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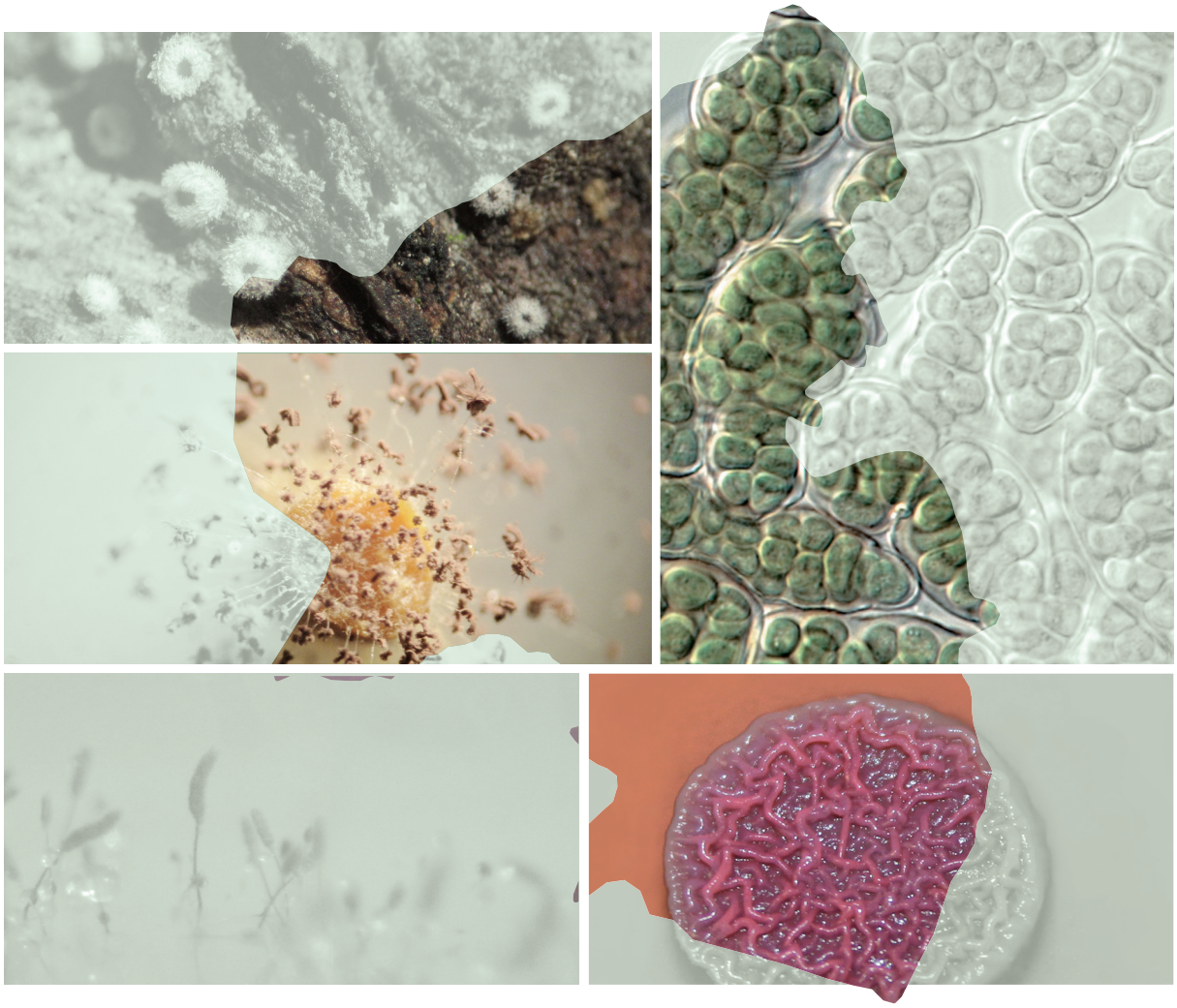

17–20 MARCH 2019 MAINZ

Johannes-Gutenberg-University

JG|U JOHANNES GUTENBERG UNIVERSITÄT MAINZ

TECHNISCHE UNIVERSITÄT KAISERSLAUTERN

ABSTRACTS

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IL01

From Cell Polarity to Bacterial Virulence Control

U. Jenal*[1]
[1]*Biozentrum, University of Basel, Switzerland*

Cyclic dinucleotides are highly versatile signaling molecules that control and coordinate a range of important biological processes in bacteria and eukaryotes. The best studied example is cyclic di-GMP (c-di-GMP), a near ubiquitous second messenger that coordinates diverse aspects of bacterial growth and behavior, including motility, cell cycle progression, virulence, and biofilm formation. In this lecture, I will give a brief overview on c-di-GMP signaling principles and will highlight examples of how c-di-GMP controls growth and behavior of different bacteria. The first illustrates how oscillating levels of c-di-GMP determine cell polarity, morphogenesis, and cell cycle progression of Caulobacter crescentus, an aqueous bacterium with a characteristic bi-modal life cycle. Work in this non-pathogenic model organism has provided a basic molecular and cellular understanding of the c-di-GMP network that has guided our studies of experimentally less tractable systems like the human pathogen Pseudomonas aeruginosa. In the second part, I will provide an update on how P. aeruginosa makes use of c-di-GMP to induce its full virulence potential. In particular, our studies have disclosed novel strategies, through which this organism optimizes host tissue colonization.

IL02

Repurposing bacterial two-component systems as sensors for synthetic biology applications

J. J. Tabor*[1]
[1]*Rice University, Department of Bioengineering, Houston, TX, United States*

Bacterial two-component histidine kinase systems (TCSs) are an important family of bacterial signal transduction pathways, and a treasure trove of sensors for synthetic biology. TCSs sense a remarkable diversity of chemical and physical inputs, from metal ions of particular oxidation states to gut polysaccharides and light. We and others have previously repurposed TCSs to engineer diagnostic gut bacteria and for a range of optogenetic applications. Despite this promise, a number of major challenges still remain. First, though tens of thousands of TCSs have been identified in bacterial genomes, the vast majority remain uncharacterized. Second, even when characterized, TCSs are often silent in heterologous hosts, subject to poorly understood output promoter cross-regulation, or too sensitive or insensitive for the application at hand.

Recently, we have developed a suite of synthetic biology technologies enabling us to reliably express and optimize the performance of bacterial TCSs in heterologous host organisms. Our methods involve 2-D expression optimization of sensor kinases (SKs) and response regulators (RRs), RR DNA binding domain (DBD)-swapping, output promoter sequence optimization, and mutation of signaling residues to tune detection thresholds. We have combined bioinformatic genome mining with large scale commercial gene synthesis and DBD swapping to construct an unprecedented library of over 500 TCSs from the human gut microbiome which we are screening against a wide range of inputs. We have improved the dynamic range of evolved TCSs from ~3 to ~600 fold, ported TCSs between Gram negative and positive bacteria, identify a novel pH sensing TCS from S. oneidensis, eliminate unwanted cross-regulation at TCS output promoters, discovered dozens of TCSs that respond to human fecal samples, and increased the sensitivity of TCSs for their ligands by hundreds of fold. We are applying these results to medical, environmental, and industrial problems. Our work is enabling unprecedented access to bacterial TCSs, a large and important family of bacterial signal transduction pathways. It has implications in fundamental microbiology and synthetic biology.

IL03

No abstract has been submitted.

IL04

Metabolic Coordination Through Metabolite-Protein Interactions

U. Sauer*[1]
[1]*ETH Zürich, Inst. für Molekulare Systembiologie, Zürich, Switzerland*

How do bacteria know what goes on in their environment and how to they make appropriate decisions? While some bona fide extracellular sensors are known, there are far more environmental conditions and cellular responses than could possibly be dealt with through dedicated sensors. Instead, most microbial responses are based on intracellular changes to environmental changes. One of the first affected networks to just about any extracellular change is metabolism that passively responds to nutritional or chemical/physical challenges. Since fluxes and intracellular metabolite levels respond within seconds, allosteric binding of metabolites to regulatory proteins and enzymes is a highly effective and rapid sensing mechanism. Different from well-establish methods to assess physical interaction between proteins and between proteins and nucleic acids, however, methods to assess metabolite-protein interactions are still in their infancy. At present we know on the order of 1500 unique regulatory metabolite-protein interactions (1), which is only the tip of the iceberg (2). Beyond mapping the regulation network, I will focus in this talk on the even more challenging and conceptual problem: understanding which of the many regulation mechanisms actually matter for a given adaptation to elicit an appropriate physiological system response. The surprising result for *E. coli* is that only very few regulation events appear to be required for a given transition, typically involving less than a handful of active regulators (3).

1. Reznik, Christodoulou, Goldford, Briars, Sauer, Segre & Noor. **Cell Reports** 20: 2666-2677 (2017).
2. Piazza, Kochanowski, Cappelletti, Fuhrer, Noor, Sauer & Picotti. **Cell** 72:358-372 (2018).
3. Kochanowski, Gerosa, Brunner, Christodoulou & Sauer. **Molecular Systems Biology** 13: 903 (2017).

IL05

How a fungus manages to colonize a plant and cause disease: what have we learned from a model system?

R. Kahmann*[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

To colonize plants successfully and cause disease, fungi need to down-regulate plant defense responses and reprogram the metabolism of the host to support pathogen growth and development. We are using the biotrophic

fungus *Ustilago maydis*, which causes corn smut disease, as a model to study these processes molecularly. During colonization *U. maydis* secretes a cocktail of several hundred effector proteins whose expression correlates with distinct phases of colonization. The majority of these proteins lack known domains and their function remains to be determined. In the few cases, where the molecular function of such novel effectors has been elucidated, they interact with plant proteins and inhibit or modulate their activity. Based on a comparative analysis of 11 smut genomes, we have now identified a set of core effectors and have systematically deleted them in *U. maydis*. 40% of the mutants were affected in virulence and in eight mutants virulence was completely abolished. These latter mutants were able to penetrate, but their growth stopped in epidermal tissue. We now show that these essential effectors, whose expression coincides with the establishment of biotrophy, reside in a protein complex. We demonstrate genetically that complex formation is critical for disease progression and speculate that the effector complex constitutes the structural part of a machinery responsible for contact and communication with the host plant.

IL06
Microbial symbioses as sources of evolutionary innovation in insects
M. Kaltenpoth*[1]
[1]*Johannes Gutenberg University, Department for Evolutionary Ecology, Mainz, Germany*

Symbiotic associations with microbes are important driving forces of evolutionary innovation. Insects are associated with an astounding diversity of symbionts that provide a wide range of functional benefits to their hosts. Highlighting a few selected insect-microbe associations, I will report on novel findings of symbiont-provided antibiotic defense and microbe-enabled digestion of plant material. These examples are intended to show the impact of symbionts on niche expansion and adaptation in insects, but also provide insights into life-style switches in bacteria as well as the process of extreme genome reduction beyond the well-known intracellular symbioses.

IL07
Small Talk – Chemical communication of entomopathogenic bacteria
R. Heermann*[1]
[1]*Johannes Gutenberg University Mainz, Institute of Molecular Physiology and Microbiology, Mainz, Germany*

Bacteria communicate via small diffusible molecules to regulate group-coordinated behaviour, a process termed quorum sensing (QS). The best-studied QS systems are those that use acylated homoserine lactones (AHLs) for communication. In their prototype these systems consist of only two components, a LuxI-like AHL synthase and a LuxR-type receptor. LuxI produces the specific AHL and the cognate LuxR receptor adapts gene expression upon receiving the AHL as signal. However, many proteobacteria possess LuxR receptors but lack any LuxI-type synthase, and thus they are referred to as LuxR solos. The insect pathogen *Photorhabdus luminescens* harbours the LuxR solo PluR, which we found not to sense AHLs but alpha-pyrones named photopyrones (PPYs) produced by the pyrone synthase PpyS [1]. The closely related insect and human pathogen *Photorhabdus asymbiotica* has a close relative to PluR, named PauR, but the bacteria lack both LuxI and PpyS. Instead of PPYs and AHLs we found that PauR senses dialkylresorcinols (DARs) that are synthesized by the DarABC pathway [2]. The PpyS/PluR as well as the DarABC/PauR system are the first two examples of LuxR solo-based QS systems, which use other "languages" than AHL. Both systems regulate cell clumping and thereby contribute to the overall pathogenicity of the bacteria. Since DarABC combined with a LuxR solo is widespread even among human pathogens, the respective QS system might be a promising target for novel antimicrobial drugs.

[1] Brachmann, A.O.; Brameyer, S.; Kresovic, D.; Hitkova, I.; Kopp, Y.; Manske, C., Schubert, K.; Bode, H.B.; Heermann, R. (2013). Nat. Chem. Biol. 9(9):573-578.

[2] Brameyer, S.; Kresovic, D.; Bode, H.B.; Heermann, R. (2015). PNAS 112(2):572-7.

IL08
Cable bacteria bridging the anaerobic and the aerobic world
L. P. Nielsen*[1,2]
[1]*Aarhus University, Center for Electromicrobiology, Aarhus, Denmark*
[2]*Aarhus University, Department of Bioscience, Microbiology, Aarhus, Denmark*

Anaerobic processes prevail in sediment and biofilm when oxygen demand exceeds the supply by diffusion, advection, and production. Reduced products of anaerobic decomposition can be re-oxidized through cascades of redox and transport processes with oxygen as the ultimate electron acceptor. Cable bacteria, however, circumvent the cascades by transporting electrons from electron donors to oxygen through electric wires in their multicellular, filamentous body, thus challenging the conventional distinction between aerobes and anaerobes. Cable bacteria in the Desulfobulbacea family often dominate sediment metabolism by coupling sulphide oxidation and oxygen reduction over centimeter distances. In this presentation, I will discuss how far we are in understanding the factors determining the success of cable bacteria in the environment. The spatial separation of oxidation and reduction processes induces pH extremes and electric fields, which may both facilitate and impede the activity of cable bacteria. Gliding behavior and exceptional mechanical strength explain how they manage to maintain optimal positioning despite disturbance by bioturbation and resuspension. Electron conduction in cable bacteria wire is indeed much more efficient than conventional biological electron transport by hopping between redox sites. Yet, a recorded voltage loss of about 13 mV per mm do suggest that wire resistance ultimately controls depth penetration in the sediment. A diversity of other bacteria flourish around active cable bacteria and might be either parasites hooking on to the cable bacteria conductors or synergists providing cable bacteria indirect access to other or more distant electron donors. Sudden crashes of cable bacteria populations indicates that other, yet unresolved biological, physical, or chemical stress factors can be important. More detailed studies of competition between co-existing cable bacteria species should help shedding new light on how these remarkable bridges between the anaerobic and aerobic world are established.

IL09

No abstract has been submitted.

IL10

Gut Microbiota Metabolism during Health and Disease

S. Winter*[1]
[1]*W. W. Caruth, Jr. Scholar in Biomedical Research, Department of Microbiology, UT Southwestern Medical Center, Dallas, TX, United States*

The development of the germ theory of disease was a milestone in our understanding of microbial-induced diseases. In the past decade, we have come to appreciate that many non-infectious diseases involve a microbial component. For example, changes in gut microbiota composition have been observed in malnutrition, obesity, autoimmunity, HIV enteropathy, necrotizing enterocolitis, and inflammatory bowel disease (IBD). In many setting the microbiota is not merely an innocuous bystander but acts as a driver of disease. These findings spawned an interest in developing novel, microbiota-based intervention strategies for non-infectious diseases. The field has now matured to a state in which mechanistic studies on functional microbe-microbe and host-microbe interactions are needed to establish causal relationships, to define the exact microbial contribution to disease, and to identify molecular therapeutic targets. In this lecture, I will provide a conceptual overview on factors that control the composition and function of the gut microbiota during homeostasis and inflammatory diseases, with a particular focus on the intersection of microbial and host metabolism. Furthermore, I will provide an example of how my lab has leveraged mechanistic insights to develop microbiota-based intervention strategies for inflammatory diseases of the intestinal tract.

IL11

How archaea swim: the archaellum

S. V. Albers*[1]
[1]*University of Freiburg, Institute of Biology, Molecular Biology of Archaea, Freiburg i. Br., Germany*

The archaeal motility structure, the archaellum, is a unique structure, which is evolutionary not related to bacterial flagella, but shows structural homology to type IV pili. The archaellum is a rotary motility structure which achieves forward propulsion of the cells by ATP hydrolysis. We have analysed the subunit interaction of archaellum components from different archaeal species and performed detailed studies on archaellum assembly in the crenarchaeon *Sulfolobus acidocaldarius* and euryarchaeon *Haloferax volcanii*. In the presentation our current understanding of archaellum assembly and how rotation of the structure is achieved, will be discussed.

IL12

Evidence for phospholipid export from the gram-negative inner membrane: time to rethink the Mla pathway?

T. Knowles*[1]
[1]*School of Biosciences, University of Birmingham, Birmingham, United Kingdom*

The Mla pathway is believed to be involved in maintaining the asymmetrical Gram-negative outer membrane via retrograde phospholipid transport. The pathway is composed of 3 components: the outer membrane MlaA-OmpC/F complex, a soluble periplasmic protein, MlaC, and the inner membrane ATPase, MlaFEDB complex. Here we solve the crystal structure of MlaC in its phospholipid free closed apo conformation, revealing a novel pivoting β-sheet mechanism which functions to open and close the phospholipid-binding pocket. Using the apo form of MlaC we provide evidence that the inner membrane MlaFEDB machinery exports phospholipids to MlaC in the periplasm. Furthermore we confirm that the phospholipid export process occurs through the MlaD component of the MlaFEDB complex and that this process is independent of ATP. Our data provides, for the first time, evidence of an apparatus for lipid export away from the inner membrane and suggests that the Mla pathway may have a role in anterograde phospholipid transport.

IL13

No abstract has been submitted.

IL14

c-di-AMP signaling in *Staphylococcus aureus:* Why Staph needs it & what regulates it

T. Tosi[1], F. Hoshiga[1], C. Millership[1], R. Singh[1], C. Eldrid[2] D. Patin[4], D. Mengin-Lecreulx[4], K. Thalassinos[2,3], P. Freemont[5], A. Gründling*[1]

[1]*Section of Microbiology and MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, UK*
[2]*Institute of Structural and Molecular Biology, Birkbeck College, University of London, Malet Street, London, UK*
[3]*Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London, UK*
[4]*Institute for Integrative Biology of the Cell, CEA, CNRS, Univ Paris-Sud and Université Paris-Saclay, Gif-sur-Yvette Cedex, France*
[5]*Section of Structural Biology, Department of Medicine, Imperial College London, London, UK*

Staphylococcus aureus is a Gram-positive opportunistic bacterial pathogen that can cause infections ranging from osteomyelitis to pulmonary diseases and sometimes endocarditis. Antibiotic-resistant strains, such as Methicillin-resistant S. *aureus* (MRSA) strains have emerged making this organism nowadays one of the leading causes of antibiotic-resistant infections in hospitals worldwide. Due to the difficulties in effectively treating *S. aureus* infections with current antibiotics, novel bacterial targets for therapeutic interventions are being explored.
The cyclic dinucleotide c-di-AMP has emerged as a novel essential signalling molecule in Gram-positive bacteria. It regulates several cellular processes, including osmotic and cell wall homeostasis. A decrease in cellular c-di-AMP levels has been shown to render *S. aureus* more susceptible to beta-lactam antibiotics. In this work, we investigated the regulation of c-di-AMP production in *S. aureus*. We focused on the interaction between the staphylococcal c-di-AMP producing enzyme DacA and the phosphoglucosamine mutase enzyme GlmM, an essential enzyme involved in peptidoglycan synthesis. We show that GlmM is a negative regulator of DacA activity both *in vitro* and *in vivo*, halting or decreasing c-di-AMP production. We also solved the crystal structures of the individual DacA and GlmM enzymes and determined the Small Angle X-ray Scattering **(**SAXS) envelope for the DacA/GlmM complex. The SAXS data of the complex revealed that GlmM likely inhibits DacA by masking the active site of the cyclase and preventing higher oligomer formation. Together these results provide an important mechanistic insight into how c-di-AMP production can be regulated in *S. aureus.*

IL15

Shaping up and Responding: Color Vision and Light-Dependent Developmental Plasticity in Cyanobacteria

B. Montgomery*[1]
[1]*Michigan State University, College of Natural Science, East Lansing, MI, United States*

Photosynthetic organisms exhibit finely tuned abilities to sense and respond to changes in their ambient environment. Photoperception and the resulting metabolic and developmental changes that occur as a response to light signals are among the most important adaptations of any organism that uses light for carbon fixation and generation of reductant. Photoperception and photomorphogenesis in the filamentous cyanobacterium Fremyella diplosiphon includes roles for the phytochrome-related biliprotein RcaE in regulating light-dependent changes in the protein composition of the photosynthetic light-harvesting complexes, i.e. phycobilisomes (PBS). RcaE also regulates light-dependent changes in cell shape and filament morphology, as well as the carbon fixation potential, in F. diplosiphon. The light-dependent changes in PBS protein composition and cellular morphology occur maximally in response to green and red light and are core features of the light-dependent acclimation process known as complementary chromatic acclimation (CCA). The molecular genetic, biochemical, and cell biological approaches used to elucidate the RcaE-dependent signaling cascade and regulation of gene expression resulting in developmental changes that are linked to optimizing organismal fitness during CCA will be discussed.

IL16

Light and stress sensing in fungi

R. Fischer*[1]
[1]*Karlsruhe Institute of Technology - KIT Institute for Applied Biosciences, Department of Microbiology, Karlsruhe, Germany*

Many bacteria and fungi are able to live under a wide range of environmental conditions. However, life under different conditions requires metabolic and/or morphological adaptations preceded usually by differential gene expression. We are studying light sensing in the filamentous fungi *Aspergillus nidulans* and *Alternaria alternata*. Light controls many processes, such as spore production, spore germination or the production of secondary metabolites. *A. nidulans* and *A. alternata* are examples for fungi which use several light receptors, such as phytochrome for red light, flavin-containing white collar proteins for blue light and opsin for green-light sensing. Whereas the blue-light sensor resides in the nucleus, phytochrome hubs into the HOG MAP kinase pathway to transmit the light signal from the cytoplasm to the nucleus. The HOG pathway is also used for osmotic and oxidative stress signaling. Hence light and stress sensing are intimately linked. The role of opsins in fungi is less well understood. In *A. alternata* it pumps protons across the membrane after green-light exposure, whereas *A. nidulans* opsin lacks a chromophore and is thus not light-activated. Recently we discovered that in *A. nidulans* phytochrome is also involved in temperature sensing, which could be an ancient function of the light receptor. Stressful conditions, such as high temperature or draught are likely to happen at the surface of substrates and hence light can be considered as a reliable alerting system indicating upcoming stresses.

Igbalajobi, O., Yu, Z. & Fischer, R. (2019) Red- and blue-light sensing in the plant pathogen *Alternaria alternata* depends on phytochrome and the white-collar protein LreA. mBio, in press.

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Yu, Z., Armant, O., and Fischer, R. (2016). Fungi use the SakA (HogA) pathway for phytochrome-dependent light signalling. Nature Microbiol *1*, 16019.

EMV01

Effect of a diet rich in gluten on the intestinal bacterial microbiota composition of mice

S. Weis*[1,2], D. Grundmann[3], V. F. Zevallos[4], S. Lehnerts[3], D. Schuppan[4], S. Schnell[2], K. H. Schäfer[3], M. Egert[1]
[1]*Furtwangen University, Institute of Precision Medicine, Villingen-Schwenningen, Germany*
[2]*Justus-Liebig-University, General and Soil Microbiology, Department of Applied Microbiology, Gießen, Germany*
[3]*University of Applied Sciences Kaiserslautern, Working Group Enteric Nervous System, Zweibrücken, Germany*
[4]*Johannes Gutenberg University , Institute of Translational Immunology, University Medical Center, Mainz, Germany*

Introduction: Gluten is a food compound that is found in various grains, such as wheat, rye and barley, and that is associated with several diseases such as celiac disease, type 1 diabetes and non-celiac gluten sensitivity[1]. Recent studies suggested a negative influence of this food compound on the microbial community in the human gut[2].

Methods & Results: For 4 weeks, 6 mice were fed a gluten rich diet (GD), while 11 received a gluten free diet (GFD). Subsequently, caecum, colon and Ileum were extracted from the animals. To identify bacterial taxa differing between GD and GFD, the V4 and V5 variable region of PCR-amplified 16S rRNA genes were sequenced using Illumina MiSeq technology.

Based on preliminary analyses of ~2.6 million sequences, bacterial diversity was significantly decreased under GD conditions. 9 genera, including *Faecalibacterium* and *Bacteroides,* were found to be significantly increased under GD conditions. In contrast, 29 genera, including *Lactobacillus*, *Streptococcus*, *Ruminiclostridium* and several groups of the family *Lachnospiraceae,* were significantly decreased under GD conditions.

Conclusion: A gluten rich diet clearly has a significant effect in modulating the gut microbiota and in reducing its diversity. Since gluten is suggested to be connected to many health issues, our findings may hint towards intestinal bacterial taxa playing a role in gluten-related illnesses.

[1] Catassi C, Bai JC, Bonaz B et al. (2013) Non-celiac gluten sensitivity: the new frontier of gluten related disorders. Nutrients 5(10): 3839–3853. doi: 10.3390/nu5103839; [2] Palma G de, Nadal I, Collado MC et al. (2009) Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects. Br J Nutr 102(8): 1154–1160. doi: 10.1017/S0007114509371767

EMV02

Gene-level modeling of *Microcystis* growth and toxin production

F. Hellweger*[1]
[1]*TU Berlin, Wasserreinhaltung, Berlin, Germany*

The problem of harmful cyanobacteria blooms in lakes, for example toxin-producing *Microcystis*, has a global dimension, increasing trend and dim future projection, and existing control methods are expensive and not always successful. At the same time, advances in microbiology are generating molecular-level understanding of the mechanisms underlying the growth and toxin production of cyanobacteria. Yet, this knowledge is generally not used in our ecosystem models, which limits their utility for research and management. Closing this gap is a grand challenge that cuts across general and environmental microbiology, and extends to lake management. The aim of this project is to incorporate this vast amount of knowledge into a dynamic, mechanistic, gene-level model of *Microcystis* growth and toxin production, and then use it to support the management of full-scale ecosystems. In this approach, individual cells are simulated explicitly using agent-based modeling (ABM). Each cell has a number of genes that are expressed to yield transcripts and proteins, which perform metabolism, toxin production and other cellular functions. The model is build using the pattern-oriented modeling (POM), based on a literature meta-analysis. Reproduction of patterns from over 300 laboratory experiments suggests the model cells are realistic representatives of their real-world counterparts. In some cases, the model reproduces and explains mechanisms underlying observed patterns, like the transient increase in microcystin content upon light intensity downshift. Present challenges include dealing with the genetic diversity of *Microcystis*, accounting for interactions with other microbes (allelopathy, Black Queen) and integrating into full-scale ecosystem models.

EMV03

Deep-cultivation, phenomics and genomics of the *phylum Planctomycetes* unveil novel, unsuspected bacterial biology

S. Wiegand*[1], M. Jogler[2], C. Boedeker[2], D. Pinto[3], J. Vollmers[4], E. Rivas-Marín[5], T. Kohn[1], S. H. Peeters[1], A. Heuer[2], P. Rast[2], S. Oberbeckmann[6], B. Bunk[2], O. Jeske[2], A. Meyerdierks[7], J. E. Storesund[8], N. Kallscheuer[1], S. Lücker[1], O. M. Lage[9], T. Pohl[10], B. J. Merkel[10], P. Hornburger[2], R. W. Müller[11], F. Brümmer[11], M. Labrenz[6], A. M. Spormann[12], H. Op den Camp[1], J. Overmann[2], R. Amann[7], M. S. M. Jetten[1], T. Mascher[3], M. Medema[13], D. P. Devos[5], A. K. Kaster[4], L. Øvreås[8], M. Rohde[14], M. Y. Galperin[15], C. Jogler[1]
[1]*Radboud Universiteit Nijmegen, Microbiology, Nijmegen, Netherlands*
[2]*DSMZ, Braunschweig, Germany*
[3]*TU Dresden, Dresden, Germany*
[4]*Karlsruhe Institute of Technology, Karlsruhe, Germany*
[5]*CABD, Sevilla, Spain*
[6]*IOF, Warnemünde, Germany*
[7]*Max Planck Institute for Marine Microbiology, Bremen, Germany*
[8]*University of Bergen, Bergen, Norway*
[9]*University of Porto, Porto, Portugal*
[10]*Freiberg University of Mining and Technology, Freiberg, Germany*
[11]*University of Stuttgart, Stuttgart, Germany*
[12]*Stanford University, Stanford, CA, United States*
[13]*Wageningen UR, Wageningen, Netherlands*
[14]*HZI Braunschweig, Braunschweig, Germany*
[15]*NIH, Bethesda, MD, United States*

For many reasons, most microbiological studies rely on several well-established model organisms. However, it remains unknown how well the current set of such model species can cover the diverse aspects of bacterial biology. To adequately address this central question, we thoroughly explored the barely studied Planctomycetes. We started by collecting samples from diverse aquatic habitats around the globe and pursued a diversity-driven deep-cultivation approach. This way, we obtained many more novel planctomycetal axenic cultures than had been available in strain collections. Such pure cultures enabled a detailed genomic and phenotypic characterization.

Genome sequencing provided the basis for constructing a multi-dimensional phylogeny supported by general genome parameters. We observed unusual gene absence/presence patterns for otherwise universal bacterial shape and cell division genes; these patterns were consistent with the proposed phylogenetic clades. In-depth comparative analyses further revealed that Planctomycetes sense their environments differently and employ novel proteins for signaling and regulation of gene expression. Many analyzed genomes also comprise a untapped potential for small molecule production as the related biosynthetic gene clusters are highly distinct from known pathways.

Phenotypic characterization by various microscopic methods revealed unexpected variations in bacterial morphology. We discovered novel types of bacterial reproduction and observed previously unseen planctomycetal amoeba-like locomotion.

Overall, we demonstrate that unobserved biology exists in the environment and that unearthing the bacterial diversity of neglected phyla can provide novel insights that are significant for future fundamental and applied research endeavors.

EMV04

Diversity of the cultivated bacteria in lakes on Tibetan Plateau

P. Xing*[1,2], H. Lu[2], Q. Wu[2]
[1]*Leibniz Institute DSMZ -- German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[2]*Nanjing Institute of Geography & Limnology, CAS, Nanjing, China*

Tibet has often been called the "Roof of the World" or "The Third Pole" due to its high altitude and extreme environments. This region comprises several thousand lakes and covers a total area of 4.5×104 km^2. Most of these lakes have been geographically isolated from each other for millions of years mainly due to the tectonic movement. As another feature, the lakes in Tibet are very diverse in their environmental conditions. The isolation and cultivation methods were applied for studying the culturable microbial diversity. Four kinds of mediums were selected in the study. In total, 1023 strains were isolated from 46 lakes. Eleven of the strains belong to the *Archaea* domain. The isolated bacterial strains were mainly assigned to the phylum *Proteobacteria*, including 284 strains within *Alphaproteobacteria*, 31 strains within *Betaproteobacteria* and 344 strains within *Gammaproteobacteria*. In addition, 189, 114 and 50 strains affiliated with the phylum *Firmicutes*, *Actinobacteria* and *Bacteroidetes*, respectively. The 16S rRNA gene of 1023 isolated strains were sequenced and 64 of them shared the similarities below 98.65 % with the known species. Strain AY8S had only 90.5% 16S rRNA gene sequence similarity with its most nearest neighbors. Although some strains shared > 99.0 % similarity of 16S rRNA gene sequence, heterogeneous characteristics were demonstrated in their nutrients utilization and hydrolytic enzyme tests. Moreover, the polyphasic taxonomy methods were applied to identify the taxonomic ranks for five strains, which were proposed as *Pelagibacterium montanilacus* CCL18^T, *Halomonas tibetensis* pyc13^T, *Roseovarius tibetensis* LM2^T,

Flavobacterium tibetense YH5^T and *Nitrincola tibetensis* xg18^T, respectively.

EMV05

Methane producing *archaea* in Siberian permafrost and their response to long-term thaw

S. Holm*[1], J. Walz[2], C. Knoblauch[2], F. Horn[1], D. Wagner[3,4], S. Liebner[3,5]
[1]*German Research Centre for Geosciences, Section 5.3 Geomicrobiology, Potsdam, Germany*
[2]*Universität Hamburg, Institute of Soil Science, Hamburg, Germany*
[3]*German Research Centre for Geosciences, Section 5.3, Potsdam, Germany*
[4]*Universität Potsdam , Institute of Earth and Environmental Science, Potsdam, Germany*
[5]*Universität Potsdam, Institute of Biochemistry and Biology , Potsdam, Germany*

The release of greenhouse gases (GHG) from thawing permafrost organic matter is called the permafrost carbon feedback (PCF). Methane is a GHG with a warming potential, at least 28 times greater than CO2. The production of methane from thawing permafrost can be substantial but often evolves after long lag phases or is lacking. We investigated permafrost cores from the islands Kurungnakh and Bolshoy Lyakhovsky in Northeast Siberia, seeking an explanation on the contrasting evolution of methane production after permafrost thaw. Samples of Holocene, Late Pleistocene to Eemian origin were investigated and subsamples were incubated anaerobically at 4°C for 3-7 years. The methane evolvement was measured by gas chromatography and the *mcrA* gene copy numbers were evaluated by quantitative PCR (qPCR). In addition, the community composition was determined by amplicon based high throughput sequencing. During incubation the α-diversity decreased and the communities were dominated by *Methanomicrobiales, Methanosarcinales* and *Methanoregula*. Based on methane production rates and the methanogenic community analysis, we conclude that long term thaw stimulated methanogenesis in permafrost environments where an initial active methanogenic community was present. Eemian permafrost serving as analogue for current global warming was found to be highly productive and even exceed other investigated epochs. High methane production coincided with a large abundance of Bathyarchaeota-affiliated sequences potentially involved in carbon turnover and methane production. In summary, the response of the methanogenic community after long-term permafrost thaw appears to depend on the conditions, under which the soil was formed; warmer and wetter conditions enhanced the activity of methanogens.

EMV06

***Ascunsovirinae*, a new *Microviridae* subfamily, infects alphaproteobacteria from terrestrial and marine environments**

F. Zucker*[1], V. Bischoff[1], B. Heyerhoff[1], A. Poehlein[2], S. Roux[3], F. Enault[4], M. Simon[1], C. Moraru[1]
[1]*University of Oldenburg, ICBM, Oldenburg, Germany*
[2]*University of Göttingen, Göttingen, Germany*
[3]*DOE Joint Genome Institute, Walnut Creek, CA, United States*
[4]*Université Clermont Auvergne, Clermont Auvergne, France*

Microviridae are ssDNA bacteriophages found in diverse habitats, for example in human gut and feces, dragonflies, stromatolites, freshwater, seawater, sewage, methane seeps, sediments and soil. They are taxonomically diverse and grouped in several subfamilies. To date all cultivated microviruses are lytic. Putative prophages have been predicted in the genomes of Bacteroidetes and Sphingomonadales. Here, we isolated and genome sequenced Sulfitobacter phage ICBM5, a lytic ssDNA phage infecting Sulfitobacter sp. SH24-1b, a marine Rhodobacteraceae. Using the ICBM5 phage proteins as bait, we identified 23 similar prophages in genomes of Rhodobacteraceae and Rhizobiales. Phylogenetic analysis of the major capsid protein placed the ICBM5 phage and the prophages in a new subfamily of Microviridae, which we named Ascunsovirinae. In all ascunsovirus genomes we identified the main proteins characterizing the Microviridae, that is the pilot protein, the major capsid protein and the replication protein, plus lysis proteins. Ascunsoviruses were present both in marine and terrestrial Rhodobacteracea, and in terrestrial Rhizobiales. Because Rhodobacteraceae have been shown to cross several times from the terrestrial to the marine environment, we propose that they are the transport vehicle for ascunsoviruses in between the terrestrial and the marine environments. Currently, we are studying the life cycle and life style of the ICBM5 phage, including the potential to lysogenize its host. For this, we are using long read sequencing technologies and fluorescence in situ hybridization with probes targeting the ICBM5 genome. With this work we expand the known taxonomic and genomic diversity of Microviridae, and we bring new insights into their life cycle and life styles.

EMV07

Unravelling microbial communities and their responses towards stormwater events in anthropogenically impacted urban estuarie using meta-omics approaches

F. Wemheuer*[1], K. Dafforn[1,2], S. Birrer[1], P. Steinberg[1], S. Simpson[3], J. Potts[4], P. Scanes[4], S. Kjelleberg[1,5], M. Doblin[6], G. Birch[7], P. Gribben[1], P. Freewater[4], T. Thomas[1], E. Johnston[1]
[1]*The University of New South Wales, Sydney, Australia*
[2]*Macquarie University, Sydney, Australia*
[3]*CSIRO, Sydney, Australia*
[4]*Office of Environment and Heritage, Sydney, Australia*
[5]*SCELSE, Singapore, Malaysia*
[6]*University of Technology Sydney, Sydney, Australia*
[7]*University of Sydney, Sydney, Australia*

Australian estuaries are impacted by multiple anthropogenic stressors including high nutrient and metal inputs caused by storm events. However, little is known how these stressors impact the sediment microbial communities and their functions. Here, we surveyed bacterial communities at four locations with large stormwater drains in Sydney Harbour, Australia, that displayed two different retention types: embayment and channel. To decipher microbial dynamics, sediment samples were taken during the Australian summer in 2014 (baseline) and after a major storm event in February 2015. At each location, three samples were taken at different distances to the stormwater drain. In addition, sediment samples were collected over 17 months in three Australian regions (Sydney, Perth and Great Barrier Reef) to decipher bacterial and eukaryotic communities and their responses towards storm events. Bacterial and eukaryotic communities were assessed by amplicon sequencing the 16S and 18S rRNA genes, respectively. Differences in gene expression patterns were studied by RNAseq analysis. Our analysis revealed differences in community function and gene expression between locations, retention types and timepoints (baseline/stormwater) as well as between eukaryotic and bacterial communities. Furthermore, trends of decreasing functional redundancy were recorded in embayments caused by the storm event. Our results have implications for future management practices in estuaries and contribute to our understanding of the relative impacts of stormwater run-off on microbial communities and processes in the sediment.

EMV08

Genetic Globetrotter - A novel plasmid-based vector for horizontal gene transfer found in marine *roseobacter* populations world-wide

J. Vollmers*[1], J. Petersen[2], V. Ringel[2], H. Brinkmann[2], C. Ellebrandt-Sperling[2], C. Spröer[2], A. M. Howat[3], J. C. Murrell[3], A. K. Kaster[1]
[1]*Karlsruhe Institute of Technology, IBG-5, Eggenstein-Leopoldshafen, Germany*
[2]*Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Brunswick, Germany*
[3]*University of East Anglia, School of Environmental Sciences, Norwich, United Kingdom*

Roseobacters are a significant diverse and abundant group of marine bacteria which are involved in the global cycles of carbon and sulfur. They are considered representative for niche adaptations in marine habitats due to their functional as well as metabolic versatility. This versatility is likely linked to the high genome plasticity of this group, thus making awareness and understanding of the involved vectors crucial for elucidating the role and impact of Roseobacters in their respective environments.

Recently, addition of lanthanum to methanol enrichments of coastal seawater facilitated the isolation of a novel methylotroph within the Roseobacter group, Marinibacterium strain La 6. Sequencing and genome analyses revealed not only the largest so far reported genome of this group, but also an exceptionally high number of replicons, consisting of 1 chromosome, 5 chromids and 7 plasmids. When analyzing these with respect to plasmid compatibility groups, we found a novel conserved plasmid system not yet recognized in Roseobacters. This plasmid system can be found, with a highly conserved backbone sequence and various recurring integrated gene cassette motifs in marine environments worldwide.

Thus, we present a new important vector of horizontal gene transfer in Roseobacters, expanding our knowledge on mechanisms for gene plasticity and increasing our understanding of niche adaptation in marine habitats.

BTV01

One amino acid – two functions: *Phaeobacter inhibens* DSM 17395 switches from antibiotic to biomass production if phenylalanine is the only available carbon source

S. Will*[1], D. Schomburg[2], M. Neumann-Schaal[1]
[1]*Leibniz-Institut DSMZ, Junior Research Group Bacterial Metabolomics, Braunschweig, Germany*
[2]*TU Braunschweig, Bioinformatics & Biochemistry, Braunschweig, Germany*

It's widely assumed that bacterial organisms producing antibacterial compounds harbor resistance mechanisms. However, the production of an antibiotic can be a burden for the producing strain either by the production or by the antibiotic itself. *Phaeobacter inhibens* DSM 17395 is a marine, gram-negative bacterium which produces the antibacterial compound tropodithietic acid (TDA). TDA leads to a disruption of the proton gradient and thus to a decreased biomass yield, even in the producing strain.

Phenylalanine is the precursor for TDA production but can also be utilized as carbon source by *P. inhibens* DSM 17395. The aim of this project was to investigate the fate of phenylalanine as precursor for TDA and as carbon source. Therefore the adaptation process of *P. inhibens* DSM 17395 to phenylalanine as carbon source was analyzed. A combined approach of metabolomics, transcriptomics and metabolic modelling was used to combine growth characteristics, metabolic and regulatory changes as well as theoretical maximal biomass yields to understand the fate of phenylalanine.

While in complex media phenylalanine is solely used as precursor for the TDA production, *P. inhibens* DSM 17395 reduces the production of TDA with each adaptation cycle and increases biomass production after transfer to medium containing phenylalanine as sole carbon source. Despite the major drawback on its own metabolism, *P. inhibens* DSM 17395 produces TDA. However, the organism is able to react to altered substrate availability and increases biomass production by routing available substrate into different pathways.

BTV02

Corynebacterium glutamicum as platform for the production of hydroxybenzoic acids

N. Kallscheuer[1], L. Milke[1], J. Marienhagen*[1]
[1]*Forschungszentrum Juelich GmbH, Institut für Bio- und Geowissenschaften, IBG-1: Biotechnologie, Jülich, Germany*

Hydroxybenzoic acids are industrially relevant aromatic compounds, which also play key roles in the microbial carbon metabolism, e.g., as precursors for the synthesis of cofactors or metal-chelating molecules. Due to its pronounced resistance to aromatics *Corynebacterium glutamicum* represents an interesting platform for production of these compounds. Unfortunately, a complex catabolic network for aromatic molecules prevents application of *C. glutamicum* for microbial production of aromatic compounds other than aromatic amino acids, which cannot be metabolized by this microorganism.

We completed the construction of the platform strain *C. glutamicum* DelAro[5], in which the deletion of altogether 27 genes in five gene clusters abolished most of the peripheral and central catabolic pathways for aromatic compounds known in this microorganism. The obtained strain was subsequently applied for the production of 2-hydroxybenzoate (salicylate), 3-hydroxybenzoate, 4-hydroxybenzoate and protocatechuate, which all derive from intermediates of the aromatic amino acid-forming shikimate pathway. For an optimal connection of the designed hydroxybenzoate production pathways to the host metabolism, *C. glutamicum* was additionally engineered towards increased supply of the shikimate pathway substrates erythrose-4-phosphate and phosphoenolpyruvate by manipulation of the glucose transport and key enzymatic activities of the central carbon metabolism. With an optimized genetic background the constructed strains accumulated 0.01 g/L (0.07 mM) 2-hydroxybenzoate, 0.3 g/L (2.2 mM) 3-hydroxybenzoate, 2.0 g/L (13.0 mM) protocatechuate, or 3.3 g/L (23.9 mM) 4-hydroxybenzoate in the supernatant.

BTV03

Fermentative production of halogenated tryptophan by metabolically engineering *Corynebacterium glutamicum*

K. H. Veldmann*[1], H. Minges[2], N. Sewald[2], J. H. Lee[3], V. F. Wendisch[1]
[1]*University Bielefeld, Genetics of Prokaryotes, Bielefeld, Germany*
[2]*University Bielefeld, Organic and Bioorganic Chemistry, Bielefeld, Germany*

[3]*Kyungsung University, Major in Food Science & Biotechnology, Busan, South Korea*

Introduction: Halogenated compounds, like 7-chloro-L-tryptophan (7-Cl-Trp), are important intermediates and bioactive substances in the pharmaceutical, and agrochemical industries [1]. About 20 % of all pharmaceutical small molecule drugs and around 30 % of all active compounds in agrochemistry are halogenated. Chemical halogenation is usually characterized by hazardous or toxic chemicals [2]. Recently, a biocatalytic process for L-tryptophan halogenation at the gram-scale has been described [3]. Many proteinogenic amino acids are produced by fermentation using *Corynebacterium glutamicum* [4].

Objectives: We developed fermentative production of halogenated tryptophan in a tryptophan overproducing *C. glutamicum* strain which heterologously expresses genes encoding for the FAD-dependent halogenase RebH and the NADH-dependent flavin reductase RebF from *Lechevalieria aerocolonigenes* [5].

Materials & methods: Various *C. glutamicum* strains were constructed and cultivated with different halide salts and carbon sources. Product concentrations were measured via HPLC.

Results: Fermentative production of 7-Cl-Trp by recombinant *C. glutamicum* to a titer of 62 ± 7 mg L^{-1} with $CaCl_2$ as halogen donor has been enabled. Optimization of the ribosome binding site of *rebH* further increased production to a titer of 108 ± 2 mg L^{-1}.

Conclusion: Fermentative production of 7-Cl-Trp was enabled and improved by modification of the cultivation media and by the enhancement of *rebH* translation.

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BTV04

Gluconobacter japonicus: a promising platform for the large-scale production of levan-based fructans

M. Hövels*[1], J. Kniewel[1], U. Deppenmeier[1]
[1]*University of Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*

Levan is a β-2,6-glycosidic linked fructan that can be used for a broad range of industrial applications. Due to its prebiotic character levan is attracting attention for large-scale production and commercial distribution. To date, no industrial process for levan production has been established. Here we present a levan production strategy based on the acetic acid bacterium *Gluconobacter japonicus*. We successfully verified the levansucrase produced by this organism as the most active levansucrase described so far. The extracellular enzyme, which is secreted by an unknown signal peptide-independent pathway, shows a specific activity of 3064 U mg-1 at 30 °C. To improve levan production the gene encoding the levansucrase was cloned into a pBBR1MCS-based expression vector, which was transformed into *G. japonicus*. The culture supernatant of the overexpression strain, containing elevated amounts of the constitutively produced levansucrase, was successfully used for the complete conversion of a saturated sucrose solution (687 g L-1 at 30 °C). A levan yield of ~200 g L-1 was achieved by the cell-free strategy, which represents a significant increase over the previously most productive process based on immobilized *Zymomonas mobilis* CCT 4494 cells (112.5 g L-1). Our second aim was the production of levan-type fructooligosaccharides (FOS). Therefore, we investigated levan-hydrolyzing endolevanases after heterologous production in *E. coli*. We found that a highly active endolevanase from a bacterium of the family Pseudomonadaceae efficiently generated levan-type FOS with a degree of polymerization ≥ 3. These findings could enable large-scale production of prebiotic levan and levan-type FOS.

BTV05

Temperature-dependent dynamic control of the TCA cycle for increased volumetric productivity of itaconic acid production by *Escherichia coli*

B. Harder*[1], K. Bettenbrock[1], S. Klamt[1]
[1]*Max-Planck-Institut für Dynamik komplexer technischer Systeme, Magdeburg, Germany*

Question: We recently engineered *E.coli* (strain ita23) for growth-coupled synthesis of itaconic acid with high yield. Here we aimed to improve the productivity by applying a two-stage process strategy with decoupled production of biomass and itaconic acid.

Methods: Based on the design of the *E. coli* strain ita23, we constructed a strain ita32, which, in contrast to ita23, has an active tricarboxylic acid (TCA) cycle. This enables the strain to grow with a fast growth rate of 0.52 h^{-1} at 37°C, thus representing an ideal phenotype for the first stage, the growth phase. To down-regulate the TCA cycle and thus to switch from growth to itaconic acid production in the second stage, we replaced the promoter of the isocitrate dehydrogenase by the Lambda promoter (*pR*). The expression of this promoter was controlled by the temperature-sensitive repressor *CI857* which is active at lower temperatures (30°C). The respective strain ita36A grew with a fast growth rate at 37°C and switched to production of itaconic acid at 28°C.

Results: To study the impact of the process strategy on productivity we performed one-stage and two-stage bioreactor cultivations with strain ita36A. The two-stage process enabled fast formation of biomass resulting in improved peak productivity of 0.86 g/L/h (+48%) and volumetric productivity of 0.39 g/L/h (+22%) after 120 h in comparison to the one-stage process. With our dynamic production strain, we also resolved the glutamate auxotrophy of ita23 and increased the itaconic acid titer to 47 g/L.

Conclusion: Here we knocked-down an essential gene in E. coli to design a two-stage process for improved volumetric productivity. The control by temperature avoids expensive inducers and has the potential to be generally used to improve cell factory performance.

BTV06

Heterotrophic and autotrophic production of acetoin in *Cupriavidus necator*

C. Windhorst*[1], J. Gescher[1]
[1]*Karlsruhe Institute for Technology, Institute for Applied Biosciences, Applied Biology, Karlsruhe, Germany*

Introduction: Independence on non-renewable resources necessitates the production of valuable chemicals via biotechnological processes. Here we report on the lithoautotrophic production of the platform chemical acetoin using the Knallgasbacterium *Cupriavidus necator*. Acetoin is ranked in the top 30 value added chemicals that can be produced from biomass. The strain was genetically engineered for the efficient production and inductor independent production of this C4-compound.

Objectives: This study concentrated on the efficient production of acetoin from H_2 and CO_2. The aim is to develop a robust production process that can be fueled with a number of different gas-sources.

Materials & Methods: For the acetoin production, the codon-optimized genes *alsS* and *alsD* from *Bacillus subtilis* were heterologously expressed on plasmids with either inducible or constitutive promotor systems. To achieve higher product yields, the carbon flux was redirected from polyhydroxybutyrate (PHB) to pyruvate and acetoin by deleting PHB synthases *phaC1* and *phaC2*. The strains were cultivated with fructose or CO_2 as sole carbon source under oxic or autotrophic conditions, respectively.

Results: Using the different expression systems, an acetoin production up to 90% of the theoretical maximum was measured. By deleting the PHB synthases, the acetoin yield increased 26 times and 19fold for the heterotrophic and autotrophic batch cultivation, respectively. In a fermenter with a continuous autotrophic gasing, an acetoin concentration of 44 mM could be measured after two weeks.

Conclusion: *C. necator* can be used for the highly efficient production of acetoin without many steps of strain optimization. Future studies will aim to improve the biotechnological process for higher space-time yields.

BTV07

The many Roads to Rome: How to overcome limitations for the production of chiral β-amino acids via microbial ω-transaminases (ω-TA)

J. Rudat*[1], O. Buß[1]
[1]*Karlsruhe Institute of Technology (KIT), BLT_II: Technical Biology, Karlsruhe, Germany*

Optically pure β-amino acids constitute interesting building blocks for a great variety of pharmaceutically important compounds. Their efficient production still poses a major challenge since methods are based on kinetic resolutions with a maximum theoretical yield of 50%. ω-Transaminases (ω-TA) hold great potential for the enzymatic synthesis of these molecules [1].

The objective of this study was to overcome different obstacles limiting the application of these enzymes by the following means:

- process control and stability by enzyme immobilization
- higher productivity by increasing heat stability
- understanding structure-function relations to access novel substrates by modifying the active site

We succeeded in a one-step purification and immobilization of an ω-TA on functionalized magnetic beads [2]. We designed variants of this enzyme, thereby realizing the first thermostabilization of a non-thermostable (*S)*-selective ω-TA by FoldX-guided site-directed mutagenesis [3].

The ω-Transaminase Engineering Database (oTAED) was established as a publicly accessible resource on sequences and structures of biotechnologically relevant ω-TA, representing a useful tool for enzyme engineering and the selection of novel ω-TA candidates with desired properties [4]. Engineered ω-TA were successfully tested for the enantioselective synthesis of *(R)*- and *(S)*-β-phenylalanine ethyl ester [5].

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BTV08

LinD-catalyzed dehydration of short-chain alkenols

M. P. Fischer*[1]
[1]*Institute of Biochemistry and Technical Biochemistry, Universitaet Stuttgart, Technical Biochemistry, Stuttgart, Germany*

Introduction: Dehydrations of short-chain alcohols are very valuable functional group interconversions for the preparation of olefins. Most of the chemical reagents applied are highly acidic or basic and therefore not practical for the dehydration of alcohols containing acid or base sensitive functionalities. Linalool dehydratase isomerase (LinD) from *Castellaniella defragrans* naturally catalyzes the stereoselective isomerization of geraniol to S-linalool as well as the dehydration of S-linalool to myrcene.

Objectives: Using site saturation mutagenesis approaches, LinD should be adapted to convert smaller alkenols such as prenol and 2-methyl-3-buten-2-ol to isoprene as well as crotyl alcohol and 3-buten-2-ol to 1,3-butadiene.

Materials and Methods: Making use of the "22c trick", an alpha-helix showing the highest b-factors in the LinD structure was targeted for saturation mutagenesis. A mid-throughput screening system was set up with 2-methyl-3-buten-2-ol as model substrate, using an *E. coli* whole cell based two phase system with direct injection out of the organic phase into GC/MS.

Results: Five variants at four positions of the alpha-helix could be identified to increase the conversion of the model substrate. Kinetic stutides of the resulting variants revealed Km to only slightly differ, while strong increase in vmax could be observed. However, combination of these positions did not result in any synergystic effects.

Conclusion: By applying site saturation mutagenesis, five positions on an alpha-helix approximately 10 A from the active site could be identified to increase the conversion of small alkenols. Therefore, making use of a two phase system without the need to extract the samples made the analysis of extremely volatile products reproducibly possible.

AMV01

On the thermodynamics of electroactive microorganisms – Revealing the microbial electrochemical Peltier heat

B. Korth*[1], T. Maskow[1], F. Harnisch[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany*

Introduction: Electroactive microorganisms (EAM) are capable of performing extracellular electron transfer (EET). It represents a unique metabolic trait enabling the transfer of electrons via cytochromes beyond the cell membrane to solid terminal electron acceptors. Thus, EAM are able to occupy anoxic ecological niches including chemically and redox-stratified environments. EAM are discussed to contribute substantially to natural processes (e.g., redox cycles of metals) and to enable promising biotechnological applications (e.g., energy-saving wastewater treatment, synthesis of chemicals).

Objective: Although EAM attracted considerable interests over the last decade, their thermodynamic characterization is still in its infancy. However, assessing the energy fluxes during EET is a prerequisite for deciphering their role in nature and for leveraging their biotechnological potential. Therefore, calorimetric measurements of the model organism *Geobacter* sp. are conducted to evaluate its energetic efficiency.

Material & methods: *Geobacter anodireducens* enrichment cultures were cultivated in the tailor-made bioelectrocalorimeter for simultaneously measuring heat dissipation and current production of EAM.

Results: By analysing redox titration experiments, the so far unknown microbial electrochemical Peltier heat was revealed. In the studied model system it amounts to a heat formation of −27±6 kJ per mole of transferred electrons.

Conclusions: This first bioelectrocalorimetric study shows that EAM have to overcome an entropic barrier at the cytochrome/solid electron acceptor interface for direct EET. This barrier lowers the microbial energy yield but also provides driving force for growth reactions. To further assess this effect, alternative electron acceptors are analysed.

AMV02

Limonene dehydrogenase hydroxylates the allylic methyl group of cyclic monoterpenes in the anaerobic terpene degradation by *Castellaniella defragrans*

E. Puentes-Cala[1], M. Liebeke[1], S. Markert[2], J. Harder*[1]
[1]*Max Planck Institut for Marine Microbiology, Bremen, Germany*
[2]*University of Greifswald, Dep. of Pharmaceutical Biotechnology, Greifswald, Germany*

Question: How are C-H bonds of monoterpenes activated by anaerobic bacteria

Methods: Enzyme purification and characterization

Results: A limonene dehydrogenase was purified from *Castellaniella defragrans* 65Phen grown on monoterpenes under denitrifying conditions in the absence of molecular oxygen. The purified limonene:ferrocenium oxidoreductase activity hydroxylated the methyl group of limonene (1-methyl-4-(1-methylethenyl)-cyclohex-1-ene) yielding perillyl alcohol ([4-(prop-1-en-2-yl)cyclohex-1-en-1-yl]methanol). The enzyme had a dithiothreitol:perillyl alcohol oxidoreductase activity yielding limonene. Mass spectrometry and molecular size determinations revealed a heterodimeric enzyme comprising CtmA and CtmB. Recently the two proteins had been identified by transposon mutagenesis and proteomics as part of the cyclic terpene metabolism (*ctm*) in *Castellaniella defragrans*and were annotated as FAD-dependent oxidoreductases of the protein domain family phytoene dehydrogenases and related proteins (COG1233). CtmAB is the first heterodimeric enzyme in this protein superfamily. Flavins in the purified CtmAB are oxidized by ferrocenium and are reduced by limonene. Heterologous expression of CtmA, CtmB and CtmAB in E. coli demonstrated that limonene dehydrogenase activity required both subunits carrying each a flavin cofactor. Native CtmAB oxidized a wide range of monocyclic monoterpenes containing the allylic methyl group motif (1-methyl-cyclohex-1-ene).

Conclusion: We characterized a novel hydrocarbon-activating enzyme. CtmAB acts as a hydroxylating limonene dehydrogenase and is the first heteromer in a family of FAD-dependent dehydrogenases acting on allylic methylene or methyl CH-bonds.

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AMV03

Aldehyde:ferredoxin oxidoreductase (AOR): kinetics, abilities and maturation

F. Arndt*[1], G. Schmitt[1], A. Winiarska[2], M. Szaleniec[2], J. Heider[1]
[1]*Philipps-Universität Marburg , Biologie, Marburg, Germany*
[2]*Jerzy Haber institute, PAS, Theoretical and Experimantal Biocatalysis, Cracow, Poland*

Tungsten-dependent aldehyde:ferredoxin oxidoreductases (AOR) directly interconvert aldehydes and the corresponding carbonic acids. In addition to the previously known archaeal AORs [1], similar enzymes are also present in bacteria, but differ in their subunit composition and other properties. An AOR from the betaproteobacterium *Aromatoleum aromaticum* was purified and found to consist of three different subunits in contrast to typical one subunit of archaeal AOR. The enzyme consists of two AOR-type subunits carrying a W-*bis*-MPT cofactor and an Fe-S cluster, two electron transfer subunits carrying four Fe-S clusters and two FAD-containing subunit. The corresponding genes are organized in an operon [2]. AOR from *A. aromaticum* has been purified in significant amounts from a constructed mutant strain which depends on this enzyme for anaerobic Phe degradation [3]. We present first results of characterization and recombinant production of bacterial AOR. The maturation of W enzymes is completely unclear. Candidate enzymes for the specific insertion of W are two additional paralogs of Mo inserting MoeA, called MoeAII and MoeAIII. First results suggest that MoeAI (Mo specific) and MoeAII (W specific) may already contain bound adenylated precursor of Moco and Wco.

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AMV04

Degradation of methoxylated aromatic compounds by a methanogenic archaeon: Unravelling central metabolism of *Methermicoccus shengliensis*

J. Kurth*[1], M. S. M. Jetten[1], C. Welte[1]

[1]Radboud University, Department of Microbiology, Nijmegen, Netherlands

The main source of the greenhouse gas methane are methanogenic archaea. This emphasizes the importance of those organisms for the global carbon cycle. Although methanogens have been studied for more than 111 years [1], a novel methanogenic pathway was recently discovered: the thermophilic methanogen *Methermicoccus shengliensis* is able to use a large variety of methoxylated aromatic compounds as substrates for methane generation [2-4]. Despite the significance and novelty of this unique archaeon a detailed analysis of its metabolism is still missing. Here, we used transcriptomic and proteomic methods to investigate the response to growth on methoxylated aromatics along with enzymological characterization of new methyltransferase enzymes. The transcriptomic analysis revealed a gene cluster highly expressed under growth on a methoxylated compound. The encoded enzymes resemble an enzyme system of acetogenic bacteria used for degradation of methoxylated aromatics and are most likely essential for methoxydotrophic methanogenesis. Four genes were chosen for heterologous expression in *E. coli* and subsequent purification by affinity chromatography. Next to this demethoxylation system, genomic and transcriptomic analysis also revealed two interesting soluble heterodisulfide reductase (Hdr) complexes to be present in *Methermicoccus*, associated with proteins resembling the F420 hydrogenase subunit beta (FrhB) and the formate dehydrogenase subunit alpha (FdhA). With bioinformatics and enzymological assays we aim to get further insight into the role of those complexes.

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AMV05

Perturbations of the microbiota support *Salmonella* growth through release of amino acids

M. Winter*[1], L. Spiga[1], E. Hughes[1], S. Taylor[1], W. Zhu[1], S. Winter[1]

[1]UTSouthwestern Medical Center, Microbiology, Dallas, TX, United States

Question:*Salmonella* serovars such as *S*. Typhimurium induce a potent, acute inflammatory response in the intestinal tract. Inflammation depletes a subset of microbiota components such as members of the Firmicutes phylum and promotes an expansion of the S. Typhimurium population. The molecular mechanisms leading to a bloom of the *Salmonella* population in the gut lumen are incompletely understood.

Methods: Here, we investigated the amino acid metabolism of *S.* Typhimurium in the streptomycin pretreated mouse model of *Salmonella*-induced colitis.

Results: Oral streptomycin treatment increased the availability of amino acids. Utilization of arginine by *S.* Typhimurium through the arginine deiminase pathway increased gut colonization. To test the idea that arginine in the large intestine is of microbial origin, we used a gnotobiotic mouse model. Mice, mono-colonized with either *C. symbiosum* (phylum Firmicutes) or *B. thetaiotaomicron* (phylum Bacteroidetes), were orally treated with streptomycin and then infected with the *S.* Typhimurium wild-type strain and an arginine deiminase deficient mutant. Streptomycin treatment eradicated *C. symbiosum* populations, which enhanced *S.* Typhimurium growth through the arginine deiminase pathway. In contrast, *B. thetaiotaomicron* populations were significantly reduced by Streptomycin treatment, yet the arginine deiminase pathway was dispensable for *Salmonella* gut colonization.

Conclusions: The results suggest that the depletion of Firmicutes leads to an increase in available arginine, possibly through the release of amino acids from lysing bacteria and bacterial debris. This work identifies arginine metabolism as a novel metabolic pathway that facilitates enteric pathogen gut colonization.

