.

The key to effectively establishing the standard as a common basis for data exchange, is to create a technical infrastructure to share these data. Customizable tools for data export and import will be developed, to simplify data transfer. Two of the largest microbial strain databases, the bacterial diversity database BacDive (bacdive.dsmz.de) and the MIRRI-IS, the microbial resource research infrastructure MIRRI database (mirri.org), have joined forces to develop a new open system that makes use of this new format and enables data contributions by researchers. The format will be developed by experts from DSMZ and MIRRI within the Bioindustry 4.0 Project (Grant agreement ID: 101094287). The data about the ~250.000 microbial strains provided by the partners, will be made available in this new data standard to enable industrial exploitation of microbial biodiversity. Beyond the project, a permanent governance structure will be established to keep the new standard flexible and up to date.

P-MDE-003
DSMZ Digital Diversity – the central gateway to Bac*Dive*, BRENDA, LPSN, SILVA and more
*J. Koblitz[1], L. C. Reimer[1], J. Overmann[1], T. D. Digital Diversity Team[1]
[1]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Bioinformatics, Brunswick, Germany*

In the field of microbiology, numerous databases contain extensive information about microorganisms, each representing a "data island". However, the true potential of this wealth of knowledge lies in their connection and integration.

The Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures is a renowned biological resource center known for its diverse microbial and cell culture collections, state-of-the-art research facilities, and key web services such as Bac*Dive*, LPSN, and TYGS. The DSMZ is building an integrated suite of scientific databases of fundamental importance to the life sciences. The databases include the BacDive bacterial phenotype database, the BRENDA enzyme database, the SILVA rRNA sequence database, the LPSN nomenclature service, and others, all developed and maintained in a coordinated

manner. The resulting data platform, DSMZ Digital Diversity, will provide an integrated resource, enabling the linking and comprehensive analysis of diverse types of scientific data from all areas of the life sciences.

For the first time, we are introducing the Digital Diversity Hub (https://hub.dsmz.de), a central gateway to the integrated resources. The Hub will facilitate research by providing multiple ways to find data across resources, explore data relationships, and exploit data through novel AI-powered tools. It will also integrate all available data on a microbial resource into a central passport and augment this information with AI-based predictions. We will show how you can already use the Hub to find tools for your research and discuss ideas for future development.

P-MDE-004
Methylation-induced errors in Nanopore Sequencing data, showcased by an Outbreak Analysis

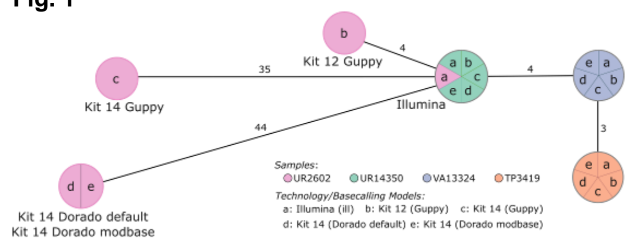
*M. Lohde[1], A. Viehweger[2], R. Ehricht[3], M. W. Pletz[4], C. Brandt[1]
[1]*University Hospital Jena, Institute for Infectious Diseases and Infection Control, Cloud Computing and Sequencing group, Jena, Germany*
[2]*University Hospital Leipzig, Institute of Medical Microbiology and Virology, Leipzig, Germany*
[3]*Leibniz-Institute of Photonic Technology, Jena, Germany*
[4]*University Hospital Jena, Institute for Infectious, Jena, Germany*

Our study investigated the accuracy of Oxford Nanopore Technology by resequencing a three-year-long *Klebsiella pneumoniae* outbreak and comparing their genomes and relationship in an outbreak context using additional Illumina sequencing data as a point of reference.

We performed whole genome sequencing using the latest chemicals (SQK-NBD114.24) from Oxford Nanopore Technologies with improved accuracy over the previous ones. The long-read data was characterized on a sequence, nucleotide, and raw signal level while evaluating alternative kits and basecalling approaches. Compared to Illumina data, base errors detected in cgMLST (see Figure) and phylogenetic analysis of Nanopore-sequenced genomes led to false exclusions of closely related strains from an outbreak. Nearby methylation sites affect the raw signal, resulting in false bases in the genomes. These errors are not limited to *K. pneumoniae* and were identified among six different bacterial orders (Actinomycetales, Xanthomonadales, Burkholderiales, Flavobacteriales, Pseudomonadales and Enterobacterales). Considering these findings, we explored PCR-based sequencing (SQK-RPB114.24) and a masking strategy, which successfully addressed these inaccuracies. Additionally, we provide a bioinformatic workflow to identify and mask problematic genome positions in a reference-free manner (MPOA is freely available on GitHub under the GNUv3 license: github.com/replikation/MPOA).

Our research identified limitations in using Oxford Nanopore Technologies for sequencing prokaryotic organisms when high accuracy is mandatory, particularly in outbreak investigation. Without further technological developments, our study recommends either PCR-based sequencing or using our provided bioinformatic workflow to mask problematic genome positions.

Fig. 1



P-MDE-005
The EDGAR platform for large-scale comparative genomics – recent developments and new features

*M. Pfister[1], L. Fenske[1], M. Dieckmann[1], S. Beyvers[1], A. Jain[1], J. Blom[1], A. Goesmann[1]
[1]*Justus-Liebig University Gießen, Bioinformatics & Systems Biology, Giessen, Germany*

Introduction
EDGAR 3.2 provides precomputed orthology databases for more than 80,000 microbial genomes in public as well as private projects. The platform allows rapid identification of the differential gene content of kindred genomes, i.e., the pan genome, the core genome, or singleton genes. Furthermore, EDGAR provides a wide range of analyses and visualization features required for phylogenomic inter- and intraspecies taxonomic analyses, like the calculation of core-genome-based phylogenetic trees and genome similarity matrices (AAI, ANI, POCP, fastANI). Since the latest update, it also offers a functional categorization of genes based on the databases KEGG, COG and GO. Thus, the software enables a quick survey of evolutionary relationships and simplifies the process of obtaining new biological insights.

Objectives
Over the last decade, the average number of genomes analysed per EDGAR project has constantly increased. As a consequence, the goal of recent EDGAR developments was twofold: First, to create the necessary infrastructure and accompanying back-end processes to manage subsequent increases in the inflow of data. Second, the continued development of the EDGAR frontend, creating new ways for scientists to examine their genomes.

Methods
The backend for the calculation of orthologs and genomic subsets has been rewritten in Rust and is now deployed on an auto-scaling Kubernetes-Cluster. For the alignment workflow, BLAST was replaced by the much faster Diamond tool. Furthermore, we are currently iterating upon the functional-category features, extending them to enable users to interactively explore KEGG subcategories and compare the abundance of GO terms within their datasets.

Results
In EDGAR 3.2, functional category data was added for all genomes along with their respective visualization features. The technical infrastructure was further optimized to be scalable with increasing query sizes, and is currently used to process more than 30,000 genomes per year. The optimizations ensure that EDGAR 3.2 remains a convenient platform for comprehensive microbial gene content analysis.

The web server is accessible at:
http://edgar3.computational.bio

P-MDE-006

Towards reliable prediction of significant changes in microbial communities based on time series data

*A. K. Dörr[1], S. Imangaliyev[1], F. Meyer[1], I. Kraiselburd[1]
[1]*University Hospital Essen, Institute for Artificial Intelligence in Medicine, Essen, Germany*

The ability to detect significant changes in bacterial communities in patients could be a step toward early detection of infectious diseases such as sepsis. This could lead to a higher chance of survival for the patient [1]. We seek to predict the changes in the abundance of bacterial genera in samples over time through analysis of 16S rRNA gene amplicon time series data. For this, we are employing Long Short-Term Memory (LSTM) [2] models for prediction and Shapley Additive Explanations (SHAP) [3] for feature importance analysis. So far, the model demonstrated a good performance for the prediction of the overall abundance range of bacterial genera in patient samples over time. Outlier detection is implemented to distinguish significant changes from normal fluctuations. As wastewater surveillance gained importance during the COVID-19 pandemic, we employed similar models on time series data from wastewater treatment plants. With the use of machine learning models on time series data for anomaly detection we hope to provide a treatment advantage for physicians and patients. In an environmental context we have the desire to help create an environmental pathogens surveillance and an early warning system. In the future we plan on testing different model architectures and data types to achieve even better results.

References:

[1] Ferrer, R. et al. Empiric Antibiotic Treatment Reduces Mortality in Severe Sepsis and Septic Shock From the First Hour. 2014. Critical care medicine.
[2] Baranwal, M. et al. Recurrent neural networks enable design of multifunctional synthetic human gut microbiome dynamics. 2022. eLife.
[3] Lundberg, S.M., Lee S.-I. et al. A Unified Approach to Interpreting Model Predictions. Part of NIPS 2017.

P-MDE-007

SubtiWiki relaunched: a brand-new foundation and novel features

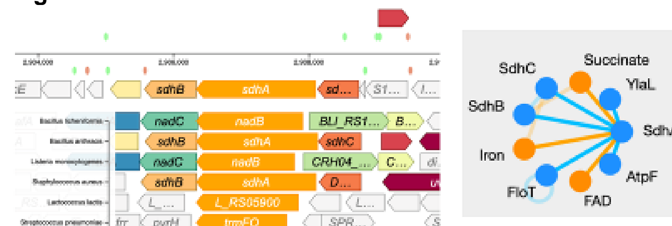
*C. Elfmann[1], V. Dumann[1], T. van den Berg[1], J. Stülke[1]
[1]*Georg-August University Göttingen, Institute of Microbiology and Genetics, General Microbiology, Göttingen, Germany*

As more and more data from microbiological research is amassed, keeping up to date with the newest findings can be difficult. For this reason, model organism databases attempt to bundle and organize all relevant information on a certain organism, enabling scientists to develop new research hypotheses. We have created *Subti*Wiki, the most popular freely available database for the model bacterium *Bacillus subtilis* (1). It holds a wealth of up-to-date curated information as well as datasets from relevant publications. In addition, it features many interactive displays to explore the presented data in an intuitive manner. But as demands on the platform increase, we decided to give *Subti*Wiki a new foundation, building upon already established web frameworks. This will serve as the basis for a range of additions to the database and its web interface. For instance, we upgraded our Genome Browser by integrating an interactive implementation of the FlaGs tool (2, see figure). It allows users to compare gene neighborhoods among selected representative bacteria, as the analysis of gene order conservation can provide important first hints about the role of a gene. Another extension is the introduction of dedicated pages for metabolites and their integration into the Interaction Browser (see figure). With this, we hope to give a more complete picture of the inner workings of *B. subtilis*. Yet another update is the implementation of a proper API (Application Programming Interface), which enables programmatic access to *Subti*Wiki's data for bioinformatics purposes.

We are confident these additions will make *Subti*Wiki even more useful for the *Bacillus* research community. In addition, we believe that the structure of our database and web application could serve as a blueprint for future databases dedicated to other microorganisms.

(1) Pedreira, T. et al. (2022) Nucleic Acids Res. 50: D875–D882
(2) Saha, C.K. et al. (2021) Bioinformatics 9: 1312-1314

Fig. 1



P-MDE-008

Digitally controlled reactors enable cultivation of fastidious microorganisms

*C. Ding[1], L. Adrian[1]
[1]*Helmholtz Centre for Environmental Research (UFZ), Molecular Environmental Biotechnology, Leipzig, Germany*

Bioreactors have been an important tool to study physiological behaviors of microorganisms. In recent years, rapid development in microprocessors, controlling unit, sensors, and software has enabled construction of reactors with more versatile functions, better performance, easier controls, and much lower prices. In our lab we have been developing our customized reactors since many years, which are based on Raspberry Pi microprocessor as the monitoring and controlling unit and syringe pumps as the pump unit. Such reactors have been successfully used in cultivation of "*Candidatus* Kuenenia stuttgartiensis" (an anaerobic ammonia oxidizing bacterium), *Dehalococcoides mccartyi* (an organohalide respiring anaerobe), and a nitric-oxide reducing *Paracoccus* strain. The reactor script was written in python and relied solely on open-source software packages. Online monitoring and data storage are achieved. Electronic lab book and git-powered script update are implemented. We believe that such reactor designs represent the future trend that calls for bioreactors with flexible functions and fully digital management at affordable prices.

P-MDE-009

NFDI4Microbiota supporting microbiome research providing data access, services, training and workflows

*M. Müller[1], C. Hege[1], B. Götz[2], K. U. Förstner[2], A. McHardy[2]
[1]*Helmholtz Center for Infection Research, Bioinformatics, Brunswick, Germany*
[2]*ZB-MED, Information Centre for Life Sciences, Köln, Germany*

Introduction: NFDI4Microbiota aims to support the microbiome research community by providing access to data, analysis services, data/metadata standards, and training. It belongs to the National Research Data Infrastructure (NFDI), which aims to develop a comprehensive research data management. Different consortia ensure a broad coverage from cultural sciences, and engineering to life sciences and natural science. NFDI4Microbiota intends to facilitate digital transformation in the microbiological community (bacteriology, virology, mycology, and parasitology).

Goals: NFDI4Microbiota aims to support the German microbiome research network through training and community-building activities, and by creating a cloud-based system that will make the storage, integration, and analysis of microbial data and (microbial) omics data, consistent, reproducible, and accessible. Thereby, NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable, and Re-usable) principles and Open Science.

Results: To enable FAIR data management, the NFDI4Microbiota consortium develops and provides computational infrastructure and analytical workflows to store, access, process, and interpret various microbiome-related data types. NFDI4Microbiota works on developing and implementing software and standardized workflows for users to analyze their data. Further, NFDI4Microbiota offers training, spanning from metagenomics, over courses about programming in R, to research data management and ELN (electronic lab notebooks). To interact with young scientists, the consortium launched an ambassador program, thereby helping to identify the needs of their local community. All relevant information and specific services are available via the web portal.

Summary: NFDI4Microbiota has established community services providing access to data, analysis services, data/metadata standards, and training thereby promoting FAIR principles and Open Science in the microbiology community.

Fig. 1

P-MDE-010

Recent updates on Bakta - a rapid & standardized software tool for the comprehensive annotation of bacterial genomes

*O. Schwengers[1], J. M. Hahnfeld[1], J. Blom[1], A. Goesmann[1]
[1]*Justus Liebig University Giessen, Bioinformatics and Systems Biology, Giessen, Germany*

Introduction
A thorough genome annotation has become key for many downstream analyses. However, focus has shifted from manual annotations of single genomes to high-throughput batch annotations. Addressing these challenges, we recently published Bakta - a command line software tool for the annotation of bacterial genomes, MAGs and plasmids. Here, we present new developments, added features and improvements.

Goals
We improved the performance of our software including better annotation results, technical maintenance, supporting common standards, and addressing the various feedback and requests of our active users.

Results
New features and enhancements have been added improving annotation results and overall software usability. First, we introduced the detection of pseudogenes. A new workflow detects conserved sequences located around de novo-predicted genes and screens elongated alignments for pseudogenization events or hints for translational exceptions. Second, Bakta now supports the annotation of MAGs adjusting internal gene prediction parameters. Third, we updated a the mandatory database incorporating the latest releases of public databases. Fourth, for the annotation of special-interest genes, an import of user-provided protein sequences has been implemented. Fifth, since de novo gene prediction tools fail to correctly predict translational exceptions, we developed a module to detect and annotate selenocysteine proteins by taking advantage of specific ncRNA motifs. Sixth, to manually correct gene coordinates, user can provide trusted feature coordinates in Genbank or GFF format. Seventh, for the streamlined bulk annotation of protein sequences only, we added a new sub command that can be used in addition to the actual whole-genome annotation pipeline. Last but not least, many enhancements have been added in terms of quality, maintenance and usability of the software following feedback and requests from the community.

Summary
We present various improvements of Bakta. This includes important new features and various usability improvements supported by the comprehensive feedback from our active user community.

P-MDE-011

From Sequence to Function: A Comprehensive Evaluation of Prokaryotic Genome Annotation Pipelines Across Thousands of Genomes

*M. Jundzill[1,2], R. Spott[1], M. Lohde[1], O. Makarewicz[3], M. W. Pletz[3], C. Brandt[1,2,4]
[1]*University Hospital Jena, Institute for Infectious Diseases and Infection Control, Cloud Computing and Sequencing group, Jena, Germany*
[2]*Leibniz Center for Photonics in Infection Research (LPI), Jena, Germany*
[3]*Institute for Infectious Diseases and Infection Control, Jena University Hospital, Jena, Germany*
[4]*InfectoGnostics Research Campus Jena, Jena, Germany*

Bacterial genome annotation is crucial for identifying genes, understanding bacterial biology, metabolic pathways, aiding in strain classification, or discovering novel treatment targets. Existing publications provide limited focus only on "benchmark organisms" and partial steps of the process, therefore lacking comprehensiveness.

We evaluated the tools' performance across the whole domain of Bacteria and Archaea by using all 14,675 different species (referential genomes) registered in Genome Taxonomy Database (GTDB). The stability of in-species annotation was checked on 24,385 *Escherichia coli* strains. The analysis was conducted on four popular annotation tools (Prokaryotic Genome Annotation Pipeline (PGAP), Prokka, Bakta, EggNOG-mapper). We annotated genomes with each tools' default or recommended settings, and the annotation quality was gauged by various metrics such as coding space, gene count, gene length, assigned GO terms, and feature count (e.g., rRNA, tRNA). Additionally, we simulated erroneous genomes with frameshifts by randomly deleting nucleotides (0.5%, 1%, 2%).

In comparison, Bakta annotates more coding space in Bacteria, but at some lower taxonomic ranks, other tools can outperform it. While in Archaea EggNOG-mapper and PGAP provide good coding space annotations. For metagenome-assembled samples (MAGs), PGAP performs better, most likely due to its taxonomic-specific annotation. For GO terms, EggNOG-mapper provides the highest count of GO terms per gene, while PGAP performs well in gene coverage with at least one GO term per feature. The simulated erroneous genomes showed that PGAP maintained stable performance.

Based on our findings, Bakta generally provides the most comprehensive annotation for the Bacteria domain. While in Archaea and MAGs, PGAP demonstrates superior performance. If functional GO annotation is important, EggNOG-mapper optimally balances GO term count while maintaining a reasonable count of hypothetical proteins. The performance of each tool may vary based on taxa, type of genome, correctness, and other factors. Nevertheless, the recommendations based on our findings apply to most use cases.

P-MDE-012
Colony Counting with Artificial Intelligence
L. Driesch[1], X. Jiang[1], F. Titgemeyer[2], *S. Fischer[2]
[1]*Universtiy Münster, Institute of Computer Science, Münster, Germany*
[2]*University of Applied Sciences Münster, Oecotrophologie · Facility Management, Münster, Germany*

Introduction: Culture-based methods and colony enumeration are central in microbiology for quality assessment in food, water and pharmaceutical analysis to ensure public health. However, manual enumeration is time-consuming, labour-intensive and subjective.

Objective: To overcome the above-mentioned limitations, we have set ourselves the goal of developing user-friendly, adaptive image processing methods by using artificial intelligence (AI) tools.

Materials & Methods: A multi-layered strategy that integrated pre-trained and novel convolutional neural networks (CNNs) was employed by including data acquisition, preparation and model training.

Results: By using a self-developed FHOTOBOX, we compiled a data set of colony images by using agar plates of diverse media showing bacterial colonies from food sample analyses. Our innovative deep learning approach led to successful segmentation of individual colonies with high accuracy, overcoming classic computer vision hurdles such as overlap and boundary errors. This strategy also allowed us to effectively process multiple image sources, demonstrating the adaptability and efficiency of deep learning. Based on the pre-trained CNNs and transfer learning, we developed a web application that can be used on a tablet for fast and reliable enumeration of bacterial colonies.

Conclusions: AI systems, especially in image processing, can facilitate time-consuming routine procedures such a bacterial colony counting in microbiologiy laboratories. It is conceivable that the technology could one day be integrated as a low-cost mobile app.

Microbial Ecology & Evolution

P-MEE-001
Xenorhabdus taiwanensis sp. nov., a symbiotic bacterium associated with the entomopathogenic nematode Steinernema taiwanensis
*C. J. Huang[1], C. T. Tzeng[1], H. Y. Shu[2], M. H. Chen[1], Y. J. Fang[1], T. L. Wu[3], Y. C. Lin[3]
[1]*National Chiayi University, Plant Medicine, Chiayi, Taiwan*
[2]*Chang Jung Christian University, Bioscience Technology, Tainan, Taiwan*
[3]*Academia Sinica, Agricultural Biotechnology Research Center, Tainan, Taiwan*

Steinernema taiwanensis is a recently identified entomopathogenic nematode (EPN) which was isolated from soil in Taiwan. In this study, we investigated an association between *S. taiwanensis* and its bacterial symbiont using a polyphasic taxonomic approach in this study. The strain TCT-1, which was isolated from *S. taiwanensis*, shared the highest sequence identity of the 16S rRNA gene to *Xenorhabdus* spp. but below the threshold of 98.7% required to recognize a new species of EPN bacterial symbionts. Multilocus sequence analysis showed that the strain TCT-1 formed a distinct sister clade to *X. griffiniae*, *X. ehlersii* and *X. thuongxuanensis* When the genome sequence of TCT-1 was compared with those of *Xenorhabdus* spp., the average nucleotide identity (ANI) scores and digital DNA-DNA hybridization (dDDH) values were below 95% and 70%, respectively. TCT-1 is closely related to *X. ehlersii* DSM 16337 with the dDDH value of 62.6% and the ANI score 94.15%. Phenotypical characterization also revealed that TCT-1 differed from other *Xenorhabdus* spp. in biochemical characteristics. Based on the polyphasic taxonomic results, we propose that the strain TCT-1T (= BCRC 81417T) represents a new species within *Xenorhabdus*, for which the name *Xenorhabdus taiwanensis*sp. nov. is proposed.

P-MEE-002
Escherichia coli and Salmonella Typhimurium isolates from the pre-antibiotic era differ from recent isolates in their ability to take up foreign DNA

*Y. Yilmaz[1], S. Simon[2], A. Flieger[2], M. Berger[1], U. Dobrindt[1]
[1]*University of Münster, Institute of Hygiene, Münster, Germany*
[2]*Robert Koch Institute, Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*

Since the discovery of penicillin by Alexander Fleming in 1928, the consumption of antibiotics in human and veterinary medicine has increased continuously. This has resulted in strong selection pressure on bacteria and the spread of multi-resistant microbes. Horizontal gene transfer (HGT) is an important process triggering bacterial evolution. Conjugation is the most important form of HGT, resulting in the rapid spread of plasmids among bacteria. We hypothesize that the increased use of antibiotics since the discovery of penicillin is a selective force that affects HGT rates in bacteria. The transfer frequency of a plasmid is known to be affected by many biotic and abiotic factors, such as growth phase, donor:recipient ratio, and environmental conditions (temperature, pH, mating time, etc.). However, factors affecting the capability of the recipient bacterial cell to take up foreign DNA are less well understood. Since the inhibition of conjugation could be a useful strategy to reduce resistance gene spread among bacteria, a better understanding of the molecular mechanisms that control plasmid spread is essential.

Here, we compared the DNA uptake efficiency of *E. coli* and *Salmonella* isolates collected in the early twentieth century, which means before the broad introduction of antibiotics into human medicine, with that of recent clinical isolates. The conjugation frequency, transformation efficiency, growth kinetics as well as the plasmid content of the selected strains was analyzed. In conjugation experiments, we examined the transfer of two different conjugative plasmids, the highly transmissible resistance plasmid RP4 and the ESBL plasmid pO104_90. *E. coli* MG1655 and *S. Typhimurium* LT2 served as donor strains. Interestingly, recent isolates often showed better recipient properties than historical isolates. These results should serve as a basis for us to learn more about the mechanisms and possible selection pressures involved in bacterial adaptation to increasing exposure to antibiotics and the emergence of multidrug-resistant strains.

P-MEE-003
Detection of human pathogenic fungi in compost using whole metagenome sequencing
*A. Pereira da Costa Filho[1], A. Barber[1]
[1]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*

Microbiome studies often overlook eukaryotic species, which have traditionally been targeted using amplicon sequencing of marker genes such as the ITS region. This method has contributed to our understanding of eukaryotic diversity in host-associated and environmental microbiomes. However, it is unable to provide strain-level taxonomic resolution or even differentiate closely related fungal species. Whole metagenome sequencing (WMS) can play a crucial role in differentiating human pathogenic fungi from closely related non-pathogenic species, which is important considering that fungal diseases are responsible for over 1.6 million deaths each year. In our study we used metagenomics to detect human pathogenic fungi (HPF) from compost, a habitat enriched for human pathogens due to its elevated temperature. Our dataset consists of 62 compost samples from public gardens in Berlin and private gardens in Jena. To enable absolute quantification of microbial abundance, we developed and employed a cell-based spike-in composed of one yeast and a gram-negative bacterium. The strains used for the spike-in were selected based on their low abundance and prevalence in European soil samples available in publicly available ITS and 16S datasets. We were able to detect fungi in our metagenomic data and 57% of our samples were positive for *Aspergillus fumigatus* and 40% were positive for other human pathogenic fungi, including *Rhizomucor*, *Rhizopus*, *Fusarium*, *Scedosporium*, and *Mucor*. Our work using shotgun sequencing data will help to better understand the dynamics of HPF in their native habitat. Ongoing work will focus on further characterizing the environmental microbial communities in which these pathogens participate to better understand their ecology and virulence during human infection.

P-MEE-004
The impact of Escherichia coli genetic background on its ability to evolve antimicrobial resistance
A. Mulkern[1], *M. Galardini[1]
[1]*Hannover Medical School, Twincore, Hannover, Germany*

Background

The rise of antimicrobial resistance (AMR) is a serious threat to global health in the 21st century. Life-threatening multi-drug resistant microbes have emerged and thus new approaches are needed to maximize the "shelf-life" of antimicrobials by reducing the risk of the emergence and spread of resistance.

Objective

Recent studies have shown how different strains belonging to the same bacterial species possess a different propensity to develop AMR ("evolvability"). The genetic determinants of differences in evolvability have however not been determined in *E. coli*; our aim is to discover them using large-scale laboratory evolution.

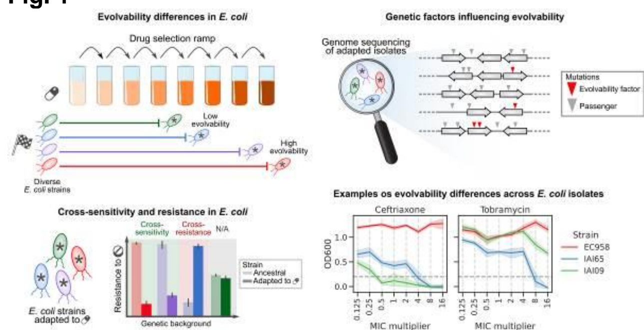
Methods

We have so far exposed 380 *E. coli* strains to 3 antimicrobials and 6 biological replicates: Amikacin, Ceftriaxone, and Ciprofloxacin, using a serial passaging protocol with daily doublings in concentration, from 1/8th of the Minimum Inhibitory Concentration (MIC) until 64X MIC. We have preserved one evolved population for each biological replicate for genome sequencing and further characterization.

Results

Using the passage at which we last observe growth as a measure of evolvability, we observed substantial variability in the ability of each strain to become resistant, which varies by genetic background and drug class, with Ceftriaxone showing the largest variance. At the genome level, we observed differences in the mutations acquired during the experiment, further underscoring the influence of *E. coli*"s genetic background on evolution. We will show data on more strains and antimicrobials, as well as patterns of cross-sensitivity in the evolved isolates, which have important implications in designing combination therapies.

Fig. 1



P-MEE-005

Within-Host evolution and adaptation patterns of oral and intestinal streptococci: Insights into the Oral-Gut Axis

*M. Abdelbary[1,2], M. Hatting[3], A. Dahlhausen[4], A. Bott[2], C. Trautwein[3], G. Conrads[2]

[1]*Robert Koch Institute, Division of Nosocomial Pathogens and Antibiotic Resistances, Department of Infectious Diseases, Wernigerode, Germany*
[2]*RWTH Aachen University, Division of Oral Microbiology and Immunology, Department of Operative Dentistry, Periodontology and Preventive Dentistry, Aachen, Germany*
[3]*RWTH Aachen University, Department of Medicine III, Aachen, Germany*
[4]*RWTH Aachen University, University Medical Center for Occupational Medicine, Aachen, Germany*

Streptococci, a versatile bacterial group, thrive in various host habitats. The intricate oral-gut axis, linking the oral cavity and gastrointestinal tract, plays a pivotal role in shaping microbial composition and host health. This dynamic interaction provides streptococci with opportunities for evolutionary adaptation. This study delves into the genetic changes and adaptive mechanisms exhibited by streptococci during colonization of both oral and intestinal habitats within the same individual.

We performed whole-genome sequencing on 218 streptococcal isolates from saliva and fecal samples of 14 Inflammatory Bowel Disease (IBD) patients and 12 healthy controls (HC). Analysis unveiled 16 distinct streptococcal species among the isolates, with *S. infantis*, *S. mitis*, *S. parasanguinis*, *S. australis,* and *S. salivarius* being the most predominant species. *S. infantis* dominated the oral habitat in both IBD patients and HC. It was the primary species in IBD fecal samples and the second most prevalent in those of HC. *S. parasanguinis* was more abundant in the gut of both groups than in their oral habitats. The analysis revealed within-host microevolution of different streptococcal species, with evidence of adaptations through recombination events and the acquisition of mobile genetic elements (MGEs). The *tetA* gene encoding tetracycline resistance was significantly more abundant in genomes of fecal origin. Streptococcal genomes with intestinal origin had a higher percentage of intact phage sequences compared to their oral counterparts. MGEs such as CRISPR-Cas and restriction modification systems had significant impact on niche-specific genotype diversities, facilitating streptococcal genome evolution through the integration of exogenous DNA. The core-genome single nucleotide polymorphisms (SNPs) analysis consistently demonstrated that oral and intestinal streptococcal genomes from the same individual exhibited distinct clustering, characterized by a significant number of core-SNPs differences. Our findings suggest emergence of distinct lineages within each habitat, and within-host streptococcal evolution is individual-dependent, potentially initiated in the oral cavity.

P-MEE-006

Cultivation strategies have a strong impact on studies of antimicrobial resistant bacteria in the environment

*S. P. Glaeser[1], S. Balachandran[2], D. Pulami[1], A. Scott[3], G. Chalmers[4], N. Ricker[4], C. Chifiriuc[5], E. Topp[6], H. Schmitt[7], P. Kämpfer[1]

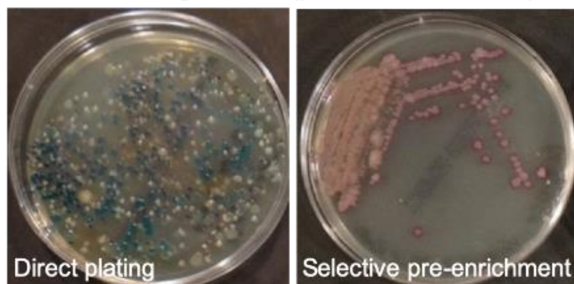
[1]*Justus-Liebig-University Giessen, Institute for Applied Microbiology, Giessen, Germany*
[2]*Justus-Liebig University Gießen, Institut für Angewandte Mikrobiologie, Giessen, Germany*
[3]*Agriculture and AgriFood Canada, London, Canada*
[4]*University of Guelph, Department of Pathobiology, Guelph, Canada*
[5]*University of Bucharest, Bucharest, Romania*
[6]*University of Burgundy, Institute for Agriculture, Food, and Environment (INRAE), Dijon Cedex, France*
[7]*National Institute for Public Health and the Environment, Bilthoven, Netherlands*

Cultivation of antimicrobial resistant bacteria (ARB) is more and more recognized as a key strategy to understand the spread of antimicrobial resistance (AMR) in the environment. For many years, extraction of environmental DNA and quantification of antimicrobial resistance genes (ARG) was used by many research groups to target environmental AMR spread. But, especially the multi-resistance status of bacteria or plasmid content cannot by covered by these methods and new resistance strategies are overseen. These important data can be obtained by using specific cultivation approaches. However, the cultivation strategies must be carefully selected and critically evaluated to get reliable results.

By selecting specific media and incubation conditions, ESKAPE bacteria can be selectively cultured. By supplementing antibiotics to these media, subgroups of resistant bacteria can be cultured. Nevertheless, there are several challenges which can strongly impact the outcome of these cultivation efforts. Growth of more abundant intrinsically resistant non-target bacteria can disturb the detection of ARGs or low abundant or viable but non culturable (VBNC) potential pathogenic ARBs in environmental samples. This may affect their direct cultivability on agar media. Non-selective or selective pre-enrichment strategies enhancing the cultivability of specific bacteria but can also influence the outcome of these studies, because some strains may have growth benefits during pre-enrichment. Resistance gene exchange during pre-enrichment may also occur.

We will give a critical overview of cultivation efforts for the research on AMR based on our knowledge we obtained in the last 15 years in our AMR research collaborative network projects (the BMBF funded projects RiskAGuA and the JPI AMR project ARMIS in which the spread of AMR from manure and biogas plant digestates were studied, and the DFG research group PARES in which the spread of AMR in Mexican soils after a shift from irrigation with untreated to treated wastewater is monitored.

Fig. 1



P-MEE-007

On the trail of oxygenic photosynthesis in ancestral Cyanobacteria on early Earth.

*M. Gehringer[1]
[1]*University of Kaiserslautern-Landau, Microbiology, Kaiserslautern, Germany*

Oxygenation of the atmosphere by early cyanobacterial oxygenic photosynthesis occurred ca 2.4 Ga, during the Great Oxygenation Event (GOE), however, geological evidence of phototrophic mats and stromatolites dates to at least 3.2 Ga [1], with the origin of Cyanobacteria estimated between 3.5 [2] & 3.4 [3] Ga. This study reports on simulation experiments utilising deep branching Cyanobacteria to gain insight into what factors may have contributed to the uncoupling of the emergence of Cyanobacteria and oxygenation of the early Earth atmosphere.

The high Fe(II) availability on early Earth may have inhibited early Cyanobacteria by encasing them in rust. We show that deep branching species that are actively photosynthesising are not encrusted by rust under Archean ocean Fe(II) concentrations [4]. The reduced growth rates observed under repeated Fe(II) exposure were offset by increased photosynthetic activity, measured by O2 release rates, when compared to modern oxygen-rich conditions.

Early oxygenic photosynthesisers may initially have endured high levels of oxidative damage, thereby restricting their expansion prior to the GOE. Diurnal expression and activity of superoxide dismutases in *Pseudanabaena* sp. PCC7367 was monitored under a simulated Archean atmosphere and compared to modern day conditions. We demonstrate significantly enhanced transcription and activity under an early Earth anoxic simulation [5].

In summary we demonstrate that iron encrustation and oxidative stress were not responsible for limiting the spread of Cyanobacteria on early Earth. Instead, we propose that bioavailability of essential metals, such as iron, phosphates and fixed nitrogen may have contributed to the uncoupling of the emergence of Cyanobacteria and oxygenation of the early Earth atmosphere.

[1] Homann et al. (2018) Nat Geosciences 11,665.
[2] Boden et al. (2021) Nat. Comms 12,4742.
[3] Fournier et al. (2021) Proc R Soc B 288,20210675.
[4] Herrmann et al. (2021) Nat. Comms 12,2069.
[5] Tamanna et al. (2024) Submitted to Geobiology)

P-MEE-008

Overlapping Genes form a gene reservoir for evolutionary novel genes in bacteria

*K. Neuhaus[1], Z. Ardern[1], S. Scherer[1]
[1]*Technical Univeristy of Munich, Core Facility Microbiome, ZIEL Institute for Food & Health, Freising, Germany*

Question. The presence of overlapping genes (OLGs) with substantial coding overlaps beyond translational coupling challenges and transforms our understanding of genomic complexity. The prevalence of overlapping genes, either fully embedded or with extended overlaps, is likely considerably underreported. Currently, only a handful of these genes have been thoroughly confirmed, but the number of prokaryotic overlapping genes is steadily expanding. Validating the presence of genes within alternate reading frames has the potential to elucidate taxon-specific evolutionary innovations.

Methods. mRNA sequencing, ribosome profiling, phenotype testing of strand-specific knockouts, and in some cases mass spectrometry was used to verify protein-coding overlapping genes. Genome-wide analysis of transcription start sites was conducted in an exemplary organism. Bioinformatic comparisons were applied to number of different datasets for published omics-data, to identify serendipitously discovered overlapping genes.

Results. Overlapping genes were identified through RNA sequencing and ribosome profiling. Bioinformatics confirmed that the identified candidate genes possess characteristic structural elements allowing their expression. Clear phenotypic effects were observed in strand-specific knock-out mutants. For some proteins, we successfully used mass spectrometry identifying expressed proteins. Specific overlapping genes are often constrained to specific taxonomic groups, but overlapping genes have been unintentionally identified in a number of different taxa.

Conclusions. Overlapping genes are more prevalent in bacterial genomes (outside prophages) than anticipated. They may contribute evolutionarily novel functionalities and could be an important contribution of evolutionary novelties in bacterial genomes.

P-MEE-009

Functional diversity of the microbiome of an activated carbon filter for wastewater treatment: perspective of bioaugmentation for micropollutant degradation

*Y. Abdullaeva[1], M. Löwe[1], J. Fleckenstein[1], M. Jochimsen[1], *B. Philipp[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

Traditional wastewater treatment methods are ineffective at removing micropollutants like pesticides, pharmaceuticals, microplastics, and nanoparticles. Advanced techniques such as activated carbon filters (ACFs) improve the elimination of trace chemicals. There is limited research on the microbiome and associated processes in ACFs despite the cognition that microbial biodegradation takes place in these filters.

Our research aims to understand the microbial communities and their functional role of specific bacterial microbiomes within ACFs with the perspective of bioaugmentation by introducing micropollutant-degrading bacteria. To achieve

this, microbiome analysis via amplicon sequencing and physiological experiments were employed.

The initial analysis of the microbiome of a newly installed ACF at a wastewater treatment plant revealed a diverse range of bacterial groups colonizing the AC over 12 months. Pairwise PERMANOVA tests indicated that the bacterial communities differed significantly (Padonis< 0.05) between sampling points. *Sphingomonadaceae*, *Chitinophagaceae*, *Hyphomonadaceae*, *Nitrosomonadaceae*, and *Comamonadaceae* consistently dominated the samples. Based on the molecular data, members of the *Chitinophagaceae* are expected to be involved in the breakdown of polysaccharides within the ACF biofilm. The concurrent presence of *Nitrosomonadaceae*, *Hyphomonadaceae*, and *Comamonadaceae* with known nitrifying and denitrifying members indicates that nitrogen elimination might take place within the ACF. Additionally, *Sphingomonadaceae* and *Comamonadaceae* might be involved in the biodegradation of organic micropollutants in the AC filter.

Physiological experiments with *Novosphingobium* and *Pseudomonas* strains indicated that *N. aromaticivorans* can actively desorb adsorbed organic compounds from AC while *P. putida* was unable. This notion aligned with molecular data indicating the absence of pseudomonads in the ACF.

These findings provide insight into the functional capabilities of specific bacterial groups within ACFs and suggest the potential of using *Sphingomonadaceae* for bioaugmentation to enhance micropollutant degradation in wastewater treatment processes.

P-MEE-010

Codon usage, a powerful tool for the phylogenetic analysis of the Mollicutes

*A. M. Ilic[1], M. Kube[1]
[1]*University of Hohenheim, Integrative Infection Biology Crops-Livestock, Stuttgart, Germany*

The massive generation of sequence data has enabled phylogenetic analyses of unprecedented quality, leading to overdue revisions in bacterial taxonomy, including the class Mollicutes. The need to consider as many informative sites as possible is reflected in the use of average nucleotide identity (ANI) and average amino acid identity (AAI) analyses. Uncertainty, and thus problems in assigning taxonomic rank, arise in rapidly evolving genomes, such as the obligate bacterial parasites of the provisional taxon '*Candidatus* Phytoplasma'.

We investigated the complementary use of qualitative scoring of percentage of conserved proteins (POCP) and signature-based approaches such as codon usage and tetranucleotide frequency analysis.

A total of 143 complete and draft genome sequences were selected and protein-coding sequences were predicted by Prodigal to ensure comparable data sets [1]. ANI, AAI, POCP, codon usage and tetranucleotide frequency analyses were carried out [2,3].

The results are consistent with the ANI and AAI analyses and the most recent taxonomic revisions. Using the taxonomy recommended by the International Committee on Systematics of Prokaryotes, the POCP analysis of species within families showed only weak significance. In contrast, genome-wide codon usage analysis shows species-specific patterns and provides a stable phylogenetic assignment. Tetranucleotide analysis also allows differentiation below the species level. Thresholds are suggested using the phytoplasma group as an example.

Codon usage analysis should be considered in future phylogenetic analyses, as their lineage-specific signatures allow reliable phylogenetic clustering.

References

[1] Vesth T, Lagesen K, Acar Ö, Ussery D (2013). CMG-biotools, a free workbench for basic comparative microbial genomics. PLoS One. 8(4):e60120.

[2] Richter M, Rosselló-Móra R, Glöckner FO, Peplies J (2016). JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics. 32(6): 929–931.

[3] Kim D, Park S, Chun J (2021). Introducing EzAAI: a pipeline for high throughput calculations of prokaryotic average amino acid identity. J Microbiol 59(5):476-480.

P-MEE-011

Random targeting of prokaryotic genomes by bacterial CRISPR-Cas systems in natural environments

*K. Sures[1], S. Eßer[1], T. L. V. Bornemann[1], C. J. Moore[1], A. R. Soares[1], J. Plewka[1], P. A. Figueroa-Gonzalez[1], S. E. Ruff[2], A. J. Probst[1]
[1]*University of Duisburg-Essen, Department of Chemistry, Essen, Germany*
[2]*Marine Biological Laboratory, Woods Hole, MA, United States*

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated *(cas)* genes provide archaea and bacteria with adaptive immunity to invading mobile genetic elements (MGEs), like viruses. Recently, we showed that CRISPR-Cas systems of uncultivated archaea do not only target MGEs, but also harbour spacers matching protospacers in their episymbiont's genome. However, this alone does not explain the tremendous diversity of spacers that is generally found in CRISPR-Cas systems from natural environments. We therefore tested the hypothesis that bacterial CRISPR spacers match genomic DNA of other prokaryotes inhabiting the same environments. Applying metagenomics, we investigated the CRISPR-Cas systems of metagenome-assembled genomes (MAGs) from (non-)hydrothermal sediments in the Guaymas Basin (Gulf of California, Mexico) and groundwater of the cold-water geyser Wallender Born (Wallenborn, Germany). We found that bacterial CRISPR-Cas systems have acquired spacers matching a large diversity of different prokaryotic genomes in their respective environment. Analyses of bacterial CRISPR-Cas systems did not reveal a subtype associated with increased uptake of spacers from prokaryotic DNA. However, our results indicate that the selectivity of spacer acquisition is influenced by the number of different CRISPR-Cas subtypes found in a genome. Our data show that the respective bacterial genomes encode for proteins potentially involved in the uptake and subsequent degradation of environmental DNA. We consequently posit that DNA uptake for either horizontal gene transfer or as a source of nutrients facilitates the acquisition of spacers from prokaryotic DNA. Our results shed new light on the diversity of spacer

populations in natural communities and provide an explanation for some of the many unmatched spacers in public databases.

P-MEE-012
Evolutionary implications of heterogeneous disinfectant tolerance

*L. Y. Sobisch[1], *N. Nordholt[1], D. V. Lewerenz[1], A. Gödt[1], O. Kanaris[1], F. Schreiber[1]
[1]*Bundesanstalt für Materialforschung und -prüfung (BAM), Department 4 - Materials and Environment, Berlin, Germany*

Introduction

Effective disinfection is crucial for hygiene and infection prevention, but phenotypic heterogeneity, particularly in heterogeneous tolerance, may lead to disinfection failure and foster resistance evolution to both disinfectants and antibiotics. However, the consequences of phenotypic heterogeneity for disinfection outcome & resistance evolution are not well understood.

Goals

This study investigates the impact of phenotypic heterogeneity on *E. coli* survival and evolution during disinfection with six commonly used substances and exploring the consequences of evolved disinfectant tolerance for antibiotic resistance.

Materials & Methods

Population heterogeneity is assessed through time-kill kinetics and mathematical modeling, with a link to the evolvability of disinfectant tolerance examined through periodic disinfection evolution experiments. The ability of disinfectant-tolerant strains to evolve antibiotic resistance is evaluated through serial transfer experiments and whole-genome sequencing.

Results

Multi-modal time-kill kinetics in three disinfectants suggest the presence of disinfectant-tolerant subpopulations (persister cells). Notably, strains with disinfectant tolerance show a reduced likelihood of developing high-level resistance to specific antibiotics compared to the sensitive ancestor, indicating a distinct antibiotic-specific outcome. WGS reveals epistatic interactions between disinfectant tolerance & antibiotic resistance mutations, preventing access to canonical evolutionary paths to resistance.

Summary

Our findings suggest that phenotypic heterogeneity can facilitate disinfection survival & the evolution of population wide tolerance, which can impact future antibiotic resistance evolution.

P-MEE-013
Application of the adaptive cycle on complex microbial communities of developing soil

*A. Bartholomäus[1], H. Zoller[1], W. zu Castell[1], D. Wagner[1,2]
[1]*GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Potsdam, Germany*
[2]*University of Potsdam, Geosciences, Potsdam, Germany*

Complex systems are systems with many components that can interact with each other. They are difficult to understand due to different types of interactions and relationships. Microbial communities in nature can contain thousands of microbial species and are an example of a complex system in biology. These microbial communities are driven by complex multi-species interactions but also interactions of species or sub-communities with the environment.

In microbial ecology, concepts that are able to describe the state of microbial communities are useful to compare communities of different origin and help interpret the community development and evolution. One concept that tries to describe complex system development is the adaptive cycle by Gunderson and Hollings (2002). It divides the evolution of systems into four major phases (exploitation, conservation, release, reorganization) by the interplay of three essential variables (connectedness, resilience and potential).

Here, we propose that the adaptive cycle is a valuable tool to study the state of microbial communities in developing soil. We applied this method on bacterial, archaeal and eukaryotic microbial community data of a glacier transect in East-Antarctica (Larsemann Hills) and found the results to match the expected developmental state of the soil.

The communities of "older" soils, that are expected to be more developed, show a higher resilience and more connectedness. In addition, our results are more robust when the combination of eukaryotic and prokaryotic microbial community data is used. Prokaryotic communities alone are less informative, probably because some prokaryotes show a much higher potential to be the absolute early pioneers and are able to live on their own with only few inter-species interactions to the environment. To sum up, the adaptive cycle can be a tool to describe developments of microbial communities in natural ecosystems.

P-MEE-014
Microbial abundance and community composition in soil depth profiles of grassland and arable lands

*A. Pausch[1], M. Speth[2], P. Kämpfer[1], A. Gattinger[3], S. P. Glaeser[1]
[1]*Institute für Angewandte Mikrobiologie/ Justus-Liebig-Universität Gießen, Mikrobiologie der Recyclingprozesse (Kämpfer), Giessen, Germany*
[2]*Department of Soil Science and Soil Conservation, Professur für Bodenressourcen und Bodenschutz, Giessen, Germany*
[3]*Institute für Pflanzenzüchtung II / Justus-Liebig-Universität Gießen, Professur für Ökologischen Landbau m.d.S. Bodennutzung, Giessen, Germany*

Soil microbial communities are crucial for ecological processes such as organic matter turnover and nitrogen cycling in agricultural soils. Studies on the soil microbiome in agricultural systems often neglect deeper soil layers and focus instead on top soils. As part of the Green Dairy LOEWE project we performed detailed studies on the soil microbiome in depth gradients from top soil layers down to one meter depth. In spring 2022, soil samples from 0-10, 10-30, 30-50 and 50-100 cm depth from one grassland and two arable plots were analyzed comparatively. On arable plot contained alfalfa at the beginning of the second year of cultivation and one had spelt and field beans as the previous

crop. We determined the total abundance of *Bacteria*, *Archaea* and fungi and specific microbial groups involved in the nitrogen cycle using quantitative PCR (qPCR) assays. We further on studied bacterial and archaeal community profiles with respect to community diversity and composition in different soil layers by 16S rRNA gene amplicon Illumine sequencing. Initial data showed that microbial abundance (*Bacteria*, *Archaea*, fungi and N cycling genes) significantly decrease with soil depth with clear differences among arable and grassland soil gradients. We expect that microbial community patterns will also show clear differences within the depth gradients with system specific differences. This study gives important information of the soil microbial communities in different soil layers of grassland and arable agricultural systems and helps to select individual soil layers for specific research questions.

P-MEE-015

Five ways soil bacteria and archaea respond to nematode grazing

*M. Tamang[1], J. Sikorski[1], M. Van Bommel[2], M. Piecha[3], T. Urich[3], K. Huber-Fischer[1], L. Ruess[2], M. Neumann-Schaal[1], M. Pester[1,4]
[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Microbiology, Brunswick, Germany*
[2]*Humboldt University of Berlin (, Institute of Biology, Berlin, Germany*
[3]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*
[4]*Technical University of Braunschweig, Institute of Microbiology, Brunswick, Germany*

Microbial communities in soil ecosystems are shaped by trophic interactions. However, this impact is not well investigated and quantified so far. We analyzed how trophic interactions modulate the bacterial community composition within a natural soil community. The experiment was set up in a microcosm system with circulating airflow filled with haplic cambisol soil, inoculated with maize litter, a bacterivorous nematode, and incubated for 32 days. Soil respiration and total bacterial and archaeal communities were determined at days 0, 4, 8, 16 and 32. Bacterial and archaeal communities were compared through 16S rRNA gene amplicon sequencing and qPCR analysis. Treatments with the bacterivorous nematode resulted in an earlier onset of soil respiration and a strong microbial community shift during the first 16 days. In total, 221 dominant amplicon sequence variants (ASVs) were underlying these community shifts and could be grouped into five distinct response types at the population level. Response types ranged from steadily decreasing over intermitted population maxima to steadily increasing. Taxonomic affiliation was not a factor that distinguished response types. Our results are an important step forward to disentangle microbial population dynamics in response to nematode grazing.

P-MEE-016

The dynamics of the upper respiratory microbiome ontogenesis in early childhood reveal an equilibrium of drift-migration

*B. Cheaib[1], S. Mayer[1], O. Sommerburg[2], A. Dalpke[1], S. Boutin[3]
[1]*Heidelberg University Hospital, Medical Microbiology and Hygiene, Centre for infectious diseases, Heidelberg, Germany*
[2]*Heidelberg University Hospital, Clinic for Peadiatrics, Heidelberg, Germany*
[3]*University of Lübeck, Klinik für Infektiologie und Mikrobiologie, Lübeck, Germany*

According to the island biogeography theory, the heterogeneous composition of the microbiota in the human respiratory tract differs in density, diversity, structure, and composition between lower and upper airways. Understanding the fundamental process of the respiratory tract microbiota ontogenesis can help us to understand the function and the development of the lung in the context of health and disease, especially in patients with Cystic Fibrosis. This study included 210 samples of nasal swabs and 210 throat swabs from a cohort of 73 patients (infants and children) with CF, where 57 infants were followed up for at least two consecutive years. A 16s rDNA amplicon approach was applied to sequence all nasal and throat samples Illumina Miseq. The Alpha and Beta diversity analyses suggest community structure and composition stabilization over time in both sites but higher stability in the throat niches. An expectation-maximization analysis for microbial source tracking found more stable microbiota in the throat over age. The network analysis of the throat microbiome across sequential ages compared to the nose"s microbiome showed a significantly higher persistence of positive interactions between commensals despite the prevalence of pathogens. The goodness of fit of a drift-migration model used to predict the microbiota spatial progression in the upper respiratory tract suggests deterministic ontogenies after 1-year age, where the infant immune system was under development. Understanding the microbiota ontogenesis from the angle of the eco-evolutionary concepts can help in preventing the episodes of such respiratory disease exacerbation in the lungs of patients for instance with CF, using the microbiome dysbiosis patterns in the community structure and function as a diagnostic tool in a preventive manner to control the impact of the disease on the lower respiratory airways.

P-MEE-017

Evolving Thermotoga maritima to thrive at sub optimal temperatures

*C. Prohaska[1], M. Basen[1]
[1]*University of Rostock, Microbiology, Rostock, Germany*

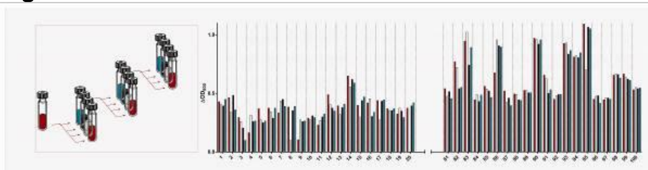
Phylogenetic studies suggest that LUCA, the last universal common ancestor of Bacteria and Archaea, was a thermophile.1 This implies that at some point in the history of Early Earth, mesophilic organisms may have evolved from thermophilic organisms, but laboratory experiments towards that are missing. The order *Thermotogales* contains mesophilic and thermophilic members with a broad temperature range[2] , and it has been hypothesized that the former may have evolved from the latter within the order.[3] *T. maritima* is one of the few known hyperthermophilic bacteria with an optimal growth temperature of 80 °C.[4] In a first attempt, we characterized the phenotype of *T. maritima* at suboptimal temperatures. Interestingly, we observed growth at 45° C, 10 K below the published T_{MIN}, at a specific growth rate of 0.01 h^{-1}. Growth was accompanied by dramatic changes in morphology, with larger cells compared to their counterparts grown at 80 °C. We analysed fermentation products at different temperatures and observed higher concentrations of lactate during incubation at 45 and 50 °C. To study adaptions towards lower temperatures, we then serially transfered *T. maritima* at 45 °C and 50 °C in an ongoing adaptive laboratory evolution experiment (ALE) (Fig: 1).

After 25 transfers at 45 °C and after 60 transfers at 50 °C two passaged populations (ATM45 and ATM50), show shifts in their growth rates compared to the type strain at different temperatures. While 80 °C seems to stay the optimum for one population (ATM50), growth rates at lower temperatures

were increased in both adapted populations. In addition, OD_{MAX} of ATM50 increased about 20-25 %. For ATM45 overall higher growth rates could be observed at all temperatures.
Currently, we are studying the molecular basis for these phenotypes. We anticipate that these adapted strains will allow us to gain insights into the evolution of thermophiles towards lower temperatures.

References
1. Weiss M., *et al*. NatMicrobiol (2016). 2. Mori K. *et al*. Microbes Environ. (2020)
3. Dipippo J. *et al*. Int J Syst Evol Microbiol.(2009) 4. Huber, R. *et al*. Arch. Microbiol. (1986)

Fig. 1



P-MEE-018
Surface and wastewater as reservoirs for a broad spectrum of health related klebsiellae? Insights into the diversity and potential impact of isolates from natural and human-associated sources in Haïti

C. Jaeckel[1], S. Baron[2], I. Garcia-Menino[1], *J. A. Hammerl[1]
[1]*German Federal Institute for Risk Assessment, Biosafety, Berlin, Germany*
[2]*French Agency for Food, Mycoplasmology-Bacteriology and Antimicrobial Unit, Ploufragan, France*

Klebsiellae are nosocomial pathogens increasingly notified by public health services. Due to their high adaptability and their ability to acquire foreign DNAs, human infections with MDR-isolates are challenging to treat. In addition, environmental klebsiellae are also reliable indicators for the dynamics in resistance acquisition in ecosystems caused by pollutions forcing the adaption of these bacteria. As comprehensive information from different ecosystems is lacking, klebsiellae from surface/wastewater were investigated in Haïti. Here, we report on 58 Klebsiella-isolates collected from 12 stations during an environmental survey of Vibrio in 2021, ten years after the beginning of the local cholera outbreak. Isolates genome profiling showed a broad variety of XbaI-PFGE pattern. Phenotypically, only some isolates exhibit resistances to the tested antimicrobials, which were shown to be in good agreement with the resistance genes determined by WGS. In addition, some of the isolates further exhibit a strong hypermucoviscity. In silico dissection of the klebsiellae genomes provides a detailed insight into the genetics and their potential impact for human health. Dissection of environmental Klebsiellae provides information about sources of antimicrobial resistance acquisition, hotspots for the evolution of the bacteria and the general occurrence of clinically relevant lineages, which may affect the health of the local human population due to the colonization of susceptible people.

P-MEE-019
Drivers of the differentiation of the human pathogen Clostridioides difficile

*P. Halama[1], T. Riedel[2], I. Schober[1], J. Wittmann[3], M. Göker[4], J. Meier-Kolthoff[5], C. Rodriguez[6], D. Paredes-Sabja[7], B. Bunk[1], U. Groß[8], J. Overmann[9]
[1]*DSMZ, Bioinformatics and Databases, Brunswick, Germany*
[2]*DSMZ, MÖD/DZIF, Brunswick, Germany*
[3]*DSMZ, BUG/Phage genomics, Brunswick, Germany*
[4]*DSMZ, Bioinformatics and Databases/Phylogenomics, Brunswick, Germany*
[5]*University Augsburg, Bioinformatics Core Facility, Augsburg, Germany*
[6]*Universidad de Costa Rica, Facultad de Microbiologia, San José, Costa Rica*
[7]*Texas A&M University, Biology, College Station, TX, United States*
[8]*Georg-August University Göttingen, Medical Microbiology, Göttingen, Germany*
[9]*DSMZ, Scientific Director, Brunswick, Germany*

Clostridioides difficile is a major causative agent of antibiotic-associated diarrhea and pseudomembranous colitis. However, most research is based on incomplete draft genome sequences mainly with clinical background. Recent findings suggest a large, but untapped genomic variability particularly of the mobilome of non-clinical and environmental isolates. However, a resolution beyond draft genomes is required for better understanding into their evolution and functional implications.

To elucidate the genomic diversity and evolutionary dynamics, our large-scale study comprised a representative dataset consisting of 175 *C. difficile* isolates which was investigated by *de novo* complete genome sequencing and comparative genomics. This allowed the prediction of lineage-specific genes, mobile genetic elements and a detailed investigation of the pan-genome. Newly sequenced complete genomes were obtained from various geographic locations and epidemiological contexts and tried to specifically cover previously underrepresented clades. This revealed a high conservation of genomic synteny across different continents and clades, despite a high variability of acquired lineage-specific genes and mobile genetic elements. Among the lineage-specific genes, several virulence factors and antiviral systems were identified. Additionally, a diverse set of phages and phage-like plasmids was detected. This is in agreement with the phylogenetic network analyses of the core-genome, which revealed different evolutionary strategies between the five clades. The selective advantage and genetic function of lineage-specific genes were determined and potential evolutionary mechanisms based on genomic evidence identified.

Based on the dataset of *C. difficile* different genomic evidences about evolving traits, the mobilome and extrachromosomal elements were identified, which one could not conduct without complete genomes. These findings contribute to our understanding of early genome evolution and the role of mobile elements on the separation, diversification and ultimately the speciation of *C. difficile*.

P-MEE-020
Far-red photosynthesis in early-branching cyanobacteria from a desert

*S. Wu[1,2], L. A. Antonaru[1], J. Brandhorst[1], N. U. Frigaard[3], M. Kühl[3], D. P. Canniffe[4], D. Nürnberg[1,2]
[1]*Free University of Berlin, Institute for Experimental Physics, Berlin, Germany*
[2]*Free University of Berlin, Dahlem Centre of Plant Sciences, Berlin, Germany*
[3]*University of Copenhagen, Department of Biology, Helsingør, Denmark*
[4]*University of Liverpool, Department of Biochemistry and Systems Biology, Liverpool, United Kingdom*

Oxygenic photosynthesis has been considered to be restricted to visible light. However, some cyanobacteria can extend their photosynthetic range into far-red light (FRL) by undergoing a complex and extensive acclimation process known as far-red light photoacclimation (FaRLiP) which leads to the formation of the red-shifted chlorophylls (chl) *d* and *f*, as well as modified photosystems and phycobilisomes. In shaded areas, FRL is usually enriched, and it has been shown that for some habitats such as stromatolites, beach rock, or caves, FaRLiP cyanobacteria are commonly found within them. Nevertheless, our understanding of the impact of FRL photosynthesis on a global scale is largely unknown. Here, we focus on Sabkha Oum Dba (Morocco), an arid habitat characterized by photosynthetic soil crusts. We prove that it contains large quantities of chl *f*, suggesting that FRL might be a key driver of photosynthesis and the carbon cycle in this environment.

By isolating several new FaRLiP cyanobacteria from this habitat, we were able to characterize their photophysiology and define their position in the phylogenetic tree. Two characteristic spectral features were observed for all FRL-grown isolates: (i) the fluorescence emission spectra were shifted to 730-750 nm and (ii) the reflection spectra showed an additional peak at 708 nm. High-performance liquid chromatography (HPLC) experiments confirmed that all FRL samples contain chl *d* and *f*. In certain cells of this order, a distinctive segregated distribution of chl *a* and chl *d/f* has been observed. All cyanobacteria consist of thin filaments with diameters of 0.9-1.4 µm. The 16S-23S ribosomal RNA (rRNA) phylogenetic tree revealed that the majority of the isolates with the capability to perform FaRLiP belong to phylogenetically early-branching cyanobacteria *Nodosilineales* (also known as *Phormidesmiales*), a largely understudied order. Our isolates are closely related to *Halomicronema hongdechloris* C2206, which is the first chl *f*-containing FaRLiP strain.

P-MEE-021
Widespread occurrence of chitinase-encoding genes suggests the Endozoicomonadaceae family as a key player in chitin processing in the marine benthos
D. M. G. da Silva[1], F. R. Pedrosa[1], M. Â. Taipa[1], R. Costa[1], *T. Keller-Costa[1]
[1]*Instituto Superior Técnico, University of Lisbon, Institute for Bioengineering and Biosciences, Department of Bioengineering, Lisbon, Portugal*

Chitin is the most abundant natural polymer in the oceans, where it is primarily recycled by chitin-degrading microorganisms. *Endozoicomonadaceae* (*Oceanospirillales*) bacteria are prominent symbionts of sessile marine animals, particularly corals, and presumably contribute to nutrient cycling in their hosts. To reveal the chitinolytic potential of this animal-dwelling bacterial family, we examined 42 publicly available genomes of cultured and uncultured *Endozoicomonadaceae* strains for the presence of chitinase-encoding genes [1]. Thirty-two of 42 *Endozoicomonadaceae* genomes harbored endo-chitinase – (EC 3.2.1.14), 25 had exo-chitinase – (EC 3.2.1.52) and 23 polysaccharide deacetylase-encoding genes. Chitinases were present in cultured and uncultured *Endozoicomonadaceae* lineages associated with diverse marine animals, including the three formally described genera *Endozoicomonas*, *Paraendozoicomonas* and *Kistimonas*, the new genus *Candidatus* Gorgonimonas, and other, yet unclassified groups of the family. Most endo-chitinases belonged to the glycoside hydrolase family GH18 but five GH19 endo-chitinases were also found. Many endo-chitinases harbored an active site and a signal peptide domain, indicating the enzymes are likely functional and exported to the environment where endo-chitinases usually act. Phylogenetic analysis revealed clade-specific diversification of endo-chitinases across the family. The presence of multiple, distinct endo-chitinases on the genomes of several *Endozoicomonadaceae* species hints at functional variation to secure effective chitin processing in diverse micro-niches and changing environmental conditions. We demonstrate that endo-chitinases and other genes involved in chitin degradation are widespread in the *Endozoicomonadaceae* family and posit that they play important roles in chitin turnover in marine invertebrates and benthic ecosystems.

[1] da Silva et al., 2023 ISME COMMUN, 3
https://doi.org/10.1038/s43705-023-00316-7

Molecular Infection Epidemiology and Prediction of Antimicrobial Resistance

P-MIPA-001
In Bavaria, Germany and Europe, where is antibiotic resistance in Enterococcus faecium heading?
*S. Heinzinger[1], G. Rutz[1], S. Jungnick[1], M. Marx[1], A. L. O. 30 BARDa laboratories[2], S. Hörmansdorfer[1], A. Sing[1]
[1]*LGL, Public Health Microbiology, Oberschleißheim, Germany*
[2]*laboratories, Oberschleißheim, Germany*

Introduction: Germany has lower antibiotic resistance rates than the European average, with the exception of *Enterococcus faecium (ENCFAC)* vancomycin (vanco).

Goal: The aim is to compare the trend of resistance of *ENCFAC* against vanco in different surveillance systems and to investigate the development of the phenotypic ratio of vanco-resistant isolates to teicoplanin (teico) resistance in Bavaria.

Material/method: From 2020 to 2023, 24 to 30 Bavarian laboratories submitted their resistance data to BARDa, evaluated according to the EUCAST standard, anonymized as SIR assessment. For each patient, only the first isolate in 90 days is included. Screening samples are excluded. Confidence intervals are calculated using Wilson score method. The resistance data of 11 bacterial pathogens are evaluated and compared with other German (ARS by RKI, ARMIN by Lower Saxony) and European (EARS-NET by ECDC) surveillance systems. For the latter, data for 2023 are not yet available.