AMV06

Genomic insight into the co-dependent hydrogenogenesis in the facultative anaerobe *Parageobacillus thermoglucosidasius*

H. Aliyu*[1], T. Mohr[1], D. Cowan[2], P. De Maayer[3], A. Neumann[1]

[1]Karlsruhe Institute of Technology, Section II: Technical Biology, Institute of Process Engineering in Life Science, Karlsruhe, Germany

[2]University of Pretoria, Centre for Microbial Ecology and Genomics, Pretoria, South Africa

[3]University of the Witwatersrand, School of Molecular & Cell Biology Faculty of Science, Johannesburg, South Africa

Hydrogen gas, regarded as a viable alternative energy source to the dwindling fossil fuel resources, hugely relies on strategies that are energy intensive and make use of non-renewable substrates. Biological hydrogen provides an alternative source of pure and clean future energy carrier with zero contaminants during production and zero CO_2 on combustion. *Parageobacillus thermoglucosidasius* is a facultative anaerobic thermophilic bacterium, which is frequently isolated from high temperature environments including hot springs and compost. Here, we present the analysis of the hydrogenogenic capacity of *P. thermoglucosidasius* via comparative genomics and gas analyses. *P. thermoglucosidasius* encodes three distinct hydrogenases, two uptake hydrogenases as well as one H_2-evolving hydrogenase linked to an anaerobic CODH. Evolutionary analysis showed that this combination of hydrogenases is unique to *P. thermoglucosidasius.* Cultures grown under 50% CO and 50% air gas atmosphere utilized CO in the water-gas shift reaction to produce an equimolar amount of H_2. Subsequent comparison of four distinct *P. thermoglucosidasius* strains (publicly available) revealed that three strains (DSM 2542T, DSM 2543 and DSM 6285) are hydrogenogenic, while the fourth strain (DSM 21625) was not. In addition to the genetic differences in CODH-NiFe hydrogenase loci among the four strains, their genomes also encode variable protein complement, which may underpin the observed differences in hydrogenogenic capabilities. The data from this study may be invaluable towards the genetic improvement of *P. thermoglucosidasius* for optimum and efficient biohydrogen production and its potential incorporation in industrial-scale production strategies.

AMV07

Anaerobic steroid catabolism: a treasure trove of unprecedented enzyme reactions

C. Jacoby*[1], M. Boll[1]
[1]*Institute for biology II, Albert Ludwig University of Freiburg, Microbiology, Freiburg i. Br., Germany*

Steroids are ubiquitous constituents of biological membranes that are nearly insoluble in water and persistent to biodegradation. Aerobic steroid-degrading bacteria employ oxygenases for isoprenoid side chain and tetracyclic steran hydroxylation. In contrast, the anaerobic steroid degrading *Sterolibacterium (Stl.) denitrificans* comprises genes uniquely found in anaerobic steroid degraders. The Mo-dependent Steroid C25 dehydrogenase (S25DH) represents a characteristic key enzyme in this pathway, catalyzing the tertiary hydroxylation of the C25 atom of cholesterol with water. The genome of *Stl. denitrificans* contains seven further homologous genes encoding S25DH-like enzymes that are involved in the hydroxylation of steroidal compounds with modified side-chain. Preliminary results indicate that the further degradation of C25-OH proceeds via an ATP-dependent elimination of the hydroxyl-group, giving a C26 alkene that is most likely hydroxylated at C26, followed by the β-oxidation of the aliphatic side-chain. The degradation of the tetracyclic steran proceeds via the oxygen-independent 2,3-*seco* pathway by hydrolytic Ring A cleavage.

For the anaerobic degradation of 17β-estradiol (E2) and estrone (E1) a different enzymology is suggested since enzymes of the 2,3-*seco* pathway cannot attack the aromatic Ring. While the aerobic degradation of E2 proceeds via the 4,5-*seco* pathway involving oxygenases that are strictly O2-dependent, metabolites of the 17β-estradiol degrading, denitrifiying *Denitratisoma oestradiolicum* strongly suggest that the degradation of E2 proceeds via an initial carboxylation, allowing the thioesterification and subsequent reduction of the aromatic ring.

AMV08

Turning a Methanogen into an Acetogen

M. Rother*[1], A. Poehlein[2], C. Schöne[1]
[1]*Technische Universität Dresden, Institut für Mikrobiologie, Dresden, Germany*
[2]*Georg-August Universität Göttingen, Institut für Mikrobiologie und Genetik, Göttingen, Germany*

Biogenic methane formation, methanogenesis, is a unique energy metabolism found only in certain members of the domain *Archaea*. A common feature of the various types of methanogenesis that have been described is that energy is conserved via chemiosmotic mechanisms, i.e., by coupling the pathway to the generation of an ion motive force. Furthermore, all methanogenic archaea (methanogens) investigated to date are obligate methanogenic, i.e., must employ methanogenesis to grow. *Methanosarcina acetivorans* is able to utilize carbon monoxide (CO) as energy substrate, and produces from it, beside methane, substantial amounts of acetate via a variant of the reductive acetyl-coenzyme A pathway. As this pathway involves ATP synthesis by substrate-level phosphorylation, the question arose if *M. acetivorans* can grow on CO without the need to produce methane. To address this question, the genes encoding the membrane-integral, energy-converting N^5-methyl-tetrahydrosarcinapterin (H_4SPT):coenzyme M (HS-CoM) methyltransferase (MTR) were deleted. The mutant still required anabolic concentrations of a methyl-group donor (e.g. methanol or trimethylamine) to grow with CO. However, this requirement was overcome by spontaneous mutation. One of the suppressor mutants isolated grew acetogenic on CO, and generated essentially no methane. Still, the strain"s ability to generate methane from other substrates was not abolished. To identify the mutation(s) that may have caused the phenotype of the suppressor, its genome was sequenced. The strain carries a large (> 177 kb) chromosomal deletion, encompassing more than 145 open reading frames. Current efforts aim at elucidating which (subset of) genes *M. acetivorans* needs to loose in order to become independent of methanogenesis.

BDV01

Similarities and Peculiarities Between Bacterial Degradation of Indole and Indole-3-acetic acid

M. Sadauskas*[1], J. Vaitekūnas[1], R. Gasparavičiūtė[1], R. Meškys[1]
[1]*Vilnius University, Life Sciences Center, Vilnius, Lithuania*

Introduction: Microbial degradation of *N*-heterocyclic compounds has proven to be a rich source of enzymes with novel activities. Indole, which is a product of tryptophan hydrolysis, recently was accepted as an interspecies signalling molecule, while a similar molecule - indole-3-acetic acid (IAA) - has been known as plant hormone auxin. Bacterial degradation of these two molecules has been explored extensively, yet key mechanisms remained unknown.

Objective: Characterization of bacterial biodegradation of indole and indole-3-acetic acid.

Materials and methods: Biodegradation genes were identified by constructing genomic libraries of bacteria able to grow on indole or IAA and screening the libraries for indigo-producing activity. Selected genes were expressed in *Escherichia coli*, purified by Ni-NTA and checked for activity in different combinations and with different indole substrates. Reaction products were analysed by HPLC/MS.

Results**:** We analysed the biodegradation of indole performed by *Acinetobacter* sp. strain O153 and revealed a full mechanism of indole mineralisation in protein level, catalysed by IifCDBA proteins. The final degradation product, anthranilic acid, is formed by IifA, an enzyme which is both structurally and functionally comparable to cofactor-independent oxygenases. We also show that Iif and Iac proteins perform analogous reactions with indole and IAA thus clarifying the mechanism of IAA biodegradation. Moreover, due to enzyme promiscuity, activities of some Iif and Iac proteins overlap, suggesting means for construction of degradation platform with improved activity or substrate spectra.

Conclusion: Biodegradation of indole and IAA in certain bacteria proceed through analogous pathways which involve novel and uncharacterized activities.

BDV03

Different roles for 7alpha-hydroxysteroid dehydratases in bacterial degradation of bile salts

F. M. Feller*[1], O. Yücel[1], J. Holert[2], B. Philipp[1]
[1]*Westfälische Wilhelms-Universität, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*
[2]*University of British Columbia, Microbiology and Immunology, Vancouver, Canada*

Bile salts are steroids in the digestive tracts of vertebrates, which enter the environment by excretion and are degraded

by aquatic and soil bacteria. Bile salts comprise mono-, di- and trihydroxy steroids, and the number, position and conformation of OH-groups influence the degradability and the metabolic pathways used in different bacteria.

While C12-OH is apparently consistently being removed in Gram-negative bacteria the fate of C7-OH is more variable. Reductive C7-OH dehydroxylation, which is initiated by the 7α-dehydratase BaiE. serves as an electron sink in strictly anaerobic intestinal bacteria. In aerobic degradation, the C7-OH is retained in *Pseudomonas* and *Comamonas* strains and is essential for degradation of the steroid skeleton in later steps. Sphingomonads, in contrast, remove this hydroxyl group by the 7α-hydroxysteroid dehydratase Hsh2. It is currently unknown whether it has to be re-introduced in later steps.

Azoarcus strain Aa7 grew with bile salts under aerobic and under aerobic conditions with nitrate as electron acceptor using the 9,10-seco and the 2,3-seco pathway, respectively. Deletion of a *hsh2* homolog adjacent to the key genes for the anaerobic 2,3-seco pathway had no phenotype under aerobic conditions while it caused a growth defect with the 7α-hydroxyl bile salts under anaerobic conditions. Apparently, removal of the C7-OH is essential for completely degrading 7α-hydroxyl bile salts via the 2,3-seco pathway in strain Aa7.

In conclusion, 7α-hydroxysteroid dehydratases obviously contribute to different steroid degradation pathways in the bacterial kingdom. For further exploring the diverse roles of Hsh2 genomes and metagenomes are currently being analyzed with first results showing its presence mainly in Sphingomonads and *Azoarcus* strains.

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BDV04

N5-functionalized flavin cofactors may facilitate versatile redox chemistry

A. Matthews*[1], R. Saleem Batcha[1], R. Teufel[1]
[1]*Uni Freiburg, Freiburg i. Br., Germany*

Flavoenzymes catalyze many (redox-) reactions in all domains of life. Some are able to transfer an oxygen atom from molecular oxygen to an organic substrate and thus function as monooxygenases. Until recently, this oxygenation chemistry was thought to be exclusively mediated by transiently formed peroxy species bound to the C4a position of the flavin. However, a different mechanism involving flavin-N5-oxide (Fl_{N5O}) as the oxygenating species was found in the bacterial flavoenzyme EncM[1,2]. We postulate that N5-funtionalized flavin cofactors are employed more widely in nature, e.g. in the reactions catalyzed by the flavoenzymes RutA and LadA that are non-homologous to EncM. Previous work on the bacterial pyrimidine monooxygenase RutA showed that the Fl_{N5O} is formed during catalysis[3]. However, the underlying biochemical mechanism has not been fully elucidated. We propose a RutA mechanism in which an N5-peroxide (Fl_{N5OO}) acts as the oxygenating species that transfers the distal oxygen to the substrate, thereby generating Fl_{N5O}. We propose a common pathway for the functionalization of N5 based on structural and biochemical analyses of the O_2 reactions sites of EncM and RutA. Remarkably, highly similar O_2 reactions sites can be found in additional enzymes. This includes the bacterial long-chain alkane monooxygenase LadA that catalyzes the terminal oxidation of alkanes to the corresponding primary alcohols[4,5]. Alkane oxidation mediated by an organic cofactor is exceptional and we envisage a radical oxidation mechanism, possibly relying on an N5-nitroxyl radical.

BDV05

Stereochemical Insights into the Anaerobic Degradation of 4-Isopropylbenzoyl-CoA in Denitrifying Bacterium Strain pCyN1

P. Becker*[1], J. Küppers[2], R. Jarling[1], M. Dörries[1,3], N. Cakic[1], M. Schmidtmann[2], J. Christoffers[2], R. Rabus[1], H. Wilkes[1]
[1]*Carl von Ossietzky Universität Oldenburg, ICBM, Oldenburg, Germany*
[2]*Carl von Ossietzky Universität Oldenburg, Institut für Chemie, Oldenburg, Germany*
[3]*Carl von Ossietzky Universität Oldenburg, HIFMB, Oldenburg, Germany*

Introduction: The denitrifier *"Aromatoleum aromaticum*" pCyN1 was previously shown to anaerobically degrade *p*-cymene to 4-isopropylbenzoyl-CoA. Further degradation was assumed to proceed in analogy to that of benzoyl-CoA, possibly requiring specific adaptations due to the *para*-positioned isopropyl-group.

Objectives: Elucidating the reaction sequence for anaerobic 4-isopropylbenzoyl-CoA degradation, focusing on the *para*-isopropyl group as a handle to obtain evidence for the involved stereochemistry.

Materials & methods: Soluble and membrane protein-enriched fractions of substrate-adapted cells were analyzed (2D DIGE, SDS-PAGE, mass spectrometry). Reference standards of two detected intermediates were stereoselectively synthesized to confirm their molecular structure and absolute configuration.

Results: Protein profiles and homology studies suggest, that the anaerobic degradation of 4-isopropylbenzoyl-CoA employed by strain pCyN1 combines a *Thauera*-type, diene-forming, reductive dearomatization with a monoene-converting *Rhodopseudomonas*-type modified β-oxidation. The unambiguous identification of (1*S**,2*S**,4*S**)-2-hydroxy-4-isopropylcyclohexane-1-carboxylate and (*S*)-3-isopropylpimelate, using synthesized pure (*R*)- and (*S*)-isomers as standards, indicates that in initial *Thauera*-type dearomatization, involving two one-electron reductions, each followed by a protonation, the second protonation step defines the absolute configuration of the formed dienoyl-CoA. Further degradation by the *Rhodopseudomonas*-type modified β-oxidation is supported by the identification of the non-hydroxylated (*S*)-3-isopropylpimelate.

Conclusion: The anaerobic degradation pathway of 4-isopropylbenzoyl-CoA employed by strain pCyN1 appears to be of a hybrid character.

BDV06

Manganese oxidizing bacteria degrade cylindrospermopsin

E. B. Martinez Ruiz*[1], M. Cooper[1], J. Fastner[2], U. Szewzyk[1]
[1]*Technical University Berlin, Environmental Microbiology, Berlin, Germany*
[2]*German Environment Agency , Section Drinking Water Treatment and Resource Protection, Berlin, Germany*

Manganese oxidizing bacteria (MOB) play an important role in the biogeochemical manganese (Mn) cycle and are commonly detected in natural and technical systems. The biogenic oxides produced by MOB are potent oxidizers of a variety of organic and inorganic compounds, including pollutants from anthropogenic origin such as diclofenac or 17α-ethinylestradiol. Aerobic neutrophilic MOB may coexist in the same environment as toxigenic cyanobacteria that are able to produce cyanotoxins, such as cylindrospermopsin (CYN). So far, there is no information about CYN degradation by MOB. In this study, we investigated the degradation of CYN by four different MOB strains. The removal of CYN by biogenic oxides in the absence of viable cells was also quantified. In addition, the effect of yeast extract and iron, and the concentration of oxidized Mn on the degradation of CYN by MOB was evaluated. The four tested organisms removed 6 to ~100% of CYN within 3 to 28 days. *Pseudomonas* sp. strain OF001 almost completely degraded 120 µg L^{-1} of CYN in 3 days. The highest degradation of CYN was observed by all the MOB, when $MnCO_3$ was added as Mn^{2+} source compared to setups without Mn^{2+}. Sterile biogenic oxides showed negligible or zero removal of CYN. The concentration of oxidized Mn did not affect CYN degradation, however, the mere active oxidation reaction of manganese seems to be the key factor for the degradation of CYN. MOB may contribute to the biodegradation of CYN in the environment and could be a promising tool for the reduction of CYN in water treatment plants.

BDV07

Bioremediation of inorganic mercury using *Exiguobacterium indicium* (MW1) isolated from the marine environment of Odisha coast

S. Satapathy*[1]
[1]*CSIR-IMMT, Environment and sustainability, Bhubaneswar, India*

Introduction: Methylmercury is more toxic than any other form of mercury and it biomagnifies in the aquatic food chain which can have lethal effect on human through contaminated fish. It has been studied in the literature that mer operon (mer A) contain mercury reductase (Mer A) which degrade MeHg to Hg(II) and methane.

Objective: We have used *E indicium* (MW1) bacterial strain as a model to study its reduction potential for MeHg to Hg(0).

Material and methods: E indicum was isolated from the marine sediment sample of east coast of India. Hg resistant bacteria was isolated using nutrient agar medium which contain 30 mg/L of Hg. Methylmercury chloride dissolved in ethanol as a source of mercury and was analyzed in mercury analyzer. Cultures were kept to inoculate at an equivalent optical density of 0.05. Cells were centrifuged and cell pellets were resuspended. The MeHg reduction of the strain was analyzed and optimized with different mercury concentrations (100–1000 mg/L). Under optimized incubation period for 160 h about 90%, 54% and 42% of reduction of mercury were noticed. The reduction occurred according to the growth of the strain. It had increased the reduction capability in the marine environment. the associated bacterial reduction was confirmed by using FTIR, UV–Vis–DRS and FESEM-EDX.

Results: Maximum reduction observed with 1% NaCl (w/v), 35°C temperature, 8.0 pH, and 120 rpm. It followed a decreasing trend with higher mercury concentration. The reduction time was in the range of 2.14×10^{-2} to 3.4×10^{-3} h−1 .

Conclusion: The isolated bacterial strain E. indicum MW1 shows noticeably resistant to toxic elements like MeHg.Therefore they can be further studied for bioremediation of marine environment.

BDV08

Preventing deterioration of cultural heritage by fungal decay of paper-based documents via application of ionic liquids with anti-fungal properties in paper restauration

K. Schmitz*[1], S. Wagner[1], C. Maier[2], J. P. Benz[1,2]
[1]*TU München, Wood Bioprocesses, Freising, Germany*
[2]*Nitrochemie AG, Aschau, Germany*

With early industrialization enabling cheap production of paper, a spiraling process of increasing application fields for paper and demand for paper-based products has led to an explosion in paper production during the last two centuries (Valente, 2010). Today, national and private archives and libraries harbor vast amounts of documents. However, due to less sophisticated production processes, most of the paper produced between the 1850s and the late 20th century was acidic in its nature (Cedzová, Gállová, & Katuščák, 2006), making it susceptible to slow degeneration of the cellulose fibers, causing brittleness and decay. Current industrial processes help to maintain the status quo of archived objects through pH neutralization, but cannot restore an intact fiber structure. A novel approach patented by the Nitrochemie AG takes advantage of the cellulose-resolving properties of ionic liquids (ILs) to precipitate intact cellulose fibers onto damaged paper surfaces. With some of these ILs having structural similarities to the well-known fungicide clotrimazole, a potential fungistatic or fungicidal property was proposed that might add additional value to this treatment (Sterflinger, 2010), which was characterized in this study.

We have evaluated the potency of four ILs with potential application in paper restauration for growth suppression of five relevant paper-degrading fungi. In plate-assays, varying anti-fungal activities could be observed. The minimal inhibitory concentration of each IL was determined, and fungal growth behavior on treated paper samples was validated. The obtained results help to guide further efforts in optimizing industrial paper-restoration processes, aiming at additional protection of historic documents against biotic degeneration through fungi.

GRV01

Molecular characterization of an adaptor protein for a major endoribonuclease in *Escherichia coli*

S. Durica-Mitic*[1], B. Görke[1]
[1]*University of Vienna, Max F. Perutz Laboratories, Department of Microbiology, Immunobiology and Genetics, Vienna, Austria*

In *E. coli*, bulk RNA turnover is initiated by RNase E, a major endoribonuclease whose activity is extensively regulated. RapZ is the first adaptor protein identified for RNase E and is essential for cleavage of small RNA (sRNA) GlmZ. Processed GlmZ is inactive and cannot upregulate its target mRNA encoding the cell envelope biosynthesis enzyme GlmS. When needed, GlmZ cleavage is countered by increased amounts of decoy sRNA GlmY, which binds and sequesters RapZ (1). Recently, RapZ structure was solved, revealing that it adopts a tetrameric assembly which is required for its function (2). RapZ interacts with the catalytic domain of RNase E and binds GlmY/Z via its C-terminal domain (CTD) (1,2) but whether both functions are required for its role as an adaptor is yet to be elucidated. In this study,

we show that globular domains of RapZ alone (NTD and CTD) are unable to form a complex with RNase E. We find that RapZ-CTD promotes GlmZ *in vitro* cleavage only marginally, further emphasizing the role of RapZ-NTD in this process, as well as the importance of interaction with RNase E. Conversely, we show that loss of RNA binding function does not prevent RapZ from promoting GlmZ cleavage provided that the interaction with RNase E is retained. In addition, we performed whole transcriptome analysis which revealed that RapZ overproduction results in altered levels of various RNase E targets. Lastly, we demonstrate more efficient *in vitro* processing of additional RNase E substrates in the presence of RapZ. Taken together, our data indicate that RapZ may act as an allosteric activator of RNase E and potentiate its activity through direct interaction.

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GRV02

Small proteins in *Sinorhizobium fredii* NGR234 – the next puzzle piece to its broad host range?

H. Duin*[1], K. Petersen[1], S. Güllert[2], N. Jacky[3], W. R. Streit[1]

[1]*University Hamburg, Microbiology and Biotechnology, Hamburg, Germany*

[2]*University of Kiel, General Microbiology, Kiel, Germany*

[3]*University of Kiel, Biochemical Ecology and Molecular Evolution, Kiel, Germany*

The Gram-negative alpha-Proteobacterium *Sinorhizobium fredii* NGR234 has evolved a diverse and unique set of molecular mechanisms to infect a very wide range of legume and non-legume plants. The vast majority of these symbiotic features are encoded on its symbiotic plasmid pNGR234*a*, with a size of 0.5 Mb and 422 annotated features. Our main goal is to understand the mechanisms of host infection and the quorum sensing-dependent regulation of copy number in pNGR234*a.* Previously we reported on the construction of a deletion strain carrying a deletion of the autoinducer synthase gene *traI* (ΔtraI627). Deletion of the pNGR234*a*-encoded autoinducer synthase *traI* (ΔtraI627) gene resulted in a 6-8-fold increased copy number of pNGR234*a (1)*. Because of the increased copy number all ORFs on this replicon were transcribed at low level. Transcriptome analyses of RNAseq data obtained in the background of the mutant identified that in addition to the 422 known ORFs at least 600-700 so called µ-ORFs with sizes ranging between 33 nts and 183 nts were differentially regulated. *In silico* prediction confirmed this finding and estimated a total of 701 µ-ORFs encoded on this replicon. For the vast majority no function could be assigned and most of these mapped in intergenic regions. Using mutagenesis approaches, heterologous expression in combination with reporter gene studies and immunogold labelling we could assign functions to two of these µ-ORFs. Deletion of the µ-ORFs, *repX* and NGR_a01725 revealed a role in plasmid stability and replication control. Both mutations resulted in a loss of pNGR234*a* and consequently mutants did not infect plants. Further work supported the observation that both µ-ORFs are involved in plasmid copy number regulation and maintenance.

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GRV03

Redefining the promoter architecture of the capsular biosynthesis gene cluster in *Staphylococcus aureus*

D. Keinhörster*[1], A. Salzer[1], A. Duque-Jaramillo[1], S. E. George[1], C. Weidenmaier[1], C. Wolz[1]

[1]*University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Tübingen, Germany*

Staphylococcus aureus produces a capsular polysaccharide (CP) which has been shown to possess anti-phagocytic properties, allowing the bacterium to persist in the infected host. However, CP synthesis is highly heterogeneous within a population and mainly detectable in non-growing cells. Capsular biosynthesis genes are encoded by a *capA-P* operon, which is regulated and transcribed by one principal promoter (P_{cap}) in front of *capA*. The interplay of different regulators influencing the early-Off/late-Heterogeneous *cap* transcription at the molecular level is poorly understood. Here, we aim to decipher the molecular architecture of the P_{cap} promoter region.

Expression and regulation of *cap* was investigated by creating various mutations of the ~ 400 bp P_{cap} promoter region fused to *yfp.* Promoter-activity of the constructs in different genetic backgrounds was assessed by microscopy and quantification of fluorescence during growth. Molecular analysis of P_{cap} revealed two new transcriptional start sites with one being directly controlled by Sigma factor B (SigB). As SigB is known to be involved in growth phase-dependent regulation this partially explains the temporal *cap* expression. Furthermore, some major regulators were shown to act on a region upstream of the SigB consensus sequence.

The late heterogeneous expression pattern of CP is likely determined by the interaction of transcriptional factors with Sigma factor B activity. Redefining the promoter architecture is a powerful first step to elucidate complex processes leading to temporal and bistable expression of cell surface polymers.

GRV04

HrrSA Orchestrates a Systemic Response to Heme and Determines Prioritization of Terminal Cytochrome Oxidase Expression

M. Keppel*[1], C. Davoudi[1], A. Filipchyk[1], U. Viets[1], E. Pfeifer[1], T. Polen[1], M. Baumgart[1], M. Bott[1], J. Frunzke[1]

[1]*Forschungszentrum Jülich GmbH, IBG1, Jülich, Germany*

Heme (iron bound protoporphyrin IX) is a versatile molecule that is synthesized and used by virtually all aerobic eukaryotic and prokaryotic cells because it serves as the prosthetic group of hemoglobins, hydroxylases, catalases, peroxidases, and cytochromes and is thereby essential for many cellular processes, such as electron transfer, respiration and oxygen metabolism. Despite its essential role, it exhibits severe toxicity at high concentrations. While this complexity of heme as a stimulus has shaped bacterial network evolution, only a small number of targets controlled by heme-responsive regulators have been described to date.

Here, we used a genome-wide approach to monitor the *in vivo* promoter occupancy of HrrA, the response regulator of the heme-regulated two-component system HrrSA of *Corynebacterium glutamicum*. Time-resolved profiling revealed dynamic, heme-induced binding of HrrA to more than 250 different genomic targets encoding proteins associated with heme biosynthesis, the respiratory chain, oxidative stress response and cell envelope remodeling. Additionally, transcriptome analysis gave insight into the regulatory role of HrrA binding. For example, we found, that

in a heme-rich environment and by repression of *sigC*, which encodes an activator of the *cydABCD* operon, HrrA prioritizes the expression of genes encoding the cytochrome *bc*1-*aa*3 supercomplex for the constitution of the respiratory chain of *C. glutamicum*.

In general, this dataset describes, for the first time, the systemic response strategy bacteria have evolved to respond to the versatile signaling molecule heme.

GRV05
1 billion years of RpaB evolution
M. Riediger*[1], J. Peisert[1], W. R. Hess[1]
[1]*Institute for Biology III, University of Freiburg, AG Hess, Freiburg i. Br., Germany*

The OmpR-type transcription factor RpaB is well conserved all over the cyanobacterial phylum and can be found in most chloroplast genomes of non-green algae, overcoming more than 1 billion years of evolution to the chloroplast genome of the charophyte algae *C. atmophyticus* and making it one of the most conserved response regulators overall. In *Synechocystis*, RpaB is essential and controls the expression of several genes related to photosynthesis on the transcriptional level but also via posttranscriptional regulation by controlling the expression of the sRNA PsrR1. RpaB binds the HLR1 sequence, consisting of two imperfect 8 nt long direct repeats separated by two random nucleotides. Depending on the distance between HLR1 and the start site of transcription, RpaB can activate or repress transcription. Despite its wide conservation, functional studies on RpaB are restricted to *Synechocystis* 6803 and *S. elongatus* 7942 due to the lack of genetic tools for most organisms. Its stunning structural conservation of important residues and the description of HLR1-like sequences in several organisms indicate similar properties for most RpaB homologues, although experimental proof for how evolution rendered the physiological role and DNA binding properties of RpaB is lacking. Therefore, cross-species researches seem promising to reveal new features on how RpaB mediated photosynthesis regulation evolved, and to understand the extent of its physiological relevance. Applying in-vitro approaches in combination with in-silico predictions and genome-wide high precision mapping data for TSS, we aimed at reconstructing how RpaB affects photosynthesis regulation over evolutionary time scales.

GRV06
Impact of the two-component regulatory system 09 on oxidative stress resistance of *Streptococcus pneumoniae*
S. Hirschmann*[1], A. Gómez-Mejia[1], U. Mäder[2], D. Driesch[3], J. Karsunke[1], S. Häußler[4], S. Hammerschmidt[1]
[1]*Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany*
[2]*Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany*
[3]*BioControl Jena GmbH, Jena, Germany*
[4]*Institute of Molecular Bacteriology, Helmholtz Centre for Infection Research, Braunschweig, Germany*

Introduction: The pathobiont *Streptococcus pneumoniae* encounters different human host departments and causes severe diseases. The success of the pneumococcus as a pathogen relies on its capacity to respond to environmental signals and regulation of its versatile repertoire of virulence factors. Pneumococcal stand-alone regulators and two-component regulatory systems (TCS) enable adaptation to changing environmental conditions.

Objectives: The aim of this study was to decipher the impact of the TCS09 on regulatory mechanisms involved in oxidative stress resistance and pathophysiological processes.

Methods: Isogenic mutants for the response regulator (Δ*rr09*), the histidine kinase (Δ*hk09*) and both components (Δ*tcs09*) were generated. Using RNAseq, real-time PCR and LI-COR® analysis, the regulatory effect of the TCS09 in general and on genes belonging to an extracellular thioredoxin repair system (CcdA1-Etrx1-MsrAB2 (CEM) system) was investigated. The survival of mutants was examined in presence of hydrogen peroxide, paraquat and post-infection of phagocytosis. Finally, *in vivo* infections using the acute pneumonia and sepsis mouse models were performed to analyze the virulence potential of TCS09-deficient pneumococci.

Results: A regulatory effect of the TCS09 on the CEM operon could be verified on the RNA and protein level. HK09- and TCS09-deficient pneumococci were more sensitive to extracellular and intracellular oxidative stress and less viable after phagocytosis. Loss of one or both components of the TCS09 substantially attenuated virulence in lung infections but not in systemic infections.

Conclusion: This study indicates that the TCS09 is involved in regulation of the CEM system and in sensing oxidative stress. Thus, the TCS09 is pivotal for pneumococcal virulence.

GRV07
Regulation of Heterologous Subtilin Expression in *Bacillus subtilis* W168
Q. Zhang*[1], C. Kobras[2,3], S. Gebhard[2,3], T. Mascher[1], D. Wolf[1]
[1]*Technische Universität(TU) Dresden, Department of Microbiology, Dresden, Germany*
[2]*Ludwig-Maximillians-Universität München, Department I, Microbiology, München, Germany*
[3]*University of Bath, Department of Biology & Biochemistry, Bath, United Kingdom*

In its native producer, *Bacillus subtilis* ATCC6633, biosynthesis of the lantibiotic subtilin occurs via the gene cluster *spaBTCSIFEGRK* (*spa*). The expression of the *spa*-locus is dependent from cell-density and thereby strongly regulated by a set of transcriptional regulators [1]. The transfer of the *spa*-locus into the well-known "workhorse for protein production" *B. subtilis* W168 successfully resulted in native subtilin production that indicates a regulatory conformity in subtilin expression in both strains W168 and ATCC6633. However, an in-depth analysis of the *spa*-locus regulation led to a great divergence compared to results from previous studies. Here, we investigated the dependency of subtilin expression and activity of the *spa* promoters, P_{spaI}, P_{spaB}, P_{spaS} and P_{spaR}, on the transition state regulators AbrB, Spo0A, SigH, and the two-component system (TCS) SpaRK in *B. subtilis* W168. Contrary to previous findings [1], the regulator pair Spo0A and AbrB control all four promoters of the *spa*-locus simultaneously. Interestingly, SigH that was thought to regulate the activity of the TCS promoter P_{spaR} [1], solely plays a role as positive regulator of P_{spaB} downstream of AbrB as a negative regulator of that promoter. Furthermore, the subtilin-induced TCS SpaRK regulates P_{spaI}, P_{spaB} and P_{spaS}, which overlaps previous findings in *B. subtilis* ATCC6633. Additionally, subtilin production in wild

type and mutants was analyzed by "online-reporter strains" and spot-on-lawn assays were done to test the potency of the lantibiotic. In summary, we identified a new regulatory pathway in subtilin biosynthesis that seems to be helpful to improve native heterologous subtilin expression in *B. subtilis* W168.

[1] Stein *et al.,* 2002, Mol Microbio. 44 (2):403-416

GRV08

The distinctive regulation of cyanobacterial glutamine synthetase

S. Klähn*[1]
[1]*Helmholtz Centre for Environmental Research, Solar Materials, Leipzig, Germany*

The rising biotechnological importance of metabolic engineering in cyanobacteria is contradicted by the current state of knowledge about the regulation of their metabolism. In this regard they differ from other bacterial groups in many aspects. This will be exemplified by the regulation of glutamine synthetase (GS), the key enzyme of bacterial nitrogen assimilation. The comprehensively characterized GS of enterobacteria is subject to exquisite regulation at multiple levels, among them gene expression regulation to control GS abundance as well as feedback inhibition and covalent modifications to control enzyme activity. The cyanobacterial GS, however, features a fundamentally different regulation which includes the interaction with small proteins, the so-called inactivating factors (IFs) that inhibit GS linearly with their abundance (Bolay et al., 2018; *Life* 8(4):52). The model strain *Synechocystis* sp. PCC 6803 harbours two of these factors called IF7 and IF17 whose abundance is mainly controlled at the transcriptional level. In addition, the regulatory RNA NsiR4 was found to interact with and to affect translation of the mRNA encoding IF7 (Klähn et al., 2015; PNAS *112*(45):E6243-E6252). Recent analyses also revealed a glutamine riboswitch as another key element that controls IF17 synthesis in a glutamine-dependent manner (Klähn et al., 2018; Nucleic Acids Res., in press). Both RNA elements are unique to but widespread among cyanobacteria and are required for sufficient control of GS activity in this major bacterial group. These intriguing regulatory differences suggest that further metabolic reactions might be controlled differently compared to other bacteria. Hence, further basic research is required to make cyanobacteria amenable for biotechnological applications.

SMV01

Design and characterization of synthetic ECF-σ /anti-σ threshold gate genetic circuit in *Escherichia coli.*

S. Vecchione*[1], A. Diehl[1], G. Fritz[1]
[1]*LOEWE-Center for Synthetic Microbiology SYNMIKRO, Biology, Marburg, Germany*

The rational design of synthetic circuits is often restricted by the limited number building blocks, consisting of a handful of transcriptional regulators, and by the ability of performing a designated function without cross-reactions (orthogonality). To overcome these restrictions we apply extracytoplasmic function (ECF) σ factors - alternative σ factors that specifically recognize distinct promoter sequences. Previously we used ECF-σ to build and characterize ECF-σ based "autonomous timer circuits" that sequentially activate a series of target genes with a defined time delay(1). In order improve the dynamic response and achieve a tunable time delay, here we introduce anti-σ factors to these synthetic circuits. Anti-σ factors are often transmembrane proteins that inactivate ECFs by sequestration. However it was shown that anti-σ factors are often toxic when expressed at high level in *E. coli*(2). To alleviate toxicity here we generated truncated, soluble versions of anti-σ factors and show that that they maintain the ability of sequestering the ECFs. Using these novel non-toxic anti-σ factors, we designed ECF-σ/anti-σ threshold gate circuits with clear-cut on/off switching behavior, and a tunable time delay. By applying these threshold gated circuits to drive the expression of the *ccdB* toxin, we generated a time-tunable kill switch circuit in *E. coli.* Our results serve as a proof of concept for the application of ECFs as building blocks for synthetic biology and could be used, in biotechnological applications, to implement a defined time hierarchy among the expression of biosynthetic pathways components.

(1) Pinto D, Vecchione S *et al.* Engineering orthogonal synthetic timer circuits based on extracytoplasmic function σ factors *Nucl. A. Res.* **46,** 7450–7464 (2018).

(2) Rhodius V A *et al.* Design of orthogonal genetic switches based on a crosstalk map of σ, anti-σ, and promoters. *Mol. Syst. Biol.* **9,**702–702 (2014).

SMV02

New gene regulatory tools to improve synthetic biology in *Synechocystis* sp. PCC 6803

A. Behle*[1]
[1]*Heinrich Heine University, Synthetic Microbiology, Düsseldorf, Germany*

Cyanobacteria are becoming more and more interesting for various research applications due to their ability to fix carbon photosynthetically. While great progress has been made with other model organisms such as *E. coli* in the field of synthetic biology, cyanobacteria currently lag behind. This is mostly due to the fact that available modules do not function in an equivalent manner when used in cyanobacteria. For example, most commonly known promoters that have been tested in cyanobacteria have a much weaker induction ratio or do not respond at all to the inducer. In addition, experimental setups differ widely in the literature, making it difficult to compare different promoters.

Here, we apply two different strategies to combat this problem. Firstly, we created a library of different inducible promoter constructs, only varying the promoter region in order to ensure comparability. Secondly, in order to take advantage of the natural polyploidy of our model organism, *Synechocystis* sp. PCC 6803, we created both plasmid-based and genomic promoter constructs.

In summary, we hope to expand the current understanding and employability of inducible promoters in order to facilitate the construction of more complex regulatory synthetic networks.

SMV03

Synthetic pathway for carbon fixation in *Synechocystis* sp. PCC 6803

E. M. Brouwer*[1], S. Song[1], M. Hagemann[1]
[1]*Uni Rostock, Pflanzenphysiologie, Plantphysiology, Rostock, Germany*

With the increase reliance on renewable energy sources and increased anthropogenic CO_2 emissions the idea rises to use photosynthetic organisms for biotechnological production of various organic compounds. Photosynthetic carbon assimilation in cyanobacteria, as well as in plants, depends on the activity of RubisCO, an enzyme with relatively low activity and affinity toward the substrate CO_2. Here we aime to implement an additional synthetic CO_2 fixing pathway to end up with a higher net carbon fixing rate. In this synthetic pathway CO_2 is fixed as formate and subsequently incorporated into carbon metabolism. Key enzymes are the *formate dehydrogenase* reducing CO_2 to formate and the *formate tetrahydrofolate ligase*. Strains, expressing heterologous enzymes for formate synthesis and assimilation, are currently used to achieve an efficient formate assimilation pathway working in parallel to the photosynthetic CO_2 assimilation. The additional fixed carbon can be used to produce organic compounds without harming growth and therefore achieving higher yields.

SMV04

An α-pheromone-based Yeast Communication Tool Kit (YCTK) for the implementation of multicellular communication networks in *Saccharomyces cerevisiae*

N. Krink*[1], A. Anders [1], V. Sourjik [1]
[1]*Max Planck Institute for Terrestrial Microbiology & LOEWE Research Center for Synthetic Microbiology (SYNMIKRO) Department of Systems and Synthetic Microbiology, Systems an Synthetic Microbiology, Marburg, Germany*

Engineering complex artificial multicellular communication networks is a challenge in Synthetic Biology. Already established systems have been mostly implemented in bacterial hosts by utilizing modules derived from bacterial quorum sensing entities. However, for *Saccharomyces cerevisiae* , the most used eukaryotic model organism in Synthetic Biology, complexities of artificial intercellular communication systems remain limited due to a lack of reliable communication modules. To close this gap and to facilitate the engineering of multicellular communication networks in *Saccharomyces cerevisiae* equivalent to quorum sensing, we are providing a yeast communication toolkit (YCTK). This kit is fully compatible with the Golden Gate cloning standard. The toolkit comprises of sender- (α-pheromones) and receiver-modules (α-pheromone receptors) from 11 different yeast species. Furthermore, it contains quenching modules (Bar1like proteases) and a set of 7 pheromone responsive promoters with different expression profiles. We characterized the toolkit by separately expressing its sender- and receiver modules in *Saccharomyces cerevisiae* and measuring cellular responses for all sender-receiver and quencher-signal combinations by flow cytometry. We found a wide range of specificities, ranging from specific to promiscuous sender- and receiver-modules as well as quenching systems. Interestingly we found an anti-correlation between pheromone and receptor promiscuity. The YCTK allowed us to design, construct and test different multicellular networks. These networks can find application in multicellular computing, in the development of novel biosensors and in metabolic engineering of communities.

SMV05

Cyclic triterpenoid production with tailored *Saccharomyces cerevisiae* – challenges and limitations

B. E. Ebert*[1], K. Walter[1], E. Czarnotta[1], J. Maury[2], S. A. Jacobsen[2], A. Lewandowski[3], H. Guo[1], T. Polakowski[3], J. Förster[2], C. Lang[3], L. M. Blank[1]
[1]*RWTH Aachen University, Institute of Applied Microbiology, Aachen, Germany*
[2]*DTU Denmark, Lyngby, Denmark*
[3]*Organobalance GmbH , Berlin, Germany*

Triterpenoids are secondary plant metabolites derived from squalene and consist of six isoprene units (C30). Many of them or their synthetic derivatives are currently being investigated as medicinal products for various diseases, including cancer, HIV or microbial infections. Despite their obvious industrial potential, the application is often hindered by their low abundance in natural plant sources. This poses challenges for a sustainable production of such compounds due to wasteful and costly product purification. Recombinant synthesis in engineered microorganism might be one solution for efficient and scalable manufacturing of these compounds.

Here, we present a tailored *Saccharomyces cerevisiae* strain optimized for the synthesis of 2,3-oxodosqualene derived triterpenoids. We showcase the potential of the strain for the synthesis of betulinic acid, a cyclic triterpenoid with antiretroviral, antimalarial, and anti-inflammatory properties and potential as anticancer agent.

The strain engineering included (i) pathway engineering by optimal gene combination and dosage, (ii) strain engineering by implementation of different push, pull and block strategies and (iii) compartment engineering. The latter effort was especially important to increase the reaction space for the highly lipophilic and non-water-soluble product.

In an optimized fermentation process, a maximum titer and productivity of pentacyclic triterpenoids of above 6 g/L and ca. 40 mg/L/h, respectively, were achieved with the final strain.

The results are discussed with respect to challenges and limitations of intracellular, lipophilic compound synthesis.

SMV06

Bacterial varieties of terpenoid building blocks

M. Kschowak[1], L. Drummond[1], H. Wortmann[1], J. S. Dickschat[2], J. Schrader[1], M. Buchhaupt*[1]
[1]*DECHEMA-Forschungsinstitut, Industrielle Biotechnologie, Frankfurt a. M., Germany*
[2]*Rheinische Friedrich Wilhelms University Bonn, Kekule-Institut für Organische Chemie und Biochemie, Bonn, Germany*

Terpenoids, also called isoprenoids constitute the largest class of natural products with more than 70,000 compounds that have been discovered so far. So far, most of these substances were characterized after isolation from plant materials, in which they often accumulate to relatively high concentrations. In general, terpenoids are synthesized from the universal precursors IPP and DMAPP. The isoprene rule states that terpenes are formally built out of isoprene units and therefore display a skeleton which has always a multiple of five carbon atoms. After the elucidation of the biosynthetic pathways of 2-methylisoborneol and 2-methylenebornane in bacteria, a GPP methyltransferase converting GPP into 2-methyl-GPP was described. This C11 prenyl diphosphate is used by certain terpene synthases to produce mainly the two different bicyclic terpenoids.

We aimed at a detailed description of the chemical diversity accessible by these C11 terpene synthases. Therefore, *E. coli* strains expressing a mevalonate pathway, a GPP synthase, a GPP-C2-methyltransferase and one of several

C11 terpene synthases were analyzed for volatile terpenoids. In addition to eleven known compounds, it was possible to detect 24 novel C11-terpenoids which have not yet been described as terpene synthase products. Four of them, 3,4-dimethylcumene, 2-methylborneol and the two diastereomers of 2-methylcitronellol could be identified. Moreover we discovered previously unknown bacterial prenyl diphosphate methyltransferase activities, e.g. IPP-C4-methyltransferases, which further expand the structure space of the terpene biosynthesis pathway.

Our study exemplarily demonstrates the broad range of unusual bacterial terpenoid building blocks and shows possibilities for biotechnological applications.

SMV07

Biosynthetic design of nonribosomal peptides

H. Kries*[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology (HKI Jena), BDN, Jena, Germany*

Nonribosomal peptide synthetases (NRPSs) that protect microorganisms against environmental threats by producing siderophores or antibiotics, for instance, are predisposed to biosynthetic engineering because of their modular molecular structure. We have explored several strategies for the redesign of NRPS specificity [1]. Notable examples are the incorporation of a clickable amino acid through targeted binding pocket mutagenesis [2] or specificity transfer through swapping of small protein fragments [3]. Incorporation of clickable amino acids has further enabled a strategy for high-throughput sorting of mutagenized NRPSs leading to a remarkable switch in substrate specificity from alpha- to beta-phenylalanine [4]. Here, we demonstrate how bottlenecks in engineered NRPSs can be identified with novel assays and repaired by site-directed mutagenesis. Screening for non-ribosomal peptides with antibiotic activity in a directed evolution setup could potentially yield new weapons for the fight against increasing antibiotic resistance.

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SMV08

Cell free production of polyketide building blocks

S. Hoefgen*[1], C. Graf[2], J. E. Kufs[1], J. Rautschek[1], J. Thiele[2], V. Valiante[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Leibniz Research Group Biobricks of Microbial Natural Product Syntheses , Jena, Germany*
[2]*Leibniz Institute of Polymer Research Dresden, Leibniz Research Group - Polymer Micro(bio)reactors, Dresden, Germany*

Due to the more and more increasing impact of infectious diseases and multi-resistant organisms the need of new active compounds is dramatically increasing, too. Polyketides are a group of natural products that are already used as cancer therapeutics and antibiotics. They are produced in many different organisms such as bacteria, fungi and plants. Genome mining approaches showed that filamentous fungi comprise the genes for the production of a high number of polyketide derivatives. Since the majority of these organisms are recalcitrant to cultivation, so far, only few of the naturally available polyketides have been characterized.

Polyketides are synthesized by highly complex multimodular enzymes named as polyketide synthases (PKSs). PKSs use small building blocks to assemble their products. In most fungi acetyl-CoA is used as starter unit which is afterwards extended by adding malonyl-CoA units or its derivatives. In contrast, plant chalcone synthases use coumaroyl-CoA as starter units. The *in vitro* synthesis of polyketides is often limited by the availability of precursors; thus, a protocol for the production of such molecules in high amount is needed.

Here we present the *in vitro* production of a high number of natural and non-natural CoA ligated building blocks using heterologously expressed and purified adenylating enzymes. For activity measurements, we optimized a molybdate-based 96-well assay to determine the amount of PPi that is released through the hydrolysis of ATP to AMP during thioesterification. Using this assay, we characterized CoA-ligating enzymes regarding their optimal temperature, kinetics and substrate promiscuity.

BIV01

Mycelia Enable Bacterial Activity in Dry and Oligotrophic Environments

A. Worrich*[1], H. Stryhanyuk[1], N. Musat[1], S. König[1], T. Banitz[1], F. Centler[1], K. Frank[1], M. Thullner[1], H. Harms[1], H. H. Richnow[1], A. Miltner[1], M. Kästner[1], L. Y. Wick[1]
[1]*UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany*

Fungi and bacteria communicate and interact with each other and form interconnected entities. These interactions contribute to many ecosystem processes in natural and constructed environments. However, their emergence and benefit under environmental stress conditions remains elusive. Here we used a spatially explicit synthetic microbial ecosystem to elucidate whether fungal mycelia reduce bacterial water and nutrient stress in a dry and oligotrophic microhabitat. We found that mycelia enabled dormant bacteria to regain activity despite the absence of water and nutrients in their direct environment. We observed germination and subsequent growth of bacterial spores (*Bacillus subtilis*) in the vicinity of the hyphae of different fungi (*Fusarium oxysporum*, *Lyophyllum* sp. strain Karsten) and the mycelium-like networks formed by the oomycete *Pythium ultimum*. Using a unique approach combining Time of Flight- and nanoscale Secondary Ion Mass Spectrometry (ToF- and nanoSIMS) coupled with stable isotope (^{13}C,^{15}N,^{18}O) labeling, we could demonstrate that spore germination is accompanied by hyphal transfer of water, carbon and nitrogen. This work provides for the first time direct experimental evidence for the stimulation of bacterial activity by mycelial supply of scarce resources in dry and nutrient-free environments. We propose that mycelia may stimulate bacterial activity and thus contribute to the stability of ecosystem functioning in environments exposed to stress.

BIV02

Interkingdom communication in the metaorganism *Aurelia aurita*

N. Weiland-Bräuer*[1], M. A. Fischer[1], N. Pinnow[1], R. A. Schmitz[1]
[1]*Christian-Albrechts-Universität zu Kiel, Institute for General Microbiology, Kiel, Germany*

Multicellular organisms can be regarded as metaorganisms, comprising of a macroscopic host interacting with associated microorganisms. Within this alliance, the host has to ensure attracting beneficial bacteria and defending against pathogens to establish and maintain a healthy homeostasis.

We hypothesize that interference of bacterial cell-cell communication (Quorum sensing, QS) namely Quorum quenching (QQ) is involved in the establishment and maintenance of a specific host microbiota in the moon jellyfish *Aurelia aurita.*

First, we identified that *A. aurita* harbors specific associated microbial communities, which differ the developmental stages. Second, we elucidated the impact of QQ within the metaorganism *A. aurita*. We obtained several lines of evidence arguing that *A. aurita* uses QQ as one host defense mechanism. Three *A. aurita*-derived proteins interfering with bacterial QS were identified. Native expression patterns of these host open reading frames (ORFs) differed in the diverse life stages pointing to a specific role in establishing the developmental stage-specific microbiota. Highly increased expression of all QQ-ORFs in germ-free animals further indicates their impact on the microbiota. Moreover, incubation of native animals with pathogenic bacteria induced expression of the identified QQ-ORFs arguing for a host defense strategy against confronting bacteria. In agreement, immobilized active QQ proteins induced restructuring of polyp-associated microbiota through changing abundance and operational taxonomic unit composition.

We conclude that QQ activities are crucial for establishing the specific and healthy microbiota and represent a host defense strategy besides the innate immune system in *A. aurita*.

BIV03

Multiple bodyguards or a single strong one? Coexistence of defensive symbiont strains in a beetle

L. Flórez*[1], J. Wierz[1], I. J. Miller[2], J. C. Kwan[2], K. Scherlach[3], C. Hertweck[3], M. Kaltenpoth[1]
[1]*Institute for Organismic and Molecular Evolution - Johannes Gutenberg University , Evolutionary Ecology, Mainz, Germany*
[2]*University of Wisconsin, Madison, WI, United States*
[3]*Leibniz Institute for Natural Product Research, Jena, Germany*

Symbiotic associations between microorganisms and animals are widespread and diverse. Until recently, the presence of related yet distinct bacterial symbiont strains within the same host individual was unexpected and often overlooked. However, there is now growing awareness that symbiont strain diversity is not rare, and might have an impact on functional and regulatory aspects of symbiotic partnerships. *Lagria* beetles live in symbiosis with antibiotic-producing *Burkholderia gladioli* bacteria that can protect their eggs from antagonistic fungi. The beetles carry coinfecting symbiont strains in specific organs and can transmit these from mother to offspring as well as acquire them from the environment. As revealed by 16S rRNA gene profiling, the relative abundance of the coexisting strains is consistent across field individuals. Interestingly, the bioactive potential as well as the genome size varies for the different strains as shown by a combination of chemical and (meta)genomic analyses. While a readily culturable strain carries a genome 8.5 Mb in size, that of the most abundant strain is estimated around one fourth of the former, and is smaller than the genome of any other *Burkholderia* strain reported so far. This dominant strain has not been amenable to *in vitro* cultivation. However, it can be transmitted from the insect to different plant species and survive in these alternative hosts. Further efforts are focused on understanding how symbiont strain composition is regulated by either the insect host or the bacteria, and whether the tripartite interaction with a plant plays a role in this process. For the beetles, ensuring a broad-spectrum defense might be the ecological driver of strain coinfections.

BIV04

Gut microbiota protect *Dysdercus fasciatus* firebug against a co-transmitted trypanosomatid parasite infection

T. Onchuru*[1], H. Salem[2], E. Bauer[1], A. Martinez[1], M. Kaltenpoth[1]
[1]*University of Mainz, Evolutionary Ecology, Mainz, Germany*
[2]*Emory University, Department of Biology, Atlanta, GA, United States*

Many animals partner with bacterial symbionts for nutrition, protection, detoxification or behavioral manipulation. The guts of *Dysdercus fasciatus* firebugs (Hemiptera: Pyrrhocoridae) harbor a relatively simple but well conserved bacterial community that supplements the host with B-vitamins. Because of their importance, the essential bacterial mutualists are transmitted vertically and horizontally to the offspring and conspecifics. The guts of these insects are also infested with *Leptomonas pyrrhocoris* (Kinetoplastea: Trypanosomatidae), an intestinal trypanosomatid parasite, that is persistently present in natural firebug populations at high frequencies. Here, through fluorescent *in situ* hybridization, and controlled experimental bioassays, we report that the parasite hitch-hikes on the symbiont transmission routes to infect host populations within and across generations. Thus, the transmission of *D. fasciatus* mutualists indirectly influences host-parasite interactions by increasing the risk of host exposure to the protist parasites. Additionally, by artificially infecting symbiont depleted and symbiont harboring firebug nymphs with cultured *L. pyrrhocoris* parasite and subsequently measuring parasite titres upon emergence, we show that *D. fasciatus* gut microbiota blocks the establishment of the co-transmitted parasite in the host gut. Symbiont depleted bugs possess significantly high parasite titres compared to symbiont harboring bugs, a clear suggestion that in addition to being nutritionally important, *D. fasciatus* gut microbes complement the host immune system in preventing pathogenic invasions. Even though risky, maintenance of stable bacterial communities in animals may be important since these bacteria can influence host interactions with other organisms.

BIV05

Biochemical and molecular insights into use of PGPR *Pseudomonas* and *Bacillus* strains for suppressing plant disease as formulated biofertilizers

M. Zubair*[1,2], S. Yasmin[2], X. Gao[1]
[1]*Nanjing Agricultural University, Plant Protection, Nanjing, China*
[2]*National Institute for Biotechnology and Genetic Engineering, Soil and Environment Biotechnology Division, Faisalabad, Pakistan*

The study was performed to develop biofertilizer using growth promoting biocontrol agents for the suppression of Bacterial leaf blight (BLB) in rice. The research was directed at basic and applied aspects of using beneficial bacteria as formulated product. Rhizospheric samples collected from rice growing areas of Punjab, Pakistan were used for the isolation of bacteria antagonistic to BLB pathogen i.e. *Xanthomonas oryzae* pv. *oryzae* (Xoo). Of 230 different isolates, nine bacterial isolates showed antagonistic activity against Xoo. *Pseudomonas* sp. ZA86 showed the maximum

inhibition of Xoo pathogen in plate assays. 16S rRNA gene sequencing and phylogenetic analysis identified these nine isolates as *Citrobacter* sp. ZA21, *Bacillus* spp. strains ZA33, ZA57, ZA17 and *Pseudomonas* spp. strains ZA20, ZA22, ZA62, ZA85 and ZA86. All the tested bacteria possessed P-solubilization and IAA production abilities. Maximum seedling vigor index observed for *Citrobacter* sp. ZA21 and *Pseudomonas* sp. ZA86 may be attributed to IAA production. Strains ZA21 and ZA86 showed effective pathogen suppression of Xoo during *In-Planta* experiment with an increase in the activity of defense related enzymes in rice plants. These bacterial strains also increased the root/ shoot length and plant weight. Root colonization by the antagonistic bacteria was observed with molecular marker such as BOX and FISH by CLSM. ZA86 capable of producing several important secondary as observed by LCMS, was used for formulation development and shelf life estimation; CMC and glycerol were tested for the development of liquid formulation. We can conclude that CMC based biofertilizer appeared best option with regard to suppression of BLB disease.

BIV06

The different morphologies of yeast and filamentous fungi trigger distinct killing and feeding mechanisms in a fungivorous amoeba

S. Radosa[1], I. Ferling[1], J. Sprague[1], F. Hillmann*[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute (HKI), Evolution of Microbial Interactions, Jena, Germany*

Size and diverse morphologies pose a primary challenge for phagocytes such as innate immune cells and predatory amoebae when encountering fungal prey. While filamentous fungi escape phagocytic killing by pure physical constraints, unicellular spores and yeasts can mask molecular surface patterns or arrest phagocytic processing. We have recently isolated the amoeba *Protostelium aurantium* from a decaying leave surface. Here we show that this amoeba acts as a fungivorous predator on a wide range of environmental and pathogenic fungi and was able to adjust its killing and feeding mechanisms to thedifferent fungal morphologies. Yeast-like fungi from the major fungal groups of basidiomycetes and ascomycetes were readily internalized by phagocytosis, except for the human pathogen *Candida albicans* whose mannoprotein coat was essential to escape recognition by the amoeba. Dormant spores of the filamentous fungus *Aspergillus fumigatus* also remained unrecognized, but swelling and the onset of germination induced internalization and intracellular killing by the amoeba. Mature hyphae of *A. fumigatus* were mostly attacked from the hyphal tip and killed by an actin-mediated invasion of fungal filaments. Our results demonstrate that predatory pressure imposed by amoebae in natural environments selects for distinct survival strategies in yeast and filamentous fungi but commonly targets the fungal cell wall as a crucial prey- and pathogen associated molecular pattern.

BIV07

Bacteriolytic properties of a predatory soil bacterium

K. Arend[1], T. Bentler[1], C. Lüchtefeld[1], C. Kaimer*[1]
[1]*Ruhr-Universität Bochum, Lehrstuhl Biologie der Mikroorganismen, Bochum, Germany*

Myxobacteria, such as *Myxococcus xanthus*, are δ-proteobacteria, which are ubiquitous in soil and display elaborate multicellular behaviors: They form large clusters, move slowly, and secrete enzymes at high concentrations to efficiently access nutrients. In addition to their saprophytic lifestyle, myxobacteria are predators that specifically kill bacteria of other species to use their biomass as energy source. It has been established that predation by myxobacteria is a complex, multi-component process that involves hydrolytic enzymes and secondary metabolites, but the molecular mechanisms of specific prey recognition and killing, as well as the identity of most lytic factors remain unknown.

In order to understand the predation behavior of *M. xanthus* on a molecular level, we aim to identify lytic factors involved in the disintegration of bacterial prey cells, and to study their functional mechanism and regulation. We compared the bacteriolytic properties of membrane, secreted, and vesicle-associated protein fractions of *M. xanthus* against live bacteria of different species. Zymography with different bacterial substrates was used to further resolve proteins and their bacteriolytic activity *in vitro*. Finally, mass spectrometry analysis was performed to identify active proteins.

We observed varying efficacy of different protein fractions against Gram positive and Gram negative prey bacteria. Moreover, zymography revealed differing lytic activity of individual proteins, suggesting that prey specificity is, at least partly, determined by the identity of lytic factors and by their delivery mode. MS-based identification of active proteins provided several putative bacteriolytic enzymes, one of which, as a proof of concept, was further characterized *in vivo* and *in vitro*.

BIV08

Biological role and host resources enable a minimal fermentative metabolism in a pectinolytic symbiont

E. Bauer*[1], M. Kaltenpoth[1], H. Salem[2,3]
[1]*Johannes Gutenberg Universität Mainz, Institut für Organismische und Molekulare Evolutionsbiologie , Mainz, Germany*
[2]*Emory University, Department of Biology, Atlanta, GA, United States*
[3]*Wissenschaftskolleg zu Berlin, College for Life Sciences, Berlin, Germany*

Reductive genome evolution in symbiotic microbes leads to organisms with minimal functional traits and high host dependency. Most genome-reduced symbionts live within host cells, enabling extensive metabolic interactions, such as the exchange of nutrients. While genome reduction is recognized for intracellular symbionts, extracellular symbionts are less well studied. Tortoise leaf beetles harbor an extracellular symbiont, "Candidatus Stammera capleta", in specialized organs, which secretes pectinases aiding the host"s breakdown of plant polysaccharides. Despite its consistent extracellular localization, this symbiont possesses an extremely reduced genome, providing the opportunity to study reductive evolution in an extracellular environment. In this study, we investigated genomic and transcriptomic data of Stammera and its host to shed light on host-symbiont metabolic integration. Given the symbiont"s demands for various nutrients, the host"s symbiotic organ shows a re-purposing of internal resources such as cuticle and trehalose to support symbiont growth, as well as a specific immune response. In the symbiont, highly consistent gene expression profiles highlight the tight regulation and metabolic streamlining to produce high amounts of pectinases for the host. Facilitated by its extracellular localization and nutritional supplementation by the host, Stammera evolved a minimal lactate fermentation pathway for energy conversion, which contrasts with the respiratory metabolism of intracellular symbionts. Our results thus provide insights into a tightly regulated and metabolically integrated symbiosis enabled by

Stammera"s pectinolytic function and extracellular host environment, expanding our understanding of the minimal metabolism required to sustain extracellular life.

BCV01
Diversity, Structure, Function, Assembly and Engineering of Bacterial Microcompartments
C. Kerfeld*[1]
[1]*MSU-LBNL, East Lansing, MI, United States*

Bacterial microcompartments (BMCs), are widespread among Bacteria; they are multienzyme-containing proteinaceous organelles bounded by a selectively permeable protein shell [1]. One example, the carboxysome is a self-assembling metabolic module for CO2 fixation found in all cyanobacteria. These large (~100-500 nm) polyhedral bodies sequester Carbonic Anhydrase and RuBisCO within a protein shell, thereby concentrating substrates and protecting RuBisCO from oxygen generated by the light reactions. Because carboxysomes and other BMCs function to organize reactions that require special conditions for optimization, including the sequestration of substrates, cofactors, or toxic intermediates and the protection of oxygen sensitive enzymes, they have received considerable attention as templates for synthetic nanoreactors in bioengineering and as metabolic modules for programming synthetic microbial consortia. There are two central challenges to building bespoke protein-based nanoreactors, design and assembly of multi-enzyme cores and engineering of the shell proteins to serve as a selectively permeable barrier, the interface between the cytosol and the encapsulated reactions.

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BCV02
Biogenesis of a prokaryotic organelle: A nightmare before xMas
R. Uebe*[1]
[1]*Universität Bayreuth, Bayreuth, Germany*

Unlike most prokaryotes, magnetotactic bacteria (MTB) are able to form membranous organelles, called magnetosomes. These unique organelles consist of membrane-enclosed magnetic iron nanoparticles and are arranged in chain-like structures by several cytoskeletal structures to enable magnetic navigation. Magnetosome biogenesis in the model MTB *Magnetospirillum gryphiswaldense* has been shown to depend on >30 specific magnetosome proteins. However, the processes and mechanisms underlying magnetosome formation are only poorly understood and it remained largely unknown how proteins are specifically targeted to magnetosomes.

Using phenotypic analyses, magnetosome proteome profiling, protein interaction, and fluorescence microscopic analyses we identified three proteins involved in the late stages of magnetosome membrane biogenesis. These so-called xMas proteins assist magnetosome assembly by targeting several magnetosome proteins involved in regulation of iron nanoparticle growth or magnetosome chain formation to the magnetosome membrane. Thus, in contrast to previous assumptions, the xMas proteins regulate iron biomineralization indirectly and are essential to coordinate iron biomineralization with magnetosome chain formation. Furthermore, the remote relationship of the xMas proteins with chloroplast assembly proteins suggests a novel mechanism of organelle biogenesis in prokaryotes.

BCV03
Living in the shade - How cyanobacteria use far-red light
D. Nürnberg*[1,2], L. Antonaru[2], J. Morton[3], S. Santabarbara[4], E. Krausz[3], A. Boussac[5], A. Fantuzzi[2], A. W. Rutherford[2]
[1]*FU Berlin, Experimental Physics, Berlin, Germany*
[2]*Imperial College London, Life Sciences, London, United Kingdom*
[3]*Australian National University, Chemistry, Canberra, Australia*
[4]*CNR, Biofisica, Milano, Italy*
[5]*CEA Saclay, Biologie Intégrative de la Cellule, Gif-sur-Yvette, France*

Oxygenic photosynthesis uses chlorophyll *a* to convert solar energy into the chemical energy that drives the biosphere. Chlorophyll *a* absorbs visible light only up to ~700 nm, the so-called "red limit". When deeply shaded by other photosynthetic species, some cyanobacteria are able to extend this limit by using modified chlorophylls. Chlorophyll *f*, is the longest wavelength and most recently discovered chlorophyll but it is assumed to play a purely light harvesting role. When the cyanobacterium *Chroococcidiopsis thermalis* is grown in far-red light it forms around 10% chlorophyll *f* in addition to chlorophyll *a*. Here, we investigated the effect of far-red light on the remodelling of membranes, photosystems and the phycobilisome complexes using various spectroscopic, microscopical and biophysical methods. We show the presence of (i) a highly efficient photosynthetic system with chlorophyll *f* as the primary electron donor in both photosystems, PSI and PSII, (ii) densely packed thylakoid membranes with phycobilisome complexes strongly reduced in quantity and size, (iii) highly oligomerised photosynthetic complexes of PSI, and (iv) the formation of bioenergetic membrane domains. The findings provide important new insights into the mechanism of light acclimation and the limits of oxygenic photosynthesis.

BCV04
Awakening of *Synechocystis* sp. PCC 6803 from nitrogen starvation requires rapid glycogen mobilization and ATP production
S. Doello*[1], K. Forchhammer[1,2]
[1]*University of Tübingen, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin Tübingen, Tübingen, Germany*
[2]*University of Tübingen, Tübingen, Germany*

Nitrogen limitation is one of the most common hurdles cyanobacteria face in nature. The non-nitrogen fixing cyanobacterium *Synechocystis* sp. PCC 6803 has developed a metabolic strategy to survive periods of nitrogen starvation by entering a state of dormancy. During the awakening from nitrogen starvation, *Synechocystis* passes through a heterotrophic and mixotrophic phase before re-entering photoautotrophic growth. Here, we investigated how resuscitation is initiated. Addition of nitrate induces an immediate switch from a maintenance metabolism, characterized by residual photosynthesis and low ATP levels, to an initial heterotrophic phase, characterized by respiration and a rapid ATP increase. This raise in ATP levels seems to depend on the activity of the nitrate reductase, since cells are unable to rapidly produce ATP when other nitrogen sources, such as nitrite or ammonium, are used to start resuscitation. Respiration relies on degradation of the glycogen that cells have accumulated during the preceding starvation phase. Glycogen catabolism requires the glycogen phosphorylase GlgP2 and the parallel

operation of the Entner-Doudoroff and the oxidative pentose phosphate pathways. Interestingly, the key enzymes for glycogen catabolism are already expressed during nitrogen starvation, rendering cells ready for rapid resuscitation. These enzymes are present in their active form, but glycogen degradation only starts after the addition of nitrate. Initiation of glycogen degradation seems to depend on dephosphorylation, and activation, of the phosphoglucomutase. Overall, our results show that resuscitation requires a rapid response that immediately allows glycogen degradation and energy production.

BCV05

A new cell cycle regulator in *Hyphomonas neptunium*

C. Reif*[1], O. Leicht[1], M. Thanbichler[1,2]
[1]*Philipps University Marburg, Faculty of Biology, Marburg, Germany*
[2]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

C. crescentus, a model organism for bacterial cell cycle studies, divides into two cell types with distinct morphologies and physiology. The DNA-binding response regulator CtrA was identified as a master regulator driving this differentiation process. CtrA is regulated by the CckA-ChpT phosphorelay, and in turn controlled by another phosphorelay, involving the histidine kinase DivJ, the phosphatase PleC, and the single-domain response regulator DivK. The complex signaling network associated with CtrA is highly conserved in α-proteobacteria. A close relative of *C. crescentus*, *Hyphomonas neptunium* is a newly introduced model organism that divides by the formation of buds at the end of its stalk. Here, we investigate the role of CtrA in the regulation of processes such as DNA segregation, morphogenesis and cell division. Moreover, we dissect the mechanisms controlling CtrA activity and present a potential new component of the CtrA-regulatory phosphorelay in this species.

BCV06

Exception to the Rule: Naturally Occurring *Vibrio cholerae* Strains with a Single Chromosome

M. Bruhn[1], D. Schindler[1], F. Kemter[1], M. Wiley[1], K. Chase[1], G. Koroleva[1], G. Palacios[1], S. Sozhamannan[1], T. Waldminghaus*[1]
[1]*Philipps-Universität, SYNMIKRO, Marburg, Germany*

Chromosomal inheritance in bacteria usually entails bidirectional replication of a single chromosome from a single origin into two copies and subsequent partitioning of one copy each into daughter cells upon cell division. However, the human pathogen *Vibrio cholerae* and other *Vibrionaceae* harbor two chromosomes, a large Chr1 and a small Chr2. Chr1 and Chr2 have different origins, an *oriC*-type origin and a P1 plasmid-type origin respectively, driving the replication of respective chromosomes. Recently, we described naturally occurring exceptions to the two-chromosome rule of *Vibrionaceae*: i.e., Chr1 and Chr2 fused single chromosome *V. cholerae* strains, NSCV1 and NSCV2, in which both origins of replication are present. Using NSCV1 and NSCV2, here we tested whether two types of origins of replication can function simultaneously on the same chromosome or one or the other origin is silenced. We found that in NSCV1, both origins are active whereas in NSCV2 *ori2* is silenced despite the fact that it is functional in an isolated context. The *ori2* activity appears to be primarily determined by the copy number of the triggering site, *crtS,* which in turn is determined by its location with respect to *ori1* and *ori2* on the fused chromosome.

BCV07

Formation of Acidocalcisomes (ACs) and Polyphosphate (PolyP) in *Agrobacterium tumefaciens*

C. Frank*[1], D. Jendrossek[1]
[1]*Institute for Microbiology, University of Stuttgart, Stuttgart, Germany*

Acidocalcisomes (ACs) are well-characterized membrane-enclosed acidic organelles in lower eukaryotes [1]. They contain large amounts of inorganic polyphosphate (polyP) together with cations such as calcium or magnesium [2]. Only two prokaryotic species, *Rhodospirillum rubrum* and *Agrobacterium tumefaciens*, have been described so far, that can also form ACs [3, 4]. The most common bacteria species produce polyphosphate (volutin) granules, but these structures are not surrounded by a membrane and therefore represent a more simple form of inclusions. In this study, we determined the *in vivo* localization of fused fluorescent proteins with an AC-specific protein (proton translocating pyrophosphatase, [HppA]) and with polyP granule-associated proteins (polyphosphate kinases [PPK]) respectively. We also analyzed the effect of *hppA* and/or *ppk* gene deletion in *A. tumefaciens*. The finding that polyP granules did not colocalize with the HppA protein and/or with MitoTracker/LysoTracker stained ACs, suggests that ACs and polyP granules are different subcellular structures. Additionally, we observed that one of the two PPKs present in *A. tumefaciens* (PPK2), unlike PPK1, is associated with polyP granules which actively move during cell cycle.

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BCV08

DNA topology in *Synechocystis sp. PCC6803*

M. Dietsch*[1]
[1]*Heinrich Heine Universität, Synthetic Microbiology, Düsseldorf, Germany*

The topology of the cyanobacterial nucleoid is highly regulated by various levels of negative supercoiling. Depending on external stimuli such as variations in temperature, osmolarity, pH or carbon source, but also the intracellular energy level, cells can quickly adapt and globally modify gene regulation by changing the levels of DNA superhelicity. In turn, supercoiling-sensitive promoters can dynamically be activated or repressed depending on the current energy state of the cell. The global level of DNA-supercoiling is mainly modulated by ATP dependent DNA-gyrase (consisting of the two subunits gyrA and gyrB), which actively introduces negative supercoils, and topoisomerase I (topA), which relaxes the DNA by removing supercoils. In this work, we show the effect of different levels of supercoiling by altering the intracellular levels of DNA gyrase and topoisomerase I by overexpression and CRISPRi-mediated repression of gene expression. Through this regulation, a growth stop of the cells is initiated and the putative released energy should result in a higher production of heterologous proteins.

EMV09

Activity of the nodule specific NCR247 peptide expressed during the *Sinorhizobium-Medicago* legume symbiosis is dependent on its enantiomeric form

M. Arnold*[1], S. Sankari[1], G. Walker[1]
[1]*Massachusetts Institute of Technology, Biology, Cambridge, MA, United States*

Bacterial pathogens and symbionts must respond to host signals in order to establish a successful infection in their eukaryotic hosts. During the nitrogen fixing symbiosis between *Sinorhizobium meliloti* and *Medicago* plant species *S. meliloti* is exposed to a cocktail of nodule-specific cysteine-rich (NCR) peptides after being endocytosed into plant root cells. These NCR peptides have resemblance to defensins and trigger the essential morphological and physiological differentiation of intracellular bacteria into bacteroids which are then able to fix atmospheric nitrogen and supply it to the plant. Interestingly, in addition to the signaling capabilities of NCR peptides, a number of them have been shown to possess antimicrobial activity at higher doses, including NCR247. The NCR247 peptide has been extensively studied and several of its physiological effects on *S. meliloti* are known.

Our main goal is to identify the molecular targets for NCR peptides and how NCR peptides mediate their essential effects on *S. meliloti*. To assess whether the cationic NCR247 peptide elicits its effects via non-specific, charge mediated mechanisms or via structure specific molecular interactions, we obtained an all D-enantiomer of NCR247. We tested the effects of the D-NCR247 peptide on *S. meliloti* by comparing its activity to the effects of the well characterized L-NCR247 peptide.

We found that the D-NCR247 enantiomer does not show significantly altered antimicrobial activity, compared to the L-enantiomer. However, D-NCR247 does not elicit the same physiological effects on *S. meliloti* as the L-NCR247 peptide.

Our findings indicate that NCR peptides have cellular targets that require structure specific interactions to elicit defined responses that are essential for the legume symbiosis.

EMV10

Colonization dynamics of *Pantoea sp.* on germ free wheat root

R. Soluch*[1], N. Hülter[1], T. Dagan[1]
[1]*Christian-Albrechts-Universität zu Kiel, Institut fur Allgemeine Mikrobiologie, Kiel, Germany*

Microbial communities inhabiting the rhizosphere and phyllosphere are ubiquitous in all plants and are often of vital importance to their host. The acquisition of endophytic bacteria residing in the interior of roots and above-ground tissues includes vertical transmission from parent plants to seedlings via seeds. In the cereal crop species wheat (*Triticum aevistivum*), a vertical transmission route seems to exist for a limited set of endophytic bacterial species. However, the dynamics of host colonization upon germination are unknown and it is not clear whether endophytic host colonization is preceded by a colonization stage on the root outer surface. In this study we investigated colonization of young wheat roots by a native endophytic bacterium. For this we isolated the enterobacterial species *Pantoea sp.* from surface sterilized seeds of German and Turkish wheat cultivars. Furthermore, we developed a protocol for the creation of germ-free wheat plants to study the colonization dynamics under controlled conditions. Inoculating roots of young seedlings with only few cells of *Pantoea sp*. enables us to document the initial stages of root colonization. Our results reveal that *Pantoea* readily settles on root tissues within 5 hours of coincubation. The colonization dynamics are characterized by logarithmic increase of the population size that is limited by the root habitat size. The population sizes per gram of fresh root material remained stable over time, indicating that growth of *Pantoea* is limited only by the root carrying capacity. Together with the genetic tractability of *Panteoa*, our results provide a framework to study the ecological principles and genetic factors that govern the colonization of wheat in a native endophyte.

EMV11

Potential of actinobacteria to enhance tolerance of tomato (*Lycopersicon esculentum*) to drought stress through production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase

K. El-Tarabily*[1], A. Al Khajeh[1], M. Al Darmaki[1], F. Khoory[1], M. Alblooshi[1], A. Albadwawi[1], A. Al Zahmi[1]
[1]*UAE University, Department of Biology, College of Science, Al-Ain, United Arab Emirates*

The objective of this work was to evaluate whether actinobacteria isolated from desert soils in the United Arab Emirates (UAE) can increase tolerance in tomato plants (*Lycopersicon esculentum*) to drought stress through the reduction in the endogenous levels of the stress hormone ethylene. Thirteen isolates of streptomycete (SA) and non-streptomycete actinobacteria (NSA) that showed

1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity were obtained from a total of 45 isolates. DNA analysis of the 16S RNA indicated that the most promising isolate selected produced the highest levels of ACC deaminase *in vitro* and was identified as *Micromonospora chalcea*. This wild type isolate (WT) significantly increased the fresh and dry weights, and root and shoot lengths of tomato plants grown under drought condition, both under gnotobiotic and greenhouse conditions. The application of the WT strain of *M. chalcea* significantly, reduced the endogenous levels of ACC, the immediate precursor of the hormone ethylene, in the roots and shoots compared with the non-inoculated control treatment. In comparison, an ACC deaminase non-producing mutant strain (MT) and *Streptomyces atrovirens* which acted as negative controls failed to reduce the endogenous levels of ACC in the roots and shoots and failed to promote plant growth under drought conditions both under gnotobiotic and greenhouse conditions. This is the first report of the production of ACC deaminase by NSA. This study is also the first published report to demonstrate the potential of actinobacteria to ameliorate the deleterious effects of high drought stress on plant growth through promotion of plant growth.

EMV12

Metagenomics reveal how rainforest conversion affects specific groups of microbial communities and microbiome functionality in soils

D. Berkelmann *[1], D. Schneider[1], A. Meryandini[2], R. Daniel[1]
[1]*Georg August University of Göttingen , Göttingen, Germany*
[2]*IPB, Bogor Agricultural University, Bogor, Indonesia*

Palm oil production in Indonesia increased substantially over the last decades which led to massive deforestation, especially on Sumatra Island. The ongoing conversion of rainforest to agricultural systems results in high biodiversity loss, while the entirety of effects on the biosphere is still not

completely understood. Prokaryotic communities and their functionality, however, are crucial factors for soil environments due to their involvement in almost all nutrient cycling pathways. Our recent studies on rainforest conversion showed that bacterial communities are indeed affected. We could demonstrate that rainforest transformation has different effects on abundances of specific taxonomic groups, leading to a decrease for example for *Rhizobiales* and *Rhodospirillales* and increasing abundances for *Frankiales* and Subgroup 2 of the *Acidobacteria*. Initial studies were based on 16S rRNA gene sequence analysis and gave an overview of the total and active bacterial community composition, while functionality was predicted to generate hypotheses. To investigate metabolisms which are associated with the previously observed and affected taxa (e.g nitrogen metabolism, methane metabolism) we employed metagenomic shotgun sequencing. Therefore, 32 metagenomes were generated from secondary rainforest, jungle rubber, rubber plantation and oil palm plantation soils. Additionally, analyses of taxonomic composition, functional potential and specific metabolisms were correlated with 16S rRNA gene and transcript data.

EMV13

Scavenging atmospheric gases for energy and the ecological implications in soil acidobacteria

S. A. Eichorst*[1], D. Trojan[1], A. Giguere[1], E. G. Robledo[2,3], S. Roux[4], C. Herbold[1], A. Richter[1], N. P. Revsbech[3], T. Rattei[1], D. Woebken[1]
[1]University of Vienna, Department of Microbiology and Ecosystem Science, Vienna, Austria
[2]Universidad de Cádiz, Departamento de Biologia, Facultad de Ciencias del Mar y Ambientales, Puerto Real, Spain
[3]Aarhus University, Microbiology, Department of Bioscience, Aarhus, Denmark
[4]Joint Genome Institute, Department of Energy, Walnut Creek, CA, United States

Terrestrial ecosystems represent habitats with unpredictable conditions, confronting soil microorganisms with suboptimal conditions for growth. Remarkably, certain microbial groups such as the *Acidobacteria* are abundant and ubiquitous across many soils, yet the reasons for their persistence remain elusive. To elucidate features that could explain their success in soil, a large-scale comparative genome analysis was performed on a diverse collection of acidobacteria, followed by experimental analysis to test suggested physiologies. In addition to a diverse repertoire for both C and N utilization, many strains harbored the genetic potential to obtain energy from atmospheric gases. Multiple soil acidobacteria harbored the potential to scavenge atmospheric H_2 concentrations due to a high-affinity hydrogenase, a proposed mechanism to create sufficient proton motive force for ATP generation under nutrient-limiting conditions. This capability was supported in growth experiments, in which H_2 was consumed in stationary phase at and below atmospheric levels in tandem with hydrogenase gene expression. Limited access to oxygen is another potential stress factor for soil microorganisms, as oxygen is often depleted e.g. in larger soil aggregates. Low- and high-affinity respiratory oxygen reductases were detected in soil acidobacterial genomes, suggesting the capacity for growing across different oxygen gradients. This physiology was further supported by respiratory kinetic experiments. This large-scale genome analysis coupled to growth-based experiments revealed traits that provide soil acidobacteria physiological and metabolic versatility, presumably allowing flexibility and thus can explain their success in soil.

EMV14

Dynamics of bacterial mineral colonization in natural grasslands

S. Vieira*[1], J. Sikorski[1], R. Boeddinghaus[2], S. Marhan[2], E. Kandeler[2], J. Overmann[1]
[1]Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Microbial Ecology and Diversity, Braunschweig, Germany
[2]University of Hohenheim, Soil Biology, Stuttgart, Germany

Bacteria in soils are frequently attached to surfaces of minerals, which are available as fresh inputs due to the process of mineral weathering. So far, the patterns and dynamics of bacterial colonization of soil minerals have not been assessed in natural settings. High throughput Illumina sequencing was employed to investigate the colonization of artificial minerals that were exposed to natural grassland soils. We studied the impact of different types of organic carbon sources (simple carbon compounds versus plant detritus) on the development of distinct communities and evaluated the temporal dynamics of bacterial colonization of soil minerals. The observed consistent temporal patterns suggest that the properties of *de novo* inserted minerals in soils drive the establishment of bacterial communities and override the effect of the type of carbon sources. Particular bacterial species exhibited distinct colonization patterns, including early, intermittent, and late colonizers. These colonization patterns do not represent phylogenetically coherent properties, however. This study highlights the impact of mineral composition on soil bacterial communities and reveals their temporal progression during soil colonization, which are important for a better understanding of bacterial community dynamics, stability and resilience during soil formation in natural ecosystems.

EMV15

Only the fit can access the Goldilocks zone

R. Jerdan[1], A. Kusmierska[1,2], N. White[1], A. Spiers*[1]
[1]Abertay University, School of Science, Engineering and Technology, Dundee, United Kingdom
[2]University of Łódz´, Department of Industrial Microbiology and Biotechnology, Łoʹdzʹ, Poland

Adaptive radiation can be investigated by experimental evolution studies using in simple microcosms where abiotic and biotic factors are readily manipulated to alter selective pressures. One such system uses populations of *Pseudomonas fluorescens* SBW25 incubated statically in vials where O_2 availability is the growth-limiting resource. In these, early colonists establish an O_2 gradient dividing the liquid column into a shallow high-O_2 region (the Goldilocks zone) above a deeper low-O_2 region. Subsequently the high-O_2 region is colonised by Wrinkly Spreaders (WS), a class of adaptive mutants able to produce a biofilm at the air-liquid (A-L) interface with a competitive fitness advantage over the non-biofilm–forming ancestor. Although the evolutionary ecology of the WS is well-understood, we have recently questioned why biofilm-formation is used to access the A-L interface when aerotaxis should be sufficient to maintain cells in the O_2-rich region. Using wild-type SBW25 and the non-motile flagella mutant FleQ, we show that cells can overcome Brownian diffusion and swim from any position in the liquid column to the high-O_2 region, but they cannot transfer from this into the A-L interface (a molecular layer at the surface which is difficult to penetrate) where they could remain in place without further energy expenditure. However, the WS mutant can achieve this as demonstrated by liquid surface tension measurements, presumably because of cell-surface alterations giving cells a more amphiphilicnature. Our results demonstrate that the key adaptive change in the WS are these cell-surface alterations allowing the initial

penetration of the A-L interface, rather than the subsequent development of the biofilm across the surface of the liquid column.

EMV16

Gnotobiotic and whole genome sequence studies reveal that *Burkholderia* sp. strain Nafp2/4-1b is a potential candidate for commercial inoculant in sustainable crop production in South Africa

A. I. Hassen*[1], Z. H. Swanevelder[2], L. S. Khambani[1]
[1]*Agricultural Research Council, Plant Health and Protection, Pretoria, South Africa*
[2]*Agricultural Research Council, Biotechnology Platform, Pretoria, South Africa*

Burkholderia strain Nafp2/4-1b, a plant growth promoting rhizobacteria (PGPR), was initially isolated from the rhizosphere of pristine grassland in South Africa. A gnotobiotic screening experiment was conducted under glasshouse condition to evaluate its potential in enhancing the growth of maize (*Zea mays* L.) and in improving the nodulation and the growth of lucern (*Medicago sativa* L.) when co-inoculated with the symbiotic *Rhizobium* strain RF14. Based on positive PGPR results from the gnotobiotic tests, a draft genome of this strain was sequenced using an Illumina HiSeq 2500 to confirm and to better understand its PGPR properties for the possible use as a biofertilizer inoculant in crop production. The draft genome sequence resulted in 92 scaffolds, with a N50 of 322,407 bp and a maximum scaffold size of 1 302 675 bp with a total draft genome size of 7, 788, 045 bp with a GC content of 66.2 %. This whole genome sequence of *Burkholderia* sp Nafp2/41b revealed the presence of essential genes that code for a number of PGPR traits including the genes for **-** aminocyclopropane -1-carboxylate (ACC) deaminase); iron binding pyoverdines siderophores biosynthesis Tryptophan synthase beta and alpha chains involved in auxin biosynthesis Ferric (Fe+3) hydroxamate siderophore – iron transporter (*FhuB* gene) (contig 28) and ATP binding cluster (ABC) transporters that mediate the uptake of iron and siderophores and phytochelatin, heavy metal complexing peptides. The study generated a very vital information in devising new strategies to screen for beneficial PGPR isolates for use in the development of microbial inoculants for use in agriculture in South Africa.

BTV09

Generation of nanomagnetic hybrid materials by genetic engineering of bacterial magnetosomes from *Magnetospirillum gryphiswaldense*

F. Mickoleit*[1], D. Schüler[1]
[1]*Universität Bayreuth, Lehrstuhl Mikrobiologie, Bayreuth, Germany*

The formation of bacterial magnetosomes is an intriguing example for a biomineralization process. They are synthesized by magnetotactic bacteria such as the alphaproteobacterium *Magnetospirillum gryphiswaldense* and consist of a monocrystalline magnetite core enveloped by the magnetosome membrane. Within the bacteria, these organelles are arranged in a chain-like manner and serve as a geomagnetic field sensor [1]. Because of their unprecedented properties (high crystallinity, strong magnetization, uniform shape and size), magnetosomes have the potential to yield biomaterials useful in numerous biomedical and biotechnological applications.

For the generation of magnetic organic-inorganic hybrid materials, genetic techniques were developed to engineer both the crystal morphology and the enveloping membrane. Abundant magnetosome membrane proteins were used as anchors for high-level expression of foreign peptides and enzymes as large hybrid proteins. This allowed the generation of multifunctional nanoparticles with maximized protein-to-particle ratios [2]. Furthermore, the surface properties (charge, hydrodynamic diameter) could be tuned, and the colloidal and enzymatic stability was improved by coating with inorganic and organic shells. In addition, the expression of nanobodies provides promising strategies for the generation of new biomaterial assemblies (e.g. by combining magnetosomes and tobacco mosaic virus particles [3]) that could be used as scaffolds for the introduction of further functionalities.

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BTV10

Identification of process parameters shaping microbial communities in biogas plants using large scale metaproteome analysis

R. Heyer*[1], K. Schallert[1], S. Püttker[1], F. Kohrs[1], P. Hellwig[1], S. Theuerl[2], A. Schlüter[3], U. Reichl[1,4], D. Benndorf[1,4]
[1]*Otto-von-Guericke Universität Magdeburg, Lehrstuhl Bioprozesstechnik, Magdeburg, Germany*
[2]*Leibniz-Institute for Agricultural, Engineering Potsdam-Bornim e.V., Potsdam, Germany*
[3]*University Bielefeld, Center for Biotechnology, Genome Research of Industrial Microorganisms, Bielefeld, Germany*
[4]*Max Planck Institute for Dynamics of Complex Technical Systems, Bioprocess Engineering, Magdeburg, Germany*

Currently, more than 9.000 biogas plants produce about 5% of the renewable energy in Germany. Within a biogas plant complex microbial communities convert biomass to methane and carbon dioxide. Biogas yield and productivity depend mainly on the metabolic abilities and performances of the single microorganisms. High resolution metaproteome analysis of 95 biogas plants was carried out to correlate process parameters with the taxonomic and functional composition of the microbial communities and to understand the mechanisms which shape the microbial communities. An improved workflow for metaproteome analysis was applied. It comprises cell lysis and phenol extraction in a ball mill followed by FASP digestion, LC-MS/MS measurements using a timsTOF Pro system, and bioinformatic data evaluation with the latest version of the MetaProteomeAnalyzer software. Clustering and principal component analysis based on the identified metaproteins, taxonomies and biological processes revealed several clusters that could be linked with process temperature, substrate feed, and biogas plant design. For all biogas plants two main types of microbial communities were observed. The first comprised communities which performed acetoclastic and hydrogenotrophic methanogenesis simultaneously. In contrast, the second type, showed strictly hydrogenotrophic behaviour but the presence of large amounts of proteins of the bacterial C1-metabolism implicated syntrophic acetate oxidation. Furthermore, it could be shown that competition and host-phage interactions affected the taxonomic and functional composition of the microbial communities. The improved understanding of the biogas process helps to develop new concepts for biogas plant design, control and operation.

BTV11

Influence of flue gas and increased CO_2-concentrations on terrestrial cyanobacteria

D. Strieth*[1], J. Stiefelmaier[1], K. Muffler[2], R. Ulber[1]
[1]*TU Kaiserslautern, Bioprocess Engineering, Kaiserslautern, Germany*
[2]*University of Applied Sceinces, Bioprocess Engineering, Bingen, Germany*

The CO_2 content of the atmosphere increased in the past 70 years from 310 to 390 ppm caused by combustion of fossil fuels. Accordingly, in industry a reduction of CO_2 emissions is essential. Terrestrial cyanobacteria are living embedded in extracellular polymeric substances (EPS) as biofilm. Due to their ability of CO_2 fixation they are good candidates for an extension of CO_2 circle. CO_2 could be bound and simultaneously dietary supplements and biocompatible products could be produced. For cultivation an emerse photobioreactor (ePBR) was developed were the medium is given as aerosol. This reactor design imitates the natural habitat of terrestrial cyanobacteria what leads to higher growth rates in comparison to submerged cultivations.

This study focuses on the surface associated cultivation of the terrestrial cyanobacterium *Trichocoleus sociatus* in the ePBR using different CO_2-concentrations (390 Vol-ppm, 2, 5 and 10 Vol-% CO_2) and a synthetic flue gas. Biomass, EPS and pigment productivity was determined and changes in EPS as well as in pigment composition were investigated. A growth inhibition was observed above 2 vol-% CO_2 and the increase of the CO_2-concentration operated anti-proportional to the BM-productivity. No EPS- and pigment-synthesis by concentrations above 2 vol-% CO_2 and with flue gas were obtained.

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BTV13

Carbon-efficient feedstock conversion for the production of bio-succinate and further reduced compounds

P. Weyrauch*[1], A. Arnold[1], R. Carr[1], M. Roger[2], F. Sargent[2], D. Smith[3], F. Brown[1]
[1]*Ingenza Ltd, Roslin, Midlothian, United Kingdom*
[2]*Newcastle University, School of Natural & Environmental Sciences, Newcastle upon Tyne, United Kingdom*
[3]*Drochaid Research Services Ltd, St Andrews, United Kingdom*

Succinate is widely recognised as a platform chemical which has already received attention within the context of industrial biotechnology as a model for sustainable manufacturing. However, the yield from traditional sugar-based bioprocesses for succinate production is constrained because a significant amount of carbon present in the bioprocess feedstock is lost as gaseous CO_2. In order to maximise carbon use efficiency, additional reducing equivalents are required which can be supplied as a co-substrate (e.g. formate) alongside glucose.

Alternatively, formate can be generated intracellularly from CO_2 and H_2 gas via the reverse reaction of the formate:hydrogen lyase (Fhl) complex. Using an *Escherichia coli* strain with upregulated Fhl expression and further genetic modifications to channel carbon flux towards succinate, we could achieve a ca. 20% improvement in molar yields for glucose to succinate conversion in small scale assays supplied with hydrogen gas. At the same time, supplying H_2 in such assays leads to an equivalent reduction in oxidised by-products (e.g. pyruvate) formation. Large scale experiments with optimised mass transfer are currently underway and we believe that these conditions should allow for an even better improvement in succinate yields.

We seek to advance our technology to deliver an enhanced bioprocess towards the production of succinate with carbon conversion efficiency beyond that possible with glucose and CO_2 alone. Using this as a model system, we believe this approach is deployable on a wider scale to minimise and eliminate CO_2 arising in other bio-based chemical manufacturing applications. This will provide an opportunity to increase CO_2 abatement even further compared to conventional sugar/biomass derived "green" fermentation processes.

BTV14

Insights into xylan utilization of thermophiles by complete genome sequencing of *Thermus brockianus* strain GE-1

C. Schäfers*[1], C. Hagedorn[1], S. Wiebusch[1], G. Antranikian[1]
[1]*Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany*

Introduction: *Thermus brockianus* GE-1 is a thermophilic, Gram-negative, rod-shaped and non-motile bacterium that was isolated from the Geysir geothermal area, Iceland. It was chosen for whole genome sequencing due to its ability to use xylan as sole carbon source [1]. With the description of the thermostable xylanase, Xyn10, we already characterized one promising biocatalyst [2]. Here we present further industrial relevant enzymes from *T. brockianus* GE-1 including a xylose isomerase for high fructose corn syrup (HFCS) production.

Objectives: To gain deeper insights into xylan utilization of thermophilic bacteria we determined biochemical properties of key enzymes involved in the xylan degradation pathway of *T. brockianus* GE-1.

Results: Here, we present the complete genome sequence of *T. brockianus* GE-1 with a genome size of 2.38 Mb, representing the very first genome sequence of *T. brockianus* [1]. The finished genome comprised one chromosome and two plasmids, including the megaplasmid pTB1. A deeper understanding of the enzymatic toolbox of thermophiles was obtained by analysis of the genomic region on pTB1, which encodes key elements for polymer degradation. Promising enzyme candidates such as the xylose isomerase XylA, which can be applied for the production of HFCS, will be described in more detail.

Conclusions: The characterization of novel extremozymes from *Thermus brockianus* GE-1 will contribute to the understanding of xylan metabolism in thermophiles. Utilization of such enzymes will enable a more efficient industrial production of HFCS.

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BTV15

Diversity mutation recombination and evolution of tomato leaf curl geminiviruses disease complex in Pakistan and its control strategies using RNAi

M. Shafiq*[1]
[1]*University of the Punjab, Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan, Lahore, Pakistan*

Tomato *leaf curl disease (ToLCD)* is an important limiting factor for tomato, vegetable and ornamental crops in the Pakistan. Symptoms of ToLCD include severe upward and downward leaf curl with cup-shape, yellowing and stunted plant growth. This disease is caused by begomoviruses (single-stranded DNA viruses (family Geminiviridae) that are transmitted by whiteflies). The begomoviruses are either bipartite (with two genomic components known as DNA A and DNA B), monopartite (with a genome homolog of DNA A component of bipartite begomoviruses) or monopartite associated with DNA satellites (mainly betasatelites). All three types of begomoviruses are main player in ToLCD complex. An overview presenting the emergence and evolution of ToLCD in Pakistan and its effect on future food security and their control is discussed. We also produced transgenic N. Benthamiana plant using transcriptional gene silencing based approach to methylate the Tomato leaf curl geminiviruses(ToLCGV). I will discuss the achievement in my lab to control ToLCGV.

BTV16

Metabolization of glycated amino acids from wort by *S. cerevisiae* leads to novel metabolites in beer

M. Hellwig*[1], A. L. Kertsch[1], T. Henle[1]
[1]*Technische Universität Dresden, Chair of Food Chemistry, Dresden, Germany*

Introduction: Products of non-enzymatic browning (Maillard reaction, glycation) are decisive for color and aroma of beer. Important products of this reaction are covalently modified ("glycated") amino acids. Effects of glycated amino acids on *S. cerevisiae* have not yet been described.

Objectives: To study the metabolization of glycated amino acids by different strains of *S. cerevisiae*.

Materials and methods: Glycated amino acids and peptides were chemically synthesized. Top- and bottom-fermenting brewer"s yeast strains as well as baker"s yeast strains were incubated in the presence or absence of defined glycated amino acids and peptides. The stability of individual glycated amino acids was measured by RP-HPLC-UV. Identification and quantification of metabolites was performed by RP-HPLC-MS/MS.

Results: Glycated amino acids are metabolized by *S. cerevisiae* to novel hydroxy acids and higher alcohols ("fusel alcohols"). Higher alcohols such as pyrralinol (up to 200 µg/L) and formylinol (up to 50 µg/mL), formed from the lysine derivatives pyrraline and formyline respectively, were quantitated especially in wheat beers. Yeast strains differed in the extent of formation of these novel metabolites.

Conclusion: Glycated amino acids are metabolized by *S. cerevisiae* through the Ehrlich pathway, a metabolic route important for the formation of key aroma compounds such as phenylethanol and 3-methylbutanol. Glycation of malt protein might therefore have an impact on the formation of these aroma compounds in beer.

ARV01

Hydrogenase 4 in *Trabulsiella guamensis* forms a labile H_2-producing formate hydrogenlyase complex

U. Lindenstrauß[1], C. Pinske*[1]
[1]*Martin-Luther-University Halle-Wittenberg, Halle a. d. S., Germany*

Trabulsiella guamensis is a non-pathogenic enterobacterium that was isolated from a vacuum cleaner on the island of Guam. It is closely related to the pathogenic enterobacterium *Salmonella typhimurium*. It has one H_2-oxidizing Hyd-2-type hydrogenase (Hyd), and encodes a H_2-evolving Hyd that is most similar to the uncharacterized *Escherichia coli* Hyd-4. This enzyme is predicted to have 5 membrane-associated and between 4-5 cytoplasmic subunits[1]. The expression of the *E. coli* operon encoding Hyd-4_{Ec} is silent and over-expression did not yield active protein[2,3]. The Hyd-4_{Ec} enzyme was proposed to form a second formate hydrogenlyase (FHL-2) complex for the disproportionation of formate to CO_2 and H_2, but the operon does not encode a formate dehydrogenase (FDH)[2].

Cloning and genetic manipulation of the entire 13 kbp Hyd-4_{Tg} operon of *T. guamensis* in the heterologous *E. coli* host have now enabled us to prove FHL-2 activity unambiguously and allowed us to characterize the Hyd-4_{Tg} complex biochemically. Its activity was dependent on the presence of *E. coli* FDH-H. Hyd-4_{Tg} has an activity similar to the *E. coli* FHL-1 complex in H_2-evolution from formate, but the complex is more labile upon cell lysis. Also, in contrast to *E. coli, T. guamensis* can ferment the alternative carbon sources cellobiose and cellulose, and we further investigated the participation of both the H_2-oxidizing Hyd-2_{Tg} and the H_2-forming Hyd-4_{Tg} under these conditions.

These experiments solve the long-standing mystery of the Hyd-4/FHL-2 complex and allow a first biochemical characterisation of *T. guamensis's* fermentative metabolism.

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ARV02

The Role of Formate in Haloalkaline Sulfate Reducing Environments

C. Plugge*[1,2], E. Koppius[1], J. Sousa[1,2,3], A. Stams[1], D. Sorokin[4,5]
[1]*Wageningen University & Research, Laboratory of Microbiology, Wageningen, Netherlands*
[2]*Wetsus, European Centre of Excellence for Sustainable Water Technology, Leeuwarden, Netherlands*
[3]*Paques Technology bv, Balk, Netherlands*
[4]*Winogradsky Institute of Microbiology, Research Centre of Biotechnology, Moscow, Russian Federation*
[5]*Delft University of Technology, Department of Biotechnology, Delft, Netherlands*

In haloalkaline environments, formate is an important intermediate in the anaerobic biodegradation of organic matter. In sulfate and thiosulfate-reducing bioreactors operated at haloalkaline conditions with H_2 as the only electron donor, formate intermediately accumulated up to a concentration of 150 mM. The high carbonate concentration (>0.5 M) in haloalkaline conditions, compared to neutral conditions, allows ~100-fold more formate formation. The formate produced from H_2 and HCO_3^-/CO_3^{2-} by the biomass in the bioreactors was subsequently used as electron donor for sulfate and thiosulfate-reducing bacteria. *Tindallia* sp.

strain JE1 was isolated from the biomass and responsible for the formate production. Strain JE1 differed from previously described acetogenic *Tindallia* species, as it grows lithotrophically with H2 and formate, without production of acetate, which is expected for an acetogen. To better understand the metabolic interaction as well as formate-hydrogen interconversion, pure and co-cultures of formate producer *Tindallia* strain JE1, and a sulfate reducer, *Desulfonatronovibrio thiodismutans* AHT10, were studied. When hydrogen was supplied as electron donor, sulfate was reduced by *D. thiodismutans* AHT10 only in the presence of *Tindallia* strain JE1, indicating a syntrophic relationship. Interspecies formate transfer occurred between the producer *Tindallia* strain JE1 and the consumer *D. thiodismutans.* The interactions were not restricted to formate transfer. Possibly, cross feeding of trace amounts of acetate or other unknown compounds produced by *Tindallia* sp strain JE1 play a role as well. We speculate that formate is produced by a *reversed* formate lyase reaction leaving the bacteria involved with an energetic challenge, even under haloalkaline conditions.

ARV03

Role of the Iron-Sulfur Flavoprotein DsrL in sulfur-oxidizing and sulfate-reducing bacteria

M. I. Löffler*[1], J. Feldhues[1], V. Klingbeil[1], S. Venceslau[2], I. A. C. Pereira[2], C. Dahl[1]
[1]*Universität Bonn, Institut für Mikrobiologie und Biotechnologie, Bonn, Germany*
[2]*Universidade Nova de Lisboa, Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal*

DsrAB-type dissimilatory (bi)sulfite reductase is a key enzyme of microbial sulfur-based energy metabolism. It occurs in sulfate/sulfite reducers and also in sulfur oxidizers, where it functions in reverse. Despite considerable progress towards its reaction mechanism [1], the long-standing question about the nature of the physiological electron acceptor/donor for DsrAB is still unresolved. All organisms encoding DsrAB also contain *dsrC* and minimally *dsrMK.* The combination of this core set with further *dsr* genes is used for predictions on physiology and activity in the environment. A gene for the iron-sulfur flavoprotein DsrL is typically found in *dsr* gene clusters from sulfur-oxidizers and indeed the protein is indispensable for sulfur oxidation in *Allochromatium vinosum*. While *dsrL* is not present in most sulfate reducers sequenced so far, it is not absolutely confined to sulfur oxidizers but appears in combination with genes for reductive-type DsrAB in several metagenomes as well as in *Desulfurella amilsii*, an organism capable of sulfite and thiosulfate reduction. Here, we show that DsrL proteins from *A. vinosum* and *D. amilsii* exhibit NAD(P)H-oxidoreductase activity. While the *A. vinosum* enzyme is strongly biased towards NADH, *D. amilsii* DsrL clearly prefers NADPH over NADH. From the phototrophic sulfur oxidizer *A. vinosum*, DsrL is co-purified with DsrAB and in the presence of DsrC this enzyme preparation catalyzes electron transfer from NADH to sulfite *in vitro*. These findings indicate NADPH as a probable electron donor for reductive-type DsrAB in those sulfate/sulfite reducers that contain DsrL. In sulfur oxidizers, DsrL likely channels at least part of the electrons stemming from reverse-acting sulfite-reductase onto NAD+.

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ARV04

The terminal oxidases of *Opitutaceae* (phylum: *Verrucomicrobia*) and their differential response to environmental oxygen concentrations

C. Feldewert*[1], D. Tegtmeier[1], A. Brune[1]
[1]*Max-Planck-Institute for Terrestrial Microbiology, RG Insect gut microbiology and symbiosis, Marburg, Germany*

The bacterial family *Opitutaceae* comprises members from aquatic, terrestrial, and host-associated environments. Reports on their oxygen relationship are controversial, but recent reports confirmed that all family members are at least microaerobic. Functional genome analysis revealed that the number of terminal oxidases in the respiratory chain varies among species. All species except *Ereboglobus luteus* have a low-affinity quinol oxidase of the *bo*$_3$-type, and each family member encodes at least one high-affinity oxidase — a *bd*-type quinol oxidase and/or a *cbb*$_3$-type cytochrome *c* oxidase. The role of the multiple terminal oxidases of *Opitutaceae* and their differential expression in response to fluctuating oxygen conditions remain unclear. We addressed these questions at the physiological and transcriptional levels. Using Clarke-type oxygen microsensors, we compared the oxygen-uptake rates of cell suspensions grown under different oxygen regimens. Because the sensitivity of the microsensors does not allow determination of oxygen affinities below 100 nM, the characterization of high-affinity oxidases requires a more sensitive system. Therefore, we are presently establishing an assay based on the oxygenation level of leghemoglobin and myoglobin. The expression levels of the terminal oxidases in response to different oxygen regimens were measured with quantitative PCR. Preliminary results suggest a versatile regulation of the oxidases in *Opitutaceae*, which allows them to adapt to changing environmental oxygen conditions.

ARV05

The cytochrome *bcc-aa*$_3$ oxidase supercomplex is required for Nar1-dependent nitrate respiration in spores of *Streptomyces coelicolor*

D. Falke*[1], M. Fischer[1], G. Sawers[1]
[1]*Martin-Luther-University Halle-Wittenberg, Institute of Biology/Microbiology, Halle a. d. S., Germany*

The ability to respire with oxygen is one of the main characteristics of energy conservation in aerobic and facultative aerobic microorganisms. To generate ATP using oxygen as terminal electron acceptor electrons must pass through a membrane-bound and proton-translocating respiratory chain, which ends with a terminal cytochrome oxidase. *Streptomyces coelicolor* A3(2) is an aerobic soil-dwelling bacterium characterized by undergoing a complex developmental cycle, including spore formation and production of both substrate and aerial hyphae. Oxygen respiration in *S. coelicolor* is performed by one of two pathways involving either a menaquinol:cytochrome *bcc* oxidoreductase (*bcc* complex) and copper-*aa*$_3$-type cytochrome *c* oxidase branch or a menaquinol:cytochrome *bd* oxidase branch. Because *S. coelicolor* lacks a soluble *c*-type cytochrome the *bcc* complex and cytochrome *aa*$_3$ oxidase must form a supercomplex, allowing electron transfer to occur. Despite requiring oxygen for growth, the bacterium can use nitrate as electron acceptor, catalyzed by any of three respiratory nitrate reductases (Nars). Surprisingly, we show that activity of Nar1, which is exclusively present and active in spores, depends on a functional *bcc-aa*$_3$-supercomplex to reduce nitrate. Furthermore, combined complementation and biochemical analyses of key *bcc* complex and *aa*$_3$ oxidase components also revealed that Nar1 activity in spores could be restored only by reintegrating the complete gene locus encoding the

bcc-aa$_3$-supercomplex. Together, our findings suggest that when oxygen becomes limiting in spores, the bacterium can redirect the electrons within the supercomplex to nitrate reductase, thus maintaining efficient proton translocation.

ARV06

A novel glycyl-radical enzyme (GRE) enables sulfidogenic taurine respiration in the human gut bacterium *Bilophila wadsworthia*

A. Burrichter*[1], S. C. Peck[2], K. Denger[1], E. P. Balskus[2], D. Schleheck[1]
[1]*Universität Konstanz, Konstanz, Germany*
[2]*Harvard University, Cambridge, MA, United States*

The anaerobic microbial metabolism of taurine (2-aminoethanesulfonate) is an important source of hydrogen sulfide (H_2S) in the human gut, for which H_2S production (sulfidogenesis) has been associated with inflammatory bowel diseases (IBD) and colon cancer (1). Here, we decipher the complete taurine desulfonation pathway in the human-gut bacterium and opportunistic pathogen *Bilophila wadsworthia* 3.1.6, using differential proteomics, *in vitro* reconstruction of the pathway with heterologously produced enzymes, and identification of its metabolites. An initial deamination of taurine to sulfoacetaldehyde by a known taurine:pyruvate aminotransferase (Tpa) (2) is followed, unexpectedly, by reduction of sulfoacetaldehyde to isethionate (2-hydroxyethanesulfonate) by a NADH-dependent reductase (SarD). Isethionate is then cleaved to sulfite and acetaldehyde by a previously uncharacterized glycyl radical enzyme (GRE), isethionate sulfite-lyase (IslA). The acetaldehyde produced is oxidized to acetyl-CoA by a dehydrogenase (AdhE), and the sulfite is reduced to H_2S by dissimilatory sulfite reductase (Dsr) for respiratory energy generation. This unique GRE is also found in *Desulfovibrio desulfuricans* DSM642 and D*. alaskensis* G20 that utilize isethionate as electron acceptor but not taurine. In conclusion, the novel radical-based C-S bond cleavage reaction catalyzed by IslA diversifies the known repertoire of GRE superfamily enzymes and enables sulfite respiration of *B. wadsworthia.* This GRE is widely distributed in gut bacterial genomes and may represent a novel target for control of intestinal H_2S production.

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ARV07

Na^+-transport by an ancient respiratory enzyme, the Rnf complex

D. Trifunovic*[1], H. Huber [2], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*
[2]*Universität Regensburg, Lehrstuhl für Mikrobiologie & Archaeenzentrum, Regensburg, Germany*

Introduction: Many anaerobic bacteria use a low potential ferredoxin (E0` ≈ -500 mV) as a common electron carrier. This low potential electron carrier can be used to drive various endergonic reactions or it can be used as an electron donor for respiration. One hypothesized ferredoxin-dependent respiratory enzyme is the Rnf complex, which couples exergonic electron flow from ferredoxin to NAD^+ to the translocation of ions across the cytoplasmatic membrane. However, biochemical evidence for this hypothesis is missing.

Objectives: To purify a Rnf complex and prove Na^+ transport.

Material & methods: The Rnf complex was purified from cell paste of *Thermotoga maritima* by chromatography and reconstituted into liposomes. Na^+ transport was measured using $22Na^+$.

Results: The Rnf complex was purified from membranes of *T. maritima* to apparent homogeneity. Gel chromatography and native PAGE revealed an active 225 kDa protein complex that harboured all six subunits of the Rnf complex. Enzyme activity was strictly Na^+ dependent. *N*,*N'*-Dicyclohexylcarbodiimide (DCCD) was identified to inhibit electron flow from ferredoxin to NAD^+**.** The Rnf complex was reconstituted into liposomes and the proteoliposomes still catalyzed ferredoxin oxidation with high rates. Ferredoxin-dependent NAD^+ reduction was coupled to vectorial Na^+ transport which was primary and electrogenic.

Conclusion: This is the first purification of an active Rnf complex and the first demonstration of ion (Na^+) transport catalysed by the Rnf complex, an ancient respiratory complex.

ARV08

Exploring the chemoautotrophic abilities of a diazotrophic sulfate-reducer methanogen

N. Maslac*[1], M. Jespersen*[1], T. Wagner*[1]
[1]*Max Planck Institute for Marine Microbiology, Microbial Metabolism, Bremen, Germany*

Hydrogenotrophic methanogens are strictly anaerobic archaea that thrive at the thermodynamic limit of life. Despite their low energy yield (0.5 ATP/formed CH_4), some can assimilate sulfate (SO_4) and fix N_2 [1,2], two high energy-consuming processes. The absence of cytochromes in these methanogens suggests unconventional electron donors for these pathways. We are using the SO_4-reducing diazothroph *Methanothermococcus thermolithotrophicus* to decipher the metabolic tricks which allow its fast growth in extreme chemoautotrophic conditions.

A combination of genome analyses, cellular physiology and biochemistry was performed to investigate these two pathways.

The enzymes involved in SO_4/N_2-assimilation have been identified in the archaeon genome and notable differences among homologs have been found. Strikingly, the last enzyme required for SO_4-reduction (DsrAB) is incomplete: DsrB, is not present. DsrA must operate by its own through an unknown mechanism. For N_2-fixation, the nitrogenase of *M. thermolithotrophicus* belongs to a different class; the operon contains a protein of unknown function and specific regulators. After adaptation on SO_4 or N_2 as sole source of sulfur and nitrogen, the minimum substrate concentrations to sustain growth were determined. During a screen of growth stimulators, molybdate (MoO_4) addition showed a boost for both N_2-fixers and SO_4-reducers. This is of interest since SO_4-reducing bacteria are strongly inhibited by MoO_4 and this archaeon seems to have a very high tolerance.

Our future goal is to structurally characterized each of the enzymes of both pathways and elucidate their catalysis, electron donors, regulations and evolutions.

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[2] Daniels *et al.*, Applied and Environmental Microbiology, 51, (1986)

GR-MSV01

Bacterial sRNAs are conserved but their targets are not - Lessons learned from and for comparative target prediction with CopraRNA

P. R. Wright[1], M. Raden[2], F. Eggenhofer[2], W. R. Hess[3], R. Backofen[2], J. Georg*[3]
[1]*University of Basel Hospital, Clinical Trial Unit, Basel, Switzerland*
[2]*University of Freiburg, Bioinformatics Group, Freiburg i. Br., Germany*
[3]*University of Freiburg, Genetics and Experimental Bioinformatics, Freiburg i. Br., Germany*

Bacterial sRNAs are important regulators of gene expression and partly control large regulons. Spot42, for example, is a central regulator of catabolite repression in *E. coli* and controls more than 25 targets. Interestingly the Spot42 synteny, sequences and promoter elements are conserved beyond the *enterobacterial* clade and reach into *Vibrionales*, *Alteromonodales* and *Aeromonadales.* Using the target prediction tool CopraRNA we asked the question if the regulons and the physiological functions are also conserved. Surprisingly, many known Spot42 targets, verified in *E. coli*, seem to be restricted to a small group of related species. Comparing the top 100 predicted genes in *E. coli*, *Yersinia pseudotuberculosis*, *Vibrio parahaemolyticus* and *Shewanella oneidensis* the overlap is remarkably low. Each organism has ≥ 67% unique targets and there is no common target appearing in all organisms. Despite the lack in the individual target conservation, a functional enrichment analysis indicates that the general function of Spot42 in the regulation of the carbohydrate metabolism is more widely retained. Only *Shewanella oneidensis* stands out. Here Spot42 seems to have switched to be a nucleotide metabolism regulator.

In the light of the missing target conservation it remained puzzling why sRNAs are nevertheless conserved over a longer evolutionary distance. We provide an explanation for this counter-intuitive phenomenon using an "*in silico*" evolution approach.

Based on the above findings we modified CopraRNA to account for patchy and narrow target conservation. The new CopraRNA has a significantly improved performance and can compete with experimental large scale methods like RIL-seq or MAPS. Additionally, the best performing methods, RIL-seq and CopraRNA 2.0, are highly synergistic.

GR-MSV02

Gene regulation in *trans* by the bacterial attenuator sRNA rnTrpL and the leader peptide peTrpL

H. Melior*[1], S. Li[1], M. Stötzel[1], R. Madhugiri[2], K. U. Förstner[2], R. Schütz[1], S. Barth-Weber[1], K. Baumgardt[1], J. Ziebuhr[3], E. Evguenieva-Hackenberg[1]
[1]*Institute for Microbiology and Molecularbiology JLU-Giessen, Gießen, Germany*
[2]*Research Center for Infectious Diseases, University of Würzburg, Würzburg, Germany*
[3]*Institute of Medical Virology, JLU Gießen, Gießen, Germany*

Transcription attenuation is widespread in bacterial biosynthesis operons. Liberated attenuator RNAs and leader peptides arising in this process are generally considered nonfunctional. Here, we provide evidence that, in *Sinorhizobium meliloti* having the tryptophan biosynthesis genes organized in three operons, the attenuator RNA rnTrpL of *trpE(G)* and the corresponding leader peptide peTrpL have functions in *trans*. We show that as a small RNA (sRNA), rnTrpL base-pairs with *trpD* mRNA to coordinate the post-transcriptional regulation of the *trpE(G)* and *trpDC* operons according to tryptophan availability. It is remarkable that this coordination is executed by the same regulatory RNA using two fundamentally different mechanisms: transcription attenuation in *cis* and base-pairing in *trans.* Further, we found that, upon exposure to tetracycline (Tc), rnTrpL destabilizes *rplUrpmA* (encodes the ribosomal proteins L21 and L27) in a peTrpL-dependent manner. Additionally, peTrpL mediates enhanced multiresistance to antimicrobial compounds. This is based on rnTrpL-independent destabilization of *smeR* mRNA (encodes the repressor of the major multidrug efflux pump genes) by peTrpL. Coimmunoprecipitation experiments revealed Tc-dependent interaction of the leader peptide peTrpL with the RNAs rnTrpL, *rplUrpmA* and *smeR*. In summary, our data demonstrate an exceptional multifunctionality of a bacterial transcription termination attenuator. We propose that in *S. meliloti* and other bacteria, the *trpL* attenuator evolved into a multifunctional mRNA leader whose products (sRNA and peptide) promote bacterial competitiveness under tryptophan limitation and exposure to antimicrobial compounds in the environment.

GR-MSV03

The involvement of the antisense RNA RSaspufL in regulated formation of photosynthesis complexes in *Rhodobacter sphaeroides*

C. M. Reuscher*[1], G. Klug[1]
[1]*Institut für Mikro- und Molekularbiologie, Gießen, Germany*

The facultative phototrophic model bacterium *Rhodobacter sphaeroides* is known for its metabolic versatility. Under microaerobic conditions it synthesizes intracytoplasmic membranes harbouring the pigment protein complexes needed for anoxygenic photosynthesis. For fast adaption to varying environmental conditions the transcription of genes in the photosynthetic gene clusters is tightly regulated. The *puf* operon comprises genes which encode proteins of the light harvesting complex I (LHI) and of the reaction centre (RC). RNA-Seq and Northern blot analysis of transcripts derived from the *puf* operon unveiled that also certain small RNAs are transcribed. Up to date two different *puf* operon associated sRNAs were characterized. The Hfq-dependent sRNA aspufL was detected antisense to the 5´region of the *pufL* gene extending into the *pufA-pufL* intercistronic region. Northern blot results confirmed the presence of aspufL (~ 180 nt) under microaerobic and phototrophic conditions. Since aspufL overstretches an RNAse E cleavage site and the Shine-Dalgarno sequence of *pufL*, we hypothesize that the antisense RNA RSaspufL contributes to the regulated processing and degradation of the *puf* mRNA in *R. sphaeroides*. An artificial increase in the amount of the aspufL by plasmid driven over-expression led to a reduction in the amount of LHI/RC-complexes. The *in vivo* reporter assay showed that aspufL influences *pufL* in an Hfq-dependent manner. Additionally, we could show that the over-expression also influences the half-live of the polycistronic *puf* mRNA. AspufL is the first characterized cis-encoded sRNA which is associated with the *puf*-operon and plays an important role in processing and degradation of its target mRNA.

GR-MSV04

NynA, a novel RNase of the Alphaproteobacteria

M. McIntosh*[1], L. Paul[1], R. Matos[2], V. Srinivasan[3]
[1]*Justus-Liebig-Universität, Institute of Biology, Gießen, Germany*
[2]*ITQB NOVA, Lisbon, Portugal*
[3]*Philipps-Universität Marburg, SYNMIKRO, Marburg, Germany*

Regulatory networks depend upon post-transcriptional control of gene expression. Key to post-transcriptional regulation are the RNAases. Investigation of quorum sensing in the Alphaproteobacterium *Sinorhizobium meliloti* uncovered an interesting "hypothetical gene" with predicted RNAse-like protein folds. The protein contains the so-called NYN domains which are strongly conserved throughout the Alphaproteobacteria. In fact, NYN domains exist in many different organisms, from archaea to humans, however their roles remain mostly uncharacterised. The best-known NYN domain-containing protein is LabA, an uncharacterised regulator of the circadian clock of cyanobacteria. The presence and orientation of Rossmann folds and metal ion binding motifs in the NYN domain hints at RNA binding/modifying activity, and we have confirmed that the *S. meliloti* NYN protein (which we have named NynA) contains RNase activity using a standard RNA oligo. Furthermore, we have elucidated its crystal structure. It forms a pentamer ring, with each monomer carrying a putative active site consisting of a metal-binding domain oriented towards the outside of the ring. These rings align with one or more additional rings to form a barrel-shaped complex. Mutation studies showed that *nynA* was important for quorum sensing in *S. meliloti* and another Alphaproteobacterium, *Rhodobacter sphaeroides*, but not in *Paracoccus denitrificans*. RNA seq data confirmed that *nynA* specifically controls transcript accumulation for multiple genes in *S. meliloti*, including *expR* and *sinRI*, which are essential for quorum sensing. Finally, using promoter-reporter fusions and ChIP-Seq, we found that the promoter of *nynA* was transcriptionally activated by a LuxR type regulator and DnaA, the master regulator of the cell cycle.

GR-MSV05

A novel mineral component of the biofilm extracellular matrix promotes antbiotic resistence

I. Kolodkin-Gal*[1], I. Karukner[1], A. Keren-Paz[1]
[1]*Weizmann Institute of Science, Rehovot, Israel*

Multicellular bacterial communities, known as biofilms, have been thought to be held together solely by a self-produced organic extracellular matrix (ECM). However, new evidence for a missed mineral constituent of ECM recently emerged from our lab. Our study of three phylogenetically distinct bacteria: *Bacillus subt*ilis, *Mycobacterium smegmatis* and *Pseudomonas aeruginosa* identified a novel mechanism crucial for proper biofilm development and architecture – an active, genetically regulated production of crystalline calcite scaffolds. We demonstrated the existence of calcite scaffolds in bacterial biofilms, their role in stabilizing biofilm structure, and in limiting penetration of small molecule solutes as antibiotics. In addition, we demonstrated that calcite mineral scaffolds play a conserved role in the assembly of complex differentiated multicellular communities. Using a transcriptome analysis, we identified several clusters of genes, among them a conserved putative calcium transporter, which actively promote biomineralization and are essential for the structure and function of bacterial biofilms. Furthermore, our results indicate that dedicated biofilm cells actively differentiate into mineral producers both *in vitro* and in clinical settings. Overall, We propose it is time for a more complete view of the ECM as a complex combination of organic and nonorganic materials, especially in light of the possible implications on treatment of biofilm infections.

GR-MSV06

Biofilm architectural breakdown in response to antibiotics facilitates community invasion

F. Díaz-Pascual*[1], R. Hartmann[1], M. Lempp[1], L. Vidakovic[1], B. Song[2], F. H. Yildiz[3], J. Dunkel[2], H. Link[1], C. D. Nadell[1,4], K. Drescher[1,5]
[1]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[2]*Massachusetts Institute of Technology, Department of Mathematics, Cambridge, MA, United States*
[3]*University of California, Department of Microbiology and Environmental Toxicology, Santa Cruz, CA, United States*
[4]*Dartmouth College, Department of Biological Sciences, Hanover, NH, United States*
[5]*Philipps University Marburg, Department of Physics, Marburg, Germany*

Question: Bacterial biofilms often encounter environment changes that lead to stress. However, it is still unclear how do they respond to these changes from a single cell level and an architectural point of view. One of these environmental fluctuations is the exposure to antimicrobial compounds, for which biofilms are highly tolerant. This work looks to address how biofilm architecture respond to antibiotic exposure from a single cell point of view.

Methods: Here we show how *Vibrio cholerae* biofilms grown in microfluidic systems respond to antibiotic stress by using confocal microscopy and single cell tracking, together with metabolomics and *in silico* simulations.

Results: We found that translational inhibition causes striking changes in biofilm structure. They cause an increase in cell volume and a decrease in cell density in comparison to untreated biofilms. These changes are due to an active mechanism. They lead to a breakdown of the cell-matrix interactions due to changes in the properties of the matrix protein RbmA, for which the putative polysaccharide lyase, RbmB, is necessary. The detachment between the cells and matrix create gaps in the biofilm structure that can be used by invader cells to colonize the biofilm with important consequences for the development and study of antibiotics, and their ecological impact.

Conclusions: Translational inhibitors cause changes in cell shape and physiology that yield large-scale alterations of biofilm architecture. The loosening of cell-matrix associations that occurs following antibiotic exposure dramatically alters community ecology by allowing community invasion. These findings open new windows into the study of bacterial collective behavior and microbiota ecology in response to antimicrobial therapeutics.