Results: From 2020 to 2023 1,855,256 isolates were included in the BARDa reports with *Escherichia coli* (712,971; 38.4%) as the most prevalent species. *ENCFAC* isolates count for 54,948 (3.0%). Resistance rates of *ENCFAC* for vanco and teico show a significant decrease in Bavaria according to BARDa data since 2022 to 17.9% and 10.1% in 2023 respectively, approaching ARMIN and ARS levels. In general, German resistance values to vanco are above the European average (17.6% in 2022). In BARDa, the phenotypic combined resistance of vanco and teico (as encoded in the *vanA* gene) increases from 45% in 2020 to 55% in 2023.

Discussion: *ENCFAC* resistance rates for vanco in Bavaria and Germany are higher than the European average, but seem to converge slowly. In 2023, a significant decrease is observed in BARDa data in invasive isolates and all materials since 2022. This may be due to an increase of the *vanA*-gene in the Bavarian *ENFAC* population. Studies at the University Hospitals of Regensburg and Erlangen, where invasive isolates from 2020 and 2022 were analyzed using (core genome) multilocus sequence typing, show that a novel *vanA* lineage was an important driver of the increasing vanco-resistant ENCFAC rates.

Fig. 1

Resistance to *Enterococcus faecium* in different surveillance systems in Germany and EARS-Net

	all materials					only invasive isolates (blood/cerebrospinal fluid)																		
																				average				
	BARDa all					BARDa invasiv					ARMIN				ARS				EARS-Net				
Antibiotics	%R				n	%R				n	%R			n	%R			n	%R			n	%R
	2020	2021	2022	2023	2023	2020	2021	2022	2023	2023	2020	2021	2022	2022	2020	2021	2022	2022	2020	2021	2022	2022	range 2022
Ampicillin	87,0%	88,0%	87,2%	87,9%	14.046	91,6%	92,1%	90,7%	91,2%	980	89,5%	93,0%	90,6%	702	93,0%	93,5%	93,1%	4.062					77,9-100%
Imipenem	87,8%	89,4%	88,4%	89,4%	13.770	92,5%	92,8%	91,1%	92,7%	1.000	90,9%	94,2%	92,1%	433	93,2%	94,4%	93,8%	4.708					
HL-Gentamicin	20,2%	22,9%	26,5%	37,7%	6.524	21,9%	22,4%	26,9%	41,8%	728	22,5%	42,2%	49,7%	559	46,6%	57,4%	54,6%	3.612					0,0-100%
Teicoplanin	14,2%	14,4%	12,4%	10,1%	12.229	11,6%	13,7%	8,9%	7,5%	918	5,9%	6,4%	5,6%	480	10,1%	9,2%	8,8%	4.325					
Vancomycin	28,7%	29,8%	24,0%	17,9%	15.257	30,6%	29,5%	24,3%	15,5%	1.074	22,5%	17,7%	16,5%	705	22,0%	21,1%	17,8%	5.057	16,8%	17,2%	17,6%	22.709	0,0-67,7%
Linezolid	0,6%	0,7%	0,6%	0,8%	14.912	1,5%	1,6%	0,5%	1,6%	1.058	0,5%	0,6%	0,9%	703	0,6%	1,1%	1,0%	5.022					

HL-Gentamicin: Gentamicin 500 (high level) — significant change, calculated with 95% confidence interval — EARS-Net: Population-weighted average

P-MIPA-002
Genomic surveillance of STEC/EHEC infections in Germany 2020-2022 permits insight into virulence gene profiles and novel O-antigen gene clusters

*A. Fruth[1], C. Lang[1], T. Groessl[1], T. Garn[2], A. Flieger[1]
[1]*Robert Koch-Institut, Division Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*
[2]*Robert Koch Institute, Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*

Question

Shiga toxin-producing *E. coli* (STEC), including the subgroup of enterohemorrhagic *E. coli* (EHEC), are important bacterial pathogens which cause diarrhea and the severe clinical manifestation hemolytic uremic syndrome (HUS). Genomic surveillance of STEC/EHEC is a state-of-the-art tool to identify infection clusters and to extract markers of circulating clinical strains, such as their virulence and resistance profile for risk assessment and implementation of infection prevention measures. The aim of the study was characterization of the clinical STEC population in Germany to establish a reference data set for STEC/EHEC molecular surveillance and detection of infection clusters.

Methods

From 2020 to 2022 1,257 STEC isolates, including 39 of known HUS association, were analyzed by PCR-based virulence gene analysis, antibiotic susceptibility testing and whole genome sequencing including bioinformatic analysis.

Results

Major serogroups in all clinical STEC analyzed were O26, O146, O91, O157, O103, and O145; and in HUS-associated strains were O26, O145, O157, O111, and O80. *stx1* was less frequently and *stx2* or a combination of *stx, eaeA* and *ehxA* were more frequently found in HUS-associated strains. Predominant *stx* gene subtypes in all STEC strains were *stx*1a (24 %) and *stx*2a (21%) and in HUS-associated strains were mainly *stx*2a (69%) and the combination of *stx*1a and *stx*2a (12.8%). Furthermore, two novel O-antigen gene clusters (RKI6 and RKI7) and strains of serovars O45:H2 and O80:H2 showing multidrug resistance were detected. By means of phylogenetic analysis, 383 isolates (30.4%) were assigned to 129 infection clusters (threshold allelic distance > 10 AD) including two to ten isolates.

Conclusions

The implemented surveillance tools now allow to comprehensively define the population of clinical STEC strains including those associated with the severe disease manifestation HUS reaching a new surveillance level in Germany. Therefore, the integration of epidemiological data and data of the competent food authorities on national and international level leads to highly efficient control strategies positively impacting public health.

P-MIPA-003
Plasmid diversity in hypervirulent Klebsiella pneumoniae isolates from German hospitals

*A. Wahl[1], A. Müller[1], M. Fischer[1], G. Werner[1], *Y. Pfeifer[1]
[1]*Robert Koch Institute, Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany*

Introduction

Klebsiella pneumoniae (*Kp*), an opportunistic bacterium in animals and humans and common cause of nosocomial infections, has evolved to a novel pathotype known as hypervirulent (hv) *Kp*. Hv*Kp* strains are associated with severe infections in otherwise healthy individuals. Virulence and resistance traits are usually located on different plasmids. Recently, convergence plasmids carrying both antibiotic resistance and virulence-associated genes have been reported in various countries worldwide.

Goals

The presence of resistance- and virulence-associated genes in *Kp* isolates from German patients, along with convergence plasmids is poorly investigated, necessitating comprehensive plasmid analyses.

Methods

Genomes of 62 *Kp* isolates with PCR confirmed presence of virulence associated genes were analysed using short and long-read sequencing. We applied bioinformatics tools (Kleborate, MOBsuite, AMRfinder) to identify multi-locus sequence types, capsule types, plasmid types, resistance and virulence genes. Whole genome alignment aided phylogenetic studies of similar plasmid types available in public databases and described as virulence, resistance or convergence plasmids.

Results

MOBsuite analyses revealed that 18/62 isolates harbored >6 different plasmids. Three major types of plasmids carrying only resistance genes were found in 38 isolates; the typical hv associated plasmid was found in 15 isolates (mainly sequence type ST23). Resistance and virulence genes were present on different plasmids in 9 isolates but in 28 isolates (mainly ST147 and ST395) convergence plasmids were identified. These plasmids carried both carbapenemase genes (*bla*NDM) and virulence associated genes (*rmpA/A2, iuc*).

Summary

This study showed the presence of typical hv*Kp* strains of ST23 with or without additional resistance plasmids in Germany. A remarkable number of isolates of known global epidemic lineages (ST147, ST395) carried convergence plasmids contributing to the pathogenic potential and antibiotic resistance burden of these strains. Our findings shed light on the intricate interplay between resistance and virulence traits that has to be investigated in future studies.

P-MIPA-004
Antimicrobial resistance of Neisseria gonorrhoeae isolated from the pharynx
*H. Tlapák[1], A. Pantke[1], K. Klaper[1], R. Selb[1], K. Jansen[1], D. Heuer[1]
[1]*Robert Koch-Institut, Berlin, Germany*

Introduction: The human restricted pathogen *Neisseria gonorrhoeae* (NG) is the causative agent of the sexually transmitted infection (STI) gonorrhoea. With over 83 million infections annually, gonorrhoea is one of the most prevalent STI on a global scale. The rise of antimicrobial resistance (AMR) to all antibiotic classes used to treat NG infections makes management and treatment of NG infections increasingly complex. The pharynx represents a potential reservoir for AMR due to its poor accessibility for antibiotics and the possibility of horizontal gene transfer of AMR determinants from commensal *Neisseria* species. Yet, few data on AMR of NG isolated from the pharynx are available as most NG infections of the pharynx are asymptomatic and culture is further impeded by often low bacterial load and surrounding microbiota.

Goals: The study Go-Pharynx aims to improve culture methodology of NG isolation from the pharynx and to gain insight into the prevalence of NG-AMR in the pharynx as well as identify potential epidemiological risk factors for NG-AMR.

Methods: Partner clinics in Berlin recruit participants that either present with a symptomatic NG infection or that tested positive for NG in an STI screening. Clinicians and participants answer clinical and epidemiological questionnaires and two pharyngeal swabs are taken. Within 24 h one swab is sent to a partner laboratory for follow-up nucleic acid amplification testing (NAAT) and the other to the RKI for culture. Culture is only pursued for NAAT positive samples. After successful isolation of NG, phenotypic AMR testing as well as whole genome sequencing (WGS) are performed. Phenotypic and genomic data are correlated to identify known and possibly knew genetic determinants of AMR. Statistical analysis of the epidemiological and laboratory data is performed to calculate the prevalence of NG-AMR in Germany and to identify predictors for its development and spread.

Summary: Determining the extent of AMR in NG isolated from the pharynx as well as identifying prognostic factors contributing to its spread is important for the implementation of public health measures for prevention and treatment of resistant pharyngeal NG.

P-MIPA-005
Plasmid analysis of NDM metallo-β-lactamase-producing Enterobacteriacea
*A. Müller[1], Y. Pfeifer[1], G. Werner[1], F. Martin[1]
[1]*Robert Koch-Institut, Department of Infectious Diseases, FG13 Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany*

Introduction

Carbapenemase-producing bacteria pose a significant threat to public health due to their broad resistance to β-lactam antibiotics and further substance classes. NDM-1 is the second most abundant carbapenemase in *Enterobacteriacae* in Germany [1]. Understanding the genetic environment of β-lactamase genes and their dissemination pathways is crucial for effective treatment and infection control strategies.

Goals

We performed comprehensive genome analyses of NDM-producing clinical *Enterobacteriaceae* to map the diversity of plasmids and to understand the inter- and intraspecific distribution of antibiotic resistance genes. Multiple isolates were obtained from patients with a recent travel history abroad.

Methods

Genome sequencing (Illumina and Nanopore) was performed for 28 NDM-producing isolates. Detected plasmids were compared with plasmids from the public available database NCBI. Susceptibilities to antibiotics (broth microdilution) and antibiotic resistance transfer (conjugation assays into *E. coli* J53) were tested.

Results

Hybrid-assemblies enabled the circularization of 24 out of 28 plasmids. The plasmids sizes ranged from 47-396 kbp and different Inc-types (IncC, IncFIA, IncFIB, IncX3, IncR) were found. Nucleotide based comparison of the plasmids show a large diversity, both within the *Enterobacteriaceae* and within individual species. Various resistance genes and several virulence associated genes were located on these plasmids. In addition to 21 *bla*NDM-1 six *bla*NDM-5 and one *bla*NDM-19 were found. No correlations were found between the place of isolation or the travel background of the patients and the *bla*NDM genetic environment of the isolates. Conjugative transfer was successful for 13 of these 28 plasmids.

Conclusions

Our analyses showed a high diversity in plasmids carrying *bla*NDM and their horizontal spread in different *Enterobacteriaceae* species. Genomic surveillance with the focus on plasmid analysis will improve our understanding on dissemination of carbapenemase genes.

[1] Pfennigwerth et al., (2023). Bericht des Nationalen Referenzzentrums für gramnegative Krankenhauserreger.

P-MIPA-006
Go-Surv-AMR: Tackling Antimicrobial Resistance in Neisseria gonorrhoeae through Comprehensive Surveillance
*K. Klaper[1], R. Selb[2], S. Buder[3], K. Jansen[2], D. Heuer[1]
[1]*Robert Koch-Institute, Unit 18: Sexually transmitted bacterial Pathogens (STI) and HIV, Berlin, Germany*
[2]*Robert Koch-Institut, Unit 34: HIV/AIDS, STI and Blood-borne*

Infections, Berlin, Germany
[3]*Robert Koch-Institute, Gonococcus reference laboratory, Sexually transmitted bacterial Pathogens (STI) and HIV, Berlin, Germany*

Introduction

In 2021, the Robert Koch-Institute in Germany reinforced the Gonococcal Resistance Surveillance for Antimicrobial Resistance (Go-Surv-AMR) program to address the urgent issue of rising resistance in *Neisseria gonorrhoeae* (NG). Increased resistance to Azithromycin and stability in resistance to the cephalosporins Cefixime and Ceftriaxone have been reported. The off-label use of doxycycline for sexually transmitted infection (STI) prophylaxis (Doxy-PEP), not licensed in Germany, is anticipated to play a critical role in impacting and altering the resistance patterns in NG. This aspect is now closely monitored within the surveillance efforts.

Methods

DNA of NG isolates was extracted and the genomes were sequenced using Illumina technology. The web-based platform Pathogenwatch was used for genotyping and to predict resistance genes.

Results

Between 2020 and 2022, 1739 NG isolates were sequenced, unveiling 116 known Sequence Types (STs) and 15 new STs. Phylogenetic analysis identified globally distributed groups associated with reduced susceptibility to azithromycin (ST9363), cefixime (ST7363), and ceftriaxone (ST7827). Genetic patterns associated with reduced susceptibility to Penicillin were identified in 95.6% of isolates and for Tetracycline in 89.8%. Regarding isolates with the co-occurrence of patterns for both antibiotics, additional patterns associated with reduced susceptibility towards Azithromycin were identified in 20.2%. In contrast, for these isolates with simultaneous genotypic resistance to Penicillin and Tetracycline, patterns associated with reduced susceptibility to Cefixime and Ceftriaxone were rarely identified (<1%).

Summary

The impact of Doxy-PEP on the emergence of AMR in NG warrants specific attention, especially considering the co-occurrence of azithromycin resistance genes. Some of these genes are known to contribute to cephalosporin resistance while also conferring a fitness benefit. Moreover, current surveillance of AMR in STIs in Germany primarily focuses on NG. Expanding the monitoring efforts to include the emergence of resistance to other antimicrobials and other bacterial species would be beneficial.

P-MIPA-007

Long-term experimental evolution of β-lactam resistance in Haemophilus influenzae

*S. Petersen[1], M. Diricks[2], H. Schulenburg[3], M. Merker[1]
[1]*Research Center Borstel, Evolution of the Resistome, Borstel, Germany*
[2]*Research Center Borstel, Molecular and Experimental Mycobacteriology, Borstel, Germany*
[3]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Evolutionary Ecology and Genetics, Borstel, Germany*

Introduction: *Haemophilus influenzae is* an opportunistic bacterial pathogen that can cause pneumonia, meningitis, and bacteremia. The increased prevalence of ampicillin resistant strains led to the endorsement of third-generation cephalosporins which overcome resistance mediated by certain β-lactamases. However, mutational resistance, and long-term bacterial adaptation under β-lactam exposure is only poorly understood, thus we established a long-term evolution experiment.

Materials/Methods: *H. influenzae* DSM 11121 was cultivated over several weeks with stepwise increasing concentrations of ampicillin, cefotaxime, and ceftriaxone. For over 300 clones we determined minimum inhibitory concentrations (MICs) and analyzed genomic variations with next generation sequencing. In addition, we measured the bacterial fitness of evolved mutants with a competitive growth assay.

Results: Presence of ampicillin and cefotaxime led to stepwise evolution of mutations in *ftsI* (encoding the main target of β-lactam antibiotics) which were associated with increased MICs to all three β-lactams. Exposure to ceftriaxone, however, independently selected for different mutations in *ompP2,* coding for an outer membrane protein. In addition, a genomic inversion affecting *ompP2* was associated with reproducible but unstable heteroresistance. Selected clones, showed variable bacterial fitness effects, and acquired possible fitness compensating mutations.

Discussion: Our data provide new insights into the evolutionary pathways of β-lactam resistance of *H. influenzae*. We highlight the role of *ompP2* as resistance determinant, and possible mechanism to induce a heteroresistant phenotype.

P-MIPA-008

WBEready - Wastewater-based epidemiology and preparedness: Research needs for a roadmap to build adaptive monitoring capacity in the Public Health Service

*I. Kraiselburd[1], F. Meyer[1], *R. Schmithausen[1], F. A. Weber[2], J. Schoth[3], M. Widera[4], S. Moebus[5], D. Schmiege[5], T. Wintgens[6], V. Linnemann[6]
[1]*Institute for Artificial Intelligence in Medicine, Data Science, Essen, Germany*
[2]*RWTH Aachen University, Research Institute for water management and cliomate future (FiW), Aachen, Germany*
[3]*Emscher-Lippe Association (EGLV), Essen, Germany*
[4]*University Hospital of Frankfurt, Institute for Medical Virology, Frankfurt a. M., Germany*
[5]*Institute for Urban Public Health (InUPH), University Hospital Essen (UME), Essen, Germany*
[6]*Institute for Water Resource and Wastewater Management (ISA), RWTH Aachen University, Aachen, Germany*

Wastewater-based epidemiology (WBE) was able to provide early indicators of an infectious event during the COVID-19 pandemic in Germany, complementing individual testing for outbreak detection. WBE also enables regional surveillance and can assist public health systems (PHS) in evaluating the effectiveness of infectious disease control measures. The German Federal Ministry of Health (BMG) sees great benefit for the PHS in further development of WBE, even beyond COVID- 19. However, in order to bring the full potential of WBE into broad application, new analytical, technical, epidemiological, and institutional research questions need to be addressed.

The overall objective of the project WBEready applied for herewith is the scientific extension of a roadmap for the development of a future-oriented WBE with adaptive monitoring capacities in the PHS. To this end, the current screening in the AMELAG (Wastewater monitoring for epidemiological situation surveillance) project is to be expanded to include numerous other pathogens in addition to SARS-CoV-2, including circulating and emerging human pathogenic viruses as well as antimicrobial resistances, and the foundations are to be laid by answering open research questions in order to prepare for new requirements in the PHS in a targeted and cost-efficient manner (preparedness).

Central questions are if new target parameters are suitable for the WBE set-up, which requirements are epidemiologically necessary and how WBE can support the institutional work of the PHS. In order to address the relevant research questions, the association area of Emschergenossenschaft and Lippeverband with focus on the Ruhr area will be investigated as a real laboratory with regard to the usable wastewater infrastructure (agglomerations, sewer networks, hospitals, wastewater treatment plants of different size classes) under consideration of socio-economic parameters.

P-MIPA-009

First insights into the plasmid landscape of clinical vancomycin resistant Enterococcus faecium

*A. Sobkowiak[1], A. Oelgemöller[1], N. Scherff[1], F. Schuler[2], V. Schwierzeck[1], V. van Almsick[1,3], A. Mellmann[1]

[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*

[2]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

[3]*University Hospital Münster, Department of Cardiology I – Coronary and Peripheral Vascular Disease, Heart Failure, Münster, Germany*

Introduction: The prevalence of vancomycin resistant *Enterococcus faecium* (VREfm) is increasing worldwide and the management of nosocomial outbreaks has become a challenge in infection control. Using whole genome sequencing (WGS), we investigated potential VREfm outbreak clusters including *vanA* plasmid clusters.

Methods: VREfm were collected as part of routine hospital surveillance in a one-year period (2022) and sequenced with a PacBio® Sequel IIe system. After *de novo* assembly, the WGS datasets were further analysis using MOB-Suite v.3.1.8 and NCBI AMRFinder Plus v.3.11.26 to characterize plasmids and to extract resistance genes. We used Ridom SeqSphere⁺ v.9.0.1 software, we extracted cgMLST allelic profiles and compared plasmids via a Mash based approach. Transmission clusters (0 alleles distance/ Mash distance ≤0.001) were further evaluated based on patient records and infection control evaluation.

Results: Of the 232 VREfm isolates analysed, 122 *vanA* and 111 *vanB* carrying isolates were identified. While *vanA* was exclusively plasmid-encoded, *vanB* was only found within the chromosome. The predominant sequence types (ST) were 80 (47% of all VREfm) and 117 (47%). 102 *vanA* were found in ST80 and 101 *vanB* in ST117. Based on cgMLST, 13 clusters with 2 to 17 isolates were identified, which harboured a *vanA* plasmid. In 7 clusters we could find an epidemiological link, indicating a nosocomial transmission.

In addition, we found 9 plasmid clusters via mash. The size of the *vanA* plasmids in the biggest cluster varied between 22 and 39 kb; the replication type was characterized as rep_889 alone or together with rep_1763 and predicted as non-mobilizable. In every plasmid, the *vanA* genes associated with transposable elements such as IS*1216E*.

Summary: The analyses of VREfm sequencing data provides a comprehensive overview of the van gene distribution and the variation of plasmids harbouring a *vanA* locus. Our results suggests that other mobile genetic elements rather than plasmids play a role in the spread of vancomycin resistance in VREfm. In the future, analysis of plasmid clusters could be used as part of outbreak investigations or to study the epidemiology of VREfm.

Microbial Metabolism & Biochemistry

P-MMB-001

Analysis of comprehensive local and global bacterial genome methylation and epigenetics in the model bacterium Helicobacter pylori detects strain-specific methylation and various internal and external influencing factors

L. Patel[1,2], F. Ailloud[1,2], F. Neukirchinger[1,2], S. Suerbaum[1,2], *C. Josenhans[1,2]

[1]*Ludwig-Maximilians University, Max von Pettenkofer Institute, Chair of Hygiene and Medical Microbiology, München, Germany*

[2]*DZIF, partner site Munich, München, Germany*

Background and Questions. Bacterial epigenetics is a recently expanding field of study. All bacterial species express methyltransferases (Mtases) which methylate genomic DNA at specific nucleotide motifs. Known functions of DNA methylation include the protection of genomic DNA integrity, DNA replication, exclusion of heterologous DNA and damage repair. Recently, DNA Mtase functions were also shown to include regulation of genomic transcripts, governing both housekeeping functions and virulence. We are using the model bacterium *Helicobacter pylori* which possesses the highest number and the most variably expressed set of DNA Mtases. We have a basic understanding of *H. pylori* epigenetics, we need to better characterize the activity, expression and basic biology of the complete set of methyltransferases under various environmental conditions. We also would like to investigate global and local genome methylation in a more quantitative manner, including single nucleotide resolution of changes in methylation.

Methods and Results. We have established novel methods, including enzymatically aided detection, biochemical methods, long-read sequencing, and mutant analyses, to quantitate specific methylation patterns of the *H. pylori* genome under various conditions. We have also expressed and purified active DNA methyltransferases. This has helped us to gather reproducible information on local quantitative methylation. DNA Mtase expression and activity are significantly strain and growth condition specific. We have also identified Mtase enzymes that show and overall high methylation, versus such which lead to a lower percentage of local methylation. Furthermore, basic conditions that modulate genome-wide nucleotide methylation in *H. pylori* include growth phase and methionine availability. We have started to use targeted intervention strategies to alter global and local DNA methylation.

Conclusions. We have obtained quantitative information on diverse global and local genome methylation in *H. pylori*

under various conditions. In the future this knowledge will support to develop novel bacterial epigenetic modulation strategies which may also have therapeutic uses.

P-MMB-002

Optimal pathway usage for growth inStaphylococcus aureus revealed by incorporatingproteomics into an enzyme constrained genomescale metabolic model

*H. Chapman[1], D. Al Nahhas[2], S. E. Wilken[1], O. Ebenhöh[1]
[1]*Heinrich Heine University Düsseldorf, Quantitative and Theoretical Biology, Düsseldorf, Germany*
[2]*Porto Conte Ricerche, Alghero, Italy*

Staphylococcus aureus is a human commensal bacteria, living in up to 30% of the population harm-free. However, in a number of cases, it can lead to opportunistic pathogenic infection causing serious harm.

The main goal of this work is to investigate the difference in metabolic pathway usage by *S. aureus* under different media conditions, simulating growth during infection as well as in minimal media. The purpose of this is to find novel drug targets against this antibiotic resistant pathogen.

We have built an enzyme constrained genome scale metabolic model (ECMM) of the livestock-associated MRSA strain ST398, in accordance with our aforementioned main goal. We have used novel analysis techniques to both predict, and offer explanations for, differential proteomic abundances under different defined media conditions. By identifying the most important pathways for growth, we also investigate potential novel drug targets against this antibiotic resistant strain.

In building an ECMM of *S. aureus*, we can increase the accuracy in phenotype predictions over the existing non-enzyme constrained genome scale metabolic models. We are able predict lethal phenotypes resulting from full or partial enzyme inhibition. These enzymes are potential drug targets.

This works highlights a promising way in which we can incorporate omics data into computational modelling for an interdisciplinary approach to fighting antimicrobial resistance.

P-MMB-003

Genome-based analysis of the inositol gene cluster in pathogenic and commensal bacteria

*M. Weber[1], T. M. Fuchs[1]
[1]*Friedrich-Loeffler-Institute Federal Research Institute for Animal Health, Institute of Molecular Pathogenesis, Jena, Germany*

Phytate is the main phosphorus storage molecule in plants and plays an important role as phosphate donor in the plant-based nutrition of animals. *Myo*-inositol (MI) is the dephosphorylated form of phytate and represents a valuable carbon and energy source for bacteria. MI degradation and the related metabolic pathway has been studied in selected species such as *Salmonella enterica* and *Bacillus subtilis*, but has not yet been analysed for its prevalence in the whole bacterial kingdom.

In this work, we analysed the MI catabolic gene cluster (IolCatGC) in 193,757 bacterial genomes, covering a total of 24,812 species, with a focus on presence, organisation, and taxonomy. To identify the presence and composition of MI gene clusters within annotated genome sequences, we established an analysis pipeline which relies on hidden markov models (HMMs) that allowed us to assign inositol gene functions. This approach was combined with a proximity criterion to detect co-localized genes. As a result, we identified 7,384 (29.8%) species to harbour the IolCatGC. Within the groups of *Actinobacteria* and *Proteobacteria*, IolCatGC is highly abundant, while *Bacteroidetes* species possess the cluster less frequently. The clusters show a high diversity with regard to gene number and composition, while the order of core genes is highly conserved on the phylum level. In summary, 111 animal pathogens, more than 200 commensals, and 430 plant pathogens utilize MI, providing strong evidence for the beneficial properties of this metabolic capacity within different environments.

P-MMB-004

L-rhamnose metabolism in Loigolactobacillus coryniformis subsp. coryniformis DSM 20001 and production of propionate-containing biopreservatives

*M. Elvan[1], K. Gravlund Fønss[1], A. Marietou[1], C. Schwab[1]
[1]*Aarhus University, Biological and Chemical Engineering, Aarhus, Denmark*

Propionate is an important antimicrobial short-chain carboxylic acid (SCCA) that prolongs the shelf life of food products. L-rhamnose is metabolized to 1,2-propanediol (1,2-PD) by the deoxyhexose pathway, this intermediate can be further converted to propionate by the 1,2-PD pathway. *Loigolactobacillus coryniformis subsp. coryniformis* DSM 20001 has been recently identified as a propionate producer, but the mechanism behind the conversion has not been revealed. The aim of this study was to investigate *L. coryniformis* L-rhamnose utilization and to demonstrate the antimicrobial activity of fermentates.

L. coryniformis was grown in bioreactors in the presence of L-rhamnose under anaerobic conditions at 30°C and pH 6.5 for 78 h. Expression of key genes related to deoxyhexose metabolism was monitored using qPCR assays. The antimicrobial properties of individual SCCAs, fermentates, and synthetic SCCA (sSCCA) mixtures mimicking the composition of fermentates against common food pathogens including *Escherichia coli, Salmonella enterica, Klebsiella oxytoca, Staphylococcus aureus,* and *Candida albicans* were investigated using 2-fold dilution assays at pH 4.5.

qPCR analysis demonstrated that genes encoding enzymes responsible for L-rhamnose utilization were expressed during the initial phase of fermentation, whereas genes involved in 1,2 PD utilization were transcribed later. From 40 mM rhamnose, up to 16 mM propionate was produced in addition to lactate (26 mM), formate (2 mM), and acetate (4 mM). Among the tested only propionate exhibited the ability to suppress the growth of the tested pathogens. The comparison with sSCCA indicated that propionate contributed to the inhibitory effect of fermentates against the tested Enterobacteriaceae.

In conclusion, our data suggested conversion of L-rhamnose to 1,2 PD and then to propionate. Our findings showed that in the *L. coryniformis* fermentates, propionate was the relevant SCCA that conferred antimicrobial properties against pathogens.

P-MMB-005

Characterizing the substrate preferences of the methanogenic archaeon Methermicoccus shengliensis and its O-demethylase system

*J. Willemsen[1,2,3], J. M. Kurth[1,2,3]
[1]*Philipps-University Marburg, Faculty of Chemistry & Microcosm Earth Center, Marburg, Germany*
[2]*Max-Planck Institute for Terrestrial Microbiology, Microcosm Earth Center, Marburg, Germany*
[3]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*

Recent studies demonstrated that the methanogen *Methermicoccus shengliensis* is capable of methane production from methoxylated aromatic compounds (MACs), which are released upon degradation of the abundant biopolymer lignin [1, 2]. A bacteria-like corrinoid dependent *O*-demethylase system (MtoABCD) enables such "methoxydotrophic" lifestyle [2]. Although resembling methyltransferases from conventional methylotrophic methanogens, a key difference is that tetrahydromethanopterin (H4MPT) acts as the terminal CH3-acceptor instead of coenzyme M (CoM). This likely results in an altered energy metabolism and redox (im)balance [2]. Interestingly, two gene isoforms encoding the first methyltransferase that cleaves the O-CH3 bond (*mtoB1* and *mtoB2*) are present in the genome of *M. shengliensis*, suggesting that these might have different substrate ranges to cover a wider range of metabolizable substrates [2]. Pioneering research showed that methane yields of grown *M. shengliensis* cultures indeed depend on the type of MAC added to the medium, as well as on the strain [1]. To further elaborate on this, we will combine *in vivo* and *in vitro* experiments: 1) For a selection of substrates, we will perform growth experiments while following production of methane (GC measurements). RT-qPCR should reveal upregulation of either of the *mtoB* genes in response to a specific substrate; 2) MtoB1/MtoB2 will either be natively purified from *M. shengliensis* based on RT-qPCR information to specifically enrich either of the isoforms. Moreover, research is ongoing to heterologously produce these two MtoB isoforms. Preliminary data obtained so far revealed that *Escherichia coli* does not properly produce MtoB, but the reason for this issue remains yet to be elucidated. In conclusion, an integration of *in vivo* and *in vitro* approaches should provide us with novel insights regarding substrate specificities and preferences of the Mto-system from *M. shenglienis*.

[1] Mayumi *et al.* (2016) *Science* 354: 222
[2] Kurth *et al.* (2021) *ISME J* 15: 3549

P-MMB-006

Deciphering substrate specificity: Lipoate- protein ligases in the spotlight of a novel lipoic acid assembly pathway in sulfur-oxidizing microorganisms

*C. Kümpel[1], T. S. Tanabe[1], E. Holz[1], C. Dahl[1]
[1]*Universtiy Bonn, Institute of Microbiology and Biotechnology, Bonn, Germany*

Many prokaryotes that oxidize reduced sulfur compounds use a pathway that involves a heterodisulfide reductase-like enzyme (sHdr) system that includes lipoate binding proteins (LbpA) as essential components [1]. The LbpA proteins are not modified by the established posttranslational lipoate assembly machinery for proteins like the glycine cleavage system protein H (GcvH) [1]. Unique maturation enzymes, including a specific lipoate-protein ligase, sLpl(AB), are encoded proximate to the genes for the sHdr system. sLpl(AB) catalyzes the ATP-dependent ligation of octanoic or lipoic acid to LbpA. LipS1 and LipS2 catalyze the insertion of sulfur into bound octanyol in the absence of free lipoic acid [2].

GcvH and LbpA are similar. It is unclear how sLpl(AB) differentiates between them in organisms that contain both lipoylation substrates. We tested lipoate:protein ligases and potential substrates from five representatives of the phyla Pseudomonadota and Aquificae, with or without the capability of sulfur oxidation. Gel shift assays and MALDI-TOF mass spectrometry confirmed that the canonical *E. coli* Lpl(AB) lipoate:protein ligase preferentially modifies GcvH proteins, but not LbpAs. In contrast, sLpl(AB) ligases from organisms that rely on the LipS1/LipS2-dependent assembly pathway prefer LbpA proteins as substrates. GcvH proteins are usually not recognized. LbpA proteins are characterized by two conserved cysteine residues, which are absent in GcvH proteins [1]. Apo-LbpA proteins with replacements of either one or both of the cysteines to alanine still serve as substrates for sLpl(AB). This indicates that these residues are not relevant for substrate recognition. Further determinants include differentially charged protein segments, as revealed by sequence alignments. GcvH proteins feature acidic amino acid patches that are replaced by basic residues in LbpAs. The interaction between lipoate-protein ligase and its substrate appears to rely on extensive hydrogen bonding between charged amino acids and is probably dictated by surface charge complementarity.

[1] Cao et al 2018 eLife 7, e37439
[2] Neti et al. 2022 ACS Bio & Med Chem Au 2, 509

P-MMB-007

Development of an *in vitro* ADP-Glucose Pyrophosphorylase Assay for the modelling of *Synechocystis* sp. PCC 6803 Glycogen Synthesis

*K. Lee[1]
[1]*University of Tübingen, Organismic Interactions, Tübingen, Germany*

The ADP-Glucose Pyrophosphorylase (GlgC) in *Synechocystis* sp. PCC 6803 catalyzes the first step in glycogen synthesis by converting glucose-1-phosphate into ADP-Glucose, which is added in turn to a growing glycogen chain by glycogen synthases (GlgA1 and GlgA2). Thus far, the *in vitro* study of GlgC was limited to using malachite green assays for free phosphate, or radiolabel-based methods1. With this work, we present our efforts in developing an *in vitro* continuous assay coupling GlgA1 to the GlgC reaction, simulating the process of glycogen synthesis *in vivo*. We also demonstrate the screening of selected metabolites and their effects on GlgC activity. We also describe in further detail the interplay between the activator and inhibitor of GlgC, 3-PGA and phosphate respectively. This new coupled glycogen assay could serve as a useful modelling tool to enable further kinetic studies on the glycogen synthesis pathway and its key players.

1. Iglesias, A. A., Kakefuda, G. & Preiss, J. Regulatory and Structural Properties of the Cyanobacterial ADPglucose Pyrophosphorylases. *Plant Physiol.* **97**, 1187–1195 (1991).

P-MMB-008

Glutathione-mediated modulation of [NiFe] hydrogenase activity and reactivation in Synechocystis sp. PCC6803 after oxygen exposure

*M. Romig[1], D. Deobald[2], A. Schmid[1]
[1]*Helmholtz center for environmental research UFZ, Solar Materials Biotechnology, Leipzig, Germany*
[2]*Helmholtz center for environmental research UFZ, Molecular Environmental Biotechnology, Leipzig, Germany*

Hydrogenases are ubiquitous metalloenzymes facilitating both hydrogen (H_2) oxidation and H_2 formation. However, their biotechnological applicability is hampered by oxygen sensitivity, particularly in aerobic or oxygen-evolving organisms, *e.g.*, the unicellular cyanobacterium *Synechocystis* sp. PCC6803 standing out as a promising candidate for photosynthetic H_2 production. It harbors Hox hydrogenase, a heteromultimeric oxygen-sensitive [NiFe] hydrogenase encoded by the *hox* operon. Hox is gradually inactivated by increasing oxygen concentrations and reactivated under a specific redox potential threshold or by electron mediators. Intracellularly, glutathione (GSH) and its oxidized form (GSSG) play a significant role in shaping the cellular redox potential.

Given that, we hypothesize an interplay between GSH and GSSG in the regulation of Hox activity, both in the presence and absence of oxygen. This was investigated through analyzing the impact of oxygen on the Hox complex *via* blue native PAGE by activity staining and complexome analysis. Additionally, H_2-driven benzyl viologen reduction activity in cell-free extracts of *Synechocystis* was analyzed *in vitro.* Oxygen exposure and different additives were applied to assess their influence on activity.

Activity staining in native gels revealed that oxygen exposure led to the formation of lower molecular weight Hox subcomplexes compared to anoxic samples. GSH addition had no impact on the staining pattern. However, at the activity level, GSH and GSSG modulated the H_2 oxidation activity of Hox in a reverse manner. While GSSG addition reduced maximal activity, GSH displayed a reactivating effect on Hox after oxygen exposure in a concentration dependent manner and increased the maximal activity. Alternative thiol agents dithiothreitol and cysteine exhibited a comparable effect to GSH on Hox activity in both anoxic and oxygen-exposed samples.

In summary, our results show that GSH and GSSG play a significant role in modulating the maximal activity and oxygen tolerance of the oxygen-sensitive Hox hydrogenase of *Synechocystis in vitro*, which enhances our understanding of the redox buffering function of GSH i*n vivo.*

P-MMB-009

The Old yellow enzyme (OfrA) responds to an integrated signal of nutrient depletion and redox imbalance in Staphylococcus aureus stress response

*E. S. Ibrahim[1], T. Wesoly[1], K. Ohlsen[1]
[1]*University of Würzburg, Institute for Molecular Infection Biology (IMIB), Würzburg, Germany*

Old yellow enzymes (OYEs) are pervasive in microbial systems, yet their impact on microbial stress response particularly during infection situations, remains elusive. In the genome of the gram-positive opportunistic pathogen, *Staphylococcus aureus* (*S. aureus*), two paralogous genes (*ofrA* and *ofrB*) encode for two OYEs. We investigated the function and the regulatory pathway of the highly conserved *ofrA* in *S. aureus* employing transcriptional, functional, and genetic analysis. Our findings reveal that OfrA is integral to *S. aureus* survival in human blood and intra-macrophages. Furthermore, *ofrA* mutation renders *S. aureus* more sensitive against reactive electrophilic, oxygen, and chlorine species (RES, ROS, and RCS). Additionally, *ofrA* responds to nutrient depletion through a tightly controlled transcriptional axis. In summary, *ofrA* significantly contributes to *S. aureus* fitness in infection conditions through stress response mechanisms.

P-MMB-010

Oxygen-independent activation of unsaturated side chains of steroids involving a radical flavin desaturase

*T. Zhan[1], C. Jacoby[1], M. Jede[1], B. Knapp[2], S. Ferlaino[3], A. Günter[4], F. Drepper[2], M. Müller[3], S. Weber[4], M. Boll[1]
[1]*University of Freiburg, Institute of Biology II/ Microbiology, Freiburg i. Br., Germany*
[2]*University of Freiburg, Institute of Biology II/ Biochemistry and Functional Proteomics, Freiburg i. Br., Germany*
[3]*University of Freiburg, Institute of Pharmaceutical Sciences, Freiburg i. Br., Germany*
[4]*University of Freiburg, Institute of Physical Chemistry, Freiburg i. Br., Germany*

Sterols are abundant hydrophobic organic compounds that persist in the environment due to their water insolubility and chemical inertness. Recently, a sterol degradation pathway was discovered in a cholesterol-grown denitrifying bacterium *Sterolibacterium* (*S.*) *denitrificans* under anoxic conditions. The initial activation of primary C26 of the isoprenoid side chain of cholesterol is catalyzed by a four-step ATP-dependent enzyme cascade involving hydroxylation to an allylic alcohol via a phosphorylated intermediate.[1] However, this degradation mechanism is not applicable to steroids with a double bond at C-22 in the isoprenoid side chain, such as the plant sterol stigmasterol. Here, we have enriched delta-24 desaturase (Δ24-SD) from *S. denitrificans*, which catalyzes the oxidation of the intermediate Δ22-en to a conjugated Δ22,Δ24-diene in the presence of an electron acceptor. Biochemical analysis suggests a $\alpha_4\beta_4$ architecture of 440 kDa for Δ24-SD, in which each subunit covalently binds an FMN cofactor to a histidyl residue. UV/vis and EPR analysis revealed the presence of both flavins as red semiquinone radicals, indicating a radical-based mechanism involving an allylic radical intermediate. Our proposed model involves a homodimeric catalytic subcomplex in which two flavin semiquinones each abstract a hydrogen atom from the substrate. Subsequently, the formed C24 double bond enables the hydroxylation of the terminal C26 with water by a heterologously produced molybdenum-dependent steroid C26 dehydrogenase 2 ($S26DH_2$). In conclusion, Δ24-SD allows for direct hydroxylation of the unactivated primary C26 of Δ22-steroids by circumventing the ATP-dependent formation of a phosphorylated intermediate.[2]

[1]Jacoby, C. *et al.*, *Nat. Commun.* **11**, 3906 (2020)
[2]Zhan, T. *et al.*, (2024) [submitted for publication]

P-MMB-011

Structure and function of radical SAM menaquinone methyltransferases

*D. Wilkens[1], *J. Simon[1]
[1]*Technical University of Darmstadt, Department of Biology, Darmstadt, Germany*

Menaquinone (MK) serves as an essential membranous redox mediator in several electron transport chains of aerobic and anaerobic respiration. In addition, the composition of the quinone/quinol pool has been widely used as a biomarker in microbial taxonomy. The class C radical SAM methyltransferases (RSMTs) MenK, MqnK and MenK2 have been shown to facilitate specific menaquinone methylation reactions at position C-8 (MenK/MqnK) or C-7 (MenK2) to synthesise 8-methylmenaquinone, 7-methylmenaquinone or 7,8-dimethylmenaquinone [1-3]. In addition, primary structure motifs of the MenK/MqnK/MenK2 family allowed a functional categorisation [4].

Here, the functional MenK and MenK2 enzymes from *Collinsella tanakaei* were produced in *Escherichia coli*. (Methyl)menaquinone species from the respective cells were separated and identified by UV/Vis spectroscopy and mass spectrometry. The activity of purified and Fe/S centre-reconstituted MenK and MenK2 was investigated using a specific *in vitro* MK methylation assay. The biochemical analysis was supported by AlphaFold structure prediction, which suggested the architecture of the catalytic site and a putative MK entry cavity, thus helping to predict a universal reaction mechanism of MK methylation.

References

1 Wilkens, D. and Simon, J. (2023) Biosynthesis and function of microbial methylmenaquinones. Adv. Microb. Physiol. **83**, 1-58.
2 Hein, S., von Irmer, J., Gallei, M., Meusinger, R. and Simon, J. (2018) Two dedicated class C radical *S*-adenosylmethionine methyltransferases concertedly catalyse the synthesis of 7,8-dimethylmenaquinone. Biochim. Biophys. Acta **1859**, 300-308
3 Hein, S., Klimmek, O., Polly, M., Kern, M. and Simon, J. (2017) A class C radical *S*-adenosylmethionine methyltransferase synthesizes 8-methylmenaquinone. Mol. Microbiol. **104**, 449-462.
4 Wilkens, D., Meusinger, R., Hein, S. and Simon, J. (2021) Sequence analysis and specificity of distinct types of menaquinone methyltransferases indicate the widespread potential of methylmenaquinone production in Bacteria and Archaea. Environ. Microbiol. **23**, 1407-1421.

P-MMB-012

Dynamic Assembly and Disassembly of NrdR Filaments in the Nosocomial Pathogen Acinetobacter baumannii Modulated by the DNA Methyltransferase AamA

*A. Elatris[1], K. Weber[1], G. Wilharm[1]
[1]*Robert Koch-Institut, Bereich Wernigerode, Wernigerode, Germany*

Acinetobacter baumannii, a Gram-negative opportunistic pathogen, poses a severe public health threat due to its increasing resistance to multiple drugs. Our research focuses on understanding the epigenetic regulation in *A. baumannii* mediated by the newly identified DNA adenine methyltransferase AamA [1,2] and its interaction with the putative transcriptional regulator NrdR [3].

Small-angle X-ray scattering analyses demonstrated the propensity of His-tagged NrdR to form extensive macromolecular assemblies exceeding 500 kDa, displaying a filamentous-like structure composed of repeating units. Furthermore, upon the introduction of AamA, NrdR starts to disassemble into smaller, likely polydisperse particles and non-specific aggregates [3].

Here, we successfully purified untagged NrdR through the concentration of semi-pure NrdR fractions using ultrafiltration. This process resulted in the formation of higher order filament structures, leading to subsequent precipitation. Following the removal of the supernatant, pure NrdR was readily solubilized in Tris-HCl buffer, achieving a concentration of 5-7 mg/mL.

Additionally, native gel analyses corroborated the interaction between AamA and NrdR as well as the capability of NrdR to form ordered filaments. Notably, NrdR displayed sequence-independent binding to nucleic acids. NrdR-specific sequence boxes remain to be discovered.

In conclusion, our hypothesis posits a crucial role for AamA in *A. baumannii* virulence through its involvement in differential methylation, facilitated by the dynamic switching of NrdR between filamentous storage-form and de-polymerized active-form.

[1] Blaschke et al. (2018) *Recombinant production of A1S_0222 from A. baumannii ATCC 17978 and confirmation of its DNA-(adenine N6)-methyltransferase activity.* Protein Expr Purif **151**:78-85
[2] Yang et al. (2023) *AamA-mediated epigenetic control of genome-wide gene expression and phenotypic traits in A. baumannii ATCC 17978.* Microb Genom **9**(8)
[3] Weber et al. (2022) *Recombinant AcnB, NrdR and RibD of A. baumannii and their potential interaction with DNA adenine methyltransferase AamA.* Protein Expr Purif **199**:106134

P-MMB-013

Comparison of 7-hydroxysteroid dehydratases in two bile acid-degrading strains from the Sphingomonadaceae family.

*J. C. Coutiño-Montes[1], J. Holert[1], B. Philipp[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

Bile acids are amphipathic steroid compounds produced in the digestive tract of vertebrates. After their excretion, these compounds can be used as carbon and energy sources by environmental bacteria. Degradation of bile acids proceeds via formation of $\Delta^{1,4}$-intermediates in Actinobacteria and γ- and β-proteobacteria. In contrast, a pathway variant found in Sphingomonads such as *Sphingobium* sp. strain Chol11 involves formation of $\Delta^{4,6}$-degradation intermediates from 7-hydroxylated bile acids. These intermediates are formed by water elimination from C7 catalyzed by the stereospecific 7α- and 7β hydroxysteroid dehydratases (Hsh2 and Hsh3, in strain Chol11) as key enzymes of this pathway variant.

The novel bile acid-degrading bacterium *Novosphingobium* sp. strain Chol12 also degrades 7-hydroxy bile acids via $\Delta^{4,6}$-intermediates but exhibits significantly different kinetics of $\Delta^{4,6}$ and $\Delta^{1,4}$-intermediate production than strain Chol11. This study aimed to compare 7α- and 7β-hydroxysteroid dehydratase activities from strain Chol11 and strain Chol12 using C_{24} and C_{19} steroid substrates (with and without acyl side chain, respectively) with different hydroxylation patterns at C7 and C12. Enzyme assays with cell extracts from both strains presented similar 7α-dehydratase activity with C_{19} substrates with a hydroxyl group in C12. In contrast, 7α-dehydratase activity was not observed in cell extracts of strain Chol12 when using the C12-hydroxylated substrate with C_{24}. This instance suggests that C12-hydroxyl group

and the presence of an acyl side chain affect the Hsh2-like activity in strain Chol12. Assays for 7β-dehydratase activities of both strains are currently being carried out in our laboratory.

Genome sequencing of strain Chol12 revealed the presence of two proteins that share high sequence identity to Hsh2 and Hsh3 of Chol11. The function and substrate specificities of these homologs are currently studied at the biochemical level by heterologous expression and enzyme assays.

These results provide novel insights into the substrate specificity of hydroxysteroid dehydratases and might be relevant for the biotechnological production of pharmaceutical steroid compounds.

P-MMB-014

The heterodisulfide reductase-like (sHdr) pathway for dissimilatory sulfur oxidation involves the novel sHdrH protein that crystallizes as a dimer

*M. Grosser[1], N. Schneberger[2], E. Ruyters[1], J. Li[1], A. Mancoglu[1], G. Hagelueken[2], C. Dahl[1]
[1]*University of Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*
[2]*University of Bonn, Institute of Structural Biology, Bonn, Germany*

Many sulfur-oxidizing bacteria and archaea pursue a dissimilatory sulfur oxidation pathway involving the sHdr system. The core proteins of this widespread system are encoded in a conserved *shdrC1B1AHC2B2* cluster in Pseudomonadota and Aquificae and resemble the subunits of the heterodisulfide reductase from methanogenic archaea. The sHdr proteins are indispensable for the use of thiosulfate as an accessory electron donor in the Alphaproteobacterium *Hyphomicrobium denitrificans* [1]. In the hyperthermophilic bacterium *Aquifex aeolicus*, the sHdr proteins have been shown to be membrane-associated [2]. On the other hand, the sHdrA subunit from *H. denitrificans* is soluble [1,3]. Our goal is to characterize the sulfur-oxidizing sHdr system from the obligately chemolithotrophic Gammaproteobacterium *Thioalkalivibrio thiocyanoxidans.* To achieve this, we conducted anion exchange chromatography followed by immunodetection. We discovered that all sHdrAB1B2C1 and -C2 proteins are soluble in this organism. Additionally, all five proteins co-purify in the same fractions upon anion exchange chromatography, indicating the formation of a heterocomplex.

The sHdrH protein is always encoded among the core *shdr* genes. It has no homologs outside of the sHdr system, indicating a special function in the context of dissimilatory sulfur oxidation. In the presence of thiosulfate, the transcription of *shdrH* is upregulated in *H. denitrificans,* along with that of the other *shdr* genes. The capacity for thiosulfate oxidation is significantly reduced in an *H. denitrificans in frame* deletion mutant lacking sHdrH, highlighting the importance of sHdrH in the overall process. In this study, we present the high-resolution crystal structure of the soluble dimeric sHdrH from *T. thiocyanoxidans*.

[1] Koch & Dahl 2018 ISME J 10, 2479
[2] Boughanemi et al. 2016 FEMS Microbiol Lett 363, fnw156
[3] Ernst et al. 2021 FEBS J 288, 1664

P-MMB-015

Unraveling methyl chloride conversion in the acetogen Acetobacterium dehalogenans

*J. Bernhardt[1,2], P. Klemm[3], S. Vuilleumier[4], J. M. Kurth[1,3,2]
[1]*Microcosm Earth Center, Max Planck Institute for Terrestrial Microbiology and Philipps-Universität, Marburg, Germany*
[2]*Microbial Physiology Lab, Department of Chemistry, Marburg, Germany*
[3]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*
[4]*Génétique Moléculaire, Génomique, Microbiologie, UMR 7156 CNRS, Straßburg, France*

Methyl chloride (MC) stands out as the most prevalent organochlorine in the atmosphere, contributing 15-20% to chlorine-catalyzed ozone depletion in the stratosphere [1]. The acetogenic bacterium *Acetobacterium dehalogenans* utilizes MC as its sole energy source [2]. While the methyltransferase systems of *A. dehalogenans* for the demethylation of methoxylated aromatics have been characterized [3], the system responsible for MC demethylation remained unidentified. This study aims to understand the metabolic changes of *A. dehalogenans* during growth on MC versus the methoxylated aromatic syringate and to identify the enzyme system catalyzing MC demethylation/dehalogenation. We analyzed the growth and substrate conversion of *A. dehalogenans* grown on MC, syringate and both substrates. Furthermore, we used comparative transcriptomics to analyze the gene expression of *A. dehalogenans* grown under these conditions. We found more than 100 highly differentially expressed genes (log2FC >3) comparing MC vs. syringate as substrate (67 genes up-, 78 downregulated) and syringate plus MC vs. syringate (164 genes up-, 43 downregulated). During growth on MC, three neighboring genes were highly upregulated (log2FC >10) that encode a yet uncharacterized corrinoid-dependent methyltransferase system. This system is most probably responsible for the transfer of the methyl group from MC to tetrahydrofolate, a C1 carrier in the Wood-Ljungdahl pathway. We are heterologously producing and purifying the corresponding proteins of *A. dehalogenans* in *E. coli* and purifying them and will subsequently perform UV-Vis spectroscopy as well as activity assays to define substrate utilization and reaction kinetics. Blast analysis further indicates that homologous genes are present in several other anaerobic bacteria, which may enable these microorganisms to convert MC by a similar enzyme system.

[1] Harper, D. B. (2000). *Nat Prod Rep*, *17*, 337-348.
[2] Traunecker, J., et al. (1991). *Arch Microbiol, 156,* 416–421.
[3] Engelmann, T. et al. (2001). *Arch Microbiol,* 175, 376–383.

P-MMB-016

Targets and properties of the two-component system MxtR/ErdR of *Pseudomonas putida* KT2440

*F. Burr[1], H. Jung[1], M. Eder[1], T. Henriquez[2], J. S. Hsu[1]
[1]*Ludwig-Maximilians University Munich, Microbiology, Planegg, Germany*
[2]*University of Siena, Medical Biotechnology, Siena, Italy*

The MxtR/ErdR two-component system of *Pseudomonas* and *Vibrio* species has been reported to control the expression of genes required for acetate utilization [1,2]. A characteristic feature of the sensor kinase MxtR is the presence of a membrane-integrated solute/sodium symporter (SSS) domain connected to domains typical of bacterial sensor kinases via a STAC domain [3]. It is hypothesized

that the SSS domain senses a yet unknown chemical signal, possibly transports the corresponding solute and influences the signal transduction process by associated conformational changes. The aims of the present work are (1) to elucidate possible additional metabolic processes controlled by MxtR/ErdR and (2) to determine the functional significance of individual domains of MxtR.

To address Aim 1, the growth of an *mxtR* mutant of the soil bacterium *Pseudomonas putida* KT2440 on different C-sources was investigated. Furthermore, electrophoretic mobility shift assays (EMSA) were performed to test the binding of the response regulator ErdR to promoters of potential target genes. To address Aim 2, domains of the sensor kinase MxtR were individually deleted and the growth of the corresponding mutants on different C- sources was examined.

Our results show that MxtR/ErdR is essential for growth on propionate as the sole carbon source in addition to acetate. EMSAs confirm that ErdR controls the expression of genes encoding enzymes involved in the activation (acyl-CoA synthesis) and degradation (methylcitrate cycle) of propionate. Furthermore, we observed that in addition to the histidine kinase (HisKA) domain, the SSS and STAC domains are essential for MxtR function. In contrast, the receiver (REC) domain of MxtR is dispensable for growth on short chain fatty acids.

The results obtained highlight the role of MxtR/ErdR in the regulation of short-chain fatty acid metabolism and demonstrate that the transporter domain of MxtR is essential for signal transduction in *P. putida* KT2440.

[1] Hang, S. *et al.* (2014) *Cell host & microbe* **16**, 592-604.
[2] Henriquez, T. *et al.* (2023) *Microbiol Spectr* **11**, e0292322.
[3] Korycinski, M. *et al.* (2025) *J Mol Biol* **427**, 3327-3339

P-MMB-017
DC electric fields promote biodegradation of a waterborne contaminant in biofilter systems
*J. He[1], L. Wick[1]
[1]*Helmholtz center for environmental research UFZ, Applied microbial ecology, Leipzig, Germany*

Biofiltration is a simple and low-cost method for the clean-up of contaminated waters. Reduced chemical availability to surface-attached degrading bacteria however may limit their efficient use. When a direct current (DC) electric field is applied to an immersed packed bed, it invokes electrokinetic processes such as the electroosmotic water flow (EOF). EOF is a surface charge-induced, plug flow-shaped movement of pore fluids. It acts at a nanometer distance above surfaces and provokes the mobilization of fluids that are typically unaffected by hydraulic flow. EOF therefore has the potential to change microscale pressure-driven flow profiles and to promote the availability of contaminants to microbial degraders. In laboratory percolation columns we here assessed the effects of a weak DC electric field (on the biodegradation of waterborne naphthalene (NAH) by glass bead attached *Pseudomonas fluorescens* LP6a. To vary NAH bioavailability, we used different NAH concentrations at $C_0 > K_m$ and hydraulic flow velocities at ranges typical for biofiltration systems (= 0.2 – 1.2×10^{-4} m·s-1). In DC free controls we observed higher specific degradation rates (*q*c) at higher NAH concentrations. At given C_0 however, *q*c decreased with increasing suggesting bioavailability restrictions depending on the hydraulic residence times. Relative to controls, DC fields increased *q*c and resulted in linearly increasing benefits up to 50% with raising hydraulic loadings. We interpret these benefits by EOF promoted NAH bioavailability to cells. As the EOF acted opposite to hydraulic flow, it modified pressure-driven flow profiles around the collector beads at microscale and, thereby allowed for better NAH provision to the cells exposed to zones of low NAH bioavailabilty. To our knowledge this is the first description of the superimposition of EOF and pressure-driven flow on the bioavailability of waterborne chemicals. Electrokinetic approaches may give rise to future technical applications that allow regulating biodegradation of waterborne contaminants.

P-MMB-018
Reduction of aromatic acids to corresponding alcohols by coupled enzyme assays
*Y. Gemmecker[1], D. Hege[1], J. Heider[1]
[1]*Philipps University of Marburg, Microbiology, Marburg, Germany*

Aromatoleum aromaticum EbN1 and other species of the genus *Aromatoleum* have been sources for many enzymes catalyzing reactions with aromatic carbohydrates. Aromatic carboxylic acids can be found in various sources from crude oil to lignin. They have a broad range of applications. So far, industrial synthesis and applications rely on lithium/aluminium catalysts amongst others. Here we present a pathway way of biocatalytic reduction from aromatic acids to corresponding alcohols utilizing recombinant enzymes from *A. aromaticum* EbN1.

The tungsten cofactor containing aldehyde oxidoreductase (AOR) is an oxygen sensitive enzyme that is able to catalyze the oxidation of a broad variety of aromatic and aliphatic aldehydes to the respective acids in presence of an electron acceptor. [1] Recent results show that AOR is also catalyzing the reverse reaction, reduction of benzoate to benzaldehyde, albeit at very low rates and under conditions strongly favoring acid reduction, e.g., low pH. Reports of thermophilic AOR orthologs also demonstrate slow reduction of organic acids directly to the corresponding aldehydes, if the thermodynamic equilibrium is made favorable for this reaction by the presence of semicarbazide or alcohol dehydrogenases removing the aldehydes from equilibrium [2, 3] Therefore, introduction of a coupled system with a benzyl alcohol dehydrogenase (BADH) also from *A. aromaticum* EbN1 enabled us to study the kinetics for the acid reduction reactions of AOR.

It has been reported that BADH is produced in various growth conditions. [4] We established recombinant expression and purification of BADH to study its kinetics for reduction of aldehydes and oxidation of alcohols. Furthermore, the BADH itself has a broad substrate spectrum of mainly aromatic compounds and favors NAD(H) as cofactor, but works with NADP(H) as well. This allows multiple experimental settings, either including or excluding cofactor.

[1] Arndt, F. et al. (2019). *Front. Microbiol.* 10:71. doi: 10.3389/fmicb.2019.00071
[2] Heider, J. et al. (1995). J. Bacteriol. 177, 4757–4764. doi: 10.1128/jb.177.16.4757-4764.1995

[3] Huber, C. et al. (1995). Arch. Microbiol. 164, 110–118. doi: 10.1007/BF02525316
[4] Wöhlbrand, L. et al. (2007). *Proteomics* 7, 2222–2239. doi: 10.1002/pmic.200600987

P-MMB-019

Insights into the Physiological Responses of the two Cyanobacterial strains Synechocystis sp. PCC 6803 and Synechococcus sp. PCC 11901 during Nitrogen Starvation

*A. Zini[1], *S. Mondal[1], K. Forchhammer[1]
[1]*Eberhard Karls University of Tübingen, Organismische Interaktionen, Tübingen, Germany*

Cyanobacteria are photoautotrophic prokaryotic Gram-negative organisms that exhibit ubiquity across diverse environments. In addition to their ability of fixing atmospheric CO2, these microorganisms produce various secondary metabolites with pharmaceutical and industrial benefits. In nature, one of the most common growth constraints is limitation of nitrogen supply both in marine and terrestrial ecosystems. Nitrogen regulation in cyanobacteria is mediated by the transcriptional regulator NtcA, in conjunction with its co-activator PipX and by the ubiquitous PII signaling protein.

In this study, two unicellular cyanobacterial strains, *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) and the newly isolated, fast growing strain *Synechococcus* sp. PCC 11901 (hereafter *Synechococcus*) are compared based on their response to nitrogen starvation conditions; the process known as "chlorosis". Under optimal growth conditions, *Synechocystis* displays a doubling time of 12h whereas the fast-growing *Synechococcus* of only 2-3h of doubling time. During chlorosis, in both species the cellular metabolism experiences a reduction in flux, marked by diminished protein synthesis and anabolic processes. Moreover, the photosynthetic apparatus is compromised, evident in the absence of chlorophyll α and phycobiliproteins, resulting in a transition from a blue-green to yellow color in *Synechocystis* and white in *Synechococcus.* Remarkably, *Synechococcus* also displays a shift in the morphology from rod-shaped to spherical. Chlorotic cells accumulate glycogen in a form of carbon and energy source, subsequently utilized during cellular resuscitation. Transcriptomic analysis of chlorotic cells in *Synechocystis* reveals heightened expression of the *nblA* gene, responsible for phycobilisome degradation, and of genes associated with glycogen metabolism.

P-MMB-020

Metabolic cross-feeding between milk bacteria and *Brucella abortus* supports the proliferation of the highly contagious food borne pathogen

A. Stephan[1], C. M. Ufermann[1], S. Gensberger-Reigl[2], M. Jarek[3], D. Maus[4], M. Blume[4], S. Al Dahouk[1,5], M. Pischetsrieder[2], *D. Hofreuter[1]
[1]*German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany*
[2]*Friedrich-Alexander-University Erlangen-Nürnberg, Department of Chemistry and Pharmacy, Erlangen, Germany*
[3]*Helmholtz Center for Infection Research, Research Group Genome Analytics, Brunswick, Germany*
[4]*Robert Koch-Institute, Metabolism of Microbial Pathogens (P6), Berlin, Germany*
[5]*German Environment Agency, Department of Environmental Hygiene, Berlin, Germany*

Brucellosis is a widespread zoonotic disease primarily transmitted by consumption of unpasteurized dairy products derived from cow's or sheep's and goat's milk contaminated with *Brucella abortus* or *Brucella melitensis*. Both *Brucella* species are viable in milk for several days, yet, the impact of milk on the metabolic activity and propagation of *Brucella* is unknown.

Whole genome sequence analyses of *B. abortus* identified a panel of proteases, peptidases and peptide transporters that may contribute to the degradation and utilization of milk proteins. We demonstrated that *B. abortus* uses milk efficiently as a substrate for growth: It proliferates in commercial cow's milk with an increase in colony counts of several magnitudes over 72 hours. To examine the proteolytic activity of *B. abortus*, the peptide changes of commercial cow's milk upon cultivation with *B. abortus* were examined by LC-MS analysis.

Since casein proteins are the major components of milk, we investigated the impact of casein, its derived peptides and free amino acids on the growth of *B. abortus*. Bacteria were cultivated in minimal medium supplemented with casein, tryptic-digested casein or casein hydrolysate as sole energy sources. While, casein peptides and free amino acids from casein hydrolysate promoted *Brucella* growth, uncleaved casein could not be catabolized by *B. abortus*.

We hypothesized that milk bacteria produce the casein-derived products utilized by *Brucella* during their growth in milk. We therefore cultivated bacteria from fresh, unpasteurized cow's milk and identified isolates by MALDI-TOF MS and NGS analyses. Among the bacterial species identified were *Staphylococcus equorum* and *S. sciuri*. The latter isolate exhibited pronounced proteolytic activity when grown on milk agar plates. We incubated the milk bacteria in minimal medium containing casein to produce spent media. *B. abortus* growth was promoted in the spent medium derived from the proteolytically active *S. sciuri*. This observation suggests that *B. abortus* takes advantage of the casein-derived amino acids and peptides as well as other metabolic products being generated through the catabolic activity of *S. sciuri* in cow's milk.

P-MMB-021

Purification and characterization of an electron-bifurcating formate dehydrogenase/hydrogenase complex from Sporomusa ovata

*J. Roth[1], V. Müller[1]
[1]*Goethe University Frankfurt/Main, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: Acetogenic bacteria are a group of strictly anaerobic bacteria that fix CO_2 and produce acetate as the main product using the Wood-Ljungdahl pathway (WLP). The first step of the WLP is the reduction of CO_2 to formate, catalysed by formate dehydrogenases, but the involved electron carriers differ among acetogens[1-3]. The model for cytochrome-containing acetogens, *Sporomusa ovata* has at least three different formate dehydrogenase genes, but the nature of the enzyme remained to be identified.

Goals: We aimed to elucidate the subunit composition, mechanism of electron transport and electron carriers involved in the reduction of CO_2 to formate in *S. ovata*.

Materials & Methods: Protein purification, biochemical characterization, enzymatic assays and bioinformatic analysis.

Results: The formate dehydrogenase of *S. ovata* was purified from the cytoplasmic fraction using three different chromatographic steps. After each purification step, the fractions containing formate dehydrogenase also showed hydrogenase activity. The purified protein catalyzed the simultaneous reduction of NAD^+ and Fd with H_2 as well as with formate as electron donor. The enzyme was able to produce formate from H_2 and CO_2 as well as H_2 from formate.

Summary: The formate dehydrogenase (FdhA) of *Sporomusa ovata* forms a complex with the electron bifurcating hydrogenase (HydABC) and the complex uses the mechanism of electron bifurcation to reduce CO_2 with electrons derived from molecular hydrogen. Ferredoxin and NAD^+ are involved as electron carriers.

[1] Li, L.F., Ljungdahl, L., Wood, H.G. (1966) J Bacteriol 92:405-412
[2] Wang, S., Huang, H., Kahnt, J., Müller, A.P., Köpke, M., Thauer, R.K. (2013) J Bacteriol 92:405-412 195:4373-4386
[3] Schuchmann, K., Müller, V. (2013) Science 342:1382-1385

P-MMB-022
Infant fecal accumulation of fucose and 1,2PD and Clostridium perfringens utilization

*L. Huertas-Díaz[1], A. Marietou[1], A. Dunkel[2], A. Feilberg[1], J. Behr[3], M. Somoza[3], C. Schwab[1]
[1]*Aarhus University, Biological and Chemical Engineering, Aarhus, Denmark*
[2]*Leibniz Institute for Food Systems Biology at the Technical University of Munich (LSB), Integrative Food Systems Analysis, Freising, Germany*
[3]*Leibniz Institute for Food Systems Biology at the Technical University of Munich (LSB), Transcriptome & Proteome Profiling, Freising, Germany*

Introduction

Strain of *Clostridium sensu stricto* are early colonizers of the infant gut that can use the human milk oligosaccharides derived fermentation metabolite fucose and 1,2-propanediol (1,2PD). Fucose can be catabolized to 1,2PD which is metabolized by enzymes of the *pdu* operon to propionate and propanol. *Clostridium perfringens* exhibits a unique response to 1,2PD metabolism. We previously confirmed fucose and 1,2PD utilization in the bacterium. This study aimed to reveal fucose and 1,2PD mechanistic pathway in *C. perfringens* FMT 1006 from infant feces.

Method

The genome was sequenced and compare to reference strains. Growth kinetics were evaluated in yeast casitone medium (YC) supplied with glucose as positive control (YC-Glc, 50 mM), propanediol (YC-1,2PD, 43 mM), and glucose (10 mM) + 1,2PD (43 mM, YC-Glc-1,2PD). Fermentation metabolites and gases were analysis with GCMS and PTR-TOF-MS. RNA samples were collected for gene expression analysis using microarrays. Membrane staining and composition analysis by LCMS were performed.

Results

Genome analysis confirmed presence of the pdu operon including pduCDE, pduQ, pduP, pduL, and pduW. Gene synteny was highly conserved compared with other strains. The growth rate with YC-Glc was 0.24 OD/h, 1.45-fold higher than growth rate of YC-Glc-1,2PD (0.17 OD/h). *C. perfringens* grown in YC-Glc formed mainly lactate (42.5 mM from 24 mM glucose) while acetate was the main metabolite (9.7 mM from 5.3 mM glucose) during growth in YC-Glc-1,2PD. While *C. perfringens* produced propanol in YC-1,2PD, propionate production was compromised, which was supported by gene expression analysis and lack of expression of pduP, pduL and pduW. During growth in YC-1,2PD, membrane lipids polarity and membrane fluidity increased compared with YC-Glc.

Conclusion

C. perfringens metabolism of 1,2PD leads 1-propanol as the main and only product. These findings set the framework to understand HMO by-product metabolism by *C. perfringens* infant isolated species.

P-MMB-023
The second messenger c-di-AMP controls natural competence via ComFB signaling protein

*S. Samir[1], S. Doello[1], E. Zimmer[1], S. V. Albers[2], K. A. Selim[1,3]
[1]*Tübingen University, Interfaculty Institute of Microbiology and Infection Medicine, Organismic Interactions Department, Cluster of Excellence “Controlling Microbes to Fight Infections”, Tübingen, Germany*
[2]*University of Freiburg, Molecular Biology of Archaea, Institute of Biology, Freiburg i. Br., Germany*
[3]*University of Freiburg, Microbiology/ Molecular Physiology of Prokaryotes, Institute of Biology II, Freiburg i. Br., Germany*

Natural competence is a conserved mechanism of horizontal gene transfer that permits massive genetic variation and genomic plasticity via uptake of extracellular DNA. Natural competence requires a contractile pilus system and an assemblage of competence-accessory proteins. Here, we provide evidence that the pilus biogenesis and natural competence in cyanobacteria are regulated by the second messenger c-di-AMP. The c-di-AMP-free (*DdacA*) mutant showed significantly lower transformation efficiency than the WT cells. Transcriptome and proteome analysis revealed a strong downregulation of specific genes and proteins involved in pilus biogenesis and DNA uptake in the *DdacA* mutant. These proteins include PilT1, an essential motor required for retraction of type IV pilus. In contrast to WT cells, transmission electron microscopy revealed that *dacA* cells possess only thick pili with hyperpiliation phenotype similar to the non-competent *pilT1* mutant. Furthermore, we show that the competence factor B signaling protein (ComFB) is a novel c-di-AMP-receptor protein, widespread in bacterial phyla. A *DcomFB* mutant was created to test if ComFB is playing a role in natural competence, and like *DdacA* mutant, it showed reduced transformation efficiency. Our findings further support that natural competence depends on c-di-AMP signaling and is controlled by a pathway that involves ComFB as a c-di-AMP receptor.

References:

Samir, S., Doello, S., Zimmer, E., Haffner, M., Enkerlin, A. M., Mueller, T., ... & Selim, K. A. (2023). The second

messenger c-di-AMP controls natural competence via ComFB signaling protein. *bioRxiv*, 2023-11.

P-MMB-024

Being lazy is not always bad: The effects of c-di-AMP signalling on glutamine toxicity in cyanobacteria

*A. M. Enkerlin[1], L. Dengler[2], M. Haffner[2], K. A. Selim[1]
[1]*University of Freiburg, Microbiology, Freiburg i. Br., Germany*
[2]*University of Tuebingen, Tübingen, Germany*

Cyanobacteria exhibit a distinctive lifestyle as photoautotrophic bacteria, necessitating rapid responses to environmental shifts that jeopardize their carbon/nitrogen (C/N) balance. To maintain homeostasis, cyanobacteria employ cyclic nucleotide second messengers, such as cyclic di-adenosine monophosphate (c-di-AMP)[1]. In cyanobacteria, c-di-AMP has now been implicated in a broader range of functions, including C/N balance regulation[2], day-night acclimation[3], and natural competence[4]. While mechanistic aspects of C-regulation have been elucidated[3], N-regulation remains unexplored. In the search for novel c-di-AMP targets, we identified the glutamine (Gln) transporter BgtAB as a c-di-AMP target. Gln was reported to be toxic for cyanobacteria[5], but its mode of action remained elusive. Surprisingly, the c-di-AMP-free mutant (Δ*dacA*) was able to grow on toxic Gln concentrations and use it as sole N-source, while wildtype (WT) died. We found that Δ*dacA* exhibited impaired Gln uptake compared to WT. Here, targeted metabolomics unveiled an overaccumulation of arginine and its intermediates in WT cells growing on Gln. In contrast, Gln metabolization was severely hampered in Δ*dacA*, highlighting a role for c-di-AMP in controlling N-metabolism. We further show that the high Gln uptake in WT induces *dacA* gene expression and c-di-AMP overproduction, which is known to be toxic for bacteria[6]. Overall, our findings suggest that c-di-AMP positively regulates BgtAB, and the resulting Gln uptake triggers c-di-AMP overproduction, establishing a lethal feedback loop. This work presents the first mechanistic explanation of Gln toxicity in cyanobacteria due to c-di-AMP signalling and showcases the role of c-di-AMP in N-metabolism.

1. **Mantovani *et al.* (2023).** *MicroLife*, 4, uqad008. DOI: 10.1093/femsml/uqad008
2. **Haffner *et al.* (2023).** *BioRxiv*. DOI: 10.1101/2023.11.14.567006
3. **Selim *et al.* (2021).** *Science Advances*, *7*(50), eabk0568. DOI: 10.1126/sciadv.abk0568
4. **Samir *et al.* (2023).** *BioRxiv*. DOI: 10.1101/2023.11.27.568819
5. **Flores & Muro-Pastor (1990).** *Archives of Microbiology*, *154*(6). DOI: 10.1007/BF00248831
6. **Gundlach *et al.* (2015).** *Journal of Bacteriology*, *197*(20), 3265–74. DOI: 10.1128/jb.00564-15

P-MMB-025

The role of polyamines in microbial interactions of intestinal bacteria in the context of acid stress

*S. Beck[1], K. Jung[1]
[1]*Ludwig-Maximilians University Munich, microbiology - AG K. Jung, Martinsried, Germany*

In the complex milieu of the human gut microbiome, understanding the intricate interactions within microbial communities is essential to unravel the dynamics that govern health and disease. This study focuses on the symbiotic relationship between *Escherichia coli* and *Enterococcus faecalis* and investigates their adaptive responses to acid stress[1,2] and polyamine cross-feeding[3] under physiological conditions. Ornithine, a polyamine crucial for this interplay[4], is produced by the enzyme SpeF, marking its significance in the context of microbiome interactions. By analyzing various pathogenic and non-pathogenic *E. coli* strains, we were able to show that the presence of regulatory elements essential for *speF* expression appears to correlate with their efficacy in defense against *Salmonella typhimurium*. To understand the role of ornithine production by SpeF in this cross-protection, we are using high-performance liquid chromatography (HPLC) to analyze polyamine secretion and uptake in selected *E. coli* strains, including the human uropathogenic UTI89 and the pathogenic Mt1B1 strain, under conditions of *E. faecalis* coexistence and acid stress. This study sheds light on the role of acid stress and polyamine cross-feeding in shaping bacterial interactions of the human gut microbiome.

References

1. Brameyer, S., Schumacher, K., Kuppermann, S. & Jung, K. Division of labor and collective functionality in Escherichia coli under acid stress. *Communications biology* **5,** 327; 10.1038/s42003-022-03281-4 (2022).
2. Ratzke, C. & Gore, J. Modifying and reacting to the environmental pH can drive bacterial interactions. *PLoS biology* **16,** e2004248; 10.1371/journal.pbio.2004248 (2018).
3. Miller-Fleming, L., Olin-Sandoval, V., Campbell, K. & Ralser, M. Remaining Mysteries of Molecular Biology: The Role of Polyamines in the Cell. *Journal of molecular biology* **427,** 3389–3406; 10.1016/j.jmb.2015.06.020 (2015).
4. Keogh, D. *et al.* Enterococcal Metabolite Cues Facilitate Interspecies Niche Modulation and Polymicrobial Infection. *Cell host & microbe* **20,** 493–503; 10.1016/j.chom.2016.09.004. (2016).

P-MMB-026

Sulfur transport and signal transduction for sulfur-dependent transcriptional regulation in Hyphomicrobium denitrificans

*J. Li[1], J. Koch[2], F. Göbel[2], N. Hager[2], H. Y. Hsu[2], C. Dahl[2]
[1]*Universtiy Bonn, Institute for Microbiology & Biotechnology, Bonn, Germany*
[2]*University of Bonn, Institute of Microbiology and Biotechnology, Bonn, Germany*

Many sulfur-oxidizing bacteria oxidize thiosulfate through a combination of initial periplasmic and downstream cytoplasmic reactions. In the case of facultative sulfur oxidizers, such as the Alphaproteobacterium *Hyphomicrobium denitrificans*, transcriptional regulation is necessary to respond to the availability of the sulfur compound. Although we have gathered a substantial amount of information on sulfur-oxidizing enzymes and two different sulfane sulfur-responsive transcriptional repressors in *H. denitrificans* [1,2], we lack knowledge about the transfer of sulfur from the periplasm into the cytoplasm and how the cytoplasmic regulator proteins, SoxR and sHdrR, detect the presence of oxidizable substrate outside of the cells.

In *H. denitrificans*, the genes for two potential sulfur compound transporters (SoxT1A and SoxT1B) are located in the same genetic island as those for sulfur oxidation (*sox* and *shdr).* Both transporters exhibit similarity to the

structurally characterized YeeE/YedE-family thiosulfate transporter from *Spirochaeta thermophila* [3]. In this study, we examined the thiosulfate oxidation capabilities and transcription levels of the indicator genes *soxXA* and *shdrA* in *H. denitrificans* mutants with different combinations of transporter and regulator gene deletions. Although *H. denitrificans* strains lacking either SoxT1A or SoxT1B alone have the same negative phenotype for thiosulfate oxidation, we can now conclude that the two transporters fulfill fundamentally different roles. SoxT1B serves as the signal transducing unit for SoxR, as evidenced by the fact that *soxXA* and *shdrA* transcription remains very low in its absence unless the gene for the repressor is also removed. Accordingly, a Δ*soxR* Δ*soxT1B* double mutant is able to oxidize thiosulfate. SoxT1A functions as a transporter that allows sulfur oxidation in the cytoplasm. Mutants lacking SoxT1A are unable to oxidize thiosulfate, despite high levels of transcription of *sox* and *shdr* genes, particularly in regulator-deficient double mutants.

[1] Li et al 2022 BBA Bioenergetics 1864, 148932
[2] Li et al 2023 Antioxidants 12, 1620
[3] Tanaka et al 2020 Sci Adv 6, eaba7637

P-MMB-027

The formate-hydrogenlyase complex of Trabulsiella guamensis catalyzes formate-dependent H2 production in vitro

*M. Hardelt[1], C. Pinske[1]
[1]*Martin Luther University Halle-Wittenberg, Institute of Biology / Microbiology, Halle (Saale), Germany*

Introduction: During mixed-acid fermentation, a number of *Enterobacteriaceae* produce hydrogen (H_2) using the formate hydrogenlyase (FHL) complex, a progenitor of complex I of the respiratory chain. The FHL complex serves to maintain pH homeostasis of the cytoplasm and possibly to translocate nH^+. The complex"s soluble arm, which is oriented towards the cytoplasm, facilitates two reactions: a formate dehydrogenase (FdhH) catalyses the oxidation of formate to CO_2 and a H^+; a hydrogenase domain receives two electrons from this oxidation via iron-sulfur clusters and reduces $2H^+$ to H_2. This soluble arm is also connected to the membrane domain, which includes a variable number of transmembrane subunits. While the well-studied FHL-1 complex in *E. coli* includes two membrane subunits (HycCD), the less well-explored FHL-2 complex has five membrane subunits (HyfB-F). The enterobacterium *Trabulsiella guamensis* has a single FHL-2_{Tg} complex.

Goals: Despite their shared phylogeny with complex I, the potential role of the membrane subunits of FHL-2_{Tg} in proton pumping and their contribution to the catalytic reactions of the soluble arm remain unresolved. To explore their possible contribution to proton translocation and catalysis, an intact, active FHL-2_{Tg} complex must be isolated.

Materials & Methods: We created plasmids carrying the *hyf* genes from *T. guamensis* and introduced them into a hydrogenase-deficient strain of *E. coli*. The FHL-2_{Tg} was synthesized and purified anaerobically from this strain using a StrepTag-based system. The analyses included activity assays, gas chromatography, Blue-Native & SDS-PAGE, as well as western blots.

Results: We successfully purified an FHL-2_{Tg} complex anaerobically that disproportionates formic acid to H_2 and CO_2. Although the aerobically purified complex lacks this activity, the individual enzymatic components retain activity when measured using viologen dyes. Structural and biochemical analyses of this complex will be reported.

Summary: Conditions have been optimized for the purification of an active FHL-2_{Tg} complex, marking the first step towards examining the potential proton-pumping activity of this complex.

P-MMB-028

Enterobacterial citramalate lyase is a prototype enzyme for a novel C-C-lyase family.

*L. Schäfer[1], *E. A. Cassens[1], L. Schmidt[1], J. Eggers[1], S. A. Simon[2], T. L. V. Bornemann[2], A. J. Probst[2], U. Demmer[3], U. Ermler[3], I. A. Berg[1]
[1]*University of Münster, Münster, Germany*
[2]*Research Center One Health, University Alliance Ruhr, University of Duisburg-Essen, Faculty of Chemistry, Essen, Germany*
[3]*Max-Planck-Institute for Biophysics, Frankfurt a. M., Germany*

Many enterobacteria are capable of fermenting glutamate, most of which are pathogenic, such as *E. coli* O157:H7 (EHEC) strains [1,2]. Glutamate fermentation in these organisms proceeds via the methylaspartate pathway, in which glutamate is isomerized to methylaspartate, which is then converted to (*S*)-citramalate and cleaved to pyruvate and acetate. Here we show that these enterobacteria possess a novel (*S*)-citramalate lyase. This enzyme is highly specific to citramalate and requires ATP for its activity, whereas ATP is not hydrolyzed during the reaction. The structural analysis of the enzyme revealed that although ATP does not directly take part in the citramalate lyase reaction, it is involved in the formation of the reaction site and in the coordination of substrate and products. We identified the corresponding amino acid residues involved in catalysis and in the binding of citramalate and ATP and confirmed their function via kinetic analysis of the respective exchange variants. The interaction of the wildtype enzyme and its exchange variants with ATP was confirmed via the thermal shift assay. Interestingly, the enzyme was also active with ADP instead of ATP, although its activity was an order of magnitude lower than with ATP. The proposed enzyme mechanism highlighted participation of ATP in the coordination of Mg2+ that is involved in the binding of citramalate. The hidden Markov model analysis revealed the existence of multiple citramalate lyase homologs that probably catalyze various reactions. Indeed, the homologous protein from *Bacillus oleivorans* was shown to catalyze ATP-dependent 2-methylisocitate cleavage. To sum up, the enterobacterial ATP-dependent (*S*)-citramalate lyase is a prototype enzyme for a novel family of C-C-lyases widespread in different bacterial groups.

[1] Kato &. Asano, *Arch Microbiol* **168**:457 (1997).
[2] Kronen & Berg, *PLOS One* **12**:e0145098 (2015).

P-MMB-029

A cytochrome c-containing periplasmic nitrate reductase in the acetogen Sporomusa ovata

*L. M. Waschinger[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: Any acetogenic bacterium known to date generates ATP *via* two ferredoxin-dependent respiratory chains, containing either the Rnf or the Ech complex[1].

These two membrane-bound respiratory enzymes do not have cytochromes. However, some species such as *Moorella thermoacetica* or *Sporomusa ovata* contain *b*- and *c*-type cytochromes respectively[2,3], whose functions remain to be identified.

Goals: To unravel the role of cytochromes in the physiology of *S. ovata*.

Materials & Methods: Bioinformatics, growth experiments, metabolite analyses, determination of transcript levels, biochemistry, cytochrome *c*-levels.

Results: Addition of nitrate led to higher optical densities and to reduced acetate concentrations in *S. ovata* when grown on fructose, methanol or H_2 + CO_2. Nitrate was reduced and nitrate reduction was CO2/bicarbonate independent. Inspection of the genome sequence revealed *nap* genes coding for a periplasmic nitrate reductase as well as genes encoding cytochrome *c* and heme biosynthesis and the genes coding for a periplasmic nitrite reductase. Transcriptome analyses revealed induction of expression of these genes by nitrate. Biochemical analyses confirmed the presence of cytochrome *c* in nitrate-grown cells.

Summary: Cytochrome *c* is apparently involved in the utilization of an alternative electron acceptor in *S. ovata*, nitrate, but not in the primary energetics. Rnf is still active in *S. ovata* under these conditions. Whether nitrate reduction produces additional ATP remains to be identified.

[1] Schuchmann, K., Müller, V. (2014) Nat Rev Microbiol 12: 809-21
[2] Rosenbaum, F. P., Müller, V. (2021) Extremophiles 25: 413-24
[3] Kremp, F., Roth, J., Müller, V. (2022) Microbiol. Spectr 10: e0138522

P-MMB-030
Genetic optimization of metabolic pathways in Phocaeicola vulgatus for enhanced succinate production
*M. Gindt[1], R. Lück[2], A. Neff[1], U. Deppenmeier[1]
[1]*Universtiy Bonn, Institute of Microbiology and Biotechnology, Bonn, Germany*
[2]*German Aerospace Center, Institute for the Protection of Terrestrial Infrastructures, Sankt Augustin, Germany*

Due to the growing challenges posed by climate change, the significance for alternative production methods to the conventional petrochemical production of bulk chemicals and the utilization of renewable materials is rapidly increasing. This includes the use of microbial fermentation and enzyme-based technologies to convert natural resources to key platform chemicals like succinate. As a salt of succinic acid, succinate serves as a fundamental component in various compounds across the food, chemical, and pharmaceutical sectors. *Bacteroides* and *Phocaeicola* species are widely distributed and prevalent members of the human gut microbiota and possess enzyme systems for the metabolization of complex plant polysaccharides. As a natural succinate producer, *P. vulgatus* (formerly *Bacteroides vulgatus*), seems to be a promising candidate for succinate production.

This study aimed to enhance succinate yields in *P. vulgatus* by channeling the metabolic carbon flow in favor of succinate formation. The methodology involved employing a markerless gene deletion method, as described in earlier studies, in combination with the shuttle vector pG106, previously identified as a suitable overexpression system for *P. vulgatus*.

Specifically, the deletion of the methyl-malonyl Co-A-mutase (Bvu_0309-0310) resulted in a 100% increase of succinate production in *P. vulgatus*, as metabolization to propionate was effectively blocked. Furthermore, deletion of the lactate-dehydrogenase (Bvu_2499) and the pyruvate-formate-lyase (Bvu_2880) has successfully eliminated the formation of fermentative end products lactate and formate. Moreover, the overexpression of the transketolase (Bvu_2318) from the pentose-phosphate shunt contributed to an additional increase in succinate production.

In summary, a total increase of the succinate yield by 150% compared to the wildtype strain has been achieved through a combination of genetic deletions and overexpression. Additionally, the formation of undesired fermentation byproducts has been successfully prevented, highlighting the potential of *P. vulgatus* as an efficient succinate producer with applications in sustainable bioproduction processes.

P-MMB-031
Investigations on bile acid catabolism in Sphingobium sp. strain Chol11 reveal an oxygenase-catalyzed hydroxylation in the degradation of the carboxylic side chain.
*A. Fuentes[1], J. Holert[1], B. Philipp[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

Bile acids are steroids produced by vertebrates in their digestive tract, and after excretion, they can be used as carbon and energy sources by environmental bacteria. Bile acids have a hydroxylated steroid skeleton with a C_5 side chain attached to C17. Two pathway variants are known for bile acid catabolism under aerobic conditions, named $\Delta^{1,4}$ and $\Delta^{4,6}$ after the unsaturation of their respective intermediates. While the $\Delta^{1,4}$-variant is common in pseudomonads and *Actinobacteria*, the $\Delta^{4,6}$-variant seems unique to the *Sphingomonadaceae* family.

Proteomic analysis of *Sphingobium* sp. strain Chol11 grown with the bile acid cholate revealed a gene cluster specifically upregulated during side-chain degradation. It contains genes for a CoA ligase (*sclA*), an acyl-CoA-dehydrogenase (*scd4AB*), an amidase, and the putative Rieske Monooxygenase (RMO) gene *nov2c228*. Notably, this cluster lacks genes for stepwise side-chain degradation, like thiolases and aldolases as described in organisms having the $\Delta^{1,4}$-variant such as *Pseudomonas stutzeri* Chol1. Additionally, strain Chol11 Δ*scd4A* cannot grow with cholate and accumulates hydroxylated metabolites with a C_5 side-chain. These instances suggest that side-chain degradation in strain Chol11 involves a hydroxylation catalyzed by Nov2c228.

The potential role of the RMO Nov2c228 was approached by gene deletion and heterologous expression. Attempts to delete the gene resulted in the apparent loss of the whole cluster and have been, thus, inconclusive. Heterologous expression in *P. stutzeri* Chol1 *ΔstdA1 ΔkstD cells,* which cannot degrade bile acids with a C_5 side-chain, showed that the $\Delta^{4,6}$-compound 3-oxo-chol-4,6-dienoate (OCDA), a

presumptive Nov2c228 substrate, was converted into a compound 16 Da heavier, suggesting that a hydroxylation took place. Ongoing studies aim to characterize this hydroxylation, including the structure of the compound.

This study provides further evidence that bile acid side-chain degradation in *Sphingomonadaceae* proceeds via an unknown pathway involving an RMO-catalyzed hydroxylation.

P-MMB-033
Exploring myo-inositol metabolizing anaerobic bacteria in the gastrointestinal tract of laying hens

*H. Naithani[1], B. Rios-Galicia[1], A. Camarinha-Silva[2], J. Seifert[1]
[1]*University of Hohenheim, Department of Functional Microbiology in Farm Animals (460c), Stuttgart, Germany*
[2]*University of Hohenheim, Department of Microbial Ecology in Farm Animals (460m), Stuttgart, Germany*

Introduction: *Myo*-inositol (MI), a cyclic polyalcohol sugar, holds significant importance in avian physiology notably impacting metabolic and regulatory functions. However, the metabolic pathway of MI in poultry remains unclear. Few studies indicate the metabolization of MI by some bacteria, raising speculation about the potential involvement of gut microbiota utilizing MI as a carbon or energy source. Although a diverse range of bacteria possess genes involved in this pathway belonging to the *iol* gene cluster, detailed information regarding specific bacterial species, core genes, their participation, and enzyme function remains limited. Furthermore, there is a need to explore the metabolism of MI within bacterial communities.

Goal: This study intends to investigate the possible involvement of anaerobic bacteria in MI metabolism. The approach involves isolating and identifying potential bacterial strains that harbor *iol* genes capable of MI degradation or utilization in the gastrointestinal tract of laying hens, either as individual strains or within a community.

Materials & Methods: Anaerobic bacterial strains were isolated from the ileum section of the laying hen. Using custom-designed minimal media and molecular techniques, a total of 107 bacterial strains were isolated, and redundant strains were identified using the ARDRA technique. Subsequently, the taxonomic diversity of 45 selected strains was determined through 16S rRNA gene sequencing.

Results: The sequencing results revealed that the isolated strains belonged to Pseudomonadota (65%), Bacillota (33%), and Bacteroidota (2%). Among these strains, 13% belonged to Risk Group 1, while 87% belonged to Risk Group 2.

Summary: These findings suggest a potential association of various bacterial taxa in MI metabolism. While further investigation to elucidate the specific roles of bacteria in MI metabolism and the underlying metabolic pathway is ongoing, these preliminary findings provide a novel glimpse into the potential interactions between diverse bacteria working synergistically which might further reveal the relationship between gut microbiota and MI metabolism in laying hens.

P-MMB-034
Characterising the molecular architecture of the archaeal ACDS superassembly

*E. Zimmer[1], T. Reif-Trauttmansdorff[1], J. Schuller[1]
[1]*University of Marburg, SYNMIKRO Research Center, Marburg, Germany*

Introduction:

The acetyl-CoA decarbonylase/synthase (ACDS) enzyme complex plays a key role in many acetogenic bacteria and methanogenic archaea. As part of the ancient Wood-Ljungdahl pathway, it catalyses the reversible formation of acetyl-CoA from CO2, a methyl group, coenzyme A and reduced ferredoxin. In acetoclastic methanogens, this reaction runs in reverse leading to the formation of a methyl group which is further transferred and reduced to build up a Na+/H+ gradient for ATP-synthesis. Distinct from its bacterial homolog, the archaeal ACDS forms a ~2 MDa superassembly of so-far unknown structure.

Goals:

We want to determine the three-dimensional structure of the archaeal ACDS complex and resolve catalytically important conformations within the assembly. In the end, we would like to understand the functional differences between the bacterial and archaeal complex.

Materials & Methods:

We are working with the ACDS complex natively and anaerobically purified from *Methanosarcina acetivorans*. Since previous approaches to determine the structure of ACDS by cryo-EM single-particle analysis yielded poor resolutions, we employ an integrative structural biology approach with cross-linking mass spectrometry and mass photometry to characterise subcomplexes of the superassembly and their interactions.

Results:

After successfully disassembling the ACDS complex into three smaller, functional subcomplexes, we found that they form different homo-oligomers. Furthermore, we identified terminal disordered regions of three protein subunits as likely being responsible for the oligomerisation patterns. Using cross-linking mass spectrometry, we resolved two catalytically relevant conformations of the acetyl-CoA synthase subcomplex bound to either one of the other two subcomplexes.

Summary:

The archaeal ACDS complex displays a molecular architecture which is clearly distinct from its bacterial counterpart. However, the exact functional difference as well as the structure of this complex are largely not understood despite three decades of research. With our work, we hope to gain further insights into this puzzle.

Further reading:
https://doi.org/10.1021/acs.biochem.2c00425

P-MMB-035
3-Hydroxypropionate production from myo-inositol by the gut acetogen Blautia schinkii

S. M. Rustler[1], *R. Trischler[1], A. Poehlein[2], R. Daniel[2], V. Müller[1]

[1]Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany
[2]Georg-August University Göttingen, Göttingen Genomics Laboratory, Göttingen, Germany

Introduction: The gut microbiome is a very complex community not only involved in the digestion of nutrients but also plays a role in developing diseases as well as in human well-being. Gut acetogens such as *Blautia* strains are often connected to the human well-being[1]. However, only little is known about the physiology of different *Blautia* species. In this study, we characterized the degradation pathway of *myo*-inositol, an abundant sugar of the human body, by the acetogen *Blautia schinkii.*

Goals: To unravel the *myo*-inositol degradation pathway of *B. schinkii.*

Materials & Methods: Growth experiments, fermentation and metabolite analyses, enzyme assays, genome analysis and analysis of gene expression

Results: *B. schinkii* can grow on *myo*-inositol as carbon and energy source. *Myo*-inositol is first oxidized by an inositol dehydrogenase and then further converted *via* a dehydratase and kinase to 2-deoxy-5-keto-D-gluconic acid 6-phosphate (DKGP). DKGP is then split by an aldolase into dihydroxyacetone (DHA) and malonate semialdehyde. DHA is metabolized to acetate and ethanol. Interestingly, malonate semialdehyde is reduced to the unusual product 3-hydroxypropionate (3HP). The highest yield of 3HP produced by *B. schinkii* was 22 mM. Furthermore, we could identify the NADH-dependent 3HP dehydrogenase, the key enzyme in 3HP production.

Summary: The human gut acetogen *B. schinkii* produces the valuable product 3-hydroxypropionate from *myo*-inositol, presenting a novel feature of acetogenic gut bacteria that might be of relevance not only for the human well-being but also for bioindustrial applications.

[1] Liu X., Mao, B., Gu, J., Cui, S., Wang, G., Zhao, J., Zhang, H., Chen, W. (2021) Gut Microbes 13: e1875796

P-MMB-036
A signal transduction cascade controlling 2-oxoglutarate dehydrogenase activity in the actinobacterial cell factory Corynebacterium glutamicum

*S. Gujar[1,2], L. Sundermeyer[1], G. Bosco[1], B. Lückel[1], J. Folkerts[1], M. Baumgart[1], O. Weiergräber[1], M. Bellinzoni[3], M. Bott[1]
[1]Research Center Juelich, Jülich, Germany
[2]Institute for Physical Biology University Düsseldorf, Düsseldorf, Germany
[3]Institut Pasteur, Université Paris Cité , Paris, France

1. **Introduction**

A lowered 2-oxoglutarate dehydrogenase (ODH) activity is crucial for production of the flavor enhancer monosodium glutamate (MSG) by *Corynebacterium glutamicum.* ODH activity is inhibited by the regulatory protein OdhI in its unphosphorylated state. Phosphorylation of OdhI by the serine/threonine kinase PknG relieves inhibition. Previous studies indicated that the activity of PknG is controlled by the secreted putative glutamine-binding protein GlnH and the integral membrane protein GlnX.

2. **Goals**

The aim was to biochemically and structurally characterize the proteins GlnH and GlnX in order to obtain a detailed molecular understanding of their function. In addition, we aimed to analyze the cellular localization of OdhI and the pyruvate dehydrogenase (PDH)-ODH supercomplex.

3. **Materials and methods**

The binding properties of purified GlnH were determined by ITC and tryptophan fluorescence quenching. The cellular localization of proteins was analyzed by fluorescence microscopy. Structural models of GlnH and GlnX were generated using the Colabfold2 pipeline by AlphaFold2.

4. **Results**

GlnH binds L-aspartate and L-glutamate with moderate to low affinity, but not L-glutamine, L-asparagine, or 2-oxoglutarate. The predicted topology of GlnX with four transmembrane helices and two large periplasmic domains was confirmed by LacZ and PhoA fusions. *Ab initio* structure prediction of GlnX by AlphaFold2 revealed a novel architecture named as Helical Tandem Module (HTM) composed of three four-helix bundles (4HBs) – with one 4HB located in the membrane and two 4HBs in the periplasm. All subunits of the PDH-ODH complex were localized in distinct spots at the cell poles and at mid-cell, possibly due to nucleoid exclusion.

5. **Summary**

Our results provided novel information on the GlnH-GlnX-PknG-OdhI-ODH signal transduction cascade, which serves to adapt the carbon flux at the 2-oxoglutarate node between ammonium assimilation and energy generation via TCA cycle to the availability of L-glutamate and L-aspartate in the environment. Furthermore, an unexpected localization of the PDH-ODH was identified.

P-MMB-037
Dissimilatory sulfur oxidation in cable bacteria involves a new sulfurtransferase

*M. G. Mohr[1], L. E. Plum-Jensen[2], T. S. Tanabe[1], A. Schramm[2], C. Dahl[1]
[1]Universtiy Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany
[2]Aarhus University, Center for Electromicrobiology, Aarhus, Denmark

Cable bacteria are multicellular filaments found in aquatic sediments that perform a unique form of energy conservation. The cells in the anaerobic zone oxidize reduced sulfur compounds, and the electrons released by this oxidation are transported by fibers in the periplasm to cells in the aerobic zone, where oxygen acts as a terminal electron acceptor. Cable bacteria, like almost all members of the *Desulfobulbaceae* family, possess genes for the rDsr pathway of sulfur oxidation, including reverse-acting dissimilatory sulfite reductase rDsrAB, DsrC and the sulfur transferase TusA [1]. However, the sulfur relay system in cable bacteria has not been fully elucidated. It shuttles sulfane sulfur to rDsrAB, where it is oxidized to sulfite. Cable bacteria have never been shown to contain genes for the DsrEFH sulfurtransferase, despite serving as a long-standing marker for sulfur oxidizers.

The aim of this study was to clarify the enzymes, transfer reactions, and potential terminal acceptors involved in sulfur trafficking in cable bacteria. We identified a candidate sulfurtransferase, DsrEFH, by comparative proteomics and transcriptomics, despite its low amino acid sequence similarity. We heterologously produced the candidate sulfurtransferase, as well as the TusA and DsrC proteins from the cable bacterium *Ca.* Electronema sp. GS in *E. coli*. The recombinant proteins were analyzed using polyacrylamide gel electrophoresis and size exclusion chromatography. A study of sulfur transfer reactions between the proteins was conducted *in vitro* using MALDI-TOF mass spectrometry.

Whether it carries a StrepTag on DsrE or DsrH, the novel DsrEFH from *Ca.* Electronema sp. GS forms heterotrimers and heterohexamers in solution, similar to canonical DsrEFH from *Allochromatium vinosum* [2]. *In vitro*, cable bacterial DsrEFH serves as a sulfur acceptor for TusA and a sulfur donor for DsrC. In conclusion, the novel *dsrEFH*-like genes encode a previously missing component in the cytoplasmic sulfur relay system of cable bacteria. These genes should be included in the canon of marker genes for sulfur oxidizers.

[1] Kjeldsen et al 2019 PNAS 116, 19116
[2] Dahl et al 2008 J Mol Biol 284, 1287

P-MMB-038

Structural and functional analysis of tRNA^{sec} from different bacterial species

*S. Schmidt[1], J. Heider[1]
[1]*Philipps-Universität Marburg, Marburg, Germany*

Selenium in the form of selenocysteine, the rare 21st amino acid, is an important component of proteins from all kingdoms of life, the so-called selenoproteins. Selenocysteine is incorporated cotranslationally at specific UGA (stop) codons by the specific tRNA^{sec} (SelC) First, the specific tRNA^{sec} is loaded with serine by seryl synthetase, which is then converted to a tRNA-bound selenocysteine by selenocysteine synthase (SelA) with selenophosphate synthetase (SelD) providing the required selenol donor (1,2). Finally, the specific translation factor SelB is required, which simultaneously recognizes selenocysteine-loaded selenocysteyl-tRNA^{sec} and a specific RNA structure close to the UGA codon that specifies the incorporation site of selenocysteine (3). This specific recognition sequence, the selenocysteine insertion sequence (SecIS), discriminates between UGA codons for selenocysteine insertion and chain termination (4). Bacterial selenoprotein synthesis has been studied mainly in *Escherichia coli* and a few other species, while selenoproteins appear to be present in many other taxonomic groups, often with divergent features in their selenocysteyl-tRNAs. Here, we investigate the functionality of several aberrant SelC-tRNAs from members of the *Bacillaceae*, Alpha- and Deltaproteobacteria, which have not been among the usual selenoprotein-forming model organisms in earlier studies. This will provide valuable data on selenocysteine metabolism and what role different structural features of SelC play in heterologous systems.

(1) Forchhammer, K. et al. (1991) *Journal of Biological Chemistry*, *266*(10), 6318-6323.
(2) Leinfelder, W. et al. (1990) *Proceedings of the National Academy of Sciences*, *87*(2), 543-547.
(3) Forchhammer, K. et al. (1989) *Nature*, *342*(6248), 453-456.
(4) Heider, J. et al. (1992) *The EMBO journal*, *11*(10), 3759-3766.

P-MMB-039

The path of the iron-sulfur clusters into the proteins: Investigation of the Sulfur Mobilization Machinery

*L. Schmück[1], J. Schuller[1]
[1]*Philipps University Marburg, Synmikro, Chemistry, Marburg, Germany*

Introduction: Iron-sulfur cluster containing proteins are ubiquitous in nature and conserved across all kingdoms of life; however, iron-sulfur clusters are not assembled within their target proteins. Instead, iron-sulfur clusters are assembled on scaffold proteins assisted by specialized assembly machinery. Currently, four iron-sulfur cluster machineries are known: the NIF (nitrogen-fixation system), ISC (iron-sulfur cluster assembly machinery), CIA (cytosolic iron-sulfur cluster assembly machinery), and SUF (sulfur mobilization machinery). The SUF machinery is the most ancient of the identified iron-sulfur cluster biogenesis systems. The SUF machinery contains the proteins SufS, SufE, SufBCD, and SufA. The SufBCD protein complex's postulated function is the assembly of iron-sulfur clusters on its SufBD subunits. SufC belongs to the highly diverse ABC ATPase-family, whose function within the iron-sulfur cluster biogenesis pathway remains unresolved. To overcome this, structures in the enzyme's conformational cycles are required to understand how the ATPase movement is related to the formation of a nascent iron-sulfur cluster. **Objective:** We aim to determine a high-resolution structure of the bacterial SUF machinery with a particular focus on the SufBCD protein complex, concentrating on the iron-sulfur cluster cofactor binding coordination and the proposed ATPase activity-induced, conformational changes. **Methods:** We utilised single-particle cryo-electron microscopy (cryo-EM) to create structural models of the purified, SufBCD protein complex. **Results:** The SUF machinery components were overexpressed, purified and cryo-EM sample preparation was carried out under redox controlled conditions. UV/Vis spectroscopy confirmed the presence of an iron-sulfur cluster in the anaerobically purified SufBCD complex. Preliminary data processing revealed SufBCD undergoes conformational changes, possibly in relation to the presence of an iron-sulfur cluster. **Conclusion:** Overall, preliminary structural information acquired by single particle cryo-EM show that the SufBCD protein complex adopts different structural conformations during iron-sulfur cluster assembly.

P-MMB-040

Oxygen-independent C-H activation by the molybdenum-containing p-cymene dehydrogenase

*R. Weßbecher[1], C. Jacoby[1], T. Seifermann[1], P. Becker[2], R. Rabus[2], M. Boll[1]
[1]*University of Freiburg, Institute of Biology II/ Microbiology, Freiburg i. Br., Germany*
[2]*Carl von Ossietzky University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany*

Hydrocarbons are major constituents of petroleum, natural gas or essential oils and are primarily degraded by bacteria. The initial activation of apolar C-H bonds of hydrocarbons represents a challenging task in chemical or enzymatic syntheses due to high activation energies. Under aerobic conditions, the hydroxylation of C-H bonds is catalysed by O2-dependent oxygenases or peroxidases, however, in the absence of oxygen, a different enzymatic strategy is

required. In denitrifying bacteria, molybdenum-dependent hydroxylases of the type II DMSO reductase family were identified that use water for the hydroxylation of unactivated C-H bonds.

In this study, we have isolated and characterised the p-cymene dehydrogenase (PCDH) from *Aromatoleum aromaticum* pCyN1[1] as a novel molybdenum-containing enzyme after heterologous production in the denitrifying host *Thauera aromatica* K172. Metal analyses and UV-vis spectroscopy of the heterotrimeric enzyme complex suggest the presence of a molybdenum cofactor, iron-sulfur clusters and a heme b. PCDH catalyses the hydroxylation of the primary carbon of the monoterpene p-cymene to its primary alcohol cuminol, and also the further oxidation to the corresponding aldehyde. Furthermore, we could show hydroxylation of a variety of aromatic hydrocarbons and terpenes to the corresponding primary alcohols, including p-xylene and limonene. This demonstrates the potential of this enzyme for the regioselective activation for a broad range of aromatic and isoprenoid compounds.

[1]Strijkstra et al, AEM, *80*(24), 7592–7603 (2014)

P-MMB-041
Unraveling the potential of styrene-degrading actinobacteria Gordonia rubripertincta CWB7 and Rhodococcus erythropolis CWB15
*S. Malcherczyk[1], M. Haarmann[1], A. Wessely[1], A. C. Ngo[1], N. Weindorf[1], D. Tischler[1]
[1]*Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany*

The widely distributed phylum *Actinobacteria* constitutes to one of the largest and most diverse groups of microorganisms in nature. It is notable for their remarkable potential in the degradation of diverse compounds. Moreover, they are versatile producers of bioactive natural products. Consequently, actinobacteria hold considerable economic significance and utility for various applications. Due to their capability to catalyze a diverse range of enzymatic reactions, uncommon in other organisms, they become attractive candidates for biotechnological applications.

Recently, the actinobacterium *Gordonia rubripertincta* CWB2 and related strains *G. rubripertincta* CWB7 and *Rhodococcus erythropolis* CWB15 were isolated. Earlier studies demonstrated strain CWB2"s ability to degrade the plastic precursor styrene and related compounds. In contrast to other styrene degraders, it employs a "hybrid" pathway, resulting in the central metabolite phenylacetic acid, a compound with high industrial demand. CWB2 also showed its ability to produce ibuprofen in a co-metabolic process. Noteworthy is the involvement of glutathione and glutathione *S*-transferases in these catabolic processes. The same degradation pathway was found in related strains CWB7 and CWB15.

To understand the role of involved enzymes, this study aims at the generation of knockout strains and the establishment of genetic tools for actinobacterial protein production. Both strains were characterized to enable an easy operation.

Therefore, genomic, morphological, and physiological analysis was initially performed. Efficient protocols for electrocompetent *Gordonia* and *Rhodococcus* cells were established. Moreover, various protocols for knockout were tested. For protein production, expression plasmids were tested with *sfGFP* as reporter gene regarding their transformation efficiency and its protein yield.

Overall, this study presents not only the morphological and physiological characterization of recently found *G. rubripertincta* CWB7 and *R. erythropolis* CWB15, but also the understanding of involved enzymes. Furthermore, these strains were explored as alternative expression strains for protein production.

P-MMB-042
Metabolic engineering of Acetobacterium woodii towards enhanced lactate production
*K. Baur[1], A. Mook[1], F. R. Bengelsdorf[1]
[1]*Ulm University, Molecular Biology and Biotechnology of Prokaryotes, Ulm, Germany*

Climate change progresses by increasing amounts of greenhouse gases such as CO2 in our atmosphere and is intensified by the production and disposal of e.g. plastics. *A. woodii* is capable of autotrophic growth using H2 + CO2, producing acetate. By plasmid-borne expression of a D-lactate dehydrogenase (*ldhD*) from *Leuconostoc mesenteriodes* and deleting the native genes encoding the bifurcating lactate dehydrogenase complex, a recombinant *A. woodii* strain was constructed, capable of D-lactate production from, using H2 + CO2 (Mook et al., 2022, doi: 10.1007/s00253-022-11770-z). D-lactate is the monomer for the bioplastic polylactic acid (Vaidya et al., 2005, doi: 10.1080/10643380590966181).

The lactate production shall be increased in *A. woodii* by expressing the pyruvate-formate-lyase (PFL) to bypass the methyl branch of the Wood-Ljungdahl pathway. PFL functions in a reversible manner in many anaerobic bacteria, converting acetyl-CoA and formate to HS-CoA and pyruvate, depending on a PFL-activating enzyme (ACT). A plasmid containing the genes *pfl* (controlled by the theophylline-inducible P*ackA*-theo promoter (Beck et al., 2020, doi: 10.1007/s00253-019-10248-9)) and *act* (controlled by the constitutive P*pta-ack* promoter (*Clostridium ljungdahlii*) from *C. pasteurianum* was constructed. *A. woodii"s* endogenous type 1-B CRISPR-Cas system was used to delete its pheA gene, using a plasmid containing the CRISPR array, spacer and homology arms needed for homologous recombination (Poulalier-Delavelle et al., 2023 doi: 10.3389/fbioe.2023.1213236). Currently the endogenous CRISPR-Cas system was successfully applied to implement a *pheA* deletion in *A. woodii,* verified via PCR. Next, *pheA* will be restored together with the *ldhD* gene regulated by the lactose inducible promoter system PbgaL from *C. perfringens* as well as by the lactate inducible promoter system PlctA from *A. woodii.* Thus, two plasmids containing the respective CRISPR array, spacer, *pheA* locus, *ldhD* and promoter systems were constructed.

These plasmids will be used to construct respective *A. woodii* strain and growth as well as production kinetics will be analyzed.

P-MMB-044

Structure-function studies towards the mechanisms of exo-lytic N-acetylmuramidases of CAZy families GH170 and GH171

M. Borisova-Mayer[1], S. Friz[1], N. Dierlamm[2], T. Stehle[2], G. Zocher[2], S. Caulton[3], A. Lovering[3], A. Titz[4,5,6], *C. Mayer[1]
[1]*University of Tübingen, IMIT - Glycobiology, Tübingen, Germany*
[2]*University of Tübingen, IFIB - Biochemistry II, Tübingen, Germany*
[3]*University of Birmingham, Institute of Microbiology and Infection, School of Biosciences, Birmingham, United Kingdom*
[4]*University of Saarland, Department of Chemistry, Saarbrücken, Germany*
[5]*Helmholtz Institute for Pharmaceutical Research, Saarbrücken, Germany*
[6]*German Center for Infection Research, Brunswick, Germany*

Lysozyme-like endo-*N*-acetylmuramidases, which catalyze the endo-lytic cleavage of the peptidoglycan cell wall of bacteria, are intensively studied and well-characterized antimicrobial enzymes. In contrast, little is known about exo-β-N-acetylmuramidases that catalyze the exo-lytic cleavage of N-acetylmuramic acid (MurNAc) and 6-phospho-MurNAc entities from non-reducing termini. We recently identified such enzymes constituting two novel families of glycosyl hydrolases, GH171 (www.cazy.org/GH171.html) and GH170 (www.cazy.org/GH170.html), respectively. NamZ of *B. subtilis* (BsNamZ)[1] and two related enzymes from *Tannerella forsythia* (TfNamZ1 and TfNamZ2)[2] were identified and classified into GH171, whereas MupG of *Staphylococcus aureus* (SaMupG)[3] and MupG-like enzymes from *Priestia megaterium* (i.e. *Bacillus megaterium*) and *Lactobacillus pasteurii* were identified and classified into GH170. Chemical synthesis of chromogenic substrates facilitated the identification and initial characterization of these enzymes and they are now used, in combination with x-ray crystallography, mutagenesis and kinetic studies of active site variants, to elucidate the structure, function and mechanism of these enzymes. We are presenting structural and kinetic data that shine a light on the mechanisms of these unique exo-lytic muramidases.

[1] Müller M, Calvert C, Hottmann I, Kluj RM, Teufel T, Balbuchta K, Engelbrecht A, Selim KA, Xu Q, Borisova M, Titz A, Mayer C (2021) The exo-β-N-acetylmuramidase NamZ from *Bacillus subtilis* is the founding member of a family of exo-lytic peptidoglycan hexosaminidases, J Biol Chem. 296: 100519.
[2] Borisova M, Balbuchta K, Lovering A, Titz A, Mayer C. (2022) NamZ1 and NamZ2 from the oral pathogen *Tannerella forsythia* are peptidoglycan processing exo-β-N-acetylmuramidases with distinct substrate specificities. J Bacteriol. 2022 Mar 15;204(3):e0059721.
[3] Kluj RM, Ebner P, Adamek M, Ziemert N, Mayer C, Borisova M. (2018) Recovery of the peptidoglycan turnover product released by the autolysin Atl in *Staphylococcus aureus* involves the phosphotransferase system transporter MurP and the novel 6-phospho-N-acetylmuramidase MupG. Front Microbiol. 9:2725.

P-MMB-045

Succinic semialdehyde reductase from autotrophic archaeon Nitrosopumilus maritimus involved in the 3-hydroxyprotionate/4-hydroxybutyrate cycle

*J. Liu[1], M. Könneke[2], S. König[3], I. A. Berg[1]
[1]*Universtiy Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*
[2]*Carl von Ossietzky University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany*
[3]*Universtiy Münster, Graduate School of Natural Products, Münster, Germany*

Ammonia-oxidizing Archaea of the phylum Nitrososphaerota are one of the most abundant microbial groups responsible for a large part of the primary production in the dark ocean. These microorganisms use the energy-efficient variant of the 3-hydroxypropionate/4-hydroxybutyrate cycle to assimilate inorganic carbon. In this variant of the cycle, only a few enzymes have been identified biochemically, while most of the specific dehydrogenases of the cycle have not yet been identified. Here, we demonstrate that gene *nmar_0523* encodes succinic semialdehyde reductase in *Nitrosopumilus maritimus*. Characterization of the heterologously produced succinic semialdehyde reductase revealed that it is a homotrimeric enzyme specific for succinic semialdehyde as a substrate. This enzyme is not homologous to other identified succinic semialdehyde reductase, highlighting the convergent evolution of the 3-hydroxypropionate/4-hydroxybutyrate cycle in hyperthermophilic Sulfolobales (Thermoproteota) and in mesophilic ammonia-oxidizing Archaea.

P-MMB-046

The intrinsic tilmicosin resistome of Escherichia coli

*Y. Ma[1], M. Pirolo[1], B. Jana[2], L. Guardabassi[1]
[1]*University of Copenhagen, Department of Veterinary and Animal Sciences, Copenhagen, Denmark*
[2]*Massachusetts General Hospital, Center for Genomic Medicine, Boston, MA, United States*

Introduction

Tilmicosin (TIL) is a macrolide antibiotic that is licensed in pig farming for treating infections caused by pathogens other than *Escherichia coli*, which is intrinsically resistant to macrolides due to outer membrane impermeability. We previously demonstrated that peptidomimetics can be used to re-sensitize *E. coli* to TIL, suggesting potential for repurposing macrolides to address the shortage of effective therapeutic options for managing pig enteritis caused by *E. coli*.

Aims

To identify genes required for expressing intrinsic TIL resistance in *E. coli*, referred to collectively as the intrinsic TIL resistome, and use these genes as targets for TIL potentiation.

Methods

A clinical enterotoxigenic *E. coli* strain (ETEC5621) and a laboratory strain (K-12 MG1655) were used to construct transposon mutant libraries. Transposon Directed Insertion-site Sequencing (TraDIS) was used to explore non-essential genes involved in intrinsic TIL resistance. Lambda red recombineering was used to construct gene deletion mutants to test the effects of gene deletion on the minimal inhibitory concentration (MIC) of TIL.

Results

TraDIS analysis identified intrinsic TIL resistome genes in ETEC5621 and MG1655 after exposure to 1/8 MIC (n=15 and 16, respectively) and 1/4 MIC (n=38 and 32,

respectively) of TIL. Genes common to both strains (n=23) were enriched in functions related to lipopolysaccharide biosynthesis, outer membrane assembly, the Tol-Pal system, efflux pump and peptidoglycan metabolism, suggesting that the integrity of cell envelope is crucial to intrinsic TIL resistance in *E. coli*. Deletion of seven of these genes led to a 64- to 2-fold reduction of TIL MIC in both strains. Notably, deletion of *surA* or *waaG* conferred the highest MIC reduction in the clinical strain, decreasing it to 16 µg/ml, a concentration that is achievable in the pig intestinal tract after oral administration.

Conclusion

Our findings contribute to a genome-wide understanding of the intrinsic TIL resistome in pig clinical *E. coli* and provide the proof of concept to re-sensitize this important veterinary pathogen to TIL by interfering with genes involved in cell envelope integrity.

P-MMB-047
Expression of a ubiquitin ligase by Simkania negevensis as a potential factor to hijack the host ubiquitin system
*E. M. Hörner[1], V. Boll[2], T. Hermanns[2], K. Hofmann[2], T. Rudel[1], V. Kozjak-Pavlovic[1]
[1]*Julius-Maximilians-Universität Würzburg, Department of Microbiology, Würzburg, Germany*
[2]*University of Cologne, Institute for Genetics, Köln, Germany*

Simkania negevensis (*Sne*) is an obligate intracellular, *Chlamydia*-like emerging pathogen, associated with respiratory diseases. It exhibits a biphasic developmental cycle with extracellular, non-replicative elementary bodies (EBs) and intracellular, replicative reticulate bodies (RBs). After invading the host cell, they multiply within a *Sne*-containing vacuole [1]. Intracellular bacteria run the risk of being combated by the host immune system. This includes the host induced ubiquitination of bacterial components. Ubiquitination is an essential eukaryotic protein modification that, depending on the ubiquitinylation pattern, regulates many cellular aspects such as proteostasis, DNA repair and NF-κB activation. Increasing evidence suggests that various bacteria introduce effectors into the host cell to hijack the host ubiquitin system and establish an infection [2]. A bioinformatics screen of the *Sne* genome identified a high number of putative ubiquitin-modifying enzymes including four ubiquitin ligases. In previously published proteomics analysis performed on day 3 post infection (pi), one of these ligases could be identified [3].

In our research, we are especially interested in the activity of this ligase and its role during infection. To analyze this, quantitative real-time PCR, protein purification, autoubiquitinylation- and ubi crest assays as well as mass spectrometry were performed. Additionally, a cell line was generated that inducibly expresses the ligase and various transfection methods were used.

From day 2 to day 4 pi, a downregulation of the expression of the ligase could be measured. Furthermore, the ligase showed no toxic effect on the host cell and the protein seems to localize in the host cytoplasm when it is overexpressed. A specificity of the ligase for K11- and K63- linked ubiquitin chains could be shown.

In the ongoing arm race between host and pathogen, *Sne* expresses a ubiquitin ligase that possibly interferes with the host ubiquitin system.

References:

1. M. Vouga et al., *Crit. Rev. Microbiol.* **43**, 62 (2017).
2. Y. Zhou & Y. Zhu, *Cell. Microbiol.* **17**, 26 (2015).
3. J. Herweg et al., *Mol. Microbiol.* **99**, 151 (2016).

P-MMB-048
Bacterial nitrate and nitrite metabolism in the human gut
*N. Hager[1], M. Gindt[1], M. Hövels[1], U. Deppenmeier[1]
[1]*University of Bonn, Institute of Microbiology and Biotechnology, Bonn, Germany*

The human gastrointestinal tract harbors a diverse population of approximately 10^{14} prokaryotic cells, predominantly residing in the colon. This gut microbiota actively interacts with the gut epithelium, influencing intestinal health, immune modulation, and disease development. While there has been extensive research on the nitrogen cycle in the environment, the nitrogen metabolism in the human intestine apart from *E. coli* is still poorly understood. Nitrogen is a crucial element for life, forming essential biomolecules, and its various oxidation states undergo important transformations. However, some of these transformations may be involved in reactions that lead to the production of carcinogenic nitroso compounds. This study aimed to clarify the enzymatic reactions and kinetic mechanisms of the nitrate and nitrite metabolism by establishing a gut model, featuring ten prevalent core species which represent the major phyla of the gut. This model utilized organisms of the phyla Bacteroidota, Bacillota, Proteobacteria, Actinobacteria, and Verrucomicrobia. During growth experiments, organisms were cultivated at 37°C for 24 hours and key parameters such as optical density and pH were recorded. The bioavailable amounts of nitrate and nitrite during cultivation were adapted to the physiological concentration of 100 µM present in the colon. The concentration of both compounds was quantified using colorimetric assays and HPLC analysis. These experiments revealed in the small intestine a significant nitrite degradation capacity for *L. reuteri*, *S. vestibularis* and *V. atypica* while in the large intestine, a reduction was predominantly observed in *E. coli*, *B. cellulosilyticus*, *B. xylanisolvens* and *P. dorei*. In contrast, nitrate degradation in the small intestine was only observed through *V. atypica* and in the large intestine through *E. coli*. This study provides insights into the diverse nitrate and nitrite reduction capabilities in prevalent gut bacteria, emphasizing the significance of *E. coli* in the intestinal metabolic landscape, as it exhibits the highest degradation rates.

P-MMB-049
Pseudomonadal itaconate degradation gene cluster encodes enzymes for methylsuccinate utilization
*L. Gonner[1], S. König[2], I. A. Berg[1]
[1]*University of Münster, Institut of Molecular Microbiology and Biotechnology, Münster, Germany*
[2]*University of Münster, Core Unit Proteomics, Münster, Germany*

Itaconate, a product of mammalian macrophages, controls immune responses and serves as an antimicrobial agent by inhibiting various reactions of carbon metabolism [1]. Some pathogens, including *Pseudomonas aeruginosa*, metabolize itaconate, detoxifying it and using it as a growth substrate [2].

Interestingly, the *P. aeruginosa* itaconate utilization operon contains three genes of unknown function in addition to the genes encoding the itaconate degradation enzymes, itaconate CoA-transferase, itaconyl-CoA hydratase and citramalyl-CoA lyase. Two of the unknown genes, encoding putative acyl-CoA dehydrogenase and MmgE protein, are found in a homologous gene cluster in *Cupriavidus necator* H16 (E6A55_22600 and E6A55_22590, respectively, referred further as *mcd* and *msi*). It has been proposed that the *P. aeruginosa* dehydrogenase catalyzes methylsuccinyl-CoA oxidation [2], whereas the corresponding MmgE protein may function as a methylsuccinate or methylsuccinyl-CoA isomerase [3]. Here, we showed that *mcd* from *C. necator* encodes a methylsuccinyl-C4-CoA dehydrogenase that catalyzes the conversion of both (*S*)- and (*R*)-enantiomers to mesaconyl-C4-CoA (k_{cat}/K_M of 1.2 and 1.4 s^{-1} mM^{-1} for (*S*)- and (*R*)-methylsuccinyl-CoA, respectively). In contrast, the *C. necator* itaconate CoA-transferase worked preferentially with (*R*)-methylsuccinate (k_{cat}/K_M of 0.7 and 64.1 s^{-1} mM^{-1} for (*S*)- and (*R*)-isomers, respectively). In its turn, the *C. necator* MmgE protein interconverted (*S*)- and (*R*)-methylsuccinate, but not methylsuccinyl-CoA, thus allowing the efficient conversion of (*S*)-methylsuccinate through the pathway. Indeed, both stereoisomers of methylsuccinate could be used by *C. necator* as the sole carbon source. We confirmed that the *P. aeruiginosa* homologs had the same function, thus revealing methylsuccinate degradation as the second function of the itaconate utilization operon in pseudomonads. Since this operon is widespread among bacteria, methylsuccinate appears to be an important natural metabolite, the source of which remains to be determined.

[1] Cordes *et al*, *Annu Rev Nutr* **35**:451 (2015).
[2] Sasikaran *et al*, Nat Chem Biol **10**:371 (2014).
[3] de Witt et al, *Metab Eng* **75**:205 (2023).

Microbial Pathogenicity

P-MP-001
Inhibition of bacterial adherence to fibronectin as a novel therapeutic concept

*S. Gottfried[1], D. J. Vaca[1], K. P. Holzhüter[2], W. S. Ballhorn[1], V. Dötsch[2], V. Kempf[1]
[1]University Hospital Frankfurt, Medizinische Mikrobiologie und Krankenhaushygiene am Universitätsklinikum Frankfurt, Frankfurt a. M., Germany
[2]Goethe University Frankfurt, Institut für Biophysikalische Chemie, Frankfurt a. M., Germany

Introduction: Bacterial infections pose a significant global health threat. The initial step involves bacterial adhesion to host cell extracellular matrix (ECM) proteins such as fibronectin (Fn). Trimeric autotransporter adhesins (TAAs) are important pathogenicity factors of Gram-negative bacteria. *Bartonella* adhesin A (BadA), a prototypic TAA from *B. henselae*, is exceptionally long (monomer: 3,974 aa) and extensively characterized, making it a prime example of a TAA. *B. henselae*, the causative agent of cat scratch disease and bacillary angiomatosis, adheres to endothelial cells and ECM proteins via BadA interaction. This study aimed to develop novel anti-virulence components to inhibit TAA and Fn interaction.

Material & Methods: BadA domains with reported Fn affinity were expressed and purified. Single domain antibodies (sybodies) binding to the purified BadA domains, were selected using a combination of ribosome and phage display technologies. Affinity and specificity of the candidate sybodies were evaluated using ELISA assays and qPCR. Binding inhibition with sybodies was evaluated by ELISA, qPCR and immunofluorescence microscopy.

Results: Specific BadA domains with reported Fn affinity were purified. Sybodies targeting a major Fn binding domain were selected and purified. Functional evaluation of the sybodies demonstrated their ability to bind the specific BadA domain and hinder BadA and Fn interaction, providing evidence of their anti-ligand potential.

Conclusion: Targeting TAA-function is a promising strategy for fighting Gram-negative infections. Sybodies seem to represent promising "antiligand" tools awaiting future assessments in various *in vitro* and *ex vivo* models. The herein described *Bartonella* spp. experiments serve as proof-of-concept, paving the way for new therapeutic concepts ("antiligands") also targeting highly drug-resistant TAA-expressing *A. baumannii* and other TAA-expressing bacteria in the future.

P-MP-002
Identification of networks counteracting a successful combination treatment of MDR Pseudomonas aeruginosa with β-lactams and ciprofloxacin

*A. Rudolph[1], O. Eggers[1], F. Smollich[1], F. Renschler[1,2], J. Müller[3], C. Engesser[3], M. Schütz[1,2], E. Bohn[1,2]
[1]Eberhard Karls University of Tübingen, Institute of Medical Microbiology and Hygiene, Tübingen, Germany
[2]German Center for Infection Research, Partner Site Tübingen, Tübingen, Germany
[3]Eberhard Karls University of Tübingen, NGS Competence Center Tübingen (NCCT), Tübingen, Germany

Multidrug resistance (MDR) of *Pseudomonas aeruginosa* (Pa) poses a growing challenge for the treatment of medical infections. Previous studies have demonstrated that combining different classes of drugs successfully reduces mortality among patients with severe MDR-Pa infection. Thus, a combination of fluorochinolones/β-lactams or aminoglycosides/β-lactams in treatment regimens can markedly enhance the susceptibility as opposed to monotherapy alone and might be a therapeutical option in some cases. Nevertheless, certain percentages of strains remain resistant.

Using the bloodstream isolate Pa ID40 which is resistant to most β-lactam antibiotics and ciprofloxacin, we aimed at elucidating secondary resistance genes that are additionally involved in promoting ciprofloxacin resistance on the one hand and resistance to a ceftazidime (CAZ)/ciprofloxacin (CIP) combination therapy on the other hand to identify potential targets.

For this purpose, a transposon directed insertion sequencing approach was used for screening. To further validate candidates derived from this screen, checkerboard assays were performed to determine minimal inhibitory concentrations (MIC) of unmarked deletion mutants.