GR-MSV07

***In vitro* evaluation of biofilm-inhibiting compounds to treat infective endocarditis**

V. Pauker*[1], D. Gottschling[1], L. Maletzki[2], A. Strohbach[2], S. Petersen[3], S. Kohse[4], N. Grabow[4], K. Riedel[1]
[1]*Institute of Microbiology, University of Greifswald, Greifswald, Germany*
[2]*University Medicine Greifswald, Clinic und Policlinic for Internal Medicine B, Researchcluster III, Greifswald, Germany*
[3]*Faculty of Engineering and Computer Science, University of Applied Sciences, Osnabrück, Germany*
[4]*Institute for Biomedical Engineering, University Medicine Rostock, Rostock, Germany*

Introduction**:** Biofilm formation on cardiovascular implants can lead to life-threatening conditions such as infective endocarditis, short IE. The major cause of IE are bacteria of the genera *Streptococcus*, *Staphylococcus* and *Enterococcus,* which colonize the implants and are able to form biofilms. Biofilms are characterized by cells embedded

in a slimy matrix composed of extracellular polymeric substances (EPS) such as polysaccharides, proteins, extracellular DNA (eDNA), and amyloid fibers, which form a protective layer between bacterial cells and environmental stress.

Objective: Infections of cardiovascular implants can lead to life threatening conditions and prevention of infection and especially biofilm formation is a prerequisite for an effective treatment. Thus, this study aimed to determine the capability of different compounds to interfere with biofilm growth.

Methods: A standard micro-titer assay and a flow-through system, with subsequent crystal violet staining or confocal laser scanning microscopy, respectively, were employed for a quantitative and qualitative evaluation of the biofilm forming capacity of the above-mentioned Gram-positive cocci. As a starting point, N-acetylcysteine, chlorhexidine digluconate and a combination of rifampicin/minocycline were tested to prevent formation and dissolve existing *Staphylococcus aureus* biofilms alone and in cocultivation with endothelial cells. An LC-MSE-based proteomics approach was used to record stress signatures of *S. aureus* biofilms treated with the different compounds.

Results and conclusion**:** The tested compounds successfully prevent biofilm formation as solutions and hence, the compounds will be further evaluated as possible coating substances for endothelialized TAVI surfaces.

GR-MSV08

Proteomics reveal enhanced oxidative stress responses and metabolic adaptation in *Acidithiobacillus ferrooxidans* biofilm cells on pyrite

S. Bellenberg[1], N. D. Huynh[2], A. Poetsch[1,3], W. Sand[2,4], M. Vera *[5]
[1]*Kalmar University, Centre for Ecology and Evolution in Microbial Model Systems, Kalmar, Sweden*
[2]*Technische Universitaet Bergakademie Freiberg, Biohydrometallurgisches Zentrum für strategische Elemente, Freiberg, Germany*
[3]*Plymouth University, School of Biomedical Sciences, Plymouth, United Kingdom*
[4]*Donghua University, College of Environmental Engineering, Shanghai, CÔte D'ivoire*
[5]*Pontificia Universidad Católica de Chile, Institute for Biological and Medical Engineering. Schools of Engineering, Biological Sciences & Medicine. Department of Hydraulic and Environmental Engineering. School of Engineering, Santiago, Chile*

Acidithiobacillus ferrooxidans is an acidophilic bacterium able to leach metal sulfides such as Pyrite (FeS_2) and Chalcopyrite ($CuFeS_2$). These metal sulfides, generate extracellular reactive oxygen species (ROS) upon exposure to acidic water. Oxidative stress management is especially important in acidophilic leaching microorganisms, especially when forming biofilms on metal sulfides in industrial biomining operations. The molecular repertoire of responses upon exposure to pyrite and the presence of ROS are not thoroughly understood in acidophiles. In this study the impact of the external addition of H_2O_2 in *A. ferrooxidans* DSM 14882^T was investigated. Iron(II)- or sulfur-grown cells showed a higher sensitivity towards H_2O_2 than pyrite-grown ones. Shot-gun proteomics was used to compare proteomes of cells grown with iron(II)-ions against biofilm cells, grown for five days in presence of pyrite as sole energy source. In total 1157 proteins were identified. 213 and 207 ones were found to have increased levels in iron(II)-grown or pyrite-biofilm cells, respectively. In total, 80 proteins involved in ROS degradation, thiol redox regulation, macromolecule repair mechanisms, biosynthesis of antioxidants, as well as metal and oxygen homeostasis were found. 42 of these proteins had no significant changes in abundance, while 30 proteins had increased levels in pyrite-biofilm cells. Proteins associated with inorganic sulfur compound (ISC) oxidation were among the latter. New insights in ROS mitigation strategies are presented for *A. ferrooxidans*^{T} biofilm cells. Furthermore, proteomic analyses provide insights in adaptations of carbon fixation and oxidative phosphorylation pathways under these two growth conditions.

SMV09

Identification of the polyketide synthase gene responsible for the synthesis of Tanzawaic acids in *Penicillium steckii* IBWF104-06

L. Antelo*[1], A. Bernal[1], S. Jacob[1,2], K. Andresen[2], A. Yemelin[1], H. Hartmann [3], E. Thines[1,2]
[1]*IBWF, Kaiserslautern, Germany*
[2]*Johannes Gutenberg-University, Mikrobiologie und Weinforschung am Institut für Molekulare Physiologie, Mainz, Germany*
[3]*BASF SE, Computational Biology, Ludwigshafen, Germany*

Microorganisms have already been used as biological control agent in agriculture for a long time, but their importance increased dramatically in the last years. The so-called biological control agents may be used as an alternative to the widespread use of chemicals against plant pathogens, even more since the use of chemical pesticides have become controversial due to their potential harmful effects.

The *Penicillium steckii* strain IBWF104-06 was patented by BASF SE for its promising results in greenhouse experiments performed against phytopathogenic fungi and oomycetes. *P. steckii* strains produce several tanzawaic acids, interesting compounds harboring a decalin moiety in its structure, which is presumably a result of a Diels-Alder reaction with the action of a polyketide synthetase. The strain produces some new tanzawaic acid derivates, some of them with antifungal activity.

The IBWF104-06 genome was sequenced and annotated. Several polyketide synthetase genes were found, among two of them (PKS1 and PKS2) have high similarity in homology and gene cluster organization to the genes responsible for lovastatin and solanapyrone synthesis, compounds structurally related to tanzawaic acids.

Tools for molecular manipulation of P. steckii were developed at the IBWF and deletion mutants of both PKS1 and PKS2 have been generated by gene replacement. The ΔPKS1 mutant is not able to produce any tanzawaic acid, whereas the ΔPKS2 mutant produces them in a similar manner than the wild type strain.

Nevertheless, both ΔPKS1 and ΔPKS2 display similar antagonistic activity against phytopathogenic fungi and oomycetes like the wild type, indicating that the plant protecting activity is inherent to other components than the tanzawaic acid production.

SMV10

Linking genomic and metabolic diversity of the myxobacterial genus *Sorangium*

F. Panter[1], M. Steglich*[2,3], N. Zaburannyi[1], A. K. Szafranska[2,3], C. Wolf[4], K. Mohr[4], J. Wink[4], R. L. Hahnke[2], J. Meier-Kolthoff[2], B. Bunk[2], J. Overmann[2], M. Göker[2], D. Krug[1], R. Müller[3,1], U. Nübel[2,3,5]
[1]*Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research and Department of Pharmaceutical Biotechnology, Saarland University, Saarbrücken, Saarbrücken, Germany*
[2]*Leibniz Institute DSMZ-German Collection of Microrganisms and Cell Cultures, MIG, Braunschweig, Germany*

[3]German Center for Infection Research (DZIF), Braunschweig, Germany
[4]Helmholtz Centre for Infection Research, Braunschweig, Germany
[5]Technical University Braunschweig, Braunschweig Integrated Center of Systems Biology (BRICS), Braunschweig, Germany

Bacterial genome sequencing in conjunction with bioinformatic 'mining' approaches has led to the detection of a multitude of hypothetical biosynthetic gene clusters (BGCs), suggesting an enormous potential for synthesis of diverse metabolites. For the vast majority of these BGCs, however, associated metabolites are currently unknown.

We have integrated a dataset of genome sequences and experimental secondary metabolomics data from 78 *S. cellulosum* strains to identify statistical associations between BGCs and the production of potentially biotechnologically useful metabolites. We detected a total of 3,658 BGCs in 78 *S. cellulosum* genomes and classified these into 954 families of unique BGCs based on their sequence similarities. By combining high-resolution mass spectrometry with our in-house myxobase data warehouse, the same strains were investigated for their metabolite production under controlled conditions, leading to the identification of 523 distinct molecular features from secondary metabolites specific for the suborder *Sorangiineae*. Associations between BGC families and metabolite features were investigated by applying a maximum-likelihood approach, considering the genome-based phylogeny of *S. cellulosum*. As a result, 110 BGC families were found to be statistically associated with specific metabolite features, including several correct links which had been fully chemically characterized previously.

Our results demonstrate that statistical association analysis is a powerful tool for accelerating the identification of links between genes and secondary metabolites, which is a prerequisite for understanding bacterial biosynthetic potential, for investigating its genetic regulation, and for using synthetic biology approaches for rational engineering of molecules.

SMV11
Heterologous production of pseudochelin in *Myxococcus xanthus*

A. Sester*[1,2], J. Korp[1,2], L. Winand[1], M. Nett[1]
[1]TU Dortmund, Technical Biology, Dortmund, Germany
[2]Hans Knöll Institute, Jena, Germany

Beside their natural biological function as iron chelating agents, many siderophores exhibit activities that are of high interest for diverse medical applications. For example, myxochelins were found to be efficient inhibitors of the human 5-lipoxygenase. Recently, the biosynthetic pathway of the novel myxochelin derivative pseudochelin A was identified. An enzyme, named MxcM, belonging to the amidohydrolase family catalyzes an intramolecular condensation in myxochelin B that forms the characteristic 4,5-dihydroimidazole moiety of pseudochelin A.

In this work, we reconstituted the biosynthesis of pseudochelin A *in vivo*. For this, different expression plasmids were constructed to achieve a heterologous expression of the *mxcM* gene in the myxobacterium *Myxococcus xanthus* DSM16526. *M. xanthus* is a natural producer of myxochelin B and thereby provides the substrate for pseudochelin A production directly *in vivo*. After transfer of the expression plasmids into *M. xanthus* DSM16526 by electroporation, all expression strains were found to produce pseudochelin A. The respective compound was purified and structurally verified by NMR. With a production titer of 16 mg per liter, we are now able to produce sufficient amounts of pseudochelin A to test its 5-lipoxygenase inhibition properties or other biological activities. For the first time, an effective plasmid-based expression of a biosynthetic gene was carried out in a myxobacterium, which demonstrates a new possibility for natural product research in myxobacteria.

SMV12
Unravelling the Biosynthetic Pathway of Polythioamide Antibiotics in Anaerobic Bacteria

K. L. Dunbar*[1], H. Büttner[1], M. Dell[1], E. M. Molloy[1], J. Kumpfmüller[1], C. Hertweck[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Biomolecular Chemistry, Jena, Germany
[2]Friedrich Schiller University, Jena, Germany

Thioamides are peptide bond isosteres where the amide oxygen has been substituted by sulfur. This seemingly simple substitution is often leveraged by synthetic chemists since it greatly changes the properties of the corresponding molecule. Although thioamide moieties are ubiquitous in the primary metabolism of organisms from all domains of life, thioamide-containing secondary metabolites are exceedingly rare. Indeed, only one thioamide-containing nonribosomal peptide has been identified to date. This compound, closthioamide (CTA), is an unprecedented symmetric peptide containing six thioamide linkages. CTA is a potent DNA gyrase-targeting antibiotic that was isolated from the soil-derived anaerobic bacterium *Ruminiclostridium cellulolyticum*. Despite being discovered nearly a decade ago, the CTA biosynthetic pathway has remained elusive until now. Based on the structure of CTA, we took a "retro-biosynthetic" approach to propose enzymatic transformations that could be needed for its formation, enabling us to pinpoint a region of the *R. cellulolyticum* genome encoding enzymes with those predicted activities. We subsequently used a combination of genome editing, heterologous expression and *in vitro* enzyme assays to deconvolute the CTA biosynthetic pathway, including the installation of the thioamide moieties. Our results demonstrate that CTA is produced by an unusual thiotemplated peptide assembly line that strikingly differs from canonical nonribosomal peptide synthases. As well as solving the long-standing mystery of CTA biosynthesis, our findings pave the way for the discovery of additional thioamide-containing nonribosomal peptides.

SMV13
***Serratia plymuthica C*-methyltransferase with novel activities: methylation and simultaneously five-ring cyclisation in the biosynthesis of the secondary metabolite sodorifen**

M. C. Lemfack*[1], S. von Reuß[2], D. Domik [3], N. Magnus [1], M. Kai[1], T. Weise[4], B. Piechulla [1]
[1]Institute for Biological Sciences/University of Rostock, Biochemistry, Rostock, Germany
[2]Institute of Chemistry/University of Neuchatel, Neuchâtel, Switzerland
[3]Biotechnology Institute/University of Minnesota, St. Paul, MN, United States
[4]Euroimmun AG, Lübeck, Germany

Microorganisms are able to produce a plethora of secondary metabolites for their defense and survival. The rhizobacterium *Serratia plymuthica* is capable to produce a unique and unusual polymethylated bicyclic secondary metabolite named sodorifen [1, 2]. This compound was found to possess toxicity against colorectal carcinoma cell lines and its production can be significantly up-regulated during

co-cultivation with other microorganisms [3]. Transcriptome analysis and genome sequencing revealed a cluster of four genes involved in the biosynthesis of sodorifen. These genes encode a terpene cyclase, *C*-methyltransferase, DXP synthase and IPP isomerase. Unlike other terpene synthases, this *S. plymuthica* terpene cyclase (*Sp* SODS) does not accept the canonical C15 substrate farnesylpyrophosphate (FPP) but the C16 pre-sodorifen pyrophosphate harboring a novel five ring structure synthesized by the *C*-methyltransferase (*Sp* FPPMT) [4]. Subsequently, the terpene cyclase performs several cyclisation cascades to produce sodorifen. To our knowledge this is the first methyltransferase, which not only catalyzes the methylation of the substrate but also a five-ring cyclisation in the biosynthesis of a secondary metabolite.

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SMV14

Flavin-dependent enzymatic synthesis of the griseorhodin A pharmacophore

B. Frensch*[1], R. Teufel[1]
[1]*University of Freiburg, Biology, Freiburg i. Br., Germany*

Griseorhodin A is a member of the rubromycin family of polyketide natural products that are produced by Actinobacteria. Most rubromycins, including griseorhodin A, feature a highly unusual spiroketal pharmacophore required for bioactivity, whose biosynthesis remains largely enigmatic to date.[1,2] The polycyclic aromatic compound collinone was proposed as direct precursor for the enzymatic spiroketalization that involves a unique oxidative rearrangement of the carbon skeleton. Based on knock-out experiments, it was proposed that these exceptional redox tailoring steps are catalyzed by several enzymes, including the flavin-dependent enzymes GrhO1, GrhO5 and GrhO6.[1,2] To gain a detailed understanding of the individual enzymatic reactions and catalytic mechanisms, we first aimed to reconstitute the spiroketal-forming steps *in vitro*. We could show that only the two flavoenzymes GrhO5 and GrhO1 are required to synthesize the spiroketal moiety, as confirmed by LCMS, MS^2 (including 18O-labelled product) as well as NMR. Subsequent in-depth analysis of the enzymatic reactions now allows us to propose a detailed route for spiroketal formation. Further experiments revealed that GrhO6 is presumably involved in a subsequent unusual isomerization step that sets up the final epoxidation by a cytochrome P450-dependent oxygenase to afford griseorhodin A.

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SMV15

New biosynthetic principles towards protease inhibitors in bacteria

F. Leipoldt[1], F. Wolf[1], A. Kulik[1], H. Gross[1], A. Truman[1,2], L. Kaysser*[1]
[1]*University of Tübingen, Microbiology/Biotechnology, Tübingen, Germany*
[2]*John Innes Centre, Microbiology, Norwich, United Kingdom*

Bacteria have been a rich source of small molecule protease inhibitors over the last decade. A frequent structural feature of protease inhibitors from nature is the presence of warheads that mediate their specific binding to the active site of target enzymes and may allow mechanism-based inhibition. We have studied the formation of various protease inhibitors in filamentous actinobacteria and have found that their corresponding warheads are not only interesting from a pharmacological perspective but often derive from unusual biosynthetic pathways. One example is the single enzyme transformation of an α-dimethyl β-keto acid precursor to the epoxyketone warhead of epoxomicin and eponemycin proteasome inhibitors via decarboxylation, dehydrogenation and epoxidation.1,2 Remarkably, a similar mechanism generates the chemical diversity in the matlystatin family of metalloproteinase inhibitors.3 We also found that the hydroxamic acid warhead of this class of compounds is assembled by an unprecedented variation of the ethylmalonyl-CoA pathway.3,4 Moreover, we discovered that construction of the β-lactone moiety of belactosin and cystargolides is reminiscent of leucine biosynthesis.5 In the presentation I will discuss our recent progress to investigate key reactions in the biological formation of protease inhibitors from bacterial sources. The potential to apply the gathered information for genome mining and bioengineering approaches will be outlined.

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SMV16

Natural Products from Predator-Prey Interactions

P. Stallforth*[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Chemistry of Microbial Communication, Jena, Germany*

The search for new bioactive natural products has prompted scientists to exploit environmental niches in which the production of these compounds is ecologically motivated. Microbial predator–prey interactions are particularly rich sources of natural products. We describe one such interaction in which bacterivorous amoebae and their prokaryotic prey meet. Amoebae are voracious and ubiquitous predators to bacteria that cause constant depletion of huge bacterial reservoirs. This puts both organisms under strong evolutionary selection pressure: the bacteria have evolved mechanisms to prevent grazing and the amoebae must counteract or surmount these mechanisms in order to survive. Here, we describe a variety of natural products that show amoebicidal activity along with investigations into their biosynthesis, evolution, and regulation.

EM-CCV01

Separation techniques for particle-associated marine bacteria

A. Heins*[1], R. Amann[1], J. Harder[1]
[1]*Max Planck Institute for Marine Microbiology, Molecular Ecology, Bremen, Germany*

In most aquatic environments particle-associated (PA) bacteria are outnumbered by free-living (FL) bacteria. However, they frequently surpass the metabolic activity of the FL fraction and can therefore not be neglected from holistic studies of the diversity and ecology of the bacterioplankton. Their isolation remains a challenging task. Fractionated filtration is the most common separation method and nominal pore sizes are used to define the respective groups. We here define PA bacteria as those retained by a 3 µm filter and those that pass through that pore size as FL. This definition places large or filamentous bacteria, e.g. *Tenacibaculum* spp., in the PA fraction, regardless of their actual attachment to a particle. Mechanosensitive particles may not survive the filtration pressure yielding PA bacteria in the FL fraction. These biases are intrinsic to the respective techniques and need to be understood to prioritize a method for particular aims. We therefore conducted an extensive method comparison. This included plankton nets, centrifugation and sedimentation as alternatives to filtration. We used all of these methods to obtain FL and PA bacteria from algal spring blooms in the North Sea. Dilution cultivation on plates and culture-independent analyses (partial 16S rRNA gene sequences) were subsequently applied to isolate PA bacteria and to evaluate the efficiencies and biases of the different fractionation methods. We were able to retrieve isolates of the flavobacterial genera *Flavobacterium, Algibacter, Olleya, Lutimonas, Cellulophaga, Winogradskyella, Polaribacter, Maribacter, Gramella, Nonlabens, Dokdonia,* and *Tenacibaculum* from the particles.

EM-CCV02

Targeted cell sorting combined with single cell genomics reveals nineteen novel Chloroflexi species from a Uruguayan winery wastewater treatment plant

H. Dam*[1], J. Vollmers[1], A. Cabezas da Rosa[2], A. K. Kaster[1]
[1]*Karlsruhe Institute of Technology, Institute for Biological Interfaces 5, Eggenstein-Leopoldshafen, Germany*
[2]*Instituto de Investigaciones Biológicas "Clemente Estable", Montevideo, Uruguay*

Chloroflexi is one of the most frequently detected bacterial phyla on earth. However, only 162 (draft) genomes of 22.363 different 16S rRNA gene sequences (0.72%) deposited in the Ribosomal Database Project are known to date. They are currently placed in 8 classes: *Anaerolineae, Caldilineae, Ardenticatenia, Thermoflexia, Chloroflexia, Thermomicrobia, Dehalococcoidia*, and *Ktedonobacteria*. In this study, draft genomes of 19 novel Chloroflexi species were reconstructed from single amplified genomes using a targeted cell sorting approach with modified Fluorescence *in situ* hybridization methods from a single sample of a Uruguayan winery wastewater treatment plant. The method was proved to be efficient in enriching Chloroflexi present at less than 1% of the total microbial community. This novel approach greatly improves the current single cell genomics pipeline, and can also be applied to study other underrepresented bacterial and archaeal phyla in the so called "microbial dark matter". Total gene content and multi locus sequence analysis placed the 19 Chloroflexi species in distinct groups with so far no cultured representatives, that also have not yet been properly classified and characterized within the phylum. The study therefore contributes to unravel genomes of unclassified Chloroflexi, which might also lead to a revision of evolutionary relationships between the bacteria in this phylum. In addition, it provides an overview about metabolic potentials of uncultured Chloroflexi species in wastewater environments.

EM-CCV03

Machine learning to decipher metabolic differences between *Photorhabdus* and *Xenorhabdus*

N. Tobias*[1], C. Parra-Rojas[2], Y. N. Shi[1], Y. M. Shi[1], A. Thanwisai[3], A. Vitta[3], N. Chantratita[3], E. Hernandez-Vargas[2], H. B. Bode[1]
[1]*Goethe University, Frankfurt a. M., Germany*
[2]*Frankfurt Institute for Advanced Studies, Frankfurt a. M., Germany*
[3]*Naresuan University, Phitsanulok, Thailand*

Although members of different genera, *Xenorhabdus* and *Photorhabdus* produce a number of shared natural products and occupy very similar ecological niches. Interestingly, the bacteria have yet to be isolated from the environment as free-living bacteria, but instead are always found in association with their respective nematodes. Despite this specificity towards a nematode host, the bacteria-nematode pairs may be isolated from the same geographical location. Recently we highlighted the extensive chemical diversity present in these genera using high-throughput genomic and metabolomic analyses. Here we perform a comprehensive metabolic screening using HPLC-MS of 175 strains (91 *Photorhabdus* and 84 *Xenorhabdus*) collected from across Thailand and explore the metabolic potential among the strains, matched with several abiotic factors. Using machine learning, we ranked the importance of individual metabolites in determining all given metadata. We show that values associated with soil pH, moisture, temperature, soil type, elevation above sea level or geographical position play no role in the chemical potential associated with individual strains. Instead, we were able to prioritize metabolites in the context of natural product investigations subsequently allowing us to accurately distinguish between *Xenorhabdus* and *Photorhabdus* strains with high accuracy based on only metabolite data.

EM-CCV04

Functional Metagenomics of the Thioredoxin Superfamily

S. Nilewski[1], M. Varatnitskaya[1], N. Lupilov[1], L. I. Leichert*[1]
[1]*Ruhr-Universität Bochum, Institute for Biochemistry and Pathobiochemistry, Bochum, Germany*

Environmental sequence data of microbial communities now makes up the majority of public genomic information. The assignment of a function to sequences from these metagenomic sources is challenging, because organisms associated with the data are often uncharacterized and not cultivable. To overcome these challenges, we created a rationally designed expression library of metagenomic proteins covering the sequence space of the thioredoxin superfamily. This library represents more than 22"000 thioredoxins found in the Global Ocean Sampling dataset. We screened this library for a functional rescue of *E. coli*mutants lacking the thioredoxin-type reductase (Δ*trxA*), isomerase (Δ*dsbC*), or oxidase (Δ*dsbA*). Our approach revealed an unusual thioredoxin-superfamily oxidoreductase. This representative of a class of thioredoxin-like proteins is highly active *in vitro*and *in vivo*and its migration on gel filtration column is consistent with a multimer. Instead of the intramolecular disulphide bond formation typical for thioredoxins, this protein forms an intermolecular disulphide between the attacking cysteines of two subunits during its catalytical cycle.

EM-CCV05

Interspecific formation of the antimicrobial volatile schleiferon

M. Kai*[1], U. Effmert[1], M. C. Lemfack[1], B. Piechulla[1]
[1]*University of Rostock, Biochemistry, Rostock, Germany*

Microorganisms release a plethora of volatile secondary metabolites. Up to now, it has been widely accepted that these volatile organic compounds are produced and emitted as a final product by a single organism e.g. a bacterial cell. We questioned this commonly assumed perspective and hypothesized that in diversely colonized microbial communities, bacterial cells can passively interact by emitting precursors which non-enzymatically react to form the active final compound. This hypothesis was inspired by the discovery of the bacterial metabolite schleiferon A. This bactericidal volatile compound is formed by a non-enzymatic reaction between acetoin and 2-phenylethylamine. Both precursors are released by *Staphylococcus schleiferi* cells. In order to provide evidence for our hypothesis that these precursors could also be released by bacterial cells of different species, we simultaneously but separately cultivated *Serratia plymuthica* 4Rx13 and *Staphylococcus delphini* 20771 which held responsible for only one precursor necessary for schleiferon A formation, respectively. By mixing their headspace, we demonstrated that these two species were able to deliver the active principle schleiferon A. Such a joint formation of a volatile secondary metabolite by different bacterial species has not been described yet. This highlights a new aspect of interpreting multispecies interactions in microbial communities as not only direct interactions between species might determine and influence the dynamics of the community. Events outside the cell could lead to the appearance of new compounds which could possess new community shaping properties.

Kai M, Effmert U, Lemfack MC, Piechulla B (2018) Interspecific formation of the antimicrobial volatile schleiferon. *Scientific Reports* 8:16852

EM-CCV06

Elucidating the mode of action of a newly discovered glycosylated lanthipeptide

J. Deisinger*[1,2], A. Müller[1,2], F. J. Ortiz-López[3], O. Genilloud[3], T. Schneider[1,2]
[1]*University of Bonn, Institute for Pharmaceutical Microbiology, Bonn, Germany*
[2]*German Center for Infection Research (DZIF), partner site Bonn-Cologne, Bonn, Germany*
[3]*Fundación MEDINA, Parque Tecnológico de Ciencias de la Salud, Armilla (Granada), Spain*

To overcome rising antibiotic resistance, novel antibiotics with unprecedented mechanisms of action are urgently needed. A newly discovered lantibiotic produced by *Streptomyces spec.* shows potent activity against Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA). A structural key feature of the compound is a not previously reported disaccharide that is attached to a tyrosine residue of the lanthipeptide. Mechanism of action investigations were performed combining whole cell assays and *in vitro* test systems. Our study reveals that the compound interferes with the lipid II cycle. A comparative analysis showed, that the molecular target differs from that of mersacidin, which is known to bind to lipid II. This indicates that the compound might exhibit a yet unknown mode of action, potentially involving the unprecedented disaccharide.

EM-CCV07

Evaluation of the antagonic and quitinolytic activity of trichoderma spp., to inhibit the growth of *Moniliophthora roreriand Moniliophthora perniciosa*

L. Galarza*[1]
[1]*Escuela Superior Politécnica del Litoral (ESPOL), Centro de Investigaciones Biotecnologicas del Ecuador (CIBE), Guayaquil, Ecuador*

Searchingfor new biological alternatives to control the moniliasis and the witch's broom caused by *Moniliophthorarorer*iand *Moniliophthoraperniciosa*, respectively, in the cocoa cultivars. Ten strains of *Trichoderma*that exhibited under *in vitro*conditions an antagonistic effect over these pathogens were studied. The percentages of inhibition obtained after performing the confrontations in dual crops varied from 89.32% to 96.69% for *M. roreri*and from 87.50% to 93.53% for *M*. *perniciosa*. The qualitatively and quantitatively production of chitinolytic enzymes, which hydrolyze the chitin cell wall of the pathogens, was determined too. It was observed that the ten isolates of *Trichoderma*spp., were efficient to utilize as the sole carbon source both the commercial colloidal chitin and the chitin isolated from the cell walls of *M. roreri*and *M. perniciosa*. The total chitinolytic activity was measured by the release of N-acetyl-*β*-D-glucosamine (NAGA) from the colloidal chitin in the presence of chitinolytic enzymes. NAGA"s concentrations were detected in the range of 51.75 to 266.75 mg/mL with the commercial colloidal chitin, from 21.75 to 94.25 mg/mL and from 24.25 to 71.75 mg/mL with the chitin isolated from the cell wall of *M. perniciosa*and *M. roreri*. Seventeen compounds with antifungal properties or with a different biological activity were detected by gas chromatography-tandem mass spectrometry (GC-MS) after analyzing the ethyl acetate extracts obtained from the different strains of *Trichoderma*that grew either individually or from the confrontations with the two causal agents of these diseases in the cocoa plantations.

Keywords: Antagonism, chitinases, N-acetil-*β*-*D*-glucosamine, metabolites.

EM-CCV08

Genomic erosion causes nutritional dependency in '*Streptomyces philanthi*', the European beewolf defensive symbiont

M. Sandoval-Calderon*[1], T. Nechitaylo[2], M. Kaltenpoth[1,2]
[1]*Institut für Organismische und Molekulare Evolutionsbiologie, Evolutionary Ecology, Mainz, Germany*
[2]*Max Planck Institute for Chemical Ecology, Insect Symbiosis Research Group, Jena, Germany*

Several insect species rely on microorganisms for defense against pathogens. Beewolves are solitary digger wasps in the tribe Philanthini that hunt bees to feed their larvae. A bacterial symbiont, named '*Streptomyces philanthi*', inhabits glands in the antennae of female beewolves and is vertically transmitted to the progeny. '*S. philanthi'* produces a cocktail of antibiotics that protect beewolf cocoons from fungal infections. Despite tight association between these symbionts and their hosts, strains of '*S. philanthi'* isolated from different beewolf species exhibit considerable variation in their metabolic capabilities.

We analysed the genome and transcriptome of '*S. philanthi'* biovar Triangulum to better understand its physiology and elucidate the metabolic interchange that occurs between the host and its symbionts. The genome of this strain does not show an A+T enrichment nor a significant reduction in size, as it is common for obligate bacterial

symbionts, but is highly eroded, accumulating frameshift mutations in more than a third of its predicted coding sequences. Some of these mutations explain the strict nutritional requirements of this strain, which reflects a stronger dependency on its host for provision of nutrients. The symbiont's transcriptome within the beewolf antennal glands highlights metabolic pathways that could be generating the building blocks for polyketide biosynthesis. It also suggests some nutrients that could be provided by the host in exchange for the production of antibiotics by the symbiont.

'*S. philanthi'* is an excellent model to study metabolic interactions in defensive symbiosis and the evolution of bacterial genomes during the establishment of an obligate symbiotic relationship.

BCV09
Surface Acclimation of the Cyanobacterium *Synechocystis* sp. PCC 6803
S. Oeser*[1], T. Wallner[1], A. Wilde[1]
[1]*University of Freiburg, Institute for Biology III - Molecular Genetics, Freiburg i. Br., Germany*

Many bacteria can exhibit planktonic as well as sessile lifestyles. We investigate the acclimation to these environmental conditions in the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*).

To this end, a microarray experiment of *Synechocystis* cells, which were incubated either on agar plates or in liquid medium, was performed. It revealed, among others, an altered transcription of genes potentially involved in extracellular polymeric substances (EPS) production. This hints at a role of EPS in surface acclimation and biofilm formation in cyanobacteria.

Furthermore, transcription level of minor pilin genes differ between the two conditions in the microarray analysis. In *Pseudomonas aeruginosa,* a role of the minor pilins in pilus formation and function is proposed. There are at least 10 genes encoding potential minor pilins in *Synechocystis*. Their functions were unknown so far. Now, our studies of minor pilin mutants reveal several different roles of these proteins. They seem to be involved in motility, attachment to surfaces and natural competence.

We show that levels of second messengers, such as c-di-GMP and cAMP, change upon acclimation to surfaces. Further, a microarray analysis demonstrates that the level of the second messenger c-di-GMP alters the minor pilin gene transcription. Moreover, other genes encoding cell surface components, such as chaperone usher pili, were differentially transcribed.

In conclusion, we propose that sensing of surfaces leads to modified levels of the second messengers c-di-GMP and cAMP, resulting in an altered transcription of minor pilin genes. Thus, we assume that several minor pilins of *Synechocystis* are involved in surface acclimation.

BCV10
Methicillin-resistant *Staphylococcus aureus* alters cell wall glycosylation to evade immunity
D. Gerlach*[1], A. Peschel[1]
[1]*Universität Tübingen, Infection biology, Tübingen, Germany*

Question: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a frequent cause of difficult-to-treat, often fatal human infections. Most humans have antibodies against *S. aureus,* but these are highly variable and often not protective in immuno-compromised patients. Previous vaccine development programs have not been successful. A large percentage of human anti-*S. aureus* antibodies targets wall teichoic acid (WTA), a ribitol-phosphate (RboP) surface polymer modified with N-acetylglucosamine (GlcNAc). It is currently unknown if the particular immune evasion capacities of MRSA are due to variation of dominant surface epitopes such as those associated with WTA.

Methods: We employed Microbiological assays such as Phage assays, Molecular cloning. Additionally we utilized Flow cytometry and in-vivo models.

Results: We demonstrate that a considerable proportion of the prominent healthcare-associated (HA) and livestock-associated (LA) MRSA clones CC5 and CC398, respectively, contain prophages that encode an alternative WTA glycosyltransferase. This enzyme, named TarP, transfers GlcNAc to a different hydroxyl group of the WTA RboP than the standard enzyme TarS, with major consequences for immune recognition. TarP-glycosylated WTA elicited lower levels of IgG in mice than TarS-modified WTA. This difference was reflected by only low amounts of antibodies against TarP-modified WTA in human sera.

Conclusions: Our study reveals a new immune evasion strategy of *S. aureus* based on averting the immunogenicity of its dominant glycoantigen WTA. It will instruct the identification of invariant *S. aureus* vaccine antigens and may enable the development of TarP inhibitors as a new strategy for rendering MRSA susceptible to human host defense.

BCV11
Cytoskeletal confinement of membrane microdomains
R. Wagner*[1,2], D. Lopez[1]
[1]*Spanish National Center for Biotechnology, Microbial Biotechnology, Madrid, Spain*
[2]*Universität Würzburg, Institut für molekulare Infektionsbiologie, Würzburg, Germany*

It has been established that the cellular membrane is composed of different protein and lipid species. These, due to their physico-chemical properties, laterally segregate leading to a heterogeneous distribution of lipids and proteins and the formation of microdomains. Microdomains are an important principle in the organization of membrane processes as it facilitates wanted protein-protein interaction and complex formation and shields unwanted interactions. Bacteria organize signaling processes in functional membrane microdomains (FMM). FMMs are highly dynamic structures that move within the membrane. The molecular mechanisms and the biological significance that underlie FMM dynamics are yet to be understood. We use live-cell imaging in combination with biochemical and molecular biological approaches to decipher FMM dynamics in the bacterial model *B. subtilis*. Physiologically targeting different cellular structures revealed the integrity of the bacterial cell envelope and its associated structures to be important for FMM dynamics, rather than the membrane itself. In eukaryotic cells, lipid rafts, the counterpart to the prokaryotic FMMs, interact with the actin cytoskeleton which is thought to form a network confining lipid rafts and associated processes. We hypothesize that FMM dynamics is controlled in a similar fashion. Namely, the cytoskeleton forming a fence-like structure acting as a barrier for FMM dynamics.

This is a new principle of membrane organization which restricts protein diffusion and thus presents a means of regulating cellular processes confined to the membrane.

BCV12

Superresolution microscopy reveals the dynamic behaviour of cell wall synthesis enzymes and of MreB during cell elongation of *Bacillus subtilis*

L. Stuckenschneider*[1], S. Dersch[1], J. Mehl[2], J. Roth[2], A. Rohrbach[2], P. L. Graumann[1]
[1]SYNMIKRO, LOEWE-Zentrum für Synthetische Mikrobiologie, Chemie, Marburg, Germany
[2]Laboratory for Bio- and Nano-Photonics, Microsystems Engineering-IMTEK, BIOSS Centre for Biological Signalling Studies, Freiburg i. Br., Germany

Most proteins involved in extension of the cell wall (CW) of *B. subtilis* and their respective activities are known, but their dynamics maintain rather unclear. We have set out to investigate the localization and mobilities of some major CW synthesis proteins (MreB, RodA and PBPs) by superresolution microscopy techniques. Especially single molecule tracking (SMT) is used to gain deeper insight into the modes of motion and insertion of peptidoglycan during cell elongation. Additionally, we extended our analysis to cells subjected to different types of stress (osmotic and antibiotic stress) to determine differences in the modes of motion of our target proteins.

In the present work, we show that MreB, RodA and PbpH are traveling alongside each other, but with differing lengths of trajectories. This suggests that RodA and PbpH bind and unbind to the perpendicular-moving MreB filaments, indicating that the peptidoglycan elongation machinery (PGEM) complex is not strictly rigid, but instead a coordinated action of independently-acting synthetic proteins. SMT suggests that diffusive modes of RodA, PBPs and MreB are incongruent. Diffusive behaviour was altered by different stress conditions in a protein-specific way. Higher salt levels lead to increasing MreB diffusion and reduction of filament formation, whereas the behaviour of PbpH did not change drastically in response to osmotic stress. CW antibiotic treatment interfered with the mode of motion of PBPs. Overall our findings indicate that peptidoglycan insertion and cell wall synthesis localisation might not be directly dependent on MreB movement and that MreB plays a role during adaption to cell wall stress in which cell wall synthesis needs to be reorganized.

BCV13

Bacteriophage genome transfer mechanisms at the Gram-negative cell wall

N. K. Broeker[1], M. S. Meyer[1], F. Kiele[1], S. Barbirz*[1]
[1]Universiät Potsdam, Physical Biochemistry, Potsdam, Germany

Tailed bacteriophages, upon initial contact with the Gram-negative cell wall, encounter a variety of biomolecular structures which they exploit during the first infection steps, ranging from receptor recognition, particle positioning to opening of a genome translocation channel. The molecular mechanisms of these steps are not understood on a molecular level despite the high prevalence of tailed phages isolated against Gram-negative pathogens for the purpose of developing new antimicrobials.

To elucidate mechanisms of initial rearrangement steps in contact with the Gram-negative cell envelope we have analyzed a set of model *Salmonella* (*S.*) model phages of all three tail morphologies, *i.e.* short or long non-contractile or contractile. They are strictly O-antigen specific on the same *S.* Typhimurium host [1]. *In vitro* particle opening studies showed that the tail morphology dictated the velocity by which the phages ejected their DNA. Whereas the contractile-tailed myovirus had O-antigen adsorption coupled with particle opening, short-tailed podoviruses stayed adsorbed to their host O-antigen receptor without genome loss at low temperature. Only permissive temperatures could then trigger rearrangements in the tail proteins leading to particle opening [2]. This illustrates that O-antigen specialized phages have developed diverse infection strategies adapted to their life style and the current life cycle status of their hosts.

[1] Broeker, N. K.; Barbirz, S., *Mol. Microbiol.* **2017,***105* (3), 353-357.

[2] Broeker, N. K.; Kiele, F.; Casjens, S. R.; Gilcrease, E. B.; Thalhammer, A.; Koetz, J.; Barbirz, S., *Viruses-Basel* **2018,***10* (6), doi:10.3390/v10060289.

BCV14

Adhesion of *E. coli* on nano-rough titanium surfaces - image and genetic analyses

N. Stefani*[1], M. Schürmann[2], V. Schroeckh[1], S. Dietrich[3], T. Wolf[4], K. D. Jandt[2], M. T. Figge[3], G. Panagiotou[4], A. A. Brakhage[1]
[1]Leibniz-Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Molecular and Applied Microbiology, Jena, Germany
[2]Otto Schott Institute of Materials Research, Otto Schott Institute of Materials Research, Jena, Germany
[3] Leibniz-Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Applied Systems Biology, Jena, Germany
[4]Leibniz-Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Systems Biology and Bioinformatics, Jena, Germany

Possibilities to control infections got more complicated in recent years due to an increase of antibiotic resistant bacterial strains. Consequently, also approaches besides antibiotics are needed. One of them is to reduce microbial adhesion on biomaterial surfaces by nano-feature equipped surfaces on biomaterials, e.g. on titanium implants for bone contact. We aim to unravel the causal relationships between nano-roughness of titanium layers and microbial adhesion. One clinical *E. coli* urosepsis isolate (sticks weakly to surfaces) and strain *E. coli* ATCC 25922 (sticks strongly to surfaces) are compared to the lab strain *E. coli* RV308 regarding their adhesive behaviour on 2 and 7 nm rough titanium surfaces. As initial characterization step, every strain was incubated on titanium surfaces to allow primary adhesion; the number of adhered bacteria was detected via fluorescence microscopy and quantified via image analysis developed in Python. To gain more insight in the mechanism of bacterial adhesion and to find involved triggers, RNA sequencing of adherent and planktonic bacteria was realised. Here fore, isolated RNA was sequenced, reads were mapped with HISAT2 following differential gene expression analysis tools. Significant antimicrobial efficacies between the two investigated nano roughnesses could not be observed, but between the *E. coli* strains themselves. Preliminary transcriptome data (i) support the expectation that bacteria sense the surface by modifying their genetic response and (ii) indicate no effect of nano-roughness on the bacteria"s adhesion to the investigated surfaces.

BCV15

Spatial organization of motility machinery in the archaeal cell

T. Quax*[1], Z. Li[1], S. V. Albers[1]
[1]*Freiburg University, Freiburg i. Br., Germany*

Bacteria and archaea can perform tactic behavior and thereby move up and down chemical gradients. This tactic behavior relies on a motility structure, which can be guided by a chemosensory system. The archaeal motility structure, the archaellum, has a fundamentally different structural organization than the bacterial motility structure, the flagellum. Environmental signals are sensed by chemosensory receptors that are organized in large arrays, from which the signals are transferred to the base of the motility structure. In bacteria, the spatial and temporal positioning of these macromolecular assemblies in the cell has been studied in detail. Several active mechanisms were identified that are responsible for exact positioning of flagella and chemosensory arrays in bacteria. In contrast, the cellular localization of archaeal proteins involved in taxis and motility has not been addressed yet.

We applied a combination of fluorescent and electron microscopy using the model euryarchaeon *Haloferax volcanii.* We found that the archaeal motility structures, archaella, are located specifically at the cell poles of actively dividing cells. Chemosensory arrays were also present at the poles of archaeal cells, and, in addition, several freely moving smaller clusters were detected at the lateral membranes. The positioning of archaeal motility machinery is precicely orchestrated and shares characteristics with several bacterial species.

This work provides the first insights into the organization of archaeal motility machinery and indicates that there might be an active mechanism responsible for cellular positioning of macromolecular protein complexes in archaea.

BCV16

Effect of different bile acids on flagella formation in *Clostridioides difficile*

N. G. Metzendorf*[1], S. Dittmann[1], N. Lainer[1], R. Schlüter[2], L. Kieper[1], K. Riedel[1], S. Sievers[1]
[1]*University Greifswald, Institute of Microbiology , Greifswald, Germany*
[2]*University Greifswald, Imaging Center of the Department of Biology, Greifswald, Germany*

Introduction: *Clostridioides difficile* is an intestinal human pathogen that infects the gut of individuals with depleted microbiota. During infection the composition of the intestinal bile acid cocktail plays an important role. However, the specific response of vegetative *C. difficile* cells to bile acids is largely unknown on the molecular level.

Methods: Proteome signatures of long-term (LT) stressed *C. difficile* cells were obtained. The four main bile acids cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and lithocholic acid (LCA) were tested and data generated by LC-MS/MS. Furthermore, bacterial morphology was determined and quantified in images obtained by electron micrographs (EM). Flagella were purified and selected proteins were analyzed by Western blot.

Results: LT-stress caused a significant and bile acid specific change in global protein signatures. The major structural flagellum protein FliC was dramatically decreased in the presence of LCA compared to control conditions, while DCA and CDCA resulted in moderate down-regulation of FliC and CA did not affect FliC expression at all. EM validated these findings: while CA treatment did not alter the number of flagella, DCA- and CDCA-stressed *C. difficile* had fewer and LCA-treated bacteria were almost devoid of flagella compared to controls.

Discussion: Lack of flagella, especially the immunogenic FliC, causes non-motility but enhanced adherence and virulence of *C. difficile* in hamster (Dingle *et al*., 2011). In our study, we found a differential impact of bile acids on the formation of flagella in *C. difficile*. This raises the question which regulatory mechanism leads to the absence of flagella during LCA stress and the ineffectiveness of CA.

EMV17

Persulfide dioxygenase from *Acidithiobacillus caldus*: Variable Roles of cysteine residues and hydrogen bond networks of the active site

P. Rühl*[1], D. Tietze[2], F. Folgosa[3], M. Teixeira[3], C. Frazão[4], A. Kletzin[1]
[1]*Technische Universität Darmstadt, Schwefelbiochemie und mikrobielle Bioenergetik, Darmstadt, Germany*
[2]*Technische Universität Darmstadt, Eduard-Zintl Institut für physikalische und inorganische Chemie, Darmstadt, Germany*
[3]*ITQB NOVA, Metalloproteins and Bioenergetics, Oeiras, Portugal*
[4]*ITQB NOVA, Macromolecular Cr ystallography, Oeiras, Portugal*

Introduction: Iron-containing persulfide dioxygenases (PDO) are abundant in Bacteria and essential for H2S detoxification in mitochondria. They catalyze the oxidation of glutathione persulfide (GSSH) to sulfite and reduced glutathione (GSH).

Objectives: We describe the biochemical, spectroscopic and crystallographic properties of the PDO from the bioleaching bacterium *Ac. caldus* (*Ac*PDO) including protein modification and an extensive mutational analysis.

Results: The activity of the purified *Ac*PDO was approximately 111 U/mg protein after expression of the gene in *E. coli*. The enzyme activity with GSH plus sulfur in the reaction mixture and colorimetric product detection followed a Michealis-Menten kinetic, whereas incubation with GSSH resulted in a sigmoidal curve, suggesting positive cooperativity. Mutagenesis showed that residues in the predicted GSH/GSSH binding site and in the central hydrogen bond networks are essential for catalysis. Two out of five cysteines are also essential, both located in disulfide bridge distance on the surface of the *Ac*PDO. Mass spectrometry analyses suggest that at least one of the cysteines becomes S-glutathionylated after incubation with substrate. Charge transfer bands were visible in UV/Vis spectra of PDO with GSSH. The results of EPR spectroscopy and X-ray crystallography will be presented.

Conclusion: The results (i) pinpoint the essential amino acids within the active hydrogen-bonding network around the iron site for substrate binding and catalysis of the *Ac*PDO and (ii) suggest that disulfide formation between the two surface-exposed cysteines and (iii) that Cys-S-glutathionylation serves as protection against thiol oxidation and the associated loss of enzyme activity.

Rühl *et al*. 2018, Front Microbiol:10.3389/fmicb.2018.01610

EMV18

Significance of MccR, MccC, MccD, MccL and 8-methylmenaquinone in *Wolinella succinogenes* sulfite respiration

J. Eller*[1], S. Hein[1], J. Simon[1]
[1]*TU Darmstadt, Biology, Darmstadt, Germany*

Reduction of sulfite to sulfide is an essential step in the biogeochemical sulfur cycle. The Epsilonproteobacterium *Wolinella succinogenes* uses the copper-containing octahaem cytochrome *c* sulfite reductase MccA to respire sulfite. MccA is encoded by the first gene of the *mcc* gene cluster, whose transcription is apparently induced by the two-component regulatory system MccRS. It has been proposed that the iron-sulfur protein MccC, the putative quinol dehydrogenase MccD, the copper chaperone MccL as well as menaquinone-6 (MK_6) and/or 8-methylmenaquinone-6 (8-MMK_6) are involved in the electron transport chain of *W. succinogenes* sulfite respiration. Non-polar *W. succinogenes* mutants were constructed that lacked MccC, MccD, MccL or the 8-MMK_6-producing MK_6 methyltransferase MqnK. Each mutant possessed a frameshift-corrected *mccR* gene, thus inducing *mcc* expression in the presence of a mixture of fumarate and sulfite as terminal electron acceptors. Under these conditions, growth by sulfite respiration of cells lacking MccC or MccD was found to be abolished. However, cells lacking MccL or 8-MMK_6 still coupled formate oxidation to sulfite reduction and grew by sulfite respiration to some extent. The results indicate that MccR, MccC, MccD, MccL and 8-MMK_6 are essential or significant components of *W. succinogenes* sulfite respiration.

EMV19

Methane-derived carbon-flow through a microbial community in a stratified lake

S. Schorn*[1], J. Graf[1], S. Littmann[1], P. Hach[1], G. Lavik[1], M. M. M. Kuypers[1], C. Schubert[2], J. Milucka[1]
[1]*Max Planck Institute for Marine Microbiology, Bremen, Germany*
[2]*Swiss Federal Institute of Aquatic Science and Technology (Eawag), Kastanienbaum, Switzerland*

Methane-oxidizing bacteria (i.e. MOB; methanotrophs) have the unique capacity to use methane as a carbon and energy source and thereby form the basis of methane-driven food webs. Compounds that are formed during methane oxidation may leak from the methanotrophs and become available for other bacteria. It is presumed that methylo- or heterotrophic bacteria feed off these methane-derived carbon compounds; thus, their growth is indirectly supported by methane.

Here we investigated the flow of methane-derived carbon through a microbial community in a stratified lake using stable isotope labeling and nanoscale secondary ion mass spectrometry (nanoSIMS). We collected samples from the permanently stratified Lake Zug (Switzerland), which is characterized by anoxic, methane-rich (> 30 µM) bottom waters. Incubations with ^{13}C-labeled methane revealed high rates of methane oxidation (up to 2 µM day^{-1}) under both oxic and anoxic (denitrifying) conditions. Using catalyzed reporter deposition fluorescence *in situ* hybridization coupled to nanoSIMS we identified active *gammaproteobacteria*l methane-oxidizing bacteria (gamma-MOB) in all investigated water depths. NanoSIMS analyses revealed increasing ^{13}C enrichment in the biomass of individual gamma-MOB over time. Interestingly, *betaproteobacterial* cells that were physically associated with the gamma-MOB also became ^{13}C-enriched over time, indicating that they grew on methane-derived carbon compounds. Our results highlight the importance of methane-derived carbon for microbial communities in stratified lakes and demonstrate the power of single-cell approaches for studying microbial interactions in the environment.

EMV20

Microbial Loop in an Anaerobic Hydrocarbon-degrading Enrichment Culture

A. Akbari*[1], R. U. Meckenstock[1]
[1]*University of Duisburg-Essen Campus Essen , Biofilm Centre, Essen, Germany*

In contaminated aquifers, high concentrations of spilled hydrocarbons often lead to a temporary boost in microbial activity, an increased biomass concentration and a depletion of background nutrients. *In-situ* necromass fermentation can potentially contribute to the recycling of carbon and nutrients in the ecosystem. However, the significance and extent of the process are unknown. This study aims to elucidate the carbon and nitrogen flow in an anoxic hydrocarbon-degrading enrichment culture isolated from a contaminated aquifer. The culture consisted of the hydrocarbon-degrading, sulfate-reducing *Desulfobacterium* N47 and the spirochaete *Rectinema cohabitans*. Stable isotope-labeling and Raman microscopy were used to determine the carbon and nitrogen incorporation at the single-cell level. An in-house code was developed for analysis of spectra including baseline correction, denoising, statistical and ordination analyses. Distinct Raman spectra for *Desulfobacterium* N47 and *R. cohabitans* cells allowed for linking the identity and function. Amendment with 13C naphthalene compared to non-labelled systems resulted in a clear shift in Raman band of phenylalanine as a marker of 13C incorporation. The ratio of 1003 to 991, 981, 968 cm-1 bands of imaged cells was significantly decreased from 1.4±0.5 in non-labelled systems to 0.37±0.1 in labelled ones. The outcomes of this study will provide insights on the extent of necromass degradation leading to carbon and nitrogen recycling in contaminated subsurface environments.

EMV21

Regulatory Response Dynamics towards *p*-Cresol in the Denitrifying Bacterium "*Aromatoleum aromaticum*" EbN1

J. Vagts*[1], A. Weiten[1], L. Wöhlbrand[1], R. Rabus[1]
[1]*Institute for Chemistry and Biology of the Marine Environment, General and Molecular Microbiology, Oldenburg, Germany*

Introduction: The denitrifying betaproteobacterium "*Aromatoleum aromaticum*" EbN1 regulates formation of proteins involved in the degradation pathway for *p*-cresol with high substrate-specificity. This process is proposed to be mediated by a two-component system consisting of a sensory protein (EbA5371), which upon effector binding autophosphorylates a δ54-dependent transcriptional activator (EbA5375).

Material and Methods: In this study, the time-resolved responsiveness of "*A. aromaticum*" EbN1 towards *p*-cresol was investigated. Cultures were grown under nitrate-reducing conditions with a limiting supply of benzoate. Upon depletion of the latter, *p*-cresol was added at varying concentrations (100 *µ*M down to 0.1 nM). Subsequently, cells were harvested for time-resolved targeted transcript profiling via qRT-PCR. Seven target genes were selected according to their function and genomic location.

Results: The obtained response profiles indicate two independently regulated transcriptional units with highly different response thresholds. Interestingly, the far more-

sensitive one (response threshold of 1 - 10 nM) contains both genes required for the conversion of *p*-cresol to the less cytotoxic 4-hydroxybenzoate. The genes coding for the CoA-ligase subsequently forming 4-hydroxybenzoyl-CoA (HbcL-1) as well as for the two-component sensor/regulator system itself appear to be regulated with a lower sensitivity (response threshold of 0.1 – 1 µM).

Conclusion: Thus, an apparent connection between physiological function and genomic organization could be revealed in an aromatic compound degrader as well as an extremely high sensitivity for an aromatic substrate. Furthermore, a newly generated unmarked *in-frame* Δ*ebA5371/5* deletion mutant of strain EbN1 is currently being characterized.

EMV22

Identification of the first glyphosate transporter by genomic adaptation

L. M. Schulz*[1], D. Wicke[1], P. Scholz[2], A. Poehlein[3], J. Gibhardt[1], R. Daniel[3], T. Ischebeck[2], F. M. Commichau[1]
[1]*Institute for Microbiology and Genetics, Department of General Microbiology, Göttingen, Germany*
[2]*Albrecht-von-Haller-Institute for Plant Sciences, Department for Plant Biochemistry, Göttingen, Germany*
[3]*Institute for Microbiology and Genetics, Department of Genomic and Applied Microbiology, Göttingen, Germany*

The soil bacterium *Bacillus subtilis* gets into contact with growth-inhibiting substances like antibiotics and herbicides, which may be of anthropogenic origin. Glyphosate is such a substance serving as a non-selective herbicide to kill weeds and other plants. Glyphosate specifically inhibits the 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase, which generates an essential precursor for *de novo* synthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan in plants, fungi, bacteria and archaea. Inhibition of the EPSP synthase by glyphosate results in depletion of the cellular levels of aromatic amino acids unless the environment provides them. *B. subtilis* is a well-studied organism. However, the information about the interaction between this bacterium and glyphosate as well as its potential to adapt to the herbicide is limiting. We have assessed the potential of *B. subtilis* to adapt to glyphosate at the genome level. Surprisingly, none of the mutants that were isolated in the presence of toxic glyphosate levels had accumulated mutations in the EPSP synthase. By contrast, all characterized mutants had inactivated the *gltT* gene encoding the high-affinity sodium-coupled glutamate/aspartate symporter GltT. Further adaptation of the *gltT* mutants to glyphosate led to the inactivation of the *gltP* gene encoding the proton/glutamate symporter GltP. We also found that GltT is involved in uptake of the herbicide glufosinate (phosphinotricin), which inhibits the glutamine synthetase. Growth experiments and metabolome analyses revealed that GltT is the major entryway of glyphosate into *B. subtilis*. To conclude, we have identified the first transporters that are involved in the uptake of glyphosate, which has become the dominant Weedkiller worldwide.

EMV23

Characterization of bacterial isolates tolerating extreme levels of the herbicide glyphosate

S. Lentes[1], A. Poehlein[2], R. Daniel[2], F. M. Commichau*[1]
[1]*Institute for Microbiology and Genetics, Department of General Microbiology, Göttingen, Germany*
[2]*Institute for Microbiology and Genetics, Department of Genomic and Applied Microbiology, Göttingen, Germany*

Glyphosate (*N*-(phosphonomethyl)glycine) serves as a non-selective herbicide and crop desiccant of formulations, which are widely used in agriculture to kill weeds and other plants that compete with crops. The herbicide is resistant to chemical hydrolysis, thermal decomposition and photolysis. Due to the fact that glyphosate is toxicologically safe and that transgenic, glyphosate-resistant crops have been introduced, the herbicide has become the dominant weedkiller worldwide. Glyphosate inhibits the 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase generating an essential precursor for *de novo* synthesis of aromatic amino acids in plants, fungi, bacteria and archaea. Inhibition of the EPSP synthase by glyphosate results in depletion of the cellular levels of aromatic amino acids unless the environment provides them. Recently, we have observed that some batches of commercially available Roundup® solution, which should consist of water, a detergent and glyphosate, contain extremely high numbers of bacterial species. The initial physiological characterization of one bacterial species that has been isolated from the Roundup® solution revealed that the bacteria rapidly grow with succinate and ammonium as carbon and nitrogen sources, respectively. We also found that the bacterial isolate tolerates glyphosate levels exceeding the solubility of the herbicide. Furthermore, we could show that the bacteria probably degrade the herbicide. Whole genome sequencing revealed that the glyphosate-resistant bacteria belong to the genus *Burkholderia*. The underlying molecular basis of glyphosate resistance of the novel *Burkholderia* species will be presented.

EMV24

Degradation of azo dyes using native and mutated azo reductase of *Rhodococcus opacus* 1CP

A. C. Ngo*[1], D. Tischler[1]
[1]*Ruhr-Universität Bochum, Microbial Biotechnology, Bochum, Germany*

Synthetic dyes have severe impact to the health and environment. Physical and chemical treatments are now used to mitigate the problem, but these options can be expensive and may lead to secondary pollution. Microorganisms have now been tapped as an alternative strategy through adsorption or use of enzymes. However, adsorption requires large quantities of biomass to be effective and disposal is another drawback. Meanwhile, enzymatic degradation leads to destruction of original structure of azo dyes achieving complete mineralization of the compounds. *Rhodococcus opacus* 1CP and related Actinobacteria are known for its capability in biotransformation of xenobiotics as such azo dyes. Studies have already been conducted to assess its azo reductase activity. However, its mechanism and substrate preference is not well understood and therefore, a number of mutants were generated and characterized.

This study, therefore, investigates AzoRo from *R. opacus* 1CP in its mutated form to degrade various synthetic azo dyes. Mutations were introduced using site-directed mutagenesis basing the changes near the active site (R139X and A121X). Seven mutants were used in this study (40 to 110 µmol/min^{-1} L^{-1}). Results showed that the mutants have higher activity as compared to the native azo reductase. The best mutants were completely characterized using different buffers, pH, temperature, and product analysis after dye degradation. Therefore, HPLC analytics was employed. The overall activity is discussed in the course of a 3D homology model of AzoRo. This research will provide insights in understanding enzyme function and for further industrial application.

BTV17

Towards a competitive expression platform: A high-throughput screen for an enhanced unconventional secretion capacity in *Ustilago maydis*

K. Hußnätter*[1,2], M. Philipp[1,2], J. Stock[1], M. Terfrüchte[1,2], M. Feldbrügge[1,2], K. Schipper[1,2]
[1]*Heinrich Heine Universität, Institut für Mikrobiologie, Düsseldorf, Germany*
[2]*Bioeconomy Science Center (BioSC), Jülich, Germany*

Introduction: Proteins often have highly specific demands on their expression host and production conditions. Therefore, a broad variety of expression platforms is required to overcome existing bottlenecks. One promising candidate host is *Ustilago maydis*. This fungus is capable of secreting proteins via an unconventional secretion pathway circumventing *N*-glycosylation. Fusions of heterologous proteins to endogenous unconventionally secreted proteins allow hitchhiking of this mechanism: Heterologous proteins are co-exported avoiding devastating effects linked to unsuitable *N*-glycosylation patterns. Previous projects focused on increasing biosynthesis and secretion of heterologous proteins. Cultivation conditions were optimized and extracellular proteases were deleted to avoid product degradation. In combination, this led to yields of about 500 µg/L.

Objectives: Yields are still too low to compete with already established expression platforms. Hence, we now focus on elucidating the molecular details of unconventional secretion by developing a high-throughput screen for an improved unconventional secretion capacity.

Methods: A screening strain for unconventional secretion is UV-mutagenized. Extracellular activity of three internal reporters present in mutants is compared to the progenitor strain. Cells with enhanced extracellular reporter activity are further characterized.

Results and Conclusion: 40000 UV mutagenized cells were investigated qualitatively based on their extracellular reporter activity. 400 promising candidates were further investigated in quantitative liquid assays. Finally, 6 candidates showed an enhanced extracellular activity of all three reporter proteins. Whole genome sequencing should now elucidate mutations responsible for the beneficial effects.

BTV18

RGB-S Reporter: a novel multi-stress reporting tool for bacterial planktonic and biofilm stress sensing

A. E. Zoheir*[1], K. S. Rabe[1]
[1]*Karlsruhe Institute of Technology (KIT), Intstitute for Biological Interfaces (IBG-1), Eggenstein-Leopoldshafen, Germany*

Bacterial stress responses are an essential component of survival strategies in changing environments. Reporting stress responses help to understand how bacteria sense and react to their surroundings, and thus reduce these adverse effects, when employing bacteria in biotechnological settings. In addition, it could be used to measure the toxicity of the stressing agents.

In this study, we used synthetic biology designing tools to develop a novel three-color whole-cell biosensor, called RGB-S Reporter, for reporting: physiological stress, genotoxicity and cytotoxicity in three fluorescence colors: red, green and blue respectively. Stress-specific promoters and engineered fluorescent protein variants with non-overlapping spectra have been selected to design a transcriptional sensing system. By screening various chemical, physical and physiological stresses, the new biosensor, hosted in *E. coli*, enabled measuring the three mentioned stress categories simultaneously and independently, providing a reliable qualitative and quantitative stress assessment. The RGB-S Reporter showed compatibility to high throughput stress quantification in bulk solutions as well as single-cell level enabling a fast screening of environmental conditions. This new multi-stress biosensor has a wide range of possible applications in biotechnology and basic science research, as amongst them environmental toxicity assessment, bioprocess and fermentation optimization as well as studying bacterial stress in planktonic and biofilm life forms. Furthermore, combining the newly developed biosensor with advanced imaging systems to understand stress states in biofilms becomes feasible. 3D imaging of stressed *E. coli* biofilm by CLSM revealed natural heterogenic organization of stress responses within biofilm.