Deletion of genes in DNA repair (*recA, recG, xseA*), efflux pumps (*mexEFoprN*), cell envelope components (*mlaA/vacJ, oprF*), as well as *amgK,* encoding an enzyme involved in peptidoglycan recycling and LPS biosynthesis, reduced MIC

values to ciprofloxacin 2- to 4-fold but did not break resistance of Pa ID40. A marked impact on MIC values regarding CIP/CAZ combination treatment resulted from single deletion of *amgK*, *recG* or *mexEFoprN,* but the MIC values still remained above the breakpoints for both antibiotics. Double deletions addressing different biological processes, such as deletion of *amgK*/*mexEFoprN* or *amgk*/*recG,* but not of *mexEFoprN*/*recG* reduced MIC values below breakpoints for both antibiotics upon CIP/CAZ treatment.

Thus, a complex network involving efflux pumps, DNA repair mechanisms and the so far in this context unrecognized N-acetylmuramate/N-acetylglucosamine kinase AmgK counteracts effective treatment of MDR-Pa strains with a CIP/CAZ combination.

P-MP-003

Transcriptional profiling of Staphylococcus aureus during the transition from asymptomatic nasal colonization to skin infection in patients with atopic dermatitis

J. Schulte[1], P. Li[1], C. Wolz[2], A. S. Yazdi[1], *M. Burian[1]
[1]RWTH Aachen University, Department of Dermatology and Allergology, Aachen, Germany
[2]University of Tuebingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany

Introduction:

Staphylococcus aureus typically colonizes the anterior nares of 20-30% of healthy individuals, with transient colonization occurring on the skin. Notably, the situation is markedly different in patients with atopic dermatitis (AD). Here, *S. aureus* is frequently isolated from skin samples, and its prevalence increases with disease severity. The regulatory processes that facilitate the transition from an asymptomatic nasal colonizer to a pathogen associated with symptomatic skin disease remain unknown.

Methods:

To gain insight into the regulatory adaption mechanisms that promote the transition from a commensal to a pathogen associated with symptomatic skin diseases, we analyzed the *in vivo* transcriptional profile of *S. aureus* colonizing the nose and non-diseased skin, as opposed to diseased skin of patients with AD, by quantitative real-time PCR.

Results:

The transcriptional profile during asymptomatic colonization of the nose or non-lesional skin closely resembled that of lesional skin samples for many of the genes studied with elevated expression of genes that encode adhesion-related proteins and proteases. In addition, genes that modify and maintain the cell wall and encode proteins that facilitate immune evasion showed increased transcriptional activity during both colonization and infection. Notably, in a subgroup of patients, the peptide quorum sensing system Agr (accessory gene regulator) and downstream target genes were inactive during nasal colonization but upregulated in lesional and non-lesional skin samples.

Conclusions:

Taken together, our results demonstrate an infection-like transcriptional profile during colonization and suggest a role for the Agr system during the transition from asymptomatic colonization to infection.

P-MP-004

Applying microfluidics to studying polar growth of infectious hyphae from Ustilago maydis

*J. Postma[1], K. Müntjes[1], M. Feldbrügge[1]
[1]Institute of Microbiology HHU, Düsseldorf, Germany

A hallmark for fungal pathogens is a morphological switch from yeast-like to hyphal growth. This hold true for human pathogens like *Cryptococcus neoformans* or *Candida albicans* as well as for plant pathogens like *Ustilago maydis.* In the latter, the morphological transition is particularly important during the formation of infectious hyphae. These are essential to penetrate the plant for infection. Long distance transport along microtubules is important for hyphal growth. Key carriers are early endosomes that serve as multi-purpose platform for the transport of numerous cargos such as mRNAs, ribosomes, heteromeric protein complexes as well as whole organelles such as peroxisomes. We uncovered that the RNA-binding protein Rrm4 is essential for endosomal transport of mRNAs and associated ribosomes. Loss of Rrm4 causes defects in polar growth i.e. hyphae exhibit aberrant bipolar growth. A limitation of our current experimental set-up is the fact that we can only observe hyphal growth for a short period of time and without additional nutrient supply. Here, we present a microfluidic approach to study endosomal RNA transport during hyphal growth in the time range of hours. Thereby, we will be able to follow this important biological process with unprecedented spatial and temporal resolution in the context of an experimentally well-controlled environment.

P-MP-005

Interaction of CihC orthologs of relapsing fever spirochetes with host-derived proteins involved in adhesion, fibrinolysis, and complement evasion

A. S. Damm[1], F. Reyer[1], L. Langhoff[1], Y. P. Lin[2], F. H. Falcone[3], *P. Kraiczy[1]
[1]University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a. M., Germany
[2]Tufts University, Department of Infectious Disease and Global Health, North Grafton, MA, United States
[3]Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany

Question

Relapsing fever (RF) remains a neglected human disease caused by different *Borrelia* species. Characterized by high cell densities in human blood, relapsing fever *Borrelia* have developed strategies to avoid recognition by the host defense mechanisms. CihC orthologs, in particular, are known to interact with host-derived proteins involved in adhesion, fibrinolysis and complement activation.

Here, we elucidate the binding properties of CihC orthologs from distinct RF spirochetes including *B. parkeri*, *B. hermsii*, *B. turicatae*, and *B. recurrentis* to fibronectin, plasminogen, and complement C1r.

Methods

Binding of CihC orthologs to human proteins was measured by ELISA. A microtiter-based approach was also performed to analyze the inhibitory capacity of borrelial proteins on complement. AlphaFold predictions were used to identified the complement-interacting residues in four CihC orthologs. Functional analyses was conducted to explore conversion of protein-bound plasminogen to proteolytic active plasmin.

Results

All CihC orthologs displayed similar binding properties to fibronectin, plasminogen, and C1r, respectively. Regarding complement inactivation, CihC orthologs and the C-terminal CihC fragment exhibited a strong inactivation capacity toward the classical complement pathway. Moreover, pre-incubation of human serum with these molecules protected serum-susceptible *Borrelia* cells from complement-mediated lysis. Utilizing Alphafold predictions, we mapped the putative key residues involved in C1r binding on the CihC orthologs attempting to explain the relatively small differences in C1r binding affinity. In addition, functional studies revealed a dose dependent binding of plasminogen to all borrelial proteins selected and conversion to active plasmin. The proteolytic activity of plasmin was almost completely abrogated by the lysine analog tranexamic acid, indicating that lysine residues are involved in the interaction.

Conclusions

Collectively, our data advance the understanding of the multiple binding properties of structural and functional highly similar molecules of diverse RF spirochetes proposed to be involved in pathogenesis and virulence.

P-MP-006

Analysis of translocated T3SS effector protein dynamics and their role in endosomal remodeling by intracellular *Salmonella enterica*

*V. N. Fritsch[1], V. Göser[1], L. Breitsprecher[1], R. Kurre[2], M. Hensel[1,2]
[1]*Universtiy Osnabrück , Mikrobiologie, Osnabrück, Germany*
[2]*Universtiy Osnabrück , Integrated Bioimaging Facility iBiOs, Osnabrück, Germany*

Introduction: *Salmonella enterica* Typhimurium (STM) relies on the translocation of effector proteins by two type III secretion systems (T3SS). While T3SS-1 is required for invasion of non-phagocytic cells, T3SS-2 allows formation of a replication-permissive niche within host cells. **Goals:** This project aims to study the translocation, targeting, and interaction of T3SS-1 and T3SS-2 effectors with host structures and to assess the individual contribution of the latter to endosomal remodeling, resulting in the formation of an extended interconnected network of *Salmonella*-induced filaments (SIF). **Materials & Methods:** Our recent work demonstrated the applicability of genetically encoded self-labeling enzyme (SLE) tags to investigate the subcellular localization and dynamics of T3SS-2 effector proteins in living cells[1]. Here, we further improve this approach by generating different HaloTag variants with improved detection sensitivity and less interference with translocation by T3SS. Additionally, we employ SPLIT-GFP-based contact site sensors for live-cell imaging and super-resolution microscopy to assess the contribution of T3SS-2 to membrane contact sites formed during SIF biogenesis. **Results:** Volumetric electron microscopy of STM-infected HeLa cells revealed that endosomal remodeling creates novel contact sites between mitochondria and the SIF network. SPLIT-GFP-based contact site sensors are used to assess the function of T3SS effectors in this process. To enable analyses of effector translocation with high spatial and temporal resolution, we identified HaloTag variants with improved T3SS translocation and high signal intensity after ligand binding. The fusion proteins were functional as effectors after translocation into host cells, allowing the analysis of T3SS effector translocation early during infections and monitoring of effector fates. **Summary:** Here, we present improved tools to study the function and translocation dynamics of STM effector proteins *in vivo* in real-time during infection.

[1]Göser V *et al.,* (2023). Single molecule analyses reveal dynamics of *Salmonella* translocated effector proteins in host cell endomembranes. Nat Commun 14(1):1240.

P-MP-007

Revealing the localization and function of the gatekeepers of the type III secretion system using optogenetic tools

*S. Großmann[1], A. L. Koida[1], B. Milne-Davies[1], A. Diepold[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Ecophysiology, Marburg, Germany*

Many Gram-negative bacteria use a type III secretion system (T3SS) to manipulate eukaryotic cells. The injection of virulence proteins into the target cells is a precisely timed process that prevents unnecessary waste of these toxins and avoids alerting the immune system. A gatekeeper complex blocks premature secretion and is essential for a successful infection. However, little is known about how this complex regulates protein secretion by the T3SS.

Using live cell microscopy, we found that in *Yersinia enterocolitica*, all gatekeeper proteins localize in the bacterial cytosol. Applying and adapting optogenetic methods, we succeeded to control individual gatekeepers with high spatial and temporal resolution in a reversible manner. Sequestration of the gatekeppers led to uncontrolled secretion, indicating that they are prevented from carrying out their regulatory function. Interestingly, this control required the sequestration of gatekeepers to a liquid-liquid pase separation compartment, while sequestration to the membrane did not impede the function of the gatekeeper proteins.

Our observations support a model in which the gatekeepers perform their regulatory role in the cytosol by a yet unexplained mechanism. This data on the localization of the complex leads to a better understanding of T3SS-regulation and host cell sensing in *Yersinia enterocolitica* infection, paving the way for possible applications, such as the cotrolled application of the T3SS or targeting the gatekeeper proteins to prevent disease.

P-MP-008

Extracellular and cytosolic proteome analyses in Streptococcus pneumoniae

*S. De[1], L. Steil[2], G. Burchhardt[1], U. Völker[2], S. Hammerschmidt[1]
[1]*University of Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany*
[2]*University of Greifswald, Department of Functional Genomics, Greifswald, Germany*

Introduction Pneumococci colonize asymptomatically the upper nasopharyngeal cavity, but upon an external trigger, pneumococci disseminate and cause severe infections. *S. pneumoniae* can express up to four different extracellular serine proteases called PrtA, HtrA, SFP and CbpG. Strain EF3030, a colonizing strain was chosen for profiling of the extracellular and cytosolic proteome during early and late log phase. Furthermore, mutants lacking these serine proteases will be studied to monitor their impact on the proteome composition.

Goals Monitoring of the abundance of intracellular and extracellular pneumococcal proteins, especially extracellular serine proteases, using global proteomic techniques.

Methods An optimized minimal medium was established for cultivation of pneumococci. Bacterial pellet and supernatant samples were taken in mid log phase and late log phase. Supernatant proteins were obtained by TCA precipitation, while pellet proteins were obtained by cell disruption using a bead mill. Both sample types were subjected to data independent LC-ESI MS/MS analysis (directDIA).

Results Around 1600 proteins, identified with more than 2 peptides, were profiled in the supernatant samples. A significant proportion of these proteins exhibited higher levels in the late log phase compared to the mid log phase. Proteins included extracellular proteases HtrA and PrtA, several choline-binding proteins, and the competence factor ComG. Proteome analysis of the pellet samples identified approximately 1600 proteins with more than 2 peptides, and the relative amount of the vast majority of these proteins remained unchanged upon entering the late log phase. Significantly reduced relative amounts were observed for proteins involved in energy metabolism, such as alpha amylase Amy, 1-phospho-fructokinase FruB, and galactokinase GalK in the late log phase. The relative amounts of HtrA and PrtA remained unchanged.

Summary We were able to monitor the abundance of intracellular and extracellular pneumococcal proteins, in particular the extracellular serine proteases, using proteomic profiling.

P-MP-009

Role of multi-drug-resistance Gram negative bacteria in the development of acute- on-chronic liver failure (ACLF).

*C. Cadoli[1], W. S. Ballhorn[1], S. Göttig[1], V. Kempf[1]
[1]*Institute for Medical Microbiology and Infection Control, Frankfurt a. M., Germany*

INTRODUCTION: Acute-on-chronic liver failure (ACLF) is the result of a dysbalance within the gut-liver axis culminating by the translocation of bacteria and bacterial compounds of the gut barrier. A chronically affected but balanced liver disease status is driven from multidrug-resistant (MDR) Gram-negative bacterial infections to an acute organ failure with high mortality rates.

METHODS: Liver cells (HepG2) and intestinal cells (Caco2) were infected with either *Klebsiella pneumoniae* ATCC 700603 and two clinical isolates (one MDR) or *Acinetobacter baumannii* ATCC 19606 and two clinical isolates (one MDR) for 6h hours, each. For identification of toxic effects on liver cells, supernatants were analyzed for secreted cytokines and released metabolites. Using proteome profiler analysis, secretion of cytokines upon infection was brodly analyzed and confirmed by specific ELISAs. Bacterial and host cell metabolites were identified using HPLC/mass spectrometry.

RESULTS: Proteome Profiler assays revealed an increased secretion of the pro-inflammatory cytokines IL-18 and MIF, the anti-inflammatory cytokine IL-13 and the primary inhibitor of tissue-type plasminogen activator SerpinE1. These results were confirmed for MIF and SerpinE1 using ELISA assays. By applying targeted and untargeted metabolomics analysis, the metabolic pathways that are affected during Gram-negative bacterial infection were identified. Fumaric acid, a metabolite of the Krebs cycle, was increased upon infection of liver and intestinal cells, and indole-3-acetic acid, a metabolite derived from aromatic amino acids cycles was increased. Interestingly, uridine and deoxycytidine, both metabolites resulting from nucleic acid metabolism, were increased during *A. baumannii* infections but decreased upon *K. pneumoniae* infections.

DISCUSSION: We speculate that the translocation of MDR bacteria or bacterial compounds or metabolites from the gut to the liver is decisive in the development of ACLF. Exact analysis of the role of inflammatory cytokines, bacterial compounds and metabolites might explain the pathogenic mechanisms underlying ACLF and might represent an attractive target for therapeutic intervention.

P-MP-010

Characterization of Actin-Binding Properties of the Virulence Factor BipC of Human-Pathogenic Burkholderiae.

*S. Klein[1], D. Dretvic[1], M. Maack[1], M. Riedner[2], S. Linder[3], W. R. Streit[1], *M. Himmel[1]
[1]*University of Hamburg, Microbiology and Biotechnology, Hamburg, Germany*
[2]*University of Hamburg, Mass Spectrometry Platform, Hamburg, Germany*
[3]*Medical Center Hamburg-Eppendorf (UKE), Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany*

Background

Human-pathogenic bacteria of the *Burkholderia pseudomallei* group in part cause severe infectious diseases. Multi-drug resistance and virulence factors complicate an efficient therapy. The intracellular lifestyle of the pathogen relies on phagosomal escape, actin-based intracellular motility, and fusion of host cell membranes. The type-III secretion system T3SSBsa is one important virulence factor promoting the intracellular persistence with *Burkholderia* invasion proteins (Bip) being an essential part of it. The translocator/effector protein BipC is homologous to *Salmonella* SipC and *Shigella* IpaC proteins, thus BipC might share the ability of interact with host cell F-actin.

Question

The work presented here focuses on the molecular characterisation of BipC to further elucidate the impact of BipC on host cell invasion and phagosomal escape with emphasises on actin binding properties.

Methods

Sequence alignment and structure prediction were used to identify structural similarities between BipC and the

homologs SipC and IpaC. The interaction with actin was performed in *in vitro* studies with recombinant BipC. Ectopic expression of fluorescently labeled BipC was performed in primary human macrophages. CRISPRi-mediated *bipC* knockdown in the model organism *B. thailandensis* E264 was used in cellular infection assays.

Results

The structural similarity between the homologous SipC and IpaC to BipC was used to select possible actin binding interfaces and design protein fragments of BipC. Also, ectopically expressed BipC-SiriusGFP in primary human macrophages binds to actin-rich podosomes and localizes at the subcortical F-actin network. The knockdown of *bipC* in *B. thailandensis* results in increased number of actin-coated intracellular bacterial cells and shows a clear reduction in the percentage of invasion and phagosomal escape.

Conclusions

The results indicate an essential contribution of the *B. thailandensis* virulence factor BipC to the pathogenicity of *Burkholderia* infections.

P-MP-011
RNase-mediated reprogramming of Yersinia virulence
*I. Salto[1], I. Meyer[1], M. Volk[1], T. Moesser[1], A. S. Herbrüggen[1], M. Rohde[2], M. Beckstette[2], A. K. Heroven[2], P. Dersch[1,3]
[1]*Institute für Infektiologie. ZMBE. Münster, Münster, Germany*
[2]*Helmholtz Center for Infection Research, Department of Molecular Infection Biology, Brunswick, Germany*
[3]*German Center for Infection Research, Partner site HZI Braunschweig and site University of Münster, Münster, Germany*

RNA degradation is an essential process that allows bacteria to regulate gene expression and has emerged as an important mechanism for controlling virulence. However, the individual contributions of RNases in this process are mostly unknown. Here, we report that the endoribonuclease RNase III and the exoribonuclease PNPase of the intestinal pathogen Yersinia pseudotuberculosis, repress the synthesis of the master virulence regulator LcrF. LcrF activates the expression of virulence plasmid genes encoding the type III secretion system (Ysc- T3SS) and its substrates (Yop proteins), that are employed to inhibit immune cell functions during infection. Loss of both RNases led to an increase in lcrF mRNA levels and stability. Our work indicates that PNPase exerts its influence via YopD, known to accelerate lcrF mRNA degradation. Loss of RNase III results in the downregulation of the CsrB and CsrC RNAs, leading to increased availability of active CsrA, which has previously been shown to enhance lcrF mRNA translation and stability. Other factors that influence the translation process and were found to be differentially expressed in the RNase III-deficient mutant could support this process.

Transcriptomic profiling further revealed that Ysc-T3SS-mediated Yop secretion leads to global reprogramming of the Yersinia transcriptome with a massive shift of the expression from chromosomal towards virulence plasmid-encoded genes. A similar extensive transcriptional reprogramming was also observed in the RNase III-deficient mutant under non-secretion conditions. This illustrates that RNase III enables immediate coordination of virulence traits, such as Ysc-T3SS/Yops, with other functions required for host-pathogen interactions and survival in the host.

P-MP-012
Screening of entomopathogenic fungi for their potential as beneficial endophytes in plants
*S. Sabu[1,2], K. Burow[2], P. Franken[1,2]
[1]*Friedrich Schiller University Jena, Microbiology, Jena, Germany*
[2]*University of Applied Sciences Erfurt, Research Center for Horticultural Crops (FGK), Erfurt, Germany*

Due to insect attack, reducing the quality of the fruits and transmitting plant pathogens, horticulture sector suffers from significant losses worldwide. The demand for more ecological alternatives to synthetic chemicals has high priority in biological control of plant pests and pathogens in recent years. Among these, endophytic entomopathogenic fungi (EEMPF) colonize plant tissues, protecting the plants against insect attack is an emerging hope. In this project we are trying to isolate potential EEMPFs against the plant pest *Bradysia difformis* (fungus gnat) which poses a serious problem in pot cultures under glass and especially highly attracted to peat free substrates.

The fungal strains including *Beauveria bassiana, Metarhizium flavoviride, Mucor hiemalis and Niesslia tinuis* from the German Collection of Microorganisms and Cell culture (DSMZ) were tested for entomopathogenic activity against *B. difformis* and for their ability to colonize *Petunia axillaris, Petunia exserta* and *Ocimum basilicum* plants. Also, several soil samples were analyzed for the presence of entomopathogenic fungi against *B. difformis*.

The preliminary findings indicate that the strain *M. flavoviride* can potentially act as an entomopathogenic fungi against *B. difformis* with an ability to colonize petunia and basil with no significant impact on plant development. The reproducibility of the results in terms of entomopathogenic activity was verified and confirmed through experimentation involving different concentrations of *M. flavoviride* spore suspension against *B. difformis*. The project is in progress, screening the new fungal isolates from various soil samples for their entomopathogenic activity against *B. difformis* and their ability to colonize plants.

P-MP-013
The thermonuclease NucA makes Staphylococcus aureus highly pathogenic
*N. Li[1], M. Deshmukh[2], F. Sahin[1], V. Leal[1], N. Hafza[1], A. Ammanath[1], S. Ehnert[1], A. Weber[1], T. Jin[2], F. Götz[1]
[1]*University of Tuebingen, Tübingen, Germany*
[2]*University of Gothenburg, Gothenburg, Sweden*

The Gram-positive bacterium *Staphylococcus aureus* is an opportunistic human pathogen that causes a wide spectrum of acute and chronic infections. Its genome encodes two thermonuclease-like homologs *nuc1* and *nuc2*. The mature form of Nuc1, known as NucA, is secreted and plays an important role in virulence. NucA degrades extracellular DNA (eDNA) as well as RNA, and contributes to bacterial escape from neutrophil extracellular traps (NET) and killing, thereby boosting staphylococcal persistence. Besides, it also disrupts e-DNA based biofilm matrix and contributes to bacterial dispersion and spreading. Here we show that uncut bacterial DNA induces production of TNF-α by murine macrophages (RAW264.7), however, pretreatment of DNA with NucA abrogated this activity. Live *S. aureus* JE2 and JE2Δ*nuc1* similarly immuno-stimulated RAW264.7, MM6 monocytes, HaCaT keratinocytes, and SAOS-2 osteoblast-like cells. However, JE2Δ*nuc1* showed decreased host cell invasion,

was killed much better than the parent strain by human primary neutrophils and induced less cytokines (TNF-α and IL10). In a septic arthritis mouse model the pathogenicity of Δ*nuc1* was largely attenuated as compared to the parental *S. aureus* Newman strain. Δ*nuc1*-infected mice showed almost no weight loss, mild symptoms of clinical arthritis, largely decreased kidney abscess score and bone destruction, and produced less cytokines. The results suggest that Nuc1 is an important virulence factor during *S. aureus* infection.

P-MP-014

Streptococcus suis Dpr provides protection from hydrogen peroxide in an iron rich environment

*S. Öhlmann[1], Y. M. Fornoville[1], A. T. Pulliainen[2], C. G. Baums[1]
[1]*University of Leipzig, Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, Leipzig, Germany*
[2]*Institute of Biomedicine, University of Turku, Turku, Finland*

Introduction: *Streptococcus suis (S. suis*) is a common porcine pathogen with zoonotic potential. It can cause meningitis, arthritis, septicemia and in humans also the streptococcal toxic shock-like syndrome. *S. suis* expresses a Dps-like peroxide resistance protein, designated Dpr. The protein prevents production of reactive oxygen species (ROS) by the Fenton-reaction ($H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+}$), as it is capable of binding iron and oxidizing Fe^{2+} to Fe^{3+}. So far, the structure and the mechanism of iron incorporation by Dpr have been studied at atomic resolution, but its relevance in the pathogenesis of *S. suis* infection remains to be elucidated.

In this study we aim to understand the role of Dpr in immune evasion as a putative virulence factor protecting against oxidative stress during bacteremia and meningitis.

Methods: We used a formerly created Dpr-deficient mutant (Pulliainen et al. 2003) to investigate its role in different biological settings: survival of *S. suis* in porcine blood, growth in porcine serum or cerebrospinal fluid (CSF) at increasing H_2O_2 concentrations and sensitivity to H_2O_2 under hemolytic conditions caused by *S. suis*-produced toxin suilysin.

Results: In culture medium H_2O_2 concentrations as low as 1 mM impaired bacterial growth even in the presence of Dpr, whereas higher H_2O_2 concentrations of 5 mM led to killing of the Dpr-deficient mutant but not of the wild type (wt). The addition of iron to the culture medium increased the toxic effect of H_2O_2 to the mutant but not the wt. Preliminary results showed that H_2O_2 concentrations as high as 5 mM did not kill *S. suis*, in porcine serum or CSF even in the absence of Dpr unless iron was added, leading to a certain level of killing of the mutant.

Summary: Our results underline the role of Dpr in protection against ROS in the presence of high iron concentrations and suggest that *S. suis* possess additional mechanisms to protect itself from killing by H_2O_2. During infection, *S. suis*-caused hemolysis might account for high iron levels in body fluids and render Dpr important for survival. Further experiments are planned to better understand its role in the pathogenesis of *S. suis* infection.

P-MP-015

Francisella tularensis ssp. holarctica is able to colonize natural aquatic ex vivo biofilms

*K. Heuner[1], K. Köppen[1], C. Schaudinn[1], K. Rydzewski[1]
[1]*Robert Koch Institute, Center for Biological Threats and Special Pathogens, Berlin, Germany*

Francisella tularensis (*Ft*) is an intracellular pathogen causing tularemia in a variety of hosts including humans and rodents. *Fth* is more frequently associated with aquatic habitats and is widely distributed throughout Eurasia. *F. tularensis* maintains viability in cold water for long periods of time. In (aquatic) natural environments, biofilm formation is known to increase the survival of bacteria.

The objective of this study was to investigate the survival of a wild type strain of *Francisella tularensis* ssp. *holarctica* (*Fth*) in natural aquatic *ex vivo* biofilms. To that purpose, we allowed *Fth* to produce its own biofilm on solid surfaces. The survival rate of biofilam and planctonic bacteria were compared. We also analyzed the ability of *Fth* to colonize naturally formed biofilms. Light- and electron microscopy showed that *Fth* WT is able to form a complex, matrix-associated biofilm. *Francisella* in its own biofilm showed longer cultivabilety in natural water when compared to planktonic bacteria. The same was true for *Fth* infecting an existing *ex vivo* biofilm. In addition, *Francisella* was also able to establish microcolonies and areas with their own exclusive biofilm architecture within the *ex vivo* biofilm. For the first time, using a *Fth* wild type strain, we can demonstrate the ability of *Francisella* to successfully colonize an aquatic multi-species *ex-vivo* biofilm. It can be assumed that *Fth* becomes more persistent in the environment when it forms its own biofilm or becomes a part of an existing one. This may have also impact on the long-term survival of *Francisella* in aquatic habitats and the infection cycles in nature.

P-MP-016

Understanding the genetic basis of resistant CTCL-related Staphylococcus aureus - a concern for individuals with mycosis fungoides

P. Licht[1,2], A. Schüffler[3], V. Mailänder[1,2], R. Heermann[4,3], *N. Dominelli[4]
[1]*University Medical Center Mainz, Department of Dermatology, Mainz, Germany*
[2]*Max Planck Insitute for Polymer Research, Mainz, Germany*
[3]*Institute for Biotechnology and Drug Research gGmbH (IBWF), Mainz, Germany*
[4]*Johannes-Gutenberg-University, Institute of Molecular Physiology, Microbiology and Biotechnology, Mainz, Germany*

Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma (CTCL), a rare subtype of non-Hodgkin lymphoma. This blood cancer variety specifically affects the skin tissue causing different types of skin lesions. MF patients can be categorized into SA-positive and SA-negative groups. In the SA-positive case, the patients skin lesion is predominantly colonized by *Staphylococcus aureus*, leading to an abnormal skin flora. Also, SA-neutral patients exhibit an abnormal skin flora on lesions, however, these are composed of different *Staphylococcus* and *Cutibacterium* strains. After isolating different bacterial strains from MF-patients, the role of *S. aureus* in affecting the skin flora and the healing process of the lesions is pivotal. The aim is to determine the genetic composition and to identify different pathogenic factors in *S. aureus* isolates from MF patients. Indeed, a high number of pathogenic factors could be

identified. Among these, genes for toxins, antibiotic resistance, antimicrobial peptides, antiseptic resistance, and proteins binding to immunoglobulins were identified. Further, for *S. aureus* MFMZ1 resistance towards different types of antibiotics, including reduced sensitivity to methicillin, and antimicrobial peptides was observed. The genome data disclosed an increased occurrence of octapeptide domains in the *Staphylococcus* Protein A (SpA) in *S. aureus* MFMZ1 compared to the control strain from a healthy patient, responsible for enhanced binding capacity of SpA towards host immunoglobulins. Moreover, *S. aureus* MFMZ1 produces a yet unidentified metabolite that may be involved in suppressing the microbiome in SA-positive patients" skin lesions.

Novel extracts from fungi were screened against *S. aureus* MFMZ1 to identify potential drug candidates, revealing promising agents that can inhibit either growth or biofilm formation. In conclusion, the main objective is to understand the role of staphylococci, especially, *S. aureus*, in MF-patients and to understand what triggers the pathogenicity of these bacteria. Here, we target first insights in the molecular basis for future treatment of *S. aureus* infected MF patients.

P-MP-017

The Small basic protein (Sbp): structure and spatio-temporal patterning in Staphylococcus epidermidis biofilm formation

*M. Savickis[1], S. W. Weißelberg[1], A. V. Failla[2], G. Abbaszade[3], S. Müller[3], M. Landau[4], H. Rohde[1]
[1]*University Hospital Hamburg-Eppendorf, medical microbiology, Hamburg, Germany*
[2]*University Hospital Hamburg-Eppendorf, UMIF, Hamburg, Germany*
[3]*UFZ, Leipzig, Germany*
[4]*CSSB, Hamburg, Germany*

Staphylococcus epidermidis is one of the most frequent nosocomial pathogens which cause implant-associated infections through biofilm formation. It's biofilm assembly relies on the production of an extracellular matrix. In effort to understand specific molecular interactions within this matrixome, a so far uncharacterized, 18 kDa protein was identified named small basic protein (Sbp).

The aim is to characterize and to elucidate the temporal-spatial distribution of Sbp in *S. epidermidis* biofilms.

To investigate the dynamic Sbp matrix three Sbp-isoforms were constructed in which Sbp is fused to an ALFA-tag located at different positions within the protein. The constructs were cloned in two plasmids (constitutive promotor and xylose-inducible promotor) and were tested (growth curves, biofilm formation, WB, CLSM).

Bioinformatic analysis revealed a structured protein head with ß-loops and α-helices, along with an unstructured N-terminal tail. Analysis of the three cloned constructs by WB revealed no degradation products and no effect on growth rate. CLSM revealed a unique spatial distribution pattern of Sbp within matrices, accumulating at early timepoints at the biofilm-substratum interface (forming a carpet-like structure). Similar patterns were observed for sbp-ALFA at positions 508 and 466. In mature biofilms, Sbp, regardless of isoform, is enriched in both the biofilm matrix and the substrate. In higher parts of the biofilm Sbp partially co-localizes with ALFA independent of tag position whereas these carpet like structure of ALFA could only be detected in sbp-ALFA 508.

All Sbp-ALFA fusion constructs are stable and reconstitute the wildtype biofilm phenotype in Δ*sbp* strains. Sbp-ALFA nt508 recapitulates wildtype Sbp distribution in biofilms the best, so this construct can be used for high resolution microscopy and to decipher specific Sbp- matrix interactions and their functional importance. At present, it is unclear whether and how Sbp can be exchanged between *S. epidermidis* sbp-producing and non-producing cells. Co-culture experiments will help understand Sbp production, repositioning, and exchange dynamics between different subpopulations and strains.

P-MP-018

Vancomycin-peptide conjugates overcome vancomycin resistance in Gram-positive pathogens

*T. Hertlein[1], E. Mühlberg[2,3], F. Umstätter[2,3], G. Braune[1], L. Dreher[1], J. Brock[4,5], F. Faber[4,5], T. Bischler[6], T. Gräfenhan[6], J. Bender[7], G. Werner[7], W. Mier[3], P. Uhl[2,3], *K. Ohlsen[1]
[1]*University Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*
[2]*Universtiy Heidelberg, Institute of Pharmacy and Molecular Biotechnology, Heidelberg, Germany*
[3]*University Hospital Heidelberg, Department of Nuclear Medicine, Heidelberg, Germany*
[4]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*
[5]*University Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[6]*University Würzburg, Core Unit Systems Medicine, Würzburg, Germany*
[7]*Robert-Koch-Institut, Wernigerode, Germany*

Infections caused by vancomycin-resistant enterococci (VRE) are increasing in most countries, including Germany. As there are very few therapeutic options, the development of novel compounds that overcome resistant phenotypes is urgently needed. Here, we report the design, synthesis and activity testing of vancomycin-peptide conjugates that are highly active against a broad range of Gram-positive pathogens, including VRE, MRSA and *Clostridioides difficile*. The most active conjugate, a hexaarginine-vancomycin conjugate designated FU002, was found to be highly active against all resistant pathogens tested, with the highest activity against *vanB E. faecium* strains. The compound has bactericidal activity, killing vancomycin resistant strains within four hours of incubation. In vivo studies in *Galleria melonella* larvae and murine systemic infection models demonstrated efficacy as indicated by increased survival of *G. melonella* larvae and reduced colony forming unit counts in organs of mice treated with FU002. The mode of action of the peptide-vancomycin conjugates differs from that of vancomycin as shown by transcriptome studies. In conclusion, vancomycin-peptide conjugates represent a promising class of novel antibiotic compounds against highly resistant Gram-positive pathogens.

P-MP-019

The type VI secretion system 3 of the insect pathogen Photorhabdus luminescens is involved in interbacterial competition

*K. Götz[1], F. Pisarz[1], R. Heermann[1,2], A. Regaiolo[1]
[1]*Johannes Gutenberg University, Institute for molecular physiology, Mikrobiologie und Biotechnologie, Mainz, Germany*
[2]*Institute for Biotechnology and Drug research gGmbH, Mainz, Germany*

Type VI secretion systems (T6SS) play a significant role in virulence, interkingdom competition and host interactions in various Gram-negative bacteria. These systems facilitate the

contractile translocation of effector proteins into prokaryotic or eukaryotic target cells. Thereby, effector translocation often relies on T6SS spike proteins like VgrG and PAAR, which are essential for T6SS function and activity. The entomopathogenic Gram-negative bacterium *Photorhabdus luminescens* has a complex lifecycle which involves the interaction with different eukaryotic hosts as well as bacterial competitors. The genome of *P. luminescens* encodes four T6SS gene clusters. However, the role of these putative T6SS for the biology of the bacteria is not yet understood. Here, we investigated the function of the VgrG spike proteins and the cognate Tle lipase effectors of the T6SS-3 in *P. luminescens*. First bioinformatic analyses identified for all VgrG proteins a DUF2345 C-terminal extension domain followed by a TTR-fold domain in VgrG6 and VgrG8. To determine their role in effector delivery, protein-protein interaction analyses were performed using BACTH, showing interactions between the VgrGs and a different subset of Tle2 and Tle4-type effector proteins. Homology modelling of the VgrG6 and Tle4A interaction site revealed that the DUF2345 and TTR-fold domain are involved in effector binding. Besides this, an interaction between VgrG6 and VgrG7 leads to the hypothesis of a multi-VgrG spike complex facilitating the delivery of a Tle effector cocktail. To provide initial insights into putative Tle4A effector activities, *in vivo* toxicity assays were performed, revealing growth inhibiting properties attributed to cell rounding and subsequent cell lysis. These findings provide preliminary insights into the roles of T6SS-3 VgrG proteins in effector binding and highlight Tle4A as an anti-bacterial effector, implying the importance of T6SS-3 for interbacterial competition.

P-MP-020

Streptococcus suis adaptation to the host environment: genome-wide identification of fitness determinants using tn-seq and Nanopore sequencing

*M. Juanpere Borras[1], J. Boekhorst[1], B. Fernandez Ciruelos[2], P. van Baarlen[1], J. Wells[1]
[1]*Wageningen University, Host-Microbe Interactomics, Wageningen, Netherlands*
[2]*Utrecht University, Utrecht, Netherlands*

Streptococcus suis is a zoonotic agent capable of inducing sepsis and meningitis in both pigs and humans, causing substantial economic losses. Cross-protective vaccines are not available and antibiotics are commonly used to control the spread of *S. suis* on farms. This increases the development and spread of antimicrobial resistance drawing attention of the global health community [1]. Alternatives to the currently most often used antibiotics are crucial, requiring research on identifying new therapeutic targets. This is a challenging goal because a large proportion of *S. suis* genes and proteins only have predicted functions. A better understanding of the virulence factors required for survival and growth in the host environments during colonization and invasive disease would be valuable strategy to identify new therapeutic targets. Here, we present the construction of a highly saturated transposon library in *S. suis* P1/7 and high-throughput transposon sequencing screen (Tn-Seq) for fitness determinants in body fluids.

The mutant strain library was screened in porcine serum and cerebrospinal fluid extracted from choroid plexis organoids, representing early and late (meningitis-associated) stages of the infection, respectively. After growing the library, mutants were collected and sequenced using Nanopore, achieving fast in-house, accurate results. The Tn-Seq data analysis pipeline was updated to incorporate nanopore reads for the identification of gene disruptions that are significantly affecting growth in these conditions.

We characterized a nucleoside ABC transporter and a novel sensory system which are essential for proliferation in serum. Additionally, a small putatively exported protein played a crucial role in growth in both fluids. Our study highlights the relevance of functional characterization of genes mediating *S. suis* survival in diverse host body fluids. Further research into the roles that the conditionally essential genes play in *S. suis* pathogenicity and survival might eventually allow us to find novel ways to control or prevent *S. suis* disease.

[1] Dechêne-Tempier et al. (2021) Microorganisms. 18;9(8):1765.

P-MP-021

Distribution of virulence genes in vancomycin-resistant Enterococcus faecium (VREfm) from bloodstream infections in North Rhine-Westphalia

*C. Böing[1], J. S. Schneider[1], A. Jurke[2], S. Kampmeier[3], A. Mellmann[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*
[2]*North Rhine-Westphalia State Health Center, Infectious disease epidemiology, Bochum, Germany*
[3]*University Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*

Question

Vancomycin-resistant *Enterococcus faecium* (VREfm) is a multidrug-resistant pathogen that can cause severe nosocomial infections including bloodstream infections. In recent years, there has been a significant increase in the incidence of multilocus sequence type (MLST ST) 117 in Germany. As the genomic content with accessory genes is highly variable, we hypothesize that the comprehensive equipment with virulence genes might be responsible for the successful spread of ST117. The aim of this study was to analyze the distribution of 32 known virulence genes in VREfm from bloodstream infections.

Methods

From October 2015 to May 2023, 187 VREfm isolates from bloodstream infections isolated in microbiology laboratories in the German federal state of North Rhine-Westphalia were included into the study. After whole genome sequencing (WGS), the MLST ST and core genome MLST (cgMLST) cluster types (CT) were extracted. In total, the sequences were examined for the presence of 32 virulence genes coding for known virulence factors of VREfm.

Results

The isolates in the study cohort had the MLST ST117 (n=50), ST80 (n=50), ST17 (n=8), ST192 (n=25), ST612 (n=7), ST1299 (n=6) and ST203 (n=41). The distribution of virulence genes in the investigated MLST sequence types were found to be distinctly variable for several virulence genes that code for adhesion factors, biofilm formation, survival and capsule formation, i.e. *scm*, *esp*, *hyl*, *ecbA*, *pilA*, *lwpC*, *swpC*, *fms15*, *tirE1*/*tirE2* and *capD*. Interestingly, the presence of these virulence genes was relatively stable for isolates with certain MLST STs and cgMLST CTs. Isolates with the same cgMLST CT therefore usually had the same set of virulence genes. ST117 exhibited the broadest

spectrum of virulence genes and particularly higher prevalence of *ecbA*, *hyl*, *capD* and *tirE1*/*tirE2* compared to the other MLST STs.

Conclusions

The distribution of virulence genes in VREfm is variable, but relatively stable for certain MLST ST and particularly cgMLST CT as surrogates for the genomic background. ST117 had the most extensive set of virulence genes, which might be a key factor for the successful distribution of ST117 in Germany.

P-MP-022

Identification of a novel biofilm-associated virulence factor in Rodentibacter heylii

*S. Kähl[1], D. Volke[2], R. Hoffmann[2], R. Ulrich[3], L. Benga[4], C. G. Baums[1]

[1]*University Leipzig, Institute of Bacteriology and Mycology, Faculty of Veterinary Medicine, Leipzig, Germany*

[2]*University Leipzig, Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Centre for Biotechnology and Biomedicine, Leipzig, Germany*

[3]*University Leipzig, Institute of Veterinary Pathology, Faculty of Veterinary Medicine, Leipzig, Germany*

[4]*Heinrich Heine University Düsseldorf, Central Unit for Animal Research and Animal Welfare Affairs, Düsseldorf, Germany*

Introduction

Rodentibacter heylii (R. heylii) is a frequently detected pathogen causing pneumonia, abscesses and mastitis in laboratory mice. In a recent infection experiment, we observed an unexpected high virulence of a strain not harboring any known virulence-associated factor. Furthermore, efficient colonization of the upper respiratory tract was detected 28 days after experimental infection. In *R. heylii*, three RTX-Proteins are described. However, the used strain harbors none of these known virulence factors, suggesting that an unknown virulence factor must be expressed by this strain.

Goals

Aim of this study was to identify a new potential virulence factor crucial for the pathogenesis of bronchopneumonia caused by *R. heylii*.

Materials and Methods

Immunogens of *R. heylii* strain were identified via immunoproteomics. Using an RhiA-specific IgY, flowcytometry and immunohistochemistry were conducted to evaluate the *in vitro* and *in vivo* expression of RhiA. Further experiments are in progress to characterize RhiA functionally.

Results

Sera from convalescent mice were immunoreactive to a specific band of a protein extract from *R. heylii* labeled to an SDS-Page. The corresponding protein was identified and designated as *R. heylii* immunogen A (RhiA). Flow cytometry confirmed expression on the bacterial surface *in vitro*, indicating that RhiA is a surface-associated protein. Immunohistochemistry showed that RhiA is expressed *in vivo* in association with putative bacterial biofilms on epithelial surfaces in bronchioli and alveoli after experimental infection. In silico analysis revealed similarity to a TISS secreted RTX agglutinin of a different strain in the family of Pasteurellaceae (NI1060).

Summary and Outlook

RhiA is an immunogenic protein, which is associated to the bacterial surface and expressed in vivo during infection. Similarities to known RTX-proteins suggests, that it is a new potential virulence factor. Further a deletion mutant of the used *R. heylii* strain without RhiA should be constructed by Crispr/Cas to analyze the function of RhiA. Therefore, several in vitro assays including adherence and biofilm formation followed by in vivo studies will be conducted.

P-MP-023

Induction and persistence of Mycobacterium avium in myeloid derived suppressor cells in the mouse model

*S. G. Worku[1], A. Pich[2], S. Lienenklaus[3], E. Goethe[1], A. Beineke[4], G. A. Grassl[5], R. Goethe[1]

[1]*University of Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany*

[2]*Hannover Medical School, Institute of Toxicology, Hannover, Germany*

[3]*Hannover Medical School, ZTL Imaging-Center, Hannover, Germany*

[4]*University of Veterinary Medicine Hannover, Institute for Pathology, Hannover, Germany*

[5]*Hannover Medical School, Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany*

Introduction-*Mycobacterium* (*M.*) *avium* subsp. *avium* (MAA) and *M. avium* subsp. *hominissuis* (MAH) belongs to non-tuberculous mycobacteria group induce different courses of disease after mice infection. Previously we have shown that severe disease caused by MAA, but not MAH, was due to the accumulation of nitric oxide (NO) expressing splenic monocytic myeloid derived suppressor cells (M-MDSC). The mechanisms by which MAA induces M-MDSC and persists in the NO-rich environment is unknown. We hypothesized the subspecies-specific genes of MAA might mediate these unique features. In the present study, we identified putative phenotypic features that enable MAA to survive in the NO environment after infection of mice and after *in vitro* NO exposure**.**

Materials and methods-C57BL/6J mice were infected intraperitoneally with $\sim 10^8$ colony-forming units of MAA 44156 or MAH 104 per mouse and mice were sacrificed on 29 day post-infection. Both MAA and MAH were purified from spleens and *ex vivo* proteomic analyses were performed using mass spectrometry**.**

Results-Our proteomic analyses identified a total of 2729 MAA proteins. Among these 78.7% were detected in both the MAA infection input and MAA from mice. 19.3% MAA proteins were detected only in input and 1.9% were detected in MAA from mice only. The latter group is suggested to be involved in the adaption of MAA to the host milieu. Interestingly, some of the MAA proteins differentially abundant in mice, such as a paralog of a predicted nitric oxide reductase subunit B, an iron-sulfur cluster repair di-iron protein and ferredoxin paralogs were found to be encoded from genes located on MAA-specific genomic islands. To further investigating the functional relevance of the *ex vivo* expressed MAA-specific proteins, we monitored the mRNA

expression of the above MAA proteins upregulated in mice after MAA exposed to NO *in vitro*. Interestingly, we found the upregulation of the respective genes which indicates a role of such proteins for MAA defense against NO stress**.**

Conclusions-Our proteomic and gene expression analyses provide new insights for a specific ability of MAA to respond to NO supporting its persistence in NO producing cells.

P-MP-024
Analysis of the emerging global pathogen Stenotrophomonas maltophilia in single vs. multispecies biofilms
*R. Moll[1], I. Alio[1]
[1]*Universtiy Hamburg, Microbiology, Hamburg, Germany*

Introduction:

Stenotrophomonas maltophilia is an opportunistic pathogen causing various infections. Its prevalence has increased, especially in cystic fibrosis patients, and its multidrug resistance poses challenges in treatment. The bacterium forms multispecies biofilms with other pathogens and is known for its role in pathogenicity in infected lungs.

Goals:

Our goal is to understand the biofilm characteristics of *S. maltophilia* in single und multispecies biofilms as well as the differential gene expression patterns within these biofilms.

Materials and methods:

In this study over 200 clinical isolates of *S. maltophilia* as well as chromosomally labeled fluorescent strains of *S. maltophilia* K279a, *P. aeruginosa* PA01, *S. aureus* SH1000 and *C. albicans* SC5314 were used. Biofilms were formed in flow and static settings and were used for RNA seq analysis. Additionally, promotor fusion constructs of selected genes were generated and were further analyzed.

Results:

Our analysis revealed strain-specific variability in biofilm formation and architecture among *S. maltophilia* isolates. RNA seq analysis of these isolates identified shared and strain-specific genes, with iron uptake being a key factor in biofilm metabolism. CLSM imaging showed that species interactions affect the structural composition of multispecies biofilms. *S maltophilia* showed alteration in lactate metabolism, propionate degradation and a switch in cytochrome oxidases in our biofilm models. The expression of virulence factors, QS signaling and cyclic diGMP was decreased in PA01 in coculture with K279a.

Summary:

Our data show that isolates of *S. maltophilia* are highly diverse on a phenotypic and genotypic level. Common genes were identified to play a crucial role in the biofilm formation of *S. maltophilia*. CLSM imaging has shown a distinct distribution and layer formation of different species within multispecies biofilms. RNA seq analysis and the application of reporter fusion constructs showed specific and different expression patterns for each species as compared to single species biofilms, suggesting that each species acknowledge and respond to the presence of others.

P-MP-025
Comparison of invasive non-typhoidal Salmonella from endemic regions in Sub Saharan Africa with invasive strains from Germany
*K. Weber[1], M. Pietsch[1], S. Simon[1], D. Dekker[2], A. Flieger[1]
[1]*Robert Koch-Institut, Unit 11: Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*
[2]*Bernhard Nocht Institute for Tropical Medicine, One Health Bacteriology, Hamburg, Germany*

Introduction: In Germany *S.* Enteritidis and *S.* Typhimurium are the most common serovars of the genus *Salmonella enterica* associated with foodborne illness. Typically, infection leads to gastrointestinal symptoms like diarrhoea, light fever and vomiting. However, also invasive non-typhoidal *Salmonella* (iNTS) infections especially in Sub Saharan Africa are known. Importantly, in recent years, an increase in the numbers of iNTS in Germany, among these infections due to *S.* Typhimurium and *S.* Enteritidis, has been observed at the National Reference Centre for *Salmonella* (NRC). Salmonellae of specific MLST sequence types (ST), such as ST 313, ST19 (*S.* Typhimurium) and ST11 (*S.* Enteritidis), are primarily responsible for iNTS cases in Sub-Saharan Africa, particularly in children under 5 years (case fatality rate of 20-25%). Additionally, these pathogens exhibit special resistance patterns, including resistance towards the antibiotic ceftriaxone, which belongs to the 3rd generation of cephalosporines.

Goals: We aimed to compare iNTS isolates from Germany with those from Sub-Saharan Africa to identify genomic signatures and infection phenotypes associated with invasive disease.

Materials and Methods: Selected *S*. Typhimurium strains isolated from blood and stool samples of Sub Saharan African and German patients were analysed for their virulence potential and genomic differences. Specifically, they were tested in cell culture infection assays to assess invasiveness and intracellular replication capacity. Further, bioinformatic analyses like creating a Gene presence/absence table (tools: PROKKA, Roary and Scoary) were performed to identify possible underlying genetic factors accounting for a difference in virulence.

Results: Within phylogeny of *S*. Typhimurium ST 19 strains, we identified a clade in which strains from Germany and Africa show higher invasiveness and intracellular replication in comparison to other strains tested. Genome comparisons are currently ongoing.

Summary: Invasive *Salmonella* strains occur in Africa as well as in Germany. Here, we would like to identify genetic factors (genes, pseudogenization, host factors) that contribute to higher invasiveness.

P-MP-026
Revealing membrane insertion mechanisms of Legionella's integral membrane effector proteins in host cells
*S. Trenz[1], S. Schminke[1]

[1]Eberhard Karls University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany

A virulence strategy used by the intracellular pathogen *Legionella pneumophila* is to manipulate host cellular processes in order to survive within phagocytic host cells. More than 300 virulent effector proteins are secreted into host cells by the specialized Dot/Icm type IV secretion system (T4SS). Many effector proteins contain hydrophobic transmembrane domains (TMDs) to fulfil their function in host cell membranes. However, the mechanisms T4-secreted TMD effectors (TMEs) use to target and insert into the correct membranes of eukaryotic hosts remain to be elucidated.

To understand the relevance of host cell targeting factors and receptors involved in membrane insertion, the subcellular localization and interacting proteome of bacterial TMEs were assessed either after T4SS-assisted injection or in-host ribosomal translation. The intracellular environment of four *Legionella* TMEs, differing in hydrophobicity and position of their TMD, was characterized using TurboID-mediated proximity dependent biotinylation. The biotinylated proteins were then captured on streptavidin beads and identified by mass spectrometry.

Proteomic analysis revealed that bacterial TMEs interact with host protein components involved in vesicle trafficking pathways between the endoplasmic reticulum (ER) and the Golgi apparatus, endosomal vesicle transport, protein folding and turnover, and mitochondrial energy metabolism. Moreover, T4-secreted and in-host ribosomal translated TMEs with their TMD located towards the C-terminus could be associated with several proteins involved in the post-translational signal recognition particle (SRP)-dependent ER targeting pathway. To investigate the mechanisms of TME integration at the ER, co-immunoprecipitation and inhibitors that specifically target post-translational pathways will be used. Additionally, fluorescence microscopy will be employed using self-labelling tags to track the route of TMEs in living cells.

These primary findings suggest that T4-secreted TMEs, depending on the position and hydrophobicity of their TMD, use eukaryotic membrane protein biogenesis pathways into the ER and are then trafficked to their specific compartment in the host.

P-MP-027

Host cytoplasmic targets of the Staphylococcus aureus cysteine protease Staphopain A involved in host cell death

K. Paprotka[1], K. Stelzner[1], A. Boyny[1], N. Enslinger[1], L. Steil[2], *M. Fraunholz[1]
[1]Julius-Maximilians University Würzburg, Microbiology, Würzburg, Germany
[2]University of Greifswald, Interfaculty Institute of Genetics and Functional Genomics, Greifswald, Germany

Staphylococcus aureus constitutes a major opportunistic pathogen of humans. *S. aureus* is readily internalized by non-professional phagocytes such as epithelial cells where it can escape from the phagosomal membrane and replicate within the host cytoplasm. Eventually, the host cells are killed whereby the bacteria exit into the extracellular environment. Previously, we had identified the staphylococcal cysteine protease staphopain A (ScpA) to be involved in this intracellular cytotoxicity in infected epithelial cells.

Targeted release of wild-typic ScpA or its active site mutant ScpA(C238A) to host cytoplasm by the otherwise non-cytotoxic laboratory strain *S. aureus* RN4220 demonstrated that the protease activity in host cytoplasm was required for accelerated cell death. Thus the *S. aureus* cysteine protease staphopain A must possess intracellular host targets. These targets, however, are currently unknown.

To identify these targets, we ectopically expressed ScpA or its proteolytically inactive variant ScpA(C238), harvested the samples at the first sign of host cell death in wild-type infected cells and compared the proteomes of infected cell cultures to each other.

We here present data on the identified target candidates as well as potential modes of action of the protease during intracellular *S. aureus* virulence.

P-MP-028

Overview of the Francisella tularensis subsp. holarctica clades, B.6 and B.12, regarding their phenotypic and pathogenic characteristics

*K. Köppen[1], K. Rydzewski[1], K. Heuner[1]
[1]Robert Koch-Institute, Berlin, Germany

Francisella tularensis is the causative agent of the zoonotic disease tularemia and is classified as a Category A select agent for bioterrorism. Tularemia can affect a broad range of animals predominantly rabbits and rodents. In humans the clinical manifestation varies from flu-like symptoms to severe pneumonia with a lethality rate of up to 60 %. In Europe, tularemia is caused by *F. tularensis* subsp. *holarctica* (*Fth*) which is further classified into two clades (B.6 and B.12) using canonical Single Nucleotide Polymorphism (canSNP) typing and whole genome SNP typing. These clades also represent two biovars according to their resistance to erythromycin (I: B.6 sensitive; II: B.12 resistant). *Fth* strains belonging to the B.6 clade are primarily isolated in Western Europe, whereas B.12 strains are mainly found in Northern, Central and Eastern Europe. In Germany, both clades are present with a comparable geographical distribution. For a long time, it was not clear if the genetic differences result in clade-specific phenotypes including a different pathogenic potential. Recently, we have demonstrated that *Fth* strains show a clade-specific growth pattern in media and a distinct protein expression profile reflecting the phylogenetic relation. Data from wild animals might reveal a more severe course of disease caused by B.6 strains suggesting a higher pathogenic potential of the B.6 clade. In humans, more cases of pneumonia caused by B.6 strains are found, but too less data are currently available to statistically confirm this assumption. Moreover, the proteomic data showed that known virulence factors, like the pathogenicity determinant protein PdpA1, Fe(2+) transporter FeoB, Biotin synthase BioB and Catalase-peroxidase KatG, are differently expressed in both clades. Using *Drosophila* S2 cells, B.6 strains showed a faster replication capacity during the first hours, but a reduced survival at later stages of infection, which corresponds with the decreased expression of ribosomal proteins observed in B.6 strains. So far, the available data do not allow a general conclusion about the putative different pathogenicity of the *Fth* genotypes; therefore, more research is needed.

P-MP-029

Role of triclosan in stringent response induced antibiotic tolerance of Staphylococcus aureus.

*P. Iyer[1,2], D. Walsh[3], A. Salzer[1], K. Hardie[2], C. Wolz[1]
[1]*University of Tuebingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*
[2]*University of Nottingham, Life sciences, Nottingham, United Kingdom*
[3]*University of Warwick, School of Life sciences, Warwick, United Kingdom*

The biocide triclosan is used extensively in both household and hospital settings. The chronic exposure to the biocide occurring in individuals that use triclosan-containing products results in low levels of triclosan present in the human body. Triclosan was proposed to induce antimicrobial resistance in bacteria (Suller & Russell, 2000). Antimicrobials have failed to control *Staphylococcus aureus* and often infection persist or relapse. Stringent response mediated by alarmones ppGpp and (p)ppGpp have been reported to induce various virulence pathways and might be involved in antibiotic resistance and tolerance (Salzer, 2023). Here we aim to analyse whether the fatty acid inhibitor triclosan impacts antibiotic tolerance in planktonic and biofilm grown *S. aureus*. We analysed different *S. aureus* strains and mutants deficient in (p)ppGpp synthesis. We show that physiological concentrations of triclosan protects *S. aureus* from bacterial killing by ciprofloxacin and vancomycin. Triclosan pretreatment also protected *S. aureus* biofilms against antibiotics as shown by live/dead cell staining and viable cell counting. In planktonic cultures the triclosan effect on antibiotic tolerance was independent of (p)ppGpp and there was no induction of the stringent response by triclosan treatment. However, in biofilms antibiotic tolerance was decreased in a pppGpp^0 mutant. This suggests that the mode of action of triclosan varies in planktonic and biofilm. RNA-sequencing should unravel the molecular mechanism of triclosan induced tolerance.

Salzer, A., & Wolz, C. (2023). Role of (p) ppGpp in antibiotic resistance, tolerance, persistence and survival in Firmicutes. *Microlife, 4*, uqad009.
Suller, M., & Russell, A. (2000). Triclosan and antibiotic resistance in Staphylococcus aureus. *Journal of Antimicrobial Chemotherapy, 46*(1), 11-18.

P-MP-030

The functional role of the cst gene cluster in S. aureus

*V. Wiemann[1], J. S. Puls[1], T. S. Tanabe[2], T. Schneider[1], C. Dahl[2], T. Fließwasser[1], F. Grein[1]
[1]*Institute for Pharmaceutical Microbiology, Bonn, Germany*
[2]*Institute for Microbiology and Biotechnology, Bonn, Germany*

Hydrogen sulfide (H2S) is ubiquitously distributed in the human body and involved as signaling molecule in a wide range of physiological processes. Despite its function as a gaseous signaling molecule, H2S is primarily known for its toxicity, which mainly arises from its ability to inhibit the respiratory chain. Thus many microorganisms, including the opportunistic pathogen *S. aureus*, protect themselves from these effects. The genome of *S. aureus* contains the *cst* gene cluster, consisting of the genes *tauE, cstR, cstA, cstB* and *sqr*. It is reported to be involved in the detoxification of sulfide. The proposed detoxification process is initiated by the oxidation of sulfide by means of the sulfide:quinone oxidoreductase (SQR), followed by the stepwise conversion into sulfite by CstA and CstB and the subsequent export of sulfite by TauE. Phylogenetic analyses revealed that the *cst* operon is widely distributed among staphylococci. However, we found that the presence of the *sqr* in the *cst* operon is essentially limited to *S. aureus* indicating that the function of the core *cst* operon in staphylococci is not the detoxification of H2S. Phenotypical analyses of a comprehensive set of *cst* deletion and complementation mutants and numerous clinical and laboratory derived strains, revealed the core cst gene cluster to be essential for the detoxification of polysulfides rather than H2S. In addition, we found a partial duplication of the core *cst* gene cluster associated with SCC*mec* in clinically relevant *S. aureus* (MRSA). Moreover, our studies revealed this cluster to be functional and to confer higher polysulfide tolerance to MRSA.

P-MP-031

Role of the alternative sigma factor SigB on antibiotic tolerance in Staphylococcus aureus

*N. Vetter[1], I. Simic[1], C. Wolz[1]
[1]*University of Tuebingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*

Staphylococcus aureus is a major human pathogen, responsible for numerous infections that, despite appropriate treatment, often become chronic or relapsing. The recurrence of infections is closely associated with the formation of bacterial persister cells. There, a subpopulation of bacteria becomes tolerant to the antibiotic treatment by entering a metabolic status of low activity (triggered persistence) or by heterogenous activation of different stress pathways (spontaneous persistence). Previous research suggested that *S. aureus* cells stochastically enter the stationary phase at any timepoint, which is accompanied by a drop of intracellular energy levels and results in persister formation. Bacteria expressing stationary phase markers such as activity of the capsular polysaccharide promotor (P*cap*) are indicative for persisters (1). Recently, we could show that P*cap* activity is mainly regulated by the alternative sigma factor B (SigB) (2). Thus, we hypothesized that SigB activity might be a trigger for persister formation in *S. aureus*. P*cap* activity as well as the prototypic SigB target gene *asp* were shown to be heterogeneously expressed within a population, with maximum expression upon entering the stationary phase. We analysed *S. aureus* strain Newman and its *sigB* mutant for persister cell formation following treatment with various bactericidal antibiotics (vancomycin, gentamycin, daptomycin and ciprofloxacin). SigB had no impact on antibiotic resistance (MIC) or on persister cell formation in the exponential growth phase. However, the *sigB* mutant was less antibiotic tolerant when bacteria were grown to stationary phase. Repeated subculturing indicated that triggered persistence in *S. aureus* is SigB dependent while spontaneous persister formation seems to be independent of SigB. We aim to further elucidate the signals leading to SigB activation and thus persister formation on the single cell level.

(1) Conlon et al. (2016)
(2) Keinhörster et al. (2019)

P-MP-032

"Novel points of attack" – Exploiting capsule biosynthesis in Streptococcus pneumoniae for antibacterial treatment

*V. Becker[1], M. Arts[1], M. Rausch[2], S. Bamyaci[3], B. Henriques-Normark[3,4], S. Normark[3], K. Blomqvist[3,4], T. Schneider[1], A. Mueller[1]

[1]University Hospital Bonn, University of Bonn, Institute for Pharmaceutical Microbiology, Bonn, Germany
[2]University Hospital Bonn, Institute for Hygiene and Public Health, Bonn, Germany
[3]Karolinska Institutet Stockholm, Department of Microbiology, Tumor and Cell Biology, Stockholm, Sweden
[4]Karolinska University Hospital Solna Stockholm, Clinical Microbiology, Stockholm, Sweden

The polysaccharide capsule is the major virulence factor of pneumococci and crucial to resist the immune system during an infection. Whereas vaccines that target pneumococcal capsules are in use for decades, neither biosynthetic reactions nor mechanisms regulating capsule expression have been investigated as potential therapeutic targets so far. Most *S. pneumoniae* serotypes produce capsular polysaccharides (CP) via the Wzx/Wzy-dependent pathway. In this case, CP repeat units are assembled on C_{55}-P at the inner side of the cytoplasmic membrane, translocated and polymerized in a non-processive manner on the exterior of the cell. Enzymes of the LytR-CpsA-Psr (LCP) family catalyze the transfer and the covalent linkage of CP to peptidoglycan (PGN) under release of the lipid carrier. Here we show the functional reconstitution of the CP biosynthesis reactions of *S. pneumoniae* ST14 *in vitro* using purified recombinant enzymes and substrates. In addition, we provide first biochemical evidence and molecular details for the post-translational regulation of involved reactions, which allows the concerted action of CP and PGN biosynthesis reactions.

P-MP-033

Inhibition of Bartonella bacilliformis hemolysis as a novel anti-virulence strategy against Oroya fever

*D. Munteh[1], A. A. Dichter[1], W. S. Ballhorn[1], V. Kempf[1]
[1]University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a. M., Germany

Question

Bartonella bacilliformis is the causative agent of Carrion's disease, a vector-borne neglected tropical disease endemic to the South American Andes. In the acute phase, known as Oroya fever, the bacteria infect erythrocytes, causing severe hemolytic anemia with a mortality rate of up to 90%. Erythrocyte infection results in hemolysis, contributing to the high mortality rate. Two genes, a porin and a phospholipase, were identified to be essentially involved and represent therefore potential drug targets. It is assumed that prevention of hemolysis might represent a promising anti-virulence strategy to treat Oroya fever patients.

Methods

To prevent hemolysis, we systematically examined a library of 41 known phospholipase inhibitors as well as peptide-based rabbit anti-porin and rabbit anti-phospholipase antibodies *in vitro*. The hemolytic activity of *B. bacilliformis* was assessed in a novel *in vitro* based hemolysis assay using human erythrocytes. Various concentrations of each inhibitor and antibody were tested to determine the optimal hemolysis-inhibitory effect.

Results

Preliminary *in vitro* results revealed a phospholipase inhibitor reducing hemolysis significantly (68% reduction at a concentration of 10 µM). Furthermore, administration of anti-porin and anti-phospholipase antibodies also reduced hemolysis significantly (porin-antibody: 77%, phospholipase-antibody: 75%).

Conclusions

Our findings indicate that one particular phospholipase inhibitor compound or anti-porin and -phospholipase antibodies may have the potential to prevent hemolysis *in vivo*. Current efforts are directed to establish an experimental mouse model to verify these findings *in vivo*. Identification of a hemolysis inhibitor and/or a therapeutic antibody would represent a novel anti-virulence strategy that prevents a key process in the pathogenicity of *B. bacilliformis*.

P-MP-034

Quality control mechanism in the type III secretion system export apparatus assembly

*E. Kim[1], M. Forberger[1], F. Weichel[1], S. Wagner[1,2]
[1]University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Tübingen, Germany
[2]German Center for Infection Research, Tübingen, Germany

The virulence-associated type III secretion system (T3SS), also known as the injectisome, is utilized by many gram-negative bacteria to translocate various effector proteins into host cells. Comprising around 200 subunits, the mega-dalton sized injectisome undergoes a highly coordinated assembly initiated by the export apparatus (EA). The EA serves as a scaffold for the inner and outer membrane rings, forming the base of the injectisome. Subsequently, the cytoplasmic compartment and needle filament are assembled.

The EA is composed of five conserved proteins, SctRSTUV, in a 5:4:1:1:9 stoichiometry, forming a unique helical cup structure. Its assembly involves distinct steps: initial assembly of pentameric SctR with one SctT, followed by recruitment of four SctS wrapping around SctR5T1 to form the core EA, and final addition of SctU and nonameric SctV to complete the assembly.

Given the size and complexity of the T3SS, its assembly is a complicated and energy-intensive task. Hence, elaborate regulatory networks exist to ensure robust assembly and prevent the waste of cellular resources. In our study, we observed that the EA has a highly conserved gene order across many T3SS-expressing pathogens. Interestingly, the assembly order (SctRTSU) does not follow the conserved gene order (*sctRSTU*). Matching assembly and gene orders is often observed in protein complex assemblies, as it allows co-translational assembly and minimizes the chance for subunit diffusion. We hypothesized that the conserved genetic organization may harbor regulatory motifs for robust EA assembly. In our analysis of *Salmonella* Typhimurium, we discovered that *sctS* and *sctT* are translationally coupled. *sctS* mRNA has a stem-loop structure which harbors the ribosome binding site of *sctT*, inhibiting *de novo* translation of SctT and allowing expression of SctT exclusively through the re-initiation mechanism. The nature of the upstream gene in the translational coupling was not shown to be a critical factor in the regulation of *sctT* expression and EA assembly. Our study presents the translational coupling between *sctS* and *SctT* as a crucial quality control mechanism in EA assembly.

P-MP-035

The fibronectin binding protein PavA is involved in ribosome quality control in Streptococcus pneumoniae

*G. Burchhardt[1], L. Jachmann[1], T. Hansen[1], T. P. Kohler[1], L. Schulig[1], S. Hirschmann[1], J. Kaur[1], S. Hammerschmidt[1]
[1]*University of Greifswald, Dept. of Molecular Genetics and Infection Biology, Greifswald, Germany*

Introduction The pneumococcal adherence and virulence factor PavA was first described as virulence factor and fibronectin-binding protein (Kanwal et al 2017). The deficiency of PavA in pneumococcal mutants abrogates adherence to human host cells and impairs mouse colonization. Moreover, PavA is required to escape phagocytosis and needed for induction of an optimal cytokine production by infected dendritic cells (Noske et al 2009). However, the biological activity associated with its crucial role in maintaining virulence remains unknown.

Methods *In silico* analysis and protein sequence comparisons revealed significant homology to the ribosome quality control protein RqcH of *B. subtilis.* The RqcH protein is part of the ribosome quality control system (RQC) recognizing stalled 50S ribosomes subunits and promotes nascent chain proteolysis. To study the involvement of PavA as part of the RQC system, protein-protein interaction was analyzed between PavA and RqcP.

Results In a pull down experiment we were able to identify binding of PavA to RqcP. Surface Plasmon Resonance (SPR) analysis revealed a significant affinity of PavA to RqcP with a Kd ranging from 0.12 to 0.22 µM depending on the provided ligand. Growth behavior of D39 and 19F strains and the isogenic *pavA* mutants were studied. No growth difference was detected between the wild-type and the *pavA* mutant during cultivation in THY or minimal medium. Furthermore, the impact of PavA on oxidative stress reduction was investigated. So far no significant difference between the wild-type and the isogenic *pavA*-mutant was observed when hydrogen peroxide or diamide was applied as oxidative agents. In addition, RqcP as part of the ribosome was mutated and the growth of single and double *pavA*, *rqcP* deletion were studied.

Summary The striking homology of PavA to RqcH of *B. subtilis* and the protein interaction suggests that PavA is part of the ribosome quality control in *S. pneumoniae*. *In vitro* assays even under infection-related stress conditions did not result in growth defects so far. Hence, the stress conditions in which PavA (RqcH) is essential for pneumococci have still to be elucidated.

P-MP-036

Unravelling the factors that shape the intracellular lifecycle of uropathogenic Escherichia coli

*H. Özer[1], M. Becht[1], K. Mukherjee[1], U. Rescher[2], J. Putze[1], U. Dobrindt[1]
[1]*UK Münster, Institute of Hygiene - microbial genome plasticity, Münster, Germany*
[2]*University Münster, institute for medical biochemistry, Münster, Germany*

Introduction Uropathogenic *Escherichia coli* (UPEC) cause the majority of uncomplicated urinary tract infections (UTIs), an increasing global disease burden prevalent among women. UPEC invasion of the bladder epithelium and development of intracellular bacterial communities (IBCs) contribute to the establishment, persistence and recurrence of UTIs. The intracellular localization renders UPEC resistant to antibiotic treatment and host clearance, leading to bacterial persistence and subsequent recurrent UTI.

Goals Our understanding of regulatory steps leading to the different stages of IBC formation and the interaction between bacterial and host cell factors during the intracellular lifecycle remains incomplete. We aim to uncover mechanistic relationships underlying recurrent UTIs by deepening the understanding of UPEC-urothelial cell interplay.

Materials & Methods We analyzed the intracellular life cycle of different clinical UPEC isolates in the human epithelial urinary bladder carcinoma cell line RT-112. We quantified the invasion rate, intracellular survival and exit behavior, and monitored the development and formation of IBCs using fluorescence microscopy.

Results Our infection protocol allows us to track the intracellular life cycle of UPEC. We show that individual UPEC isolates differ in their invasion rate, intracellular replication/survival, exit efficiency as well as in the morphology of their intracellular communities. Furthermore, the functionality of the endosomal compartment and intracellular transport affect the intracellular life cycle of UPEC.

Summary Our results show that UPEC isolates differ in certain aspects of the intracellular lifecycle. This could also affect the ability of UPEC isolates to persist in the urinary tract and cause recurrent urinary tract infections.

P-MP-037

Inhibition of Legionella pneumophila ProA affects bacterial FlaA degradation, TLR5-induced NF-κB signaling and hydrolysis of human collagen IV.

*T. Tiedeken[1], L. Scheithauer[1], A. Hirsch[1], J. Haupenthal[1], M. Steinert[1]
[1]*Technical University of Braunschweig, Institute of Microbiology, Brunswick, Germany*

Legionella pneumophila, an environmental bacterium and intracellular pathogen of protozoa, is also a significant human pathogen, causing Legionnaires' disease and resulting in long-term lung damage. This bacterium possesses numerous virulence factors, of which ProA, an important extracellular zinc metalloprotease, plays a central role. ProA exhibits a broad substrate spectrum, including collagen fibers and immunogenic flagellin. This study focused on inhibiting ProA as a strategy to counteract *L. pneumophila* pathogenicity. The employed zinc-binding inhibitors effectively suppressed ProA cleavage of bacterial flagellin FlaA, which activates the TLR5 receptors of the host cell and consequently stimulates the pro-inflammatory NF-κB signaling pathway. Successful inhibition of this ProA-mediated immune evasion by FlaA degradation was confirmed *in vitro* using HEK-Blue™ hTLR5 cells. Moreover, the tested inhibitors demonstrated the ability to reduce the proteolytic degradation of native human collagen IV, a constituent of the basal lamina in human lungs. This suggests potential therapeutic approaches for reducing tissue damage associated with Legionellosis. In summary, this research provides insights into potential inhibitors to mitigate the effects of *L. pneumophila* infection and the importance of targeting ProA-mediated pathogenic

mechanisms, particularly immune evasion and tissue damage.

P-MP-038

Stay inside or leave? The impact of glucose availability on uropathogenic Escherichia coli intracellular bacterial communities.

*J. Putze[1], J. Becher[1], U. Dobrindt[1]
[1]*University Münster, Institute for Hygiene, Münster, Germany*

Introduction Uropathogenic *E. coli* (UPEC) account for the majority of urinary tract infections (UTI). Despite antibiotic treatment, recurrence of infection is frequently reported. Invasion of superficial bladder cells by UPEC and subsequent development of intracellular bacterial communities (IBCs) is one reason of recurrent UTI. Intracellular UPEC are protected from the host immune system and are more resistant to antimicrobial agents. Reservoir formation and subsequent reactivation and exit of UPEC from infected cells contribute to recurrent UTI.

Goals Our aim is to identify (1) if there are any stimuli which trigger bacterial exit from the cells and (2) which bacterial factors are involved in the intracellular life cycle of UPEC especially during the exit from infected cells.

Materials & Methods We use an *in vitro* bladder epithelial model employing RT-112 bladder epithelial cells. After infection of RT-112 cells with UPEC, the invasion, intracellular survival and the escape of the bacteria from the cells were quantified. The formation of IBCs and the localization of bacteria inside the cells was monitored by fluorescence microscopy.

Results We studied the influence of different glucose concentrations on the development of IBCs. We infected bladder epithelial cells with two different UPEC model isolates. Our results show that these UPEC strains respond differently to varying glucose concentrations in *in vitro* infection studies. Different glucose concentrations do not only impact the intracellular replication, but also the exit from the host cells by UPEC differentially. Treatment of the cells with either inhibitors of glycolysis or inhibitors of glucose transporters complements the effects seen with different glucose concentrations.

Discussion Our results show that the intracellular life cycle of UPEC is affected by the availability of the carbon source glucose. Interestingly, the two model UPEC strains also differ in growth behavior with glucose as sole carbon source, but show similar multiplication rates in more complex growth media. The mechanism and consequences how glucose affects the UPEC intracellular life cycle and exit remains to be elucidated.

P-MP-039

Inhibition of *Legionella pneumophila* virulence factor Mip with FK506-like inhibitors

*F. Leitner[1], M. S. Karagöz[2], R. Deutscher[3], F. Hausch[3], M. Steinert[1]
[1]*Technical University of Braunschweig, Institute for Microbiology, Brunswick, Germany*
[2]*National Institutes of Health, National Heart, Lung and Blood Institute, Bethesda, MD, United States*
[3]*Technical University of Darmstadt, Department of Chemistry, Darmstadt, Germany*

Legionella pneumophila, an opportunistic bacterial pathogen, is responsible for the mild-symptomatic Pontiac fever as well as the life-threatening Legionnaires' disease, which has a lethality rate of 10 %. The macrophage infectivity potentiator (Mip) was the first genetically identified virulence factor of *L. pneumophila.* This peptidyl-prolyl-*cis/trans*-isomerase is involved in the early stages of infection, tissue migration and enhancement of flagellation. Due to its important role for bacterial pathogenesis, inhibition of Mip could be a promising approach to improve the treatment of Legionellosis and reduce severe pneumonia. Six novel inhibitors, based on the immunosuppressive PPIase-binding protein FK506, were first tested in minimal inhibitory concentration and cytotoxicity assays and were later assessed in cell line infections with THP-1 and A549. While the macrolide FK506 evokes cytotoxic effects at concentrations of 50 µM, those novel inhibitors did not show cytotoxicity for lung epithelial cells in a resazurin-based assay. Five out of six compounds were functional inhibitors of bacterial growth and intracellular replication at a concentration of at least 12.5 µM. The best two candidates showed particularly high efficacy as they reduced the bacterial cell count during infection more than seven fold, similar to that of a *L. pneumophila* Δ*mip* deletion strain. Interestingly, in previous studies derivatives of FK506 already showed additional off-target effects, which cannot be explained by Mip binding and may also play a role in this study. Thus, the novel non-immunosuppressive FK506 variants seem to be great candidates for a therapeutic application to reduce the bacterial proliferation during *L. pneumophila* infection, but also for future examination of not yet known off-target binding partners in *L. pneumophila* or the human host.

P-MP-040

Differences in mode of action and target recognition of lipid II binding antibiotics

*M. Arts[1], I. Kotsogianni[2], P. Hendricks[3], Y. Thoma[4], A. M. Krüger[5], I. Helmle[6], A. Mueller[1], G. Hagelueken[3], H. Groß[6], U. Kubitscheck[5], H. Brötz-Oesterhelt[4], H. Hashizume[7], M. Geyer[3], N. I. Martin[2], T. Schneider[1]
[1]*University Hospital Bonn, University of Bonn, Institute for Pharmaceutical Biology, Bonn, Germany*
[2]*Leiden University, Biological Chemistry Group, Institute of Biology Leiden, Leiden, Netherlands*
[3]*University of Bonn, Institute of Structural Biology, Bonn, Germany*
[4]*University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Dept. of Microbial Bioactive Compounds, Tübingen, Germany*
[5]*University of Bonn, Clausius Institute of Physical and Theoretical Chemistry, Bonn, Germany*
[6]*University of Tübingen, Department of Pharmaceutical Biology, Pharmaceutical Institute, Tübingen, Germany*
[7]*Institute of Microbial Chemistry, Department of Microbiology, Tokyo, Japan*

Infections with gram-positive pathogens exert immense pressure on health care systems worldwide. Especially (multi-)drug resistant bacteria are the cause for numerous hospitalizations and deaths. As resistances against approved antibiotics are spreading rapidly, the discovery of new antimicrobial compounds as well as research on potential cellular target structures are urgently necessary.

We compare the modes of action of two structurally similar antibiotic compounds. They were both found to target a broad spectrum of gram-positive bacteria. However, besides their structural similarities they significantly differ in antimicrobial potency. To determine differences in the effects on bacterial cells and on compound:target interaction, we applied a combination of *in vitro* and *in vivo* systems, as well

as fluorescence microscopy. We obtained proof that both compounds target cell wall biosynthesis and bind the ultimate peptidoglycan precursor lipid II. However, we determined substantial differences in the interaction with additional target molecules. Combined with structural analysis describing the architecture of the compound:lipid complex by crystallization, we resolved the characteristics of target recognition.

P-MP-041

Elucidation and characterization of Yersinia enterocolitica YopP phosphorylation

*B. Berinson[1], N. Mohr[1], M. Aepfelbacher[1], K. Ruckdeschel[1]
[1]*Institute for Medical Microbiology, Virology and Hygiene, Hamburg, Germany*

Introduction

The major virulence system of pathogenic *Yersinia* spp. is a type III secretion system that mediates the translocation of effector proteins (Yersinia outer proteins; Yops) into infected cells. These Yops interact with host proteins and exhibit manifold functions in modulating host cell immune responses. YopP is an acetyltransferase, which, via acetylation of NF-kB and MAPK pathway-related kinases, disrupts central innate immunity pathways within the host cell. Phosphorylations are common post-translational modifications, which may alter the function and activity of the modified protein. Our results indicate that YopP is phosphorylated by the host cell.

Materials & Methods

A fusion construct between a peptide tag (ALFA-Tag) and YopP was created and introduced into a knockout Yersinia lineage. J774 macrophages were infected and cell lysates were analyzed by immunoblotting. ALFA-tagged YopP was immunoprecipitated and analyzed via Mass spectrometry (MS) to identify YopP phosphorylation sites. Point mutations of the identified sites were inserted in YopP in order to study the relevance of YopP phosphorylation in host cell infection.

Results

Yersinia infection of J774 macrophages followed by immunoblotting of cell lysis revealed the appearance of additional YopP bands with slower electrophoretic mobility. These additional YopP bands disappeared by phosphatase treatment, but were stabilized by the phosphatase inhibitor Calyculin A, indicating being a result of a phosphorylation. After SDS-PAGE, a band corresponding to phosphorylated YopP was analyzed via MS, which revealed Serin-6 and -10 of YopP as phosphorylated by the host cell, starting as early as 15 mins post infection. Then, phospho-mimicking (S6/10D) and phospho-nil (S6/10A) YopP mutants were created, which resulted in expectedly altered electrophoretic mobility patterns in westernblot experiments.

Summary

Our results show that *Y. enterocolitica* YopP is phosphorylated at S6 and S10 by yet unknown host cell kinases. Studies are ongoing in order to assess the physiological relevance of these phosphorylation events on YopP functions and their consequences for the host cell immune response.

P-MP-042

UPEC establishes intracellular bacterial communities and persister-like cells in prostate organoid cells

M. Guedes[1], A. Joshi[1], *C. Aguilar[1]
[1]*IMIB, Würzburg, Germany*

Introduction:

Invasion into the host cell is one of the main pathogenic mechanisms of uropathogenic Escherichia coli (UPEC), enabling them to evade clearance by immune cells and antibiotic treatment. UPEC has developed two ways of surviving within the urothelium: I) by rapidly replicating inside the cell, forming the so-called intracellular bacterial communities—highly tolerant to antibiotics, and II) by remaining quiescent within the cell until conditions become more suitable for intracellular growth. These two mechanisms are essential for UPEC pathogenesis and are directly linked to its ability to induce recurrent infections in the bladder. During a urinary tract infection, UPEC can also infect the prostate; however, whether the bacterium invades these cells and replicates within is not clear so far.

Goals:

To unravel the pathogenesis mechanisms employed by UPEC to invade prostate cells. Through this exploration, we aim to identify previously undiscovered host factors that play a pivotal role in regulating these infections.

Material & Methods:

To study UPEC pathogenesis in a physiological way, we have developed an organoid-based model of the prostate epithelium. We use this model in conjunction with in vitro infection assays (e.g., gentamicin protection assays, immunofluorescence, ligand-capture assays).

Results:

Here, we demonstrate that UPEC can invade prostate organoid cells, and this invasion is dependent on the bacterial adhesin FimH. FimH interacts with different membrane structures than those observed in bladder cells. Additionally, we observe intracellular bacterial communities in these primary prostate cells, as well as solitary bacteria that can survive intracellularly for at least 7 days, similar to what is observed in the bladder epithelium.

Summary:

Overall, we have developed a model that accurately recapitulates the cell structure and composition of the prostate epithelium. Using this model, we are elucidating the tissue-specific mechanisms by which UPEC causes infection of the prostate, holding exciting potential to deepen our understanding of urogenital diseases.

P-MP-043

Peptidoglycan hydrolases of the MepM family have a crucial function in multidrug-resistance and virulence traits of P. aeruginosa

*M. Schütz[1,2], F. Renschler[1,2], K. Vöhringer[1,2], S. Aidoo[1], J. Kemper[1,2], F. Paland[1], A. Schäfer[1], L. Brenner[1], E. Bohn[1,2,3]

[1]*University Hospital Tübingen, Institut für Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*
[2]*German Center for Infection Research, Tübingen, Germany*
[3]*Excellence Cluster "Controlling Microbes to fight infections", (CMFI), Tübingen, Germany*

Multidrug-resistant (MDR) *P. aeruginosa* (*Pa*) are a pressing threat in healthcare, so novel approaches to fight them are urgently needed. The cell envelope of Gram-negatives consists of an outer and inner membrane. The interjacent periplasm contains the peptidoglycan (PGN) sacculus. PGN is crucial for cell stability and shape, and is made of short overlapping glycan chains. Glycan consists of disaccharides connected to muropeptides, crosslinked to each other and thereby forming a mesh. These crosslinks can be cleaved by PGN hydrolases, to allow for cell growth and division. In *E. coli* (*Ec*) it was shown that three such hydrolases (*Ec*MepM (YebA), *Ec*MepH and *Ec*MepS) are essential for the assembly of PGN into the cell wall[1]. *Pa* encodes for three putative MepMs, namely *Pa*MepM1, *Pa*MepM2, *Pa*MepM3, which are phylogenetically highly related to *Ec*MepM. Using Tn-directed insertion sequencing of an MDR *Pa* bloodstream isolate, we screened for genes important for β-lactam resistance[2]. Among others, 3 genes encoding PGN hydrolases, namely *mepM1*, *mepM2*, and *mepH2*, were identified, and we could corroborate their contribution to β-lactam resistance. We also tested which virulence-associated phenotypes are influenced by MepM proteins in this MDR clinical *Pa* isolate. Thus, we investigated the impact of *mepM1*, *mepM2*, *mepM3* deletions on biofilm formation, salt sensitivity, motility and cell shape, and found especially for the triple deletion mutant *Pa* Δ*mepM123* (beside the break of resistance to β-lactam antibiotics) a change in morphology (cells were more curved in the mutant compared to wildtype), and a significant reduction in biofilm formation, salt sensitivity and motility. Interestingly, reduced motility was not due to lowered FliC abundance or lack of flagella, but was at least partially caused by a higher abundance of the efflux pump MexEFOprN, indicating a more global function of MepM proteins for cell physiology. In summary, PGN endopeptidases of the MepM family are promising targets to develop novel drugs as antivirulence agents and to sensitize MDR pathogens to β-lactam treatment.

1Park et al. 2020; 10.3389/fmicb.2020.565767; 2Sonnabend et al. 2020; 10.1128/AAC.01771-19

Membranes and Transport

P-MT-001

Exploring novel protein-protein interactions and functions of selected *Helicobacter pylori* Cag Type 4 Secretion System (CagT4SS) outer proteins

F. Metz[1], J. Beilmann[1], M. Camboni[1], B. Sedlmaier[1], S. Bats[1], W. Fischer[1], *C. Josenhans[1]
[1]*Ludwig-Maximilians University, Max von Pettenkofer Institute, Chair of Hygiene and Medical Microbiology, München, Germany*

Background and Questions. The *Helicobacter pylori cag* pathogenicity island (*cag*PAI) is an important virulence factor of the chronic gastric pathogen *H. pylori* and encodes a complex type IV secretion system (CagT4SS). Our earlier work has helped us to generate hypotheses concerning exposed, variable outer proteins that can interact with host factors [1,2,3]. Recently, structural information of the CagT4SS has been substantially improved by cryo-EM [4]. However, important details, in particular on the outer membrane complex (OMC) and on protein interactions within the OMC and the surface complex of the CagT4SS, consisting of T4SS outer proteins, are missing. This also concerns functional details on putative active and inactive conformations of the export complex, and on the transport functions in general.

Methods and Results. Using bacterial two hybrid system (BACTH) and biophysical characterization methods, we have enhanced our knowledge on protein-protein interactions in the CagT4SS outer proteins. This includes interactions of outer membrane proteins, of the VirB2 homolog CagC [1] and of the outer protein CagN [5] of yet unknown function. We have determined and quantitated homo-dimerization of CagC and CagN, elucidated novel interactions of all tested proteins by BACTH, and confirmed them using protein purification and interaction analysis, for instance by Octet biolayer interferometry analysis.

Conclusions. Novel interactions of *H. pylori* outer membrane proteins and CagT4SS outer proteins have been found and further characterized using biochemical and biophysical methods under different conditions. This will help to refine structural and functional details of the CagT4SS transport and translocation complex and machinery, also in contact with the human cell.

1. Andrzejewska, J., J Bacteriol 2006; doi: 10.1128/JB.00060-06.
2. Boenig, T., et al., Sci Rep 2016; doi: 10.1038/srep38101.
3. Pham, K.T., et al., Plos One 2012; doi: 10.1371/journal.pone.0035341.
4. Hu, B., et al., 2019; doi: 10.1128/JB.00060-06.
5. Bats, S.H., et al., Int J Med Microbiol 2018; doi: 10.1016/j.ijmm.2018.02.005.

P-MT-002

Molecular architecture of Anaerobic Ammonium Oxidizer

*D. Dallemer[1], L. Dietrich[2], A. Dietl[3], M. S. M. Jetten[4], B. Kartal[5], L. van Niftrik[4], T. R. M. Barends[3], K. Parey[1]
[1]*Universitat Osnabrueck, Department of biologie, Osnabrück, Germany*
[2]*Max Planck Institute of Biophysics, Frankfurt a. M., Germany*
[3]*Max Plank Institute for Medical Research, Heidelberg, Germany*
[4]*Radboud University, Nijmegen, Netherlands*
[5]*Max Planck Institute of Marine Microbiology, Bremen, Germany*

The discovery of anammox bacteria in the 1990s changed our understanding of the global nitrogen cycle (1). These extraordinary microorganisms with unusual morphology derive their energy from the oxidation of ammonium coupled with nitrite reduction, which relies on highly toxic intermediates such as hydrazine and nitric oxide (2). Approximately 50% of the dinitrogen gas released is produced by anammox bacteria. In biotechnology, the Anammox process is being used as a sustainable alternative to current wastewater treatment systems for the removal of nitrogen compounds.We have made significant contributions to elucidating the nature of the catabolic pathway and characterizing the key soluble enzymes. Central to the harvesting of energy from hydrazine is the hydrazine dehydrogenase complex, which converts hydrazine to dinitrogen gas, releasing four extremely low-potential electrons (-750 mV) (3). In addition, anammox bacteria obtain additional reducing equivalents from the oxidation of

nitrite to nitrate, catalyzed by a nitrite oxidoreductase (NXR) (4). However, little is known about the membrane complexes at the heart of this bioenergetic process. Nature of the membrane complexes will be elucidated using cryo-electron tomography, sub-tomogram averaging and segmentation. This technique will provide insights into the native landscape at molecular resolution and the bioenergetics of this largely unexplored organism. In particular, protein complexes in the respiratory chain can be differentiated and their organization studied in situ.

(1) Kuypers, M. M. M., et al. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* 16, 263-276 (2018)
(2) Kartal, B., et al. (2013) How to make a living from anaerobic ammonium oxidation. *FEMS Microbiol. Rev.*37, 428-461
(3) Akram, M., et al. (2019) A 192-heme electron transfer network in the hydrazine dehydrogenase complex. Sci Adv 5, eaav4310
(4) Chicano, T. M., et al. (2021) Structural and functional characterization of the intracellular filament-forming nitrite oxidoreductase multiprotein complex. *Nat. Microbiol.* 6, 1129-1139

P-MT-003

Functionality of the Na$^+$-translocating NADH: quinone oxidoreductase and quinol:fumarate reductase from Prevotella bryantii inferred from homology modeling

*S. Herdan[1], J. L. Hau[1], L. Schleicher[1], J. Simon[2], J. Seifert[3], G. Fritz[1], J. Fritz-Steuber[1]
[1]*University of Hohenheim, Institute of Biology, Cellular Microbiology, Stuttgart, Germany*
[2]*Technical University of Darmstadt, Department of Biology, Microbial Energy Conservation and Biotechnology, Darmstadt, Germany*
[3]*University of Hohenheim, Institute of Animal Science, Stuttgart, Germany*

Members of the family *Prevotellaceae* are Gram-negative, obligate anaerobic bacteria found in animal and human microbiota. In *Prevotella bryantii*, the Na$^+$-translocating NADH:quinone oxidoreductase (NQR) and quinol:fumarate reductase (QFR) interact using menaquinone as electron carrier, catalyzing NADH:fumarate oxidoreduction. *P. bryantii* NQR establishes a sodium-motive force, whereas *P. bryantii* QFR does not contribute to membrane energization. To elucidate the possible mode of function, we present 3D structural models of NQR and QFR from *P. bryantii* to predict cofactor-binding sites, electron transfer routes and interaction with substrates. Molecular docking reveals the proposed mode of menaquinone binding to the quinone site of subunit NqrB of P. bryantii NQR. A comparison of the 3D model of *P. bryantii* QFR with experimentally determined structures suggests alternative pathways for transmembrane proton transport in this type of QFR. Our findings are relevant for NADH-dependent succinate formation in anaerobic bacteria which operate both NQR and QFR.

P-MT-004

Bidirectional formic acid translocation by FocA and its contribution to pH homeostasis during fermentation

*C. Erdmann[1], M. Kammel[1], G. Sawers[1]
[1]*Martin-Luther University Halle-Wittenberg, Institute of Biology/Microbiology, Halle (Saale), Germany*

Introduction: Formate is a central intermediate in enterobacterial mixed-acid fermentation of glucose. During exponential-phase growth, formic acid passes from the cytoplasm to the periplasm via the pentameric channel, FocA. Upon entry into the late exponential-phase of growth, or upon a decrease in external pH below 6.5, FocA re-imports formate or formic acid into the cytoplasm, where it is disproportionated into CO2 and H2 by the membrane-associated formate hydrogenlyase (FHL) complex. Uptake of formic acid by FocA is dependent on an active FHL complex, suggesting coupling between both systems. Each protomer of the FocA pentamer has a narrow hydrophobic pore through which neutral formic acid can pass. Conserved histidine (H209) and threonine (T91) residues are required to control uptake of formate, implying that the pore of FocA does not function simply as a "channel". Objectives: FocA is suggested to have two functions: 1, as a bidirectional channel for formic acid; 2, as a formate importer. It is proposed that FocA channel/import functions are coupled to the FHL complex to maintain pH homeostasis and optimize fermentative growth. We tested this hypothesis in the current study.

Methods: We use a formate-responsive reporter system, FocA amino acid-exchange variants, together with *fhl* mutants to assess various physiological parameters, including H2 production and formate translocation to test the dependence of FocA on the FHL complex.

Results: FocA variants, respectively, either rapidly translocate formate out of the cell (H209N variant) and produce low levels of H2, or accumulate formate intracellularly (T91A variant). Mutants that lack an FHL complex fail to produce H2 and also show poorer early exponential-phase growth than the parental strain.

Conclusion: Together, our findings indicate that FocA functions together with the FHL complex to maintain relatively constant intracellular formate levels, to improve growth by helping maintain pH homeostasis, and possibly also to contribute to energy conservation.

P-MT-005

Investigating the protein machinery involved in vesicle formation in Pseudomonas aeruginosa

*S. Holland[1,2,3], A. Arce-Rodriguez[4,5], D. Jahn[3,4], J. M. Borrero-de Acuña[4,5], M. Neumann-Schaal[2,3]
[1]*Technical University of Braunschweig, Institute of Biochemistry, Biotechnology and Bioinformatics, Brunswick, Germany*
[2]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Department Services and Research Group Metabolomics, Brunswick, Germany*
[3]*Braunschweig Integrated Centre of Systems Biology (BRICS), Brunswick, Germany*
[4]*Technical University of Braunschweig, Institute for Microbiology, Brunswick, Germany*
[5]*Universidad de Sevilla, Departamento de Microbiologia, Sevilla, Spain*

Pseudomonas aeruginosa is an opportunistic human pathogen, capable of colonizing a wide range of tissue causing acute and chronic infections. The microorganism significantly contributes to morbidity and mortality in respiratory infections among immunocompromised patients with cystic fibrosis. Besides, the gram-negative pathogen is a common cause of nosocomial infections, attributed to the secretion of an extensive array of virulence factors contributing to a successful infection strategy. Bacteria have evolved mechanisms for the secretion of such virulence factors, typically enabling bacterial colonization via direct contact with the host cells. Moreover, *P. aeruginosa* produces extracellular membrane vesicles (MVs), delivering

a wide variety of molecules into the eukaryotic host cell, thereby contributing to its pathogenicity, regardless of being in direct contact. Hence, these membrane-encapsulated structures are considered to represent a novel bacterial secretion pathway (T0SS), not only being involved in infection but also crucial in inter- and intra-species cell communication. Recent studies revealed the existence of several routes underlying MV biogenesis, resulting in MVs with different biochemical cargos, which in turn affect their biological function. Despite their relevance in virulence, the mechanisms governing MV biogenesis and the cellular machinery restructuring the bacterial membrane remain poorly understood. Herein, we report the identification of two protein families as key players in restructuring events of both the inner and outer membrane. Deletion and overexpression of the genes encoding these proteins strongly impacted the vesiculation in *P. aeruginosa*. MV characterization of the constructed strains was conducted by multiple methods encompassing Nanoparticle Tracking Analysis (NTA), fluorescent membrane staining and electron microscopy analysis. Understanding the processes of vesicle formation and elucidating the protein machinery responsible for membrane remodeling offers valuable insights for developing targeted therapeutics against infections caused by *P. aeruginosa*.

P-MT-006

Molecular basis of siderophore secretion by Pseudomonas putida KT2440

*N. Stein[1], F. Burr[1], M. Eder[1], *H. Jung[1]
[1]*Ludwig-Maximilians University Munich, Biocenter, Microbiology, Martinsried, Germany*

Siderophore-mediated iron uptake is essential for the survival of many bacteria in soil and water and during infection. Pyoverdine (PVD) is a siderophore produced by fluorescent pseudomonads [1]. Using the soil bacterium *Pseudomonas putida* KT2440 as a model system, we previously identified the tripartite efflux pumps PvdRT-OpmQ (ABC type) and MdtABC-OpmB (RND type) as being involved in PVD secretion [2]. Here, we aim to (1) identify additional efflux systems contributing to PVD secretion and (2) provide biochemical evidence for the interaction of PVD with PvdRT-OpmQ.

In a screen, genes encoding components of other tripartite efflux systems were individually deleted in a *P. putida* KT2440 strain lacking functional PvdRT-OpmQ and MdtABC-OpmB systems. The consequences of the deletions on PVD secretion were analyzed. Furthermore, the inner membrane component PvdT and the periplasmic adaptor protein PvdR were purified and characterized with respect to ATPase activity and interactions using DRaCALA and surface plasmon resonance (SPR).

The screen revealed that the RND efflux system ParXY is critical for growth under iron limitation when PvdRT-OpmQ and MdtABC-OpmB are inactivated. Additional inactivation of ParXY increases the amount of PVD accumulated in the periplasm and further inhibits PVD secretion. In contrast, individual inactivation of ParXY has no significant effect [3]. DRaCALA and SPR revealed that PVD directly interacts with PvdRT. Furthermore, the ATPase activity of solubilized PvdT was stimulated by interaction with PvdR but not by addition of PVD [4].

We conclude that PvdRT-OpmQ is the major system for PVD secretion in *P. putida* KT2440, whereas MdtABC-OpmB and ParXY contribute only conditionally to this process. Given the importance of iron uptake for cellular metabolism, these overlapping activities ensure the survival and growth of bacteria under the common conditions of iron deficiency.

[1] Ringel, MT & Brüser, T (2018) *Microb Cell* **5**, 424-437.
[2] Henriquez, T *et al.* (2019) *Environ Microbiol Rep* **11**, 98-106.
[3] Stein, NV *et al.* (2023) *Microbiol. Spectr.* **11**, e02300-02323
[4] Stein, NV *et al.* (2023) *FEBS Lett.* **597**, 1403-1414.

P-MT-007

A formate transporter in the acetogenic bacterium Thermoanaerobacter kivui

*Y. Burger[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: Formate is an important intermediate in the metabolism of different anaerobic bacteria and can be used as feedstock to produce valuable chemicals. The thermophilic acetogenic bacterium *Thermoanaerobacter kivui* grows by producing acetate from formate, but formate can also exclusively be oxidized to H_2 + CO_2 [1] and *vice versa*, by the action of the hydrogen-dependent CO_2 reductase (HDCR) [2]. Thus, *T. kivui* is an ideal production platform in a formate-based bioeconomy but also in biological hydrogen production and storage [3]. One missing link in formate metabolism of *T. kivui* was the nature of formate uptake/export.

Goals: To identify and characterize a possible formate transporter in *T. kivui.*

Materials & Methods: Bioinformatic analysis, generation of deletion mutants, growth experiments and cell suspension experiments.

Results: Inspection of the genome sequence revealed a gene, *fdhC*, adjacent to the *hdcr* gene cluster whose product is similar to the *Escherichia coli* formate transporter FocA. The gene was deleted by allelic replacement. The deletion mutant had no phenotype at pH 7.0 and 150 mM formate, conditions used to grow the wildtype. However, at high pH and low formate concentrations, formate consumption by the mutant was drastically reduced.

Summary: We identified the formate transporter of *T. kivui* and its role in the physiology of *T. kivui* is discussed.

[1] Burger Y., Schwarz F. M. and Müller V. (2022) Biotechnol Biofuels 15:48
[2] Schwarz F. M. and Müller V. (2020) Biotechnol Biofuels 13:32
[3] Müller V. (2019) Trends Biotechnol 37:1344-1354

P-MT-008

Protein aggregates delay persister cell recovery

*F. Leinberger[1], B. Berghoff[1]
[1]*Justus Liebig University Giessen, Institute of Microbiology and Molecularbiology, Giessen, Germany*

Introduction:

In *Escherichia coli*, the type I toxin-antitoxin system *tisB/istR-1* is part of the SOS response. Toxin gene *tisB* is strongly induced as soon as DNA damage occurs. TisB is a small hydrophobic protein located in the inner membrane, causing growth stasis due to depolarization and ATP depletion. TisB-induced dormancy favors formation of transiently multidrug-tolerant cells, so-called persisters.

Objectives:

The aim of this study was to investigate the role of protein aggregation in the recovery of persister cells in *E. coli* wild type MG1655.

Material and Methods:

We used the ScanLag method to monitor survival and recovery of TisB-dependent persister cells after ciprofloxacin treatment. In addition, protein aggregates were induced via heat stress (46°C). Fluorescence microscopy was performed using an *ibpA-msfGFP* reporter strain to verify protein aggregation.

Results:

We are able to demonstrate that ciprofloxacin triggers protein aggregation and that this process depends on TisB. Furthermore, we observe that persister cells of a *tisB* deletion strain resume growth faster than wild-type persisters. If heat stress is applied to induce protein aggregation in the *tisB* deletion strain, growth resumption is delayed and more similar to the wild type.

Conclusions:

Our experiments suggest that protein aggregates delay the recovery from the TisB-induced persister state. We conclude that formation of protein aggregates provides a means to stabilize persistence and probably dormancy in general.

P-MT-009
Interaction of cyclic di-GMP with the motor ATPase PilF of Thermus thermophilus: role in type IV pilus function and natural transformation

*M. Essig[1], B. Averhoff[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: *Thermus thermophilus* is a model organism to study the structure and function of natural transformation systems (1). Previously we identified a hexameric motor ATPase PilF powering the natural transformation machinery and type IV pili assembly in *T. thermophilus.* Structurally, PilF is particularly remarkable because its N-terminus harbours three successive GSPII domains. Our recent studies revealed that two of these domains bind the second messenger c-di-GMP albeit with significantly different affinities (2,3).

Goals: Next we aimed to elucidate the functional importance of c-di-GMP binding to PilF *in vivo* and *in vitro.*

Materials & Methods: The functional importance of c-di-GMP binding to PilF was analysed by site directed mutagenesis, physiological characterization of mutants and biochemical characterization of PilF variants.

Results: Site directed mutagenesis of PilF followed by NMR spectroscopy analysis in collaboration with Jens Wöhnert (BMRZ, Goethe University Frankfurt) unravelled four glutamine residues in PilF essential for c-di-GMP binding. A glutamine quadruple *pilF* mutant was significantly reduced in adhesion to solid surfaces and completely defect in movement on solid surfaces and natural transformation. Biochemical analyses of PilF variants provided insights into the role of c-di-GMP binding in PilF complex formation and ATPase activity.

Summary: Here we report on the role of c-di-GMP binding to the motor ATPase PilF in adhesion, motility on solid surfaces and natural transformation of *T. thermophilus.*

[1] Averhoff, B. (2009) FEMS Microbiol Rev 33:611-626

[2] Keller, H., Kruse, K., Averhoff, B., Duchardt-Ferner, E., Wöhnert, J. (2019) Biomol NMR Assign 13:361-366

[3] Neißner, K., Keller, H., Duchardt-Ferner, E., Hacker, C., Kruse, K., Averhoff, B., Wöhnert, J. (2019) Biomol NMR Assign 13:383–390

P-MT-010
Non-essential TraO? - a T4SS surface adhesin in conjugative plasmid transfer among Gram-positive bacteria

*C. Michaelis[1], R. Tahmaseb[1], K. Kuhlmann[2], T. Berger[2], W. Keller[2], E. Grohmann[1]
[1]*Berliner Hochschule für Technik, Berlin, Germany*
[2]*University of Graz, Graz, Austria*

The rise of antibiotic resistance poses a growing global health challenge, complicating the treatment of bacterial infections. This issue is intensified by horizontal gene transfer, specifically through conjugative plasmids, carrying not only antibiotic resistance genes but also the necessary machinery for their transfer. A suitable model for studying this mechanism is the conjugative plasmid pIP501, encoding a type IV secretion system (T4SS) which consists of 15 transfer genes (*traA* - *traO*) and is arranged in a single operon. pIP501 serves as a valuable model for investigating Gram-positive bacterial conjugative transfer.
Our study employed markerless gene knockouts in pIP501, utilizing *in vivo* biparental mating assays with *Enterococcus faecalis* and other Firmicutes strains to understand the functions of transfer proteins. Results highlighted the essential nature of 13 out of 15 transfer proteins. Interestingly, TraO, a putative surface adhesin, was found non-essential, demonstrating only a transfer rate reduction. Nevertheless, TraO is assumed to play a crucial role in facilitating contact between donor and recipient cells prior to conjugative transfer.
To broaden our understanding of TraO across bacterial species, we generated new pIP501 and *traO* deletion strains. Diverse donor-recipient combinations enabled a comparative analysis of transfer rates among strains, encompassing such as *Enterococcus* and *Streptococcus*. Future investigations should focus on the role of TraO domains, necessitating complementation experiments with truncated TraO variants in inter- and intraspecies biparental mating assays.

Furthermore, understanding TraO's structure can provide valuable insights into its localization, fold and domain functions.

P-MT-011
Same, yet different: characterization of bacterial S-type and R-type phospholipid N-methyltransferases

*I. Shevyreva[1], L. S. Fritsch[1], A. Effing[1], M. Aktas[1]
[1]*Ruhr-University Bochum, Microbial Biology, Bochum, Germany*

Phosphatidylcholine (PC) was long thought to be a solely eukaryotic phospholipid. Its presence was only recently discovered in several prokaryotic organisms, where the amount of PC ranges from a few percent of total membrane lipids in *Xanthomonas campestris* to about 70% in *Acetobacter aceti*. Interestingly, many of the bacterial species with PC in their membrane have been demonstrated to engage in either symbiotic or pathogenic microbe-host interactions.

One of the most common PC biosynthesis pathways in bacteria is a threefold *S*-adenosylmethionine (SAM)-dependent methylation of phosphatidylethanolamine (PE) to PC catalyzed by phospholipid *N*-methyltransferases (Pmts). Pmts are classified based on their sequence in two groups: *Sinorhizobium* (*S*) and *Rhodobacter* (*R*). Regardless of their type, Pmt enzymes display different substrate preferences. For instance, the *S*-type *Agrobacterium tumefaciens* PmtA catalyzes all three methylations of PE, while another *S*-type *Thermobifida fusca* Pmt only executes the first two methylation steps. So far, *S*-type PmtA from *A. tumefaciens* is the best-characterized Pmt with extensively studied membrane binding sites, SAM-binding motif and order of reaction steps. However, the molecular details of substrate selectivity and the nature of the active site and the catalytic mechanism remain poorly understood. Furthermore, very little is known about *R*-type Pmts in general.

The main goal of this project is a comparative analysis of *S*-type and *R*-type Pmts. To identify conserved and unique enzyme regions responsible for the substrate selectivity and enzymatic activity, we employ a mix of biochemical and computational research, as well as site-directed mutagenesis experiments.

National Reference Centers and Consiliary Laboratories

P-NRC-001
Laboratory surveillance of invasive *Haemophilus influenzae* infections in Germany 2023

*T. T. Lam[1], K. Mohort[1], M. Krone[1], H. Claus[1]
[1]*University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*

Introduction

The National Reference Laboratory for Meningococci and *H. influenzae* (NRZMHi) carries out laboratory surveillance of invasive *H. influenzae* (Hi) in Germany. The year 2023 was the first post COVID-19 pandemic year, which led to an epidemiology similar to pre-pandemic years.

Materials and Methods

The aim was to present epidemiological data of invasive *H. influenzae* infections from 2023. Isolates from blood and cerebrospinal fluid (CSF) are considered invasive and must be reported in Germany, but submission to the NRZMHi is voluntary. The NRZMHi performed species confirmation, serotyping and susceptibility testing for ampicillin and cefotaxime on all submitted isolates.

Results

In 2023, invasive Hi were confirmed in 1317 cases; 1274 isolates were from blood, 38 from CSF only. Laboratory surveillance coverage was estimated 80% by comparing NRZMHi submissions with notified cases at the RKI. Most cases were caused by nontypeable *H. influenzae* (NTHi, 1150 isolates 87.3%), followed by Hif as the most common capsular serotype (90 cases; 6.8%). Hia, Hib, and Hie were at a comparable level with 25 cases (1.9%) for Hia, 24 cases (1.8%) for Hib, and 28 cases for Hie (2.1%). Thus, Hia was found more frequently than in previous years.Hic and Hid were not found in 2023.Among the analyzed cases, patients aged > 40 years were most affected (1136 cases; 88.6% of all cases). Ampicillin susceptibility testing revealed that 325 (20.1%) isolates were ampicillin-resistant (MIC > 1 µg/mL) and 212 (16.3%) showed β-lactamase production.

Discussion

The epidemiology of invasive Hi infections in Germany 2023 was comparable to pre-pandemic years, in particular 2019, when case numbers were 955. As previously, NTHi in elderly patients dominated the cases. The increasing trend of Hia cases and augmenting rates of ampicillin resistance need to be further monitored.

P-NRC-002
Toxigenic Corynebacterium ulcerans infection in a cat owner

*A. Berger[1], A. Dangel[2], K. Bengs[1], V. Melnikov[1], M. Ranzinger[3], S. Kandlbinder[3], S. Pfeiffer[3], F. Wiedemann[4], E. Spindler[5], D. Haug[5], S. Böhm[6], A. Sing[1]
[1]*Bavarian Health and Food Safety Authority, National Consiliary Laboratory for Diphtheria & WHO Collaborating Centre for Diphtheria, Public Health Microbiology, Oberschleißheim, Germany*
[2]*Bavarian Health and Food Safety Authority, NGS Core Unit, Public Health Microbiology, Oberschleißheim, Germany*
[3]*Landratsamt Freyung-Grafenau, Sachgebiet 34 – Gesundheitswesen, Waldkirchen, Germany*
[4]*NVZ Labor Passau, Passau, Germany*
[5]*District Office Freyung-Grafenau, Veterinary and Consumer Health Protection, Department 33, Freyung, Germany*
[6]*Bavarian Health and Food Safety Authority, Task Force Infectiology;TFI2: Infektionsepidemiologie und Surveillance, Oberschleißheim, Germany*

Objectives

Diphtheria is still rarely observed in industrialized countries, but we observe an increase of zoonotic *Corynebacterium (C.) ulcerans* infections in humans. *C. ulcerans* may (similarly to *C. diphtheriae*) harbour lysogenic beta-corynephages bearing the *tox* gene encoding diphtheria toxin (DT). Toxigenic *C. ulcerans* may cause classical respiratory diphtheria or diphtheria-like syndromes as well as cutaneous diphtheria. Companion cats and dogs, other domestic and wild animals, serve as a reservoir and a source of zoonotic

C. ulcerans infection. We report on a patient with chronic skin ulcerations due to circulatory disorders, complicated by wound diphtheria. The patient´s asymptomatic three cats were examined in order to find the potential source of infection.

Materials & Methods

Strain identification was performed by biochemical differentiation and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Germany). Susceptibility testing was performed according to EUCAST guidelines. Toxigenicity was verified by real-time PCR, the optimized modified Elek-test and a recently published Lateral Flow Immunoassay providing a more rapid detection of diphtheria toxin producing strains. Whole generation sequencing (WGS) was carried out on Illumina systems and data analysis by core genome Multi Locus Sequencing (cgMLST).

Results

A toxigenic *C. ulcerans* strain was cultivated in the patient´s wound swab in a mixed culture together with *Enterobacter cloacae* and *Klebsiella oxytoca*. Pharyngeal carriage of a toxigenic *C. ulcerans* strain occured in one of the three investigated asymptomatic cats. Analysis of WGS, epidemiological and clinical data indicate strain transmission of the toxigenic *C. ulcerans* from the pet cat to its owner via direct contact.

Discussion

Since 15 years zoonotic *tox+ C. ulcerans* has outnumbered the "classical" pathogen *C. diphtheriae* related cases in Germany – with the exception of the ongoing European 2022 diphtheria outbreak among refugees.Our experience responding to a zoonotic incident caused by *C. ulcerans* has highlighted the need for more robust surveillance and understanding of this emerging pathogen in both animals and humans.

P-NRC-003

Genome-based surveillance of Salmonella enterica serovar Enteritidis at the National Reference Center (NRC) for Salmonella and other bacterial enteric pathogens

*S. Simon[1], M. Pietsch[1], E. Trost[1], A. Meinen[2], J. Fischer[3], M. C. Lamparter[3], A. Flieger[1]
[1]*Robert Koch Institute, Unit for Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*
[2]*Robert Koch Institute, Unit of Gastrointestinal Infections, Zoonoses and Tropical Infections, Berlin, Germany*
[3]*German Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany*

Introduction: *Salmonella* (*S.*) Enteritidis, the most prevalent clinical *Salmonella* serovar worldwide, is responsible for ~50% of human salmonellosis cases in Europe (~4,000 reported cases p.a. in Germany) and frequently causes large, often multinational food-borne outbreaks. Due to the inherent clonality of this serovar unambiguous case assignment and source identification was often impeded by the insufficient discriminatory power of former subtyping methods.

Objectives: Therefore, *S.* Enteritidis is one of the prioritized organisms for a comprehensive genome-based surveillance at the NRC, aiming to enhance the discrimination, thus resulting in a more accurate differentiation of potential outbreak events and definite identification of the causative (food) source.

Methods: Bacterial genomic DNA is prepared for Illumina short read sequencing. Raw Reads are analyzed with Ridom SeqSphere+. Core genome (cg)MLST (EnteroBase scheme) is used to determine the phylogenetic relationship and potential cluster affiliation. Genomes from clinical clusters are compared with strains of non-human origin, especially from the food production chain (provided by the NRL for *Salmonella* at BfR) on a regular basis.

Results: In the period 2018-2023 about 3,900 *S.* Enteritidis genomes have been analyzed at the NRC. Applying a max. pairwise distance of 3 AD, cgMLST revealed 186 genomic clusters (24 of them comprising >30 isolates and 80 <5 isolates). The biggest cluster contains 282 isolates. Out of those, matching food or animal isolates were identified for 29 clusters. Nevertheless, even cgMLST sometimes provides ambiguous results, highlighting the need for confirmatory epidemiological data.

Summary: WGS-based methods for depicting relationships within the *S.* Enteritidis population give a comprehensive overview about the circulating lineages and in general reliably point out developing clusters allowing more focused outbreak investigations and facilitating subsequent control measures and preventive activities. Here, we share our experiences from six years of WGS-based surveillance for *S.* Enteritidis, illustrating the benefits but also describing the challenges that evolved with this new method.

P-NRC-005

Application of NGS as method for typing and monitoring of extensively drug-resistant Salmonella enterica serovar Typhi isolates in Germany

*E. Trost[1], J. Enkelmann[2], S. Simon[1], M. Pietsch[1], A. Flieger[1]
[1]*Robert Koch-Institut, National Reference Centre for Salmonella and other enteric pathogens, Wernigerode, Germany*
[2]*Robert Koch-Institut, Focal Point for the Public Health Service, Crisis Management, Outbreak Investigations and Training Programmes, Wernigerode, Germany*

Introduction: Typhoid fever is a severe and potentially life-threatening bacteraemic disease caused by *Salmonella enterica* serovar Typhi (*S.* Typhi). In November 2016 an outbreak of extensively drug-resistant (XDR) *S.* Typhi was described originating in Pakistan. XDR *Salmonella* Typhi isolates are characterized by their resistance to first-line antimicrobials, fluoroquinolones, and third-generation cephalosporins. Since then, the emergence and spread of these isolates has been documented in different regions of the world, posinge significant challenges to public health, surveillance, antimicrobial resistance monitoring, and the development of alternative treatment strategies.

Objectives: Monitoring *S.* Typhi cases and isolate cluster occurring in Germany, especially to monitor the spread of resistant isolates.

Methods: Therefor, we sequenced all *S.* Typhi isolates submitted to the NRC from 2015 to 2023 and established cgMLST as routine typing method for cluster detection using

an *ad hoc* scheme based on the reference strain CT18 comprising 3.687 loci and the EnteroBase wgMLST scheme in SeqSphere+ . Furthermore, we used the NGS data to identify and compare isolates of lineage H58 and XDR, that are characterized by 44 SNPs and multiple antibiotic resistances.

Results: In total we sequenced 408 *S.* Typhi isolates and were able to identify the XDR specific SNPs in 18 XDR isolates. In all cases these isolates were associated with travel to Pakistan. Our analysis showed an increase in multidrug-resistant *S.* Typhi isolates in Germany, especially since the emergence of the first XDR isolates.

Summary: We present the sequence-based surveillance of *S.* Typhi isolated in Germany to identify potential NGS clusters and the emergence of multidrug-resistant pathogens that represent an increasing challenge in the treatment of *S.* Typhi infections.

P-NRC-006
A rare complication in humans of a rare disease in Germany: A case of bilateral *Brucella* tubo-ovarian abscesses

M. H. Pillukat[1], K. Wohlfart[2], A. Stich[3], H. P. Maidhof[4], E. Mantel[1], M. Seidel[1], D. Lang[1], *S. Zange[1]
[1]*Bundeswehr Institute of Microbiology, München, Germany*
[2]*Klinikum Fürth, Department of General Surgery, Fürth, Germany*
[3]*Medical Mission Hospital, Department of Tropical Medicine, Würzburg, Germany*
[4]*Klinikum Fürth, Institute of Laboratory Medicine, Department of Microbiology, Fürth, Germany*

Brucellosis, caused by the gram-negative bacterium *Brucella* spp. (*B. melitensis*, *B. abortus*, *B. suis* or *B. canis*) is a neglected zoonotic disease. The global number of human cases is underestimated due to the lack of comprehensive surveillance systems in endemic regions. In Germany, brucellosis is a notifiable disease and case counts are stable at low level with around 30 to 40 cases per year. Almost all cases are imported from endemic regions like in the Mediterranean basin and the Middle East.

The different *Brucella* spp. are pathogenic for a wide variety of animals and a typical infection localization are the reproductive organs (both in female and male animals). In humans, involvement of the female genito-urinary system is rare and only a few case reports are available from literature. Here, we report of a bilateral *Brucella* tubo-ovarian abscess with infection spread to the appendix in a 29-year-old Bosnian woman. Two years after the first diagnosis of brucellosis she suffered from abdominal pain and undulating high fever. Ultrasound and a MR scan showed extensive tubo-ovarian abscesses. Explorative laparoscopy was performed and confirmed bilateral tubo-ovarian abscesses with a dermoid cyst located at the right ovary. Additionally, a severe peritonitis and a secondary phlegmonous appendicitis were observed. An appendectomy and bilateral ovarian cystectomy were performed. *Brucella melitensis* was cultured from specimens collected during surgery and confirmed by PCR and whole genome sequencing.

Our report discusses the lessons learned from this rare complication of brucellosis in a non-endemic region and includes a detailed molecular epidemiological analysis of the strain.

P-NRC-007
Update on National Reference Centers and Consultant Laboratories in Germany

*J. Seifried[1], N. Litzba[1], O. Hamouda[2]
[1]*Robert Koch-Institute, Office for the Scientific Advisory Board on Public Health and Microbiology, Department for Infectious Disease Epidemiology, Berlin, Germany*
[2]*Robert Koch-Institute, Infectious Disease Epidemiology, Berlin, Germany*

The National Reference and Consultant Laboratories in Germany play a valuable role for the scientific progress in the field of medical microbiology. They cover the entire spectrum of infectious agents from prions, viruses and bacteria to parasites and contribute substantially to laboratory capacity in Germany. The continuous development of the reference laboratory structure is of key importance to ensure public health microbiology. Here, we aim to inform the scientific community of the structure of the German National Reference and Consultant Laboratories and give an update on their activities in the surveillance of pathogens in Germany.

P-NRC-008
Using WGS as surveillance strategy for Legionella pneumophila.

*L. Kieper[1], A. Laubner[2], M. Petzold[1]
[1]*University Hospital Dresden, Consultant laboratory for Legionella, Dresden, Germany*
[2]*University Hospital Dresden, Institute of Medical Microbiology and Virology, Dresden, Germany*

Introduction: Legionella pneumophila is an intracellular pathogen, best known for causing a Pneumonia – Legionnaires´ disease. As it is ubiquitously found in the environment and genetically very divers, progress in typing and sequencing technologies contributes increasingly to understand its general distribution and specifically outbreaks. The seven gene sequence-based typing scheme (SBT), as the currently most widely used method for genotyping, bears limitations regarding the discrimination of several sequence types (STs). Therefore, sequence comparisons from modern DNA sequencing methods and genomic sequence analysis correlated with epidemiological information could indicate connections, such as common sources or unnoticed contacts.

Goals: Our goal was to generate a survey of all patient isolates from 2023, to depict the current surveillance status of legionellae infections. We aimed to extract the STs and mAb-types from the generated WGS data. Using WGS, possible matches of patients and water isolates (possible infection sources) are more definite confirmable or deniable than by only using mAb-typeing and SBT.

Methods: The mAb-type of L. pneumophila colonies (152 samples) was validated via ELISA (Dresden-Panel) and the ST was determined via sanger sequencing. All confirmed colonies from 2023 were sequenced by WGS (Illumina, coverage >30). Generated sequences were analyzed in the Ridom SeqSphere+ software (version 4.0). WGS data were correlated to L. pneumophila mAb-types and STs, generating a clonal map of isolates from 2023.

Results: In 2023 the majority of confirmed L. pneumophila belongs to SG1 (88%, diverse mAb-types). The most abundant mAb-type (32%) was Knoxville, belonging to the

highly virulent Pontiac-Group. Among the 137 determined STs (at least 33 different), ST9 was most abundant. The samples of ST9 cluster in diverse clouds, having up to 200 different alleles revealing thereby limitations of the SBT with regard to a clear identification infection sources. We obtained water isolates corresponding to 38 patients to look for possible matches. Only for 14 of the isolates, a match in mAb-type and ST with the corresponding water sample was confirmed.

P-NRC-009

Influence of ESBL PER-7 on cefiderocol susceptibility in OXA-23 or OXA-72 carbapenemase producing A. baumannii.

*P. Turowski[1], M. Cremanns[1], N. Pfennigwerth[1], S. G. Gatermann[1]
[1]*Ruhr-University Bochum, Department of Medical Microbiology, Bochum, Germany*

Question

In 2022, an OXA-23 or OXA-72 carbapenemase was detected in approximately 90 % of the *A. baumannii* isolates sent to the NRC for Multidrug-resistant Gram-negative bacteria. About 3 % of the isolates carried *bla*$_{NDM-1}$. While the majority of *A. baumannii* producing OXA-23 or OXA-72 show an inhibition zone diameter of ≥ 17 mm for cefiderocol, this is often significantly smaller for *A. baumannii* producing metallo-β-lactamases. But nevertheless, some OXA-23/OXA-72 producing isolates show an inhibition zone diameter of < 17 mm. In four out of five of these isolates, whole genome sequencing revealed the additional presence of the extended spectrum β-lactamase (ESBL) PER-7. The focus of this study is to identify the influence of PER-7 on cefiderocol susceptibility in OXA-23 or OXA-72 carbapenemase producing *A. baumannii.*

Methods

42 OXA-23 or OXA-72 carbapenemase producing *A. baumannii* isolates with cefiderocol inhibition zone diameters < 17 mm were selected from the NRC strain collection and screened for the presence of PER-7 by PCR and sequencing. To test whether the increased resistance to cefiderocol was due to the presence of PER-7, the *per-7* gene was inserted into an expression plasmid. This was transformed into OXA-23 or OXA-72 producing *A. baumannii* with cefiderocol inhibition zone diameters ≥ 17 mm to allow heterologous expression. The clinical isolates and transformed complementants were screened for altered cefiderocol susceptibility using phenotypic tests such as microdilution and agar diffusion assays.

Results

PCR and sequencing results showed that 25 out of 42 *A. baumannii* isolates with cefiderocol inhibition zone diameters < 17 mm carried the *per-7* gene. In addition, the heterologous expression of the *per-7* gene in OXA-23 or OXA-72 producing, cefiderocol susceptible *A. baumannii* increased cefiderocol resistance.

Conclusion

The majority of clinical *A. baumannii* isolates with *bla*$_{OXA-23}$ or *bla*$_{OXA-72}$ and a cefiderocol inhibition zone < 17 mm produced a PER-7 ESBL. Heterologous expression of *per-7* in OXA-23 or OXA-72 producing *A. baumannii* isolates led to a significantly lower susceptibility to cefiderocol than in the absence of this ESBL.

P-NRC-010

Diphtheria in the setting of homeless people and drug abusers in Germany

*A. Berger[1], A. Dangel[2], K. Bengs[3], V. Melnikov[3], I. Tammer[4], V. Forsbach-Birk[5], U. Schuhmacher[5], M. Hogardt[6], J. Haller[7], U. Goetsch[7], I. Friedrichs[8], A. Hansel[9], A. Sing[3]
[1]*Bavarian Health and Food Safety Authority, Public Health Microbiology; Consiliary Laboratory for Diphtheria, Oberschleißheim, Germany*
[2]*Bavarian Health and Food Safety Authority, NGS Core Unit, Public Health Microbiology, Oberschleißheim, Germany*
[3]*Bavarian Health and Food Safety Authority, National Consiliary Laboratory for Diphtheria & WHO Collaborating Centre for Diphtheria, Public Health Microbiology, Oberschleißheim, Germany*
[4]*Synlab MVZ Berlin GmbH, Abteilung Mikrobiologie, Berlin, Germany*
[5]*MVZ Labor Ravensburg, Labor Dr. Gärtner, Ravensburg, Germany*
[6]*University Hospital, Goethe University, Institute of Medical Microbiology and Infection Control,, Frankfurt a. M., Germany*
[7]*Public health institute Frankfurt am Main, Infectiology, Frankfurt a. M., Germany*
[8]*Laborarztpraxis Rhein-Main MVZ, Frankfurt a. M., Germany*
[9]*Helios Clinic Hildesheim, Dermatology, Hildesheim, Germany*

Background

Occurrence of nontoxigenic *Corynebacterium (C.) diphtheriae* wound and bloodstream infections in underprivileged and homeless people is a rising problem in Germany. In the last few years the National Consiliary Laboratory of Diphtheria observed an upsurge of infections due to toxigenic strains among homeless people, alcohol and/or drug abusers in Germany. We aimed to study microbiological data, epidemiological data and molecular epidemiology of diphtheria cases in this setting in order to find potential sources of infections.

Materials & Methods

Strain identification was performed by biochemical differentiation and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Germany). Susceptibility testing was performed according to EUCAST guidelines. Toxigenicity was verified by real-time PCR, the optimized modified Elek-test and a recently published Lateral Flow Immunoassay. Whole generation sequencing (WGS) was carried out on Illumina systems and data analysis by core genome Multi Locus Sequence Typing (cgMLST).

Results

Twelve patients with wound infections were included, harbouring 11 *tox*+ *C. diphtheriae* isolates and 1 *tox*+ *C. ulcerans* strain, respectively. In addition, a *tox*+ *C. diphtheriae* strain was isolated from a positive blood culture in a septic patient. WGS by the NCLoD suggested 7 affected patients had acquired a toxigenic *C. diphtheriae* strain belonging to the ongoing European 2022 diphtheria refugee outbreak without having a correspondent migration history. The most probable source of infection in the *tox*+ *C. ulcerans* case was the stray dog belonging to the homeless person.

Conclusions

We observed 13 diphtheria cases in the setting of homeless and drug abusing people in Germany in the last three years. Among these cases we identified members of one distinct *C. diphtheriae* cluster belonging of the ongoing European refugee outbreak. Therefore, we recommend increased awareness among clinicians, microbiologists, and institutions working with homeless people and intensified sample collection from wounds and laboratory diagnostics.

P-NRC-011
Purification and enzyme kinetics of newly discovered metallo-beta-lactamase NWM-1
*L. M. Höfken[1], S. G. Gatermann[1], N. Pfennigwerth[1]
[1]*Ruhr University Bochum, Medical Microbiology, Bochum, Germany*

Question

The worldwide increase of multidrug-resistant gram-negative bacteria has become an important clinical challenge. Resistance against carbapenems is of particular concern. It can be caused by a variety of mechanisms, however the worldwide spread of carbapenemases is especially important. A worrying trend is the dissemination of Ambler class B metallo-beta-lactamases (MBL). In 2020, a carbapenem-resistant clinical *P. aeruginosa* isolate was referred to the National Reference Laboratory for Multidrug-resistant Gram-negative Bacteria. This isolate harboured the novel bla_{NWM-1} MBL gene (North Rhine-Westphalia metallo-beta-lactamase).

Methods

The carbapenem-resistant *P. aeruginosa* isolate NRZ-63282 was subjected to Illumina whole-genome sequencing and the genome was screened for beta-lactamase-encoding genes. The bla_{NWM-1} MBL gene was cloned into the pASG-IBA vector and transformed into *E. coli* JM83. Purification of NWM-1 was performed by heterologous expression in *E. coli* JM83 and cell disruption via sonification. The lysate was then purified via two-step fast protein liquid chromatography (FPLC), including Strep-tag affinity chromatography and gel filtration. Kinetics were measured with the Eppendorf BioSpectrometer kinetic as previously described in 50 mM HEPES buffer with 50 µM zinc sulfate (pH 7.5) with 10 mm UV cuvettes (Sarstedt, Nümbrecht, Germany) or 2 mm cuvettes (Eppendorf, Hamburg, Germany) with three independent protein preparations.