BTV19

Integrated omics: A tool for the discovery of biocatalysts

P. Busch*[1], M. Suleiman[1], C. Schäfers[1], G. Antranikian[1]
[1]*Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany*

Introduction: A closer look into microbial communities, with its diversity and physiology, has been accelerated and facilitated by optimizing next generations sequencing (NGS) methods and bioinformatic analysis. Nowadays, the use of integrated omics is a desirable approach for a more efficient in-depth analysis.

Objectives: In this study, enrichment cultures from an Azorean hot spring were used for the analysis of the microbial composition and its enzyme portfolio by an integrated omic approach of metagenomics and metatranscriptomics.

Methods and methods: An Azorean hot spring sample was used as inoculum for enrichment cultures, containing 0.75 % plant biomass as substrate. After incubation for 3 days at 60 °C, genomic DNA and total RNA were isolated, sequenced and analyzed.

Results: Diversity was conducted by the metagenomic dataset. Lower abundance of Archaea was obtained in the enrichment culture (5 %) compared to the original sample (15 %). The functional annotation of the enrichment culture showed 90,342 CDS of which 29,957 were assigned as hypothetical proteins and 28,112 were assigned to an EC class. The metatranscriptomic dataset was used to give all CDS a value determining the expression levels. Furthermore, genes with the highest expression value, of its own EC class, were chosen for cloning.

Conclusion: In this study an integrated omic approach was used to exploit the natural enzymatic potential of living organisms as well as the diversity of an enrichment culture, adapted to plant biomass. The multi-omic approach led to four genes with high expression levels within its own EC class. The genes were cloned and characterized. This strategy represents a more efficient and rapid methods to discover robust biocatalysts for biomass degradation.

BTV20

New versatile plasmids for genetic analyses of mycobacteria

P. Dornbusch[1], G. Landázuri[1], J. Blázquez[1], T. Munder[2], S. Gola*[2,1]
[1]*CNB-CSIC, Madrid, Spain*
[2]*Ernst-Abbe-Hochschule Jena - University of Applied Sciences, FB Medizintechnik und Biotechnologie, Jena, Germany*

Introduction: Among the members of the genus *Mycolicibacterium*, which are mostly environmental and non-pathogenic bacteria that were until recently assigned to the genus *Mycobacterium*, several species of interest for industrial applications can be found. These comprise, for example, pigment producers as *Mycolicibacterium phlei* or organisms, as *Mycolicibacterium chlorophenolicum*, that have specific metabolic capacities of interest for bioremediation. Yet, to investigate these potentially industrially relevant organisms, genetic tools are scarce.

Objectives: To pave the way for the exploitation of these unconventional mycobacteria, we aimed at developing new versatile genetic tools. In order to provide particularly a read-out for proliferation proficiency, the assembly of the cell division machinery was addressed by the localisation of essential cell division proteins.

Materials & Methods: New modular replicative and integrative plasmids that can be selected by zeocin and expression vectors encoding new fluorescent proteins were designed.

Results: Several unconventional mycobacteria that were up-to-now not genetically tractable could be transformed by our *Escherichia coli*/mycobacterial shuttle vectors. Genes for new fluorescent reporter proteins could also be expressed. Specifically, we localised the mycobacterial divisome, the multi-protein machinery whose assembly is essential for bacterial proliferation and that has been shown in mycobacteria to depend on the interaction of the proteins FtsZ and SepF.

Conclusion: This new set of plasmids is a useful genetic tool to address the biology of unconventional and potentially industrially important mycobacteria.

BTV21

Identifying and developing novel carbene-transferring enzymes

M. Weissenborn*[1]
[1]*Leibniz-Institut für Pflanzenbiochemie & MLU Halle-Wittenberg, Bioorganic Chemistry, Halle a. d. S., Germany*

In the Leibniz Research Cluster multifunctional micro production units shall be produced, which will serve to develop cell-free compound formations. As part of this cluster, we work on developing novel non-natural enzyme activities to be applied in this artificial cell systems – with focus on carbene-transfer reactions. To identify protein-scaffolds, which catalyse or enhance non-natural reactions, we have developed a medium/high throughput protocol including rapid multi-site mutagenesis (Golden Mutagenesis[1]), split-GFP, multi-injection GC/MS as well as R-based automated data evaluation. With this setup, all iron-containing proteins present in *Escherichia coli* have been screened for carbene-based reactions. We have identified the dye-decolorizing peroxidase YfeX, which showed a promiscuous activity for a carbonyl olefination reaction with diazo-compounds as substrates.[2] The enzyme and its reaction has been further studied and could be improved by directed evolution. By investigating the reaction scope of YfeX in collaboration with the Koenigs-group (RWTH Aachen), an indole-functionalisation reaction with a novel diazoacetonitrile-compound could be shown with up to 200 turnovers.[3]

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BTV22

From microbes to microproduction units: Magnetically recoverable, thermally controllable and reusable biocatalyst for polyketide synthesis

J. Cui*[1], B. P. Krishnan[1], L. O. Prieto-López[1]
[1]*INM - Leibniz Institute for New Materials, Switchable Microfluidics, Saarbrücken, Germany*

Malonyl Coenzyme A (malonyl-CoA) and methylmalonyl-CoA are two of the most generally used building blocks for biosynthesis of polyketides which are structurally diverse large classes of natural products with a wide range of biological and pharmacological properties such as antibacterial, anticholesterol, antifungal, anticancer, antiparasitic and immunosuppressive properties. The class of bacterial malonyl-CoA synthetase enzyme catalyzes the production of malonyl-CoA and methylmalonyl-CoA directly from malonate and methylmalonate. However, enzymes usually have poor thermal and operational stability as well as limited reuse cycles, severely limiting their practical applications and thus requiring time to immobilize such an enzyme on the solid surface. In this presentation, I will introduce our work on the immobilization of MatB enzyme (malonyl-CoA synthetase) on the magnetic nanoparticles that were modified by a biocompatible polymer, poly(TEGMA) using atom transfer radical polymerization (ATRP) technique. I will also demo the recovery and reusability of FeO-poly(TEGMA)-MatB conjugate upon magnetic separation.

BTV23

Immobilization of enzymes in polysaccharide microgels for production of polyketide building blocks

C. Graf*[1], S. Höfgen[2], M. Männel[1], N. Hauck[1], A. Kaufmann[1], T. Köhler[1], V. Valiante[2], J. Thiele[1]
[1]*Leibniz Institute of Polymer Research Dresden (IPF), Polymer Micro(bio)reactors, Dresden, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology-Hans Knöll Institute (HKI), Biobricks of Microbial Natural Product Syntheses, Jena, Germany*

Secondary metabolites, as structurally complex polyketides, are assembled by consecutive steps of decarboxylative Claisen condensations. The enzymes catalyzing these condensation reactions, known as polyketide synthases (PKS), are commonly consuming coenzyme A (CoA)-activated acyl groups like acetyl-CoA and malonyl-CoA, which can be formed by acetyl-CoA synthetase and malonyl-CoA synthetase (MatB), respectively.

To support the cell-free synthesis of non-natural polyketides, which may serve as novel approach in drug development, we provide a microfluidics-based approach to synthesize key

building blocks, particularly malonyl-CoA in a tailored, cell-free environment. We prepare polymer microgels with tailored size and porosity by droplet microfluidics. The integration of a nitrilotriacetic acid (NTA) moiety loaded with nickel ions into the gel particles enables immobilization of His-tagged enzymes by forming a metal-chelate complex. To investigate the enzymatic activity, we establish an enzymatic assay based on pyrophosphate (PPi) detection as well as a HPLC-protocol monitoring the two-step reaction of MatB.

The MatB-functionalized microgels are filled into a microfluidic reaction chamber to perform the enzyme-catalyzed reaction to malonyl-CoA in a defined environment under continuous flow, where reaction conditions (e.g. temperature) can be tuned. Different binding studies indicate the high binding affinity of the His-tagged protein to Nickel-NTA-modified gels. By employing a fluorescent fusion protein of MatB, we can determine encapsulation efficiency and enzyme distribution within microfluidically prepared microgels via fluorescence intensity. The use of microreactors to study optimal conditions of immobilization efficiency and enzyme activity in the conversion of malonate to Malonyl-CoA is an important step towards the cell-free production of novel non-natural polyketides on the scale of a few microliters.

BTV24

Screening for novel protein synthesis inhibitors within the 'Tübingen strain collection'

F. Handel*[1], W. Wohlleben[1], Y. Mast[1]
[1]*Interfacultary Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbiology / Biotechnology, Tübingen, Germany*

Antibiotics are our most important weapon in the treatment of bacterial infections, including life-threatening hospital infections. Approximately 70% of all known antibiotics are produced by actinomycetes. The bacterial ribosome is a hotspot for the action of numerous successful antibiotics, like doxycycline, one of the most prescribed therapeutics in the United States. However, not all promising binding sites are therapeutically exploited. The aim of project TTU 09.812, funded by the German Center for Infection Research (DZIF), is to identify and characterize novel protein synthesis inhibitors.

The Tübingen strain collection harbors >2.000 natural product producers, mostly actinomycetes. Based on the genome sequence information of the about 100 sequenced strains, we established a computational screening approach to select for potential protein synthesis inhibitor producers. It uses the bioinformatic tools AntiSMASH[1] in order to identify potential antibiotic gene clusters and ARTS[2], which allows prioritization of gene clusters due to the occurrence of potential resistance genes. The selected candidate strains are grown in different culture media following the OSMAC strategy to allow antibiotic production. Culture extracts are analyzed in *in vitro* transcription/translation assays to confirm protein synthesis inhibitor activity and HPLC-MS/MS for secondary metabolite detection. Producers of unknown substances will be analyzed in detail.

With this strategy we were able to identify five protein synthesis inhibitors, which makes it an effective method to select possible producer strains of novel protein synthesis inhibitors.

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MTV01

The cryo-EM structure of respiratory complex I at work

K. Parey*[1], U. Brandt[2], H. Xie[3], D. Mills[4], K. Siegmund[1], J. Vonck[4], W. Kühlbrandt[4], V. Zickermann[1]
[1]*Goethe University Frankfurt, Medical School, Institute of Biochemistry II, Frankfurt a. M., Germany*
[2]*Radboud University Medical Centre, Department of Pediatrics, Nijmegen, Germany*
[3]*Max Planck Institute of Biophysics, Department of Molecular Membrane Biology, Frankfurt a. M., Germany*
[4]*Max Planck Institute of Biophysics, Department of Structural Biology, Frankfurt a. M., Germany*

Mitochondrial complex I is a 1 MDa membrane protein complex with a key role in aerobic energy metabolism [1,2]. Redox-linked proton translocation by complex I contributes about 40% of the proton motive force that drives mitochondrial ATP synthesis. We report the cryo-EM structure of complex I from the aerobic yeast *Yarrowia lipolytica* in the deactive form and captured during steady state turnover [3]. We provide evidence for an alternative binding position of ubiquinone and propose that a conformational switch of the ubiquinone reduction site is pivotal for energy conversion in the catalytic cycle of complex I.

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MTV02

Evidence for a switch in TatA interactions as trigger of Tat transport by TatABC systems

H. Geise*[1], T. Brüser[1]
[1]*Leibniz University Hannover, Institute of Microbiology, Hannover, Germany*

The twin-arginine translocation (Tat) system transports folded proteins across the cytoplasmic membrane of prokaryotes. In *Escherichia coli*, the Tat components TatA, TatB and TatC assemble to specific complexes that can bind Tat substrates. So far, no complex corresponding to the stage at which bound substrates are translocated has been identified. The two major complexes that can bind Tat substrates migrate at 440 and 580 kDa, as judged by BN-PAGE analysis, and the corresponding substrate-bound complexes migrate at 500 and 600 kDa, respectively [1].

We analyzed Tat complexes by using TatC variants carrying single amino acid exchanges by the photo-crosslinking amino acid *p*Bpa (*p*-benzoyl-L-phenylalanine). We found that one of these variants, TatC-I50*p*Bpa, caused the Tat complexes to shift to the size of approximately 660 kDa. *p*Bpa crosslinked neighboring TatC protomers, indicating that this region of the first periplasmic loop is involved in TatC-TatC interactions. However, the aromaticity of *p*Bpa rather than the crosslink induced the shift. A closer examination revealed that the complex depended on TatA association and contained no substrate. In the absence of TatA, the complex shifted to a smaller, substrate associated TatBC complex (TatBC+S). It is thus likely that the 660 kDa TatABC complex represents the stage at which transport has already

taken place. With TatC-I50*p*Bpa, the dynamic equilibrium between the complexes that occur during transport is altered, as TatA dissociation seems to be impeded. The tight association of TatA correlates with the disappearance of substrate, most likely due to transport.

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MTV03

Cryo-EM structures of KdpFABC reveal K^+ transport mechanism via two inter-subunit half-channels

C. Stock[1], L. Hielkema[2], I. Tascon[1], D. Wunnicke[1], G. T. Oostergetel[2], M. Azkargorta[3], C. Paulino[2], I. Hänelt*[1]
[1]Goethe University Frankfurt, Institute of Biochemistry, Frankfurt a. M., Germany
[2]University of Groningen, Groningen, Netherlands
[3]CIC bioGUNE, Proteomics Platform, Derio, Spain

KdpFABC is a key player in prokaryotic K^+ homeostasis[1]. When faced with externally low micromolar K^+ concentrations the constitutively expressed K^+ uptake systems KtrAB and TrkAH fail to maintain the intracellular K^+ concentration[2,3]. Instead, KdpFABC is produced, which actively transports K^+ into the cells. KdpFABC consists of four subunits and is often referred to as P-type ATPase, since subunit KdpB belongs to this superfamily. However, KdpB is believed to solely hydrolyze ATP, while K^+ translocation is accomplished by the channel-like subunit KdpA. The periplasmatically oriented single TM subunit KdpC, and the lipid-like single spanner KdpF complete the unique complex[4]. Till now, the coupling mechanism between the spatially separated energizing and transporting units remained elusive and various transport cycles have been proposed[4,5].

By single particle cryo-EM we solved two structures of the detergent-solubilized, inhibitor-stabilized KdpFABC complex at 3.7 Å and 4.0 Å resolution, respectively. In combination with pulsed EPR spectroscopy measurements the structures were assigned to an AMPPCP-bound E1 and an AMPPCP- and AlF_4^--bound E2-P conformation, respectively. Unexpectedly, the structures disclose a new translocation pathway through two half-channels along KdpA and KdpB, uniting the alternating-access mechanism of actively pumping P-type ATPases with the high affinity and selectivity of K^+ channels. This way, KdpFABC functions as a true chimera, synergizing the best features of otherwise separately evolved transport mechanisms.

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MTV04

Structural and functional analyses of a bacterial protein translocation domain that has adopted diverse pathogenic effector functions within host cells

A. Wagner*[1], N. Dietz[2], M. Huber[2], C. E. M. Seyfert[1], C. Tittes[1], T. Schirmer[2], C. Dehio[1]
[1]Biozentrum, University of Basel, Department of Microbiology, Basel, Switzerland
[2]Biozentrum, University of Basel, Structural Biology and Biophysics, Basel, Switzerland

Introduction: *Bartonella* spp. are facultative intracellular pathogens that can cause acute and chronic infections in mammals including humans. These pathogens translocate *Bartonella* effector proteins (Beps) via a type-IV-secretion (T4S)-system into the host to subvert the host cell metabolism. Most Beps (e.g. Bep1, BepA) are formed by a catalytic domain followed by a bipartite T4S-signal that is composed of a *Bartonella* intracellular delivery (BID)-domain and a C-terminal stretch. The BID domain is crucial for Bep translocation and is supposed to interact with the membrane-bound T4S-coupling protein (T4CP). Interestingly, BID domains are responsible for most known *Bartonella* mediated host cell subversions. For instance, the BID domain of BepA from *Bartonella henselae* inhibits apoptosis by interacting with human adenylyl cyclases (AC). Our overall objective was to structurally and functionally elucidate the dual role of the BID domain in T4S and apoptosis inhibition.

Results: Here, we for the first time biophysically show the interaction of a full-length T4S-effector- Bep1- with its cognate T4CP by using Microscale thermophoresis. Our data support the importance of the BID domain in the interaction with the T4CP. Furthermore, we present the crystal structure of Bep1, thereby revealing new structural features of the bipartite T4S-signal of Beps. We will also report on our progress to characterize the interaction of BepA-BID with human AC. Currently, we are deciphering the key elements of the BID domain in T4S (Bep1) and apoptosis inhibition (BepA) respectively, by using protein translocation- and cellular infection assays.

Conclusion: Summarizing, our study provides first strutural insights into how BID domains sustain a dual role in T4S and in effector function.

MTV05

Mechanisms of secretion substrate selectivity of the flagellar type-III secretion system

A. Guse*[1], M. Erhardt[1]
[1]Humboldt-Universität zu Berlin, Berlin, Germany

Question: The bacterial flagellum used for locomotion and the type-III injectisome used for translocating effectors into host cells are essential for *Salmonella*"s pathogenicity. Both share evolutionary and structurally related type-III protein secretion systems. Despite decades of research, it remains a mystery how the flagellar type-III secretion system selects few substrate proteins out of the many thousand cytoplasmic proteins and ensures correct hierarchy and stoichiometry of the exported substrate to assemble a functional flagellum.

Methods: We used bacterial genetics, microbiological and biochemical approaches as well as fluorescence microscopy to analyze the mechanisms of substrate selectivity for protein export via the flagellar type-III secretion system.

Results: The flagellar assembly hierarchy is divided into two modes of secretion as well as types of substrates, early and late. The early secretion mode comprises the assembly prior to hook completion, whereas the late secretion mode is enabled after hook completion and the thereby occurring substrate specificity switch. We investigated the mechanisms of substrate selectivity by developing a synchronized reporter strain and analyzed the secretion capability in early and late mode. We could show that the flagellar type-III secretion system secretes only early substrates in the early mode, but is able to secrete both substrate types, early and late, in the late secretion mode.

Conclusion: The flagellar type-III secretion system appears to widen its substrate recognition by including the late substrates in addition to the early substrates and does not differentiate entirely between the substrate classes as postulated before.

MTV06

Conformational flexibility of an ECF-type cobalt transporter as a basis for metal binding

F. Finkenwirth*[1], M. Gäde[1], C. Hermann[2], I. Orban[2], S. N. Pecina[1], A. Ricke[2], M. Sippach[2], H. J. Steinhoff[2], T. Eitinger[1]
[1]Humboldt-Universität zu Berlin, Biologie, Berlin, Germany
[2]Universität Osnabrück, Physik, Osnabrück, Germany

Energy-coupling-factor (ECF) transporters are an abundant class of prokaryotic ABC importers for micronutrients such as vitamins and the transition metal ions Ni^{2+} and Co^{2+}. ECF transporters share a common architecture comprising two ATPases, a membrane-embedded substrate-specific (S-) unit and a coupling membrane protein. Distinctive features of the metal-specific systems include an additional N-terminal helix in the S-unit of which Met1 and His2 provide three out of four metal ligands, and an additional membrane protein with an extensive extracytoplasmic loop. The latter (CbiN) is essential for transport activity of the $CbiMNQO_2$ Co^{2+} transporter but does not copurify with the $CbiMQO_2$ core complex. Previous work had uncovered that CbiN plus the S-unit CbiM form a minimal transport unit as individual proteins as well as in a fused state. Active Cbi(MN) exists in two SDS-resistant conformations which may represent stable conformers formed by dynamic transitions. Replacement of metal-coordinating residues in CbiM as well as deletions within the loop of CbiN eliminated one of the two states. As revealed by EPR spectroscopy upon site-specific spin labeling, deletions in the CbiN loop correlated with restricted flexibility of the CbiM N-terminal region. Distinct interactions between segments of the CbiN loop and small loops in CbiM, predicted in a structural model and confirmed by site-specific crosslinking, may contribute to opening and closure of the metal-binding pocket of CbiM.

MTV07

Identification of small proteins in the membrane-associated Proteome of *Pseudomonas aeruginosa* strain PA01

E. Lehmann*[1,2], M. Kucklick[1,2], J. Wissing[3], L. Jänsch[3], S. Fuchs[4], S. Engelmann[1,2]
[1]TU Braunschweig, Department of Microbiology, Braunschweig, Germany
[2]Helmholtz Centre for Infection Research, Microbial Proteomics, Brausnchweig, Germany
[3]Helmholtz Centre for Infection Research, Cellular Proteome Research, Braunschweig, Germany
[4]Robert Koch-Institut, Wernigerode, Germany

Previous annotation projects often neglected proteins smaller than 100 amino acids (aa). However, in the last few years a variety of small proteins with regulatory and virulence-associated functions have been detected, most of these being membrane associated. In the human pathogen *P. aeruginosa*, over 300 open reading frames encoding proteins with up to 100 aa (sORFs) are known but over 70% are still functionally uncharacterized.

To identify yet undiscovered sORFs in *P. aeruginosa* we started a proteogenomics approach. At first we established new *P. aeruginosa* protein databases for strain PA01 by using two different algorithms: six frame translation and sORF finder. For the first one we translated the whole genome sequence of *P. aeruginosa* PA01 into peptides of ten or more amino acids by using all six reading frames from STOP codon to STOP codon (TRDB). For the second we applied the sORF finder algorithm and used sequences of stable RNAs as a negative training set to predict the coding potential of intergenic regions. Afterwards, the predicted new sORFs were included in the PA01 protein database derived from NCBI (PRDB). To identify small proteins we than prepared the membrane proteome of strain PA01 under aerobic and anaerobic conditions using a GeLC-MS and Membrane Shaving approach combined with U(H)PLC-MS/MS analyses.

Using the GeLC-MS approach, we identified 39 small proteins of which 14 have not been annotated so far. Nine of these proteins were significantly changed during anaerobiosis: 1 small protein was strongly induced and another 4 were only identified under anaerobic conditions. Currently we are doing transcriptional analyses of the identified small proteins in addition to an interaction study with proteins important for denitrification.

MTV08

Functional characterization of a lipid anchored protein in *Staphylococcus aureus*

J. Bosselmann*[1,2], M. Kucklick[1,2], S. Engelmann[1,2]
[1]TU-Braunschweig, Institute of Microbiology, Braunschweig, Germany
[2]Helmholtz Centre for Infection Research, Microbial Proteomics, Braunschweig, Germany

Staphylococcus aureus is a human commensal bacterium whose lifestyle can become pathogenic, especially in immunocompromised patients, which can lead to skin infections, endocarditis, etc. Nowadays the ability of *S. aureus* to form biofilms and the increasing incidence of antibiotic resistant variants cause serious problems not only in hospitals. Therefore, it is essential to understand the biology of this bacterium. However, the bacterium still expresses many proteins with unknown functions. Discovering the functions of these proteins might have the potential to find possible new targets that can be used for novel pharmaceutical strategies. In this study, we characterize the functionally unknown protein NWMN_0364, which is lipid-anchored on the outside membrane and contains two PepSY domains. To estimate the potential function of this hypothetical protein, we generated a deletion mutant of the *S. aureus* Newman strain and analysed the extracellular proteins with a GeLC-MS/MS approach. With this method, we identified 157 proteins that are secreted or surface associated. Among them, the amount of 42 proteins was significantly repressed in the mutant strain for at least 1.5 fold. Interestingly, many virulence factors that are regulated by the two-component system SaeRS as well as substrate binding proteins of ABC-transporters were reduced in the deletion mutant. Moreover, an interaction study, based on a strep-tagged pull down in vivo, revealed possible interactions of NWMN_0364 with substrate binding proteins of ABC-transporters for Mn, Mo, methionine and amino acids. Additional growth experiments in Mn limitation showed a better adaptation of the mutant strain in contrast to the wild type. All these results point toward an ABC-transporter regulatory function of NWMN_0364.

AR-FPV01

Towards the catalytic and protection mechanism of [Fe]-hydrogenase

G. Huang*[1], T. Wagner[1], K. Ataka [2], E. Bill[3], U. Ermler[4], S. Shima[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Microbial protein structure research group, Marburg, Germany*
[2]*Freie Universität Berlin, Physics, Berlin, Germany*
[3]*Max Planck Institute for Chemical Energy Conversion, Mülheim a. d. Ruhr, Germany*
[4]*Max Planck Institute of Biophysics, Frankfurt a. M., Germany*

[Fe]-hydrogenase reversibly catalyzes the hydride transfer from H_2 to methenyl-tetrahydromethanopterin (methenyl-H_4MPT^+). Its iron-guanylylpyridinol (FeGP) cofactor plays a key role in H_2 activation. Under air, H_2O_2 is generated by the catalytic reaction and it decomposes the FeGP cofactor. O_2 reduction to H_2O_2 requires a reductant, which can be a catalytic intermediate transiently formed during the [Fe]-hydrogenase reaction. The most probable candidate is an iron-hydride species; its presence has already been predicted by theoretical studies of the catalytic reaction [1]. In addition, we report a natural protection mechanism for [Fe]-hydrogenase from *Methanothermobacter* against light and oxidative stress [2]. In the hexameric [Fe]-hydrogenase from *Methanothermobacter marburgensis*, an expanded oligomerization loop is detached from the dimer core and intrudes into the active site of a neighboring dimer. An aspartate residue from the loop ligates to Fe of the FeGP cofactor and thus blocks the postulated H_2-binding site. At higher enzyme concentrations, as present in the cell, the enzyme is predominantly in the inactive hexameric state thereby stabilized in a resting state. These experiments suggested that the iron site protected by hexamerization is the site for H_2 and hydride binding.

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[2] Wagner, T., Huang, G., Ermler, U. & Shima, S. How [Fe]-hydrogenase from *Methanothermobacter* is protected against light and oxidative stress. *Angew. Chem. Int. Ed.* 2018, 57, 15056-15059.

AR-FPV02

Deletion studies on putative pilin genes in *Halobacterium salinarum* R1

G. Losensky*[1], F. Pfeifer[1]
[1]*Technical University Darmstadt, Microbiology and Archaea, Darmstadt, Germany*

Introduction: *Halobacterium salinarum* R1 is able to form biofilms, *i.e.* multicellular communities attached to surfaces. Biofilm formation is initiated by adhesion of the cells, which is facilitated by specialized type IV pili. The *pil-1* locus is essential for the formation of the adhesion pili. It encodes an assembly ATPase and a transmembrane protein, but lacks genes coding for pilins, that are assembled into the filaments.

Objectives: We focused on the identification of the *Hbt. salinarum* R1 adhesion pilins.

Materials & methods: Bioinformatic analyses of the *Hbt. salinarum* R1 proteome were performed to identify putative type IV-like pilins. qRT-PCR was used to test the transcription of the pilin gene candidates in planktonic and adherent cells. Gene deletion mutants were generated based on a *pop-in*/*pop-out* strategy. Gene deletions were verified by PCR, sequencing and Southern analyses. The deletion mutants were characterized by microscopy, fluorescence-based adhesion assays and swimming assays.

Results: More than 30 proteins containing N-terminal type IV pilin signatures were identified. Several putative pilin genes showed increased transcript amounts in adherent cells. Single deletions of these genes were generated, as well as combinations of two or three genes together. While growth was not impaired in consequence of the gene deletions, various effects on motility, adhesion and biofilm formation were observed.

Conclusion: *Hbt*. contains a large number of putative pilin genes with so far unknown functions. Our gene deletion studies suggested roles associated with motility, adhesion and biofilm formation. However, further examinations are required, especially with regard to the constituents of the adhesion pili and the interplay between cell motility and adhesion.

AR-FPV03

Investigation of methanogen-induced microbiologically influenced corrosion (Mi-MIC) using simulated marine environments under flowing conditions

B. A. An[1], A. Hermann[1], S. Kleinbub[1], A. Koerdt*[1]
[1]*Federal Institute for Materials Research and Testing , Division Biodeterioration and Reference Organisms , Berlin, Germany*

Microbiologically influenced corrosion (MIC) of iron is usually attributed to sulfate-reducing microorganisms (SRM) in offshore industries, such as the oil and gas pipelines, due to the high concentrations of sulfate in the seawater. SRM act upon the metal by the reactiveness of hydrogen sulfide (HS^-), and by withdrawal of the available electrons ($Fe \rightarrow Fe^{2+} + 2e^-$; $E° = -0.47$ V) in electrical contact with the metal (EMIC). However, methanogenic archaea can also cause MIC. Due to, they do not produce corrosive metabolic products like hydrogen sulfide, withdrawal of electrons may be their main corrosive mechanism; however, mechanistic details and kinetics of the overall process are poorly understood.

To investigate the corrosion potential of methanogens, we studied the EMIC methanogenic strains isolated from marine sediments (*Methanobacterium*-affiliated strain IM1) and crude oil tanks (*Methanococcus maripaludis* Mic1c10), in an in-house developed flow-through cell to simulate a fluctuating environment. A co-culture of *M. maripaludis* and *D. alaskensis* was also established to study the effect of syntrophic growth on metal corrosion that may occur in industrial pipelines. Results indicate that the rates of iron corrosion due to coupled methanogenesis (up to 0.4 mm/yr) are higher to that caused by the marine SRM *Desulfovibrio alaskensis* (0.15 mm/yr). Surface analyses of the metal showed severe pitting with high methane production. Genomic analysis of the EMIC methanogen *M. maripaludis* Mic1c10 will provide an insight on the mechanisms of MIC. Such knowledge and deeper understanding also from an electrokinetic point of view may not only provide further models in microbial electrophysiology, but also contribute to mitigation strategies in MIC.

AR-FPV04

Assigning functions to unknown proteins in Archaea - α/β hydrolases from the Thaumarchaeon *Nitrososphaera gargensis*

J. Chow*[1], D. Danso[1], M. Ferrer[2], W. R. Streit[1]

[1]*Universität Hamburg, Microbiology and Biotechnology, Hamburg, Germany*
[2]*CSIC- Consejo Superior de Investigaciones Científicas, Institute of Catalysis, Madrid, Spain*

Archaeal genomes generally harbor large numbers of protein coding genes that do not fall in clusters of orthologous groups (COGs). Furthermore, annotations may not be fully correct and include many putative enzymes. The Thaumarchaeon *Nitrososphaera gargensis* Ga9.2 has 1,676 genes without known function (47% of its genome). In our study, we systematically analyze the function of genes encoding putative α/β hydrolases within this organism.

Therefore, putative ORFs were cloned and expressed in *E. coli* Rosetta-gami 2 (DE3) for detailed characterization, substrate fingerprinting and crystallization experiments. Two carboxylesterases, namely EstN1 and EstN2, had temperature optima at 40°C with an optimal pH at 7. On a collection of 96 substrates, they hydrolyzed over 16 short- to medium-chained esters (C2-C6). Interestingly, the two esterases did not show any homology based on blast-searches to already known esterases of other genera than *Nitrososphaera* and only low identity to putative α/β hydrolases from *N. evergladensis* SR1 and *N. viennensis* EN76. In search for cellular functions, both esterases were employed in genetic complementation tests of deletion mutants of various bacteria.

Surprisingly, EstN1 complemented *bioH*/*bioZ* deletions in *E. coli* and *Mesorhizobium loti* and provided first experimental evidence that Archaea carry functional biotin synthesis genes.

Concerning EstN2, immunofluorescence and transmission electron microscopy (TEM) with immunogold labeling indicates an intra- and extracellular localization in its native host *N. gargensis*. EstN2 expression in *Pseudomonas aeruginosa* as reporter strain strongly enhanced the pyocyanine production. The crystal structure of EstN2 was solved and diffracted X-rays to 1.5 Å resolution.

AR-FPV05
Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*
S. Bohnert[1], L. Antelo[1], C. Grünewald[2], A. Yemelin[1], K. Andresen[2], E. Thines[1,2], S. Jacob*[1,2]
[1]*Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Kaiserslautern, Germany*
[2]*Johannes Gutenberg-Universität (JGU) Mainz, Institut für Molekulare Physiologie, FB10, Mainz, Germany*

The question of how the remarkably diverse array of eukaryotic signaling networks may have evolved is of enormous scientific relevance. In this respect, we provide new insights into a rapid adaptation phenomenon of signaling networks in the filamentous fungus *Magnaporthe oryzae*. The "loss of function"(lof)-mutants of the high osmolarity glycerol (HOG) pathway *ΔMohog1*, *ΔMopbs2*, *ΔMossk2*, *ΔMossk1* and *ΔMoypd1* are impaired in osmoregulation and resistant to the fungicide fludioxonil. Long-term cultivation upon high osmolarity resulted in stable mutants arising as individuals restored in osmoregulation outgrowing from each of the lof-mutants. The major compatible solute produced by these "adapted" strains to cope with salt stress was found to be glycerol whereas it is arabitol in the wildtype strain. Interestingly, all of the "adapted"-mutants are again sensitive to fludioxonil, indicating that the HOG pathway or even its function has been rewired independently in each adapted strain. The phenomenon has been further investigated by DNA and RNA sequencing of *ΔMohog1(adapted)* resulting in a set of candidate genes may be responsible for the rewiring of the osmoregulation pathway. This is the first report of such a stable rapid adaptation by rewiring a signaling pathway by producing a different product, i.e. glycerol instead of arabitol. We aim to interact synergistically to combine expertise of theoretical approaches to integrate sequencing data from genomics and transcriptomics with modern quantitative (phospho)-proteomics techniques. Furthermore, reversed molecular genetics will be used to validate the candidate genes or even other factors (e.g. phosphorylation patterns) found to putatively promote or constrain rapid evolutionary adaptation.

AR-FPV06
Systematic analysis of cysteine residues within the preprotoxin of yeast viral killer toxin K1 reveals novel insights in heterodimer formation and immunity
S. Gier*[1], M. Lermen[1], M. J. Schmitt[1], F. Breinig[1]
[1]*Saarland University, Molecular and Cell Biology, Saarbrücken, Germany*

Killer toxin K1 is expressed and secreted by *Saccharomyces cerevisiae* strains co-infected with two double-stranded RNA viruses persisting in the cytoplasm of infected host cells. This A/B toxin acts as an ionophore by forming cation-selective pores into the plasma membrane and eventually disrupting the proton transmembrane gradient. Killer yeast necessitate a particular self-immunity mechanism against their own toxin as they possess the same target structures as sensitive cells. To date, neither the exact molecular mechanism underlying the toxic effect of K1 nor the molecular events critical for immunity have been elucidated completely. As the postulated disulfide bonds linking the K1 subunits α and β are solely based on sequence similarity and ultimately lacking experimental evidence, we aimed to investigate the precise cysteine pairing as well as their contribution to toxicity and immunity of K1-expressing yeasts. Therefore, mutational analyses were conducted using appropriate single and multiple cysteine mutants of the K1 precursor. Our findings suggest the formation of only one disulfide bond in the mature and biologically active toxin, thereby contradicting the current model. Interestingly, substitution of cysteines C95 and C107 in the α subunit of the precursor led to a loss of immunity, underlining the importance of this subunit not only in the toxic mechanism but also in the confirmation of proper immunity.

AR-FPV07
A novel player in archaeal cell-division: CdvD
M. van Wolferen*[1], G. Tarrason Risa[2], S. Schwarzer[1], B. Baum[2], S. V. Albers[1]
[1]*University of Freiburg, Molecular Biology of Archaea, Freiburg i. Br., Germany*
[2]*University College London, MRC Laboratory of Molecular Cell Biology, London, United Kingdom*

Within the domain of archaea, different cell division mechanisms can be found: Euryarchaeota divide in an FtsZ-dependent manner whereas most Crenarchaeota divide using the Cdv (cell division)-system. Interestingly, some of the Cdv-proteins are homologous to components of the eukaryotic ESCRT-III complex. Because of this evolutionary remarkable aspect, the Cdv-system triggered a lot of attention over the past decade.

The known components of the crenarchaeal divisome are: CdvA, an archaea-specific protein that recruits CdvB to the membrane; CdvB, an ESCRT-III homolog that forms a division ring; and CdvC a Vps4 homolog that triggers the disassembly of CdvB and thereby constricts the CdvB-ring. Remarkably, it was shown that the Cdv-system can be reconstituted in vitro, allowing the membrane constriction of giant unilamellar vesicles. The fact that this constriction occurred in a random manner led us to believe that in vivo, additional divisome components function in the correct positioning of the Cdv-system.

In our search for other divisome components in S. acidocaldarius, we discovered a conserved gene (now called cdvD) encoding a small membrane protein that shows a cyclic expression pattern throughout the cell-cycle. A deletion mutant of cdvD resulted in a widened cell-size distribution, and an unequal separation of the chromosomes, this phenotype could be complemented with the same gene on a plasmid. Using immunofluorescence localization we moreover observed atypical or asymmetric ring-formation of CdvB. These and other results suggest that in absence of CdvD, the cells can still divide, but in a random, often asymmetric fashion. We therefore propose that membrane protein CdvD is a novel player of the crenarchaeal divisome that plays a role in positioning the Cdv ring.

AR-FPV08

The AFP family of antifungal peptides – Similar, but not the same

S. Jung*[1], L. Heber[1], N. Paege[1], D. Warnecke[2], T. Utesch[1], M. A. Mroginski[1], V. Meyer[1]
[1]*Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany*
[2]*Universität Hamburg, Hamburg, Germany*

Objectives: Smarter antifungals, which affect multiple cellular targets, are one strategy to prevent resistance development. Of special interest for us is the exploitation of the AFP family of antifungal antimicrobial peptides including its founding member AFP from *Aspergillus giganteus*. This is a highly potent inhibitor of chitin synthesis and plasma membrane integrity in many human and plant pathogenic fungi without adverse effects to bacteria, plants or mammalian cells.

New results: We have so far identified more than 70 orthologs of AFP across species barriers in almost 60 different *Ascomycetes* species but also in plants. This number is still growing. Despite commonly shared structural characteristics family members show highly diverse modes of action, species specificities and biological activities. However, the analysis of the mode of action of the AFP led to the identification of specific membrane lipids whose modification mediates/prevents resistance to AFP in filamentous fungi and yeast. We could further improve the model describing the AFP-membrane interaction using molecular dynamics. We identified for the first time how a unifying structural theme which is present in thousands of antimicrobial peptides, the gamma-core motif, mediates membrane binding. Moreover, a transcriptomic meta-analyses of *afp* encoding genes predict a role for these peptides during slow growth, stress response and nutrient recycling. Comparable data exists for AFPs from plants.

Conclusions: Our observation prompts the idea that AFPs might actually act not only as defense but also as fratricide molecules (similar to the cannibal toxins of bacteria), facilitating the survival of *A. niger* and *A. giganteus*, **indicating** a novel way of fighting pathogenic fungi and resistance development.

MSV01

Where no sugar has gone before – The formation of an arginine glycoconjugate on the bacterial translation elongation factor EF-P

R. Krafczyk*[1,2], J. Macošek[3], P. K. A. Jagtap[3], W. Volkwein[1,2], D. Gast[4,2], S. Wunder[4,2], P. Mitra[5], A. K. Jha[5], J. Rohr[5], A. Hoffmann-Röder[4,2], J. Hennig[3], K. Jung[1,2], J. Lassak[1,2]
[1]*Ludwig-Maximilians Universität München, Biologie I/Mikrobiologie, Planegg-Martinsried, Germany*
[2]*Ludwig-Maximilians-Universität München, Center for Integrated Protein Science Munich, München, Germany*
[3]*Structural and Computational Biology, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany*
[4]*Ludwig-Maximilians-Universität München, Chemie, München, Germany*
[5]*University of Kentucky, Pharmaceutical Sciences, Lexington, KY, United States*

Glycosylation is a universal strategy to post-translationally modify proteins. We discovered a new form of N-linked glycosylation that activates the polyproline specific bacterial translation elongation factor EF-P. Reportedly, N-linked glycosylation occurs almost exclusively at asparagine residues. In contrast, EF-P is rhamnosylated on an arginine side chain. Our characterization of the EF-P arginine rhamnosyltransferase EarP aims to elucidate the molecular mechanism of this poorly understood form of protein glycosylation. We performed X-ray crystallography to analyze the structure of EarP from *Pseudomonas putida.* NMR spectroscopy was utilized to characterize the rhamnosylated product and to determine interaction sites. Bioinformatic and biochemical analyses were used to study structural and sequential prerequisites for donor- and acceptor interaction and catalysis. We show that EarP is an inverting GT-B glycosyltransferase that catalyzes the formation of α-rhamnosylarginine from TDP-β-L-rhamnose. The activated nucleotide sugar donor is bound within a conserved binding pocket in the EarP C-domain. The KOW-like EF-P N-domain of the acceptor is sufficient to be recognized by a mostly structure-driven mode of interaction. The inverting transfer reaction is catalyzed by two aspartate residues that activate the acceptor arginine by properly aligning it with the donor substrate. This reaction represents the first incident of arginine glycosylation in bacteria. Furthermore, EF-P is the first example of a cytosolic bacterial protein that is activated by glycosylation. Based on our findings, we propose a mechanistic model for inverting arginine glycosylation. As EarP is essential for pathogenicity in *P. aeruginosa,* our data provides the basis for targeted inhibitor design.

MSV02

Targeting ribosome rescue in bacteria: the proteomic response

M. Vázquez-Hernández*[1], A. Yayci[1], J. Alumasa[2], K. Keiler[2], J. Bandow[1]
[1]*Ruhr-Universität Bochum, Applied Microbiology, Faculty of Biology and Biotechnology, Bochum, Germany*
[2]*Pennsylvania State University, Philadelphia, PA, United States*

One of the most important cellular processes in all living organisms is Translation. However, as in all processes, things can go wrong; when this happens, ribosomes get stalled, unable to dissociate from the mRNA being translated. In these cases, the cells rely on to ribosome rescue, which results in the release of the ribosome, the tRNA and the mRNA. In the majority of bacteria, *trans*-translation is the main mechanism of ribosome rescue; in some bacteria, there is a backup system mediated by the ribosome rescue factor ArfA or ArfB. The impact on cellular physiology ribosome rescue systems are blocked is yet unknown. Several molecules called KKLs were developed for

a chemical biology approach to studying the physiology of ribosome rescue inhibition. Six KKLs (KKL-35, KKL-40, KKL-55, KKL-63, KKL-588, KKL-896), which primarily inhibit *trans*-translation, were used to study the proteomic response of *Bacillus subtilis* 168 when one of the mechanisms for ribosome rescue is blocked. 2D-gel based proteomics was used in conjunction with pulse-labeling to identify differentially regulated proteins. The Alkylhydroxiperoxidase reductase subunit related to redox homeostasis was upregulated in all cases. Among the marker proteins for all KKLs were proteins of the oxidative stress response and metal homeostasis. However, KKL-896, elicited a distinct proteomic response, including proteins involved in cysteine biosynthesis and the general stress response. Although these results provide first insights into the cellular response, we will complement this study with a gel-free LC-MS/MS analysis. A deep understanding of the physiology of ribosome rescue could open a new opportunity in antibiotic development, as ribosome rescue is essential for bacterial survival.

MSV03

Osmoadaptation in the halophilic bacterium *Halomonas elongata*: mechanosensitive channels are not essential for the export of ectoine

J. Vandrich*[1], G. Alfaro-Espinoza[1], H. J. Kunte[1]
[1]*Bundesanstalt für Materialforschung und -prüfung, Material und Umwelt, Berlin, Germany*

Changes in osmolarity require bacteria to adjust their internal solute pools to maintain turgor pressure. The halophilic bacterium *Halomonas elongata* produces the compatible solute ectoine, which allows the cells to thrive over a wide range of osmolarities. In general, mechanosensensitive channels (Msc) play an essential role in the regulation of turgor pressure in bacteria. Here, we investigated the role of the mechanosensitive channels for the survival of *H. elongata* under varying osmolarities and for ectoine export.

One MscK and three MscS were identified in *H. elongata* and their expression levels were determined under low, optimum and high salt. We found, that the expression of all Msc is severely reduced with rising osmolarity. Moreover,. *H. elongata* exhibited drastically lowered transcript levels of all Msc after a hyperosmotic shock, while a hypoosmotic shock had only minor influence.

For many bacteria, mechanosensitive channels are essential for survival of osmotic shocks. After deletion of the msc channels in *H. elongata* we found that, mutants lacking one of the channels survive hypoosmotic shocks, while the mutant lacking all four channels (Δ*msc*) had a reduced survival rate. In addition, the mutant Δ*msc* had an increased growth rate under high salt conditions in comparison to the wildtype. Interestingly, ectoine excretion under steady state conditions is only slightly reduced in this mutant and does not differ from the wildtype after an hypoosmotic shock.

In summary, the mechanosensitive channels seem to be a burden under high salt and are therefore less expressed and result in an advantage in growth when deleted. The Msc in *H. elongata* play a role in the constant outflow of internal solutes, however they are not the main route for the excretion of ectoine.

MSV04

Different strategies to defend against photooxidative stress in *Rhodobacter*

G. Klug[1], M. K. Licht*[1], A. Konzer[2]
[1]*Justus-Liebig-Universität Gießen, Microbiology and Molecular Biology, Gießen, Germany*
[2]*Max-Planck-Institute for Heart and Lung Research, Biomolecular Mass Spectrometry, Bad Nauheim, Germany*

Alphaproteobacteria of the genus *Rhodobacter* are Gram-negative, mostly aquatic species with high metabolic versatility. Their shifting between aerobic respiration and phototrophy needs tight gene regulation to avoid so-called photooxidative stress: excited bacteriochlorophyll a in the photosystems in *Rhodobacter* can transfer energy to molecular oxygen, producing cytotoxic singlet oxygen ($^1O_2$).

Our studies revealed surprising differences in the $^1O_2$-response between the closely related *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*. We aim to further investigate these differences to outline the $^1O_2$-response in *R. capsulatus*.

Exponentially growing *R. capsulatus*- and *R. sphaeroides*-cultures were shifted from semiaerobic conditions in the dark to various oxidative stress conditions, including photooxidative stress through aeration, metyhlene blue and high light (800 W m^{-2}). We analyzed samples of stressed cells via e.g. physiological assessments, omics technologies or zone of inhibition assays.

Compared to *R. sphaeroides*, *R. capsulatus* showed upon $^1O_2$-stress a similar growth rate while producing less reactive oxygen species (ROS). Species-specific traits could explain a more efficient ROS-defense in *R. capsulatus,* e.g. a stronger emphasis on quenching and proteolysis. A salient difference is the continuous accumulation of the cobalamin biosynthase protein CbiX in *R. capsulatus*. Another curiosity involves the sigma/anti-sigma factors RpoE/ChrR known to regulate the $^1O_2$-stress response in *R. sphaeroides*: their structures are very dissimilar to their homologues in *R. capsulatus* (25 % and 13 % identity), which could imply further differences in the stress response.

Further studies aim to place these findings in the context of the $^1O_2$-defense in *R. capsulatus.*

MSV05

The MhqR repressor confers resistance to quinone-like antimicrobials in *Staphylococcus aureus*

V. N. Fritsch*[1], V. V. Loi[1], T. Busche[1,2], A. Sommer[1], J. Kalinowski[2], J. Bernhardt[3], H. Antelmann[1]
[1]*Freie Universität Berlin, Berlin, Germany*
[2]*Bielefeld University, Center for Biotechnology, Bielefeld, Germany*
[3]*Universität Greifswald, Department for Microbial Physiology and Molecular Biology, Greifswald, Germany*

Introduction: *Staphylococcus aureus* is a major human pathogen which can cause several diseases, including life-threatening diseases. During infection, *S. aureus* has to cope with reactive oxygen and reactive electrophilic species (ROS and RES). ROS and RES can cause different post-translational thiol-modifications, such as reversible disulfide formation and irreversible S-alkylations. Quinones have antimicrobial properties and were shown to act as electrophiles and oxidants in bacteria.

Objectives: We aimed to elucidate the quinone stress response in *S. aureus.* The MhqR regulon was most strongly induced under methylhydroquinone (MHQ) stress in the

RNAseq transcriptome. Thus, we were interested in the role of MhqR in the quinone stress response and antimicrobial resistance in *S. aureus*.

Methods: We used genetic, biochemical and phenotype analyses to characterize the novel quinone-sensing MhqR repressor which regulates the *mhqRED* operon and confers quinone resistance in *S. aureus*.

Results: Transcriptional analysis showed that the *mhqRED* operon responds specifically to MHQ and to quinone-like antimicrobials, such as pyocyanin and ciprofloxacin. The *mhqR* mutant was resistant to MHQ, pyocyanin and ciprofloxacin. It was shown, that the non-conserved Cys95 of the MhqR repressor is not required for DNA binding and quinone sensing *in vitro* and *in vivo*. Phenotype analyses of another quinone-sensing regulator QsrR revealed that the QsrR and MhqR regulons confer independently quinone and antimicrobial resistance in *S. aureus*.

Conclusion: These studies identified a link between quinone and antimicrobial resistance via the MhqR repressor in *S. aureus*. Our current studies are directed to elucidate the quinone sensing mechanism of MhqR using biochemical and crystal structure methods.

MSV06

Deciphering antimicrobial drug combinations using high-throughput approaches

A. R. Brochado*[1], A. Typas[1]
[1]*University of Würzburg, Biocentre/ZINF, Würzburg, Germany*

Question: What drives synergy and antagonism in bacteria? My work focus on understanding the outcome of multidrug treatment using systems biology tools.

Methods: We profiled ~3000 pairswise drug combinations against 3 Gram-negative bacterial species: *Echerichia coli, Salmonella* Typhimurium and *Pseudomonas aeruginosa*.

Results: Synergy and antagonism are surprisingly species-specific, even though antibitic targets are highly conserved. Anatgonism is very prevalent and often related to decreased intracelular drug concentrations. We use our dataset to derive the mode of action of the food compound vanillin in bacteria. Finally, we found 8 new synergies with activity agains multi-drug resistant E. coli and Klebsiella pneumoniae in vitro and in simple infection models.

Conclusions: We generated the largest dataset on drug combinations against Gram-negative bacteria available to date, and uncovered new principles driving synergy and antagonism. We further estalished that combinatorial treatments are a real alterantive for fighting the currently antibiotic resistance scenario.

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MSV07

Synthesis, uptake and excretion of ectoines in the alpha-proteobacteria *Hyphomonas neptunium* and *Novosphingobium* sp. LH128

L. Czech*[1], S. Smits[2], E. Bremer[1]
[1]*Philipps University Marburg, Molecular Microbiology, Marburg, Germany*
[2]*Heinrich Heine University Düsseldorf, Biochemistry, Düsseldorf, Germany*

A broad range of bacteria either synthesizes the compatible solutes ectoine and hydroxyectoine *de novo* or employ specialized transporters for their uptake during high osmolarity conditions. Through bioinformatical analysis of all sequenced microbial genomes more then 4000 putative ectoine producing bacteria were identified (Czech *et al.*, 2018). A subset of these putative ectoine producers encodes different types of transporters in the direct gene neighborhood of the core genes (*ectABCD)* for the production ectoines. Using *Hyphomonas neptunium* and *Novosphingobium* sp. LH128 as model organisms, we analyzed the synthesis, uptake and excretion of ectoines as they possess novel types of *ect* gene clusters that harbor a different set of transporters, and in the case of *Novosphingobium* also a MscS-like mechanosensitive channel. Here we show that *H. neptunium* harbors a novel sodium solute superfamily importer with a broad substrate spectrum for a variety of compatible solutes (EctI), while *Novosphingobium* sp. LH128, a pure hydroxyectoine producer, employs a major facilitator superfamily importer with substrate-specificity for ectoine and hydroxyectoine (EctU). We were able to study the substrate binding site of EctI and EctU for the substrates glycine betaine, ectoine and 5-hydroxyectoine by modeling and docking of the putative 3D-structure, and through site-directed mutagenesis. In case of EctU we were able to show that a knock-out mutant in *Novosphingobium* sp. LH128 resulted in an hydroxyectoine secreting strain, highlighting the role of this specific importer in the recycling of the compatible solute hydroxyectoine.

Czech, L., Hermann, L., Stöveken, N., Richter, A. A., Höppner, A., Smits, S. H. J., Heider, J. & Bremer, E. (2018). *Genes (Basel)* **9**, 1–58.

MSV08

Homeostasis of the Escherichia coli biosynthetic metabolome under diverse conditions

D. Rados*[1], S. Donati[1], M. Lempp[1], H. Link[1]
[1]*MPI for Terrestrial Microbiology, Synmikro, Marburg, Germany*

Environmental conditions affect gene expression and protein abundance in microbes. A particularly strong relationship exists between the growth rate, promoter activity and protein levels. Here we examined if metabolites show a similar condition dependency.

For this purpose we used an LC-MS/MS method to quantify >100 primary metabolites in Escherichia coli in 19 environmental conditions.

The dataset covers all nucleotides, 19 out of 20 amino acids, as well as a large number of intermediates in central metabolism and biosynthetic pathways. Metabolite pools were stable across conditions, especially in the case of biosynthetic end-products (amino acids and nucleotides). Additionally, we found that nucleotide precursors accumulate in 2 out of 3 tested E. coli laboratory strains, due to a known bottleneck in pyrimidine biosynthesis.

These data highlight the homeostatic nature of metabolism under steady state growth conditions, provide a resource for basic research on bacterial physiology, and can inform metabolic engineering approaches.

SeSiV01

Cyclic nucleotides in Archaea: Cyclic di-AMP in the archaeon *Haloferax volcanii* and its putative role

F. Braun*[1], L. Thomalla[1], C. van der Does[1], T. Quax[1], T. Allers[2], V. Kaever[3], S. V. Albers[1]
[1]*Universität Freiburg, Biologie II, Freiburg i. Br., Germany*
[2]*University of Nottingham, School of Life Sciences, Nottingham, United Kingdom*
[3]*Hannover Medical School, Research Core Unit Metabolomics, Hannover, Germany*

The role of cyclic nucleotides as second messengers for intracellular signal transduction has been well described in bacteria. One recently discovered bacterial second messenger is cyclic di-AMP, which has been demonstrated to be essential in bacteria, pointing towards its involvement in vital functions of the cell. Compared to bacteria, significantly less is known about cyclic nucleotide second messengers in archaea, the third domain of life. This study presents the first evidence of the *in vivo* presence of c-di-AMP in an archaeal species.

Performing LC-MS/MS on whole cell pellet extracts the euryarchaeal model organism *Haloferax volcanii* was demonstrated to produce c-di-AMP under various growth conditions. Its genome encodes one diadenylate cyclase, named DacZ, which was shown to produce c-di-AMP *in vitro* using thin-layer chromatography and [α-32P]-labeled ATP as substrate. Similar to bacteria, the *dacZ* cyclase gene is essential and homologous overexpression of DacZ leads to cell death, suggesting the need for tight regulation of intracellular c-di-AMP levels. Such tight regulation often indicates the control of important regulatory processes. A central target of c-di-AMP signaling in bacteria is the osmotic homeostasis of the cell. The results presented here suggest a comparable function in *H. volcanii*. A mutant strain with an exchanged promotor for *dacZ* and thereby decreased c-di-AMP levels exhibited an increased cell area in media with low salt concentration, implying impaired osmoregulation.

In summary, this study expands the field of research on c-di-AMP and its physiological function to archaea. In addition, it indicates that the regulation of osmohomeostasis is likely to be a common function of c-di-AMP signaling in bacteria and archaea.

SeSiV02

The role of the c-di-GMP-specific phosphodiesterase NbdA in biofilm dispersal of *Pseudomonas aeruginosa*

K. Gerbracht*[1], M. Rüger[1], S. Zehner[1], N. Frankenberg-Dinkel[1]
[1]*Technische Universität Kaiserslautern, Microbiology, Kaiserslautern, Germany*

The opportunistic human pathogen *Pseudomonas aeruginosa* causes a broad range of acute and chronic infections often involving the formation of biofilms. In *P. aeruginosa* the c-di-GMP (bis-(3"-5")-cyclic dimeric guanosine monophosphate)-dependent switch between its sessile and motile lifestyle is triggered by several environmental cues, including non-lethal concentrations of nitric oxide (NO) [1]. The phosphodiesterase (PDE) NbdA (NO-induced biofilm dispersion locus A), consisting of the conserved domains MHYT-AGDEF-EAL, was found to play a role in NO-induced biofilm dispersal [2].

Functional complementation of an *E. coli* phosphodiesterase deficient strain with NbdA and a truncated NbdA variant confirmed PDE activity *in vivo*. Deletion of NbdA in *P. aeruginosa* did not impact motility or biofilm formation, whereas overexpression of the PDE revealed deficiencies in both, motility and biofilm formation. For better understanding of the NbdA function, regulation of gene expression was investigated using promoter *lacZ*-fusions in the background of several mutants strains and conditions. Expression of *nbdA* only occurs under aerobic conditions and is induced in stationary growth phase due to regulation by the alternative sigma factor RpoS. The obtained data will be presented and discussed with respect to the current model of NO-induced biofilm dispersal in *P. aeruginosa.*

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[2] Li Y, *et. al.* (2013)

SeSiV03

GGDEF-domain of PdeB governs its polar localization and mediates the interaction with the polar landmarkprotein HubP

T. Rick*[1], F. Rossmann[2], D. Mrusek[3], G. Bange[3], K. Thormann[1]
[1]*JLU, Department for molecular microbiology, Gießen, Germany*
[2]*Imperial College, Department of Life Sciences, London, Germany*
[3]*Philipps University Marburg, Marburg, Germany*

Bacterial cells maintain a high degree of intracellular organization, which requires the spatial and temporal organization of cellular components. One of the most important ways to obtain this strictly regulated organization is the recruitment of proteins by so called landmarkproteins such as HubP, which localizes not only the chemotaxis system but also a variety of other protein complex to the cell pole. A strict arranging is especially crucial in signaling pathways to assure proper signal transduction.

We show that the phosphodiesterase PdeB, which is critical for function and expression of the flagellar systems by altering the c-di-GMP level, localizes in a HubP dependent manner at the flagellated cell pole. Using truncated and tagged versions of PdeB we could show that PdeBs GGDEF-domain is crucial for this process. Additionally we were able to show that this localization is HubP dependent and that PdeBs GGDEF-domain interacts with HubPs C-terminal FimV-domain. A mutation of the GGDEF-motif didn"t lead to a delocalization of PdeB but altered the affinity to HubPs FimV-domain drastically, leading to the hypothesis that PdeBs localization may be controlled by c-di-GMP binding to its GGDEF-domain.

SeSiV04

CyaC, a respiration linked and redox controlled AC of *Sinorhizobium meliloti* with a succinate dehydrogenase-like membrane anchor

J. Wissig*[1], J. Grischin[2], J. Schultz[3], G. Unden[1]
[1]*Johannes Gutenberg University, Institute of Molecular Physiology, Mainz, Germany*
[2]*Max Planck Institute for Developmental Biology, Proteine Evolution, Tübingen, Germany*
[3]*Eberhard Karls University, Pharmaceutical Institute, Tübingen, Germany*

ClassIII-adenylate cyclases are known from eukaryotes and prokaryotes, but function and regulation of the prokaryotic class III AC has not been characterized in detail (1). Adenylate cyclase CyaC belongs to the IIIb subgroup and is one of 26 adenylate cyclases in *Sinorhizobium meliloti*. The 6TM region contains the tetra-histidine signature that is present in the membrane anchor of bacterial diheme-B succinate-quinone oxido-reductases (SQOR) (2,3).

Detergent solubilized CyaC was purified. It contains >1.4 mol heme B/ mol CyaC. The heme is oxidized or reduced by dithionite and ferricyanide including ubiquinone analogs (Q_0 or Q_0H_2). In vivo, CyaC is able to confer cAMP production and stimulation of β-galactosidase expression to a strain of *E. coli* deficient for *E. coli* adenylate cyclase.

Mutational replacement of the His residues resulted in the loss of heme B in isolated CyaC and inactivates CyaC (cAMP production) *in vivo* and in membranes. In membranes, CyaC activity responds to oxidation and reduction by quinone analogs, indicating, that the 6TM anchor is redox controlled by quinones.

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(3) Lancaster CR (2002) Biochim. Biophys. Acta. 1565: 215-31.

SeSiV05

Inter-kingdom-signaling by *Photorhabdus luminescens* - sensing eukaryotic hosts by PAS4-LuxR receptors

J. Brehm*[1,2], N. Neubacher[3], H. B. Bode[3], R. Heermann[1,2]
[1]*Johannes Gutenberg-Universität Mainz, Institut für Molekulare Physiologie, Mikrobiologie, Mainz, Germany*
[2]*Ludwig-Maximilians-Universität München, Mikrobiologie, Martinsried, München, Germany*
[3]*Goethe-Universität Frankfurt, Fachbereich Biowissenschaften, Frankfurt a. M., Germany*

Many Gram-negative bacteria use a well-known cell-cell-communication system based on LuxR-type receptors detecting specific signaling molecules to regulate gene expression. These receptors contain a C-terminal DNA binding domain (DBD) and an N-terminal signal binding domain (SBD). The Gram-negative entomopathogen *Photorhabdus luminescens* harbors the remarkably high number of 40 LuxR-type receptors, most of them harbor a "PAS4"-SBD1. Knockouts of *PAS4-luxR* gene clusters showed a specific decrease in pathogenicity against larvae from different insect species. Therefore, the PAS4-LuxR receptors are supposed to play a central role in inter-kingdom-signaling between the bacteria and their eukaryotic hosts. The "PAS3"-SBD of the insect juvenile hormone binding regulator Met of *Drosophila melanogaster* is structurally very homologous to the "PAS4" domains of the LuxR solos Plu2019 and Plu2018. This fact suggested that the Plu2018/Plu2019 "PAS4" domains might also sense insect hormone-like molecules. Among others, *plu0258* was identified as a target gene of Plu2018 and/or Plu2019. P*plu0258* activity could be specifically induced by *Galleria mellonella* insect homogenate revealing that the signal, which is sensed by Plu2018/Plu2019, is derived from this eukaryotic host. We then isolated the Plu2018/Plu2019 signaling molecule from *G. mellonella* insect homogenate and curtailed the number of possible ligands to a few comparatively hydrophobic small molecules. Via mass spectrometry we found a small number of molecules in the mass range of 250-800 m/z, which are supposed to be hormones and are putatively sensed by the inter-kingdom-signaling PAS4-LuxR solos. Since PAS4-LuxR solos are also present in human pathogens, they might represent promising targets for novel antimicrobial drugs.

SeSiV06

P_{II} controls multiple nitrogen uptake systems in *Synechocystis* PCC 6803

N. Neumann*[1], B. Watzer[1], P. Spät[1], M. Koch[1], O. Hennrich[1]
[1]*University of Tübingen, Microbiology/Organismic interactions, Tübingen, Germany*

PII signal transduction proteins are widely spread among all domains of life where they regulate a multitude of carbon and nitrogen metabolism related processes (Chellamuthu et al., 2013; Forchhammer, 2004; 2008). In recent years, several studies demonstrated an involvement of the cyanobacterial P_{II} protein in regulation of ammonium, nitrate/nitrite and cyanate uptake (Chang et al., 2013). However, these informations are largely scattered in literature. In this study, we aimed to further investigate the dependence of P_{II} for nitrogen uptake regulation in the unicellular and non-diazotrophic cyanobacterium *Synechocystis* sp. PCC 6803. By performing pulldown and bacterial-two-hybrid studies in combination with physiological approaches we could demonstrate that P_{II} regulates ammonium uptake by interacting with the ammonium permease Amt1; similar to the regulation of the ammonium permease AmtB by the P_{II} homologue GlnK described for *E. coli* (Conroy et al., 2007). We could further clarify that P_{II} mediates the light and ammonium dependent inhibition of nitrate uptake by interacting with the NrtC and NrtD subunit of the nitrate/nitrite transporter NrtABCD. We were able to show that both of these interactions require the large surface exposed T-loop structure of P_{II}. In addition, we also identified the ABC-type urea transporter UrtABCDE as a novel P_{II} target by showing an interaction with the UrtE subunit in a T-loop independent manner. Overall, this study underlines the great importance of the P_{II} signal transduction protein in the regulation of nitrogen utilization in cyanobacteria.

SeSiV07

Ratiometric population sensing by the RapA-PhrA "quorum" sensing system in *Bacillus subtilis*

H. Babel[1,2], P. Naranjo*[1,3,2], S. Trauth[1,2], S. Schulmeister[2], G. Malengo[1], V. Sourjik[1], I. Bischofs-Pfeifer[1,2]
[1]*Max Planck Institute for Terrestrial Microbiology, AG Bischofs, Marburg, Germany*
[2]*Heidelberg University, BioQuant & ZMBH, Heidelberg, Germany*
[3]*Pontifical Catholic University of Ecuador, Microbiology, Quito, Ecuador*

Cell-to-cell communication by diffusible signaling molecules facilitates higher level organization of cellular populations in time and space. Gram-positive bacteria use signaling peptides which are either sensed at the cell surface or probed by intracellular receptors after being pumped into the cytoplasm. While the former can be used for monitoring cell density, the functions of pump-probe networks are less clear. Here we show that pump-probe networks facilitate proportional, integral and ratiometric signal integrations. We used FRET to study signal processing by the prototypical PhrA-RapA system in *Bacillus subtilis* and quantified its extra- to intracellular signal conversion and signal transduction properties with the help of the pump-probe model. We find that the intracellular FRET response tracks fluctuations in extracellular PhrA concentrations rather

poorly; instead cells accumulate and strongly amplify the signal in a dose-dependent manner. This suggests that the PhrA-RapA system, and presumably others, have evolved to sense the community status of heterogeneous populations and infer the ratio of signal producing and non-producing cells.

SeSiV08

Modulation of virulence adaptation by the cytoplasmic membrane bound phospholipase PlaF disclosed by proteomics

M. Caliskan*[1], D. Waldera-Lupa[2,3], K. Stühler[2,3], F. Kovacic[1], K. E. Jaeger[1,4]
[1]*Heinrich Heine University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Universitaetsklinikum Düsseldorf, Institute of Molecular Medicine, Düsseldorf, Germany*
[3]*Heinrich Heine University, Biologisch-Medizinisches Forschungszentrum, Düsseldorf, Germany*
[4]*Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences (IBG-1), Jülich, Germany*

Phospholipases A (PLA) have essential functions for membrane and energy homeostasis. The mechanisms by which bacterial pathogens use intracellular PLAs to alter their own membranes for virulence adaptations are largely unknown.

Previously, we have identified the PLA PlaF as a novel virulence factor of *P. aeruginosa* with function for modification of bacterial membranes by hydrolysis of endogenous phospholipids. To understand the molecular response of *P. aeruginosa* to PlaF-mediated membrane alterations, we have performed a whole cell comparative-proteomics study of *P. aeruginosa* PA01 and a Δ*plaF* mutant strain grown in the biofilm. The results indicate that 422 proteins are differentially produced in these two strains with most of the dysregulated proteins having membrane-associated functions (transport, signal transduction, cell wall and membrane biogenesis).

Among strongly dysregulated proteins is a two-component signaling system, PprAB, a key regulator for the transition between planktonic and biofilm lifestyle. This, together with additional 36 dysregulated biofilm-related proteins might be an explanation for the lower amount of biofilm produced by the Δ*plaF* mutant. The proteomics analysis additionally revealed the dysregulation of 18 cell motility-related proteins providing a hint for the impaired swimming motility of this mutant. Finally, downregulation of proteins involved in pyoverdine biosynthesis and recycling in the Δ*plaF* mutant could be confirmed by quantification of extracellular pyoverdine and slower growth indicating a reduced pyoverdine-mediated iron uptake.

Our results suggest that the PlaF-mediated alteration of the membrane phospholipid composition modulates the production of proteins with function for virulence adaptation in *P. aeruginosa*.

MEV01

Abundance and diversity of *Bacteria* and *Archaea* within deep-sea sediments along a Pacific transect from New Zealand to Alaska

M. Pohlner*[1], J. Degenhardt[1], B. Wemheuer[2,3], B. Schnetger[1], B. Engelen[1]
[1]*University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment (ICBM), Oldenburg, Germany*
[2]*Centre for Marine Bio-Innovation, The University of New South Wales, Sydney, Austria*
[3]*Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Göttingen, Germany*

Environmental conditions shaping benthic microbial communities are mostly investigated at relatively eutrophic coastal sites or high-energy systems, e.g. hydrothermal vents. In contrast, information on the effect of abiotic factors in oligotrophic deep-sea sediments is still limited. Therefore, sediment samples (0 and 20 cmbsf) were collected during a unique transect along the 180° meridian from New Zealand to Alaska covering the main oceanic provinces of the central Pacific. We hypothesized distinct community compositions linked to nutrient availability within the sediments and productivity of the water column. Sediments were characterized regarding their geochemical composition and exoenyzme assays were performed to evaluate the microbial activity at the seafloor. Lowest cell numbers were found in the nutrient depleted mid-ocean gyres and increased towards the highly productive Bering Sea. Exoenyzme activities of aminopeptidase and phosphatase were also increased at sites with elevated nutrient concentrations. Sequencing of 16S rRNA genes and transcripts showed that *Bacteria* dominated the benthic communities over *Archaea* at most sites. Within the surface sediments at the two northernmost sites and the present communities of the deep samples, *Archaea* were more abundant. Members of the *Nitrosopumilaceae* (*Thaumarchaeota*) were predominant in the archaeal communities, while in the bacterial communities mainly *Betaproteobacterales* and *Caulobacterales* were found. The compositions of the bacterial and archaeal communities were both strongly influenced by the primary production within the water column. Our results provide new insights into bacterial and archaeal communities in Pacific sediments and the environmental factors affecting their diversity, distribution and function.

MEV02

Internal energy stores and secondary metabolites are physiological peculiarities for bacteria prevailing in long-term enclosure experiments

D. Herlemann*[1,2], S. Markert[3,4], C. Meeske[2], A. Andersson[5], C. Hentschker[6], F. Unfried[3,4], D. Becher[6], K. Jürgens[2], T. Schweder[3,4]
[1]*Estonian University of Life Sciences, Centre for Limnology, Vehendi küla, Estonia*
[2]*Leibniz Institute of Baltic Sea Research, Department of Biological Oceanography, Rostock, Germany*
[3]*University of Greifswald, Department of Pharmaceutical Biotechnology, Greifswald, Germany*
[4]*Institute of Marine Biotechnology, Symbiosis Group, Greifswald, Germany*
[5]*KTH Royal Institute of Technology, SciLifeLab, Stockholm, Sweden*
[6]*University of Greifswald, Department of Mass Spectrometry, Greifswald, Germany*

Enclosure experiments are frequently used to investigate the impact of changing environmental conditions on microbial assemblages. Yet, the question how the incubation itself challenges complex bacterial communities remains unanswered. We used metaproteomic profiling, 16S rRNA gene analysis and cell counts to monitor bacterial communities, derived from marine, mesohaline and oligohaline conditions, during long-term batch incubations.

Our results showed that early in the experiment, bacterial communities were highly diverse and their composition differed significantly between marine, mesohaline and oligohaline conditions. A manipulation of the enclosures with recalcitrant carbon (RC) showed differences to the control enclosures at the early phase of the experiment. In contrast, after 55 days, bacterial communities in both manipulated and original marine and mesohaline enclosures were more similar to each other but dominated by gammaproteobacterial *Spongiibacter*. In the oligohaline enclosure, the actinobacterial hgc-I was still abundant in the late phase of the incubation. Metaproteome analysis suggested that individual capacities, e.g. production of putative bioactive compounds and the ability to access alternative carbon sources, either by using internal energy stores (*Spongiibacter*) or the acquisition of RC (hgc-I) are important to successfully prevail during long-term incubations. Hence, carbon storage capacities strongly influence bacterial communities in long-term batch incubations experiments after labile carbon sources are extinguished. Enclosure experiments with complex natural microbial communities can be excellent tools, but species-specific properties like internal storage and production of secondary metabolites can cause manipulation-independent effects.

MEV03

Naturally isolated ecosystems help unraveling the impact of chemo diversity on microbial communities

L. Kroll*[1], V. Brauer[1], A. Akbari[1], H. Müller[1], R. U. Meckenstock[1]
[1]*University Duisburg Essen, Biofilm Centre - Aquatic Microbiology, Essen, Germany*

The world's largest natural asphalt deposit Pitch Lake in Trinidad and Tobago, harbors water droplets in microliter size (1-3 µL), originating from the oil reservoir deep below. These water droplets contain diverse and metabolically active microbiomes [1]. In this study we make use of this system to investigate the impact of chemo diversity on microbial communities. Chemo diversity is here understood as the concentrations of all measured anions (F-,HCOO-,Cl-,NO2-,Br-,NO3-,PO42-,SO42-) and cations (Li+,Na+,NH4+,K+,Mg2+,Ca2+) in each droplet. To this aim, 24 water droplets were isolated from different sampling sites of the Pitch Lake asphalt and analyzed in technical duplicatkes for their 16S rRNA gene composition by Illumina MiSeq platform and the mothur pipeline. Ion concentrations were measured via ion chromatography. Afterwards, multivariate statistics were applied to examine the correlation between sampling site, chemo diversity and single OTUs as well as the whole droplet community. For all of the 24 water droplets, more or less different patterns of taxonomic and ion composition were detected. Statistically significant correlation between the variables and OTUs was found, e.g. the relative abundance of OTU1 (family *Clostridiales Incertae Sedis XI* from the phylum *Firmicutes*) was correlated with sulfate concentration (coefficient= -0.3, $p < 0.05$). Furthermore, the relative abundances of 60 % of OTUs (with relative abundances > 0.1 %) was significantly correlated with the measured chloride level in the droplets. Our findings provide novel insights into these isolated ecosystems and into the role of chemo diversity on microbial community assembly.

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MEV04

Uncultured *Cloacimonadaceae* are abundant syntrophic propionate-oxidizing bacteria in anaerobic digestion

S. Dyksma*[1], C. Gallert[1]
[1]*Hochschule Emden-Leer, Mikrobiologie/Biotechnologie, Emden, Germany*

Propionate oxidation in methanogenic environments is a niche process that relies on microbial cooperation between syntrophic propionate-oxidizing bacteria (SPOB) and *Archaea*. Propionate can be a major intermediate in the carbon flow to methane; however, only very few SPOB have been isolated and described so far. In this study we enriched *Candidatus* Syntrophosphaera thermopropionivorans, a novel syntrophic bacterium affiliating with the candidate phylum *Cloacimonetes*. Methane production from propionate was almost stoichiometrically in the enrichment cultures, while *Ca*. S. thermopropionivorans contributed 63% to total bacterial cells. The draft genome (>95% complete) recovered from metagenomic analysis supported a syntrophic lifestyle and propionate oxidation using the methylmalonyl-CoA pathway. Phylogenetically, *Ca*. S. thermopropionivorans was most closely related to the uncultured *Cloacimonadaceae* W5-group that has been previously identified in oxygen minimum zones, sediments and anaerobic digestion systems. Sequencing of 16S rRNA gene amplicons from 12 full-scale biogas reactors together with a newly designed, genus-specific quantitative PCR assay revealed *Ca.* Syntrophosphaera/*Cloacimonadaceae* W5 as integral part of the syntrophic microbial community, outnumbering the usual suspects of SPOB such as *Smithella*, *Syntrophobacter* and *Pelotomaculum*. Our study provides new insights into the dark matter of the biogas microbiome by the identification of a novel player in syntrophic propionate metabolism that is abundant and widely distributed in anaerobic digestion.

MEV05

Antimicrobial screening and metabolomic profiling of the lichen *Usnea* from Malaysia and the Philippines and its associated endolichenic fungi

K. A. Santiago*[1], T. E. dela Cruz[2], R. Edrada-Ebel[3], Y. L. Cheow[1], A. S. Y. Ting[1]
[1]*Monash University Malaysia, School of Science, Bandar Sunway, Malaysia*
[2]*University of Santo Tomas, Biological Sciences, Manila, Philippines*
[3]*University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom*

Lichens and a group of microorganisms analogous to plant endophytes living within the lichen thalli, termed endolichenic fungi (ELF), are important producers of unique and chemically-diverse secondary metabolites. To identify novel and bioactive compounds from these microorganisms, a metabolomic approach was conducted. Here, three species of *Usnea*,*U. baileyi, U. bismolliuscula* and *U. pectinata*, were surface-sterilized and plated onto Malt Yeast Extract agar for the isolation of ELF. Of the 211 ELF isolates, nine commonly-occurring ELF together with their host lichens were extracted and selected for antimicrobial assay. Large-scale production of the secondary metabolites by the isolated ELF was done via solid-state fermentation. Both the lichen extracts and ELF crude culture extracts showed a broad-spectrum, moderate to high inhibitory activities against the test bacteria *S. aureus, B. cereus, E. coli* and *P. aeruginosa* and the yeast *Candida albicans*. Extracts with high bioactivities were fractionated and subjected to metabolomics analyses using high resolution LC-MS and NMR for dereplication purposes. A PCA model was constructed prioritizing *U. bismolliuscula* and the ELF *Xylaria venustula* based on their chemical profiles and bioactivities. OPLS-DA showed the presence of 8 and 9 metabolites,

some of which are reported for the first time. These may be responsible for the bioactivities exhibited by *U. bismolliuscula* and *X. venustula*. The potential of metabolomics approach in the discovery of novel natural products of lichen and lichen-associated fungi is highlighted by this study.

MEV06

C. elegans microbiome dynamics and the influence of predatory bacteria

J. Johnke*[1], P. Dirksen[1], H. Schulenburg[1]
[1]*CAU Kiel, Evolutionary Ecology and Genetics, Kiel, Germany*

The microbiome of natural isolates of the nematode *Caenorhabditis elegans* has been shown to be diverse and sample specific on the bacterial OTU level. In order to validate and expand these results we took *C. elegans* and associated substrate samples from the same location at multiple time points in 2016 and 2017. 16S amplicon sequencing revealed changes in the worm"s microbiome over time with Proteobacteria representing the dominant phylum. Bacterial community composition of the substrate differed from the worm"s microbiome composition and did not change substantially over time. Co-occurrence analyses revealed that OTUs from worm microbiomes show a higher percentage of significant negative co-occurrences than OTUs from substrate samples. Additionally, worm samples show a more neutral OTU composition than substrate samples. Additionally, we found that the majority of *C. elegans* microbiome samples contained predatory bacteria that belong to the *Bdellovibrio*-and like organisms (BALOs). Worms that contained BALOs were further characterized by an overall more diverse and more neutral microbiome. Lastly, worms fed with a fluorescently labeled food bacterium showed a dramatic decrease of labeled bacteria in their gut after only three hours of incubation with *Bdellovibrio bacteriovorus* HD100. BALOs can be therefore considered as predictors and potentially drivers of microbiome diversity and thereby host fitness in cases of *C. elegans*.

MEV07

Sorting phage-infected cells using direct-geneFISH and flow cytometry

J. D. Brüwer*[1], N. Heinzmann[1], L. Zeugner[1], A. Grieb[1], R. Amann[1], B. M. Buchs[1]
[1]*Max Planck Institute for Marine Microbiology, Molecular Ecology, Bremen, Germany*

Marine phages are diverse, outnumber their bacterial hosts by an order of magnitude in surface seawater, and are known to play a critical role in bacterial mortality. Despite their great impact, we have only started to record the phage host range and infection dynamics, as well as the influence on the host and, on a global scale, ocean biogeochemistry. The advances in super-resolution microscopy in combination with recent developments in labeling of individual genes by fluorescence *in situ* hybridization (direct-geneFISH), allow us to detect and visualize phage genes during the infection of bacterial host cells. We designed gene targeted polynucleotide probes to specifically target a phage that is recurring during spring phytoplankton blooms in the North Sea. We aim to follow infection dynamics of a respective isolate with direct-geneFISH and super-resolution microscopy. Using fluorescence-activated cell sorting (FACS), we intend to distinguish phage-infected from non-infected bacterial host cells from a pure culture and to sort for different infection states. To simulate for more complex systems, we will try to sort infected host cells from an artificial bacterial community. Future experiments on marine water samples will allow us to sort infected host cells from the sea providing us a powerful tool to study the impact of phages on their host *in situ*. Further, it can be used to target phages that are only known from metagenomes thereby revealing their bacterial hosts.

MEV08

The effect of smoking on the respiratory microbiom

S. Pfeiffer*[1,2], C. Herzmann[3,4], K. Gaede[5,6], S. Krauss-Etschmann[6,7,8], T. L. Consortium[9], M. Schloter[1,2,6]
[1]*Helmholtz Zentrum Munich, COMI - Department of Comparative Microbiome Analysis, Neuherberg, Germany*
[2]*Technical University Munich, ZiEL - Institute for Food & Health, Freising, Germany*
[3]*Research Center Borstel, Leibniz Lung Center, Center for Clinical Studies, Borstel, Germany*
[4]*German Center for Infection Research (DZIF), Clinical Trials Unit, Borstel, Germany*
[5]*Research Center Borstel, Leibniz Lung Center, BioMaterialBank North, Borstel, Germany*
[6]*German Center for Lung Research (DZL), Airway Research Center North (ARCN), Borstel, Germany*
[7]*Research Center Borstel, Leibniz Lung Center, Early Life Origins of Chronic Lung Disease, Borstel, Germany*
[8]*Christian-Albrechts-Universitaet zu Kiel, Institute for Experimental Medicine, Borstel, Germany*
[9]*Lung Microbiome and Environment, a consortium of the German Leibniz Association, Members of the LuMEn Consortium are Draginja Kovacevic (Research Center Borstel), Wiebke Behrens, Hiren Ghosh, Jörg Overmann (all Leibniz Institute DSMZ, Braunschweig), Mehul Chhallani, Christina Wichmann (both Leibniz Institute of Photonic Technology, Jena), Petra Rösch (Friedrich-Schiller-University Jena), Borstel, Germany*

Recent studies using culture-independent, high-throughput microbiological sequencing techniques, revealed that the human lung harbors a complex microbial community. The aim of "LuMEn" is to reveal the dynamics and epithelial interactions of bacteria in the respiratory system of healthy individuals in response to one of today's major health challenges: tobacco smoking. We examined the bacterial community structure of the upper respiratory system using nasal swabs (NSW) and oropharyngeal swabs (PHS), and the lower respiratory system via bronchoalveolar lavage (BAL), in a group of healthy smokers, ex-smokers, and never-smokers through the application of Illumina MiSeq 16S rRNA gene amplicon sequencing. Despite personal responses, our findings revealed an inner structure of the respiratory microbiome that included core microbial genera which were found consistently in NSW, PSH and BAL samples. Further, we identified genera and OTUs of the Proteobacteria that were enriched or exclusively found in BAL. We observed different influences of cigarette smoke on the bacterial community structure of NSW, PHS and BAL. While the NSW microbial community was strongly influenced by the current smoking status, changes in bacterial PHS community were driven in addition by smoking intensity. We discovered an enrichment of gammaproteobacterial and betaproteobacterial genera (*Acinetobacter, Stenotrophomonas*, *Serratia* and *Cupriavidius*) in correlation with smoking duration and intensity. We could show for the first time that smoking changes the microbial composition of lungs of healthy adults over the long term, leading to an enrichment of genera that were linked with the progression of lung diseases such as COPD.

BC-LPV01

Identification of a novel cell division protein in Gram-positive bacteria

S. Wamp*[1], J. Rismondo[1,2], L. Möller[3], S. Halbedel[1]
[1]*Robert Koch-Institute, Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany*
[2]*Imperial College London, Section of Microbiology, London, United Kingdom*
[3]*Robert Koch-Institute, ZBS4 - Advanced Light and Electron Microscopy, Berlin, Germany*

The late cell division protein GpsB is required for cytokinesis and cell wall biosynthesis of the human pathogen *Listeria monocytogenes.* These effects are explained by an interaction of GpsB with the major penicillin binding protein PBP A1, which is responsible for polymerization and cross-linking of the peptidoglycan strands during cell wall biosynthesis. We noticed that a *L. monocytogenes* Δ*gpsB* mutant rapidly acquires mutations suppressing the Δ*gpsB* phenotype. Genome sequencing of selected *gpsB* suppressor mutants led to the identification of hitherto uncharacterized genes putatively associated with GpsB function.

One out of the analyzed *gpsB* suppressor genes turned out to affect cell division, leading to clearly elongated cells upon deletion in the Δ*gpsB* background. This phenotype could be complemented by introducing an IPTG-inducible allele of either one of the genes, which was verified by cell length measurements. Further results show that the way of suppression exerted by this gene is different from previously known mechanisms of Δ*gpsB* phenotype suppression. Combination with clean deletions of other cell division proteins identify genetic interactions of this novel gene and led to the assumption of an involvement in processes related to the Min-system. This gene is widely conserved among the bacteria and recent results on the genes role in division site selection and cell division as well as the new mechanism of Δ*gpsB* phenotype suppression will be presented here.

BC-LPV02

Microindel mutations caused by intragenomic or horizontally acquired DNA single-strands

K. Harms*[1]
[1]*UiT-The Arctic University of Norway, Department of Pharmacy, Tromsø, Norway*

Mutagens are agents that can cause inheritable genomic changes. We found that single-stranded (ss) DNA molecules can cause mutations by local annealing with fully heterologous genomic DNA at microhomologies and subsequent genomic integration in the course of DNA replication, acting as primer for Okazaki fragments. These integrations by "short-patch double illegitimate recombination" (SPDIR) result in clustered nucleotide polymorphisms or microindels (about 3 to 80 bp exchanged). In the bacterial model organism *Acinetobacter baylyi* ADP1, such mutations are rare (<10-12 per genome and locus). In mutants lacking ssDNA-binding functions (RecA, DprA) or cytoplasmic ssDNA exonucleases (RecJ, ExoX), SPDIR-mediated mutations are increased (>7,000-fold in a *recA recJ exoX* triple mutant). These results suggest a physiological function for the ubiquitous, natural transformation- mediation protein DprA in non-transformable bacteria, avoiding illegitimate recombination with ssDNA of intragenomic origin. Moreover, genotoxic stress such as exposure to ciprofloxacin (sub-MIC) or UV light increase SPDIR mutations by orders of magnitude. *A. baylyi* can take up free DNA from the environment, and uptake of isogenic or heterologous DNA also increase SPDIR mutations by up to 50-fold. The taken-up DNA can be frequently integrated at microhomologies, confirming that natural transformation can be mutagenic through the SPDIR pathway.

SPDIR mutations, although rare and typically more destructive than SNP mutations, can help exploring the fitness landscape and may be a previously unrecognized force in evolution.

Pairwise comparisons of closely related genomes suggest that SPDIR is a common mutation mechanism in prokaryotes and eukaryotes and may be involved in cancer progression.

BC-LPV03

***Piscirickettsia salmonis* – insights into the biology of an important fish pathogen through comparative genomics**

I. Schober*[1], B. Bunk[1], T. Riedel[1], C. Spröer[1], J. Overmann[1]
[1]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*

The marine Gammaproteobacterium *Piscirickettsia salmonis* currently causes substantial commercial losses in the salmon farming industry, particularly in Chile. This fish pathogen is the causative agent of salmonid rickettsial septicemia or piscirickettsiosis, a disease with high mortality in different salmon species. Even though it has been thirty years since the first isolation and characterization of the pathogen, the evolutionary mechanism underlying its diversity, different virulence and antibiotic resistances are still only poorly understood. We therefore sequenced the complete genomes of 21 Chilean and one Norwegian *P. salmonis* strain which, together with 19 available public genomes allowed us to perform a detailed comparative *in silico* analysis of the largest set of closed genome sequences so far available. All Chilean strains form two phylogenomically clearly separated groups, the EM and the LF genogroup. No geographical separation could be recognized between both genogroups. Whereas homologous recombination occurs only very rarely between the EM and LF genogroups, recombination was detected frequently within each group. Notably, the single isolate from Norway was related to the LF genogroup but formed a distinct lineage. This indicates that additional, so far unrecognized genomic diversity exists. While strains of the EM genogroup harbor two distinct Dot/Icm type IVB secretion systems as distinct virulence factors, the Norwegian strain and the LF genogroup strains contain an additional third one. The phylogenetic placement of the Norwegian isolate shows that the Chilean *P. salmonis* strains do not form a monophyletic group. This suggests that the splitting of the two genogroups occurred before the bacterium was introduced to Chilean waters.

BC-LPV04

Is growth and metabolism of *Prevotella bryantii* B_14 depending on short chain fatty acid supplementation?

A. Trautmann*[1], S. Deusch[1], J. Steuber[2], J. Seifert[2]
[1]*University of Hohenheim, Feed Gut Microbiota Interaction, Stuttgart, Germany*
[2]*University of Hohenheim, Microbiology, Stuttgart, Germany*

Prevotellaceae is the most abundant bacterial family of the rumen microbiota and is described to be one of the enterotypes found in the human gut and oral cavity. Metabolisms and growth requirements like the need for short chain fatty acids (SCFA) are still not fully understood. The focus in this study was to cultivate *Prevotella bryantii* B_14, an isolate of the cow rumen, in defined media containing single SCFAs. Growth characteristics and changes in the proteome

were investigated. SCFA consumption and production was determined by gas chromatography measurements. The proteome was analyzed by processing the data of tandem mass spectrometry via label-free-quantification. The combined results gained from proteomics and cultivation data showed changes in the growth and acetate production depending on the SCFA used in the media. The proteomics data indicate metabolomic changes accompanied by diverse shifts in the enzymatic repertoire of the primary metabolism and outer membrane proteins. The data showed that SCFAs are essential for the growth of *P. bryantii* B14 more in a sense of micronutrients instead of a macronutrient or energy source.

BC-LPV05
The structure of the archaellum machinery investigated by high-resolution cryoEM
B. Daum*[1]
[1]*University of Exeter, Exeter, United Kingdom*

Archaea move through and interact with their environment by means of specialised flagella-like filaments, called archaella. The archaellum machinery acts as a cellular multi-tool. Through ATP hydrolysis, the archaellum is capable of rapid gyration and thus allows cells to swim through liquid media. In its resting state, the archaellum filament serves as an adhesion organelle and enables biofilm formation and communication between cells. To understand the mode of action of this versatile and fascinating molecular complex, we have recently solved the in situ structure of the archaellum machinery from Pyrococcus furiosus, using a combination of cryo electron tomography, single particle cryoEM and helical reconstruction. Our research provides novel insights into archaellum assembly and its working mechanism, furthering our understanding of archaeal motility, adhesion, biofilm formation ad cell-cell communication.

BC-LPV06
Identifcation of new components of the Type IV pilus machine
A. Treuner-Lange*[1], Y. W. Chang[2], M. Herfurth[1], T. Glatter[1], G. Jensen[2], L. Søgaard-Andersen[1]
[1]*Max-Planck Institut für terrestrische Mikrobiologie, Abteilung Ökophysiologie, Marburg, Germany*
[2]*California Institute of Technology, Pasadena, TX, United States*

Bacterial Type IV pilus machines (T4PMs) are the strongest molecular machines known so far and are spanning the complete envelope of a Gram-negative bacterial cell. Using electron-cryotomography (ECT) we elucidated the architecture of the T4P machine in *M. xanthus* and mapped 10 well-known proteins of the T4P machine to this architecture. We found that the T4PM comprises an outer membrane pore, four interconnected ring structures in the periplasm and cytoplasm, a cytoplasmic disc and dome, and a periplasmic stem. One density could not be assigned to the known proteins and we asked if minor pilins and/or PilY1 proteins might be responsible for that density in the sub tomogram averages.

The genome of *M. xanthus* encodes three gene clusters, each containing at least four minor pilin genes and a *pilY1* gene. We systematically deleted minor pilin and/or *pilY1* genes and analyzed for T4P-dependent motility and T4P formation in the mutants. We learned that either cluster 1 or 3 is sufficient for T4P-dependent motility and T4P formation and that minor pilins and PilY1 proteins are essential for T4P-dependent motility and T4P formation in *M. xanthus*.

We used ECT on intact cells of a nine-fold minor pilin mutant, a triple *pilY1* mutant as well as a mutant expressing PilY1-sfGFP and analyzed for missing or additional densities in the sub tomogram averages. We also performed B2H-analyses as well as GFP- and Flag-trap approaches to study protein interactions.

Our data suggest that the major pilin PilA together with four minor pilins and a PilY1 protein are forming a complex which is essential for T4P formation and which is an integral part of the T4PM. The T4PM consists therefore of at least 15 proteins and might never be purifiable in a native state.

BC-LPV07
Analysis of allosteric interactions in a multi-enzyme complex by ancestral sequence reconstruction
M. Schupfner*[1], K. Straub[1], R. Merkl[1], R. Sterner[1]
[1]*Universität Regensburg, Biochemie Zentrum Regensburg, Regensburg, Germany*

Tryptophan synthase (TS) is a heterotetrameric αββα complex. It is characterized by the channeling of the reaction intermediate indole and the mutual activation of the α-subunit TrpA and the β-subunit TrpB via a complex allosteric network. We have analyzed this allosteric network by means of ancestral sequence reconstruction (ASR), which is an *in silico* method to resurrect extinct ancestors of modern proteins. In a first step, the sequences of TrpA and TrpB from the last bacterial common ancestor (LBCA) were computed by means of ASR. The corresponding LBCA TrpA and TrpB proteins were then produced in *Escherichia coli*, purified, and characterized. The results showed that LBCA TS is reminiscent of modern TS by forming a αββα complex with indole channeling taking place. However, LBCA TrpA decreases the activity of LBCA TrpB by a factor of 5 whereas, for example, the modern ncTrpA from *Neptuniibacter caesarensis* increases the activity of ncTrpB by a factor of 1000. In order to identify those amino acid residues that are responsible for this large difference, all six evolutionary TrpA and TrpB intermediates that stepwise link LBCA TS with *N. caesariensis* TS were produced and characterized. Remarkably, the switching from TrpB-inhibition to TrpB-activation by TrpA occurred between two successive TS intermediates. The comparison of these intermediates and the mutual exchange of residues by iterative rounds of site-directed mutagenesis allowed us to identify four (out of altogether 420) residues from TrpB that are necessary and sufficient for its allosteric activation by TrpA. Our results demonstrate that ancestral sequence reconstruction can efficiently identify residues essential for allosteric communication and contribute to our understanding of large multi-enzyme complexes.

BC-LPV08
Immunogold labelling of serial thin-sections for the high-resolution localization of bacterial proteins within single cells
I. Petersen[1], R. Schlüter[1], K. Riedel[1], J. Pané-Farré*[1]
[1]*University of Greifswald, Microbial Physiology and Molecular Biology, Greifswald, Germany*

Proper function of proteins is not only dependent on their timely expression but also on their correct location within the cell. A common strategy to visualize the location of proteins within cells is to fuse the protein of interest with a fluorescent tag, e.g. the green fluorescent protein, and image the cells with a fluorescence microscope. A possible limitation of that

strategy is that the GFP-tag can influence the function of the native protein including its catalytic activity, stability, the ability to interact with other proteins or even induce oligomerization.

A possibility to circumvent uncertainties introduced by the tag is the use of immunogold labelling of the wildtype version of the proteins and the analysis of thin-sections by transmission electron microscopy. Although this strategy benefits from higher resolution of the electron microscope compared to conventional fluorescence microscopy, spatial information is usually lost.

Therefore, we developed a protocol to generate 50 nm thick serial thin-sections of bacterial cells suitable for immunogold labelling and subsequent reconstruction of the spatial protein distribution within individual cells. We demonstrate the usefulness of this approach to provide evidence that the alkaline shock protein 23 (Asp23) of*Staphylococcus aureus*forms long oligomers *in vivo*.

YV-FG01

Specialized box C/D snoRNPs act as antisense guides to target RNA base acetylation

S. Sharma*[1]

[1]*Harvard Medical School, Massachusetts General Hospital Centre for Cancer Research and Department of Medicine, Boston, MA, United States*

Base-pairing of box C/D small nucleolar RNAs (snoRNAs) with the target RNA guides site-specific ribose methylation by fibrillarin (Nop1), a highly conserved methyltransferase. Some box C/D snoRNAs, like yeast U3, U14 and U8 in higher eukaryotes are not involved in methylation but are essential for pre-rRNA processing. Biochemical and computational analyses have led to the identification and characterization of most box C/D snoRNA targets. Several snoRNAs classified as "orphan" snoRNAs lack a guide sequence or have complementarity to RNA that is not characterized. Considering the recent number of studies underscoring association of box C/D snoRNAs with genetic disorders including Prader–Willi Syndrome (PWS), cancer and obesity, it is feasible that apart from ribose methylation and RNA processing, these snoRNAs are involved in several other cellular processes. Here, we report a novel and unexpected function for two yeast orphan box C/D snoRNAs, snR4 and snR45, in site-specific base acetylation. We demonstrate that these box C/D snoRNAs guide cytosine acetylation by Kre33, a factor essential for assembly and processing of the 90S pre-ribosome. Kre33 is known to acetylate a subset of tRNAs with the help of the adaptor protein Tan1. However, here we show Kre33 dependent acetylation of 18S rRNA is guided by orphan snoRNAs. Our results expand the functional repertoire of box C/D snoRNPs and highlight yet another remarkable cellular modularity, where a single enzyme targets different substrates by means of either protein or RNA adaptors.

YV-FG02

***Saccharomyces cerevisiae* hxt0 strains serve as research and high-throughput ligand screening platform for the human glucose transporters GLUT1-5**

S. Schmidl*[1], J. Tripp[1], C. V. Iancu[2], E. Boles[1], J. Y. Choe[2], M. Oreb[1]

[1]*Goethe-Universität Frankfurt am Main, Biowissenschaften, Frankfurt a. M., Germany*

[2]*Rosalind Franklin University of Medicine and Science, Department of Biochemistry and Molecular Biology, North Chicago, IL, United States*

Glucose transporters (GLUT, SLC2 gene family) mediate the uptake and therefore the first step of glucose usage in mammalian cells. Malfunction or altered expression pattern of GLUTs in humans is associated with several widespread diseases including cancer, diabetes and severe metabolic disorders. Their high relevance in the medical area makes these transporters valuable drug targets and potential biomarkers. However, few compounds that selectively manipulate GLUTs have been discovered, so far. The heterologous expression of certain human GLUTs in the *Saccharomyces cerevisiae* strain EBY.VW4000 which lacks all endogenous hexose transporters (hxt0) allows specific investigations on GLUT transport kinetics and offers a platform for a convenient and high-throughput screening system for GLUT-specific ligands. Functional expression of human GLUTs in the hxt0 yeast strain requires further modifications either in the genome of the host or in the transporter"s sequence. Therefore, two further evolved hxt0 yeast strains, carrying mutations that most likely affect the membrane lipid composition of the strain, were developed enabling the functional expression of GLUT1, GLUT3 and GLUT4. Furthermore, GLUT5 and most recently GLUT2 were successfully activated in the hxt0 yeast strain by insertion of moderate alterations in the amino acid sequence of the transporters. These successes will not only enable further investigations on the transporter"s kinetics and molecular characteristics but also push forward the development of pharmaceuticals for GLUT related diseases.

YV-FG03

Biotransformation of the pectin degradation product D-galacturonic acid to L-galactonate in *Saccharomyces cerevisiae*

S. Harth*[1]

[1]*Goethe Universität Frankfurt, Institut für Molekulare Biowissenschaften, Frankfurt a. M., Germany*

As one of the most abundant polysaccharides in nature and a waste product of sugar industry, pectin ranks as one of the major potential second-generation feedstocks (non-food plant material) for biotechnological production of different compounds, such as fuels, fine chemicals and additives for cosmetic and nutraceutical industry. Pectin mainly consists of α-1,4-glycosidically linked D-galacturonic acid units (D-GalA), which is naturally not metabolized by the prominent biotechnological production host *Saccharomyces cerevisiae*. We aim to engineer this yeast for biotransformation of D-GalA to L-galactonate (L-GalOA), which has potential applications as chelator, moisturizer, pH-stabilizer and leaving agent in food and cosmetic industry. This requires the expression of heterologous D-GalA transporters and reductases (Benz et al., 2014), but also extensive interventions into the central carbon metabolism of the host cell. Previous attempts to construct D-GalA utilizing *S. cerevisiae* strains have revealed that the higher oxidation state of D-GalA (compared to sugars) is one of the challenges for its funneling into the endogenous metabolism of yeast (Biz et al., 2016), since surplus reduction equivalents are required. These can be derived from the fermentation of sugars present in the pectin biomass, mainly glucose, galactose and arabinose by blocking the ethanol formation. In this way, a nearly complete valorization of the pectin feedstock, which is currently underused, can be achieved.

YV-FG04

Integrated stress response associated with loss of tRNA modification in yeast

A. Bruch*[1], T. Laguna[2], F. Butter[2], R. Schaffrath[1], R. Klassen[1]
[1]*Universität Kassel, Kassel, Germany*
[2]*Institute of Molecular Biology, Mainz, Germany*

Recent studies have shown that simultaneous removal of tRNA anticodon loop modifications such as 5-methoxycarbonylmethyl-2-thiouridine (mcm5s2U34), pseudouridine 38/39 (Ψ38/39) or cyclic threonyl-carbamoyladenosine (ct6A37) can induce synthetic negative phenotypes and translational infidelity in yeast. To shed light on these physiological changes, we further characterized proteomic and transcriptomic changes in the composite modification mutants, in particular those previously shown to affect decoding by tRNALysUUU or tRNAGlnUUG. While translational inefficiencies of mRNAs highly enriched for either of the cognate codon could be demonstrated, the most significant changes occurred at the transcriptional level. In addition to a defect in the unfolded protein response, the analyzed mutants surprisingly displayed integrated stress response signatures typical of higher eukaryotes; these include transcriptional upregulation of general amino acid control (GAAC), carbohydrate catabolite and nitrogen catabolite repressed (NCR) pathways irrespective of whether tRNALysUUU or tRNAGlnUUG were affected. Moreover, all composite modification mutants inadequately induced autophagy under nutrient replete conditions, suggesting that the modification states of different tRNAs are intimately linked with sensing of nutrient availability. Suppression of the transcriptional starvation responses was achieved by overexpression of hypomodified tRNAs in different modification mutants, indicating that the changes of the transcriptome likely occur in response to translational inefficiency. Our data thus suggest that tRNA modification defects trigger common starvation and stress signatures in a fashion independent of the decoding specificity of the hypomodified tRNAs.

YV-FG05

H/KDEL receptors mediate host cell intoxication by a viral A/B toxin in yeast

B. Becker*[1], M. J. Schmitt[1]
[1]*Saarland University, Molecular and Cell Biology, Saarbrücken, Germany*

A/B toxins such as cholera toxin, Pseudomonas exotoxin and killer toxin K28 contain a KDEL-like amino acid motif at one of their subunits which ensures retrograde toxin transport through the secretory pathway of a target cell. As key step in host cell invasion, each toxin binds to distinct plasma membrane receptors that are utilized for cell entry. Despite intensive efforts, some of these receptors are still unknown. Here we identify the yeast H/KDEL receptor Erd2p as membrane receptor of K28, a viral A/B toxin carrying an HDEL motif at its cell binding β-subunit. While initial toxin binding to the yeast cell wall is unaffected in cells lacking Erd2p, binding to spheroplasts and in vivo toxicity strongly depend on the presence of Erd2p. Consistently, Erd2p is not restricted to membranes of the early secretory pathway but extends to the plasma membrane where it binds and internalizes HDEL-cargo such as K28 toxin, GFP(HDEL) and Kar2p. Since human KDEL receptors are fully functional in yeast and restore toxin sensitivity in the absence of endogenous Erd2p, toxin uptake by H/KDEL receptors at the cell surface might likewise contribute to the intoxication efficiency of A/B toxins carrying a KDEL-motif at their cytotoxic A-subunit(s).

YV-FG06

Importance of diphthamide for translational accuracy and competitive cell growth in yeast

H. Hawer*[1], K. Ütkür[1], L. Adrian[1], R. Schaffrath[1]
[1]*Universität Kassel, Mikrobiologie, Kassel, Germany*

Diphthamide is a conserved posttranslational modification of translation elongation factor 2 (EF2) in archaea and eukaryotes. The name diphthamide refers to the lethal inactivation of EF2 upon ADP ribosylation by diphtheria toxin, which specifically targets the diphthamide residue and kills cells by inhibiting translocation during mRNA translation. Although this emphasizes a pathological role, precisely why cells need to make diphthamide, is not entirely clear. EF2 (*EFT1-EFT2*) and diphthamide (*DPH1-DPH7*) gene networks cluster within the genetic interaction landscape from *Saccharomyces cerevisiae*. In line with this, our assays show that reduced EF2 amounts (*eft2*Δ) combined with loss of Dph2 (*dph2*Δ), a factor mediating the first step of diphthamide biosynthesis, interferes with cell viability of the composite mutant (*dph2*Δ *eft2*Δ) under standard and stress conditions. Moreover, EF2 undersupply together with loss of diphthamide significantly reduces competitive growth of *dph2*Δ *eft2*Δ cells in relation to strains maintaining diphthamide modified EF2 pools. Measuring programmed -1 frameshift errors, we show that diphthamide modified EF2 significantly contributes to reading frame maintenance and translational accuracy and we propose that the synthetic growth phenotypes typical of the composite *dph2*Δ *eft2*Δ mutant likely results from impaired translational capacity and error-prone translation in tandem. Thus, under conditions of reduced translational capacity, cell viability becomes dependent on the diphthamide modification. Our data strongly suggest that apart from its pathological role, the EF2 modification with diphthamide is indeed physiologically relevant, most likely through positive effects on mRNA translation.

YV-FG07

Role of N1-methyladenosine 25S rRNA base modification in ribosome biogenesis and metabolism

K. D. Entian*[1]
[1]*Goethe Universität Frankfurt, Institut für Molekulare Biowissenschaften, Frankfurt a. M., Germany*

The entire chemical modification profile of ribosomal RNAs in yeast along with the corresponding enzymes and enzyme complexes has been recently identified. Nevertheless, in many cases the precise role of these chemical modifications in ribosome structure, function and regulation remains still an open question. Previously, we demonstrated that yeast Rrp8 methylates m1A645 of 25S rRNA in yeast. Here, using mung bean nuclease protection in combination with RP-HPLC and primer extension, we report that 25S/28S rRNA of *S. pombe*, *C. albicans* and humans also contain a single m1A methylation in the helix 25.1. Using complementation analysis and siRNA mediated depletion as well, we characterized nucleomethylin (NML) as a human homolog of yeast Rrp8 and demonstrated that NML catalyzes the m1A1322 methylation of 28S rRNA in humans. Our *in vivo* structural probing of 25S rRNA, using both DMS and SHAPE, revealed that the loss of the Rrp8 catalyzed m1A modification alters the conformation of domain I of yeast 25S rRNA, thus causing translation initiation defects – halfmers formation, likely because of incompetent loading of 60S on the 43S-preinitiation complex. Quantitative proteomic analysis of the yeast Δ*rrp8* mutant strain using 2D-DIGE, revealed that loss of m1A645 impacts specific set of proteins involved in carbohydrate metabolism, translation and ribosome synthesis. These findings suggest that Rrp8 mediates a cross-talk between ribosome biogenesis and

metabolism, a role which has also been shown for human NML. Apart from underscoring the role of m1A in maintaining optimum 60S conformation, our study highlights an inbuilt "allostery" in ribosome structure and function.

EMV-FG01

"The unique cell structure and genomic organization of the world's largest freshwater bacterium, *Achromatium oxaliferum*"

D. Ionescu[1], M. Bizic-Ionescu[1], N. De Maio[2], H. Cypionka[3], H. P. Grossart*[1,4]
[1]*Leibniz Institute of Freshwater Ecology and Inland Fisheries, Department of Experimental Limnology, Stechlin, Germany*
[2]*Oxford Martin School, University of Oxford, Institute for Emerging Infections, Oxford, United Kingdom*
[3]*University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany*
[4]*Potsdam University, Institute of Biochemistry and Biology, Potsdam, Germany*

Achromatium is the largest freshwater bacterium known and lives in sediments of lakes or estuaries located at the oxic-anoxic boundary. Like other large sulfur bacteria, *Achromatium* contains a high number of chromosomes (polyploidy) and in our study we counted up to 300 DNA spots per cell. Yet, it remains unclear whether each of the spots contains one or more chromosomes. Since it is not available in culture, our goal was to use metagnomics to get a better understanding on its physiology and ecological role in the environment. For this, we isolated and cleaned roughly 10,000 cells from a few grams of sediment from the shore of Lake Stechlin, Germany, and sequenced their metagenome. Thanks to our cleaning step the metagenome was almost free of non-*Achromatium* sequences even though these cells are typically covered with ectosymbionts. However, the *Achromatium* rRNA obtained from the metagenome suggested the presence of several clades spreading widely across the known *Achromatium* sequences. In addition, copies of protein-coding genes were as distant as if coming from different bacterial families rather than the same species. Gene synteny in operons was not always conserved, and a large number of transposases were identified. Using FISH revealed that most cells were stained with two or three different oligonucleotide probes indicating that they individual cells contain several 16S r RNA gene copies. Therefore, we sequenced a fragment of the 16S rRNA as well as the whole genome from individual cells. The results revealed that the 16S diversity was extremely large even after applying the most stringent bioinformatics algorithms. Additionally, copies of the same protein coding genes were as distant in single cells as across our metagenomic data. Based on our findings, we derived a hypothetical model that could explain this phenomenon relying on the physical separation of chromosome clusters inside each cell. Future studies including transcriptome analyses could reveal a deeper insight into the underlying molecular mechanisms allowing for the observed high genetic diversity through multiple but different copies of genomes in a single polyploidic bacterial cell. We propose that this form of polyploidy could be another, yet overseen, route to the emergence of multicellular organisms.

EMV-FG02

EMV-FG02

A new picture of major players in the nitrogen cycle: Genome resolved metagenomics as the key to surprising discoveries

H. Daims*[1]
[1]*University of Vienna, Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Vienna, Austria*

Nitrogen (N), an essential chemical element for all organisms, is made bioavailable and recycled into the atmosphere by microorganisms in the biogeochemical N-cycle. A major N-cycle process is nitrification, the oxidation of ammonia via nitrite to nitrate. Aside from being the main biological source of nitrate, nitrification is indispensable for wastewater treatment. However, it also causes N-loss from fertilized soils and contributes globally to nitrous oxide emissions. Traditionally, the nitrifying microbes were regarded as specialized organisms, whose activity and growth are exclusively linked to aerobic nitrification. Moreover, a paradigm of N-cycle microbiology stated that ammonia and nitrite oxidation are catalyzed by different microorganisms in cooperation. For decades, nitrification research in natural habitats, agricultural soils, and engineered systems relied on these firm assumptions.

Recent studies based on genome resolved metagenomics and targeted physiological experiments have dramatically changed this picture. Alternative lifestyles of nitrifiers outside the N-cycle were identified, showing that nitrifiers are more versatile than assumed earlier and may even thrive anaerobically. In extreme habitats, new nitrifiers adapted to harsh alkaline conditions or high temperatures were found. The limited knowledge of marine nitrification was expanded by the characterization of novel nitrite oxidizers from seawater and sediment. Most surprisingly, complete ammonia oxidizers (comammox) were discovered that carry out full nitrification alone, are widely distributed in nature, and predominate in various engineered systems. This talk will give an overview of these recent findings and show how (meta)genomics is transforming our perspective of a key environmental process.

EMV-FG03

Pirating the pirates: Metagenome-informed microscopy reveals infections of *Altiarchaeota* in deep subsurface ecosystems

A. J. Probst*[1], J. Rahlff[1], K. Schwank[1], T. L. V. Bornemann[1], C. Moraru[1]
[1]*University of Duisburg-Essen, Essen, Germany*

Archaea of the Candidatus phylum Altiarchaeota can dominate terrestrial subsurface ecosystems as the main primary producer, yet little is known about their interaction with biological entities in the deep subsurface. Here, we performed a detailed metagenome-informed study of members of this archaeal phylum utilizing samples from ecosystems across the globe. Using genome prediction and linking spacers of Altiarchaeota CRISPR systems to protospacers, we identified thirteen genetically distinct mobile genetic elements, most of which are predicted to be viruses. Importantly, the high abundance of one putative virus enabled us to demonstrate its active infection of Altiarchaeota in the ecosystem by applying Direct-gene fluorescence *in situ* hybridization (FISH) based on probes designed off the putative viral genome. In addition, we identified a strong co-correlation of Altiarchaeota with archaea of the Candidatus phylum Huberarchaeota in one specific ecosystem. Subsequent metagenome-informed

microscopy using FISH identified a close association of these two archaea. Genome analysis suggest that Huberarchaeota have a very reduced metabolic capacity and are thus symbiotic parasites that live off metabolites from their Altiarchaeotal host. Our analyses demonstrate that Candidatus Altiarchaeota are important primary producers in terrestrial subsurface ecosystems and function as carbon sources for parasites or for other community members due to viral cell lysis.

EMV-FG04

PhenDB: Deciphering the microbiome: Large-scale prediction of microbial roles and traits

P. Hyden*[1], J. Geijo[1], L. Lüftinger[1], F. Piewald[1], P. Peneder[1], T. Rattei[1]
[1]*University of Vienna, Department of Microbiology and Ecosystem Science, Vienna, Austria*

Introduction: With the increasing sequencing capacities and rapidly improving computational tools, more and more near-complete genomes are sequenced and binned from whole-metagenome shotgun sequence data of microbial communities. The read-centric approaches are replaced by genome centered metagenomics, as whole genomes are more informative in terms of function and ecological role. The rapid, automatic annotation and comparative analysis of high numbers of metagenomic bins, as well as the reconstruction of microbial interaction networks, requires novel bioinformatics tools that are specifically adapted to these problems.

Objectives: Here we present PhenDB, a freely available resource to analyse entire collections of metagenomic bins for microbial traits. PhenDB provides a first taxonomic and functional overview of a bin collection and thereby simplifies the identification of interesting metagenomic bins for follow-up analysis.

Material & methods: Training and prediction are performed by PICA, which uses a support vector machine algorithm (SVM). The samples are represented as binary vectors of protein family presence/absence, based on EggNOG 4.5. Completeness/contamination assessment is derived from the EggNOG annotation using 34 universal marker genes. Taxonomy is inferred based on the same genes using a lowest-common ancestor algorithm similar to MEGAN.

Results & Discussion: To date, we collected training data for 45 models for microbial traits. We implemented a rapid completeness/contamination check, which matches the accuracy of CheckM. PhenDB is run on the life science cluster (LiSC) in Vienna, which allows to return results for an average metagenomic bin within 2 minutes. We are providing pre-calculated predictions for 5,563 RefSeq genomes. The training data used for each trait model is browsable on phendb.org.

Conclusions: PhenDB provides a user-friendly interface with several browsable tables simplifying identification of interesting findings. The models used in PhenDB are constantly improved by training data gathered using text-mining. We believe, that this resource will be useful to a large scope of scientists as a first-line, easy to use, analysis tool.

EMV-FG05

Genome-centric views on decomposer activity

S. F. M. Wahdan[1], W. Purahong[1], A. Heintz-Buschart*[1,2]
[1]*Helmholtz Centre for Environmental Research – UFZ, Department of Soil Ecology, Halle a. d. S., Germany*
[2]*German Centre for Integrative Biodiversity Research (iDiv), Bioinformatics Unit, Halle a. d. S., Germany*

Soils play pivotal roles in terrestrial ecosystem functions such as nutrient cycling, sustaining plant growth, litter decomposition, and carbon storage. Diverse microbial communities drive these critical functions and are strongly influenced by soil conditions, climate, and human activities, with feedbacks on plant productivity and aboveground biodiversity.

However, soil microbial communities are particularly challenging to study using meta"omic tools due to their diversity and the complexity of the members" genomes. Furthermore, because soils offer not only spatial but also temporal niches, a large proportion of detectable organisms may be inactive at a given point in time. While the presence of dormant microbes is informative on long time scales, it can blur interpretations of current processes. We therefore study a specific process – decomposition of a defined plant residue – and elucidate active microbiota, comparing different conditions in a field experiment that manipulates land use and climate according to relevant scenarios to investigate the impact of the factors on fungal and bacterial community dynamics.

Our bioinformatics approach integrates different *de novo* generated data sets at the level of (partially) reconstructed genomes. Using this approach, we compare a set of wet- and dry lab techniques to elucidate the activity of microbial decomposer communities – including labeling of active DNA, whole shotgun metagenomics, and metatranscriptomics. By means of complementing functional and taxonomic annotation, we furthermore gain insight into community structure, as well as the potential and realized microbial activity.

The presentation will give insights into the capacities of the different methods as well as persisting challenges.

EMV-FG06

Linking meta-Omics with processes - why and how?

T. Urich*[1], H. Wang[1], A. Söllinger[1]
[1]*University of Greifswald, Institute of Microbiology, Centre for Functional Genomics of Microbes, Greifswald, Germany*

It has been for a long time challenging, if not impossible, to integrate meta-Omics (e.g. metagenomics and -transcriptomics) and ecosystem process data (e.g. greenhouse gas production and consumption) in complex microbiomes. A root cause is the nature of meta-omics data being comprised of relative abundance counts. We will provide examples from recent studies (e.g. Söllinger et al., 2018, Santruckova, 2018), where the normalization of meta-Omics read counts to units of biological relevance has been successfully achieved and provided crucial insights into "who is doing what" for ecosystems of global relevance. These examples comprise the application of (1) quantitative metatranscriptomics on methanogenic microbiomes of ruminants, (2) quantitative metagenomics in soil heterotrophic CO_2 fixation, and (3) quantitative co-occurrence network analysis in permafrost soil microbiomes. Opportunities, challenges and pitfalls will be discussed.

CAV-FG01From visualisation of metabolic machinery architecture and biosynthesis to synthetic engineering

L. Liu *[1]
[1]*University of Liverpool, Institute of Integrative Biology, Liverpool, United Kingdom*

Self-assembly and compartmentalisation are the fundamental biosynthesis processes of natural biological systems at different scales, ranging from molecules, proteins, macromolecular structures and organelles to whole cells. Their well-defined structures are of fundamental significance for performing and modulating cellular metabolism in space and time. Particularly versatile paradigm in cyanobacteria is the specialized bacterial microcompartment, the carboxysome, which serves as the primitive self-assembling organelle that fixes CO2. Carboxysomes sequester the CO2-fixing enzymes ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrases from the cytoplasm, using an icosahedral protein shell formed by multiple paralogous proteins. Selective permeability of the shell allows passage of bicarbonate into the carboxysome and carbonic anhydrases convert bicarbonate into CO2. These ensure a high level of CO2 around RuBisCO, thereby enhancing carbon fixation. Here, I will describe the recent studies on the biosynthesis, structure, and regulation of carboxysomes as well as bioengineering of carboxysome structures in heterologous organisms, including higher plants, with the intent of enhancing photosynthetic performance and productivity. Comprehensive understanding of carboxysome self-assembly and architecture is pivotal for rational design and construction of new bio-nanoreactors and molecular scaffolds for biotechnological applications.

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CAV-FG02

The distinct properties of thylakoid membranes control IM30-mediated membrane fusion

C. Siebenaller*[1], A. Thurotte[1], J. Heidrich[1], D. Schneider[1]
[1]*Johannes Gutenberg-Universität Mainz, Institut für Pharmazie und Biochemie, Mainz, Germany*

The thylakoid membranes (TMs) of chloroplasts and cyanobacteria are essential for oxygenic photosynthesis. Nevertheless, not much is known about the formation and maintenance of these special membrane system. The *inner membrane-associated protein of 30 kDa* (IM30/Vipp1) seems to be crucially involved in inner membrane dynamics in chloroplasts and cyanobacteria. However, the exact physiological function of this protein is still unclear, although several potential functions were postulated within the last years. Our group has shown that *in vitro* upon membrane binding, IM30 of the cyanobacterium *Synechocystis* sp. PCC 6803 destabilizes the membrane structure and finally mediates fusion of liposomes in presence of MgCl2. Such membrane fusion processes are likely crucial for inner membrane dynamics *in vivo*.

As TMs have a very special lipid composition and architecture, we have now analyzed, which membrane properties are crucial for the fusogenic activity of IM30. Therefore, we used an *in vitro* FRET-based fusion assay and investigated fusion of liposomes with different lipid compositions and membrane curvatures. Our observations suggest that the chloroplast and cyanobacteria specific, non-bilayer-forming galactolipid MGDG, as well as negatively charged lipids are essential for IM30-mediated membrane fusion. Furthermore, also the membrane curvature clearly has an impact on the fusion rates, as membrane fusion is enhanced when highly curved liposomes fuse. This indicates that membrane fusion might happen at highly curved TM margins *in vivo*. Interestingly, this is exactly where IM30 has been found to localize in high concentrations in *Synechocystis*. Thus, we propose a special activity of IM30 at TM boundaries in chloroplasts and cyanobacteria.

CAV-FG03

Gated septal junctions allow cell-cell communication in filamentous cyanobacteria

A. K. Kieninger*[1], G. Weiss[2], M. Pilhofer[2], K. Forchhammer[1], I. Maldener[1]
[1]*University of Tuebingen, Interfaculty Institute of Microbiology and Infection, Organismic Interactions, Tübingen, Germany*
[2]*ETH Zurich, Institute of Molecular Biology and Biophysics, Zürich, Switzerland*

Multicellular organisms require cell-cell communication in order to differentiate specialized cells. Filamentous cyanobacteria, like *Anabaena* sp. PCC 7120, differentiate N_2-fixing cells called heterocysts, representing a model organism for multicellularity. The septal peptidoglycan (PG) between neighboring cells comprises a nanopore array of 80-150 pores[1], drilled by participation of the cell wall lytic amidase AmiC1[2]. Septal junctions (SJs) are protein complexes connecting the cells as intercellular communication system[3] and likely traverse the PG discs thru the nanopores.

By cryo-focused ion beam milling and imaging with electron cryotomography we could solve the architecture of the SJs in a nearly native state[4]. SJs consist of a tube traversing the septal PG. Each tube end comprised a plug embedded in the membrane, which is covered by a cytoplasmic cap. Upon disruption of the membrane potential, a conformational change in the cap structure leads to closure of the SJs and inhibition of molecular exchange as we found via fluorescence recovery after photobleaching experiments. Closed SJs reopen in favorable conditions, which makes them a gated and dynamic channel in analogy to metazoan gap junctions[4]. Mutants of the septal localized proteins FraC and FraD were suggested to be involved in SJs[5]. We found that a *fraC/fraD* double mutant lacked the plug-cap structure and was unable to close its SJs.

In conclusion, our study shows that filamentous cyanobacteria possess gated, dynamic septal junctions allowing protection of the filament in case one cell is injured or dying.

1 Nürnberg, *et al.* (2015), *mBio*

2 Bornikoel, *et al.* (2017), *Front Cell Infect Microbiol*

3 Mullineaux, *et al.* (2008), *EMBO J*

4 Weiss, *et al.* (2018), *bioRxiv*

5 Flores, *et al.* (2016), *Trends Microbiol*

CAV-FG04
Outer Membrane Vesicles in *Synechocystis* sp. PCC 6803 are enriched with small plasmid DNA
C. Taubenheim*[1], J. Taubenheim[2], N. Hülter[1], T. Dagan[1]
[1]*Christian-Albrechts-University Kiel, Institute of Microbiology, Genomic Microbiology, Kiel, Germany*
[2]*Christian-Albrechts-University, Institute of Zoology, Kiel, Germany*

Outer membrane vesicles (OMVs) are secreted by many bacterial species, where they are involved in various extracellular functions as well as intercellular communication. OMVs secreted by various microbial species contain DNA and have been reported to transfer DNA with a similar efficiency to transduction. Nonetheless, the mechanisms involved in OMV-mediated lateral DNA transfer remain unknown. Here we study OMVs abundance and genomic content in the freshwater cyanobacterium *Synechocystis* sp. PCC 6803 under various environmental conditions. We isolated vesicles from stationary growth cultures by filtration and ultracentrifugation. Transmission electron microscopy showed the OMVs as discrete, spherical blebs having a bilayer membrane with a diameter ranging between 20 and 120 nm. We found that the OMV abundance is significantly higher in bacteria cultured under heat– or high salt– stress in comparison to the control conditions. Furthermore, sequencing of the OMV genomic content revealed the presence of DNA in the vesicles. This included most of the chromosomal genome that was present in low abundance and two small cryptic plasmids – pCA2.4 and pCB2.4 – that were highly overrepresented in all tested conditions. Additional larger *Synechocystis* plasmids (ranging between 44 and 120 kb) were present in the OMVs in low abundance as well. Our results indicate that the two *Synechocystis* small plasmids are more likely to be packed into the OMVs in comparison to chromosomal and large plasmid DNA, independently of the culturing conditions. We hypothesize that the enrichment for small plasmids in the vesicles is a result of their packed topology and high diffusibility. Our observations implicate OMVs as a transfer mechanisms of small plasmids.