Results

The NWM-1 protein was purified successfully via affinity chromatography and gel filtration. Initial measurements of the kinetics have been carried out, but the analyses have not yet been finalized. But first results are showing hydrolysis of penicillin G, ampicillin, piperacillin, ertapenem, imipenem and meropenem, but not for aztreonam. Michaelis-Menten kinetics will be presented at DGHM & VAAM 2024.

Conclusions

The newly discovered metallo-beta-lactamase NWM-1 shows typical hydrolyzation patterns for subclass B3 MBLs. Further studies are necessary to analyze the IC_{50} values for a variety of inhibitors, which will also be presented at DGHM & VAAM 2024.

P-NRC-012
Antimicrobial resistance patterns and molecular epidemiology of Burkholderia cepacia complex species from cystic fibrosis patients
K. Brößner[1], T. Burbach[1], A. Schröder[1], S. Schubert[2], S. Besier[1], H. Scholz[2], *M. Hogardt[1]
[1]*University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a. M., Germany*
[2]*Ludwig-Maximilians-University Munich, Max von Pettenkofer Institute, München, Germany*

Outline: Chronic infection with the *Burkholderia cepacia* complex (BCC) in cystic fibrosis patients is associated with increased morbidity and mortality and thus represents a significant challenge in the treatment of patients suffering from CF. The BCC currently comprises at least 22 closely related species that are typically of a multi-resistant phenotype. Moreover, no antibiotic regime exists neither for eradication nor for the antibiotic treatment of chronic BCC lung infection. **Question:** The aim of this study was to assess the susceptibility of BCC to the new cephalosporines ceftolozane/tazobactam (CTB), ceftazidime/avibactam (CZA) and cefiderocol (FDC). Further, using whole genome sequencing (WGS) we want to get more insights into the molecular epidemiology and the relatedness of German BCC isolates. **Methods:** Antimicrobial activities of CZA, CTB and FDC were tested against BCC isolates (n = 100) recovered from individual CF patients by using the gold standard broth microdilution (BMD). BCC species of this study were *B. cenocepacia* (n=39), *B. multivorans* (n=35), *B. ambifaria* (n=1), *B. cepacia* (n=4), *B. contaminans* (n=4), *B. diffusa* (n=1), *B. stabilis* (n=8), *B. vietnamiensis* (n=6), and *B. arboris* (n=2). BCC isolates (the first/earliest available isolate of each individual patient was selected) derive from CF patients treated at University hospital Frankfurt/Munich (including isolates submitted to the respective Consiliary Laboratory of CF Bacteriology). For WGS, DNA of cultured bacteria was extracted using DNeasy UltraClean 96 Kit. Library preparation and sequencing was performed by a commercial service provider (Novogene, Cambridge,UK) using Illumina chemistry. **Results:** Finally, CTB, CZA and FDC susceptibility rates were 75%, 92%, 91% (by using EUCAST breakpoints available for *P. aeruginosa)*. Sequence analysis based on a cgMLST sheme is currently under way, but determination of the standard seven gene MLST sheme showed that 32% of isolates belong to so far unknown MLST types that will currently be newly assigned. **Conclusion:** CTB, CZA and FDC are highly active against BCC species from CF patients and may be effective alternative therapeutic options.

Phages and Microbial Defense Systems

P-PMD-001
Systematic mapping of Phage-specificity in Staphylococcus aureus to predict phage infection
*J. Krusche[1], C. Beck[1], E. Lehmann[2], D. Gerlach[3], C. Wolz[2], A. Peschel[1]
[1]*University of Tübingen, Infection Biology, Tübingen, Germany*
[2]*University of Tübingen, Institute of Medical Microbiology, Tübingen, Germany*
[3]*Ludwig-Maximilians University Munich, Faculty of Biology, Martinsried, Germany*

Question: *Staphylococcus aureus* can cause life-threatening infections that are often resistant to many different antibiotics. One way to combat these antibiotic resistant infections are bacteriophages. The host range of *S. aureus* phages is determined by the species-specific structure of wall teichoic acids (WTA), the only known *S. aureus* phage receptor. Nearly all *S. aureus* strains possess WTA consisting of ribitol-phosphate repeats. This study aims to investigate the binding capabilities of *S. aureus* ribitol-phosphate binding phages to their host cells through the identification of receptor-binding proteins (RBPs). Through this, we want to understand the underlying mechanism leading to adsorption and thus infection of the phage, which might enable us to predict the host range of *S. aureus* phages.

Methods: We used bioinformatic analysis to identify putative RBPs necessary for phage adsorption. Over 350 *S. aureus* phage genomes were analyzed to identify *S. aureus* ribitol-phosphate binding RBPs, which were then classified based on amino acid homology. Protein fusion constructs were created by addition of a fluorescent N-terminus to the phage RBPs, and the binding specificity of these proteins to different *S. aureus* WTA-mutants was investigated via flow cytometry and fluorescence microscopy.

Results: We found various RBPs necessary for phage adsorption and identified several different clusters of *S. aureus* ribitol-phosphate binding phages based on their predicted RBPs. Analysis of the binding specificity revealed distinct binding patterns for each RBP cluster. The created phage clusters allow prediction of phage adsorption to different WTA glycosylation types during the initial stage of phage infection.

Conclusion: This study provides insights into the host range of both known and novel phages that are useful for the development of phage-based therapeutics against *S. aureus*. The findings suggest that *S. aureus* ribitol-phosphate binding phages can be classified into different groups based on their RBPs, which can be used to predict their binding capabilities and success in phage adsorption to different WTA glycosylation types.

P-PMD-002

CRISPR-independent targeting of endogenous RNAs by Cas9 in foodborne-pathogen Campylobacter jejuni

*S. Sharma[1], C. M. Sharma[1]

[1]*Institute of Molecular Infection Biology, Molecular Infection Biology II, Würzburg, Germany*

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) systems are naturally occurring adaptive immune systems found in bacteria and archaea. There has been emerging evidence that Cas9 might have cellular roles beyond genome defence and can impact gene regulation or virulence. In our lab, we previously identified two distinct and unexpected modes of CjCas9 binding to endogenous RNAs. These are crRNA-dependent RNA targeting [1] and the discovery of non-canonical CRISPR RNAs (ncrRNAs), which are generated by novel mode of RNA-binding via duplex formation between *trans*-activating crRNA (tracrRNA) and cellular RNAs from outside the CRISPR-Cas9 locus [2]. Despite this novel discovery of ncrRNAs, it remained unclear whether formation of different cellular CjCas9 ribonucleoprotein (RNP) complexes play any role in endogenous gene regulation. Knowing that Cas9-mediated RNA cleavage requires crRNA-mRNA interactions *in-vivo*, we performed RIP-seq on *C. jejuni* mutant strains harbouring deletions of either individual crRNA, tracrRNA or the entire CRISPR locus. These analyses revealed endogenous RNAs that are exclusively enriched in these deletion strains, and those for which the binding to CjCas9 appeared to be independent of both crRNAs and tracrRNA. Further, using RIP-seq in complementation strains for wild-type and catalytically dead (dCas9) Cas9, we identify previously "hidden" RNA targets. We are currently examining these targets and exploring the possibility of complex tertiary structures on these RNAs aiding in their recognition by CjCas9, as exemplified by the presence of a unique triple-helix structure in native tracrRNA scaffold from *C. jejuni*. Overall, our findings provide new insights into cellular targets of CjCas9, uncovering its indelible impact on endogenous gene regulation, CRISPR biology and technologies.

References

[1] Dugar et al. (2018) *Molecular Cell 69, 893–905.*
[2] Jiao et al. (2021) *Science 372, 6545.*

P-PMD-003

Developing a rapid phenotypic assessment of phage susceptibility using a nanomotion technology platform

A. Luraschi Eggemann[1], A. Vocat[1], G. Resch[2], *A. Sturm[1]

[1]*Resistell AG, Muttenz, Switzerland*

[2]*CHUV, CRISP, Lausanne, Germany*

Background: Due to the spread of antimicrobial resistance and the slow development of new antibiotics, alternatives, such as bacteriophages, are coming into focus. Besides clear evidence of the clinical interest of phage therapy, its implementation requires fast and reliable phage susceptibility testing (PST) to select the right phage(s) to treat the patients.

Methods: As an alternative to complex and time-consuming standard Drop Tests Assays (DTA), we developed a new technology[1-4], based on real time measuring of living cell vibrations in response to a phage. These vibrations or nanomotions are recorded with a micromechanical sensor bearing a cantilever and the Phenotech device that is currently in clinical studies for antibiotic susceptibility testing (AST) in bloodstream infections.

Results: We present the first data obtained with the Phenotech device measuring the response of *Pseudomonas aeruginosa* ATCC-15442 to two different phages. at different concentrations (MOI, Multiplicity Of Infection). Our recordings show the dose-dependent lysis of cells in real-time. In a second step, we moved to several clinical isolates mostly from cystic fibrosis patients and developed a classification model for clear and turbid phenotypes referenced to empirical DTA. In only 6 h, the Phenotech PST was able to discriminate between clear and turbid phenotypes with an accuracy of 89%.

Conclusion: We plan to extend our experiments to a larger set of *P. aeruginosa* patient isolates and phages and continue training classification models with supervised machine learning based on common features from the nanomotion signal. A nanomotion-based PST could fill the current gap in fast PST, helping clinical implementation of phage therapy.

1 Longo, G. et al. Nat Nanotechnol 2013
2 Kasas, S. et al. Antibiotics 2021

3 Villalba, M. I. et al. Small 2018
4 Vocat, A. et al. Microbes and Infection2023

P-PMD-004
A coordinated SOS-response is inducing the auto-lysis of E. coli K-12 MG1655 by ΦO104
*M. Berger[1], P. Berger[1], G. Koudelka[2], U. Dobrindt[1]
[1]*University Muenster, Münster, Germany*
[2]*University of Buffalo, Buffalo, NY, United States*

Infections with enterohemorrhagic *Escherichia coli* (EHEC) can result in life-threatening haemolytic uremic syndrome (HUS) and ultimately in death. The German outbreak strain from 2011 *E. coli* O104:H4 was unusual, because the infections affected mainly adults and progressed in ~25% of the cases to HUS. The exceptional virulence of the strain was attributed to its "hybrid" virulence gene content, as it encoded aggregative adherence fimbria characteristic for enteroaggregative *E. coli*, as well as a Shiga toxin (STX) 2 encoding phage, the hallmark of EHEC. However, little is known about the role of the specific patient microbiome on the course of the disease. Mixed cultures of EHEC and STX phage-susceptible *E. coli* were shown to produce higher amounts of STX than pure EHEC cultures, indicating a potential role of the microbiota to overall toxin production. Moreover, it was shown that certain STX phages, including ΦO104, have replication origins different from lambdoid EHEC phages and a less stable lysogenic state. We show here that ΦO104 and the EHEC O157:H7-derived phages PA2 and PA8 are unstable in *E. coli* MG1655. We show that the stability of the phages is sensitive to environmental factors, e.g. growth temperature, growth medium composition and carbon source, and partially to genetic factors, e.g. *luxS* and *lsr*. This indicated that the auto-activation of the phages in *E. coli* MG1655 is a regulated process, however different from the respective wild types, in which the lysogenic state is much more stable. By using *recA* mutants, we show that the SOS response is the essential signal for phage activation in *E. coli* MG1655. By using an SOS reporter, we show that coordinated SOS response in the absence of an external inducer – that is completely absent in *E. coli* O104:H4 - precedes the lysis of *E. coli* MG1655. Moreover, we show that all environmental factors that are stabilizing the phage are as well reducing the extent of the SOS response in the lysogens. We discuss the potential mechanism underlying the phage activation, the role of the specific type of phage in the outcome of the disease, and potential therapeutic options for this aspect of the disease.

P-PMD-005
Viral dynamics at the Central Arctic air-water interface
*J. Rahlff[1,2], G. Westmeijer[1], A. Antson[3], K. Holmfeldt[1]
[1]*Linnaeus University, Department of Biology and Environmental Science, Kalmar, Sweden*
[2]*Friedrich Schiller University Jena, Jena, Germany*
[3]*York Structural Biology Laboratory, University of York, Department of Chemistry, York, United Kingdom*

In polar environments, aquatic viruses are challenged with low host availability and harsh environmental conditions. Due to restricted ecosystem accessibility, knowledge about viruses from near-atmosphere aquatic ecosystems around the North Pole is lacking. Here, our aim was to investigate viral diversity, adaptations, and host interactions at the air-water interface in the Central Arctic. Aquatic samples were collected from ~60 cm depth and the submillimeter surface microlayer (SML) during the Synoptic Arctic Survey expedition on icebreaker Oden in summer 2021. Samples were taken from a melt pond in the ice and the ocean before undergoing size-fractioned filtration. Genome-resolved metagenomics and cultivation were applied to investigate prokaryotes and viruses. Diversity of the melt pond was lower compared to open water and dominated by *Aquiluna* sp. (phylum Actinobacteriota) whose reconstructed small genome encoded for a limited metabolic potential. From 1154 viral operational taxonomic units (vOTUs), two-thirds were predicted bacteriophages. Auxiliary metabolic genes (AMGs) were detected in 17.2% of all vOTUs, of which 26.6% carried more than one AMG. Abundant AMGs with functions in amino acid, porphyrin, and glycan polymer metabolism were detected. Glycerol-3-phosphate cytidylyltransferase with a potential role in the host"s cryoprotection was encoded by 16 vOTUs. The presence of vOTUs at several stations was more strongly correlated to vOTU abundance in the SML than in the underlying water, suggesting dispersal to be mediated by the air-sea boundary layer. More prophages occurred in SML-derived genomes, and a prophage of the bacterial strain *Leeuwenhoekiella aequorea* Arc30 was inducible with mitomycin C. Electron microscopy revealed that the induced phage vB_LaeS_Arctus_1 was a siphovirus. Arctus_1 had a 42.7 kb circular genome, shared little protein similarities with known phages, placing it in a novel genus. The results demonstrate that Arctic viruses have elaborate strategies to endure in remote, inhospitable, and host-limited environments.

P-PMD-006
Exploring the host specificity of archaeal viruses HRTV-7 and HRTV-10
*E. R. Sensevdi[1]
[1]*University of Groningen, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Molecular Microbiology, Groningen, Netherlands*

Archaea, constituting a distinct domain of life, exhibit unique characteristics such as the composition of their cell envelope, which is different from that of bacteria and eukaryotes.

As a consequence of this distinctiveness of the cell envelope archaeal viruses attempting to infect host cells face different challenges compared to viruses infecting other domains of life. In recent years, several receptors have been identified that are utilized by some archaeal viruses. However, our comprehension of the virus-receptor interaction in archaea remains rather scarce, despite the prevalent diversity and abundance of archaeal viruses in the environment.

Understanding the interplay between archaeal viruses and receptors is paramount to studying viral host range. Archaeal viruses may exhibit a broad (viruses can successfully infect multiple hosts of the same or even different species) or a narrow host range (they only infect closely related strains/species). The capability of certain archaeal viruses to interact with a wide array of host surface receptors may be to attributed to the exchange of host-specific genetic modules for RBPs (Receptor Binding Proteins), which was also observed in T-even bacteriophages, thereby enhancing their ability to infect a broad host range 1.

The study aims to elucidate the mechanism of host recognition utilized by tailed archaeal viruses *Halorubrum* tailed virus 7 (HRTV-7) and *Halorubrum* tailed virus 10 (HRTV-10). Both viruses infect the haloarchaeal model

organisms *Haloferax gibbonsii LR2*-5, in addition to their original archaeal host strain *Halorubrum sp.* B2-2.

To achieve this objective, a combinatorial approach encompassing adsorption assays, one-step growth curves, genome sequencing, and transmission electron microscopy is employed. With this approach, we want to identify the host cell receptors required for host cell recognition and virus binding. This will aid to understand the infection mechanism of these viruses, and it will gain insight into host range determinants of tailed archaeal viruses.

P-PMD-007

Bacteriophage infection and processing within a glycan-based biofilm

*A. Nowicki[1], N. K. Bröker[2], S. Chiantia[3], S. Barbirz[1]
[1]*MSB Medical School Berlin, Berlin, Germany*
[2]*HMU Health and Medical University, Potsdam, Germany*
[3]*Universtiy Potsdam, Potsdam, Germany*

Phages and biofilms are ubiquitous in natural environments, and their interaction has contributed to accelerate the evolution of phages and their bacterial hosts in these particular niches. Biofilms have represented a protective mode of microbial life against harsh environments for millions of years, and it appears that biofilms are also a beneficial trait in pathogenesis, as the majority of chronic infections are caused by cells organized into biofilms. A characteristic property of the mature biofilm is that it has antibiotic resistance. Moreover, biofilm bacteria become inaccessible to immune cell attacks. Most bacterial species have the ability to colonize inorganic or biological surfaces and embed themselves in a gel formed by polysaccharides, DNA and proteins they excrete, the extracellular polymeric substance (EPS). EPS regulates the selective transport of molecules and is a diffusion barrier for bacteriophages.We chose Salmonella P22 podovirus as a model to analyze hydrodynamic behavior of bacteriophages inside biofilms, using a polysaccharide matrix produced by *Pantoea stewartii* as model for a glycan-based biofilm. To this end, the hindrance factor of the phage as well as beads of different sizes were determined using single particle tracking (SPT) and fluorescence correlation spectrometry (FCS). We describe bacteriophage diffusion in relation to physicochemical properties of the stewartan matrix like polysaccharide concentration, the solvent pH and the tracer properties [1]. In addition, we show that the degradation of stewartan by glycan-depolymerizing enzymes is an important regulatory mechanism that can rapidly modulate particle dynamics within the matrix. In the following, we will link bacteriophage diffusion properties to host infection dynamics to understand how bacteriophages address their associated pathogens depending on the hydrodynamic properties of viscous biofilms.

[1] Dunsing V, Irmscher T, Barbirz S, Chiantia S. Purely Polysaccharide-Based Biofilm Matrix Provides Size-Selective Diffusion Barriers for Nanoparticles and Bacteriophages. Biomacromolecules. 2019;20(10):3842-54.

P-PMD-008

Infection initiation of Salmonella bacteriophages: Characterization of viral particles interaction with host membranes and DNA release

*C. Arnaud[1], H. Pace[1], M. Bally[1], N. Broeker[1], S. Barbirz[1]
[1]*Health and Medical University Potsdam, Potsdam, Germany*

In this project, we are developing *in vitro* systems to study the interactions between *Salmonella* phage particles and host outer membrane as well as rates of DNA ejection from the viral capsid to understand initiation of the infection and how different phage tail architectures influence this process [1]. We use total internal reflection fluorescence microscopy (TIRF-M) to observe bacteriophages on supported lipid bilayers (SLBs) that contain LPS or fragments of native *Salmonella* outer membranes. Phage particle tracking on these SLBs yields quantitative information on particle attachment and detachment and on their diffusive behavior [2]. We characterize membrane compounds and SLBs with dynamic light scattering, TIRF-M and quartz crystal microbalance with dissipation monitoring. Moreover, we analyze DNA release from phages in presence of various membrane systems with fluorescence spectroscopy and TIRF-M. We observed that phage mobility on host membranes changed over time, with populations showing varying mobility, most probably a consequence of receptor depletion on the surface due to enzymatic activity of the phage tailspikes. In addition to provide information on the mechanism of infection initiation, model systems of the Gram-negative outer membrane will allow to connect the single phage particle behaviors to biological effects such as competition of phages within a phage cocktail.

[1] Andres D et al. (2012) Tail morphology controls DNA release in two *Salmonella* phages with one lipopolysaccharide receptor recognition system. Mol. Microbiol. 83: 1244-1253.
[2] Bally M et al. (2021) Physicochemical tools for studying virus interactions with targeted cell membranes in a molecular and spatiotemporally resolved context. Anal Bioanal Chem, 413:7157-7178.

P-PMD-009

Phage induced Strain Replacement within Microbiomes

*M. Gaissmaier[1], B. Stecher[1], M. S. Matchado[1], A. von Strempel[1], L. Michel[1], M. Salvado Silva[1], S. Woelffel[1], L. Cardoso[1], J. Wittmann[2], G. Werner[3], T. Clavel[4]
[1]*Ludwig-Maximilians University, Max von Pettenkofer Institut, München, Germany*
[2]*DSMZ, Brunswick, Germany*
[3]*NRZ, RKI, Berlin, Germany*
[4]*RWTH Aachen University, Aachen, Germany*

In recent decades, microbiome research has gained increasing relevance in the field of public health. It vastly contributed to understanding the pathogenesis of major non-communicable human diseases but also generated knowledge on the emergence and spread of antibiotic resistant bacteria (ABR). One crucial forthcoming task involves formulating strategies for microbiome editing, specifically targeting the elimination of harmful or antibiotic-resistant bacteria from the human gut. The objective is to achieve this without compromising or disturbing the overall composition of the microbiota.

To address this question, we focus on a new strategy termed "phage induced strain replacement within microbiomes", which aims at combining phage- and probiotics-based therapy within one treatment. This approach is grounded in previous observations made in gnotobiotic mice colonized with the Oligo-MM12 synthetic bacterial community (Von Strempel et al. 2022; Brugiroux et al. 2016). We aim at posing a fitness disadvantage on the targeted strain by a strain-specific phage cocktail while introducing a second, closely related, but phage-resistant niche competitor at the

same time. Ideally, this approach leads to strain replacement and elimination of the targeted strain without disturbing the overall gut microbiota composition. We focus on the three species *Escherichia coli*, *Enterococcus faecalis* and *Phocaeicola vulgatus* (formerly *Bacteroides vulgatus*). We confirmed our theory in batch culture setups showing that phage induced strain replacement is possible. Presently, we are building phage and competitor strain libraries for *E. faecalis*. These will be utilized in subsequent experiments conducted under various environmental conditions, both in vitro and in vivo. The aim is to further assess the potential efficacy of strain replacement as a novel therapeutic approach for addressing antibiotic resistance (ABR) and chronic intestinal diseases.

P-PMD-010

Reduction of Listeria monocytogenes in meat products by commercially available bacteriophage product

*K. Willenbücher[1], T. Algusta[2], F. Hille[1], R. Nitzsche[2], N. Biere[1], C. Hertel[2], C. Franz[1]
[1]*Max-Rubner Institute, Department of Microbiology and Biotechnology, Kiel, Germany*
[2]*DIL German Institute of Food Technologies, Biotechnology, Quakenbrück, Germany*

Introduction

Although foodborne infections with the ubiquitous bacterium *Listeria* (*L.*) *monocytogenes* are comparatively rare, they are of great importance due to their severity and high mortality rate which averages at ca. 7%. In addition to the various measures taken to reduce *L. monocytogenes* during the production of meat products, new methods for lowering bacterial contamination, such as non-thermal high-pressure treatment and biopreservation with bacteriophages, offer an opportunity to further reduce the risk of *L. monocytogenes* occurring in meat products. Biocontrol strategies with highly effective lytic bacteriophages can also be utilised to control *L. monocytogenes*.

Goal

Combining *L. monocytogenes*-specific phages with a mild high-pressure treatment will ensure adequate protection against *Listeria*. This way, the existing limitations of the respective methods for reducing *L. monocytogenes* in sausage products can be countered. Here, results of the infection effects of the two commercial phage products used in this study to infect *Listeria* at temperatures of 7°C are presented. In addition, the impact of food components such as salt, nitrite and spices on phage infection efficiency and Listeria reduction are investigated.

Results

A protocol for extraction of the bacteria and the two phage preparations from the sausage medium was successfully developed and can be used in future experiments. The tested *L. monocytogenes* strains could grow at both tested temperatures and, as expected, displayed a significantly increased growth rate at 37°C. In addition, the infection efficiency of both phages was also temperature-dependent and the countable plaques were drastically reduced for some strains at 7°C when compared to 37°C. The influence of bacterial growth and phage effectiveness were not noticeably influenced by the food components which included spice mixtures and sodium nitrite. The phage products have good infection efficiency. In combination with the high-pressure process, interesting results will be obtained.

Summary The phage products show a different infection rate at lower temperatures, which is essential information that will impact further treatments.

P-PMD-011

Bacteria conjugate ubiquitin-like proteins to interfere with phage assembly

*J. Hör[1], S. G. Wolf[1], R. Sorek[1]
[1]*Weizmann Institute of Science, Rehovot, Israel*

Multiple immune pathways in humans conjugate ubiquitin-like proteins to virus and host molecules as a means of antiviral defense. Here, we studied an anti-phage defense system in bacteria, comprising a ubiquitin-like protein, ubiquitin-conjugating enzymes E1 and E2, and a deubiquitinase. We show that during phage infection this system specifically conjugates the ubiquitin-like protein to the phage central tail fiber, a protein at the tip of the tail that is essential for tail assembly as well as for recognition of the target host receptor. Following infection, cells encoding this defense system release a mixture of partially assembled, tailless phage particles and fully assembled phages in which the central tail fiber is obstructed by the covalently attached ubiquitin-like protein. These phages exhibit severely impaired infectivity, explaining how the defense system protects the bacterial population from the spread of phage infection. Our findings show that conjugation of ubiquitin-like proteins is an antiviral strategy conserved across the tree of life.

P-PMD-012

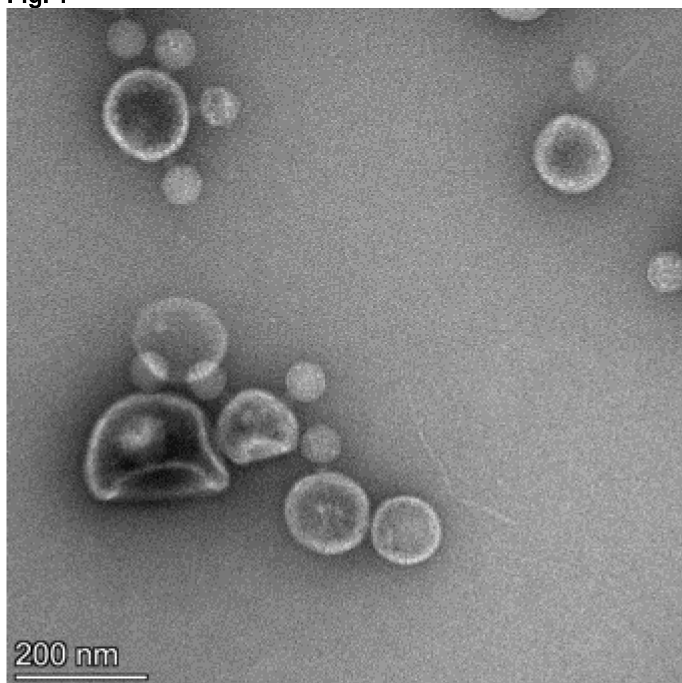
Outer membrane vesicles as a transport system after bacteriophage treatment

*H. M. Monks[1], N. K. Bröker[2], M. Anding[3], P. Wendler[3], S. Barbirz[1]
[1]*Medicalschool Berlin, Medicine, Berlin, Germany*
[2]*HMU Health and Medical University, Medicine, Potsdam, Germany*
[3]*University Potsdam, Biochemistry, Potsdam, Germany*

Bacteria use a variety of defence systems to control bacteriophage infection, amongst others extracelluar vesicles (EVs) [1, 2, 3]. Such EVs demonstrate a proficient capacity to bind and reduce the number of infectious bacteriophage particles within a bacterial population [2]. However, the precise mechanism underlying the interaction between vesicles and bacteriophages is still not fully understood. Outer membrane vesicles (OMVs) of Gram-negative bacteria like *Salmonella* (*S.*) contain a variety of surface receptors used in bacteriophage infection. Bacteriophage infection or antibiotic treatment triggers vesicles production [3]. We have recently shown that phage P22 binds to *S.* Typhimurium OMVs and injects its DNA into the vesicle lumen [2]. Aim of this study is to analyse the properties of these "phage infected" OMVs. We employed P22 bacteriophages carrying GFP-genes as reporters for successful *Salmonella* infection to study the fusion between OMVs and bacteria and explore potential new mechanisms of gene transfer between bacteria and phages. Here, we also want to study the fate of bacteriophage particles that have ejected their DNA but remain bound to the OMV surface. We compare different OMVs obtained from full membranes or isolated from culture supernatants under different conditions, and characterize them in complex with phages using dynamic light scattering, fluorescence spectroscopy and electron microscopy.

[1] Adam Kulp and Meta J Kuehn. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annual review of microbiology, 64:163–184, 2010.
[2] Mareike S Stephan, Nina K Broeker, Athanasios Saragliadis, Norbert Roos, Dirk Linke, and Ste-fanie Barbirz. In vitro analysis of O-antigen-specific bacteriophage P22 inactivation by Salmonella outer membrane vesicles. Frontiers in Microbiology, 11:510638, 2020.
[3] Ethan Hicks, Nicholas MK Rogers, Christine Ogilvie Hendren, Meta J Kuehn, and Mark R Wiesner. Extracellular vesicles and bacteriophages: New directions in environmental biocolloid research. Environmental Science & Technology, 57(44):16728–16742, 2023.

Fig. 1



P-PMD-013
Identity, infection strategy, and biogeochemical impact of nitrifier-infecting viruses

*J. Papendorf[1], D. K. Ngugi[1], N. Reimann[1], J. Wittmann[1], R. L. Hahnke[1], M. Pester[1,2]
[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Brunswick, Germany*
[2]*Technical University of Braunschweig, Institute of Microbiology, Brunswick, Germany*

Nitrification, the two-step transformation of ammonia via nitrite to nitrate plays a significant role in the global nitrogen cycle. Ammonia oxidation, the first and rate-limiting reaction, is typically being mediated by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA), followed by nitrite oxidizing bacteria (NOB) converting nitrite to nitrate. Relatively recently, specific bacteria of the genus *Nitrospira* were found to be capable of complete ammonia oxidation (comammox). While nitrification is used to reduce the concentration of nutrients in wastewater treatment plants, it can also lead to eutrophication of water bodies by transforming fertilizer-derived ammonia to leachable nitrate. Furthermore, nitrification is a source of the potent greenhouse gas nitrous oxide.

This project focuses on understanding nitrifier-virus interactions in a range of habitats and aims to characterize the impact of viruses on their respective hosts and the underlying ecosystem function. We isolated a bacteriophage infecting the nitrifying bacterium *Nitrosomonas europaea* and are now investigating its infection cycle and its effect on ammonia oxidation. The virus has a typical podoviral morphology with a capsid sized approximately 60 nm in diameter. It possesses a ca. 42 kb large genome, causes lytic infections which severely alter host-morphology and belongs to the family *Autographiviridae*. Recently, we were able to isolate two additional bacteriophages infecting *Nitrosomonas europaea* or *Nitrosospira multiformis*, respectively. Currently, we are in the process of isolating a fourth virus infecting a novel proposed type-strain of the genus *Nitrosomonas*.

P-PMD-014
You shall not flip! A strategy for biosynthetic production and structural investigation of the lytic phage protein pinholin

*D. Winkler[1], T. H. Walther[2], A. S. Ulrich[1,2]
[1]*Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry (IOC), Department of Biochemistry, Karlsruhe, Germany*
[2]*Karlsruhe Institute of Technology (KIT), Institute of Biological Interfaces (IBG-2), Department of Molecular Biophysics, Eggenstein-Leopoldshafen, Germany*

Lytic phages, such as lambdoid enterobacteriophage φ21, infect bacterial hosts in order to exploit their biosynthetic machinery for reproduction. The subsequent release of virions is ensured by a complex interplay of effectors resulting in the lysis of the host cell. This process is orchestrated by a small, helical, amphiphilic membrane protein called pinholin, via time-specific formation of homo-oligomeric pores in the host cell membrane – the eponymous pinholes.[1] While translocation of a transmembrane domain to the membrane surface (flipping) has been proposed to convert pinholin into its active, pore-forming state [2,3], sufficient experimental verification is lacking. Here, we aimed to produce sufficient amounts of pinholin for future solid-state NMR- and cryogenic transmission electron microscopy-based structural investigation. To overcome its intrinsic cytotoxicity, we opted to trap the protein in its non-flipped, inactive conformation by means of intramolecular cyclisation, utilising the SpyTag and SpyCatcher fusion tags.[4] After tag cleavage and chromatographic purification, functionality and correct fold of the protein were verified via a fluorescence-based vesicle leakage assay and circular dichroism spectroscopy, respectively. Cyclisation of pinholin was succesful and significantly reduced its toxicity. Upon optimisation of the fusion construct and expression conditions, milligram-amounts of functional and correctly folded pinholin could be purified to apparent electrophoretic homogeneity. In summary, we could establish a method for the biosynthetic production of large amounts of functional pinholin, which can be used in future experiments to elucidate the structural basis of its activation mechanism.

1. Pang, T. *et al.* PNAS 110, E2054–E2063 (2013)
2. Steger, L. M. E. *et al.* PNAS 117, 29637–29646 (2020)
3. Pang, T. *et al.* PNAS 106, 18966–18971 (2009)
4. Schoene, C. *et al.* Methods Enzymol 580, 149–167 (2016)

P-PMD-015
Insights into CRISPR-Cas systems of Streptoalloteichus tenebrarius, an aminoglycoside antibiotic producer

*L. Mitousis[1], E. M. Musiol-Kroll[1], W. Wohlleben[1]
[1]*University of Tübingen, Microbiology/Biotechnology, Tübingen, Germany*

Many *Actinomycetes* are important producers of secondary metabolites with potent properties like antimicrobial, anti-tumor or immunosuppressive activity. However, their genetic engineering is often prohibited by mechanisms that prevent uptake and/or stable establishment of foreign DNA. CRISPR-Cas systems, for example, are known as adaptive immunity systems and recently also the involvement of CRISPR-Cas in other cellular processes including DNA-repair or cell-differentiation was described. Nevertheless, the knowledge about the function of CRISPR-Cas in *Actinomycetes* is still very limited. The goal of this project is the functional analysis of CRISPR-Cas systems in antibiotic-producing actinomycetes. This includes adaptive immunity as well as functions beyond immunity, such as a potential role of CRISPR-Cas in regulation of antibiotic production. For this, the industrial aminoglycoside antibiotic producer *Streptoalloteichus tenebrarius* is used as model strain. It is particularly interesting, as its genome has 33 predicted biosynthetic gene clusters and three CRISPR-Cas systems (Region 1-3). In order to perform phage infection assays, an extensive screening for phages infecting *S. tenebrarius* was conducted. It was shown, that *S. tenebrarius* is not included in the host range of the tested phages. Investigation of the immunity function using primed assays requires the identification of the PAM sequence. Since genetic manipulation of *S. tenebrarius* is challenging, the CRISPR-Cas systems of *S. tenebrarius* were heterologously expressed in *Escherichia coli*. The cells were transformed with a PAM-library and the abundance of the individual PAM sequences after *cas*-gene induction was evaluated. No decrease in abundance was observed for all tested PAM sequences, suggesting that no PAM-recognition took place. Therefore, PAM-assays using *Streptomyces coelicolor* as a more closely related host are planned. In addition, CRISPR-Cas deletion mutants in *S. tenebrarius* were generated. The deletion of CRISPR-Cas Region 1 led to a sporulation-deficient phenotype. This suggests an involvement of CRISPR-Cas in cell-differentiation processes in *S. tenebrarius*.

P-PMD-016

Legionella pneumophila phospholipase PlaB is a cell death effector of an NADase toxin

P. Aurass[1], C. Lang[1], W. Blankenfeldt[2], *A. Flieger[1]
[1]*RKI, Wernigerode, Germany*
[2]*Helmholtz Center for Infection Research, Brunswick, Germany*

NAD+ depletion is a defense strategy of both eukaryotic cells and bacteria against viral infection. We recently uncovered the unusual mechanism which controls activity of the Legionella pneumophila phospholipase and virulence factor PlaB. Specifically, physiological concentrations of NAD+ stabilize inactive PlaB tetramers, and contrarily, low NAD+ triggers release of two active protein dimers unleashing fierce phospholipase activity. Previous work further established bacterial surface association of PlaB by a noncanonical two-stranded β-sheet. Although PlaB homologs can be widely found in environmental bacteria, it was surprising that conservation of the respective β-sheet is low (Diwo et al. 2021: https://doi.org/10.1073/pnas.201704611). Therefore, we presumed a second intrinsic function of PlaB in altruistic cell death, which is also observed in abortive phage defense, apart from acting as a surface-exposed virulence factor. Consistent with NAD+ being central for regulation of PlaB`s activation status, in gene co-occurrence studies, we found high conservation of plaB with candidate NAD+ consuming toxin and cognate antitoxin genes suggesting a functional link. Indeed, co-expression studies conducted in E. coli and L. pneumophila revealed rapid cell death when PlaB was present only when NAD+ dropped to sub-physiological levels as a consequence of toxin induction. Gene expression analysis in L. pneumophila uncovered apparent co-regulation of plaB, toxin and antitoxin genes, all of which being induced for example upon prolonged stress. Our results support a model in which PlaB together with an NADase effector module is involved in stress response of L. pneumophila eventually facilitating cell death to benefit the population. NADase toxin and antitoxin might furthermore constitute elements of Legionella anti-bacteriophage response compiling a novel phospholipase-driven abortive infection system. In summary, our data indicate that PlaB is a cell death effector of an NADase toxin found in – but not limited to - Legionella pneumophila.

P-PMD-017

Identifying the components of the Shewanella phage LambdaSo lysis system

*D. Fischer[1]
[1]*Justus-Liebig-Universität Gießen, Microbiology and molecular biology, Giessen, Germany*

Double-strand DNA bacteriophages usually disrupt their hosts" envelope with a set of lysis proteins characterized by a holin, an endopeptidase and a spanin system. By a combination of mutant screening, protein structure predictions and microscopy, we found that *Shewanella oneidensis* prophage LambdaSo uses a pinholin/signal-anchor-release (SAR) endolysin system to induce proton-leakage and degradation of the cell wall. Further insight into the lysis cassette of LambdaSo lead to the identification of a two-component spanin modul encoded by two extensively nested open reading frames. In addition, we discovered another small transmembrane protein strictly required for LambdaSo-induced cell lysis, potentially acting at the level of the pinholin. Thus, LambdaSo-mediated cell lysis requires at least four protein factors (pinholin, SAR-endolysin, spanin, Lcc6).

P-PMD-018

The stress of carrying CRISPR

*D. Haider[1], R. Bauer[1], A. Grempels[1], R. Roscher[1], C. C. Aslan[1], S. Mauerer[1], B. Spellerberg[1]
[1]*Institute of Medical Microbiology and Hospital Hygiene, Ulm University Medical Center, Ulm, Germany*

Streptococcus anginosus (*S. anginosus*) is a commensal that can cause severe invasive bacterial infections, but the molecular genetic background is sparsely investigated. A considerable percentage of *S. anginosus*strains harbor CRISPR-Cas systems, which apart from being a bacterial immunity system can play an important role regarding the adaptation to environmental stress. The functionality of *S. anginosus* CRISPR-Cas systems has previously not been investigated. To address this, we created a set of deletion mutants in the CRISPR-Cas type II-A system of the *S. anginosus* SK52 type strain, targeting the nuclease Cas9 and the CRISPR array. Testing these strains in a plasmid clearance assay, we were able to confirm CRISPR-Cas activity. Furthermore, the role of the *S. anginosus* CRISPR-Cas system was investigated under various stress conditions such as resistance against UV light and hydrogen peroxide exposure as well as high-temperature in wildtype *S. anginosus* and CRISPR-Cas mutant strains. Under these conditions, survival was significantly lower in strains carrying *cas9,* like for example the wildtype strain SK52*,* when compared to a *cas9* deletion mutant. We could also show

that bacterial growth in liquid broth and metabolic activity in Alamar blue assays was negatively affected by the presence of *cas9* in *S. anginosus*. In summary we found that the presence of a functional CRISPR-Cas system in *S. anginosus* leads to measurable metabolic and fitness costs for the wildtype strain. Carrying *cas9* was associated with an impaired stress response in our experiments and may thus explain, why many strains of this species lack CRISPR-Cas.

P-PMD-019

Towards understanding epitranscriptional regulation of RNA stability in phage-host interactions

*H. Keuthen[1], K. Höfer[1]

[1]*Max Planck Institute of terrestrial microbiology, Marburg, Germany*

Bacteriophages, viruses that specifically target and kill bacteria, hold great potential as antimicrobial agents against multidrug-resistant bacterial infections. However, the advancement of phage therapy is hindered by a lack of understanding regarding the molecular mechanisms underlying bacteriophage-mediated hijacking of host gene expression machinery. Our group´s recent results suggest previously unknown host transcriptional profiles and active transcription of *E. coli* genes during infection. Here, we evaluate the influence of various viral and host factors, including nucleases, RNA structures, protein-RNA interactions, and RNA modifications, on the regulation of RNA stability and degradation during T4 phage infection of *E. coli*.

Our lab's experiments, employing dual RNA sequencing, unveiled the dynamic regulation of RNA stability in both host and phage transcripts during the infection process [1]. For the first time, these investigations allowed us to simultaneously and comprehensively analyse the transcriptomes of the T4 phage and its bacterial host, *E. coli*. To elucidate the specific molecular mechanisms responsible for RNA processing during T4 infection, we examined the functional roles of potential candidates, such as nucleases and RNA-binding proteins, in regulating RNA stability. We utilized diverse host strains lacking the expression of RNA processing enzymes and conducted phenotype screenings, including lysis and burst size assays. We identified several host RNA processing enzymes that show specific regulation of phage infection.

Further investigations including transcriptomics and proteomics of the identified candidates could deepen our understanding of the mechanism of phage infection, crucial for advancing phage therapy. This study makes significant contributions to understand the functional role of RNA-processing enzymes in host-phage interactions, potentially paving the way for innovative approaches to combat bacterial infections, particularly those caused by drug-resistant strains.

[1] Wolfram-Schauerte, M., Pozhydaieva, N., Viering, M., Glatter, T., & Höfer, K. (2022). Integrated Omics Reveal Time-Resolved Insights into T4 Phage Infection of E. coli on Proteome and Transcriptome Levels. Viruses, 14(11), 2502.

P-PMD-020

Characterization of the type III Druantia (DTIII) anti-phage defense system

*T. Gaudin[1]

[1]*HIRI, RSYN, Würzburg, Germany*

Mobile genetic elements and their bacterial hosts are in a never-ending evolutionary dance, giving rise to a multitude of defense and evasion systems for protection and infection. Most of these defense systems are still uncharacterized or poorly understood, and a substantial number of them have conserved domains of unknown function, with the potential to uncover completely new molecular mechanisms. Understanding the molecular mechanisms of these defense systems could lead to the development of unique new biotechnological tools, as has been the case for other bacterial defense systems such as CRISPR-Cas and restriction/modification systems.

The objective of this project is to characterize the molecular mechanism by which Druantia type III (DTIII) defense system protects bacteria against bacteriophage infection. This system consists of a two-gene operon, *druE* and *druH*. The *druE* gene, characteristic of the Druantia family, encodes a large putative protein composed of ATP-dependent SF2 helicase domains, whereas the *druH* gene has no known protein domains. So far, we characterized a set of phages that are sensitive to DTIII defense system, both by expression in a heterologous strain and by deletion/complementation of the defense system inside the native strain. Most of those phages are known to be sensitive to restriction modification systems, and present only few epigenetic modifications. Moreover, mutations in the predicted helicase domains of DruE lead to a loss of the defense activity. Using recombinant DruE, we could show that the latest interacts with ssDNA and presents an uncommon mode of nuclease activity depending on the size of its substrate. Those results suggest a defense mechanism based on recognition and cleavage of the invading phage DNA expanding our understanding of the many recently discovered defenses encoded by bacteria.

References

1.Doron, S. *et al.* Systematic discovery of antiphage defense systems in the microbial pangenome. *Science* **359**, eaar4120 (2018).

P-PMD-021

"Phage around the world": Use of bacteriophages for natural biocontrol of livestock-associated methicillin-resistant Staphylococcus aureus CC398 strains

*A. D. Knipper[1], C. Jäckel[1], T. Lienen[1], G. Shakeri[1], B. A. Tenhagen[1], J. A. Hammerl[1]

[1]*Federal Institute for Risk Assessment (BfR), Berlin, Germany*

MRSA CC398 is wide spread among livestock and humans in contact with food-producing animals are at high risk for colonization and infection. Natural biocontrol of MRSA in livestock could be achieved using virulent phages. However, it is challenging to compose preparations consisting of a diverse and safe spectrum of phages that complement each other in terms of efficacy and host range and avoid bacterial phage-resistance development. In the JPIAMR project 'Phage-Ex', virulent phages are being tested for their suitability for *S. aureus* biocontrol.

Lytic phage activities were determined using spot assays on overlay agar supplemented with 20 representative *S. aureus* isolates from German pig farms. The suitability and safety of the recovered phages were evaluated using *in vitro* biocontrol assays and genomic analysis. The suitability of different *S. aureus* strains for phage propagation was tested

to avoid cross-contamination of the preparations with spontaneously-induced prophages.

Although the sample material originated from a range of German pig farms and slaughterhouses, predominantly closely related podoviruses of the PSa-type were identified. These were highly efficient with similar host ranges covering ~70-75% of the 60 methicillin-resistance livestock MRSA isolates tested. Whole-genome sequencing and bioinformatics analysis show that the recovered phages are free of genes causing undesired effects. They are considered safe for application and *S. aureus* biocontrol. However, the selection of host bacteria needs further attention, as the propagation of high-titer phage lysates partially leads to the release of temperate phages encoding staphylococcal enterotoxins (SE).

Safe polyvalent *S. aureus* phages are ubiquitous in animal-associated sample materials, but are represented by a narrow spectrum of types. This makes it difficult to prepare compositions with broad bacterial receptor specificities to avoid the development of phage resistance in bacteria. Stress-induced prophage induction during propagation can lead to cross-contamination of the composition with temperate phages carrying SEs. This increases the likelihood of transduction of DNA from the bacterial host.

P-PMD-022

PhagoFlow – Practicability of Phage Therapy in Germany

*I. H. E. Korf[1], M. Häfner[2], S. Wienecke[1], C. Rohde[3], J. Wittmann[3], M. Leddin[4], W. Wenzel[4], K. Halama[5], D. Garbe[5], M. Stichling[2], H. Ziehr[1], C. Willy[2]

[1]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Department of Pharmaceutical Biotechnology, Brunswick, Germany

[2]Military Academic Hospital Berlin, Department Trauma & Orthopedic Surgery, Berlin, Germany

[3]Leibniz Institute DSMZ, Brunswick, Germany

[4]Military Academic Hospital Berlin, Department of Microbiology, Berlin, Germany

[5]Military Academic Hospital Berlin, Hospital Pharmacy, Berlin, Germany

Question:

The use of phages, viruses that specifically kill bacteria, is a promising approach to treating infections, as numerous successful individual cases show. However, phage therapy has not yet been established as standard medicine in Germany.

Therfore we aimed at the investigation of the practicability of phage therapy with focus on ESKAPE pathogens under the infrastructural conditions available in Germany in compliance with legal requirements.

Methods:

The general procedure is as follows: After isolation, characterisation, and selection of phages / bacterial hosts that allow an efficient production, phage active pharmaceutical ingredients (API) are manufactured according to quality standards assigned in consultation with the authorities. Before bulk APIs are delivered to the pharmacy, GMP-compliant release testing must be carried out to verify the identity, purity and content of the APIs. Phages are formulated by the pharmacy according to the indication, as specified by the microbiology and the treating physician .

Results:

Three phages against *P. aeruginosa* out of 140 were selected for production based on host range analysis, genetic properties, and lysis efficacy by the DSMZ. A platform-like manufacturing process has been established for phage-APIs, in which essential process parameters in cultivation and purification must be adapted to the individual phage to ensure successful production by Fraunhofer ITEM. Requirements for phage APIs in terms of documentation, specifications and analytics were agreed with the relevant authorities. After their release, the first three phage-APIs were delivered to the clinical partners. Sensitivity testing (phagogram) was performed by the department of microbiology, phage cocktails were formulated by the hospital pharmacy and patient treatment was performed by different clinical departments of the Military Hospital Berlin.

Conclusion:

Current requirements for phage GMP production necessitate a time-consuming process, which has led to a lack of available phages in the project. To make phage therapy accessible to many patients, it will be necessary to expand production and sensitivity testing capacities.

P-PMD-023

MetSVORF12's potential to promote viral replication of MetSV in Methanosarcina mazei

*B. Barüske[1], F. Gehlert[1], K. Weidenbach[1], M. Balsera[1], R. A. Schmitz[1]

[1]Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for General Microbiology, Kiel, Germany

Archaeal viruses are still the most enigmatic and unstudied part of microbiology. In recent years, novel archaeal viruses were isolated and characterized demonstrating a high diversity not only in morphology but also in genome structure. One of these was the Methanosarcina spherical virus (MetSV), a lytic virus of the *Tectiviridae* specifically infecting the methanoarchaeal model organism *Methanosarcina mazei*. MetSV's linear dsDNA genome has a size of ~ 10.5 kbp encoding 23 ORFs, of which 12 are classified as small ORFs[1,2] (sORFs). For a better understanding of the virus-host interaction, we searched for anti-defense proteins thus focusing on the sORFs and looked for possible interactions of the encoded proteins with the hosts proteome or DNA molecules revealing MetSVORF12s ability to bind DNA. Overexpression of MetSVORF12 in the host *M. mazei* showed a strong phenotype pointing towards an interference with its replication. Recently, we have established a genetic system for MetSV now allowing genetic approaches for functional characterizations[2]. Consequently, we created a deletion of ORF12 by side-directed mutagenesis in the plasmid-derived virus. Reinfection with ΔMetSVORF12 through liposome-mediated transformation into *M. mazei* resulted in culture lysis indicating a nonessential function but still reducing the virus efficiency compared to the wildtype. Currently, we are studying more potential interaction partners. Based on our results we propose a role of MetSVORF12 in the MetSV replication.

1. Weidenbach, K. *et al.* Methanosarcina Spherical Virus, a Novel Archaeal Lytic Virus Targeting Methanosarcina Strains. *J Virol* **91**, e00955-17 (2017).
2. Gehlert, F. O. *et al.* Newly Established Genetic System for Functional Analysis of MetSV. *IJMS* **24**, 11163 (2023).

P-PMD-024
Harnessing synthetic small RNAs to unravel phage resistance mechanisms

*G. Smyrlis[1], M. Siemers[1,2], K. Papenfort[1,2]
[1]*Friedrich Schiller University Jena, Microbiology, Jena, Germany*
[2]*Friedrich Schiller University Jena, Microverse Cluster, Jena, Germany*

Vibrio cholerae is a major human pathogen and the causative agent of the life-threatening diarrheal disease, Cholera. *V. cholerae*"s lifestyle is tightly linked to bacteriophages (or short phages), which are viruses that infect bacteria. Unlike lysogenic phages, such as phage CTXφ, which has a major impact in the development of toxigenic *V. cholerae* strains, lytic phages control cholera outbreaks by diminishing *V. cholerae* populations in the environment. One of these lytic phages, the vibriophage N4 (*Podoviridae* family), is able to infect and lyse various *V. cholerae* variants, however, the underlying molecular mechanisms are currently unknown.

In this project, we identified and characterized host factors required for N4 infection of *V. cholerae*, as well as defense mechanisms that inhibited this process. To this end, we employed a recently developed library of synthetic sRNA variants that we harnessed to screen for phage-resistance. Indeed, using high-throughput sequencing, we discovered and validated several sRNAs providing phage-resistance. Follow up experiments identified resistance mechanisms that either inhibited phage adsorption or interfered with different functions of the phage infection process.

Detailed characterization of the interactions of a highly abundant sRNA variant with its respective target transcripts revealed a role of the *V. cholerae"s* O-antigen lipopolysaccharide (LPS O-antigen) in the N4 phage adsorption process. Additionally, the resistance provided from other sRNA variants indicate their involvement in downstream regulatory processes occurring after phage DNA entry in the cell. Our data demonstrates that synthetic sRNAs are a powerful tool to characterize phage-host interactions and the mechanisms underlying phage resistance.

P-PMD-025
Mapping staphylococcal prophage release and horizontal gene transfer in the human nares

*D. Gerlach[1], L. Camus[2], S. Heilbronner[1]
[1]*Ludwig-Maximilians University Munich, Biocenter Microbiology, Martinsried, Germany*
[2]*Universtiy Tübingen, Tübingen, Germany*

Staphylococcus aureus is a major human pathogen. Nasal colonization by *S. aureus* is a significant risk factor for severe infections. Staphylococcal phages expand the genetic repertoire by horizontal gene transfer (HGT) and often carry genes benefitting the bacterial host such as resistance and immune evasion factors. Phage-mediated HGT in mainly *S. aureus* has been studied comprehensively, emphasizing the importance of phage-receptor interactions and anti-viral defence systems. Despite that little is known how and to what degree the natural ecological niche of *S. aureus*, the human nose, and its microbiome govern prophage induction and phage-mediated HGT.

To investigate this question, we assembled a bacterial collection of more than 290 bacterial isolates from 11 different communities, which we are currently characterising. We probed the ability of nasal commensals (n=150) and selected xenobiotics to modulate *S. aureus* prophage induction by using a lacZ-based reporter strain. Furthermore, we analysed the genomic viral content of 40 staphylococcal commensals from different nasal communities from the nasal collection and looked for occurrences of intra-communal HGT.

On the one hand we found no evidence for the capacity of nasal commensals or xenobiotics to directly influence *S. aureus* prophage induction. On the other hand, examining the shared genetic content of predicted staphylococcal prophages we observed indicators for genetic exchange within nasal communities among staphylococci. In further research we will investigate how staphylococci and other nasal commensals drive and participate in HGT leading to the development of countermeasures to prevent dissemination of virulence and resistance genes.

P-PMD-026
The enemy of my enemy is my friend: MDR bacteria and their natural enemies in process-/ wastewater from livestock slaughterhouses

C. Jaeckel[1], *J. A. Hammerl[1]
[1]*German Federal Institute for Risk Assessment, Biosafety, Berlin, Germany*

Wastewater is considered a hotspot for MDR bacteria and plays a role in their spread and impact on human, animal and environmental health (One-Health). As detailed information on the contribution of each sector is lacking, we investigated the occurrence of ESKAPE-/ESBL-producing bacteria from wastewater of livestock slaughterhouses and their municipal wastewater-treatment plants. The target species were ubiquitously identified along the slaughter and wastewater chains, but showed a wide variety of resistance patterns. Clinically important isolates were mainly recovered from municipal wastewater (i.e. CRE). Wastewater-treatment plants appear to be failing to eliminate these bacteria, resulting in their discharge into the preflooder and subsequent dissemination into the environment. However, further analysis of the wastewater also revealed the presence of natural enemies (bacteriophages) of the target bacteria. Lytic phages of different species were found to specifically attack their target bacteria and are able to significantly reduce the number of bacteria in-vitro. The genetic diversity of the phages obtained against a target species (e.g. *K. pneumoniae*) allows the development of suitable/safe cocktails with phage-members targeting different receptor binding proteins on the surface of the bacteria to avoid the development of phage-resistant clones. In addition, careful adaptation of the phage application to the specific purposes is necessary to develop efficient application strategies. Identified phage-members did not seem to be limited to wastewater treatment purposes, as bacteria for different sectors could be tackled using them in different combinations out of the developed phage-collection and can therefore be adapted to a wide variety of One Health approaches.

P-PMD-027
Isolation of novel bacteriophages against rare actinomycetes

*C. Rolland[1], I. Nouioui[1], S. Peter[1], Y. Mast[1], J. Overmann[1], J. Wittmann[1]
[1]*Leibniz Institute DSMZ, Brunswick, Germany*

Actinomycetes are a heterogenous group of Gram-positive bacteria belonging to the phylum *Actinomycetota*. They constitute one of the largest bacterial phyla and are present in terrestrial and aquatic ecosystems, mainly in soil. Some genera such as *Streptomyces* are well known as a rich source for novel antibiotics, insecticides and other secondary metabolites that could be useful in medicine, biotechnology, and agriculture, which makes them valuable bacteria for research and applications [1]. However, the phylum also includes pathogenic species, the most well-known is *Mycobacterium tuberculosis*, the causative agent of tuberculosis. Other species can induce diseases in plants, such as *Streptomyces scabies,* or in humans and animals, such as *Nocardia asteroides,* which causes Nocardiosis and mycetoma [1,2]. One approach to control these pathogenic agents might be to use bacteriophages. For rare actinomycetes, bacteriophages have been used as a selective tool to isolate novel natural product producer rather than as potential therapeutic agents [3]. In this regard, we aimed to isolate phages against rare actinomycetes from the DSMZ collection. Ten species of actinomycetes were selected, including *Lechevaliera rhizosphaerae, Actinomadura meyerae,* and *Salinispora pacifica.* We used soil samples (compost, fields, sediments) as well as water samples from rivers, lakes or sewage treatment plants. Two phages were isolated from compost samples, one against *Actinomadura meyerae* and the other against *Lechevaliera rhizosphaerae*. Characterization of these phages is in progress with host range determination and genomic analysis.

References

1. Barka EA, Vatsa P, Sanchez L, et al. Taxonomy, Physiology, and Natural Products of Actinobacteria [published correction appears in Microbiol Mol Biol Rev. 2016 Nov 9;80(4):iii]. *Microbiol Mol Biol Rev*. 2015;80(1):1-43. Published 2015 Nov 25. doi:10.1128/MMBR.00019-15
2. Verma P, Jha A. Mycetoma: reviewing a neglected disease. *Clin Exp Dermatol*. 2019;44(2):123-129. doi:10.1111/ced.13642
3. Kurtböke DI. Exploitation of phage battery in the search for bioactive actinomycetes. *Appl Microbiol Biotechnol*. 2011;89(4):931-937. doi:10.1007/s00253-010-3021-5

P-PMD-028
Exploring novel bacterial species and phage host spectrum within the Comamonadaceae Family

*I. Friedrich[1], D. Binder[1], J. Hollensteiner[1], R. Daniel[1]
[1]*University of Göttingen, Genomic and Applied Microbiology, Göttingen, Germany*

The *Comamonadaceae* family stands as a captivating focus of study due to its diverse array of bacterial species and their ecological significance. Comprising genera such as *Variovorax*, *Acidovorax*, *Comamonas*, and *Delftia*, this family is known for thriving in various environments, including eutrophic ponds and reed surface water.

This study unveils that strain DAIF25 is a novel species within the *Variovorax* genus, along with the isolation and characterization of *Acidovorax*, *Comamonas*, and *Delftia* species. These strains were isolated from a eutrophic pond and reed surface water in Göttingen (Germany) and thoroughly examined morphologically and physiologically. Through 16S rRNA sequencing DB30, DB31, and DB46 were identified as members of *Delftia*, DB36 as *Acidovorax*, and DB45 *as Comamonas*, whereas DAIF25 was characterized by whole genome sequencing.

This study reveals three spontaneously inducible prophages in the genome of DAIF25 and investigates the host range of these phages by demonstrating their ability to infect a variety of strains of the *Comamonadaceae* family using the overlay plaque assay. In summary, this research contributes to our understanding of *Variovorax* and the *Comamonadaceae* family, shedding light on their unique characteristics and associated phages.

P-PMD-029
Streptomyces development during phage infection and the role of the phage-encoded regulator WhiB

*T. Luthe[1], J. Frunzke[1]
[1]*Research Center Juelich , IBG-1: Biotechnology, Jülich, Germany*

Bacteria of the genus *Streptomyces* undergo a complex life cycle starting from single spores developing into a branched mycelium that is followed by formation of aerial hyphae and maturation of spores. Sporulation and related cell division in *Streptomyces* is controlled by a regulatory network involving WhiA and WhiB transcriptional regulators, which co-regulate genes critical to development. In this study, we focused on investigating the impact of phage infection on cellular development using the model species *Streptomyces venezuelae*. We observed that phage infection on solid media triggered the development of aerial hyphae and sporulation at the infection interface of the plaque. Analysis of mutant strains defective at different stages of *Streptomyces* development further confirmed the importance of cellular development for the establishment of transient phage resistance promoting the containment of viral infections.

Genome analysis of phages infecting actinobacteriophages showed that WhiB-like proteins represent the most abundant transcriptional regulator in these viruses. Specifically, 31 % of phages infecting *Streptomyces* harbor *whiB* including many containing further developmentally relevant genes. RT-qPCR reveals early expression of phage-encoded WhiB during infection and global transcriptome analysis suggest a suppression of cellular development in the early stages of infection. Expression of phylogenetically diverse phage-encoded *whiB* genes in an *S. venezuelae* Δ*whiB* strain are unable to complement the phenotype of this strain. These findings indicate that development is playing an important role during phage infection, both as host-specific phage defense and as target for phage-driven host manipulation. Altogether, our studies emphasize cellular development and the emergence of transient phage resistance as an important layer of *Streptomyces* antiviral immunity.

RNA Biology

P-RNA-001
Commonalities and differences of an sRNA tandem in Caulobacter crescentus

*M. Thamm[1], L. N. Vogt[1], K. Fröhlich[1]
[1]*Friedrich Schiller University Jena Institute of Microbiology, Jena, Germany*

Small RNAs (sRNAs) contribute significantly to post-transcriptional regulation in bacteria and influence stability and translation of target mRNAs through direct base-pairing interactions. The oligotrophic Alphaproteobacterium *Caulobacter crescentus* encodes more than one hundred sRNAs however the cellular role of the majority of these non-coding regulators is currently unknown. We have used genetic and biochemical approaches to characterize two *Caulobacter* sRNAs, ChvX and ChvY, which are encoded in tandem. Both sRNAs share significant similarity in sequence and structure, and are transcriptionally regulated by the conserved two-component system (TCS) ChvIG. Global transcriptome analyses upon ChvX and ChvY pulse expression revealed a largely overlapping pool of target candidates for both sRNAs, but also a small set of mRNAs only responding to either ChvX or ChvY. Employing a GFP-reporter system we have verified direct post-transcriptional regulation of targets, and confirmed predicted base-pairing interactions through compensatory exchanges in both RNA interaction partners.

P-RNA-002
Dissecting interactomes and functions of the RNA binding proteins KhpA and KhpB in the gastric pathogen Helicobacter pylori

*S. Konikkat[1], C. M. Sharma[1], S. Eisenbart[1], C. Lenz[2,3], H. Urlaub[2,3]
[1]*University of Würzburg, Institute of Molecular Infection Biology, Würzburg, Germany*
[2]*University Medical Center Göttingen, Department of Clinical Chemistry, Bioanalytics Group, Göttingen, Germany*
[3]*Max Planck Institute for Multidisciplinary Sciences, Bioanalytical Mass Spectrometry Group, Göttingen, Germany*

RNA binding proteins (RBPs) play central roles in gene regulation in all kingdoms of life. In bacteria, very little is known about globally acting RBPs beyond the well-studied RBPs Hfq, ProQ, and CsrA. Many bacteria, including the gastric pathogen *Helicobacter* pylori, lack homologs of Hfq and ProQ. This raises the question: Are there other RBPs with global regulatory roles in bacteria lacking these well-studied RBPs? Recently, two KH domain-containing proteins, KhpA and KhpB, which are widely conserved across many bacterial species, have been suggested as a new class of globally acting RBPs. RNA-immunoprecipitation and sequencing (RIP-seq) in *Streptococcus pneumoniae* and *Clostridiodes difficile* revealed that KhpA/B bind to a large number of RNAs, including sRNAs and mRNAs. KhpA/B are associated with cell division, virulence, and toxin production in these bacteria as well. In *H. pylori,* KhpB was shown to interact with and modulate the activity of the ATPase of the type 4 secretion system (T4SS), a key virulence factor required to inject the effector protein CagA into the host cell. The RNA binding activity of KhpA/B and their potential roles in regulating the cellular processes of *H. pylori*, including T4SS activity, remain unexplored. To understand the roles of *H. pylori* KhpA/B, we identified their RNA interaction partners using RIP-seq. Our data indicate that *H. pylori* KhpA/B interact with 5"UTRs and coding sequences of genes, several of which can be validated by electrophoretic mobility shift assays (EMSA). Protein interactome capture of KhpA/B by co-immunoprecipitation of FLAG-tagged proteins followed by mass spectrometry indicates potential interactions of KhpA/B with a larger number of proteins associated with different layers of bacterial physiology. Our preliminary results show that the deletion of *khpA/B* impacts *H. pylori* stress responses and gene expression, suggesting that they could play important roles in *H. pylori* cellular processes and potentially, infections.

P-RNA-003
Sibling Team Work – Interplay of a family of regulatory RNAs with an RNA sponge in Caulobacter crescentus

*L. N. Vogt[1], M. Velasco Gomariz[1], M. Siemers[2], K. Papenfort[2,3], K. Fröhlich[1,3]
[1]*Bacterial RNA Biology Group, Institute of Microbiology, Friedrich-Schiller University Jena, Jena, Germany*
[2]*General Microbiology, Institute of Microbiology, Friedrich-Schiller University Jena, Jena, Germany*
[3]*Microverse Cluster, Friedrich-Schiller University Jena, Jena, Germany*

Small regulatory RNAs (sRNAs) contribute significantly to the post-transcriptional control of gene expression in bacteria. sRNAs may influence mRNA translation and stability by engaging in direct base-pairing interactions with target transcripts. In some cases, a given bacterium expresses multiple, seemingly homologous sRNAs which are called sibling sRNAs. The physiological advantage of encoding several copies of nearly identical regulators or how their activity is integrated into established regulatory circuits is generally not well understood.

The α-proteobacterium *Caulobacter crescentus* encodes four sibling sRNAs of the αR8 RNA family sharing conserved sequence elements and a characteristic secondary structure. The αR8 sRNAs are expressed from independent genomic loci under different environmental conditions, including carbon availability and iron deficiency, indicating sibling-specific transcriptional input. To determine the target spectra of each family member we performed comparative transcriptome analyses and identified both shared as well as individual target interactions. Additionally, by performing RIL-seq (RNA interaction by ligation and sequencing) we recovered interactions between the the αR8 sRNAs and the sRNA CrfA. CrfA is induced in response to carbon starvation and acts as a sponge RNA that binds to and facilitates the turn-over of its paralogous interaction partners. Using both *in vivo* as well as *in vitro* approaches, we have untangled a complex post-transcriptional network modulating carbon metabolism during adaptation to changes in nutrient availability.

P-RNA-004
Investigation of temperature-regulated mechanisms in Yersinia pseudotuberculosis

*D. Meggers[1], S. Javadi[1], S. Pienkoß[1], F. Narberhaus[1]
[1]*Ruhr University Bochum, Microbial Biology, Bochum, Germany*

As a pathogenic bacterium, *Yersinia pseudotuberculosis* is confronted with constantly changing conditions during infection of warm-blooded hosts and after excretion into the environment. In order to adapt quickly to these changing stresses, the bacterial cell uses sophisticated regulatory mechanisms at the DNA, RNA and protein level. Among the

various environmental influences, temperature fluctuations play a prominent role. Bacteria are able to monitor the ambient temperature by *cis*-acting RNA thermometers (RNATs), which are localized in the 5'-UTR and form a complex RNA secondary structure. These RNA structures gradually melt as the temperature rises, liberating the ribosome binding site and thus regulating the expression of the downstream open reading frame. In contrast to such gradually melting RNATs, "cold thermosensors" undergo a complete rearrangement of the RNA structure and expose the ribosome binding site (RBS) at low temperatures. In *Y. pseudotuberculosis*, we have described several RNATs that regulate virulence genes coding for LcrF, the master regulator of virulence, or for components of type 3 secretion system and and an exotoxin.

Parallel analysis of RNA structures (PARS) has identified an ok RNAT candidate upstream of *yopH*, coding for an essential effector protein of the T3SS. Translational control by this RNAT was demonstrated by reporter gene studies. Point mutations were introduced to create open and closed RNAT versions and tested for functionality. In addition, the structure and RNAT-like melting of the RBS was shown by *in vitro* structure probing.

In addition, we search for cold thermosensors that regulate gene expression once the bacterium leaves the host and re-enters the environment. Several candidates have emerged from our global RNA structuromic studies and will be subject to structure-function analyses.

P-RNA-005

Development of novel formulations for dsRNA based on cationic interpolyelectrolyte complexes

B. Moorlach[1], *D. Jakobs-Schönwandt[2], M. Poranen[3], A. Patel[1]
[1]*Bielefeld Universtiy of Applied Sciences, Bielefeld Institute for Applied Materials Research, Bielefeld, Germany*
[2]*Westphalian University of Applied Sciences, Bioengineering and Sustainability, Recklinghausen, Germany*
[3]*Universtiy of Helsinki, Faculty of Biological and Environmental Sciences, Helsinki, Finland*

Growing concerns over negative effects of pesticides on the environment and on human health are the cause of an increased demand for environmentally sustainable plant protection agents. A promising substitute for synthetic pesticides is double stranded RNA (dsRNA). Its application triggers RNA-induced gene silencing, also known as RNA interference (RNAi), a cellular mechanism of eucaryots to protect themselves against pathogens.

The aim of our investigation is to develop a spray-induced gene silencing approach with dsRNA artificially produced in a bacterial host system and formulated with biologically degradable components to stabilize and protect the dsRNA and increase its uptake in planta.

To overcome plant barriers such as size-exclusion limits and charge and to provide protection, we developed a cationic dsRNA-biopolymer carrier formulation based on the electrostatic interaction of selected cationic and anionic biopolymers. To moderate the plant uptake, biopolymers with appropriate characteristics, e.g. molecular weight and charge, were chosen and formulated with the optimal parameters, such as charge ratio, polymer concentration, and energy input, to achieve a formulation with a positive zeta potential, indicating charge masking of negatively charged dsRNA.

The hydrodynamic diameter of our formulations was determined by dynamic light scattering and was 93.7 +- 2.9 nm with a PDI of 0.214 ± 0.024 at the optimal charge ratio of 1.25 (+/-) and at a total polymer concentration of 0.005 %. Scanning electron microscopy analysis confirmed the diameter and revealed spherical particles. Furthermore, energy-dispersive X-ray spectroscopy verified dsRNA integration into the particles by the presence of a phosphorous peak. Moreover, electrophoretic light scattering measurements indicated a positive zeta-potential of +20 to +50 mV, which confirms that the dsRNA was successfully masked.

By optimizing loading ratio and polymer concentration during the process, dsRNA was formulated in sherical particles with a hydrodynamic diameter of < 100 nm and a positive zeta potential, which should improve the uptake of dsRNA in plants.

P-RNA-006

RNA structures as targets for antimicrobial compounds

*D. Scheller[1], R. N. Das[2,3], K. Islam[4], S. Sandberg[5], E. Chorell[2,3], N. Arnberg[4], J. Johansson[1]
[1]*Umeå University, Molecular Biology, Umeå, Sweden*
[2]*Umeå University, Chemistry, Umeå, Sweden*
[3]*Chemical Biology Consortium Sweden, Umeå, Sweden*
[4]*Umeå University, Clinical Microbiology, Umeå, Sweden*
[5]*Umeå University, Medical Biochemistry and Biophysics, Umeå, Sweden*

Regulatory RNA structures play an important role for pathogens. Many virulence-related genes encode a temperature-sensitive mRNA element, a so-called RNA thermometer (RNAT) in their transcript. Upon entry into a warm-blooded host, this RNAT structure melts open, allowing translation of the downstream gene. The virulence regulator PrfA of *Listeria monocytogenes* is controlled by such an RNAT. Further important RNA structures can be found in RNA viruses, such as the viral frameshift element. The viral frameshift element leads to a frameshift during translation, allowing different expression ratios of viral polyproteins located in the ORF1a and ORF1ab open reading frames. Disturbing this ratio of frameshifting, severely cripples coronavirus replication. Due to the importance of RNA structures for virulence, targeting by antimicrobial compounds could be a suitable alternative to classical protein-targeting antibiotics. By different Förster Resonance Energy Transfer (FRET) approaches we will screen a library aiming to identify compounds altering RNA structures. As a proof-of-concept approach, we have used aminoglycosides and validated their inhibitory effect on viral translation by a dual-luciferase reporter system. Furthermore, we could observe a reduction of viral infection by determining the viral titer after infection. Therefore, targeting RNA structures seems to be a promising way to treat clinically relevant infections.

P-RNA-007

Identification of sRNAs in the response to DNA damage in Klebsiella pneumoniae

*E. Ruhland[1], K. Fröhlich[1]
[1]*Friedrich Schiller University Jena, Microbiology, Jena, Germany*

The opportunistic human pathogen *Klebsiella pneumoniae* has recently gained attention due to the spread of hypervirulent and multidrug-resistant strains. *Klebsiella's* ability to acquire resistances to different antibiotics depends

on the assimilation of additional genetic material and on *de novo* mutations. The risk of genomic mutations increases in response to DNA damage, when error-prone polymerases are expressed as part of a coordinated program termed the SOS response. While the SOS pathway is well-studied for many bacterial species, little is known about the regulon and the associated transcriptome in response to DNA damage in *Klebsiella*.

To fill this gap, we have combined dRNA-seq to define transcription start sites and unannotated small regulatory RNAs (sRNAs) with RNA co-immunoprecipitation (RIP-seq) to analyze interactions with the major RNA-binding protein Hfq, and RIL-seq to analyze the Hfq-mediated RNA-RNA interactome of a multidrug-resistant *Klebsiella* strain. In addition, we performed ChIP-seq to map the set of genes directly controlled by the key SOS regulator, LexA. Together, our datasets revealed a rich landscape of coding and non-coding transcripts induced in response to DNA damage and we present results on the identification and characterization of LexA-controlled sRNAs in *K. pneumoniae*.

P-RNA-008

Recording isoform usage at single-cell resolution in macrophages and monocytes in diseased tissues

*A. Grinko[1], A. E. Saliba[1,2]
[1]*Helmholtz Institute for RNA-based Infection Research, SIGA, Würzburg, Germany*
[2]*Institute of Molecular Infection Biology (IMIB), Würzburg, Germany*

The advances of single-cell RNA-sequencing (scRNA-seq) allow to characterize the myeloid cell landscape providing a comprehensive map of monocyte/macrophage transitions in diseased tissue with unprecedented resolution. However, the short read length of conventional sequencing and the selective targeting of the 3" or 5" end of RNA molecules hinders the capture of full-length RNAs. Consequently, isoform elucidation remains elusive and valuable splicing information is lost. In contrast, long-read sequencing as provided by Pacific Biosciences conserves the full-length of the transcripts and detects multiple isoforms of the same gene. Here we hampered single-cell MAS-ISO-sequencing (scMAS-ISO-seq), a long-read sequencing approach based on the concatenation of cDNA fragments into long arrays [1]. We applied scMAS-ISO-seq to profile the transcriptomic landscape of murine monocyte-derived macrophages in diseased tissues. Using scMAS-ISO-seq, we unveiled cell-type-specific isoform utilization and differential splicing events among macrophage and monocyte populations. Additionally, our approach resolved differential isoform usage temporarily, correlating distinct isoforms with pseudotime ordering. We demonstrated scMAS-ISO-seq's potency in read length, throughput, and cell type identification and captured thousands of unique isoforms. scMAS-ISO-seq potentially emerges as a robust technology for investigating isoform dynamics in immunology and infection research, offering an unprecedented level of transcriptomic information.

P-RNA-009

RNA-Protein conjugation in pathogen-host interactions – towards an atlas of RNAylations

*M. Weber[1,2], D. Staiger[3], K. Höfer[1,2]
[1]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*
[2]*Max-Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[3]*Universtiy Bielefeld, Bielefeld, Germany*

Introduction:

ADP-ribosyltransferases (ARTs) are found in all domains of life. A common function of ARTs is acting as a toxin in pathogen-host interactions. ARTs use NAD+ (NAD) as a substrate to attach ADP-ribose to their target proteins – a post-translational protein modification called ADP-ribosylation. Interestingly, NAD has also been discovered to be attached to specific transcripts at the 5´-terminus, forming "NAD-capped RNAs".

Recently, an ART of the T4 phage was shown to be used in addition to NAD and NAD-capped RNA as a substrate, resulting in a covalent connection between RNA and proteins in a reaction called RNAylation. This discovery connects the realms of protein- and RNA-modification. However, the biological role of RNAylation is still not fully understood. As RNAylation is a newly discovered reaction, it only describes the interaction of T4 phages and *E. coli*, where it was first discovered. However, the ubiquity of both ARTs and NAD-capped RNA raises the question of whether RNAylation occurs in other organisms or interactions.

Objectives:

This project addresses the question of whether RNAylation is a niche phenomenon or a widespread biological concept. We aim to screen for ARTs in various organisms or pathogen-host interactions beyond T4 phage-infected *E. coli* that can catalyse an RNAylation reaction. The focus is on bacterial ARTs, which modify host proteins. The ability of these enzymes to perform RNAylation *in vitro* and *in vivo* is investigated. Potentially occurring RNAylations are analysed for their target proteins, site selectivity, and their competition with ADP-ribosylations.

Materials and Methods:

To tackle these questions, we employ a combination of biochemical methods, enzyme assays using purified proteins and/or host lysates and proteomics.

Results:

We unveil the ability of a bacterial ART to not only ADP-ribosylate, but also RNAylate target-proteins *in vitro*. This discovery proves that more than one ART possesses RNAylation activity. Future studies will focus on the ability of this ART to perform RNAylations in host lysates to evaluate its biological significance.

References:

Wolfram-Schauerte et al., *Nature* 620.7976 (2023): 1054-1062.

P-RNA-010

The RNA-binding protein RbpB is a central regulator of polysaccharide utilization in gut Bacteroides

*A. S. Rüttiger[1,2], D. Ryan[1], L. Spiga[3], V. Lamm-Schmidt[1], G. Prezza[1], S. Reichardt[1], L. Barquist[1,4], F. Faber[1,2,5], W. Zhu[3], A. J. Westermann[1,2,6]
[1]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*
[2]*University Würzburg, Institute of Molecular Infection Biology, Würzburg, Germany*

[3]Vanderbilt University, Department of Pathology, Microbiology, and Immunology, Nashville, TN, United States
[4]University of Würzburg, Faculty of Medicine, Würzburg, Germany
[5]University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany
[6]University of Würzburg, Biocentre, Department of Microbiology, Würzburg, Germany

Paramount to human health, symbiotic bacteria in the gastrointestinal tract rely on the breakdown of complex polysaccharides to thrive in this sugar-deprived environment. Gut *Bacteroides*, as metabolic generalists, deploy dozens of polysaccharide utilization loci (PULs) to forage diverse dietary and host-derived glycans. The expression of the multi-protein PUL complexes is tightly regulated at the transcriptional level. However, how PULs are orchestrated at translational level in response to fluctuating substrate levels is unknown. Here, we identify the RNA-binding protein RbpB and a family of noncoding RNAs as key players in post-transcriptional PUL regulation. Ablation of RbpB in *Bacteroides thetaiotaomicron* compromises colonization in the mouse gut, dependent on the host diet. Current dogma holds that individual PULs are regulated by dedicated transcriptional regulators. We demonstrate that RbpB acts as a global RNA binder interacting with several hundred cellular transcripts. This includes a paralogous noncoding RNA family comprised of 14 members, the FopS (family of paralogous sRNAs) cluster. Through a series of *in-vitro* and *in-vivo* assays, we reveal that FopS sRNAs repress the translation of a SusC-like glycan transporter when substrates are limited —an effect antagonized by RbpB. Together, this study implicates RNA-coordinated metabolic control as an important, yet overlooked, factor contributing to the *in-vivo* fitness of *Bacteroides* in dynamic nutrient landscapes.

P-RNA-011
Functional characterization of a pair of KH-domain containing RNA-binding proteins in Campylobacter jejuni

*M. Narayan[1], S. Eisenbart[1], S. Sharma[1], L. Hadjeras[1], F. König[1], T. Bischler[2], C. Lenz[3,4], H. Urlaub[3,4], C. M. Sharma[1]
[1]Institute of Molecular Infection Biology (IMIB), Department of Molecular Infection Biology II, Würzburg, Germany
[2]University of Würzburg, Core Unit SysMed, Würzburg, Germany
[3]Max Planck Institute for Multidisciplinary Sciences, Bioanalytical Mass Spectrometry, Göttingen, Germany
[4]University Medical Center Göttingen, Bioanalytics, Department of Clinical Chemistry, Göttingen, Germany

RNA-binding proteins (RBPs) are emerging as integral regulators of RNA networks controlling the physiology and virulence-associated processes in bacterial pathogens. Homologs of well-studied RBPs like Hfq and ProQ are missing in several bacteria, including *Campylobacter jejuni,* the leading cause of bacterial gastroenteritis in humans. The RBP players involved in post-transcriptional regulation of such pathogens remain to be discovered. Using a novel unbiased RBP capture method, our lab identified several new RBP candidates in *C. jejuni*, including a largely unexplored KH-domain protein, KhpB. Reciprocal co-immunoprecipitation (coIP) revealed that KhpB interacts with another KH-domain protein, KhpA. Both KH-domain proteins are widely conserved among many bacteria. Hence to gain insights into the cellular functions of these KH-domain proteins, we examined the phenotype of *khpA/B* deletions and their molecular interactome in *C. jejuni*. Notably, deletion of *khpA* or *khpB* decreased the pathogen"s abilities to adhere and invade Caco-2 cells. Using RNA co-immunoprecipitation and sequencing (RIP-seq) of epitope-tagged KhpA and KhpB, we identified many distinct and overlapping sets of mRNAs and a few sRNAs as potential RNA binding partners of KhpA/B, suggesting their roles as RBPs. RNA binding of KhpA/B to specific RNAs was further demonstrated using *in-vitro* binding assays like electrophoretic mobility shift assays (EMSA). RIP-seq upon deletion of partner Khp protein reveals loss of ~97% of the co-purified RNA targets, thus highlighting their inter-dependent RNA-binding property. Moreover, mass spectrometry-based protein interactome analysis of KhpA/B uncovered several potential protein interaction partners of KhpA/B. Overall, our findings suggest that KhpA/B fulfill diverse cellular functions in bacteria harboring this class of RNA-binding proteins.

P-RNA-012
Identification and characterization of novel RNA-binding proteins in Salmonella Typhimurium

*J. Kullmann[1], T. Prossliner[1], S. Eisenbart[1], M. Alzheimer[1], E. Fiore[1], C. M. Sharma[1]
[1]Institute of Molecular Infection Biology, University of Würzburg, Department of Molecular Infection Biology II, Würzburg, Germany

RNA binding proteins (RBPs) are important effectors of post-transcriptional control, affecting the stability, maturation, translation, or localization of bound RNAs. They thereby exert a crucial role in bacterial physiology, pathogenicity and stress. Although some bacterial RBPs are well-characterized, discovery of novel RBPs in bacteria has been largely serendipitous. Only recently global approaches for RNA-binding proteome (RBPome) capture in prokaryotes have been developed, revealing many previously unidentified RNA-interacting proteins. The increasing number and diversity of potential RBPs further adds to the complexity of the bacterial RBPome and underscores the importance of identifying and characterizing RBPs. Our group recently developed an approach termed CoCAP to globally capture RBPs in bacteria. Applying this method to the enteropathogenic proteobacteria *Salmonella* Typhimurium and *Campylobacter jejuni,* we were able to capture known RBPs, such as RNA chaperones Hfq and ProQ (in *Salmonella*) as well as RNases, translation factors and ribosomal proteins. Importantly, we also identified a considerable number of novel RBP candidates, many of which lack known RNA binding domains and have well-established cellular functions unrelated to RNA. These include diverse processes including cell cycle control, metabolism, replication, and virulence. We speculate that such unconventional RBPs may moonlight as RNA regulators in addition to their canonical roles in response to distinct conditions, or bound RNAs act as riboregulators and impact on the proteins' function, stability or localization. To ensure that the identified candidates are bona fide RBPs, we have been validating selected candidates by applying diverse techniques. We now aim to further characterize their interaction with RNA and elucidate the regulatory consequences of these interactions. Expanding our novel RBP-capture approach to a variety of infection- and stress-relevant conditions will further broaden our knowledge of the RBP repertoire of bacteria and aid our understanding of post-transcriptional regulation and its effects on bacterial physiology.

P-RNA-013
Studying the impact of post-transcriptional gene regulation for natural competence in Vibrio cholerae

*J. J. García Yunge[1], S. Krautwurst[1], K. Papenfort[1,2]

[1]Friedrich Schiller University Jena, Microbiology, Jena, Germany
[2]Microverse Cluster, Jena, Germany

Outside of the human host the pathogen *Vibrio cholerae* is commonly found in aquatic environments as biofilms attached to chitinous surfaces, which serve as an environmental niche and as a food source. Chitin sensing induces various physiological changes through the activation of multiple genetic pathways, one of which is natural competence, a state during which bacteria are able to acquire exogenous DNA from the environment to be used for nutrition, horizontal gene transfer, and DNA repair. While there are various processes known to induce competence in *V. cholerae*, the regulatory process leading to competence are still not fully explored. One key regulator is the chitin-induced, Hfq-dependent small RNA (sRNA) TfoR, which activates *tfoX* mRNA, encoding the master regulator of competence in *V. cholerae* [1]. Usually regulatory sRNAs have multiple targets, yet for TfoR just a small number of partners have been identified [2], suggesting there might be more to uncover.

To better understand the regulatory role of TfoR in *V. cholerae* and its role in DNA uptake, we explored the global RNA-RNA network using RIL-seq (RNA-interaction-by-ligation-and-sequencing) approach under competence-inducing conditions to uncover new RNA-RNA interactions. Our RIL-seq results show dozens of new interacting partners, with TfoR occupying approximately 23% of all Hfq mediated interactions under chitin conditions. We have also confirmed many targets, which are related to carbon metabolism or transporters. Additionally, our results revealed TfoR is cleaved by RNase E producing two sRNA isoforms with apparently different regulatory functions.

References

[1] Yamamoto, S., *et al.,* (2011). Identification of a chitin-induced small RNA that regulates translation of the *tfoX* gene, encoding a positive regulator of natural competence in *Vibrio cholerae*. Journal of bacteriology, 193(8), 1953-1965.

[2] Huber, M., *et al.,* (2022). An RNA sponge controls quorum sensing dynamics and biofilm formation in *Vibrio cholerae.* Nature Communications, 13(1), 7585.

P-RNA-014
Functional characterisation of the RaiA noncoding RNA in Clostridioides difficile

*J. Sulzer[1], T. Lence[1], V. Lamm-Schmidt[1], A. S. Gribling[2], R. Smyth[2], L. Barquist[2], F. Faber[1,2]
[1]University Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany
[2]HIRI, Würzburg, Germany

In all bacteria there are non-coding RNAs (ncRNAs) that fulfill a variety of cellular functions, e.g., as catalysts of chemical reactions, as structural components in multiprotein complexes or to control gene expression at the transcriptional and post-transcriptional level. Some RNA regulators display exceptionally broad conservation across bacterial phyla, and these fulfill fundamental cellular functions, such as the ribosomal 5S, 16S and 23S RNAs. Using RNA-seq based transcriptome annotation in *C. difficile*, we recently observed the expression of a ncRNA that belongs to a family of uncharacterized RNA motifs with complex secondary structure and high conservation across two bacterial phyla, which is termed RaiA (1, 2).

The structural complexity and high conservation point towards a sophisticated biochemical function and an important physiological role, which motivated us to perform a detailed characterization in *C. difficile*.

To determine the physiological relevance, we created *raiA* deletion mutants that display an overgrowth phenotype that is particularly pronounced upon addition of monosaccharides. In line with the growth phenotype, transcriptome analyses reveal that deletion of *raiA* alters expression of many amino acid and carbohydrate transport genes and elevates the levels of many tRNAs during stationary phase. We find that a *raiA* mutant shows strongly suppressed sporulation while producing elevated levels of toxins, directly implicating RaiA in *C. difficile* virulence. Complementation of the *C. difficile raiA* mutant with orthologues from different Firmicutes families restored sporulation and toxin production to wild type levels, indicating that the ncRNA function is preserved across species. In summary, we have established RaiA as substantial regulator of physiology in *C. difficile.*

(1) Lamm-Schmidt et al., (2021) Microlife
(2) Weinberg et al., (2017) NAR

P-RNA-015
Mechanistic insights into sRNA::mRNA interaction: the thermo-dynamic RNA structures and lithe interplay between CyaR and ompX in Yersinia pseudotuberculosis

*D. Guanzon[1], S. Pienkoß[1], J. Röder[1], A. Dietze[1], F. Narberhaus[1]
[1]Ruhr-University Bochum, Microbial Biology, Bochum, Germany

Bacteria adapt rapidly and continuously to their ever-changing environment by employing precise regulatory mechanisms at all levels—from transcription to translation, and protein degradation. Temperature as stimulus is particularly important for pathogens, which transition from cool ambient temperatures to an abrupt warm environment and back. We are gradually piecing together the intricate details of how *Yersinia pseudotuberculosis* responds to temperature changes.

In this study, we explored how *trans*-regulatory elements, such as the small RNA CyaR, structurally respond to temperature and how it would affect its regulatory efficiency. Our previous structuromic datasets reveal compelling conformational changes at 25 and 37°C in CyaR, where the seed region is hidden by a secondary structure that could impede further interactions with its regulatory target. Our initial comparative transcriptome analysis between *Yersinia* WT and a ΔCyaR mutant shows *ompX* is differentially regulated between the two temperatures in exponential phase. Complementation assays validate *ompX* as the first direct interaction partner of CyaR in *pseudotuberculosis.*

Moreover, in-line probing experiments provide compelling mechanistic insights into the structural interplay of CyaR::*ompX* interactions at 25 and 37°C. Our results suggest that intramolecular folding of the *ompX* 5'-UTR is favored at lower temperatures. As both transcripts breathe open at higher temperatures, intermolecular interactions between *ompX* and CyaR start to prevail. This in turn affects the ability of the 30S to form translation initiation complexes. Effective competition of CyaR with the ribosomal subunit

reduces translation efficiency at 37°C. Two additional regulatory levels, the reduced stability of the *ompX* transcript and the OmpX protein at elevated temperatures contribute to the net result that OmpX production is higher at 25°C.

P-RNA-016

RNA interaction and localization of NudC, the NAD-RNA decapping enzyme in E. coli

*D. Rodríguez Méndez[1], K. Höfer[1]
[1]Max-Planck Institute for Terrestrial Microbiology, Bacterial Epitranscriptomics, Marburg, Germany

Introduction: 5´-NAD-RNA capping was described as the first transcriptional modification in bacteria, in which the NAD-cap provides transcript protection against endonuclease degradation, like RNAse E.

In E. coli, NudC was originally described as a Nudix hydrolase, hydrolyzing NAD into AMP and NMN. However, NudC is the main NAD-decapping enzyme, since it has a stronger affinity towards 5´-NAD-RNA, hydrolyzing it into 5´-P-RNA and NMN.

Although the NudC structure has been resolved, no RNAbinding motif has been identified, and the mechanisms for the NudC-RNA interaction remain elusive.

Intrigued by these observations we hypothesized that the NudC-RNA interaction promotes NudC catalytic activity, such interaction might further result in the association with the RNA degradosome complex to facilitate RNA turnover.

Objectives:

• Identify the residues of NudC required for RNA interaction.

• Elucidate NudC localization and analyze changes upon RNA depletion.

• Demonstrate the association of NudC with the RNA degradosome.

Material and Methods:

• To identify residues required for NudC-RNA interaction, Hydrogen-Deuterium exchange (HDX) assays were performed. Site-directed mutagenesis was performed on the identified residues, recombinant proteins were purified and used for i*n vitro* NAD-decapping assays.

• To analyze subcellular localization, NudC was fused to the fluorescent protein mNeongreen, and single molecule tracking (SMT) assays were performed.

Results: HDX assays led to identifying two main residues of NudC, which showed a significatively reduced activity towards NAD-RNA when these residue were substituted. Furthermore, it was observed that NudC displays precise confinement towards the membrane and a static behavior, which was severely modified upon RNA depletion. Both phenotypes were lost when NudC no longer had the ability to interact with RNA, displaying cytosol localization and fast diffusive behavior.

Conclusion: Altogether, the presented data demonstrate the mechanisms by which NudC interacts with RNA. Such interaction promotes its decapping activity and results in NudC confinement to the membrane and association with the RNA degradosome.

P-RNA-017

Studies on the biological significance of RNA editing in filamentous fungi

U. Lipka[1], M. Skendrou[2], J. Grygosch[2], L. Bleeken[1], *I. Teichert[1]
[1]Georg-August University Göttingen, Forest Botany and Tree Physiology, Göttingen, Germany
[2]Ruhr University Bochum, General and molecular botany, Bochum, Germany

RNA editing is the selective insertion, deletion, or substitution of nucleotides and is conserved in all domains of life. RNA editing of protein-coding transcripts leads to sequence changes in the transcript as well as the protein that could alternatively be directly encoded in the DNA. In filamentous ascomycetes, adenosine (A) to inosine (I) RNA editing was recently detected to occur in protein-coding transcripts during sexual reproduction. It is supposed to be generally adaptive and has been hypothesized to be required for ascospore formation and / or ascospore germination. However, many open questions about fungal editing remain.

To gain insight into the biological role of editing, we analyzed genes whose transcripts are affected by editing in the ascomycete *Sordaria macrospora*. Deletion of several *efd* genes indeed revealed a function of these genes during ascospore formation and/or discharge. Complementation studies with mutations of the native stop codon to a *TGG* (always long protein) or a *TAA* (always short protein) revealed possible functions for the editing sites in ascospore formation. Studies on the function of editing during ascospore germination in different physiological conditions are underway. Further, we study mutants unable to form ascospores for the detection of editing regulators. Taken together, these studies should provide insight into ascospore formation in fungi, but also into the widespread phenomenon of mRNA editing also found in metazoans and bacteria.

P-RNA-018

Deciphering the tRNA Epitranscriptome: Implications for Pseudomonas aeruginosa Pathogenicity

*Y. N. Frommeyer[1], N. O. Gomez[2], M. Preusse[2], A. A. Rodriguez[2], K. Neubauer[2], B. Kennepohl[1,3], J. F. Witte[1,3], J. Erdmann[1,2], A. Pich[3], H. Bähre[4], D. P. Depledge[5,6,7], S. Häußler[1,2,7]
[1]TWINCORE – Centre for Experimental and Clinical Infection Research GmbH, Institute of Molecular Bacteriology, Hannover, Germany
[2]Helmholtz Center for Infection Research, Institute of Molecular Bacteriology, Brunswick, Germany
[3]Hannover Medical School, Research Core Unit Proteomics and Institute for Toxicology, Hannover, Germany
[4]Hannover Medical School, Research Core Unit Metabolomics, Hannover, Germany
[5]Hannover Medical School, Institue of Virology, Hannover, Germany
[6]German Center for Infection Research, Hannover, Germany
[7]Hannover Medical School, Excellence Cluster 2155 RESIST, Hannover, Germany

Introduction

Transfer RNAs (tRNAs) are crucial adapter molecules facilitating protein synthesis by decoding mRNA sequences. They harbor a diverse range of conserved chemical

modifications, collectively known as the tRNA epitranscriptome, which play pivotal roles in maintaining translational accuracy and structural integrity. Perturbations in tRNA modification levels can disrupt protein homeostasis and impact bacterial virulence phenotypes on a global scale. [1], reviewed in [2].

Aim of the study

Here, we elucidated the biosynthesis of 5-carboxymethoxyuridine (xo^5U) tRNA modifications in the opportunistic human pathogen *P. aeruginosa*. New insights into the nature and dynamics of post-transcriptional tRNA modification promise to expand our current understanding of gene regulation mechanisms shaping bacterial behavior with potential implications for antimicrobial therapy development.

Results

In the first part, we identified genes responsible for the biogenesis and characterized the epitranscriptomic landscape of xo^5U modifications in *P. aeruginosa* tRNA using LC-MS/MS and direct tRNA nanopore-sequencing. Mutant strains lacking xo^5U modifications exhibited altered virulence and secondary metabolite production, highlighting the significance of these modifications in bacterial physiology. Furthermore, we applied a multi-omics approach to unravel the molecular basis for xo^5U tRNA modification-dependent translational regulation.

Key methods used in this study

- Targeted LC-MS/MS
- Direct tRNA nanopore-sequencing (nano-tRNAseq [3])
- Transcriptomics/Translatomics/Proteomics analyses
- Phenotypic characterization assays (Virulence, secondary metabolite quantification, bacterial growth monitoring)

References

[1] Y. H. Chionh *et al.*, "TRNA-mediated codon-biased translation in mycobacterial hypoxic persistence," *Nature Communications*, doi: 10.1038/ncomms13302.
[2] V. de Crécy-Lagard *et al.*, "Functions of Bacterial tRNA Modifications: From Ubiquity to Diversity," *Trends in Microbiology*, doi: 10.1016/j.tim.2020.06.010.
[3] M. C. Lucas *et al.*, "Quantitative analysis of tRNA abundance and modifications by nanopore RNA sequencing.," *Nature biotechnology*, 2023, doi: 10.1038/s41587-023-01743-6.

Regulation & Small Proteins

P-RSP-001
Redirecting carbon flux towards products in cyanobacteria by engineering the regulatory mechanism of the 2,3-bisphosphoglycerate-independent phosphoglycerate mutase reaction

*F. Hufnagel[1], N. S. Becker[2], K. Forchhammer[2], S. Klähn[1]
[1]*Helmholtz Centre for Environmental Research (UFZ), Solar Materials Biotechnology, Leipzig, Germany*
[2]*University Tübingen, Microbiology and Organismic Interactions, Tübingen, Germany*

Introduction

Among prokaryotes oxygenic photosynthesis is a unique feature in cyanobacteria. In the context of establishing a CO_2-neutral, sustainable bioeconomy, they receive growing interest as biocatalysts in photo-biotechnological applications. To rationally engineer cyanobacteria and to direct metabolic fluxes towards chemical products, it is important to consider native molecular processes that control metabolism. Recently, the 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (PGAM) has been shown to serve as a central control point determining the carbon flux from the Calvin-Benson-Bassham cycle (CBB) to lower glycolysis. In particular, PGAM activity is controlled by the small protein PirC that also binds to the central regulatory protein P_{II}. Upon PirC-binding, PGAM is inactivated leading to glycogen accumulation within the cell.[1]

We aim to target the PirC-PGAM switch in the model organism *Synechocystis* sp. PCC 6803 to gain control over photosynthetically driven biocatalytic processes. In particular, flux through PGAM shall be blocked to increase product yields that derive from gluconeogenic routes.

Material and Methods

Three different methods to turn off the PGAM activity were developed all of which are controlled by inducible promoters. First, an approach to overexpress *pirC* is used. Second, the expression of *pgam* is downregulated. Finally, overexpression of *pirC* and downregulation of *pgam* are combined. Once all strains are constructed, Northern Blots are performed to check changes in transcription levels of both *pirC* and *pgam* after the induction of the promoters. Besides that, the glycogen concentration in each strain is measured using an enzymatic assay to determine which approach works best to inactivate PGAM.

Results

A rise in glycogen amount can be seen once the activity of PGAM is turned down in *pirC*-overexpression strains. In addition, we demonstrate that our approach also has implications on other product pathways, e. g. for sucrose, that derive from the CBB.

[1] T. Orthwein, J. Scholl, P. Spät, S. Lucius, M. Koch, B. Macek, M. Hagemann, K. Forchhammer. PNAS, 2021, 118(6): e2019988118

P-RSP-002
First insights into the role of second messengers for host adaptation of Photorhabdus luminescens

*F. Platz[1], H. Bähre[2], R. Seifert[2], R. Heermann[1,3]
[1]*Johannes Gutenberg University Mainz, Institute of Molecular Physiology (IMP), Microbiology and Biotechnology, Mainz, Germany*
[2]*Hannover Medical School, ZFA Metabolomics, Hannover, Germany*
[3]*Institute for Biotechnology and Drug research gGmbH (IBWF), Mainz, Germany*

The entomopathogenic bacterium *Photorhabdus luminescens* is a symbiont of nematodes and plants and is likewise highly pathogenic against insects. The bacteria exist in two phenotypic different variants called primary (1°) and secondary (2°) cells. The 1° cells live in close symbiosis with the nematodes, whereas 2° cells cannot interact with them anymore. However, the 2° cells are

believed to be free-living cells in the soil specifically interacting with plants. Both cell forms can infect and kill insects. Since *P. luminescens* colonizes different eukaryotic hosts, the cells need to precisely adapt to the different interaction partners regulating symbiosis and pathogenicity. In the last years the role of second messengers for regulation of various phenotypes and host adaptation in bacteria came more and more into focus. Here, we investigated the role of second messengers for the host adaptation of *P. luminescens*. In general, we identified 2',3'- and 3',5'-cyclic nucleotide monophosphates (cNMPs) in both variants of the bacteria. We found that the concentrations of these second messengers altered when they were exposed to signaling molecules derived from nematodes, insects or plants. Moreover, the concentration of cNMPs differed between 1° and 2° cells, while the general amount of 2',3'-cNMPs was significantly higher compared to 3',5'-cNMPs under all tested conditions and in both cell variants. This indicates a central role of the tested second messengers for phenotypic switching of *P. luminescens* as well as for host adaptation. As described for various other bacteria, formation of biofilms is regulated by cyclic di-GMP. However, *P. luminescens* forms biofilms, but cyclic di-GMP was not found to be involved in regulating motility and sessility. This is in accordance with the uncommon finding that *P. luminescens* does not have any proteins containing a PilZ- and GGDEF-domain responsible for cyclic di-GMP synthesis or binding in other bacteria. Overall, our studies suggest a central role of several second messengers in adaptation to different eukaryotic hosts of this biotechnological relevant bacterium.

P-RSP-003

Exploring the relationship between the two-component system CroRS and the second messenger c-di-AMP in regulating β-lactam tolerance in Enterococcus faecalis

*L. Wiens[1], S. Morris[2], S. Gebhard[1]
[1]*Johannes Gutenberg-Universität Mainz, Mainz, Germany*
[2]*University of Bath, Bath, United Kingdom*

Antimicrobial resistance is a widely recognized issue, but less attention has been paid to antimicrobial tolerance. The opportunistic pathogen *Enterococcus faecalis*, which is responsible for serious hospital-acquired infections, has a high level of intrinsic antibiotic resistance, especially to β-lactam antibiotics. *E. faecalis* uses the CroRS two-component system to provide antibiotic resistance to cell-wall targeting antibiotics, such as the β-lactam ampicillin. Previous genome profiling studies exploring the CroRS regulon did not show the exact genes responsible for the CroSR dependent ß-lactam resistance. To gain further insights into the mechanistic link between CroRS regulation and β-lactam resistance, we here used experimental evolution to restore ampicillin resistance in a *croRS* deletion strain. The resulting strains exhibited a 10-fold increase in ampicillin resistance, which is equivalent to that of the wild type. Genome sequencing showed that the only mutations common to all four evolved lines were in genes encoding for enzymes responsible for the synthesis and degradation of the second messenger c-di-AMP. Deletion of *croRS* led to a marked increase in c-di-AMP levels, while the evolved strains exhibited a decrease in c-di-AMP levels similar to the wild type. Changes in cellular c-di-AMP concentration have been found to be correlated with changes in β-lactam resistance, where excessive second messenger production coincided with β-lactam sensitivity. To gain a better understanding of the mechanistic basis of the correlation between c-di-AMP concentration and β-lactam resistance, targeted mutagenesis of c-di-AMP metabolic genes was performed in combination with phenotypic characterization. These findings will help to improve our understanding of how CroRS regulation controls β-lactam tolerance in *E. faecalis*.

P-RSP-004

The role of small DUF1127 proteins in phosphate metabolism can be explained by protein-protein interactions

*D. Remme[1], L. J. Tilg[2], Y. Pfänder[2], F. Narberhaus[1,2]
[1]*Ruhr-University Bochum, Biology and Biotechnology, Bochum, Germany*
[2]*Ruhr University Bochum, Biology and Biothechnology, Bochum, Germany*

In any given organism, the function of one-third of all proteins is unknown. Some of these proteins have a domain of unknown function, such as the arginine-rich DUF1127. Approximately 20,000 proteins with a DUF1127 domain have been identified in about 5,000 bacteria. The plant pathogen *Agrobacterium tumefaciens,* encodes seven DUF1127 proteins: three small ones (47 and 48 amino acids) and four long ones (72 to 101 amino acids). Our current study aims at identifying the interaction partners and biological role of DUF1127 proteins in *A. tumefaciens* and other bacteria.

A triple *A. tumefaciens* mutant having all three small DUF1127 genes deleted, exhibits a variety of phenotypes, including a growth defect in stationary phase, upregulated siderophores and altered phosphate uptake. Complementation with any of the small DUF1127 genes restores the wildtype phenotypes. Moreover, DUF1127 genes from a range of bacteria including *Escherichia coli*, *Rhodobacter sphaeroides*, *Rhizobium rubi*, *Sinorhizobium meliloti* and *Neorhizobium galegae* can complement the phenotypes of the triple deletion mutant. Our results suggest that the function of small DUF1127 proteins is conserved across different species. One major group of upregulated genes in the *A. tumefaciens* triple mutant is involved in phosphate uptake and belongs to the *pst* operon. The transcription of this operon is regulated by the two-component system PhoB-PhoR. We provide evidence that small DUF1127 proteins interact with the sensor kinase PhoR. Given that the phosphate metabolism in an *E. coli* DUF1127 mutant also is mis-regulated, we hypothesize that DUF1127 proteins have a conserved function in this process that is mediated through protein-protein interactions.

P-RSP-005

The human microbiome-derived antibiotic lugdunin self-regulates its biosynthesis in a quorum-sensing like fashion

L. Reetz[1,2,3,4], *J. Mößner[1,3,4], B. Krismer[1,3,4], A. Peschel[1,3,4], T. Kronenberger[5,3,4], K. A. Selim[6,3], T. Schaefle[7,3], T. Dema[7,3], A. Zipperer[1,3,4], A. Poso[5,3,4], S. Grond[7,3,4]
[1]*University of Tübingen, Infection Biology, Tübingen, Germany*
[2]*Fraunhofer ITMP, München, Germany*
[3]*Cluster of Excellence EXC 2124 Controlling Microbes to Fight Infections, Tübingen, Germany*
[4]*German Center for Infection Research, partner site Tübingen, Tübingen, Germany*
[5]*University of Eastern Finland, Faculty of Health Sciences, Kuopio, Finland*
[6]*University of Tübingen, Organismic Interactions Department, Tübingen, Germany*
[7]*University of Tübingen, Institute of Organic Chemistry, Tübingen, Germany*

Many human microbiome members inhibit bacterial competitors by production of antimicrobial compounds whose expression needs to be tightly controlled to balance costs and benefits of compound biosynthesis. The nasal commensal *Staphylococcus lugdunensis* outcompetes *Staphylococcus aureus* using the antimicrobial lugdunin. The lugdunin biosynthetic gene cluster (BGC) encodes two potential regulators whose role has remained unknown. Deletion of the regulator genes *lugR* or *lugJ* led to increased lugdunin production and/or immunity. Whereas LugR was found to repress transcription of the biosynthetic *lugRABCTDZ* operon, LugJ repressed the *lugIEFGH* export and immunity genes. Both regulators bound to different inverted repeats in the controlled promoter regions. Notably, both repressors were released from cognate promoters to allow transcription upon addition of exogenous lugdunin. Even minor structural changes disabled lugdunin derivatives to induce expression of its BGC, which is consistent with inferior binding to the predicted LugR and LugJ binding pockets. Thus, lugdunin controls its own biosynthesis in a quorum-sensing-like fashion probably to avoid futile production.

P-RSP-006

Endoribonuclease-mediated regulation of the balance between RNA degradation and transcription.

*J. Pino[1], J. Stülke[1]

[1]*Georg-August University Göttingen, General Microbiology, Göttingen, Germany*

RNase Y is an endoribonuclease that has been shown to be a key component involved in RNA metabolism in *B. subtilis* (1). The deletion of its gene (*rny*) results in the appearance of suppressor mutants under specific growth conditions. These mutants carry mutations in *rpo* genes encoding RNA polymerase subunits (2). This mechanism allows *B. subtilis* to grow even in absence of the RNase Y. However, the appearance of specific mutations in *rny* and their respective effect in RNA polymerase subunits genes have not been studied yet.

In order to identify the relation between the RNase Y and RNA polymerase mutants with their respective consequences in growth and adaptation, we want to identify couples of these proteins by making random mutations in *rny*, studying their activity and screening strains with suppressor mutations in *rpo* subunits of the RNA polymerase to be analyzed functionally and structurally. Considering that previous results have indicated that other components of the transcription machinery can also be affected (2), it is important to investigate their role in achieving the transcription-degradation trade-off and identify possible alterations on them.

These results will allow us to provide an approach to understand the link between transcription and RNA degradation.

(1) Commichau, F. M. et al. (2009) Novel activities of glycolytic enzymes in Bacillus subtilis: interactions with essential proteins involved in mRNA processing. Molecular & Cellular Proteomics. 8:1350-1360.

(2) Benda, M et al. (2021) Quasi-essentiality of RNase Y in Bacillus subtilis is caused by its critical role in the control of mRNA homeostasis. Nucleic Acids Research. 49:7088-7102.

P-RSP-007

Dissecting stress response networks in Campylobacter jejuni using a chemical-genomics approach

*A. Zannoni[1], R. Olayo Alarcon[2], M. Amstalden[3], A. R. Brochado[4], C. L. Müller[2], C. M. Sharma[1]

[1]*Institute of Molecular Infection Biology, University of Würzburg, Würzburg, Germany*

[2]*Institute of Statistics, Ludwig-Maximilians-Universität München, München, Germany*

[3]*Department of Microbiology, Biocenter, University of Würzburg, Würzburg, Germany*

[4]*Eberhard Karls University of Tübingen, Tübingen, Germany*

Pathogens are constantly exposed to a variety of environmental stimuli, originating from their host, the microbiome, food, antibiotics, and other drugs. Adaptation to their host niche requires the concerted expression of different stress response pathways, regulated by transcription factors and small RNAs (sRNAs). However, the external cues and chemical stimuli that trigger specific pathways are still largely elusive, as well as how these regulatory cascades impact bacterial virulence and sensitivity to antibiotics.

Here we aim to explore signals (stressors) and regulatory pathways controlling host adaptation and antibiotic sensitivity in the foodborne pathogen *Campylobacter jejuni*, the main cause of bacterial gastroenteritis. We designed a unique chemical-genomics approach with two time-resolved readouts, where we profile both growth and transcriptional response of a transcriptional reporter-strain library of ~30 sRNAs, global stress regulators, and effectors challenged with ~2400 host-related chemical stimuli (small molecules).

After profiling ~16000 interactions, we detected a higher sensitivity of *C. jejuni* to multiple human-targeted drugs previously not classified as antibiotics (~4% tested compounds inhibited growth) compared to another major intestinal pathogen, *Salmonella enterica*. These compounds show potential to be used as adjuvants in combination with standard antibiotic treatments against *C. jejuni*. In addition, preliminary data already show a few stressor-regulator pairs where the reporter expression appears to be modulated in the presence of the chemical compound, accounting for ~0,5% hit rate. The majority of the identified compounds, including several commonly taken drugs, activated the expression of the *cmeABC* major efflux pump of *C. jejuni*, which plays a crucial role in antibiotic resistance. Overall, our data highlight the impact of non-antibiotic drugs on *C. jejuni* growth and potentially on modulating its ability to resist antimicrobial treatments.

P-RSP-008

Redox-responsive LysR-type transcription factors in the phytopathogen Agrobacterium tumefaciens

*J. J. Schmidt[1], *R. Fiedler[1], L. R. Knoke[2], V. Brandenburg[3], L. I. Leichert[2], F. Narberhaus[1]

[1]*Ruhr-Universität Bochum, Microbial Biology, Bochum, Germany*

[2]*Ruhr-University Bochum, Microbial Biochemistry, Bochum, Germany*

[3]*Ruhr-Universität Bochum, Bioinformatics Group, Bochum, Germany*

Agrobacterium tumefaciens, a ubiquitous soilborne phytopathogen, continuously encounters reactive oxygen species (ROS) generated endogenously during respiration or exogenously during host infection. Consequentially, adaptive protection against ROS is inevitable for survival and pathogenicity. A broadly known redox-responsive regulator is

the ubiquitously found LysR-type transcription factor OxyR, which directly senses peroxide stress through redox-active cysteine residues. Interestingly, *A. tumefaciens* harbors a distinct LysR-type transcription factor called LsrB, which putatively plays a role in the oxidative stress response. Unlike OxyR, LsrB is solely found in Rhizobiales and encoded in a polycistronic operon with the thioredoxin reductase gene *trxB*. We asked if and how LsrB has a similar or even overlapping function with OxyR.

We found that deletion of *lsrB* and *oxyR* leads to increased sensitivity to a broad spectrum of reactive oxygen species. Genome-wide transcriptome profiling under hydrogen-peroxide stress revealed that both regulators control a complex network of oxidative stress responsive genes, such as antioxidant systems, as well as numerous small RNAs. Redox state profiling via roGFP biosensors further indicated a more oxidized intracellular redox environment in the *lsrB* and *oxyR* mutant, which is in line with the dysregulation of antioxidant systems. *In silico* structure modeling and functional studies identified redox-active cysteine residues in LsrB, suggesting a similar mode of action as OxyR, highlighting the intricate interplay between the two regulators. In summary, our findings uncover the pivotal role of OxyR and LsrB in orchestrating the adaptive protection against ROS in *A. tumefaciens.*

P-RSP-009

The light-dependent antirepressor LdaP of Dinoroseobacter shibae activates the expression of the photosynthetic gene cluster by specific interaction with the PpsR repressor

*S. Pucelik[1], S. K. Hotop[2], M. Brönstrup[2], D. Jahn[3], E. Härtig[1]
[1]*Technical University of Braunschweig, Institute of Microbiology, Brunswick, Germany*
[2]*Helmholtz Institute for RNA-based Infection Research, Department of Chemical Biology, Brunswick, Germany*
[3]*Technical University of Braunschweig, Braunschweig Integrated Centre of Systems Biology (BRICS), Brunswick, Germany*

Introduction: In the marine bacterium *Dinoroseobacter shibae* the photosynthetic gene cluster (PGC), encoding all components for aerobic anoxygenic photosynthesis, is regulated in a light-dependent way. In our current regulatory model, PpsR inhibits the transcription of the PGC under white & blue light conditions by binding to promoter sequences. These sequences partially overlap with the -10 and -35 promoter regions. This prevents RNA polymerase binding. Under light or blue light conditions LdaP is in its inactive form and unable to bind to PpsR. Transcriptional activation under dark conditions is mediated by the interaction of dark state LdaP with PpsR preventing repressor function. In addition, the antirepressor PpaA is able to interact with PpsR in the presence of cobalamin and act as a second antirepressor of PpsR (Pucelik et al., 2024, *Front. Microbiol.* 15:1351297. doi: 10.3389/fmicb.2024.1351297).

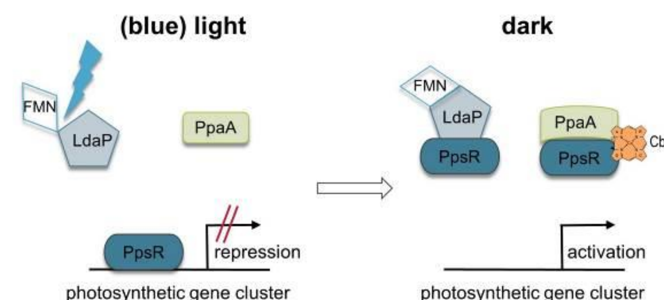
Goals: Characterising the light-dependent interaction of the antirepressor LdaP with the PpsR repressor and its influence on DNA binding properties of PpsR.

Methods: Homologous produced & purified LdaP protein was incubated under blue light or dark conditions with SPOT peptide arrays representing the PpsR protein sequence and vice versa. Specific interactions were detected using LdaP or PpsR specific antisera. The DNA binding properties of PpsR were analysed by EMSA using a 100 bp *bchF* promoter DNA fragment and recombinantly produced PpsR.

Results: The SPOT peptide arrays showed an interaction of LdaP with PpsR at the DNA binding domain of PpsR, specifically under dark conditions. EMSA analyses revealed specific PpsR binding to the conserved binding motif 5´-TGT-N12-ACA-3". Addition of LdaP did not abolish PpsR binding. Simultaneous EMSA and Western blot analyses revealed that only PpsR is present in the detected shift complex.

Summary: LdaP mediates expression of the PGC presumably by acting as an antirepressor of PpsR. Specific binding regions within the LdaP and PpsR proteins were identified. A light-dependent binding of PpsR in the presence of LdaP was not detected by *in vitro* binding studies. Specific binding of PpsR to the *bchF* promoter was determined.

Fig. 1



P-RSP-010

Exploring the hidden world of small proteins reveals a novel dual-function RNA in Vibrio cholerae

*L. M. Luke[1], D. Vega[1], M. A. Rahman[1], S. L. Svensson[2], C. M. Sharma[2], K. Papenfort[1]
[1]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*
[2]*University of Würzburg, Department of Molecular Infection Biology II, Würzburg, Germany*

Dual RNA regulators are a unique group of genes (sRNAs) encoding both: a base-pairing small regulatory RNA (sRNA) and an mRNA encoding a small protein. Despite the large spectrum of regulatory functions described for sRNAs ranging from stress responses, antibiotic tolerance, virulence, carbon metabolism, and intercellular communication, only little is known about dual RNA regulators and small proteins. The problem is mainly due to the small size of SPs (≤ 50 amino acids), poor annotation, and the lack of genetic screens aiming to characterize SPs in bacteria.

To address this problem in the major human pathogen *Vibrio cholerae*, we performed ribosome profiling to identify potential small open reading frames at the genome-wide level. These experiments revealed 65 potential sORFs in *V. cholerae*, of which we validated 21 SPs by Western Blotting. Characterization of SPs led us to discover the *V. cholerae* dual RNA and protein (VcdRP) harboring the VcdP SP and the VcdR sRNA, which together synchronize carbon uptake and central metabolism in *V. cholerae* (1). Following the search for additional dual regulators pivotal during carbon utilization, we identified an additional dual RNA regulator in *V. cholerae* named VcsRP (V. cholerae carbon starvation dual RNA and Protein). The *vcsRP* gene encodes the VcsP SP and the VcsR sRNA, which is processed from 3" UTR by

ribonuclease E. Expression of *vcsRP* is activated under carbon limitation and is repressed under glucose-rich conditions. Further, we obtained evidence showing that VcsR acts post-transcriptionally and in concert with Hfq to inhibit the expression of enzymes involved in galactose metabolism and oxidative phosphorylation.

Reference:

1. K. Venkat *et al.*, A dual-function RNA balances carbon uptake and central metabolism in *Vibrio cholerae. EMBO J* **40**, e108542 (2021).

P-RSP-011

Translated small ORFs in Sinorhizobium meliloti: a new regulatory upstream ORF and a second S21 ribosomal gene

J. Kothe[1], *T. Dietz[1], T. Sauerwein[2], K. U. Förstner[2], E. Evguenieva-Hackenberg[1]

[1]*Justus Liebig University Giessen, Institute of Microbiology and Molecularbiology, Giessen, Germany*

[2]*ZB MED - Information Centre for Life Sciences, Science and Services, Köln, Germany*

Introduction: Small genes can have important function but are often poorly annotated. Recently we applied ribosome profiling (Ribo-seq) and mass spectrometry to detect translated sORFs in the soil-dwelling plant symbiont *Sinorhizobium meliloti* 2011 and to curate its annotation.

Goals: Two of the translated sORFs were further analyzed in order to understand their function.

Materials & Methods: We used reporter fusions, applied mutagenesis, and performed RNA-Seq, Northern blot, qRT-PCR, and Western blot analyses.

Results: The first analyzed small gene is a 29 aa novel sORF (sORF26) located upstream of a DUF1127 gene; the latter showed strongest induction after exposure to tetracycline. We found that inhibition of sORF26 translation is responsible for the induction of the DUF1127 gene. Distinct elements in sORF26 and in the intergenic region that are necessary for this posttranscriptional regulation were identified. The second analyzed small gene is a *rpsU* paralog (*rpsU2*), which was missed in the original genome annotation. Under standard laboratory conditions, expression of *rpsU2* was stronger than that of the originally annotated *rpsU1*. Most recent data on the suggested ribosome heterogeneity in *S. meliloti* will be shown.

Summary: The novel sORF26 is a regulatory upstream ORF, translation of which is needed for repression of the downstream DUF1127 gene under standard conditions and strong induction upon exposure to translation inhibiting antibiotics. Existence and differential expression of two S21 genes in S. *meliloti* suggests ribosome heterogeneity, which is under investigation.

References:

Hadera"s L, Heiniger B, Maaß S, Scheuer R, Gelhausen R, Azarderakhsh S, Barth-Weber S, Backofen R, Becher D, Ahrens CH, Sharma CM, Evguenieva-Hackenberg E. (2023) Unraveling the small proteome of the plant symbiont *Sinorhizobium meliloti* by ribosome profiling and proteogenomics. Microlife 10;4:uqad012. doi: 10.1093/femsml/uqad012

P-RSP-012

Investigation of unique expression properties of the novel Staphylococcus aureus serine protease Jep

*J. Tebben[1], G. Wockenfuß[1], H. Wolfgramm[1], L. M. Fernandes Hartzig[2], L. Busch[1], S. Peringathara[2], M. Schaffer[1], A. Reder[1], K. Surmann[1], U. Mäder[1], B. M. Bröker[2], S. Holtfreter[2], U. Völker[1]

[1]*University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany*

[2]*University Medicine Greifswald, Greifswald, Germany*

Introduction: Extracellular proteases are important virulence factors in *Staphylococcus aureus.* This also comprises a set of serin protease-like proteins (Spls) whose role in infection is still poorly understood. In the mouse-adapted *S. aureus* strain JSNZ, we recently identified a novel secreted serine protease, JSNZ extracellular protease (Jep). Jep shares significant sequence homology and a conserved catalytic triad with the Spls, making it an interesting candidate for investigating the role of serine proteases in murine *S. aureus* infection models.

Goals: To conduct in-depth analyses of Jep's function, we aim to characterise its expression and secretion in *in vitro* settings, comparing key features with those of Spls.

Materials and Methods: Using the mouse-adapted *S. aureus* strain JSNZ, we analysed the expression of *jep* during growth in TSB and the plasma-mimicking minimal medium RPMI via northern and western blotting. Regulatory motifs were predicted *in silico*. Additionally, the effect of the 3'-UTR was studied using the conditional mutants JSNZ Δ*jep* pTripleTREP_*jep* and RN4220 pTripleTREP_*jep*, allowing the insertion of different *jep* sequences. Signal peptide cleavage after secretion was investigated using N-terminomics.

Results: Jep shares similarities with SplB in terms of expression and secretion. Expression starts in the transient phase and the protease accumulates in the culture supernatant during the stationary phase. We were also able to prove the same specific cleavage of the signal peptide after secretion. However, Jep reaches a much higher relative proportion in the secretome of murine *S. aureus* isolates than Spls in known clinical isolates. This is partially achieved by a positive influence of a terminator structure in the 3'-UTR on the stability of *jep* mRNA.

Summary: Our study provides a baseline characterisation of the newly identified extracellular *S. aureus* protease Jep, revealing both similarities and differences compared to known Spls. The remarkably high abundance of Jep in the secretome is likely linked to an observed transcript stabilisation by a terminator structure in the 3'-UTR of *jep.*

P-RSP-013

Post-translational glutamine synthetase regulation in Methanosarcina mazei comes in different colours

*E. Herdering[1], T. Reif-Trauttmansdorff[2], A. Kumar[2], T. Habenicht[1], G. Hochberg[3], S. Bohn[4], J. Schuller[2], R. A. Schmitz[1]

[1]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for General Microbiology, Kiel, Germany*

[2]*Philipps-University Marburg, Center for Synthetic Microbiology (SYNMIKRO) Research Center and Department of Chemistry,*

Marburg, Germany
[3]*Philipps-Universität, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[4]*Helmholtz Center Munich, Institute of Structural Biology, München, Germany*

Glutamine synthetases (GS) are central enzymes essential for nitrogen metabolism across all domains of life. Consequently, they have been extensively studied for more than half a century. Based on the ATP-dependent ammonium assimilation generating glutamine, GS expression and activity are strictly regulated in all organisms. In the methanogenic archaeon *Methanosarcina mazei*, it has been shown that the metabolite 2-oxoglutarate (2-OG), which is the central signal for nitrogen starvation, is directly inducing the GS activity (Ehlers et al., 2005). Moreover, modulation of GlnA1 activity by protein-protein interactions with the two small proteins GlnK1 and small protein 26 (sP26) has been described (Ehlers et al., 2005; Gutt et al., 2021). Thereby, GlnK1 was found to interact with GlnA1 under nitrogen upshift by pull-down approaches, pointing towards an inhibitory function of GlnK1 under shifting conditions from nitrogen limitation to sufficiency (Ehlers et al., 2005). Additionally, the 2.5 kDa sP26 was described to interact with GlnA1, stabilizing the complex and enhancing GlnA1 activity (Gutt et al., 2021). Here, we aim to clarify the previously reported contradictory effects of GlnK1 on *M. mazei* GlnA1 by mass photometry (MP) analysis. The underlying molecular mechanism of the strong 2-OG activation of *M. mazei* GlnA1 was elucidated using MP. Moreover, we determined the cryo-electron microscopy single-particle structure of the purified GlnA1 dodecamer arguing for a unique regulation of *M. mazei* GS.

Ehlers, C., Weidenbach, K., Veit, K., Forchhammer, K., Schmitz, R.A, 2005. Unique mechanistic features of post-translational regulation of glutamine synthetase activity in *M. mazei* strain Gö1 in response to nitrogen availability: Regulation of glutamine synthetase activity in *M. mazei*. Mol. Microbiol. 55 https://doi.org/10.1111/j.1365-2958.2005.04511.x

Gutt, M., Jordan, B., Weidenbach, K., Gudzuhn, M., Kiessling, C., Cassidy, L., Helbig, A., Tholey, A., Pyper, D.J., Kubatova, N., Schwalbe, H., Schmitz, R.A., 2021. High complexity of Glutamine synthetase regulation in M. mazei: Small protein 26 interacts and enhances [...] FEBS J. https://doi.org/10.1111/febs.15799

P-RSP-014

sORFdb – A database for sORFs, small proteins, and small protein families in bacteria

*J. M. Hahnfeld[1], O. Schwengers[1], A. Goesmann[1]
[1]*Justus Liebig University Giessen, Professorship for Bioinformatics and Systems Biology, Giessen, Germany*

Small proteins with fewer than 100, particularly fewer than 50, amino acids still need to be explored. Nonetheless, they are essential to bacteria's often ignored genetic repertoire. In recent years, the development of ribosome profiling protocols has led to an increasing number of previously unknown small proteins. Despite this, they are still frequently overlooked by automated genome annotation pipelines, and often, no functional descriptions can be assigned due to a lack of known homologs.
The current abundance of small proteins in existing databases was evaluated to understand and overcome these limitations, and a new dedicated database for small proteins and their potential functions was created.
To this end, small proteins were extracted from annotated bacterial genomes in the GenBank database. Subsequently, they were quality-filtered, compared, and complemented with proteins from the Swiss-Prot, UniProt, and SmProt databases to ensure a reliable identification and characterization of small proteins. Families of similar small proteins were created using bidirectional best BLAST hits followed by Markov clustering.
Analysis of small proteins in public databases revealed that their number still needs to be improved due to historical and technical limitations. Additionally, functional descriptions were often missing despite the presence of potential homologs. As expected, a taxonomic bias demonstrated an overrepresentation of clinically relevant bacteria. This new and comprehensive database is accessible via a feature-rich website providing specialized search features for sORFs and small proteins of high quality. It also includes Hidden Markov Models for small protein families and information on taxonomic distribution and other physicochemical properties.
In conclusion, the novel small protein database sORFdb is a specialized, taxonomy-independent database that improves the findability of sORFs, small proteins, and their functions in bacteria, thereby supporting their future detection and consistent annotation. All sORFdb data is freely accessible at https://sorfdb.computational.bio.

Single Cell Microbiology

P-SCM-001

Enhancing resolution in microbial imaging and RNA detection: Integrating expansion microscopy with multiplexed FISH.

*L. Cecchino[1,2], A. E. Saliba[1,2]
[1]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*
[2]*Julius-Maximilians-Universität Würzburg, Institute of Molecular Infection Biology (IMIB), Würzburg, Germany*

Transcriptomic analysis of bacterial microcolonies has been drastically limited by the use of bulk approaches that average thousands of different bacteria and ignore spatial heterogeneity. A new generation of imaging-based spatial transcriptomics methods is emerging as a powerful approach for studying gene expression at the single-cell level. Many sophisticated methods based on fluorescence in situ hybridization (FISH) have been developed in recent years, that are capable of capturing individual transcripts, but their implementation for studying bacteria is still limited. Furthermore, the inherently small size of bacteria makes the imaging challenging. Here, we aim to integrate FISH with expansion microscopy [1] to detect individual mRNAs with high spatial resolution.

We have successfully implemented expansion microscopy, enhancing the resolution of our imaging. This technique was seamlessly combined with HCR-FISH, providing a comprehensive approach to visualize RNA within individual bacterial cells. Future efforts involve optimizing gel chemistry to increase the expansion factor and applying this technique to investigate the dynamics of microcolonies formed on the intestinal epithelium by enteropathogenic E. coli.

Furthermore, we want to extend the protocol to explore diverse sample types, including gram-positive bacteria, biofilms, and infected cell cultures. Utilizing Par-Seq-FISH [2]

will additionally allow us to increase the throughput of RNA detection in the future.