RSV-FG01
Linking single cell behaviors to the formation of multicellular patterns in a predatory bacterium
T. Mignot*[1]
[1]*CNRS, Marseille, France*

A current challenge in developmental biology is to bridge molecular and multicellular scales. This task is especially complex in animals given that the dimension gap spans several orders of magnitude. In this context, multicellular microbes can be especially powerful because their lifecycle rarely exceeds a few days and it can be captured over relatively small surfaces in devices as simple as a petri dish. In addition, these organisms allow sophisticated genetic manipulations and imaging approaches. In our laboratory, we study *Myxococcus xanthus* for its ability to predate and develop collectively over other microbial preys. During this presentation, I will present an interdisciplinary approach combining genetics, imaging and mathematical modeling to decipher how single *Myxococcus* cells direct their movements and cooperate to develop collectively over prey bacteria. In general, the findings suggest that symmetry breaking and pattern formation arise by short range interactions and propagation from discrete sites in the community.

RSV-FG02
A multifunctional protein in the Bacillus subtilis biofilm matrix
N. Stanley-Wall*[1]
[1]*University of Dundee, Dundee, United Kingdom*

Biofilms formed by the Gram-positive soil-dwelling bacterium Bacillus subtilis manifest as architecturally complex, hydrophobic communities on an air-agar interface. Both the structure and the hydrophobicity of the biofilm is the consequence of the cells producing an extracellular matrix. The matrix comprises fibres formed by the secreted protein TasA, an extracellular polysaccharide, and a secreted protein BslA. Together the matrix components surround and protect the resident cells from environmental stress. I will overview the remarkable properties of BslA that have been uncovered through a cross discipline collaboration. We have uncovered that it is a bifunctional protein with roles in both biofilm hydrophobicity and structuring.

RSV-FG03
Regulated Stochasticity in Phosphorelay signaling
M. Goulian *[1]
[1]*University of Pennsylvania, Philadelphia, PA, United States*

E. coli, like many other facultative anaerobes, can use a variety of compounds for respiration in addition to oxygen. Most of the anaerobic respiratory systems are not expressed aerobically, presumably because oxygen is the most energetically favorable terminal electron acceptor. Trimethylamine oxide (TMAO) respiration is a surprising exception: the genes that enable TMAO reduction are expressed both aerobically and anaerobically. We have found that oxygen functions as an environmental cue for this pathway but, surprisingly, affects only the cell-to-cell variability of the system while leaving the mean output unaffected. I will describe our work to understand how oxygen regulates the phosphorelay controlling expression of the TMAO respiration machinery and the consequences of regulated cell-to-cell variability. In addition, I will present recent work exploring how a class of bacteriophage also modulate this phosphorelay. Our results reveal a novel mechanism for bacteria to regulate phenotypic diversity in a clonal population and the potential advantages of this behavior for surviving a rapid drop in oxygen availability

MCV-FG01
New insights into bacterial chemoreceptor arrays by electron cryotomography
W. Yang[1], C. K. Cassidy[2], S. Ringgaard[3], S. Parkinson[4], A. Briegel*[1]
[1]*Leiden University, Institute of Biology, Leiden, Netherlands*
[2]*University of Illinois at Urbana-Champaign, Department of Physics, Beckman Institute, Urbana, United States*
[3]*Max Planck Institute for Terrestrial Microbiology, Department of Ecophysiology, Marburg, Germany*
[4]*University of Utah, Biology Department, Salt Lake City, United States*

Most motile bacteria contain a highly sensitive and adaptable sensory system composed of clusters of chemoreceptors. This chemosensory system is used to detect changes in nutrient concentrations, allows the cells to navigate towards

preferential environments and is also involved in host infection by some pathogenic bacteria. While it is one of the best understood signallig systems to date, unraveling structure and function of the bacterial chemotaxis system remains challenging. High-resolution analysis, using methods like X-ray crystallography or nuclear magnetic resonance (NMR), are inherently limited to structural fragments and rely on specimens taken out of their natural environment. Thus, they lack the larger context of the native system. Here, we use Electron Cryotomography (ECT) to study the three-dimensional architecture of the bacterial chemoreceptor arrays. ECT, in combination with genetics, proteomics and molecular dynamics flexible fitting methods provides a powerful toolbox to unravel structure and function of the bacterial chemotaxis system.

MCV-FG02

Mechanisms of bacterial cell wall synthesis and repair

W. Vollmer*[1]

[1]*Newcastle University, The Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle upon Tyne, United Kingdom*

The peptidoglycan sacculus resides in the periplasm of Gram-negative bacteria. Its net-like structure made of glycan chains connected by short peptides protects the cell from bursting due to the turgor and maintains the shape of the cell. During growth and cell division the sacculus is enlarged by the combined and coordinated activities of peptidoglycan synthases and hydrolases. However, the molecular mechanisms underlying peptidoglycan growth and its regulation are poorly understood.

Cytoskeletal proteins and associated cell morphogenesis proteins control peptidoglycan synthesis from inside the cell, within large cell envelope assemblies called elongasome and divisome. In *Escherichia coli* peptidoglycan growth is also regulated by outer membrane-anchored lipoproteins. LpoA and LpoB span the periplasm and are required to activate the inner membrane anchored peptidoglycan synthases, PBP1A and PBP1B, respectively. PBP1B-LpoB are involved with the synthesis of septal peptidoglycan during cell division, and a complex regulatory input from members of the Tol complex coordinates peptidoglycan synthesis with the constriction of the outer membrane during cell division. Our recent work discovered an additional function of PBP1B-LpoB. Together with the LD-transpeptidase LdtD and the carboxypeptidase PBP6a, PBP1B-LpoB participate in a peptidoglycan repair mechanism, which is essential for maintaining sacculus integrity and cell survival when the assembly of the outer membrane is compromised. Our data illustrate the intricate mechanisms by which the cell regulates peptidoglycan synthesis and how it repairs the sacculus under stress conditions, to achieve robust cell envelope stability.

MCV-FG03

Coordination of *S. aureus* capsule biosynthesis into cell wall biosynthetic networks

T. Schneider*[1]

[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Pharmazeutische Mikrobiologie, Bonn, Germany*

Staphylococcus aureus is an opportunistic bacterial pathogen responsible for a diverse spectrum of human diseases. Most bacteria that cause invasive diseases produce extracellular capsular polysaccharides (CP), which protect the pathogen from opsonophagocytosis and thereby enhance virulence. Despite its importance for pathogenicity, little is known about the biochemistry underlying capsule biosynthesis in *S. aureus*. Moreover, it remained largely elusive how the different cell wall biosynthesis pathways, such as peptidoglycan and wall teichoic acid, which share building blocks and membrane carriers with capsule biosynthesis, function in a coordinated and integrated fashion. *In vitro* reconstitution of the capsule pathway identified crucial enzymatic checkpoints controlled by reversible phosphorylation, allowed for a detailed biochemical analysis of downstream processing, like attachment of capsule to a peptidoglycan acceptor substrate and unraveled yet unexpected enzyme functions.

MCV-FG04

Bacteria's different ways to recycle their own cell wall

C. Mayer*[1], M. Borisova[1], I. Hottmann[1], M. Mühleck[1], R. Kluj[1], A. Walter[1], S. Unsleber[1]

[1]*Eberhard Karls Universität Tübingen, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin Tübingen IMIT, Mikrobiologie/Biotechnologie, Tübingen, Germany*

The cell wall protects bacteria from adverse impacts of the environment and from disruption due to osmotic shock, thus it has to be sufficiently rigid. At the same time, however, it needs to be highly flexible, allowing bacteria to constantly adjust the mechanical properties of their cell wall and their cell shape during growth and differentiation. For this reason, the structuring component of the bacterial cell wall, the peptidoglycan, is constantly remodeled and degraded. Ultimately, cell wall turnover products are recovered in a process called cell wall or peptidoglycan recycling, which has been extensively studied in the Gram-negative model bacterium *Escherichia coli*. In this organism, in one generation as much as half of the peptidoglycan is recovered, involving a distinguished recycling enzyme, the *N*-acetylmuramic acid 6-phosphate (MurNAc 6P) etherase MurQ. Quantification of MurNAc 6P accumulation in *murQ* mutant cells allowed to determine the rate of peptidoglycan recycling in Gram-negative and Gram-positive bacteria. During the last years we revealed, however, that the cell wall recycling routes of bacteria often significantly differ from the *E. coli* paradigm. Particularly, most Gram-negative bacteria lack an ortholog of MurQ and instead harbor a pathway, absent in *E. coli*, that channels cell wall sugars directly into peptidoglycan biosynthesis, which provides intrinsic resistance to the antibiotic fosfomycin. In contrast, Gram-positive bacteria mostly possess one or more MurQs and, in addition, contain an arsenal of different cell wall-lytic enzymes targeting the entire peptidoglycan-teichoic acid cell wall complex. Thus bacteria apply different strategies to break down and recover their cell walls, which will be reviewed.

ISV-FG01

Matrix assisted laser desorption ionization time of flight (MALDI TOF) mass spectrometry - a promising tool for microbial species identification in veterinary microbiology

J. Spergser*[1]

[1]*University of Veterinary Medicine Vienna, Institute of Microbiology, Vienna, Austria*

In the past few years MALDI TOF MS has approached diagnostic veterinary microbiology, enabling rapid and accurate species identification of a vast majority of microorganisms encountered in routine diagnostic laboratories. However, problematic discrimination is still

observed with some closely related species within the family *Pasteurellaceae* and the genera *Streptococcus* and *Staphylococcus*, requiring additional phenotypic and/or genetic tests for definite species identification. Considering the huge number of different microorganisms inhabiting animals, the discovery of new microbial species of potential veterinary importance, and the broad range of matrices and animal species targeted in diagnostic veterinary microbiology, available reference databases are still far from being complete. Therefore, continuous extension and upgrading of databases is required in order to address questions arising in diagnostics, taxonomy and phylogeny. For example, an excellent performance of MALDI TOF MS in microbial identification and differentiation has been achieved by the establishment and application of a large in-house reference database containing type and well-defined field strains (n=720) of almost all known species of the genera *Mycoplasma*, *Acholeplasma*, and *Ureaplasma* that have been isolated from animals so far. Using this extended in-house reference database, MALDI TOF MS appeared to be a superior and powerful tool for the identification and taxonomic resolution of these microorganisms. Consequently, establishing robust, reproducible and reliable MALDI TOF MS reference databases should be a major target for future investigations, in order to comply with demands of modern diagnostic veterinary microbiology.

ISV-FG02
Identification and characterization of microorganisms by FTIR spectroscopy: perspectives and limitations of the method
M. Wenning*[1]
[1]*Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany*

Fourier-transform infrared (FTIR) spectroscopy is a whole-organism fingerprinting technique based on the characteristic and individual absorption of infrared light by the different biochemical components such as proteins, lipids, and polysaccharides that make up intact microbial cells. Infrared spectra of cells grown under standardised conditions provide specific fingerprints, which can be used for identification purposes by comparison to reference spectra of known species.

What makes FTIR spectroscopy even more attractive is the fact that spectral information can also be exploited for strain typing purposes, which is particularly important for epidemiological analyses or source tracking purposes where the sources and routes of contamination need to be discovered. It may furthermore be used for technological applications, e.g. the control of strain identity of starter cultures or in biotechnology. Additionally, FTIR spectroscopy is applied to characterize microbial properties that are e.g. linked to virulence or serotype and therefore represents a rapid and comparably easy technique to assess pathogenicity factors or classify below the species level.

ISV-FG03
Potential and Limitations of Whole Genome Sequences for Identification of Prokaryotes
B. Bunk*[1]
[1]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*

In the last decade the use of Whole Genome Sequencing (WGS) is rising and gains increasing importance for the identification of bacteria. In contrast to the commonly used marker genes such as 16S rRNA or housekeeping genes, the use of WGS provides the highest resolution of all available molecular techniques. The impact of WGS on identification has particularly increased through massive advancements of sequencing technologies, especially since Next Generation Sequencing entered into the market. Currently, the available short read (e.g. Illumina, IonTorrent) and long read (e.g. Pacific Biosciences, Oxford nanopore) technologies are leading to microbial genomes of quite different price, completeness and quality, and accordingly have different value for identification of microbial species.

Besides the evaluation of sequencing technologies this talk will assess the commonly used assembly procedures. Widely used estimators of genome similarity as Average Nucleotide Identity (ANI) or digital DNA-DNA-Hybridization (dDDH) will be introduced and how those can be applied for species identification. The importance of closed genomes will particularly be evaluated. Pitfalls such as the different evolutionary trajectories of each gene within a bacterial genome, but also genetic exchange by horizontal gene transfer or large-scale conjugation events will be demonstrated. Also, common limitations such as the accessibility of genome data for comparison and missing authenticity of the biological material used for sequencing of taxonomic references, e.g. bacterial type strains, will be addressed.

BTV-FG01
Computational design of new and improved enzymes
O. Kheronsky*[1]
[1]*Weizmann Institute of Science, Department of Biomolecular Sciences, Rehovot, Israel*

Enzymes are potent biocatalysts that are widely used in biotechnology, but their function often has to be altered or optimized. Enzyme evolution and engineering are constrained by epistatic relationships among the positions that make up an active site. A further constraint is due to stability-function tradeoffs, whereby accumulated mutations reduce protein stability and functional expression. To address these problems, we have developed several new methods that use bioinformatics and Rosetta atomistic simulations to stabilize enzymes, improve their activity and make new enzymes by modular backbone assembly.

BTV-FG02
Conformational heterogeneity in enzyme design
C. Curado-Carballada[1], F. Feixas[1], J. Iglesias-Fernández[1], S. Osuna*[1,2]
[1]*Universitat de Girona, Institut de Química Computacional i Catàlisi (IQCC) and Departament de Química, Girona, Spain*
[2]*ICREA, Barcelona, Spain*

Enzymes exist as an ensemble of conformations important for their function. By introducing mutations to the enzyme sequence, the populations of the different conformational states can be gradually tuned for allowing novel function. In this talk, the population shift induced by distal and active site mutations introduced along a series of laboratory-evolved enzymes1-3 is presented. Microsecond time-scale Molecular Dynamics (MD) simulations in combination with correlation-based analysis, Markov State Models (MSM), and enhanced sampling techniques are applied to elucidate the changes in the conformational landscape of laboratory evolved variants. Dramatic changes in the conformational dynamics of active site loops involved in substrate entrance and product release

are revealed, which provide a rationalization for the enhancement in catalytic activity of the new evolved variants.3 Most importantly, our new tools based on inter-residue correlations observed along the microsecond-scale MD simulations provides a strategy to identify the amino acid positions that influence the dynamic properties of laboratory-evolved enzymes.1 Our method is therefore able to rationalize, but most importantly to predict which residues situated far away from the active site can have a large impact on the enzyme catalytic activity.1

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BTV-FG03

innov"SAR: a new approach for protein engineering and screening* * Patented by Peaccel (FTO)

F. Cadet*[1], N. Fontaine[1], M. Ng Fuk Chong[1], R. Pandjaitan[1]

[1]PEACCEL, Protein Engineering ACCELerator, Paris, France

We present a strategy that combines wet-lab experimentation and computational protein design for engineering polypeptide chains. The protein sequences were numerically coded and then processed using Fourier Transform (FT). Fourier coefficients were used to calculate the energy spectra called "protein spectrum". We use the protein spectrum to model the biological activity/fitness of protein from sequence data. We assume that the protein fitness (catalytic efficacy, thermostabilty, binding affinity, aggregation, stability…) is not purely local, but globally distributed over the linear sequence of the protein. Our patented method does not require protein 3D structure information and find patterns that correlate with changes in protein activity (or fitness) upon amino acids residue substitutions. A minimal wet lab data set sampled from mutation libraries (single or multiple points mutations) were used as learning data sets in heuristic approaches that were applied to build predictive models. We show the performance of the approach on designed libraries for different examples1 and discuss how our approach can tackle epistatic phenomena2. We can screen up to 1 billion (109) protein variants in a very short time.

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BTV-FG04

***In silico* design and screening of enzyme libraries**

D. B. Janssen*[1]

[1]University of Groningen, Biochemical Laboratory, Groningen Biomolecular Sciences and Biotechnology Institute, Groningen, Netherlands

The discovery and tailoring of enzymes for biocatalytic applications and synthetic biology often requires screening of large libraries of enzyme variants. Such high-throughput screening is costly and time-consuming, whereas the outcome is uncertain if applied to genetic diversity generated by random methods. Recent developments in computational design and molecular simulations make it possible to replace large parts of this laboratory work by computation methods. To increase thermostability, we explored a strategy called FRESCO (framework for rapid enzyme stabilization by computational library design) that now has been used to enhance the stability of 6 different enzymes by 14 to 35ºC (epoxide hydrolase, two dehalogenases, aminotransferase, peptide amidase, HMF oxidase, xylanase), and other applications are ongoing. The simultaneous enhancement of cosolvent resistance and tolerance to high substrate concentration shows that FRESCO can be considered a general enzyme robustness engineering tool. We also investigate a strategy called CASCO (catalytic selectivity by computational design and screening) that uses Rosetta design, docking and molecular dynamics simulations to discover enzyme variants with desired selectivity. This was applied to a dehalogenase and an epoxide hydrolase. Virtual screening of small molecule libraries has become a powerful method for in silico discovery of small molecules that bind a specific ligand. We foresee that the further development of such computational tools will allow also the screening of (mutant) enzyme libraries enzyme to discover variants with desired catalytic properties.

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SIV-FG01

On the search for host proteins mediating host-endosymbiont interaction

E. Nowack*[1]

[1]Heinrich-Heine-Universität Düsseldorf, Emmy Noether-Gruppe "Microbial Symbiosis and Organelle Evolution", Düsseldorf, Germany

Bacterial endosymbionts can provide eukaryotic hosts with a large variety of new biochemical capacities. The establishment of strict vertical transmission of the endosymbiont from one host generation to the next, leads over prolonged time, to reductive genome evolution in the

endosymbiont. We work on two obligately endosymbiotic model systems, the amoeba *Paulinella chromatophora* (Cercozoa, Rhizaria) with photosynthetic cyanobacterial endosymbionts and the trypanosomatid *Angomonas deanei* (Kinetoplastea, Excavata) with β-proteobacterial endosymbionts that provide the host with several amino acids and vitamins. In both systems the endosymbiont genome is considerably reduced compared to free-living relatives; and the gene repertoire encoded by the host and endosymbiont are highly complementary, indicating an intricate integration of metabolic processes between the partner organisms. Additionally, host and endosymbiont cell cycle are tightly synchronized. Through proteomic characterizations of the endosymbionts, we identified in both systems host proteins that are specifically targeted to the symbionts. Currently, we are analyzing the cellular functions of these symbiont-targeted proteins and elucidate their roles in host-symbiont interactions.

SIV-FG02

NanoSIMS: a quantitative approach to study metabolism in symbioses

E. Gibbin*[1], G. Banc-Prandi[2], M. Fine[2,3], A. Meibom[1,4]
[1]École Polytechnique Fédérale de Lausanne (EPFL), Laboratory for Biological Geochemistry, School of Architecture, Civil and Environmental Engineering, Lausanne, Switzerland
[2]The Interuniversity Institute for Marine Sciences, Eilat, Israel
[3]Bar-Ilan University, The Mina and Everard Goodman Faculty of Life Sciences, Ramat-Gan, Israel
[4]University of Lausanne, Center for Advanced Surface Analysis, Institute of Earth Sciences, Lausanne, Switzerland

All symbioses depend on the reciprocal exchange of metabolites between host and symbiont. Nanoscale secondary ion mass spectrometry (NanoSIMS) combined with stable isotopic labelling, is a powerful means of visualizing and quantifying this metabolic interplay *in situ* and *ex vivo*. Our model is the emblematic symbiosis that exists between reef-building corals and dinoflagellate algae (genus *Symbiodinium*). This association is characterized by the translocation of photosynthates and their immediate derivatives (sugars, lipids, and amino acids) from the symbionts to their host. Previous studies conducted by our lab have used ^{13}C-labeled bicarbonate and ^{15}N-labeled nitrate to quantify the transfer of labelled photosynthates, focusing on the performance of the algal symbiontS. In this talk I will show how ^{13}C-labeled pyruvate and ^{15}N-labeled ammonia can be used as a metabolic marker of host performance. Two forms of labelled pyruvate: pyruvate-1-^{13}C (P1) and pyruvate-2,3-^{13}C (P23) were used to assess the viability of this approach. P1 is cleaved off (as CO_2) by pyruvate dehydrogenase before it enters the hosts TCA-cycle. Any labelling in the host tissue thus first has to have been fixed photosynthetically by the symbiont and translocated back to its host. In contrast, P23 enters the host TCA-cycle producing diffuse anabolic ^{13}C enrichment throughout the host tissue. CO2 is lost at many stages in the TCA-cycle, so the signal becomes mixed (i.e. assimilated ^{13}C can be a result of host metabolism, or of translocated material fixed by the algal symbiont). To overcome this problem we applied the photosynthetic inhibitor DCMU to isolate the symbiont contribution to its host"s metabolism. This method allowed us to assess: (i) the contribution of the symbionts to host tissue metabolism, (ii) resource allocation between different tissue layers and cell types, and (iii) how host metabolism changes according to the diel light cycle. Our method provides a new means of studying the metabolic interactions that occur between partners involved in complex holobiont systems.

SIV-FG03

Host-microbe interactions in marine thiotrophic symbioses

R. Ponnudurai[1], T. Hinzke[2], T. Schweder[2,3], S. Markert*[2,3]
[1]European Molecular Biology Laboratory, Structural and Computational Biology Unit, Heidelberg, Germany
[2]Institute of Marine Biotechnology, Greifswald, Germany
[3]University of Greifswald, Institute of Pharmacy, Department of Pharmaceutical Biotechnology, Greifswald, Germany

Chemosynthetic symbioses of marine invertebrates with sulfur-oxidizing bacteria are a widespread phenomenon in sulfidic habitats around the world. Their most prominent and well-studied examples thrive at deep-sea hydrothermal vents: Bivalves of the genus *Bathymodiolus* and the "giant tubeworm" *Riftia pachyptila* harbor highly specialized intracellular bacterial symbionts, which supply their hosts with organic carbon. Hosts and symbionts cooperate in an intricate and delicately tuned network of molecular interactions. Many of these processes have not been deciphered to date, which is – to a considerable extent – due to the uncultivability of the bacterial symbionts. Physiological proteomics circumvents this problem: A culture-independent global proteome analysis allows for comprehensive and very detailed metabolic profiling of the individual symbiotic partners and of the symbiosis as a whole. We therefore pursued a proteogenomic approach to investigate symbiont physiology and microbe-host interactions in two *Bathymodiolus* hosts from different vent sites as well as in *Riftia* tubeworms. Our results revealed a remarkable degree of metabolic flexibility and interlocking in these symbioses. In *Bathymodiolus*, the host concentrates CO_2 as well as reduced sulfur for its symbionts and provides TCA cycle intermediates, which the thiotrophic symbiont cannot produce autonomously. In turn, the symbionts appear to counterbalance the host"s deficiency to synthesize essential amino acids. Our findings furthermore suggest that the digestion of symbionts by the host in *Bathymodiolus* is not only the main mode of carbon acquisition for the host, but may also be a means of maintaining the symbiotic balance, ensuring host control over the symbionts" population size. An arsenal of virulence-related symbiont proteins, on the other hand, might facilitate colonization of host tissue or may even enable the symbionts to evade host digestion. In *Riftia*, we identified individual symbiont subpopulations within the same tubeworm host. These symbiont "morphotypes" vary not only in cell size, but also display distinct metabolic strategies. This may allow them to play different roles in the consortium, while enhancing the flexibility of the holobiont. This enormous metabolic versatility on the symbionts" side, combined with the hosts" ability to effectively buffer unsteady ambient conditions, might be the key to success for these remarkable symbioses.

SIV-FG04

Phenotypic heterogeneity of the bee gut symbiont *Frischella perrara* - an important trait for host colonization?

K. Schmidt*[1], O. Emery[1], Y. El Chazli[1], P. Engel[1]
[1]University of Lausanne, Department of Fundamental Microbiology, Lausanne, Switzerland

The gut microbiota of honey bees consists of a relatively simple, but highly specialized and experimentally amenable bacterial community of eight species. The longstanding evolutionary association between these bacteria and the host has resulted in a spatially structured community in which microbe-microbe and microbe-host interactions are likely of great importance to maintain homeostasis. This makes the honey bee gut microbiota an excellent system to analyze

molecular mechanisms underlying symbiotic interactions in a natural gut ecosystem.

One member of the honey bee gut microbiota, the gammaproteobacterium *Frischella perrara*, colonizes a small region in the gut where it induces a characteristic melanization immune response that is reminiscent of a pathogen. Fitness costs for the host have not been linked to *F. perrara*, neither has any molecular mechanism been elucidated underlying the specific interaction with the host. Interestingly, *F. perrara* exhibits phenotypic heterogeneity when cultured *in vitro,* forming two distinct colony morphotypes: yellow and white. While bacteria typically switch between forming white and yellow colonies, we managed to isolate a mutant strain that is locked in the white state. Strikingly, this mutant of *F. perrara* had significantly lower gut colonization success than its wild-type strain and also did not induce the melanization anymore. In addition, *F. perrara* wild type switched towards the yellow phenotype over time during colonization. Interestingly, RNA sequencing showed that genes known to be involved in host-microbe interactions were upregulated in *F. perrara yellow* compared to *white*, including Pilus-formation and Type VI secretion genes. Indeed, *F. perrara* was able to kill another gut microbiota member, *Gilliamella apicola*, during *in vitro* co-culturing experiments in a phenotype-dependent manner. Differential expression of these genes may be needed either for specific stages during colonization or to cope with changes in the microenvironment faced by the bacterium within the host. Therefore, we hypothesize that phenotypic heterogeneity might play an important role for this symbiont to colonize the honey bee. We are currently constructing fluorescent single cell reporter assays enabling the analysis of the relevance of both phenotypes *in vivo*.

FGV-FG01

Drugs from dirt: Mining and expressing biosynthetic gene clusters from soil metagenomes

M. Liles*[1,2], A. Pereira[1], M. Sandoval-Powers[1], Y. Zhang[3], A. I. Calderón[3], D. Mead[2]
[1]Auburn University, Biological Sciences, Auburn, AL, United States
[2]Varigen Biosciences Corporation, Madison, WI, United States
[3]Auburn University, Drug Discovery and Development, Auburn, AL, United States

Introduction: Most soil microbes are recalcitrant to cultivation under laboratory conditions. In this study a large-insert soil metagenomic library (113 Kb avg insert) was mined for biosynthetic gene clusters (BGCs).

Objective: To identify and express cloned BGCs to express bioactive metabolites.

Materials and Methods: We identified BGCs using PCR, targeting Polyketide Synthases (PKS), or using a NGS strategy in which clone DNAs were pooled, sequenced with Illumina, and contigs were screened for BGCs using antiSMASH4.0. Clones that contained PKS and/or non-ribosomal peptide synthetase (NRPS) clusters were conjugally transferred into *Streptomyces coelicolor* M1154 and screened for antimicrobial activity, followed by LC/MS characterization of active clones to identify metabolites with bioactivity.

Results: We identified 726 BAC clones that contained a PKS and/or NRPS pathway among 1,656 BGCs identified. The cloned BGCs were divergent from known pathways, with the nearest BLAST hit of keto-synthase domains ranging from 22 to 80% amino acid identity. BGCs identified via PCR (n=84) were a subset of the clones identified via NGS, which were both numerically more abundant and included highly divergent clades. BAC clones expressed in *S. coelicolor* M1154 were identified that inhibited the growth of multidrug-resistant bacterial or fungal pathogens. Clones expressing antimicrobial activity were further characterized by LC/MS analysis and other methods to associate secondary metabolites with their respective bioactivity.

Conclusion: These results indicate that novel biosynthetic gene clusters can be cloned intact from complex metagenomes and heterologously expressed to produce bioactive secondary metabolites, thereby expanding our available resources for natural product discovery.

FGV-FG02

Tax4Fun2: a rapid R-based tool for the prediction of habitat-specific functions and functional redundancy based on 16S rRNA gene marker gene sequences

F. Wemheuer[1,2], J. Taylor[3], R. Daniel[4], E. Johnston[1,2], P. Meinicke[5], T. Thomas[3], B. Wemheuer*[3]
[1]The University of New South Wales , Evolution and Ecology Research Centre, Sydney, Australia
[2]Sydney Institute of Marine Science, Sydney, Australia
[3]The University of New South Wales , Centre for Marine Bio-Innovation (CMB), Sydney, Australia
[4]Institute of Microbiology and Genetics, Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Göttingen, Germany
[5]Institute of Microbiology and Genetics, Department of Bioinformatics, Göttingen, Germany

High-throughput sequencing of 16S rRNA genes has become a powerful and widely used approach to study the composition and structure of microbial communities in a variety of environments. However, numerous questions in biogeochemistry and ecosystem ecology require knowledge of community functions rather than the taxonomic composition. Hence, assessing the functional capability and redundancy of a microbial community is a major challenge in environmental microbiology. To address this challenge, we developed Tax4Fun2, a R-based tool for the rapid prediction of functional profiles of prokaryotic communities based on 16S rRNA gene sequences. Functional profiles predicted by Tax4Fun2 for different ecosystems were highly correlated to functional profiles derived from associated metagenomes. One feature of Tax4Fun2 is that it allows users to incorporate their own habitat-specific reference data sets, which substantially enhances the accuracy of predicted functional profiles. Another key feature of Tax4Fun2 is that it enables researchers to calculate afunctional redundancy index (FRI) with respect to single community functions, The FRI reflects the redundancy and phylogenetic distribution of a specific function within the investigated community. The calculation of the FRI enables researchers to identify functions which might be lost under environmental perturbation. Tax4Fun2 provides researchers with a unique tool to predict and investigate functional profiles of prokaryotic communities based on 16S rRNA gene data. With its user-friendly, simplified workflow, Tax4Fun2 will assist researchers considerably in the functional analysis of microbial communities.

FGV-FG03

Fate of antibiotic-resistant bacteria in German wastewater treatment systems

D. Schneider*[1], S. Zachmann[1], M. Karnstedt[1], T. Böer[1], D. Zühlke[2], F. Stressmann[3], K. Premke[3], K. Riedel[2], R. Daniel[1]
[1]*Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology, Göttingen, Germany*
[2]*Institute of Microbiology, Microbial Physiology and Molecular Biology, Greifswald, Germany*
[3]*Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Chemical Analytics and Biogeochemistry, Berlin, Germany*

Antibiotic resistances of (pathogenic) microorganisms are increasing due to excessive use of antibiotics in medical care and livestock breeding. The full potential of the increasing resilience to commonly used antibiotics and involved systems for dispersal are not fully understood and distribution mechanisms remain to be unraveled. We aim to assess the antibiotic risk potential in urban wastewater treatment systems with a multi-omics approach.

The microbial community dynamics of hospital wastewater, different purification stages of wastewater treatment plants and wastewater recirculation into the environment were investigated. Sampling was performed quarterly over two years in Göttingen and Greifswald. The microbial community structure was assessed by marker gene analyses and direct sequencing-based metagenomic approaches. The metagenomic approach is complemented by metaproteomics and hydrochemical analyses.

The microbial community composition and relative abundance of potential pathogenic microbes is altered along the wastewater treatment stages. Predominant bacterial taxa are Epsilonproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacteroidetes and Firmicutes. A general reduction of potential pathogens as well as introduction of small amounts of wastewater originated bacteria into the environment is indicated. Antibiotic resistance genes mainly comprise genes of efflux pumps and genes conferring resistance against beta-lactames and aminoglycosides. An overall reduction of antibiotic resistance genes in the effluent of wastewater treatment plants was detected.

However, our understanding of the effects of continuous introduction of small quantities of dangerous genes into the environment and associated potential health risks for humans has to be deepened.

FGV-FG04

The fate of antibiotic resistances and antibiotic resistant bacteria during wastewater treatment assessed by metaproteomics

D. Zühlke*[1,2], D. Schneider[3], S. Maaß[4], D. Becher[4], R. Daniel[3], K. Riedel[1,2]
[1]*University of Greifswald, Department of Microbial Physiology and Molecular Biology, Greifswald, Germany*
[2]*Institute of Marine Biotechnology, Greifswald, Germany*
[3]*Georg-August-University Göttingen, Department of Genomic and Applied Microbiology, Göttingen, Germany*
[4]*University of Greifswald, Department of Microbial Proteomics, Greifswald, Germany*

Urban wastewater treatment plants (UWTP) have been considered as hot spots for the emergence and dissemination of antibiotic resistant bacteria (ARB). The high bacterial density in these facilities promotes horizontal gene transfer and therefore the exchange of antibiotic-resistance determinants between bacteria. In addition, the presence of antibiotics can select for resistant bacteria. Using a metaproteomics approach, we aim at the identification and quantification of metabolically active and antibiotic-resistant bacteria as well as resistance determinants at different sites of the UWTP.

Samples from different stages of the treatment process as well as hospital effluent were analyzed by GeLC-MS/MS. For protein identification sequence databases based on metagenomic data were created. Antibiotic resistance proteins were predicted using Resfams. In addition, culture-dependent methods were applied to determine the proportion of ARB and metaproteome data were validated using FISH.

Composition of the bacterial community changes during sewage treatment. Members of the human gut microbiome and bacteria related to infections of the gastro-intestinal/urinary tract that are known to carry antibiotic resistances dominate the raw sewage community. Activated sludge processes introduce bacteria involved in nutrient degradation. Treated wastewater contains significantly decreased bacterial numbers but still contains ARB and pathogens. Accordingly, proteins associated to antibiotic resistances are found at all stages of wastewater treatment.

Sewage treatment leads to reduction but not clearance of ARB and potential pathogens. Further treatment steps could prevent their release into the environment and therefore spreading of antibiotic resistances.

MPV-FG01

No abstract has been submitted.

MPV-FG02

Insights into the autotransport process of a trimeric autotransporter, *Yersinia* Adhesin A (YadA)

N. Chauhan[1], D. Hatlem[1], M. Orwick-Rydmark[1], K. Schneider[1], M. Floetenmeyer[2], B. van Rossum[3], J. C. Leo[1], D. Linke*[1]
[1]*University of Oslo, Department of Biosciences, Oslo, Norway*
[2]*the University of Queensland,The Centre for Microscopy and Microanalysis, St. Lucia Queensland, Australia*
[3]*Forschungsinstitut für Molekulare Pharmakologie, Department of NMR-Supported Structural Biology, Berlin, Germany*

Trimeric autotransporter adhesins (TAAs) are a subset of a larger protein family called the type V secretion systems. They are localized on the cell surface of Gram-negative bacteria, function as mediators of attachment to inorganic surfaces and host cells, and thus include important virulence factors. *Yersinia* adhesin A (YadA) from *Yersinia enterocolitica* is a prototypical TAA that is used extensively to study the structure and function of the type V secretion system. A solid-state NMR study of the membrane anchor domain of YadA previously revealed a flexible stretch of small residues, termed the ASSA region, that links the membrane anchor to the stalk domain. Here, we present evidence that single amino acid substitutions in this ASSA region produce two different conformers of the membrane anchor domain of YadA; one with the N-termini facing the extracellular surface, and a second with the N-termini located in the periplasm. We propose that TAAs adopt a hairpin intermediate during secretion, as has been shown before for other subtypes of the type V secretion systems. As the YadA transition state intermediate can be isolated from the outer membrane, future structural studies should be possible to further unravel details of the autotransport process.

MPV-FG03

Life after secretion - stop of T3SS-based protein translocation in response to external signals and its effect on *Yersinia enterocolitica*

A. Diepold*[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

Many pathogenic bacteria use a type III secretion system "injectisome" to manipulate host cells by injecting virulence-promoting effector proteins into host cells. Often, T3SS-active bacteria stop dividing and in some cases sacrifice for T3SS-inactive siblings. The enteric pathogen *Yersinia enterocolitica* utilizes the T3SS to prevent phagocytosis and inhibit inflammatory responses. Unlike other species, almost all *Y. enterocolitica* are T3SS-positive at 37°, which raises the question, if and how these bacteria stop secretion and restart division.

Using a fast and quantitative *in vitro* secretion assay, we have examined the initiation and termination of type III secretion. We found that just as secretion starts immediately when the activating signal, low [Ca2+], is present, bacteria stop to secrete effectors once this signal is removed. This behavior is independent of needle formation. Following secretion, the bacteria recover and resume division when introduced to a non-secreting environment, and the same bacteria are able to reinitiate secretion at later time points.

Our results indicate that *Yersinia* selectively use their type III secretion system to promote their survival when necessary, and are able to quickly switch their behavior towards replication afterwards, gaining an advantage during infection.

MPV-FG04

Minimal SPI1-T3SS effector requirement for *Salmonella* enterocyte invasion and intracellular proliferation *in vivo*

K. Zhang*[1], A. Riba[1], M. Nietschke[2], N. Torow[1], U. Repnik[3], A. Pütz[1], M. Fulde[4], A. Dupont[1], M. Hensel[2], M. Hornef[1]
[1]*RWTH University Hospital, Institute of Medical Microbiology, Aachen, Germany*
[2]*University of Osnabrück, 2Division of Microbiology, Osnabrück, Germany*
[3]*University of Oslo, Department of Biosciences, Oslo, Norway*
[4]*Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*

Non-typhoidal *Salmonella* represent a major causative agent of gastroenteritis worldwide. Hallmark of the pathogenesis is their ability to actively invade the intestinal epithelium by virtue of their type III secretion system (T3SS) that delivers bacterial virulence factors directly into the host cell cytosol. The role of these virulence factors during enterocyte entry and intraepithelial growth has only been intensively investigated *in vitro.* However, the limitation of immortalized cell lines is lack of the overlaying mucus layer, final cell lineage differentiation, apical-basolateral polarization as well as continuous migration along the crypt villus axis. Thus, the role of virulence factors during the *Salmonella* infection *in vivo* has remained largely undefined. Currently we established a novel neonatal mouse infection model which allowed us to follow *Salmonella* infection and visualize intraepithelial *Salmonella*. Here, we took the advantages of this model using wildtype and MyD88-deficient mice in combination with *Salmonella* Typhimurium strains carry various single and multiple mutant on major *Salmonella pathogenicity island* (SPI)1 encoded virulence factors. For the first time, we here systematically analyze the importance of individual SPI1 effector molecules for epithelial invasion, intraepithelial microcolony formation and mucosal translocation *in vivo*. Our results demonstrate the essential but redundant role of SipA and SopE2 for enterocyte invasion and mucosal translocation. In contrast, the formation of intraepithelial microcolonies, the recruitment of Lamp1 to *Salmonella* positive endosomes and SPI2 reporter activity, require expression of SipA and SopB or SopE2 but are independent of SopA as well as host MyD88 signaling. Finally, we define the requirement of individual SPI1 effector molecules for mucosal translocation and intraepithelial microcolony formation *in vivo* and thus provide important insight in the early course of the enteric *Salmonella* infection *in vivo*.

MPV-FG05

Epigenetic reprogramming of human macrophages by pathogenic *Yersinia*

I. Bekere*[1], M. Schnapp[1], L. Berneking[1], J. Huang[1], T. Günther[2], M. Aepfelbacher[1]
[1]*Universitätsklinikum Hamburg-Eppendorf, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Hamburg, Germany*
[2]*Leibniz-Institut für Experimentelle Virologie, Heinrich-Pette-Institut, Hamburg, Germany*

The pathogenic *Yersinia* species *Yersinia pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* suppress the host immune response by downregulating the transcription of inflammatory genes. Here we investigated whether epigenetic histone modifications play a role in the global modulation of inflammatory gene transcription by these unique pathogens, which inject effector proteins (Yops) into host cells via their type three secretion system. We infected primary human macrophages with *Y. enterocolitica* strains (wild type, its ΔYopM and ΔYopP mutants and virulence plasmid deficient WA-C) and performed Chromatin Immuno-Precipitation (ChIP)-seq employing anti-H3K4me3, -H3K27ac, -H3K27me3 and -H3K4me1 antibodies in combination with RNA-seq at 6 h post infection. This allowed us to assess the effect of *Y. enterocolitica* and its effector protein mutants on histone modifications at gene promoters and -enhancers and to correlate these modifications with gene transcription. Surprisingly, wild type induced activation of ~3000 promoters (increased H3K4me3- and H3K27ac modifications at promoters) which dose dependently paralleled transcription of the associated genes. Further, wild type and avirulent *Yersinia* strongly upregulated H3K27ac at enhancers of more than 6000 and 3000 genes, respectively. Integration of all data revealed that wild type but not WAC upregulated promoters and enhancers of genes for chromatin organisation and metabolism (i.e. fatty acid metabolism). In addition, wild type suppressed activation of promoters and enhancers of immune response genes, many of which were upregulated by WA-C. YopM did not to play a significant role while YopP showed a small contribution to the wild type effects, suggesting that Yops other than YopP or YopM or the interplay of all Yops contribute to this phenomenon. In summary, we provide evidence for a profound epigenetic reprogramming of macrophage metabolism and inflammatory response by virulent *Y. enterocolitica*.

MPV-FG06

Modulation of ER stress signaling by the *Coxiella burnetii* type IV effector protein CaeB

A. Friedrich*[1,2], P. A. Beare[3], S. Sonnewald[2], A. Lührmann[1]
[1]*Universitätsklinikum Erlangen, Mikrobiologisches Institut – Klinische Mikrobiologie, Immunologie und Hygiene, Erlangen, Germany*
[2]*Friedrich-Alexander Universität Erlangen-Nürnberg, Lehrstuhl für Biochemie, Erlangen, Germany*
[3]*National Institutes of Health, Coxiella Pathogenesis Section, Laboratory of Bacteriology, Rocky Mountain Laboratories, National*

Institute of Allergy and Infectious Diseases, Hamilton, MD, United States

Coxiella burnetii is an obligate, intracellular Gram-negative pathogen and the causative agent of the zoonosis Q fever. Like many other pathogens *C. burnetii* has evolved multiple mechanisms in order to invade and survive within host cells. Its pathogenicity depends on a functional type IV secretion system and, thus, on the translocation of effector proteins into the host cell. *C. burnetii* has a large repertoire of about 150 effector proteins. The effector protein CaeB was shown to exhibit anti-apoptotic activity. However, the mode of action is so far unknown. Here we demonstrate that CaeB co-localizes with the ER when ectopically expressed. Since the homeostasis of the ER is decisive for cell fate, we investigated whether CaeB interferes with ER stress signaling. Indeed, CaeB is able to inhibit ER stress-induced cell death. Our data indicate that CaeB selectively manipulates the signaling of the conserved ER stress sensor IRE1. Thus, the activity of the IRE1 RNase was increased, resulting in enhanced XBP1 mRNA splicing. Importantly, in cells infected with *C. burnetii* the RNase activity of IRE1 was also increased after ER stress induction. Using a *C. burnetii* strain lacking CaeB, we confirmed that enhanced splicing of XBP1 during ER stress depends on CaeB activity. However, in contrast to cell ectopically expressing CaeB, infected cells showed additionally an altered PERK signaling, indicating that *C. burnetii* developed multiple mechanisms to manipulate signaling of the unfolded protein response.

Taken together our data demonstrate that *C. burnetii* is able to manipulate ER stress signaling and that the modulation of IRE1 signaling during ER stress depends on the activity of CaeB.

MPV-FG07

No abstract has been submitted.

MPV-FG08

Subversion of retromer function by the *Legionella* effector RidL

K. Bärlocher*[1], H. Hilbi[1]
[1]*University of Zürich, Institute of Medical Microbiology, Zürich, Switzerland*

Legionella pneumophila naturally replicates in amoeba and also parasitizes macrophages in the lung, possibly leading to a severe pneumonia called "Legionnaires" disease". Hereby, the formation of a replication-permissive compartment, the *Legionella*-containing vacuole (LCV), is a crucial process. LCVs extensively communicate with the endosomal, secretory and retrograde vesicle trafficking pathways, but avoid fusion with bactericidal lysosomes. The retrograde vesicle trafficking pathway recycles cargo receptors along the endosomal route back to the Golgi apparatus and the endoplasmic reticulum (ER). A key component of the retrograde trafficking machinery is the retromer complex, consisting of the heterotrimeric cargo-selective subcomplex (Vps26, Vps29, Vps35) and heterodimeric membrane-deforming sorting nexins. A functional retrograde pathway restricts intracellular replication of *L. pneumophila* and other intracellular pathogens.

LCV formation is dependent on the Icm/Dot type IV secretion system (T4SS), which translocates approximately 300 different "effector" proteins into the host cell, where they modulate cellular processes. The Icm/Dot substrate RidL (Retromer interactor decorating LCVs) binds to Vps29, interferes with the retrograde vesicle trafficking pathway and is required for efficient intracellular replication of *L. pneumophila*. Recently, we have shown that the 29 kDa N-terminal domain of RidL adopts an unprecedented foot-like fold comprising a protruding β-hairpin at its heel. The RidL-Vps29 interaction in eukaryotic cells and *in vitro* is dependent on the β-hairpin and leads to the displacement of the Rab7 GTPase activating protein (GAP) TBC1D5 from the retromer and LCVs. Accumulation of retrograde cargos on LCVs in the absence of RidL suggests subversion of retrograde trafficking by RidL with the LCV as an acceptor of transport vesicles. Our data support a model, where RidL modulates retromer function in a β-hairpin-dependent manner, thereby promoting intracellular replication of the pathogen.

MPV-FG09

Virulence-associated protein secretion by the gastric pathogen *Helicobacter pylori*

S. Backert*[1]
[1]*Friedrich-Alexander-Universität Erlangen-Nürnberg Friedrich-Alexander-Universität Erlangen-Nürnberg, Department Biologie, Erlangen, Germany*

Many Gram-negative and Gram-positive bacterial pathogens harbour type IV secretion systems (T4SSs), which translocate virulence factors into host cells to hijack cellular processes for multiple purposes.1 *Helicobacter pylori* is a human-specific bacterium causing persistent infections in the stomach associated with pathologies ranging from chronic gastritis, peptic ulceration to gastric cancer. Development and progress of these gastric disorders are determined by various bacterial factors. The bacteria express numerous well-known adhesins, vacuolating cytotoxin VacA, serine protease HtrA, urease, and four chromosomally encoded T4SSs. The pathology of *H. pylori* strongly depends on the T4SS encoded by the *cag* (cytotoxin-associated genes) pathogenicity island. This T4SS forms a needle-like pilus which is induced upon host cell contact, and its assembly is accomplished by multiple protein-protein interactions and various pilus-associated factors. The delivered substrates of the T4SS are the effector protein CagA and the LPS biosynthesis metabolites heptose 1,7-bisphosphate (HBP) and ADP-heptose.2 Various T4SS receptors have been described on the host cell, including integrins, CEACAMs, phosphatidylserine and cholesterol, suggesting that effector molecule delivery is a highly coordinated process. We demonstrate that *H. pylori* T4SS pilus formation during infection of polarized gastric epithelial cells occurs at basolateral membranes, and not at apical sites.3 Secreted HtrA paves the way for *H. pylori* by opening the cell-to-cell junctions through cleaving host cell junctional proteins such as E-cadherin, claudin-8 and occludin, followed by paracellular transmigration of the bacteria across the polarized cell monolayer.3 In this fashion, *H. pylori* travels to basolateral membranes and inject CagA. After delivery, CagA becomes phosphorylated by oncogenic tyrosine kinases of the Src and Abl families, and mimics a host cell factor for the activation or inactivation of some specific intracellular signalling pathways. Several CagA-dependent and CagA-independent signalling capabilities of the T4SS have been identified, which include the induction of membrane dynamics, actin-cytoskeletal rearrangements and changing cell polarity, as well as pro-inflammatory, cell cycle-related and anti-apoptotic transcriptional responses. The contribution of these signalling pathways to pathogenesis during *H. pylori* infections is discussed.

1. Grohmann E, Christie PJ, Waksman G, Backert S (2018) Type IV secretion in Gram-negative and

Gram-positive bacteria. *Mol Microbiol* 107(4):455-471.
2. Pachathundikandi S, Backert S (2018) Heptose 1,7-Bisphosphate Directed TIFA Oligomerization: A Novel PAMP-Recognizing Signaling Platform in the Control of Bacterial Infections. *Gastroenterology* pii: S0016-5085(18)30021-0.
3. Tegtmeyer N, Wessler S, Necchi V, Rohde M, Harrer A, Rau TT, Asche CI, Boehm M, Loessner H, Figueiredo C, Naumann M, Palmisano R, Solcia E, Ricci V, Backert S (2017) *Helicobacter pylori* Employs a Unique Basolateral Type IV Secretion Mechanism for CagA Delivery. *Cell Host Microbe* 22(4):552-560.e5.

QAV-FG01
Miniaturization, Automation and Digitalization in Biotechnology: Incremental Progress or Disruptive Change?
W. Wiechert*[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

In recent decades, **miniaturization** has driven groundbreaking developments in biotechnology with great impact on fundamental research, development of industrial bioprocesses, or biomedicine. In general, making experimental and analytical systems smaller allows producing data in less space and with much higher throughput.

However, miniaturization is only half of the story, because high throughput experimentation and the exploration of huge parameter spaces also necessitates the **automation** of experiments. Lab robots have to take over the work that cannot be done manually anymore. For example, only automated devices have the required precision needed to achieve reproducible quantitative results in very small scales. Another driving force, synthetic biology with its modularization approaches and interfacing concepts strongly favors automated lab environments.

This, finally, leads to the third building block of a triad, i.e. **digitalization**. In an automated lab environment, the time needed to evaluate data by far exceeds the time needed to generate it. Only the overarching digitalization of all facilities, experimental and analytical procedures in an industrial development lab will raise the full potential of miniaturization and automation. In fact, first companies are already working with fully automated biotechnological development platforms.

Implementing the triad "Miniaturization, Automation and Digitalization" in biotechnology will result in strongly changed working environments both in academia and industry. Some questions concerning the academic, industrial and societal **consequences** are: Will this be an incremental or disruptive change? Are we prepared for this development? Are we educating future biotechnologists in the right way? These issues will be discussed and several examples from running projects at the Jülich biotechnology institute IBG-1 will be given.

Reference

DECHEMA Positionspapier "Neue Schubkraft für die Biotechnologie: Miniaturisierung, Automatisierung und Digitalisierung revolutionieren die Entwicklung biotechnologischer Prozesse und Produkte" (2018) www.dechema.de

QAV-FG02
Smart and connected - towards the lab of the future
F. Hauer*[1]
[1]*labfolder GmbH, Berlin, Germany*

The steps towards upgrading your lab to a future-proof digital data hub in which smart workflows help you to save time and money in optimising your processes will be presented. We will discuss opportunities and challenges in using technologies like artificial intelligence and big data applications and show how you can make the digital transformation process a success story.

QAV-FG03
How lab automation can contribute to the design of digital cells and the development of bioprocesses
P. Neubauer*[1], S. Hans[1], M. N. Cruz-Bournazou[1]
[1]*Technische Universität Berlin, Fachgebiet Bioverfahrenstechnik, Institut für Biotechnologie , Berlin, Germany*

Currently, the use of computer-aided mathematical cell models is limited by the identification of specific cell parameters under specific environmental conditions. Small variations, e.g. of the genetic system or culture conditions, can alter most parameters. In this context, mathematical models can play an important role e.g. in the identification of phenotypic properties or the characterization and optimization of bioprocesses. For a broader application, the development of efficient and automated methods for the fast and statistically valid parameter identification and calibration of models is essential for a breakthrough in cell digitization.

Laboratory robots in combination with analytical devices and intelligent supervisory software can help in the planning, execution and evaluation of experiments to support the digital description of cells. We developed strategies to perform such fully automated experiments that are operated by adaptive design algorithms. An experimental cycle includes i) designing optimal dynamic experiments, ii) using the generated data during the experiment, iii) storing and handling all generated data online, iv) adapting the model parameters to the data (learning from the experiment), and v) redesigning the optimal experimental strategy1. The integration of all units into a model-based framework is the key to creating an intelligent laboratory for rapid characterization of biosystems under industrially relevant conditions2,3.

We show that this approach is particularly useful for the description and characterization of genetic mutants and recombinant expression systems with two case studies.

References:

1 Cruz-Bournazou et al. 2017. Biotechnol Bioengin 114(3), 610-619.

2 Nickel et al. 2017. Engin Life Sci. 17(11) 1195-1201.

3 Sawatzki et al. 2018. MDPI Bioengineering 2018, 5(4), E101

QAV-FG04

Sens-o-Spheres: Mobile sensor probes for biotechnological processes

T. Lauterbach*[1], F. Lenk[1], T. Walther[1]
[1]*Technische Universität Dresden, Institute of Natural Materials Technology, Dresden, Germany*

Sensors in biotechnological processes are used for control and quality assurance purposes. While there are no space constraints on sensor technology for production-scale reactor systems, the introduction of instrumentation in laboratory-scale culture systems can be challenging. The integration of rod-shaped, wired probes is a problem when it comes to reactors in which there are no sensor connections due to constructive limitations (e.g. shaking flasks, tube or flat panel bioreactors etc.).

We propose a wireless, mobile and miniaturized approach as an alternative to state-of-the-art sensors for biotechnological processes, the so-called Sens-o-Spheres. Having the sensor packaged in a small sphere, which is only 8 mm in diameter, allowing a flexible usage in very different reactor types. The re-usable sphere consists of a data and a charging antenna, a micro-controller for the functional coordination, a rechargeable battery and all within a laser-welded encapsulation for the use in biotechnological processes. Onto this sensor platform, a temperature sensor is mounted. This spherical measurement device moves passively through the reactor volume and transmits the recorded measuring values wirelessly via radio frequency waves to a receiver.[1]

Here we present data using the Sens-o-Sphere system under laboratory conditions in different lab-scale bioreactors. Even under such harsh conditions as a bacterial cultivation in a stirred tank reactor, the mobile sensors maintain a stable radio signal which is consistent with the standard probes.

Literature[1] Tobias Lüke und Tim Lauterbach, "Ortsungebundene Aufnahme von Prozesssignalen - Neue Messmethode in State-of-the-Art-Bioreaktorsystemen", TechnoPharm, Bd. 7, Nr. 5, S. 254–259, 2017.

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LRC01

Living therapeutic materials: an alternative for personalized delivery of biopharmaceuticals?

A. del Campo[1], S. Sankaran*[1]
[1]*INM-Leibniz Institute for New Materials, Saarbrücken, Germany*

The concept "living therapeutics" refers to the vision of introducing engineered bacteria in the human body to produce drugs on-site. In spite of expected therapeutic advantages for long-term delivery and production cost savings by simplifying the drug synthesis/isolation/encapsulation sequence, real applications of living therapeutics in humans have not been realized yet. A number of important issues are associated with the delivery of engineered bacteria into the body which remain to be solved. Engineered living materials, where active bacteria are encapsulated into a synthetic matrix that sustains bacterial activity, regulates proliferation and prevents bacteria scape, might overcome many problems associated with the use of living organisms in a biomedical context. Progress in this field will be presented.

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LRC02

Dynamics of cells in confinement and microflow: From drug testing of unicellular parasites to surface sensing of bacteria

T. Pfohl*[1]
[1]*University of Freiburg, Institute of Physics, Freiburg i. Br., Germany*

Motility is a key factor for pathogenicity of unicellular parasites and a huge number of bacteria. The combination of swimming, attachment and surface colonization enable the pathogens and parasites to infiltrate and evade host cells and tissues. State of the art methods of motility analysis rely on a combination of optical tweezers with high-resolution microscopy and microfluidics. With this technology, propulsion forces, energies and power generation can be determined in order to shed light on the motion mechanisms, chemotactic behavior as well as specific survival and colonizing strategies of unicellular parasites and pathogens. With these new tools at hand, we can elucidate the mechanisms of motility and force generation of trypanosomes, a unicellular parasite responsible for devastating diseases in sub-Saharan Africa, in different environments and in response to numerous external stimuli, and can identify ways to manipulate and eventually to inhibit them.

Moreover, we are able to characterize surface sensing and biofilm-formation of the bacterium *Caulobacter crescentus*. Using optical tweezers and microfluidic controlled flow conditions as a mimic of natural environments, we demonstrated that bacterial surface attachment is mediated by a rotary flagellum and filamentous appendages called pili. Directly after surface contact, pili reorient cells into an upright position. This pili-mediated positioning of the cell poles with the rotary flagellum close to the surface initiates motor-mediated mechanical sensing and promotes long-term attachment for colonization.

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LRC03

Analysis in miniaturized enzymatic reaction systems

U. Münchberg*[1], P. Ebersbach[1], M. Jender[1], D. Maehler[1], S. Hoefgen[2], V. Valiante[2], E. Freier[1]
[1]*Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Leibniz Research Group Biobricks of Microbial Natural Product Syntheses , Dortmund, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Leibniz Research Group Biobricks of Microbial Natural Product , Jena, Germany*

Secondary metabolism is the focus of natural product research. Nature produces an enormous variety of natural products, and their biosynthetic pathways simultaneously are a valuable source for enzymes that may be applied to produce non-natural compounds. The research in this field is expected to yield new drugs and facilitate the (semi-) synthesis of active natural products. However, the work is often limited by the capability to isolate sufficient material for handling or for the **necessary analyses. By reducing the needed amounts, micro-scaling would facilitate natural product** research. Therefore, downscaling to microreactors

with integration of handling, separation, and analysis of minute amounts of substances may be an option to further this research.

We will present several different methodological developments that allow us to work with enzymes in miniaturized format. For the characterisation of enzymatic reactions, educts and products are measured simultaneously, both in identity and in quantity. This is achieved either by capillary zone electrophoresis or by spectroscopic techniques. Both methods also enable a directly following of the time course of the reaction from just one batch. For separation and purification of reaction products the technique of micro-Free Flow Electrophoresis (µFFE) is presented, allowing to recycle enzymatic cofactors or unused educts.

Our results aim at establishing enzymatic reactions on a micro scale, which will help to increase the reaction throughput.

LRC04
Design and implementation of *de novo* biosynthetic cascades
S. Flitsch*[1]
[1]*University of Manchester, School of Chemistry & MIB, Manchester, United Kingdom*

The combination of sequential biocatalytic reactions in non-natural synthetic cascades is a rapidly developing field and leads to the generation of complex valuable chemicals from simple precursors. As the toolbox of available biocatalysts continues to expand, so do the options for biocatalytic retrosynthesis of a target molecule, leading to new routes employing enzymatic transformations. The implementation of such cascade reactions requires careful consideration, particularly with respect to whether the pathway is constructed *in vitro* or *in vivo*. This lecture will showcase three successful *de novo cascades* and discuss the relative merits of *in vitro*, *in vivo* or hybrid approaches to building biocatalytic cascades and analytical challenges. Biocatalysts were obtained either directly from genomic libraries, or by re-design of enzyme activity to suit requires substrate specificity and selectivity.

Recent references: ACS Catalysis 2017, 7, 710; ACS Catalysis 2017, 7, 2920; JACS 2017, 139, 1408; ACIE 2017, 56, 1; ACIE 2017, 56, 14498; Nature Comm 2017, 8, 973; ACIE 2016, 55, 1511; Nature Chemistry 2014, 6, 65; JACS 2012, 134, 4521.

LRC05
Cryo-EM - The path to visual proteomics
D. Bollschweiler*[1]
[1]Max-Planck-Institut für Biochemie, Martinsried, Germany

In recent years, cryo-EM has developed into a widely established method in the field of biological structure analysis. Currently, a combination of expertise and techniques from the materials and life sciences offers novel approaches for structural research through in-situ studies of complex macromolecular interactions. This ongoing development paves the way for the imaging of an atlas with native proteins of whole microbes and sections of eukaryotic cells in an approach called visual proteomics. This contribution aims to give a brief methodological overview of cryo-EM, its current applications and the potential it offers to address questions that have been extremely difficult to answer so far.

AMP001
Growth and metabolism of the rumen bacterium *Prevotella bryantii*
L. Schleicher*[1], D. Stegmann*[1], A. Trautmann[2], J. Breining[1], J. Seifert[2], J. Steuber[1]
[1]*University of Hohenheim, Microbiology, Stuttgart, Germany*
[2]*University of Hohenheim, Animal Science, Stuttgart, Germany*

Prevotella is the predominant bacterial genus in the rumen of ruminants and can contribute up to 50 % to the bacterial community [1]. It is known that these bacteria degrade starches, other non-cellulosic polysaccharides, amino acids and simple sugar [2]. The major fermentation product is succinate, which is important to maintain the level of propionate in the rumen and to support glucose homeostasis in the ruminant [2]. Nevertheless, it is not known, which catabolic processes are catalyzed exactly and how the anaerobic energy conservation is working. In this study, we compared the growth of *P. bryantii* in medium with rumen fluid and yeast extract with the growth in medium without these components. We could replace the rumen fluid with a short chain fatty acid mix and the yeast extract with a vitamin mix. We analyzed the consumption of glucose and production of succinate during growth. The reduction of pH and consumption of short chain fatty acids were measured as well. Anaerobic respiration by *Prevotella copri* was proposed to involve the Na+ -translocating NADH:quinone oxidoreductase (NQR) and the fumarate reductase [3]. Here we demonstrate expression of the FAD domain of subunit NqrF from *P. bryantii* in *E. coli*.

[1] Deusch S, Seifert S. 2015. Catching the tip of the iceberg – evaluation of sample preparation protocols for metaproteomic studies of the rumen microbiota. Proteomics 15, 3590-3595.

[2] White BA, Lamed R, Bayer EA, Flint HJ. 2014. Biomass utilization by gut microbiomes. Annual review of microbiology 68, 279-296.

[3] Franke T, Deppenmeier U. 2018. Physiology and central carbon metabolism of the gut bacterium *Prevotella copri*. Molecular Microbiology 109 (4):528-540.

AMP002
Purification of 2-naphthoate:CoA-Ligase, the second enzyme of the anaerobic naphthalene degradation pathway
M. Arnold*[1], I. Heker[1], R. U. Meckenstock[1]
[1]*Universität Duisburg-Essen, Fakultät für Chemie/Biofilm Centre/Aquatische Mikrobiologie, Essen, Germany*

Introduction: Anaerobic degradation of naphthalene is initiated by carboxylation to 2-naphthoic acid followed by a thioesterification by the 2-naphthoate:CoA-Ligase (2-NA:CoA-Ligase). This CoA-Ligase belongs to the superfamily of ATP-dependent adenylation enzymes, similar to the benzoate:CoA-Ligase from *Thauera aromatica*. The ATP-dependent and oxygen insensitive activity of the 2-NA:CoA-Ligase was measured in cell free extracts of the sulfate-reducing cultures N47 and NaphS2.