[1] Cheng Z, Stefani C, Skillman T, Klimas A, Lee A, DiBernardo EF, K. Brown KM, Milman T, Wang Y, Gallagher BR, Lagree K, Jena BP, Pulido JS, Filler SG, Mitchell AP, Hiller NL, Lacy-Hulbert A, Zhao Y; MicroMagnify: A Multiplexed Expansion Microscopy Method for Pathogens and Infected Tissues. Adv. Sci. 2023, 10 (30)
[2] Dar D, Dar N, Cai L, Newman DK; Spatial transcriptomics of planktonic and sessile bacterial populations at single-cell resolution. Science 2021, 373 (6556)

P-SCM-002
A fluorescently labelled quaternary ammonium compound (NBD-DDA) to study resistance mechanisms in bacteria

*N. Nordholt[1], K. O'Hara[1,2], U. Resch-Genger[2], M. A. T. Blaskovich[3], B. Rühle[2], F. Schreiber[1]
[1]Federal Institute for Materials Research and Testing (BAM), Department of Materials and Environment, Division Biodeterioration and Reference Organisms (4.1), Berlin, Germany
[2]Federal Institute for Materials Research and Testing (BAM), Department of Analytical Chemistry, Division of Biophotonics (1.2), Berlin, Germany
[3]The University of Queensland, Centre for Superbug Solutions, Institute for Molecular Bioscience, Brisbane, Australia

Introduction: Quaternary ammonium compounds (QACs) are widely used as active agents in disinfectants, antiseptics, and preservatives. Despite being in use since the 1940s, there remain multiple open questions regarding their detailed mode-of-action and the mechanisms that can make bacteria less susceptible to QACs, including phenotypic heterogeneity.

Goals: To develop a fluorescent quaternary ammonium compound and to use it to study the mechanisms underlying QAC susceptibility in single bacterial cells.

Methods: A fluorescent quaternary ammonium compound, N-dodecyl-N,N-dimethyl-[2-[(4-nitro-2,1,3-benzoxadiazol-7-yl)amino]ethyl] azanium-iodide (NBD-DDA), was synthesized. Chemical structure and purity of NBD-DDA was confirmed by mass spectrometry and NMR spectroscopy. Antimicrobial activity against *S. aureus, P. aeruginosa* and *E. coli* was assessed by determination of minimum inhibitory concentrations and time-kill assays. Uptake, heterogeneity and efflux of NBD-DDA in wild-type and efflux-deficient mutants (*ΔtolC*) were determined, using flow cytometry and fluorometry. Sub-cellular localization of NBD-DDA was determined using confocal laser scanning microscopy.

Results: NBD-DDA was readily detected by flow cytometry and fluorescence microscopy with standard GFP/FITC-settings. NBD-DDA retained antimicrobial activity comparable to the structurally similar, widely used QAC benzalkonium chloride (BAC). Characteristic time-kill kinetics and increased tolerance of a BAC tolerant *E. coli* strain against NBD-DDA suggest that the mode of action of NBD-DDA is similar to that of BAC. NBD-DDA was preferentially localized to the cell envelope, which is a primary target of BAC and other QACs. Leveraging NBD-DDA"s fluorescent properties, it was demonstrated that reduced cellular accumulation underlies BAC tolerance in a BAC tolerant *E. coli* strain and that NBD-DDA is subject to efflux mediated by TolC.

Conclusion: Overall, NBD-DDA"s antimicrobial activity, its fluorescent properties, and its ease of detection render it a powerful tool to study mechanisms of QAC susceptibility and mode-of-action in bacteria, both on the level of populations and single-cells.

P-SCM-003
Construction of a fluorescence-based reporter gene system for in vivo analysis of differential gene expression and subpopulation-analysis in Staphylococcus aureus

*H. Wolfgramm[1], G. Wockenfuß[1], M. Harms[1], P. Hildebrandt[1], S. Michalik[1], K. Surmann[1], U. Völker[1], A. Reder[1]
[1]University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany

Introduction Analysing gene expression of pathogenic bacteria during infection at single cell level is a challenge. Most studies have to cope with averaged data, levelling subpopulations and loss of spatial information. This is especially problematic when considering the adaptations to diverse intracellular niches throughout various infection stages. The use of fluorescent reporters enables the temporal and spatial tracking of individual cell gene expression and allows isolation of observed subpopulations for subsequent downstream analyses.

Goals We aimed to develop a fluorescent reporter vector for *Staphylococcus aureus* to examine up to three different physiological states during *in vivo* infection. The vector should enable the easy adaptation of the system to the respective question, but must be transmitted stably and provide an internal reference.

Methods The reporter vector pTricolor contains the three codon-optimized fluorescent reporter genes *dtomato*, *gfp* and *mcerulean*. One reporter is constitutively expressed from an optimized *pgi*-SigA promoter and allows copy-number correction and normalization between different experiments. The remaining two reporter genes can be placed under control of target promoters of interest. Reporter gene expression is monitored by fluorescence microscopy.

Results We demonstrate that pTricolor exhibits stable transmission for at least eight days *in vitro* and 24 hours during infection under non-selective conditions. The fluorescent reporters can be expressed independently of each other and genetic modifications can be easily implemented. In an initial study, the reporter genes were placed under the control of a SigB dependent (*clpL)* and a CodY dependent promoter (*saHPF*), respectively. The detected reporter signals revealed subpopulations of intracellular *S. aureus* in an infection experiment using S9 host cells.

Summary pTricolor enables the analysis of differential gene expression for up to two *S. aureus* target genes during internalization and long-term infection in a standardised and spatiotemporal manner. Thus, it is a suitable reporter vector to investigate subpopulations and face central questions of virulence regulation.

Secondary Metabolites and Natural Products

P-SMNP-001

A new biosynthetic route enlarges the terpene portfolio of bacteria

*B. Piechulla[1], N. Schmidt[1], K. Baer[1], S. von Reuss[2]
[1]*University of Rostock, Institute for Biological Sciences, Rostock, Germany*
[2]*University of Neuchatel, Institute of Chemistry, Neuchatel, Switzerland*

Introduction: The class of terpenoids remain one of the largest groups of natural products (ca. 80,000 compounds, 1), which are structurally and functionally diverse (e.g. rubber, cholesterin, carotenoides, gibberellin, caryophyllene, geraniol, limonene), and many have pharmacological activities (e.g. artemisinin, taxol). Their biosyntheses are based on the reactions of C5 building blocks through head-to-tail, head-to-head, head-to-middle to generate terpene backbones (prenylpyrophosphates) of C10, C15, C20 etc. (2, 3). These backbones can be further rearranged and cyclized by terpene synthases. Furthermore, the regular length compounds can be modified by tailoring enzymes, e.g. methylations, hydroxylation, oxygenation or degradation to reveal irregulare terpenes.

Recently we could show that in bacteria an alternative biosynthetic pathway produces irregulare, non-canonical terpenes via C16 and C17 prenylpyrophosphates (terpene backbones). Two enzyme classes with unusual catalytic capacities and substrate specificities are required to synthesize these non-canonical homo- and bishomo-sesquiterpenes, e.g., sodorifen, chloroaphen (4, 5). The first enzyme is a SAM-dependent bifunctional FPP-methyltransferase with cyclization activity, the second enzyme is a species-specific terpene synthase that accepts (only) cyclic prenylpyrophosphates as substrates.

Results and Discussion: Here we present the state of the art of this new biosynthetic route and demonstrate its presence in α-, β- and γ-proteobacteria and a Streptomyces species. Alltogether this biosynthetic pathway contributes to the diversity of the microbial natural terpenomes and we propose that the 136-year-old isoprene dogma should be modified. Furthermore, this non-canonical terpene biosynthesis inspires potential applications (6).

References

1. D.W. Christianson (2017). Chem Rev 117, 11570–11648.
2. O. Wallach (1887). Justus Liebig's Annalen der Chemie 238: 78–89.
3. L. Ruzicka (1953). Experientia 9: 357–367.
4. S. von Reuss et al. (2018). J Am Chem Soc 140, 11855–11862
5. N. Magnus et al. (2023). Ang Chem Int Ed Engl e202303692
6. R. Chen et al. (2024). Trends in Biotech. 2447

P-SMNP-002

Regulation of the biosynthesis of the interkingdom signaling arginoketide azalomycin F requires multiple LuxR regulators

*S. Edenhart[1], L. Zehner[1], P. Hortschansky[1], M. Rosin[1], T. Netzker[1], V. Schroeckh[1], A. A. Brakhage[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute (HKI), Molecular and Applied Microbiology, Jena, Germany*
[2]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*

The Gram-positive soil bacterium *Streptomyces iranensis* is able to produce the secondary metabolite azalomycin F, an arginine-derived polyketide. Molecules of this group of arginoketides, have a broad antimicrobial activity, ranging from activity against Gram-positive bacteria, to anti-fungal and algicidal activity. Interestingly, azalomycin F exhibits not only an antimicrobial activity, but also affects various organisms at sublethal concentrations. The single-cell green alga *Chlamydomonas reinhardtii* for example reacts to sublethal dosis of azalomycin F by forming protective, multicellular structures, so called gloeocapsoids[1] and by hiding in fungal mycelia[2]. In the fungi *Aspergillus fumigatus* and *A. nidulans*, sublethal concentrations of azalomycin F induce the expression of various secondary metabolite gene clusters[3, 4]. Since azalomycin F is able to affect a broad variety of organisms and thus may have a significant impact on structuring microbial consortia in soil, we are interested in finding out what triggers the production of this compound in *S. iranensis*. So far, we could link the *bld*-regulon, which is involved in both the *Streptomyces* life cycle and natural product biosynthesis, to the production of azalomycin F. Furthermore, we identified a cluster of LuxR regulators controlling the azalomycin F biosynthesis.

[1] Krespach et al. (2021) Algicidal marginolactones induce the formation of gloeocapsoids, novel protective multicellular aggregates of *Chlamydomonas reinhardtii. PNAS,* 118, e2100892118.
[2] Krespach et al. (2020) Lichen-like association of *Chlamydomonas reinhardtii* and *Aspergillus nidulans* protects algal cells from bacteria. *The ISME Journal,* 14, 2794-2805.
[3] Stroe et al. (2020) Targeted induction of a silent gene cluster of *Aspergillus fumigatus* encoding the bacteria-specific spore germination inhibitor fumigermin by *Streptomyces rapamycinicus. eLife*, 9, e5254.
[4] Krespach et al. (2023) Ubiquitous bacterial polyketides mediating cross-kingdom microbial interactions. *Nature Microbiology,* 8, 1348-1361.

P-SMNP-003

A heterologous expression platform in Aspergillus nidulans for enhanced production of a marine leucine-rich non-ribosomal peptid

*H. Maier[1], *J. Bischof[1], H. Groß[2], W. Wohlleben[1]
[1]*University Tübingen, Microbiology/Biotechnology, Tübingen, Germany*
[2]*University Tübingen, Pharmaceutical Biology, Tübingen, Germany*

The marine filamentous fungus *Asteromyces cruciatus* 763 (Pleosporaceae, Ascomycota), collected from the coast of La Jolla (San Diego, USA), produces a leucine-rich cyclic pentapeptide named lajollamide A. The chemical structure was previously determined by HPLC-MS and NMR (Gulder *et al.*, 2012). Combination of whole genome sequencing and comprehensive bioinformatic analysis enabled the

identification of the putative biosynthetic gene cluster (BGC) and a prediction-based proposal of the corresponding biosynthetic pathway. The BGC consists only of a single intron-containing non-ribosomal peptide synthetase (NRPS) gene. The NRPS catalyzes the synthesis of lajollamide A from l-leucine (3x), N-methyl-l-leucine (1x) and l-valine (1x). Leucine-rich cyclic pentapeptides, such as lajollamide A, are underexplored with regards to their biological activity. To fully explore the potential of lajollamide A, a sustainable production is required. The optimization of the natural producer does not represent a reasonable approach as several attempts to transform *A. cruciatus 763* only led to unstable transformants. Consequently, increased production titers were attempted in a genetically manipulable heterologous host that generally enables a successful expression of fungal BGCs. In order to create a high-producing strain, the NRPS gene was cloned under a strong constitutive promoter and transferred into *Aspergillus nidulans* GR5 by PEG-mediated transformation. The transformants generated by multiple random ectopic integration of the introduced plasmid-DNA were subjected to a small-scale chemical screening process. The extracts obtained from the culture broth of the modified *A. nidulans* strains were analyzed by HPLC-MS. A total of 15 lajollamide A-producing strains were identified from 50 transformants. The successful reconstruction of the lajollamide A biosynthetic pathway demonstrates that *A. nidulans* can generally serve as a heterologous host for the expression of fungal gene clusters from the genus *Asteromyces.* The generation of this heterologous expression platform laid the foundation for a faster and more cost-effective microbial production of lajollamide A.

P-SMNP-004

Microbiological profiling and mode of action studies of polyketomycin

*L. Reinke[1,2], H. P. Fiedler[1], H. Brötz-Oesterhelt[1,3]
[1]*Interfaculty Institute of Microbiology and Infection Medicine, Microbial Bioactive Compounds, Tübingen, Germany*
[2]*International Max Planck Research School (IMPRS) 'From Molecules to Organisms' at the University of Tübingen, Tübingen, Germany*
[3]*German Center for Infection Research, Tübingen, Germany*

Multi-drug resistant bacteria are becoming increasingly common, emphasizing the urgent need for new antimicrobially active compounds. The discovery and investigation of natural products as antibacterial agents is a promising approach towards identifying new substances and new modes of action (MoA). Because of their chemical diversity and potency, natural products form a great resource for discovering new bacterial targets and mechanisms to treat even multi-resistant pathogens. Polyketomycin is an antibacterial tetracyclic quinone glycoside produced by *Streptomyces diastatochromogenes* Tü 6028 (Paululat et al., 1999) whose MoA is unknown. In production screening to find a reliable cultivation medium, we could detect polyketomycin in various settings and purify it, as confirmed by HPLC-MS. In addition, we improved a previously reported purification procedure (Paululat et al., 1999). Microbiological and cytotoxic profiling confirmed strong activity against a broad range of Gram-positive bacteria while eukaryotic cell lines were somewhat less susceptible. Moreover, we could show that polyketomycin is bactericidal and remarkably fast-acting. Exploring the mechanism further, we discovered that it apparently affects the cell envelope of Gram-positive bacteria in a concentration-dependent manner, causing pore formation and lysis at high concentrations and cell wall impairment already at low concentrations. Detailed mode of action studies are in progress to clarify the effects on the molecular level.

References:

Paululat, T., Zeeck, A., Gutterer, J. M., & Fiedler, H. P. (1999). Biosynthesis of polyketomycin produced by Streptomyces diastatochromogenes Tü 6028. *The Journal of antibiotics*, *52*(2), 96–101. https://doi.org/10.7164/antibiotics.52.96

P-SMNP-005

A naturally isolated co-culture of Streptomyces species displays antifungal synergy

*G. Sohl[1], D. Iliasov[1], T. Mascher[1]
[1]*Technical University of Dresden, Institute of Microbiology, General Microbiology, Dresden, Germany*

Fungal pathogens pose unique threats to modern healthcare: clinical treatment is currently limited to four antifungal drug classes and many drugs produce adverse side effects in patients. Exploration of new environments could lead to the isolation of novel antifungal-producing microorganisms.

Here, we report the isolation and characterization of a mixed isolate of two *Streptomyces* species from dried moth larvae (*Cirina forda*) that exhibited synergistic antifungal activity.

Diluted samples were plated on Gauze agar. Colonies were picked based on morphology and cell structure. Mature colonies were cultivated on MYM and overlayed with soft agar containing a bacterial or fungal species. Whole genome sequencing (WGS) was performed for phylogenetic identification and characterization. Activity of the colonies and cell extracts was further characterized by a panel of whole-cell biosensors, which contain the luciferase cassette under the control of an antibiotic-inducible promotor. Potential biosynthetic gene clusters were identified by genome mining.

The isolated actinobacterium displayed antibacterial and antifungal activity. Variation in colony morphology led to the subculturing of two morphologically different isolates; these experienced a decrease in antifungal activity. Both sub-strains were identified by WGS as *Streptomyces albidoflavus.* Multiple genomic variations found between the strains by comparative analysis could be responsible for differences in strain physiology. Biosensor tests indicated potential production of antifungal polyene macrolide-like molecules in all three isolates. Further characterization of the synergistic antifungal activity between the co-isolated strains is currently ongoing.

We were able to demonstrate the co-isolation of antifungal-producing organisms from a niche habitat displaying a naturally synergistic relationship. Further research towards these associations could aid the discovery and development of new and better tolerated antifungals.

P-SMNP-006

Discovering a novel mode of action of an underexplored antibacterial natural product

*D. Schneider[1], A. Esch[1], A. Strüder[1], E. Stegmann[1], C. C. Hughes[1], H. Brötz-Oesterhelt[1]
[1]*University Tübingen, Mikrobielle Wirkstoffe, Tübingen, Germany*

An obstacle during natural product screening is the rediscovery of known compounds. To increase chemical diversity, producer species can be cultivated under different growth conditions. Induction of silent biosynthetic gene clusters allows access to new, or underexplored, agents. We identified a new producer of the underexplored isonitrile compound hazimycin. For hazimycin, antibiotic activity against Gram-positive and Gram-negative bacteria was described[1,2] but mode of action information was lacking. In the last decades, naturally occurring diisonitrile compounds were discovered from fungi, bacteria, and sponges, but only a few of them were investigated for their mode of action[3]. To increase knowledge on the effects of diisonitrile compounds, we studied the mode of action of hazimycin.

Hazimycin inhibited the growth of *Bacillus subtilis* and *Escherichia coli* but did not prevent growth completely. Derivatives confirmed that the isonitrile moiety is essential for bioactivity. Copper binding could be proven for hazimycin, in accordance with the mechanism of other isonitriles[4], and binding constant and stoichiometry were determined by isothermal titration calorimetry. The impact of hazimycin on *B. subtilis* was profiled by fluorescence microscopy and the production of reactive oxygen species (ROS) and ATP levels were analyzed. The mechanism of action of hazimycin appears pleiotropic involving ROS and potential impairment of the respiratory chain.

[1]Marquez, J. A. *et al.* The hazimicins, a new class of antibiotics. Taxonomy, fermentation, isolation, characterization and biological properties. *J Antibiot (Tokyo)* **36**, 1101-1108 (1983).
[2]Wright, J. J. K. *et al.* X-Ray crystal structure determination and synthesis of the new isonitrile-containing antibiotics, hazimycin factors 5 and 6. *Journal of the Chemical Society, Chemical Communications*, 1188-1190 (1982).
[3]Zhu, M. *et al.* Diisonitrile-Mediated Reactive Oxygen Species Accumulation Leads to Bacterial Growth Inhibition. *J Nat Prod* **83**, 1634-1640 (2020)
[4]Massarotti, A., Brunelli, F., Aprile, S., Giustiniano, M. & Tron, G. C. Medicinal Chemistry of Isocyanides. *Chem Rev* **121**, 10742-10788 (2021)

P-SMNP-007

Investigation of the polyether biosynthetic machinery and its products in Streptomyces chartreusis.

*M. M. Ashraf[1], S. Heinrich[1], J. Santos-Aberturas[2], A. W. Truman[2], J. E. Bandow[1]
[1]Ruhr University Bochum, Applied Microbiology, Bochum, Germany
[2]John Innes Centre, Molecular Microbiology, Norwich, United Kingdom

S. charteusis is a gram-positive bacterium that produces a divalent cation ionophore named calcimycin that inhibits growth of gram-positive bacteria and is a widely used biochemical tool to study calcium signalling. However, of the well-characterized calcimycin biosynthesis gene cluster a few genes, such as *calU1* and *calU2*, remain catalytically uncharacterized. Moreover, in the light of calcimycin transporting manganese and iron and 4-Br-calcimycin transporting copper, we hypothesize that naturally occurring calcimycin derivatives offer altered divalent cation transport properties [1].

We aim to elucidate catalytic roles of the unknown genes and to generate calcimycin derivatives through mutasynthesis to explore divalent cation transport properties. To this end, knockout mutants of the corresponding genes were generated by Redirect technology [2].

In the *calU1* deletion mutant an intermediate accumulated, suggesting CalU1 to be a putative novel enzyme to release the polyketide chain. Bioinformatics analysis and literature review indicate CalU2 is a putative spirocyclase [3]. A feeding experiment with 3-hydroxy anthranilic acid deletion mutant produced a new derivative (m/z: 470.2638 [M+H]+) identified by LC-MS.

The characterization of the unknown genes will provide a more thorough understanding of the biosynthesis of this class of polyether ionophores that consists of three ring systems, only one of which is synthesized by a PKS. The generation of calcimycin derivatives by modifications at the benzoxazole moiety might expand the capability of this ionophore class as biochemical tools.

1. Senges, Christoph HR, et al. "Effects of 4-Br-A23187 on *Bacillus subtilis* cells and unilamellar vesicles reveal it to be a potent copper ionophore." *Proteomics* 22.17 (2022): 2200061.
2. GUST, B., et al. PCR-targeting system in *Streptomyces coelicolor*. *John Innes Center, Norwich, United Kingdom*, 2003.
3. BILYK, Oksana, et al. Enzyme-catalyzed spiroacetal formation in polyketide antibiotic biosynthesis. *Journal of the American Chemical Society*, 2022, 144. Jg., Nr. 32, S. 14555-14563.

P-SMNP-008

Antimicrobial activity of Micromonospora spp. strains isolated from termite mounds

*L. X. Celis Bautista[1], T. Mascher[1]
[1]Technical University of Dresden, General microbiology, Dresden, Germany

With the emergence of antimicrobial-resistant microorganisms, daily infections that in the past were easily treatable have become difficult to control or, in some cases, impossible to treat. Thus, antimicrobial resistance has become a top global public health and development threat. Consequently, the need for new antibiotics is a critical action point in the strategy to control the issue. Isolates from exceptional and challenging habitats offer a rich source for discovering new species with antimicrobial potential.

This study evaluates the antimicrobial activity of three bacterial strains isolated from soil samples of Angola termite mounds. Identification, using phylogenetic analysis based on 16S rDNA sequences, indicates that they are three potentially new species belonging to the genus *Micromonospora*, which is recognized for its potential to produce natural bioactive compounds.

In vivo assays, whole-cell biosensors, and genome mapping was then applied to determine their potential as antibiotic producers. The antimicrobial activity was evaluated using indicator microorganisms of the Gram-positive (*Bacillus subtilis*), Gram-negative (*Escherichia coli*), and fungi (*Penicillium chrysogenum*). A collection of *Bacillus subtilis* whole-cell biosensors was screened to elucidate the mechanism of actions of the antimicrobial response. Antimicrobial activity against Gram-positive bacteria was observed for the three isolates, showing their potential as producers of antibiotic compounds, and studies are carried

out to determine the mechanism of action. *In vivo* antimicrobial assays with pathogenic microorganisms, as well as polyphasic taxonomic studies and genome mining for secondary metabolite gene clusters of the three isolates are currently ongoing.

P-SMNP-009

The bioactive sugar 7dSh and its effect on carbon metabolism

K. Forchhammer[1], *N. von Manteuffel[1]
[1]*Universtiy Tübingen, Deparment of organismic interactions, Tübingen, Germany*

We previously described the effects of the bioactive sugar 7-deoxy-sedoheptolose (7dSh) that was isolated from culture supernatant of *Synechococcus elongatus.* 7dSh inhibits the growth of various phototrophic organisms including species of cyanobacteria, as well as the model plant organism *Arabidopsis thaliana.* Untargeted metabolome analysis of 7dSh treated organisms showed a strong accumulation of 7-deoxy-D-arabino-heptulosonic acid 7-phosphate (DHAP) the substrate of the 3-dehydoquinate synthase (DHQS) a key enzyme of the shikimate pathway. This indicates that 7dSh inhibits the shikimate pathway, an assumption that was confirmed through inhibition assays with purified DHQS and 7dSh. These findings explained the herbicidal activity of 7dSh, since the shikimate pathway is essential for bacteria, fungi, and plants. Additional experiments were undertaken with the non-diazotrophic cyanobacterium *Synechocystis sp.* (*Synechocystis). Synechocystis* is well-studied for its ability to perform chlorosis, a survival mechanism under nitrogen starvation. During chlorosis, cells accumulate glycogen, disassemble their photosynthetic apparatus, and enter a dormant state until they encounter a nitrogen source. Upon addition of a nitrogen source, cells regreen in a short period of time, thereby consuming the previously accumulated glycogen. Here, we show that 7dSh inhibits the ability to carry out the chlorosis process as well as the recovery of freshly chlorotic cells in the dark, a process that strictly depends on efficient glycogen catabolism. Our current data suggest that 7dSh has an immediate effect on the carbon metabolism by inhibiting glycogen accumulation and consumption. Our findings imply an active inhibition of the carbon metabolism by 7dSh.

Brilisauer, K et al. (2019). Cyanobacterial antimetabolite 7-deoxy-sedoheptulose blocks the shikimate pathway to inhibit the growth of prototrophic organisms. Nature Communications, 10 (545).

Rapp, Johanna et al. (2021). "In Vivo Inhibition of the 3-Dehydroquinat Synthase by 7 Deoxysedoheptulose Depends on Promiscuous Uptake by Sugar Transporters in Cyanobacteria." Frontiers in Microbiology 12: 1720.

P-SMNP-010

Investigation of a biosynthetic genecluster from the proteaseinhibitor bestatin, isolated from Streptomyces olivoreticuli MD976-C7

*J. Klerch[1], P. Fink[2], L. Kaysser[1]
[1]*institut of drug development, pharmaceutical biology, Leipzig, Germany*
[2]*Interfaculty Institute for Microbiology and Infection Medicine, Microbiology / Organismic interactions, Tübingen, Germany*

Bestatin is a natural product isolated from *Streptomyces olivoreticuli* MD976-C7 that acts as a protease inhibitor for aminopeptidase B, N or leucine aminopeptidase (Umezawa et al. 1976a; Umezawa et al. 1976b; Suda et al. 1976a; Suda et al. 1976b; Nishizawa et al. 1977). The natural product has been studied in numerous clinical trials and is already being used pharmaceutically. It has been shown to be an approved therapeutic agent for cancer and bacterial infections, by enhancing the immune response (Fontijn et al. 2006; Ishii et al. 2001; Shang et al. 2018; Abe et al. 1985; Aozuka et al. 2004; Li et al. 2020).

The aim of this work was the identification of a biosynthetic gene cluster and the establishment of heterologous expression of bestatin. In silico analysis using antiSMASH (Medema et al. 2011) identified a putative gene cluster in the *Streptomyces olivoreticuli* MD976-C7 genome sequence (Zhang et al. 2019). Following this, a fosmid library (pCC1FOS-based) was generated. Furthermore, positive clones containing the putative gene cluster were genetically modified by integrating the int_neo cassette (Bierman et al. 1992; Kaysser et al. 2012) to enable heterologous expression in *Streptomyces coelicolor* M512. The gene cluster was introduced into the expression strain using λ-Red- mediated recombination (Gust et al. 2003). After cultivation of the heterologous host and extraction of the natural product, analytical measurement (LC-MS) indicated the presence of a mass for bestatin. These results suggest that a gene cluster for bestatin has been identified. Further verification, such as the generation of knockouts, is required.

P-SMNP-011

Antimicrobial activity and natural product biosynthetic potential of Antarctic actinomycetes

*U. Tarazona Janampa[1,2], J. P. Gomez Escribano[1], I. Nouioui[1], R. Olaechea Alejo[2], J. Wink[3], Y. Mast[1]
[1]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH, Bioresources for Bioeconomy and Health, Brunswick, Germany*
[2]*Universidad Científica del Sur, Comunidades Acuáticas, Lima, Peru*
[3]*Helmholtz Center for Infection Research, Mikrobielle Stammsammlung, Brunswick, Germany*

Actinomycetes are among the most important sources for novel natural compounds. Recent efforts in actinomycetes bioprospecting have focused on unique environments to increase the probability of finding structurally novel secondary metabolites [1]. Antarctica is one of the most pristine environments. The diversity of Antarctic benthic ecosystems is expected to be a reservoir for unique communities, partly due to geographical isolation and the harsh environmental conditions [2]. The aim of this study is to investigate the biosynthetic potential of Antarctic actinomycetes to produce bioactive natural compounds and correlate their bioactivity with the uniqueness of the environment.

Marine sediment samples were collected from six locations in Antarctica with depths ranging from 140 to 1150 m. The isolation protocol was stepwise adapted by testing different parameters, such as sample acclimatization, pH, salinity, temperature, and culture media. In total, nine filamentous actinomycetes, including species from the genera *Streptomyces*, *Nocardiopsis, Micromonospora,* and several non-actinobacterial microorganisms were isolated. The actinomycetes were cultivated in ten different media and antimicrobial activity was assessed against a panel of test strains. Eight strains showed bioactivity against at least one test strain. A prioritized sub-set of strains was selected for genome sequencing. Phylogenomic analyses were carried

out using the Type Strain Genome Server [3] to identify closely related non-Antarctic neighbours. Genome analysis was completed for two isolates: *Micromonospora ureilytica* DB10_83 and *Streptomyces* sp. DSM 109305, the latter being a potential new species.

In summary, novel Antarctic actinomycetes were isolated and proved to produce bioactive compounds. In further studies co-cultivation of the Antarctic actinomycetes and non-actinobacterial co-isolates will be performed. Metabolic analyses will be carried out to investigate the interaction and biosynthetic profile of Antarctic microorganisms.

[1] R. Subramani, D. Sipkema, Mar Drugs. **17** (2019).
[2] S. Soldatou *et al.*, Mar Drugs. **19** (2021).
[3] J. P. Meier-Kolthoff, M. Göker, Nat Commun. **10** (2019).

P-SMNP-012
Heterologous Expression of a lasso peptide from Rothia aeria from the human lung microbiome

*F. Semmler[1], M. Regis Belisario Ferrari[1], F. Nehring[1], L. Kaysser[1]
[1]*Institute for Drug Discovery, Leipzig, Germany*

The functions of ribosomally synthesized and post-translationally modified peptides (RiPPs) have already been studied in detail in various contexts of the human microbiota, as they represent a widely distributed class of natural products in bacteria (Arnison et al. 2013; Barbour et al. 2022; Benjdia und Berteau 2021; Bushin et al. 2020; Clark et al. 2022; Li und Rebuffat 2020; McIntosh et al. 2009). These peptides are known for their antimicrobial properties in competitive and regulatory processes in the microbiome (Barbour et al. 2022; Li und Rebuffat 2020). Some members of the lasso peptide subgroup, which are marked by their intriguing spatial structure and resulting chemical properties, have already been identified as antibiotic peptides (Bayro et al. 2003; Salomón und Farías 1992; Tan et al. 2019). In this context, we are investigating a gene cluster encoding a lassopeptide found in *Rothia aeria*, an organism of the oral and respiratory microbiome (Sonehara et al. 2021; Tsuzukibashi et al. 2017). The corresponding natural product was produced by heterologous expression in *E. coli* and in two different *Streptomyces* species and then comprehensively characterized. Particular attention was paid to its role in the lung microbiome, which was investigated in detail in a previously established microbiome model.

Sensing, Signaling & Communicating Microbes

P-SSCM-001
Ca^{2+} signaling in filamentous heterocyst forming cyanobacteria

*T. Müller[1], K. Forchhammer[1], K. A. Selim[2]
[1]*University of Tübingen, Microbiology/ Organismic Interactions, Tübingen, Germany*
[2]*University of Freiburg, Microbiology/ Molecular Physiology of Prokaryotes, Freiburg i. Br., Germany*

Free calcium (Ca^{2+}) ions can be highly toxic as they can precipitate phosphate ions, which are important for various metabolic pathways. Therefore, cyanobacteria regulate their intracellular Ca^{2+}-concentration $[Ca^{2+}]_i$ via pumps, channels and calcium binding proteins.[1,2] In multicellular cyanobacteria, Ca^{2+} signaling also plays an important role in heterocyst differentiation, a cellular compartment required for atmospheric N_2 fixation.[3] Shortly after nitrogen depletion, a condition that triggers heterocyst differentiation, a transient increase of $[Ca_{2+}]_i$ [4] in pro-heterocysts have been reported.[3] In the filamentous cyanobacterium *Nostoc* sp. PCC 7120 two calcium binding proteins have been identified: CcbP (cyanobacterial calcium binding protein)[3] and CSE (Ca^{2+} Sensor EF-hand).[5] CcbP is mainly known for its buffer property in capturing free Ca^{2+} ions. CSE binds Ca^{2+} via two characteristic Ca^{2+} sensor EF hand domains.[5] CSE appears unfolded in the absence of calcium and undergoes a strong conformation change upon Ca^{2+} binding. CSE is strongly downregulated during nitrogen depletion.[5,6] Although there is a strong connection between Ca^{2+} signaling and heterocyst differentiation, the specific functions of both calcium binding proteins, CcbP and CSE, remain elusive. Our aim is to further investigate the role of calcium signaling and calcium homeostasis in multicellular cyanobacteria with respect to heterocyst formation and photosynthesis. Therefore, we investigate the characteristics of knockout or overexpressing mutants of those proteins in *Nostoc* sp. with respect to cell-cell-communication, growth under nitrogen limitation and heterocyst formation.

1. D.E. Clapham, *Cell*, **2007**,*131*, 1047–1058.
2. V. Norris *et al*., *J. Bacteriol.*, **1996**, *178 (13)*, 3677–3682.
3. Y. Zhao *et el., Proc. Natl. Acad. Sci. U. S. A.*, **2005**, *102 (16)*, 5744–5748.
4. I. Torrecilla *et al., Microbiol.,* **2004**, *150*, 3731-3739.
5. J. Walter *et al., BBA Bioenerg.,* **2019**, *1860 (6)*, 519-532.
6. J. Walter *et al., BMC Microbiol.*, **2020**, *20:57.*

P-SSCM-002
EXCRETE provides unpredecented insights into the extracellular landscape of cyanobacteria

*D. A. Russo[1], D. Oliinyk[2], G. Pohnert[1], F. Meier[2], J. A. Z. Zedler[3]
[1]*Friedrich Schiller University Jena, IAAC, Jena, Germany*
[2]*Jena University Hospital, Functional Proteomics, Jena, Germany*
[3]*Friedrich Schiller University Jena, Matthias Schleiden Institute, Jena, Germany*

Secreted molecules mediate interactions with the environment and the surrounding microbiome, and numerous biotechnological applications take advantage of the secretion machinery. Bacterial protein secretion has mainly been studied in pathogenic and polymer-degrading bacteria due to their importance in human health and the environment. However, many globally abundant prokaryotes, such as cyanobacteria, remain understudied. This is mainly due to challenges associated to mass spectrometric analysis of extracellular samples where proteins are highly diluted in matrices rich is salts, lipids and polysaccharides. Here, we introduce EXCRETE (enhanced exoproteome characterization by mass spectrometry), a workflow that enables in-depth analysis of microbial exoproteomes from only a few hundred microliters of culture medium. EXCRETE benchmarking demonstrated an almost 60% increase in identified exoproteins in less than a third of the time typically requited to prepare exoproteomics samples. We then show that EXCRETE can be miniaturized and adapted to a 96-well-plate high-throughput format. Finally, EXCRETE was then applied to the exoproteome of cyanobacteria from different habitats: the freshwater *Synechocystis* sp. PCC 6803; the marine *Synechococcus* sp. PCC 11901; and the terrestrial *Nostoc punctiforme* PCC 73102. Our approach identified up to 7.5x more exoproteins than previous studies,

thus demonstrating the robustness of the method independent of the composition of the medium or extracellular matrix. Functional analysis of the three secretomes revealed a predominance of proteins involved in cell envelope maintenance, protein turnover, and nutrient acquisition. Collectively, these findings challenge the general belief that cyanobacteria lack secretory proteins and point to a functional conservation of the secretome across freshwater, marine and terrestrial species. This workflow should be broadly applicable to microbes from a wide range of habitats, with the potential to open new avenues of investigation in microbial exoproteomics.

P-SSCM-003
Identification of an a-factor-like peptide mating pheromone secreted by the heterothallic ascomycete Aspergillus fumigatus

*S. Krappmann[1], E. Gabl[1], T. Pazen[1], A. Heizmann[1], S. Pöggeler[2], M. Nowrousian[3]
[1]*Friedrich-Alexander-University Erlangen-Nürnberg, Institute for Clinical Microbiology, Immunology and Hygiene erlanegn, Erlangen, Germany*
[2]*Georg-August University Göttingen, Göttingen, Germany*
[3]*Ruhr University Bochum, Bochum, Germany*

Fungal sexuality accompanied by the formation of fruiting bodies that contain fertile meiospores relies on a complex sequence of events. In heterothallic ascomycetes, mating-type systems serve as regulatory means to secure that compatible isolates of opposite gender fuse to enter the sexual phase in their life cycle. This intricate process requires reciprocal secretion and recognition of pheromones, small peptides that are processed from precursors to become secreted into the cellular vicinity. Identification of mating pheromones of fungal origin with their cognate receptors is generally achieved by genome mining and homology searches, based on considerable conservation on the protein sequence level. In the taxonomic class of the Eurotiomycetes this approach had failed for peptides that would resemble a-factor-like pheromones due to their small size and low sequence conservation. Accordingly, the existence and nature of an a-factor-like peptide secreted by the heterothallic mould *Aspergillus fumigatus* had not been revealed to date. Sexuality of this opportunistic human pathogen is genetically determined by a bipolar mating-type system encoding master regulators in an exclusive manner. By making use of consistent transcriptional profiling data, we could identify an unannotated candidate gene *ppgB* (*p*heromone *p*recursor *g*ene *B*) encoding the presumed but so far elusive a-factor pheromone of *A. fumigatus*. The deduced peptide is 24 amino acids in length and comprises a canonical CaaX box motif at its C-terminus. Transcription patterns of *ppgB* and functional analyses of its hydrophobic product by employing a suitable test system that is based on pheromone-sensitive yeast cells strongly support the hypothesis that PpgB serves as prototype for the long-sought a-factor like pheromone of the aspergilli. The identification of *A. fumigatus* PpgB closes a substantial knowledge gap with respect to cellular recognition and sexual propagation of Eurotiomycete fungi.

P-SSCM-004
Bacterial symbiosis in the phototrophic consortium \`Chlorochromatium aggregatum´ involves an interspecies transfer of giant virulence-factors and adhesins

*S. Kuzyk[1], P. Henke[1], F. Burkart[1], J. Müller[1], C. Jogler[1], G. Wanner[2], J. Overmann[1]
[1]*DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Mikrobielle Ökologie und Diversitätsforschung, Brunswick, Germany*
[2]*Ludwig-Maximilians-Universität München, Planegg-Martinsried, Botany Section, Department Biologie I, München, Germany*

During infection, pathogenic bacteria often transfer virulence factors to host cells and utilize adherence mechanisms to persist. We detected closely related adhesion and virulence factor-like proteins within the multicellular phototrophic symbiotic consortium \`*Chlorochromatium aggregatum*´, indicating a specific role in mutual interactions. This highly developed microbial symbiosis between two species of different phyla represents the only bacterial model consortium that is experimentally accessible to date. Within \`*C. aggregatum*´, the phototrophic epibiotic *Chlorobium* encodes three unique RTX-like or hemagglutinin-like proteins, each found to be directly involved in the interaction. Two proteins are of giant size, up to 36,000 amino acids long, thereby representing the largest proteins yet discovered in any organism, surpassing the previous record of titin protein in humans. Growth kinetic-dependent transcriptomics substantiated the expression of both giant proteins and respective secretion systems during the symbiotic state. Notably, immunoblotting, superresolution immunofluorescence microscopy, and immunogold labelling localized each epibiont-produced symbiosis factor predominantly at either the junction regions with its heterotrophic partner or within the cells of the latter. Structural and homology prediction of the RTX-like protein as a biofilm alginate lyase (allowing nutrient exchange between bacteria) in addition to its structural adhesion potential, was also supported by enzymatic assays from heterologous over-expression extracts. Analogous genes were further found in uncultured metagenomes of similar consortia "*Pelochromatium roseum*". Together, our results suggest that hemagglutinin-like and RTX-like proteins are also involved in bacterial symbioses, extending far beyond known specific pathogenic interactions with eukaryotes, thereby broadening our general understanding of the evolution of bacterial virulence factors.

P-SSCM-005
A pathoblocker of Pseudomonas aeruginosa affects bacterial interactions

*O. Kelting[1,2], E. Kosche[3,4,5], M. Empting[3,4,5], D. Unterweger[1,2]
[1]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute for Experimental Medicine, Kiel, Germany*
[2]*Max Planck Institute for Evolutionary Biology, Plön, Germany*
[3]*Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS) Helmholtz Centre for Infection Research (HZI), Saarbrücken, Germany*
[4]*Saarland University, Department of Pharmacy, Saarbrücken, Germany*
[5]*German Center for Infection Research, Brunswick, Germany*

The diminishing effectiveness of traditional antibiotics necessitates alternative treatment strategies. One promising novel approach is the use of pathoblockers. These compounds inhibit bacterial virulence factors without effecting bacterial growth. In this study, we explore the use of a compound that interferes with quorum sensing of *Pseudomonas aeruginosa*, a pathogen that commonly causes severe lung infections. The tested compound blocks the *Pseudomonas* quinolone signal and thereby downregulates virulence factors including toxins with anti-prokaryotic activity[1]. We hypothesize that the pathoblocker acting on *P. aeruginosa* may have effects on bacterial interactions with other coexisting pathogens.

In order to test this hypothesis, we performed spent media and cocultivation experiments of *P. aeruginosa* and a panel of microbes, which are commonly isolated from patients (*Staphylococcus aureus*, *Serratia marcescens, Escherichia coli*), in presence and absence of the compound. Furthermore, we used qPCR and RNA sequencing to elucidate the underlying mechanisms of pathoblocker treatment. Our results demonstrate a significant impact of the pathoblocker on *P. aeruginosas'* ability to reduce the number of living cells of *S. aureus* and *E. coli* in spent media. Additionally, the treatment leads to increased survival of *S. aureus* during cocultivation with *P. aeruginosa*.

In conclusion, this study highlights the importance of bacterial interactions in the context of drug development.

(1) Hamed, M. M.; Abdelsamie, A. S.; Rox, K.; Schütz, C.; Kany, A. M.; Röhrig, T.; Schmelz, S.; Blankenfeldt, W.; Arce-Rodriguez, A.; Borrero-de Acuña, J. M.; Jahn, D.; Rademacher, J.; Ringshausen, F. C.; Cramer, N.; Tümmler, B.; Hirsch, A. K. H.; Hartmann, R. W.; Empting, M. Towards Translation of PqsR Inverse Agonists: From In Vitro Efficacy Optimization to In Vivo Proof-of-Principle. *Adv. Sci.* **2023**, *10* (5), 2204443. https://doi.org/10.1002/advs.202204443.

P-SSCM-007
Lipid rafts in Aspergillus nidulans and their importance in organismic communication
*M. Kawashima[1], A. A. Brakhage[1]
[1]Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie, Molecular and Applied Microbiology, Jena, Germany

Lipid rafts are tight assemblies of proteins and lipids in a biological membrane and are thought to be involved in many physiological processes such as immune signalling and host-pathogen interactions. However, due to their small sizes exceeding the resolution limit of conventional light microscopy, direct measurement and characterisation of lipid rafts in living membranes remains to be a challenge. Most studies on lipid rafts have been carried out on mammalian cells, but utilising lower eukaryotes is advantageous as the general properties of their plasma membrane organisation are similar to that of mammals while they are also easier to manipulate genetically. In this project, we aim to gain insight on the characteristics of lipid rafts in the model filamentous fungus *Aspergillus nidulans*, with a particular focus on the involvement of the SPFH protein, FloA. This protein was first tagged with GFP and observed as punctuated structures in the plasma membrane by Takeshita et al. (2011), but its functions are still unknown. Here, we show through use of a nanoluciferase fusion that FloA is highly expressed when *A. nidulans* is co-cultivated with the bacterium *Streptomyces iranensis*, which lives in soil as does the fungus. Interestingly, a mutant of the bacterium which is unable to produce secondary metabolites cannot induce the high expression of FloA, indicating that this upregulation is attributable to a secondary metabolite produced by *S. iranensis*. This finding could lead to the uncovering of the regulatory mechanisms as well as the biological functions of FloA and possibly its importance in organismic communication.

P-SSCM-008
The phosphodiesterase (PDE) activity exhibited by NbdA is dependent on the presence of heme
*N. Bäuerle[1], J. Rehmel[1], M. T. Agbadaola[1], M. Schösser[1], E. Pérez Patallo[1], N. Frankenberg-Dinkel[1], S. Zehner[1]
[1]University of Kaiserslautern-Landau, Microbiology, Kaiserslautern, Germany

The sensor phosphodiesterase (PDE) NbdA from the opportunistic human pathogen *Pseudomonas aeruginosa* comprises three domains, an MHYT inner membrane domain, and the cytosolic tandem GGDEF-EAL domain. Previous experiments have demonstrated that the cytosolic domain of NbdA is capable of degrading the second messenger cyclic di-GMP (Li *et al*. 2013). This molecule regulates virulence and biofilm formation in *P. aeruginosa (Römling et al. 2013)*. Recombinantly produced and purified full-length protein NbdA from *E. coli* BL21(DE3) did not show any phosphodiesterase activity. Interestingly, the full-length protein demonstrated activity when produced in *E. coli* Nissle 1917 in the presence of heme and iron ions.

The aim of this study is to elucidate the putative binding of heme to purified full-length NbdA and the dependency of PDE activity. The solubilization of the full-length protein from *E. coli* Nissle 1917 membrane fractions using polymer nanodiscs enabled spectroscopic characterization of heme binding to the protein. Absorption spectra of oxidized and reduced NbdA revealed characteristic Soret bands for heme proteins at 412 nm (oxidized) and 424 nm (reduced). Results from the pyridine-hemochrome method suggest a non-covalent binding of heme to NbdA.

In summary, *in vivo* supplementation of heme during heterologous production leads to PDE activity of NbdA. Combined with spectroscopic investigations, it is inferred that heme is specifically bound by NbdA. Further enzyme assays regarding NbdA's dependence on heme are currently underway.

References

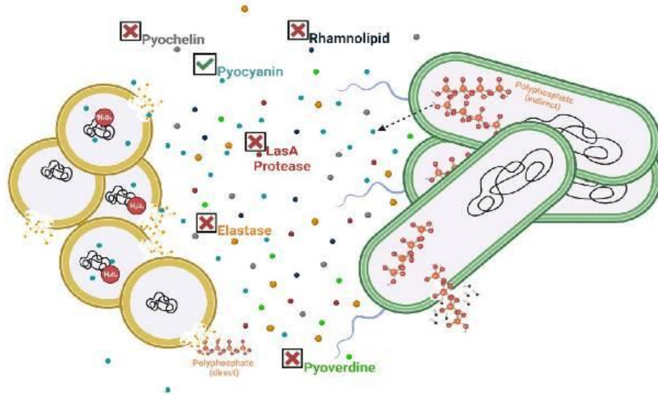
Li *et al.* (2013) *J. Bacteriol.* 195 (16), 3531–3542.
Römling *et al.* (2013) *Microbiol. Mol. Biol. Rev.* 77 (1), 1–52.

P-SSCM-009
Pseudomonas aeruginosa kills Staphylococcus aureus in a polyphosphate-dependent manner
R. Shah[1], O. Jankiewicz[1], C. Johnson[1], B. Livingston[1], *J. U. Dahl[1]
[1]Illinois State University, School of Biological Sciences, Normal, IL, United States

Due to their frequent coexistence in many polymicrobial infections, including in patients with burn or chronic wounds or cystic fibrosis, recent studies have started to investigate the mechanistic details of the interaction between the opportunistic pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *P. aeruginosa* rapidly outcompetes *S. aureus* under *in vitro* co-cultivation conditions, which is mediated by several of *P. aeruginosa*"s virulence factors. Here, we report that polyphosphate (polyP), an efficient stress defense system and virulence factor in *P. aeruginosa*, plays a role for the pathogen"s ability to inhibit and kill *S. aureus* in a contact-independent manner. We show that *P. aeruginosa* cells characterized by low polyP level are less detrimental to *S. aureus* growth and survival while the gram-positive pathogen is significantly more compromised by the presence of *P. aeruginosa* cells that produce high level of polyP. Using fluoremetric approaches and fluorescence microscopy, we show that the polyP-dependent phenotype could be a direct effect by the biopolymer, as polyP is present in the spent media and causes significant damage to

the *S. aureus* cell envelope. However, more likely is that polyP"s effects are indirect through the regulation of one of *P. aeruginosa"s* virulence factors, pyocyanin. We provide evidence that pyocyanin production in *P. aeruginosa* occurs polyP-dependent and harms *S. aureus* through membrane damage and the generation of reactive oxygen species, resulting in increased expression of antioxidant enzymes. In summary, our study adds a new component to the list of biomolecules that the gram-negative pathogen *P. aeruginosa* generates to compete with *S. aureus* for resources.

Fig. 1



Technical Hygiene, Environmental Sustainability Regarding the Climate Change

P-TEC-001
The attempt to improve barn hygiene of weaned pigs with the aim of reducing the spread of antimicrobial resistance

*M. Jaleta[1], U. Nübel[2], V. Junker[2], B. Kolte[2], M. Börger[3], C. Dolsdorf[4], J. Schwenker[5], C. Hölzel[5], T. Amon[1], T. Kabelitz[1]
[1]*Leibniz Institute of Agricultural Engineering and Bioeconomy (ATB), Sensor and Modeling, Potsdam, Germany*
[2]*Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Microbial Genome Research unit, Brunswick, Germany*
[3]*Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany*
[4]*Teaching and Research Farm for Animal Breeding and Husbandry, Ruhlsdorf, Teltow, Germany*
[5]*Christian-Albrecht University Kiel and University Medical Center Schleswig-Holstein, Institute of Animal Breeding and Husbandry - Animal Hygiene, Animal Health and Food Hygiene, Kiel, Germany*

The spread of antimicrobial-resistant pathogens in animal husbandry is usually attributed to poor hygiene and biosecurity. We therefore conducted an experimental trial to improve hygiene management in weaned pig houses and assessed the impact on the spread of antimicrobial resistance. For each of the two groups, the so-called experimental group and the control group, three batches of piglets were taken from the same pig breeder and kept in pre-cleaned flat decks. Hygiene conditions (cleaning, disinfection, dust removal and fly control) were improved on the flat decks of the experimental group, while regular procedures were followed in the control groups. The extent and spread of antibiotic resistance (AMR) were determined in *Escherichia coli* (*E. coli*; resistance indicator) using cultivation-dependent (CFU) and -independent (qPCR) methods as well as genomic sequences in various samples, including feces, flies, food, dust and swab. Surprisingly, there were no significant differences in the prevalence of resistant *E. coli* between the flat decks managed with conventional hygiene management and those managed with improved methods. Ampicillin - and sulfonamide-resistant *E. coli* were common, while cefotaxime-resistant *E. coli* was detected in rare cases and ciprofloxacin was absent in both groups. Factors such as colonization of piglets with AMR *E. coli* before arrival at fattening farm and frequent treatment of bacterial infections with antibiotics overshadowed the impact of hygiene management. The biocide tolerance test also showed no development of resistance to the disinfectants regularly used for house cleaning. It became clear that further attempts at hygiene management alone could not lead to a reduction in AMR in the piglet house. The multifactorial nature of AMR prevalence in the piglet house highlights the need for a holistic approach to AMR control strategies.

P-TEC-002
Building bridges between local public health authorities and university medicine - experiences from Germany

*A. Jack[1], M. Tröger[1], C. Hornberg[1]
[1]*Bielefeld University, Sustainable Environmental Health Sciences, Medical Faculty Bielefeld, Bielefeld, Germany*

Pandemics have shown that health crisis-management is a task that goes beyond individual institutions and sectors. Evidence-based forecasts suggest that pathogen-related scenarios will occur again (Jones et al. 2008; Marani et al. 2021) - driven and amplified by various impacts of anthropogenic environmental changes such as intensive land use, loss of biodiversity and climate change (Lefrançois et al. 2022). Accordingly, the role of public health systems will become increasingly important. This will inevitably be accompanied by increased requirements that an individual public healthcare system is currently only able to fulfil to a limited extent with its own capacities due to numerous reasons. Here, we share our experiences in expanding the collaboration between academic medicine and public health authorities to address these challenges. In terms of infection tracing and control, the value of this cooperation was demonstrated in an early period of the SARS-CoV-2 pandemic: initial successes with integrated outbreak management supported by genomic data was achieved (a study of an outbreak in a large meat-processing facility). Second, supra-regional interdisciplinary networks for genomic surveillance have subsequently been established, which have promoted further cooperation between public health sites and academic medicine. Effective participation of our local public health network in the German University Medicine Network (initially founded in response to the pandemic) emphasizes the importance of integrating public health authorities in research. Further, we identified numerous critical obstacles which need to be overcome for achieving sustainable competence clusters.Networking between academic medicine and public health institutions on various levels is an important factor for health and infection management in general. Our experiences from collaborations with the public health sector during pandemic scenarios demonstrate both the benefits and challenges of collaborative infection management, thus highlighting the importance to further develop "bridges" between public health authorities and academic sites in the face of upcoming local and global health crises.

P-TEC-003

Industrial side streams as basic feed for black soldier fly farming and the production of organic fertilizer

*D. Kreft[1,2], F. Gurusinga[1,2], R. A. Düring[3], S. Hurka[1,2,4], D. Tegtmeier[1,2], A. Vilcinskas[2,4,5]
[1]*BMBF Junior Research Group in Bioeconomy (BioKreativ), SymBioEconomy, Giessen, Germany*
[2]*Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Branch for Bioresources, Giessen, Germany*
[3]*Institute of Soil Science and Soil Conservation, Justus Liebig University, Research Centre for BioSystems, Land Use and Nutrition (iFZ), Giessen, Germany*
[4]*LOEWE Centre for Translational Biodiversity Genomics (LOEWE-TBG), Frankfurt a. M., Germany*
[5]*Justus Liebig University Giessen, Institute for Insect Biotechnology, Giessen, Germany*

Introduction

Larvae of the black soldier fly (*Hermetia Illucens*) can be used for the production of protein and fat for poultry farming and aquaculture. Larval biomass can be obtained by utilizing local side streams from agricultural or industrial production as insect feed. The residue from black soldier fly larvae (BSFL) farming, so-called frass, consists of insect excrements and feed residues. Frass is produced as a side stream itself and can be used as a valuable organic fertilizer.

Goals

The aim of our study was to evaluate different side streams as feed for BSFL and how the frass differs chemically and microbially depending on the infeed substrate. We also investigated the effect of thermal hygienization at 70°C to reduce microbes, as required by the EU directive, with regard to the effects on the microbiome that promotes plant growth. The findings should contribute to the development of frass as fertilizer for agriculture.

Methods

Our larvae were fed with potato peelings, potato pulp, apple pomace and rapeseed press cake to study their growth behaviour, while substrate, larval and feed samples were determined by elemental analysis. The frass was hygienized and the count of colony forming units (CFU) on selective and non-selective media was compared with that of an untreated sample. Subsequently, microorganisms were isolated and identified by Sanger sequencing. Amplicon sequencing of the frass samples was carried out for comparison.

Results

In the feeding trials, substrate-dependent differences in BSFL growth and chemical composition of the frass were found, with chicken feed showing the best results, also with regard to its use as NPK fertilizer. Hygienization was shown to have the desired effect, namely a reduction in CFU. The amplicon data so far showed recognizable variations in the microbiome, depending on the feed used.

Summary

Overall, we were able to show that not every industrial side stream is suitable for optimal larval growth characteristics. We were able to demonstrate the CFU-reducing hygienization effect in frass. We also found chemical differences and a clear variance in the microbiome depending on the feed used for BSFL rearing.

P-TEC-004

GENTRAIN – genome-based infection chain analysis to increase digitalization level in the public health sector

*M. Tröger[1], A. Jack[1], A. Dilthey[2], C. Hornberg[1]
[1]*Universtiy Bielefeld, Medical Faculty, Bielefeld, Germany*
[2]*Heinrich Heine University Düsseldorf, Düsseldorf, Germany*

Monitoring the dynamics of pathogen spread by public health authorities is a core element for infection prevention during pandemics but also in inter-pandemic phases. Infection management measures that are adaptable to individual and local circumstances can be highly advantageous. One example of this is genome-based or integrated infection chain analysis. This concept combines methods of classic contact tracing with genomic sequence data from molecular pathogen analysis. This enables high resolution infection chain management within the general population or in diffuse local outbreak scenarios. During the SARS-CoV-2 pandemic, we successfully demonstrated applications for digitally supported infection chain analysis, showing its valuable potential (Walker et al. 2022). The aim of the GENTRAIN project is to develop digital applications for the use of genomic-based infection chain analysis by public health authorities in Germany for further relevant pathogens beyond SARS-CoV-2. In order to contribute to a sustainable increase in digital development, the project primarily aims to enhance the dimensions of data interoperability, software and data management as well as the area of employee competence within the German public health authorities. The project expanded the tools of integrated genomic surveillance to analyze genomes of other public-health relevant pathogens (e.g. antibiotic-resistant bacteria). For sustainable improvement of data management capacities in public health authorities, we identified and describe several key factors. For instance, adaptation of existing software is essential with special regard to needs and capacities of local public health authorities. Availability of interfaces for routine data exchange is highly significant, as well as ongoing training for public health personnel. The utilization of this potential by public health authorities is essential, especially for predicted pandemic situations and the threat of increasing antibiotic resistance. Perspectively, this will contribute to more precise, evidence-based and faster reporting systems to support political decisions in terms of preventive measures - at municipal and state level.

Teaching Approaches & New Medical Approbation Regulations

P-TNAR-001

Bedeutung der curricularen Lehre im Fach Medizinische Mikrobiologie

*C. Brandt[1], F. Mattner[2]
[1]*University Hospital Frankfurt, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, Frankfurt a. M., Germany*
[2]*University Witten/Herdecke, Faculty of Health and Institute of Hygiene, Köln, Germany*

Hintergrund:

Neben den gesetzlichen Anforderungen durch eine Novellierung der ärztlichen Approbationsordnung stellen die Weiterentwicklung moderner Lernformate, die Digitalisierung und der demographische Wandel ständige Herausforderungen für die Ausbildung von Studierenden der Humanmedizin dar. Der aktuelle Stand des Lehrangebots im Fach "Medizinische Mikrobiologie" an deutschen Fakultäten wurde als Ausgangslage vor anstehenden Veränderungen erhoben.

Methoden:

In einer im September 2023 erhobene Umfrage (Fragenkatalog) über das curriculare Lehrangebot an deutschen Universitätsklinika nahmen 32 der 40 angefragten Standorte teil. Die Email-basierte Kommunikation erfolgte über die Geschäftsstelle der Deutschen Gesellschaft für Hygiene und Mikrobiologie.

Ergebnisse:

Alle Standorte sind aktiv in die Ausbildung von Studierenden der Humanmedizin eingebunden. Dabei werden an ca. der Hälfte der Standorte jährlich 300 bis 400 Studierende, an vier Standorten sogar jährlich mehr als 400 Studierende ausgebildet.

An neun der Universitätsklinika ist ein Modellstudiengang etabliert. An 31 der 32 Universitäten wird die curriculare Lehre in der Medizinischen Mikrobiologie und Hygiene in Form eines Praktikums und einer begleitenden Hauptvorlesung angeboten. Fakultative Lehrangebote, wie z. B. die Möglichkeit zur Famulatur oder einem PJ-Wahltertial, bestehen an 26 Universitätsklinika.

Zusammenfassung:

Das historisch gewachsene und bewährte Lehrangebot im Fach "Medizinische Mikrobiologie" stellt eine wichtige Säule für die nachhaltige Vermittlung der Lerninhalte und die wissenschaftliche Weiterentwicklung des Faches. Ziel eines zeitgemäßen und zukunftsfähigen zukünftigen Curriculums muss sein, dass die Absolventen und Absolventinnen nicht nur eine evidenzbasierte und finanziell abbildbare Medizin ausüben, sondern die Medizin auch weiterhin wissenschaftlich geprägt ausüben.

P-TNAR-002

Wie wird an medizinischen Fakultäten aktuell das Fach Hygiene gelehrt?

*F. Mattner[1]

[1]*Cologne Merheim Medical Centre, Institute of Hygiene, Köln, Germany*

Die gemeinsame Entwicklung des NKLM zusammen mit dem IMPP unter Mitwirkung zahlreicher Experten hat zur Formulierung von Lernzielen sowie der Festlegung von prüfungsstufenrelevanten Kompetenzen geführt, die von denen des alten Gegenstandskatalogs abweichen.

Während der über Jahre geführten Entwicklungen, haben sich die Studiendekanate teilweise bereits an neue Anforderungen angepasst oder stehen jetzt davor, Anpassungen vornehmen zu müssen. Das Fach Hygiene umfasst Hygiene und Umweltmedizin; wird aber – wie auch alle anderen Fächer nicht mehr als Fach dargestellt, sondern in Lernziele runtergebrochen, die beispielsweise durch das Fach Hygiene gelehrt werden können.

Krankenhaushygiene sollte möglichst interdisziplinär und praktisch vermittelt werden. Dabei besteht die Gefahr, dass die Lehrinhalte von anderen Fächern vereinnahmt werden und personelle, räumliche und apparative Kapazitäten aus den Krankenhaushygienischen Mitteln herausgenommen werden. Damit würde die Krankenhaushygiene an den Universitäten nicht gestärkt, sondern weiter geschwächt und das politische Ziel der Medizinerausbildung zu mehr und besserer Prävention noch schwerer erreichbar werden.

Hier werden aktuelle Daten einiger Universitäten gezeigt, wie die Lehre derzeit in Hinblick auf Lernziele tatsächlich umgesetzt oder auch nicht umgesetzt werden – ggf. in Vergleich zur zwischenzeitlich verabschiedeten neuen Approbationsordnung.

P-TNAR-003

Die experimentelle Medizin und die klinisch-theoretischen Institute

*J. Jantsch[1]

[1]*University Hospital Cologne, Institute of Medical Microbiology, Immunology and Hygiene, Köln, Germany*

Die ärztliche Aufgabe umfasst ein weites Spektrum verschiedener Tätigkeiten. Der Patientenbezug und eine weitere Praxisorientierung stehen im öffentlichen Fokus. Weniger beachtet wird jedoch, dass auf wissenschaftlicher Grundlage fußende Therapien aus der experimentellen Medizin erwachsen und dass die experimentelle Medizin in sehr großem Maße von den Wissensbeständen und Praktiken profitiert, die in den theoretischen Instituten und klinisch-theoretischen Einrichtungen gelehrt und durchgeführt werden. Die zukünftige Anpassungsfähigkeit und Innovationskraft in der Medizin ist somit sehr stark an Infrastrukturen geknüpft, die von theoretischen und klinisch-theoretischen Instituten unterhalten werden. Dies unterstreicht den fortwährenden Nutzen der theoretischen und klinisch-theoretischen Medizin. Es erscheint daher ratsam, Strategien zu entwickeln, um die Funktionalität dieser Einheiten langfristig zu sichern.

P-TNAR-004

Teaching antibiotic stewardship through interaction - a gamified learning approach

*S. Driesnack[1], N. Dietze[1], A. Viehweger[1]

[1]*University Hospital Leipzig, Medical Microbiology and Virology, Leipzig, Germany*

Introduction

Medical teaching often focuses on standardized tests instead of applied skills, which regularly leads to frustration when young doctors note a mismatch between what they learned and what is required on the job. Specialized courses are a solution, but we argue that without interactive practice, they risk limiting the long-term sustainability of the knowledge gained.

Goals

We created an elective course on ABS for medical students, substantially increasing the practice students received. We used gamified learning, a chatbot using artificial intelligence, and learning methods such as flashcards to do this. The goal was to create an output-oriented learning experience - where learners apply their gained knowledge - and to study its impact using several tests taken before, during and after the course.

Materials & Methods

We studied students across three runs of an ABS student elective. We assessed factual skills and qualitative aspects, such as perceived security in selecting an antibiotic therapy option. Students were also exposed to various analog games, practicing pattern recognition and reinforcing concepts from the course. Knowledge was further reinforced using flashcards on a spaced repetition schedule. We followed students after the course at three, six, and twelve months.

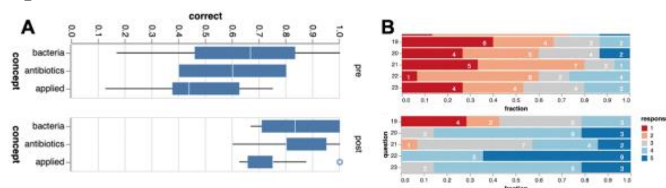
Results

All 37 enrolled students improved substantially, both on the factual questionnaire (A) and on the perception of their ability on a qualitative (Likert) scale (B). For example, while less than half of the questions on applied therapy aspects were answered correctly before the course, after the course, the mean accuracy increased to over 75%. Also, before, most students were insecure about their antibiotic prescriptions. Afterward, most students felt at least somewhat secure, as assessed on a 5-step Likert scale.

Summary

More dynamic, adaptive, and interactive teaching methods can empower medical students and young doctors to actively use the knowledge gained throughout the course. We demonstrated that this can be implemented in infectious diseases, leading to solid operating algorithms, long-term memory, and safety when prescribing antibiotics.

Fig. 1



ISBN: 978-3-948023-39-3