Objectives: Here we wanted to purify the 2-NA:CoA-Ligase from the sulfate-reducing cultures N47 and NaphS2 to homogeneity. The enzyme should be characterized and the substrate specificities of the 2-NA:CoA-Ligase determined.

Materials & Methods: After ammonium sulfate precipitation different chromatographic steps were used for the native purification of the 2-NA:CoA-Ligase. Additionally, the production of the His-tagged enzyme was started in *E. coli*. LC-MS analysis of 2-NA:CoA-Ligase assays was used to identify fractions containing the desired enzyme and determine the substrate specificity.

Results: The 2-NA:CoA-Ligase could be further purified using ammonium sulfate precipitation and affinity chromatographic methods. Different *E. coli* strains and growth conditions were tested to obtain active enzyme. The ligases showed a narrow specificity for 2-NA and a few other carboxylic acids.

Conclusion: The identified substrate specificities show similarities to the benzoate:CoA-Ligase, indicating a similar reaction mechanism and conformational change of the 2-NA:CoA-Ligase. However, the enzyme exhibits highest activity with 2-NA and is specific for the anaerobic naphthalene degradation pathway.

AMP003

Heterologous production of 1,4-butanediol using the acetogen *Eubacterium limosum*

M. Flaiz*[1], F. R. Bengelsdorf[1], P. Dürre[1]
[1]*Ulm University, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany*

Introduction: The strict anaerobic acetogen *Eubacterium limosum* is a promising bacterium for the recombinant production of chemicals from non-petrochemical resources, since it is genetically accessible and metabolizes several C1 carbon sources. *E. limosum* can metabolize the gases CO and CO_2 as well as the liquid C1 compound methanol. As a fluid, methanol has several advantages compared to the commonly used C1 gases. Methanol does not suffer from gas to liquid mass transfer problems, and it can be stored and transported easily. Moreover, methanol can be produced from syngas, which can be biomass-derived or part of the waste stream from industrial processes.

Objectives: The aim of this study is the production of the platform chemical 1,4-butanediol from methanol using recombinant *E. limosum* strains.

Materials and methods: The recombinant strains *E. limosum* [pMTL83251_P_{tet}_AA_CAMI], *E. limosum* [pMTL83251_P_{tet}_AA_CBEI], and *E. limosum* [pMTL83251_P_{tet}_AA_CAMI] were constructed by transformation of plasmids harboring the heterologous genes *abfD* (encodes 4-hydroxybutyryl-CoA dehydratase) and *adhE2* (encodes bifunctional alcohol/aldehyde dehydrogenase) under the control of the tetracycline inducible *tet* promoter. The gene *adhE2* originates from *Clostridium acetobutylicum*, and the gene *abfD* originates either from *C. aminobutyricum*, *C. beijerinckii*, or *C. kluyveri*.

Results and conclusion: The recombinant *E. limosum* strains were characterized in growth experiments using methanol as carbon source. The analysis and validation of the data obtained from growth experiments will reveal whether the production of BDO is feasible in *E. limosum* and might unveil which *abfD* gene leads to the highest amount of 1,4-butanediol.

AMP004

***Caprobacter fermentans* gen. nov., sp. nov., a new caproic acid producing bacterium**

M. Flaiz[1], T. Baur[1], S. Stibitsky[1], S. Brahner[1], A. Poehlein[1,2], R. Daniel[2], F. R. Bengelsdorf*[1]
[1]*Universität Ulm, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany*
[2]*Georg-August University Göttingen, Genomic and Applied Microbiology & Göttingen Genomics Laboratory, Göttingen, Germany*

Introduction and objectives: A strictly anaerobic, caproate producing bacterial strain termed EA1^T was isolated from anaerobic digester content. Albeit, caproate fermentation is known since the discovery of *Clostridium kluyveri* (enriched by H. A. Barker in 1937, CA, USA) the topic barely exists in microbial text books. Strain EA1^T is proposed to represent a novel taxon within the family *Ruminococcaceae* and was accordingly investigated.

Materials & methods: In total, a variety of morphological, physiological, genetic, and phylogenetic analyses have been performed to elucidate the taxonomic position of strain EA1^T. Special emphasis was set to anaerobic growth characteristics and formation of metabolic end products, which were analyzed by suppling a wide range of substrates as well as under varying pH and temperature conditions.

Results: Cells of EA1^T are motile, spore forming rods (1 to 3 µm x 0.4 to 0.8 µm) and stained Gram-negative. Growth of strain EA1^T was observed at 30 and 37 °C and within a pH range of 5 to 9. Strain EA1^T fermented several mono and disaccharides. Metabolic end products from fructose were acetate, butyrate, caproate, and lactate. Furthermore, ethanol, CO_2 and H_2 were identified as products. The classical fermentation balances (carbon recovery, oxidation/reduction (O/R) balance, and balance of available hydrogen) could be closed. The genome consists of a 3.9 Mbp chromosome with a G + C content of 51.25 %. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain EA1^T represented a novel taxon.

Conclusion: Strain EA1^T represents a novel genus and a novel species within the family *Ruminococcaceae* and the proposed name is *Caprobacter fermentans* gen. nov., sp. nov. The type strain is EA1^T (DSM 107079).

AMP005

Amino Acid Exchanges in the Membrane-Integral HybB Protein that Distinguish the H_2-Oxidizing from the *pmf*-Driven H_2-Evolving Function of Hydrogenase 2

D. Lubek*[1], C. Pinske[1]
[1]*Martin-Luther-University Halle-Wittenberg, Biology/Microbiology, Halle a. d. S., Germany*

Hydrogenases catalyse the reversible conversion of hydrogen (H_2) to protons and electrons. The [NiFe]-hydrogenase 2 (Hyd-2) of *Escherichia coli* couples H_2 oxidation with menaquinone reduction and thereby contributes to the generation of the proton motive force (*pmf*)[1]. Hyd-2 has four subunits, HybOABC[2]. Here we focus on the cofactor-free, and the postulated proton-pumping membrane subunit HybB[2].

In order to elucidate how Hyd-2 contributes to energy conservation, we have developed an assay employing the reverse reaction of Hyd-2, where the enzyme uses the *pmf* to drive hydrogen evolution. Using this assay we have been able to identify amino acids within the HybB subunit that are important for *pmf*-dependent H_2 activation.

The exchange of predicted functional and conserved amino acids to alanines showed that the reverse reaction (H_2 evolution) is absent when the conserved amino acids E133, H184 or E228 are substituted. Strikingly, these variants retained H_2-oxidizing activity, which generates a *pmf* in the forward direction, when analyzed using both an H_2-electrode and artificial redox dyes as electron acceptors. In addition, the experimental data were combined with an *in silico* analysis of HybB and a putative water-filled cavity within the protein, which might be important for proton translocation, was identified.

Our data demonstrate that proton translocation can be uncoupled from electron transfer to the quinone pool during H_2 oxidation, but raise new questions regarding the mechanism of energy conservation by cofactor-free membrane components of such ancient enzyme complexes.

[1] Pinske et al. *J Bacteriol* 197:296-306; **2015**

[2] Beaton et al. *Biochem J* 475:1353-1370; **2018**

AMP006

IaaF, an unusual acyl-CoA dehydrogenase involved in anaerobic auxin metabolism

K. Schühle*[1], J. Heider[1]
[1]*Universität Marburg, Mikrobiologie, Marburg, Germany*

Acyl-CoA dehydrogenases (ACADs) are a large and biologically important family of flavoenzymes. They catalyze the α.β-dehydrogenation of acyl-CoA esters producing *trans*-enoyl-CoA. ACADs are predominantly involved in fatty and amino acid catabolism and homologs are found in all three domains of life. The structural and catalytic features are highly conserved among the family members. The catalytic mechanism is divided in two half-reactions: in the reductive partial reaction, the FAD co-factor is reduced by the substrate; a proton is abstracted at Cα of the substrate by a conserved Glu residue in the active center, followed by hydride transfer from Cβ to FAD. In the oxidative partial reaction $FADH_2$ is re-oxidized. ACADs usually use electron-transferring flavoprotein (ETF) as electron acceptor and show relatively low direct reactivity with oxygen. In contrast, the closely related subfamily of acyl-CoA oxidases (ACOXs) transfer electrons directly to O_2, producing H_2O_2.

The bacterial acyl-CoA dehydrogenase IaaF described here shows high sequence similarity to ACADs, but exhibits several unusual characteristics. IaaF catalyzes not only the dehydrogenation of its substrate, benzylmalonyl-CoA, but also its simultaneous decarboxylation to cinnamyol-CoA. The eliminated carboxy group is mechanistically equivalent to the Cα proton of usual substrates, and IaaF seems to require an exchange of the conserved Glu to a Gly to catalyze this reaction. Additionally, IaaF is readily re-oxidized by molecular oxygen under the formation of H_2O_2, but does not react with ETF. These unique catalytic features necessitate adjustments in the catalytic center of IaaF. Recent catalytic, biochemical and structural data are discussed in reference to the postulated reaction mechanism.

AMP007

Investigating the RNF-complex and Its Impact on the Energy Metabolism of the Acetogen *Clostridium ljungdahlii*

C. M. Klask*[1], B. Molitor[1], L. T. Angenent[1]
[1]*Universität Tübingen, Geoscience, Tübingen, Germany*

The biotechnological use of acetogenic bacteria provides a promising platform to convert waste gases into bio-based chemicals. These bacteria use the Wood-Ljungdahl pathway (WLP) to fix carbon under autotrophic conditions. However, the WLP is a non-ATP generating pathway. One mole of ATP is invested for the activation of one mole of CO2 in the methyl branch of the WLP, while one mole of ATP is regenerated during the dephosphorylation of acetyl-phosphate into acetate. The membrane-located RNF-complex plays a critical role in the energy conversion in these bacteria. The only way an acetogen such as *C. ljungdahlii*, can generate ATP for its anabolism under autotrophic conditions is based on the RNF-complex activity. The RNF-complex shares high similarities with respiratory oxidoreductases, and was first characterized in ***R**hodobacter sphaeroides* while investigating its **N**itrogen **F**ixation pathway. The RNF-complex in *C. ljungdahlii* catalyzes the oxidation of Fd2- and the subsequent reduction of NAD+ while protons are transferred over the membrane and used to drive an ATP synthase. The implementation of new metabolic pathways, especially for higher value fermentation products, such as butanol or hexanol, is often strongly dependent on the availability of sufficient cellular energy. Elucidating the RNF-complex in detail will help to reveal the full potential of *C. ljungdahlii* for biotechnology. Therefore, different recombinant strains overexpressing *rnf* genes were generated and tested for growth. In addition, a full RNF knock-out mutant will be generated and complemented by an inducible *rnf*-gene expression system. This will further our knowledge on the metabolism of acetogens, and provide a powerful molecular tool to fine-regulate the RNF activity of *C. ljungdahlii.*

AMP008

Laughing gas respiration of *Wolinella succinogenes*: composition and function of the electron transport chain

S. Hein*[1], V. Mijić[1], S. Witt[1], C. Werner[1], J. Simon[1]
[1]*TU Darmstadt, Microbial Energy Conversion and Biotechnology, Darmstadt, Germany*

Microbial reduction of nitrous oxide (N_2O) is an environmentally significant process in the biogeochemical nitrogen cycle. However, it has been recognized only recently that the gene encoding N_2O reductase (*nosZ*) is organized in varying genetic contexts, thereby defining clade I (or "typical") and clade II (or "atypical") N_2O reductases and *nos* gene clusters. Clade I and clade II *nos* gene clusters contain several mutually exclusive *nos* genes, some of which predict electron transport proteins. Here, we focus on the enzymology of the clade II Nos system from *Wolinella succinogenes*, a nitrate-ammonifying and N_2O-respiring Epsilonproteobacterium that contains a cytochrome *c* N_2O reductase (*c*NosZ). The characterization of single non-polar

nos gene deletion mutants demonstrated that the NosG, -C1, -C2, -H and -B proteins were essential for N_2O respiration. Moreover, cells of a *W. succinogenes* mutant lacking a putative menaquinol-oxidizing Rieske/cytochrome *bc* complex (QcrABC) were found to be incapable of N_2O respiration. Proton motive menaquinol oxidation by N_2O is suggested, supported by the finding that the molar yield of *W. succinogenes* cells grown by N_2O respiration using formate as electron donor exceeded that of fumarate respiration by about 30%. An updated electron transport chain model underlying N_2O respiration in *W. succinogenes* and other clade II N_2O reducers is presented.

AMP009

Laughing gas production by the nitrate-ammonifying bacterium *Wolinella succinogenes*

V. Mijić*[1], D. Mania[2], L. Bakken[2], î Frostegård[2], J. Simon[1,3]
[1]*TU Darmstadt, Microbial Energy Conversion and Biotechnology, Darmstadt, Germany*
[2]*Norwegian University of Life Sciences, Ås, Norway*
[3]*TU Darmstadt, Darmstadt, Germany*

Nitrous oxide (laughing gas, N_2O) is an effective atmospheric greenhouse gas and contributes to ozone depletion in the stratosphere. Microbial N_2O generation from nitrate under anoxic conditions results from denitrification or dissimilatory nitrate reduction to ammonium (DNRA) and multiple enzymes have been reported to produce N_2O via nitrite and nitric oxide (NO) in respective microorganisms as part of respiratory and/or detoxifying metabolic pathways. Previous work by Luckmann and colleagues [Microbiology (2014) 160, 1749-1759] indicated that the DNRA model bacterium *Wolinella succinogenes* cells produced small amounts of NO (most likely from nitrite) and N_2O during anaerobic growth by nitrate respiration in nitrate-sufficient medium. Here, a set of *W. succinogenes* single, double and triple gene deletion mutants has been characterized to elucidate the enzymology of NO turnover (detoxification) and N_2O formation. The mutants lacked the cytochrome *c* nitrite/NO reductase complex NrfHA, the flavodiiron protein Fdp and/or the putative NO reductase NorH. DNRA growth parameters and substrate/product concentrations (nitrate, nitrite, NO, N_2O and N_2) obtained with *W. succinogenes* wild-type and mutant cells will be reported.

AMP010

First steps in anaerobic phenanthrene degradation

I. Kaplieva-Dudek*[1], R. U. Meckenstock[1]
[1]*Universität Duisburg-Essen, Biofilm Center (Aquatische Mikrobiologie), Essen, Germany*

Introduction: Polycyclic aromatic hydrocarbons (PAHs) constitute a class of hazardous contaminants that occur in the environment due to anthropogenic activities as well as natural processes. Only microorganisms can completely degrade PAHs. Mineralization of PAHs has been well studied for aerobic organisms. However, due to high oxygen consumption during biodegradation, PAHs end up in anoxic habitats. So far, anaerobic degradation of PAHs has been mostly investigated for two fused aromatic rings, naphthalene and 2-methylnaphthalene.

Objectives: In this work anaerobic degradation of phenanthrene, a PAH with three fused aromatic rings, is studied in the novel sulfate-reducing enrichment culture TRIP. The aim was to elucidate the enzymatic steps in phenanthrene degradation after the initial carboxylation reaction, a putative CoA-ligation followed by the reduction of the aromatic ring.

Materials & Methods: Enzyme assays were performed with crude cell extracts of TRIP culture and 2-phenanthroic acid and 2-phenanthroyl-CoA as substrate. Potential reductases involved in dearomatization of the rings were cloned in *E.coli* and will be analyzed for their function in the reduction of 2-phenanthroyl-CoA.

LC-MS was used to detect the formation of 2-phenanthoyl-CoA from 2-phenanthroic acid or the consumption of 2-phenanthoyl-CoA and formation of reduced products.

Results: 2-phenanthroic acid was converted to 2-phenanthroyl-CoA in presence of ATP indicating a ligase reaction. Potential reduction of 2-phenanthroyl-CoA will be performed with crude cell extract and expressed proteins.

Conclusion: The detected product of the enzymatic assay indicates that first steps in the degradation of phenanthrene occur in a similar way as for anaerobic naphthalene degradation.

AMP011

Probing potential mechanisms bypassing the energy-converting methyltransferase MTR in *Methanosarcina acetivorans*

C. Schöne*[1], M. Rother[1]
[1]*TU Dresden, Dresden, Germany*

Methanogenesis is a unique energy metabolism carried out by members of the domain *Archaea*. While most methanogenic archaea employ only a single methanogenic pathway, *Methanosarcina acetivorans* is able to grow on methylated compounds, acetate or carbon monoxide (CO), which are metabolized via distinct but overlapping pathways. For the use of any methanogenic substrate, the membrane-integral, energy-converting N^5-methyl-tetrahydrosarcinapterin (H_4SPT):coenzyme M (HS-CoM) methyltransferase (MTR) is important, because it energetically couples the transfer of methyl groups between H_4SPT and HS-CoM with the translocation of sodium ions across the membrane. In previous studies it was proposed that cytoplasmic activities exist in *M. acetivorans,* that can bypass the reactions catalyzed by MTR. To address this issue, conversion of different energy substrates by an *mtr* deletion mutant was analyzed. No methyl-transfer from H_4SPT to HS-CoM could be detected with CO or acetate as the electron donor. In contrast, formation of methane and CO_2 from methanol or trimethylamine indicated the presence of an MTR bypass in the oxidative direction. As methanethiol and dimethylsulfide were transiently produced during methyl-dependent methanogenesis, involvement of the methylsulfide-dependent methyl-transfer system (Mts) was analyzed in a strain lacking both the Mts system and MTR. It could be unequivocally demonstrated that the oxidative MTR bypass does not involve the Mts system. Alternative mechanisms for CO_2 formation from methanol will be discussed in light of current experimental results.

AMP012

Enantiospecific Hydroxylation of Ethylbenzene and related Compounds by Ethylbenzene Dehydrogenase from *Aromatoleum aromaticum* EbN1

D. Hege*[1], J. Heider[1]
[1]*Philipps-Universität Marburg, FB Biologie, Marburg, Germany*

Ethylbenzene dehydrogenase is an enzyme isolated from *Aromatoleum aromaticum* EbN1 responsible for the first reaction of anaerobic degradation of ethylbenzene. It belongs to the DMSO reductase subfamily 2 and catalyzes the enantiospecific hydroxylation of ethylbenzene to (S)-1-phenylethanol.

EBDH consists of three subunits. The α-subunit (96 kDa) contains the active site, where a Mo-bis(molybdopterin guanidine dinucleotide) cofactor is located. The hydroxylation of the substrate uses a water molecule, while the electrons are transferred to a Fe_4S_4-cluster. The β-subunit (46 kDa) contains further iron sulfur clusters (3 Fe_4S_4, 1 Fe_3S_4) that allow the subsequent transport of the electrons to the γ-subunit (23 kDa), where a heme b molecule mediates the final transfer to cytochrome c [1,2].

After translation and assembly of the enzyme in the cytoplasm, the complete heterotrimer is translocated into the periplasm via the Tat pathway. Assembly and transport of EBDH require the chaperone EbdD, which is encoded in the same operon [1].

EBDH has been shown to be active on a broad range of more than 30 substrates including propylbenzene and derivatives, substituted ethylbenzenes like ethylphenols or ethylanilines and even some bicyclic substrates e.g. indane, which makes it an interesting candidate for applications in enantioselective synthesis of chiral compounds [3].

This work focuses on the development of a recombinant expression system for EBDH in order to produce single site mutants that can be used for probing of mechanistic details. First results will be shown.

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AMP013

Biodiversity of bacterial menaquinone methyltransferases, a novel family of class C radical SAM enzymes

D. Wilkens*[1], S. Hein[1], J. Simon[1]
[1]*Technische Universität Darmstadt, Department of Biology, Microbial Energy Conversion and Biotechnology, Darmstadt, Germany*

The membranous quinone/quinol pool is essential to the majority of life forms and has been widely used as an important biomarker in microbial taxonomy. In the anaerobic world, the most important quinones are menaquinone (MK) and its methylated derivatives 8-methylmenaquinone (8-MMK) and 7,8-dimethylmenaquinone (DMMK), which are anticipated to serve in low-potential electron transport chains involved in anaerobic respiration. Recently, homologues of class C radical SAM methyltransferases have been shown to synthesize 8-MMK or DMMK [Hein *et al.* (2017) *Mol. Microbiol*. 104, 449-462; Hein *et al*. (2018) *BBA Bioenergetics* 1859, 300-308]. Such enzymes, designated MenK, MenK2 or MqnK, are encoded by many bacteria (and some archaea) that either possess the classical MK biosynthesis pathway (Men) or the futalosine pathway (Mqn). Here, we report the identification and phylogenetic analysis of 508 known or putative (methyl)menaquinone methyltransferases. Many of these enzymes were retrieved from organisms whose capacity to synthesize methylated menaquinones has not been reported. As proof of principle, the putative *menK* gene from *Syntrophus aciditrophicus* was expressed in *Escherichia coli*. The corresponding cells were found to produce 8-MMK, thus providing evidence of the first deltaproteobacterial MK methyltransferase. In general, the presented approach is considered to be valuable in predicting the menaquinone status of a microbial species from its genome sequence.

AMP014

Analysis and characterization of mutated variants of benzylsuccinate synthase (BSS) for advanced degradation of BTEX compounds

A. Alhaj Zein*[1], J. Heider[1]
[1]*Philipps-Universität Marburg, Biologie, Marburg, Germany*

Despite the high toxicity and adverse health effects of the monocyclic aromatic hydrocarbons (benzene, toluene, ethylbenzene and xylenes, summarized as BTEX) acting as critical groundwater-contaminants, this problem remains largely ignored and unresolved [1]. It has been established that BTEX can be degraded enzymatically even in oxygen-free environments which develop rapidly in groundwater [2]. One of the most important type of enzyme reacting anaerobically with these compounds are the trimeric fumarate-adding glycyl radical enzymes [3]. The model enzyme of this family, benzylsuccinate synthase (BSS), initiates the anaerobic degradation of toluene by addition of a fumarate cosubstrate to the methyl group [4] [5]. We show here that a single mutation is sufficient to generate a variant capable of converting *m*-xylene in addition to toluene. In our ongoing study, we are also creating and characterizing additional mutant BSS variants to understand more of the ongoing mechanism and to expand or alter the substrate range. Moreover, we are also trying to overexpress and purify other fumarate-adding enzymes for further biochemical and structural characterization.

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AMP015

Characterization of *Buckelia termitida* (gen. nov., sp. nov.) and taxonomic revision of the *Clostridium sphenoides* group (*Clostridiales*; *Lachnospiraceae*)

S. Griesel*[1], M. A. Gonzalez Vera[1], K. Meuser[1], T. Heimerl[2], R. Radek[3], A. Brune[1]
[1]*MPI für terrestrische Mikrobiologie, Marburg, Germany*
[2]*LOEWE-Zentrum für Synthetische Mikrobiologie, Marburg, Germany*
[3]*Freie Universität Berlin, Institut für Biologie, Berlin, Germany*

The family *Lachnospiraceae* (Clostridiales) includes numerous taxonomically misplaced members of the clostridial Cluster XIVa. Here, we report the isolation of *Buckelia termitida* gen. nov. sp. nov. from the gut of the wood-feeding termite *Microcerotermes indistinctus*, a novel pectinolytic species that falls into the *Clostridium sphenoides* group and represents the first member of *Lachnospiraceae* isolated from termite guts. *B. termitida* is a rod-shaped, spore-forming diazotroph that produces hydrogen but differs from its closest relatives in polysaccharide utilization and fermentation product profile. Comparative analyses of genomic potential and physiological properties revealed common characteristics that separate members of the *Clostridium sphenoides* group from the genus *Hungatella*. Based on this evidence, we propose a reclassification of all members of the group, including the taxonomically misplaced *Clostridium celerecrescens*, *Clostridium xylanolyticum*, and *Desulfotomaculum guttoideum* (which definitely cannot reduce sulfate), to the genus *Buckelia*.

AMP016

Automated high-resolution determination of anaerobic atmospheric trace gas (H_2, NO, N_2O, CO_2, CH_4) production kinetics in small-scale incubations

S. Henjes*[1], M. A. Horn[1]
[1]*Institute of Microbiology, Leibniz University Hannover, Hannover, Germany*

The regulation of anaerobic microbes metabolizing atmospheric trace gases is important in the context of climate change. Thus, we developed a system facilitating the anoxic incubation of pure cultures or microbial communities under controlled conditions with a simultaneous, automated headspace analysis. Our setup consists of a gas chromatograph coupled to a robotized incubation system. We use two molecular sieve columns, one coupled to a pulsed discharge helium ionization detector (PDHID) in tandem with an external chemiluminescence detector (CLD), and the other to a thermal conductivity detector (TCD). The incubation system comprises 64 seats for 10 or 20 ml vials that are temperature controlled in the range of 4-70°C as well as an agitator. A robotized arm moves samples between the incubation trays and the agitator, and draws gas samples prior to injection into the split/splitless injector of the gas chromatograph. The gases H_2, NO, N_2O, CO_2 and CH_4 were baseline separated on the first molecular sieve column and detected by the PDHID or the CLD (for NO) within eight minutes, while O_2 and N_2 were directed via the second molecular sieve column to the TCD. The limits of detection and quantification (LOD and LOQ, respectively) in ppm were 2/4 for H_2, 0.05/0.1 for NO, 2/4 for CH_4 and 0.1/0.5 for N_2O at an injection volume of 250 µl. The LOQ for CO_2 was 100 ppm, and 1% for O_2 and N_2. The coefficients of variation for H_2, CH_4, CO_2 and N_2O were between 0.19 and 0.67% at a mixing ratio of 500 ppm. This setup is ideal to study the effect of environmental conditions on the kinetics of denitrification and methanogenesis in soil slurry incubations and gain a better understanding of the microbial regulation of associated greenhouse gas emissions.

AMP017

Degradation of prebiotics, sugars and sugar alternatives by the human intestinal microbiota

J. Schiessl*[1], U. Deppenmeier[1]
[1]*Institut für Mikrobiologie und Biotechnologie, Universität Bonn, Bonn, Germany*

Obesity and diabetes which are in part caused by high-calorie food and the excessive consumption of sugars belong to the major health problems of today"s society. It became evident that the elevated intake of these compounds may lead to an altered microbiota composition in the human gut. Hence, the analysis of the physiology and the growth requirements of the corresponding bacteria is of major interest. One aim of this study was to investigate the impact of prebiotics, natural sugars and low-caloric sugar alternatives on the growth of a variety of intestinal microorganisms. The data showed that the major taxa of the human intestinal microbiota were able to expectably metabolize prebiotic substances and common sugars, whereas the tested low-calorie sugar substitutes are not used as carbon source for growth. Another aim of this study was to examine the degradation of the low-calorie sugar substitute 5-keto-D-fructose (5-KF). Several gut bacteria were tested for their ability to use 5-KF as carbon and energy source. We identified different 5-KF reductases that are able to reduce 5-KF to fructose which is then channelled into the central catabolic pathways. Such enzymes were found in *Tatumella morbirosei* and *Gluconobacter oxydans* which do not belong to the intestinal microbiota. Strong homologies were also detected in anaerobic organisms like the soil bacterium *Clostridium pasteurianum* and the closely related intestinal microorganism *Clostridium leptum*. The enzymes of both *Clostridium* species were heterologously produced in *E. coli* and purified. Both enzymes were able to reduce 5-KF with specific activities of 62,9 ± 2 U/mg protein and 1.5 ± 0,14 U/mg protein, respectively. However, only *C. pasteurianum* was able to metabolize 5-KF in liquid culture.

AMP018

Biochemistry of mannitol-1-phosphate dehydrogenase and acetate production by *Thermoanaerobacter kivui* during mannitol metabolism

J. Moon*[1], N. Merz[1], M. Basen[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: The thermophilic acetogenic bacterium *Thermoanaerobacter kivui* converts one mole of hexose into three moles of acetate. We discovered that *T. kivui* grows on the more reduced sugar alcohol mannitol and possesses a PTS gene cluster for mannitol utilization (1). Recently, we found that *T. kivui* produces more than three moles of acetate per one mole mannitol in carbonate-buffered medium.

Objectives: We aimed to biochemically characterize the key enzyme for mannitol utilization, mannitol-1-phosphate dehydrogenase (MtlD). Moreover, we studied the physiology of *T. kivui* during mannitol conversion.

Materials & methods: We heterologously produced MtlD, purified it *via* affinity chromatography and studied its biochemical properties. Mannitol utilization and product formation were studied in cell suspension and growth experiments in the presence and absence of carbon dioxide/sodium bicarbonate.

Results: MtlD carried out NADH-dependent reduction of fructose-6-phosphate, optimally at 70 °C. In cell suspensions

with mannitol, *T. kivui* produced acetate with a ratio of 3.1 (acetate/mannitol) in bicarbonate-buffered medium. Less acetate, but much more H_2 (47 mM) was formed in bicarbonate-free medium.

Conclusion: A thermostable MtlD catalyzes the NAD^+-dependent oxidation of mannitol-1-phosphate. The generated NADH during mannitol oxidation leads to increased CO_2 reduction to acetate in the presence of bicarbonate, whereas reductant is transferred to protons to form H_2 in the absence of CO_2.

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AMP019

Methanogenic and Acidogenic Communities Reveal a Trade-off between *n*-Caproate Production and Competitive Pathways in H_2/CO_2-Based Carboxylate Chain Elongation

F. C. Freire Baleeiro*[1], H. Sträuber[1], S. Kleinsteuber[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology - UMB, Leipzig, Germany*

Introduction: Microbial production of *n*-caproate by anaerobic fermentation of biomass is limited by the amount of ethanol or lactate available in the fermenter. Recent research revealed that *n*-caproate can be produced by microbial consortia fed solely with H_2 and CO_2. However, potentials and limitations of H_2/CO_2-based production of medium-chain carboxylates by microbiomes are not yet fully understood.

Objective: This study aimed to compare the production of carboxylates from H_2/CO_2 by two different inocula.

Materials & Methods: Effluents from a biogas reactor and from an acidogenic reactor were fed with H_2/CO_2 in duplicate serum bottles. Liquid and gas-phase composition was assayed by HPLC-RID and GC-TCD, respectively. Community composition was analyzed by 16S rRNA gene amplicon sequencing.

Results: In cultures with the methanogenic community, specific consumption rates of H_2 were up to 2849±192 mg L^{-1} d^{-1} and hydrogenotrophic methanogenesis dominated along most batches even at high acetate concentrations (200 mM) and low pH (5.5). Despite most of the H_2 being consumed to produce CH_4, the methanogenic community could produce 198±42 mg L^{-1} of *n*-caproate, in comparison to 27±17 mg L^{-1} by the acidogenic community (concentration values after subtracting *n*-caproate from H_2-free controls). Bottles with the acidogenic microbiome formed no CH_4, consumed up to 355±20 mg L^{-1} d^{-1} of H_2 and *n*-butyrate formation (1425±494 mg L^{-1}) predominated.

Conclusion: The methanogenic community revealed to be a double-edged sword as it had the functions to produce *n*-caproate from H_2/CO_2 but was dominated by resilient hydrogenotrophic methanogens. The acidogenic community, on the other hand, could barely produce *n*-caproate but produced *n*-butyrate from H_2/CO_2 with no losses of H_2 to CH_4.

AMP020

Structure and Function of an ATP-dependent Benzoyl-CoA Reductase

J. Fuchs*[1,2], O. Tiedt[1], U. Demmer[3], U. Ermler[3], M. Boll[1,2]
[1]*Institute of Biology II / University of Freiburg, Microbiology, Freiburg i. Br., Germany*
[2]*University of Freiburg, RTG1976 - Cofactors, Freiburg i. Br., Germany*
[3]*Max-Planck-Institute for Biophysics, Molecular Membrane Biology, Frankfurt a. M., Germany*

Anaerobic bacterial degradation of monoaromatic compounds proceeds via the central intermediate benzoyl-CoA (BCoA), catabolized by dearomatising benzoyl-CoA reductases (BCRs). Class I BCRs couple the reduction of the substrate to cyclohexa-1,5-diene-1-carboxyl-CoA to a stoichiometric ATP hydrolysis[1]. A "Birch-like" reaction mechanism via radical intermediates was proposed to achieve substrate reduction at $E^{0'}$= −622 mV, one of the most negative redox potentials of a redox couple in biology[2].

During studies on 3-methylbenzoate degradation in several denitrifying bacteria, a class I BCR was identified[3,4] with an unprecedented substrate spectrum including methylated and chlorinated substrates[5]. We succeeded in heterologous production of the enzyme in *Escherichia coli* and solved the crystal structure of the catalytic subunits at 1,8 Å. The result provides evidence for the proposed radical-based mechanism at an active site [4Fe-4S]-cluster.

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AMP021

Investigation of the corrosion potential by oral microorganisms related to periodontitis and peri-implantitis

S. Kleinbub*[1], B. A. An[1], H. Gabriele[1], î Özcan[2], M. Schenderlein [2], H. Dommisch[3], D. Wagner[4,5], A. Koerdt[1]
[1]*Federal Institute for Materials Research and Testing, Biodeterioration and Reference Organisms, Berlin, Germany*
[2]*Federal Institute for Materials Research and Testing, Interfacial Processes and Corrosion, Berlin, Germany*
[3]*Charité, Department of Periodontology and Synoptic Denistry, Berlin, Germany*
[4]*GFZ German Research Centre for Geosciences, Geomicrobiology, Potsdam, Germany*
[5]*University of Potsdam, Institute of Earth and Environmental Science, Potsdam, Germany*

Since the early 19th century microorganisms were known on their capabilities of causing microbiologically influenced corrosion (MIC) of metals. The most studied ones are sulfate-reducing bacteria (SRB), but others can corrode metals as well, e.g. acid-producing bacteria or methanogenic archaea (MA). However, these studies were mostly focused on metals related to the petroleum industry but metals for other industries, e.g. dentistry, are also susceptible to corrosion. The inert Titanium (Ti) is often used as an implant material, but it is a base metal. The formation of a passivating oxide layer allows Ti to be corrosion resistant at normal conditions.

Nonetheless, scanning electron microscope images on dental implants from patients with acute peri-implantitis

showed clear signs of corrosion. Currently, the corrosion mechanism of dental implants is unknown, but many indications suggest that oral microorganisms, including MA (*Methanobrevibacter oralis*) and SRB (*Desulfomicrobium orale*), could be involved.

To determine if MA or SRB can corrode Ti (pure Ti or Ti-6Al-4V alloy), corrosion rate, methane and sulfide concentrations were analyzed. Electrical potential measurements using in-house developed electrochemical cells indicated a potential change on Ti in the presence of a corrosive MA strain compared to an abiotic control.

Microbial composition comparison will be analyzed using samples from dental pockets of 150 infected patients by considering the quality of the implant and 50 healthy people by means of amplicon sequencing. Enrichments and isolation of pure cultures from the dentals samples are also examined for their corrosion behavior. Overall, this is the first study investigating the susceptibility of dental implant material to corrosion using human related MA.

AMP022

Physiological function of citrate synthase isoenzymes in *Desulfurella acetivorans*

E. Pettinato*[1], L. Steffens[1], W. Schulz[1], A. Mall[1], I. A. Berg[1]
[1]*University of Münster, Institute for Molecular Microbiology and Biotechnology, Münster, Germany*

Desulfurella acetivorans is a sulfur-reducing anaerobic deltaproteobacterium capable to grow autotrophically or by acetate oxidation using the tricarboxylic acid (TCA) cycle either in the reductive or oxidative direction, respectively. Although the classical reductive TCA cycle uses ATP-dependent citrate lyase for citrate cleavage, the pathway variant functioning in *D. acetivorans* [reductive oxidative TCA (roTCA) cycle] recruits citrate synthase (CS), making this conversion ATP-independent [1]. This reaction was previously considered thermodynamically impossible under physiological conditions. Another organism using the roTCA cycle, *Thermosulfidibacter takaii*, possesses a single CS gene [2]. The characterization of *T. takaii* CS revealed that it has high affinity to its substrates citrate and CoA (in µM range), with K_m values being two orders of magnitude lower in compare to porcine CS. The citrate and CoA concentrations in *D. acetivorans* cells are maintained in the millimolar range to effectively pull the flux through the roTCA cycle [1], questioning the requirement of specific adaptations of its CS for the functioning in the roTCA cycle. Nevertheless, *D. acetivorans* harbors three genes for CS. Phylogenetic analysis shows that one of those genes shares a high sequence similarity with CS from various auto- and heterotrophic organisms, while the other two genes form a distinct phylogenetic branch. It suggests that different isoenzymes are adapted to catalyze citrate cleavage or citrate synthesis and function under different growth conditions. Here we characterize three CS isoenzymes from *D. acetivorans* and discuss their adaption to functioning in the oTCA or roTCA cycle.

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AMP023

Effect of CO or syngas overpressure on growth and product formation of *Clostridium ljungdahlii*

A. Infantes*[1], A. Neumann[1]
[1]*KIT (Karlsruher Institut für Technologie), Karlsruhe, Germany*

Anaerobic acetogenic fermentation uses gaseous compounds (CO2, CO and/or H2) to obtain commodity chemicals, mainly acetate, ethanol and 2,3-butanediol. Engineered strains are also capable of producing other products, like butanol.

The process has, though, a mass-transfer limitation due to the poor solubility of the gaseous substrates. Improving the productivity of this bioprocess is of growing interest due to its potential in a bio-economy scenario.

By using higher pressure on the cultivation system, a higher dissolved concentration in the medium can be achieved, augmenting the amount of available substrate for the microorganism.
However, CO has been reported to potentially have inhibitory effects [1].
To date, experiments have been done with up to 7 bar of a gas mixture containing H2 and CO2 [2].

This work aims to study the effect of high partial pressures of CO. To this purpose, *C. ljungdahlii* was cultivated with either pure CO or Syngas (32,5 % CO, 32,5% H2, 16 % CO2 in N2) at pressures ranging from 1 to 10 bar(a).

The differences in growth profile and product formation, as well as transcription of selected genes by qPCR were studied, and will be presented in this poster.

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AMP024

Itaconate fermentation in *Pelosinus* spp.

W. Schulz*[1], I. Plugge[1], I. A. Berg[1]
[1]*WWU Münster, Institute for Molecular Microbiology and Biotechnology, Münster, Germany*

Itaconic acid (IA; methylensuccinic acid) is an unsaturated C_5-dicarboxylic acid produced by a broad variety of soil fungi but also by mammalian macrophages, where it controls inflammatory response and serves as an antimicrobial agent. Being synthesized at considerable amounts, IA appears to be a valuable carbon source for a number of soil bacteria, but also for pathogens. Indeed, many bacteria are capable to use IA as a sole carbon source under aerobic conditions, and aerobic IA metabolism is well understood [1, 2]. Nevertheless, to the best of our knowledge, anaerobic degradation of IA has never been shown before. We screened various soil samples to find organisms which are capable of fermentation of IA and isolated ten strains belonging to the genus *Pelosinus*. Our biochemical analysis revealed that the *Pelosinus* spp. use a different catabolic pathway for IA utilization than the one known for aerobes. Interestingly, aerobic IA degradation proceeds through CoA esters, whereas anaerobic degradation is performed without

additional activation of intermediates. Based on results of genome sequencing and biochemical studies, we propose a pathway of anaerobic IA degradation.

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AMP025

Genetic and physiological studies of a *Thermoanaerobacter kivui* mutant lacking *hdcr*

S. Jain*[1], V. Müller[1], M. Basen[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: *Thermoanaerobacter kivui* is a thermophilic acetogen which utilizes the Wood-Ljungdahl pathway (WLP) to reduce 2 molecules of CO_2 to acetate. *T. kivui* grows autotrophically by using H_2 or CO as an electron donor or heterotrophically by deriving electrons from carbohydrates, alcohols, methyl groups or formic acid. The hydrogen-dependent CO_2 reductase (HDCR) catalyzes the reduction of CO_2 to formate, the first step of the methyl branch in WLP. We hypothesized that WLP (including HDCR) might not be essential in sugar oxidation in *T. kivui*, as reductant produced may be oxidized by electron bifurcating hydrogenase, running in reverse to produce H_2.

Objective: We aimed to study the role of HDCR in *T. kivui* metabolism.

Materials and methods : The *hdcr* gene cluster containing genes encoding a hydrogenase, a formate dehydrogenase and two small electron-transferring subunits were deleted in *T. kivui*. Then, comparative growth studies were performed with wild type (WT) *T. kivui,* the *hdcr* deletion mutant (Δ*hdcr*) and strain complemented with *hdcr* (strain HDCR*c*).

Results: We deleted the *hdcr* gene cluster in *T. kivui* and found that *T. kivui* Δ*hdcr* strain did not grow on formate, H_2+ CO_2 (1 bar), glucose or any other substrate, whereas re-insertion of the *hdcr genes* into the genome restored growth. It was observed *T. kivui* Δ*hdcr* strain grows well in the presence of these substrates when formate was added as an electron acceptor.

Conclusion: HDCR and the WLP as terminal electron accepting pathway are essential for acetogenic growth of *T. kivui.*

1. Basen M, Geiger I, Henke L, Müller V. 2018 *Appl. Environ. Microbiol.* **84**: e02210–2217.

AMP026

Structural basis of extremely low potential hydride transfer during enzymatic naphthoyl-ring reduction

M. Willistein*[1], M. Boll[1], J. Fuchs[1], S. Estelmann[1], M. Müller[2], S. Lüdeke[2], J. Haas[2], S. Ferlaino[2], D. Bechtel[3], A. Pierik[3], C. Müller[4], V. Schünemann[4], L. Heimann[4], U. Demmer[5], U. Ermler[5], M. Ullmann[6]
[1]*Albert-Ludwigs-Universität Freiburg, Microbiology, Freiburg i. Br., Germany*
[2]*Albert-Ludwigs-Universität Freiburg, Pharmaceutical sciences, Freiburg i. Br., Germany*
[3]*University of Kaiserslautern, Biochemistry, Kaiserslautern, Germany*
[4]*University of Kaiserslautern, Biophysics, Kaiserslautern, Germany*
[5]*Max-Planck Institute, Biophysics, Frankfurt a. M., Germany*
[6]*University of Bayreuth, Computational biochemistry, Bayreuth, Germany*

Introduction: Polyaromatic hydrocarbons (PAH) are pollutants of high persistence. The removal of PAH from contaminated sites is of great concern due to their toxicity and potential cancerogenity. Mechanisms of enzymatic anaerobic PAH-degradation remain elusive and so far the only characterized enzyme involved in anaerobic PAH degradation is the 2-naphthoyl-CoA reductase (NCR) from the enrichment culture N47. After carboxylation and activation of naphthalene to 2-naphthoyl-CoA (NCoA), the non-activated ring of NCoA is reduced by NCR at an unusually low potential of E0" = -493 mV.

Objectives: While the key role of NCR in anaerobic PAH degradation was clearly identified, the reaction mechanism and most of the properties of NCR remained unclear. The determination of the crystal structure as well as the absolute product configuration were the main goals to elucidate this remarkable dearomatization at a flavin cofactor.

Materials & methods: We heterologously expressed NCR in *E. coli* and the absolute stereospecificity was solved by vibrational circular dichroism (VCD) spectroscopy after substrate conversion in D2O. The structure of NCR was solved at 2.2 Å with and without bound substrate NCoA.

Results: We unambiguously identified the absolute configuration of the NCoA product after conversion by NCR using VCD spectroscopy. The structure of NCR was determined and reveals unique features that enable hydride transfer at an unprecedented low redox potential; a structure-based mechanism is presented.

Conclusion: NCR is the first characterized enzyme involved in anaerobic PAH metabolism. The crystal structure provides a rationale for hydride transfer at this unprecedented low redox potential.

AMP027

Hydrogen cycling is essential for growth of the acetogen *Acetobacterium woodii*

A. Wiechmann*[1], S. Ciurus[1], F. Oswald[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: Acetogenic bacteria are characterized by the Wood-Ljungdahl pathway (WLP) of CO_2 fixation that allows lithotrophic growth on H_2+CO_2 or homoacetogenesis from organic substrates such as glucose. The first step in the methyl branch of the WLP is catalyzed by the hydrogen-dependent CO_2 reductase (HDCR) that, *in vitro*, uses H_2 but also reduced ferredoxin as reductant.

Objectives: Clarification of the role of H_2 as electron shuttle between glycolysis and WLP while addressing the physiological importance of the electron-bifurcating hydrogenase in *A. woodii*.

Materials & methods: The genes encoding the two major subunits of the electron-bifurcating hydrogenase were deleted and the phenotype of the mutant was studied with a focus on hydrogen evolution and uptake.

Results: The mutant no longer grew on H_2+CO_2 but also not on fructose, indicating that hydrogenase and thus H_2 formation is essential for heterotrophic growth. Indeed, wild type cells grown on fructose produced H_2 whereas the mutant did not. Addition of H_2 to the medium restored growth of the mutant. These data demonstrate that H_2 is the electron

donor for the HDCR *in vivo*. In accordance with this, caffeate respiration, that does not depend on H_2, was not affected in the mutant.

Conclusion: In summary, we present evidence that hydrogen cycling is essential for carbonate respiration (CO_2 fixation) in the model acetogen *A. woodii*.

AMP028
Modified degradation of ethanol and methanol in the thermophilic anaerobe *Thermacetogenium phaeum*
A. Keller*[1], B. Schink[1], N. Müller[1]
[1]*University of Konstanz, Biology, Konstanz, Germany*

Growth and metabolism of the anaerobic bacterium *Thermacetogenium phaeum* with ethanolamine, methanol, ethanol and acetate were investigated in axenic and in syntrophic cultures. Proteome data were analyzed, comparing all four substrates. Activities of key enzymes of the Wood-Ljungdahl pathway such as CO- dehydrogenase, hydrogenase and formate dehydrogenase and activities of enzyme systems leading to production or degradation of acetaldehyde such acetate kinase, phosphate acetyltransferase, alcohol dehydrogenase and aldehyde:ferredoxin oxidoreductase were detected in cell-free extracts. Accounting of fermentation stoichiometries and growth yields revealed that ethanol and methanol oxidation follow basically the same pathways and stoichiometries as previously documented for *Acetobacterium. woodii*. Yet, growth yields in *T. phaeum* were substantially lower than those reported for *A. woodii*. *T. phaeum* shows a profound difference in its genomic abilities compared to *A. woodii*, e. g. no Rnf complex is encoded in the genome of *T. phaeum*. This missing enzyme system could be the key for understanding the different growth behavior. On the basis of this data modified pathways of methanol and ethanol degradation are proposed.

AMP029
Pyrite formation from FeS and H_2S is mediated through a novel microbial redox activity
J. Thiel*[1], J. Byrne[2], A. Kappler[2], M. Pester[1,3]
[1]*University of Konstanz, Biology, Konstanz, Germany*
[2]*University of Tübingen, Tübingen, Germany*
[3]*Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany*

Pyrite is the most abundant iron-sulfur mineral on Earth"s surface and its formation from FeS and H_2S or polysulfides (Sn^{2-}) influences biogeochemical cycling of oxygen, carbon, iron and sulfur throughout geological time. It was postulated to play an important role as an early form of energy conservation on primordial Earth. Although there are hints towards a direct involvement of microorganisms in pyrite formation, it is still considered a purely geochemical reaction. We introduce microbial enrichment cultures growing with FeS, H_2S, and CO_2 as sole substrates that produce FeS_2 and CH_4. Cultures grew to cell densities of up to $2–9\times10^6$ cells mL^{-1} over periods of three to eight months. We followed transformation of FeS with H_2S to FeS_2 by ^{57}Fe Mössbauer spectroscopy and showed a clear biological temperature profile with maximum activity at 28°C and decreasing activities towards 4°C and 60°C. Besides FeS_2, CH_4 was formed, showing the same temperature dependence. Addition of either penicillin or 2-bromoethanesulfonate inhibited both FeS_2 and CH_4 production, indicating a syntrophic coupling of pyrite formation to methanogenesis. A 16S rRNA gene-based phylogenetic analysis identified at least one archaeal and five bacterial species and supported the hypothesis of syntrophic interactions in microbial pyrite formation. The archaeon was closely related to the hydrogenotrophic methanogen *Methanospirillum stamsii* while the bacteria were most closely related to sulfate-reducing *Deltaproteobacteria*, as well as uncultured *Firmicutes* and *Actinobacteria*. Our results show that pyrite can be formed according to the Wächtershäuser reaction through a microbially catalyzed redox process at low temperatures.

AMP030
On the diversity of 5,10-methylene-THF-reductases in acetogenic bacteria
C. Öppinger*[1], F. Kremp[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: Acetogenic bacteria grow lithotrophically on H_2+CO_2 but the energetics of this reaction is at the thermodynamic limit of life. Energy (ATP) is conserved by ferredoxin-dependent respiratory chains. Ferredoxin is a low potential electron donor that can be reduced by electron bifurcation with H_2 as electron donor. A second, ferredoxin-reducing, electron-bifurcating reaction of the pathway may be the reduction of methylene-THF with NADH ($\Delta G_0'$ = -23 kJ/mol).

Objective: The objective of this study was to analyse the subunit composition of MTHFRs from different acetogens and check for possible electron bifurcation.

Material & methods: Anaerobic organisms were grown heterotrophically, cytoplasm was prepared and the MTHFRs were purified anaerobically *via* different chromatographic steps.

Results: We have purified the MTHFRs from *Acetobacterium woodii, Clostridium ljungdahlii, Eubacterium limosum* KIST612 and *Sporomusa ovata.* They all differ in genomic organization and in subunit composition. The core complex of MTHFR consists of MetF and MetV, that catalyzes methylene-THF reduction. In *A. woodii*, an NADH binding and oxidizing subunit is connected to the core-unit enabling the use of NADH as reductant. This subunit is missing in the enzyme from *C. ljungdahlii* and *E. limosum* and those enzymes do not use pyridine nucleotides as electron donors, but ferredoxin could be used as electron donor. In none of these enzymes, electron bifurcation could be demonstrated.

Conclusion: Although the subunit composition of MTHFRs in acetogenic bacteria is very different and reflects the use of different electron donors, ferredoxin reduction and electron bifurcation could not be demonstrated in any of these enzymes.

AMP031
ATP synthesis in Phosphite Oxidation
Z. Mao*[1], J. Frey[1], B. Schink[1]
[1]*Uni-Konstanz, Biology, Konstanz, Germany*

Desulfotignum phosphitoxidans strain FiPS-3 is a lithoautotrophic bacterium which can utilize phosphite as an electron donor to gain metabolic energy and CO2 as the only carbon source growth in the anaerobic marine medium. The phosphite oxidation (with very low redox potential -650mV)

pathway for assimilation purposes has been well studied, but very little is known about the energetic side of the process. We do phosphite oxidation enzyme activity assays in cell-free extract and use isotope tracking method to find the pathway in the cell. This project"s objective is to clarify the dissimilatory phosphite oxidation (DPO) process in *Desulfotignum phosphitoxidans.*

AMP032

Energy conservation in the rumen bacterium *Pseudobutyrivibrio ruminis*

A. Katsyv*[1], M. C. Schoelmerich[1], J. Dönig[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: The rumen bacterium *P. ruminis* converts different sugars to butyrate and conserves ATP trough SLP. Interestingly, genome sequences revealed that these class of organisms harbour gene clusters encoding Ech, Rnf and two highly similar F_1F_O ATP synthases. The crucial difference is that one is apparently specific for Na^+, since it harbours a characteristic Na^+ binding motif, whereas the other is probably H^+-dependent. We suggest Ech and Rnf could function in concert with the NifJ protein and Bcd-Etf complex, permitting unprecedented ATP yield during glucose fermentation to butyrate.

Objectives: We aimed to elucidate whether these organisms indeed produce and use Rnf and Ech simultaneously as coupling sites.

Materials & methods: Glucose and xylose utilization and product formation under Na^+-containing and -deprived conditions were studied in growth experiments. Biochemical and molecular experiments were performed to identify the key enzymes: Rnf, Ech and ATPases during metabolism.

Results: Growth experiments revealed that *P. ruminis* grows on C_6 (glucose) or C_5 (xylose) sugars and both growth rate and final optical density were decreased to approximately 50% in the absence of Na^+ on either energy source. Gene expression analyses also revealed differences in the expression level of *ech*, *rnf* and both *atpase* gene clusters under Na^+-containing and -deprived conditions. In addition, the Fd:NAD^+ oxidoreductase-, ATP-hydrolyse-activity and Fd^{2-}-dependent H_2 evolution were detected and compared in membrane fractions of *P. ruminis*.

Conclusion: The experimental and bioinformatic data substantiate the presence of membrane-embedded Ech, Rnf and ATPases. In addition, it was revealed that P. ruminis uses Na+ bioenergetics for additional ATP synthesis.

AMP033

Bacterial microcompartments from acetogenic bacteria *Acetobacterium woodii*

N. Pal Chowdhury*[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology and Bioenergetics, Institute for Molecular Biosciences, Frankfurt a. M., Germany*

Introduction: Bacterial microcompartments (BMCs) are highly specialized protein-based organelles found in microbes, encasing metabolic enzymes for complex intermediate enzyme catalysis. BMCs are of two types, anabolic and catabolic. Anabolic BMCs found in cyanobacteria are used for CO2 fixation. Catabolic BMCs found in heterotrophs (*Salmonella*), are involved in metabolism propanediol and ethanolamine. Key feature of the BMC shell is to selectively control movement of substrates and products (toxic/volatile) between the BMC interior and bacterial cytoplasm until further processed by downstream enzymes, reducing cytotoxicity.

Objective: *Acetobacterium woodii* grows heterotrophically on organic substrates such as fructose, ethanol, propanediol, butanediol or ethyleneglycol which induces BMC (catabolic) formation. Similar to *Salmonella*, the structural and metabolic genes for propanediol utilization are found in a single gene cluster of 20 genes. Unlike *Salmonella*, *A.woodii* genome encodes four unique genetic islands each encoding BMC structural and functional genes. Here we explore and study the role and function of BMCs in *A.woodii*.

Material and methods: *A.woodii* was grown on different substrate and gene expression analyzed by qPCR. Purified BMCs was characterized by electron microscopy and MALDI analysis.

Results: Transcriptional and qPCR analysis revealed that only the single 20-gene cluster was involved in BMC formation. Further, we could purify the BMCs successfully and identify an alcohol dehydrogenase involved in the metabolism of propanediol.

Conclusion: *A.woodii* uses the classical Pdu gene cluster for growth on different substrates. However, it is interesting to investigate how the same structural proteins identify different substrates and gates entry within the BMC.

AMP034

Tartrate is a nutrient during early stages of *Salmonella* Typhimurium infection

L. Spiga*[1], M. Winter[1], T. Furtado de Carvalho[2], E. Hughes[1], R. Chanin[1], W. Zhu[1], F. Faber[3], R. Santos[2], H. Andrews-Polymenis[4], M. McClelland[5], S. Winter[1]
[1]*UTSouthwestern Medical Center, Microbiology, Dallas, TX, United States*
[2]*Universidade Federal de Minas Gerais, 2 Departamento de Clínica e Cirurgia Veterinárias, Belo Horizonte, Brazil*
[3]*University of Wuerzburg, Research Center for Infectious Diseases, Würzburg, Germany*
[4]*College of Medicine Texas A&M University, Microbial Pathogenesis and Immunology, Bryan, TX, United States*
[5]*University of California School of Medicine Irvine, Microbiology and Molecular Genetics, Irvine, CA, United States*

Question: Through ingestion, the mammalian gut microbiota is exposed to a number of xenobiotics, molecules that are thought to be foreign and not naturally produced by the host or the microbiota. For example, 2*S*,3*S*-tartrate (formerly L-(+) tartrate) and 2*S*,3*R*-tartrate (meso-tartrate) are common plant secondary metabolites, while 2*R*,3*R*-tartrate (formerly D-(-) tartrate) is not known to occur naturally. Interestingly, a number of enteric bacteria can utilize all three isomers.

Methods: Here, we used the enteric pathogen *Salmonella* Typhimurium to investigate the origin of tartrate in the murine intestinal tract. Tartrate was scarce in the healthy large intestine but increased markedly upon *Salmonella* infection.

Results: *In vitro*, tartrate isomers are common side products of the monosaccharide oxidation by hypochlorite in the presence of catalytic amounts of nitric oxide radicals (Anelli"s reaction). In the mouse, tartrate accumulation

during *Salmonella* infection was dependent on inducible nitric oxide synthase (iNOS) activity, indicating that tartrate is produced through the oxidation of sugars with inflammatory reactive oxygen and nitrogen species. *Salmonella* degraded 2*S*,3*S*-tartrate and 2*R*,3*R*-tartrate to generate acetate and to support fumarate respiration in the reductive branch of the TCA cycle.

Conclusion: The finding that tartrate isomers are produced as chemical byproducts of inflammatory radical metabolism provides an explanation for the ability of gut bacteria to degrade superficially not naturally occurring compounds.

AMP035

The monofunctional cobalamin biosynthesis enzyme precorrin-3B synthase (CobZRR) is essential for anaerobic photosynthesis in *Rhodospirillum rubrum* but not for aerobic, dark metabolism

R. Ghosh*[1], E. Roth[1], K. Abou-Aisha[2], R. Saegesser[2], C. Autenrieth[1]
[1]*Institute for Biomaterials and Biomolecular Systems, Bioenergetics, Stuttgart, Germany*
[2]*German University in Cairo, Department of Microbiology and Biotechnology, Cairo, Egypt*

The *in vivo* physiological role of the gene *cobZ*, encoding precorrin-3B synthase, which catalyzes the initial porphyrin ring contraction step of cobalamin biosynthesis via the *cob* pathway, has been demonstrated here for the first time. Cobalamin is essential for an early step of bacteriochlorophyll biosynthesis in anoxygenic purple bacteria. The *cobZ* (*cobZRR*) gene of the purple bacterium *Rhodospirillum rubrum* was localized to a 23.5 kb insert of chromosomal DNA contained on the cosmid pSC4. pSC4 complemented several mutants of bacteriochlorophyll and carotenoid biosynthesis, due to the presence of the *bchCX* and *crtCDEF* genes at one end of the cosmid insert, flanking *cobZRR*. A second gene, *citB/tcuB*, immediately downstream of *cobZRR*, shows homologies both to a tricarballylate oxidoreductase (*tcuB*) as well as a gene (*citB*) involved in signal transduction during citrate uptake. CobZRR shows extensive homology to the N-terminal domain of the bifunctional CobZ from *Rhodobacter capsulatus*, and the *R. rubrum citB/tcuB* gene is homologous to the CobZ C-terminal domain. A mutant, SERGK25, containing a terminator-less kanamycin interposon inserted into *cobZRR*, could not grow by anaerobic photosynthesis, but grew normally under dark, aerobic and microaerophilic conditions with succinate and fructose as carbon sources. The anaerobic *in vivo* activity of CobZ indicates that it does not require oxygen as a substrate. The mutant excreted large amounts of protoporphyrin IX-monomethylester, a brown precursor of bacteriochlorophyll biosynthesis, and was complemented either by the *cobZRR* gene *in trans*, or when exogenous cobalamin was added to the medium. A deletion mutant of *tcuB*/*citB* did not exhibit the *cob* phenotype. Thus, cobalamin is only required for anaerobic metabolism in *R.rubrum.*

AMP036

Increasing the electron transfer in *Shewanella oneidensis* MR-1 using a tailored periplasmic protein composition

V. Palma Delgado*[1], C. Paquete[2], J. Gescher[1]
[1]*Karlsruhe Institute of Technology, Applied Biology, Karlsruhe, Germany*
[2]*Universidade NOVA de Lisboa, Instituto de Tecnologia Química e Biológica, Lisbon, Portugal*

For exoelectrogenic microorganisms periplasmic *c*-type cytochromes are often essential for the transport of respiratory electrons between the cytoplasmic and the outer membrane. Either the small tetraheme cytochrome (STC) or the periplasmic fumarate reductase (FccA) seem to be necessary for this electron transfer (ET) step in *Shewanella oneidensis* MR-1. Nevertheless, the organism expresses besides the necessary cytochromes for the ET to an anode or ferric iron also several cytochromes that are either part of other ET chains or have not an assignable function yet.

In this study, we improve the ET rate and modify the metabolism of *S. oneidensis* by means of genetic engineering in order to allow an efficient production of platform chemicals. We followed the hypothesis that the elimination of potential other ET pathways and the simultaneous overexpression of STC could lead to an accelerated ET.

Replacing the cytochrome genes encoding NrfA, CcpA and NapAB by the *stc* gene led to a twofold increased ferric iron reduction rate and 23% more current in a bioelectrochemical system. This increase in current production is potentially due to a higher content of a suitable cytochrome for the conductive connection of inner and outer membrane. Moreover, the mutant had a higher periplasmic flavin content, which might indicate that it produced also a second periplasmic electron carrier system. In contrast to its periplasmic flavin content, the extracellular flavin concentration was insignificantly higher compared to the wild type. Interestingly, the substitution of FccA with STC resulted in a strain with reduced ferric iron reduction rates. This correlated with a lower concentration of extracellular flavin suggesting that FccA is indirectly involved in the release of flavin molecules.

AMP037

Gene knock-outs and their effects on product formation of the butanol producer *Clostridium saccharoperbutylacetonicum*

S. Nick*[1], T. Baur[1], A. Baker[2], H. Smith[2], E. Jenkinson[2], B. Schiel-Bengelsdorf[1], F. R. Bengelsdorf[1], P. Dürre[1]
[1]*Universität Ulm, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany*
[2]*Green Bologics Ltd., Abingdon, United Kingdom*

Clostridium saccharoperbutylacetonicum was first isolated in 1959 and was found to be a "hyperproducer" for butanol (Hongo, 1960). During the following years, the strain was used for butanol production in a commercial plant (Hongo et al., 1966). In acidogenic growth phase, *C. saccharoperbutylacetonicum* produces butyric and acetic acid, which are subsequently converted into butanol, ethanol, and acetone (solventogenesis).
The ERACoBioTech project "Bioprocesses for the optimized, integrated production of butyryl esters from sustainable resources" (BESTER) aims to synthesize butyryl esters from different acids and butanol. Therefore, genetically engineered strains will produce either butyric acid or acetic acid. These acids will then be linked to butanol in an enzymatic esterification. Afterwards, the respective esters will be purified. *C. saccharoperbutylacetonicum* serves as chassis strain within this project. As a natural "hyperproducer" of butanol, the strain is anticipated to be also a "hyperproducer" of acids after knock-out of genes encoding enzymes responsible for the conversion of acids into their corresponding alcohols. Several copies of genes encoding enzymes accomplishing the acids and alcohols production were identified in that strain by a differential transcriptome analysis. After data evaluation, five genes

were chosen for construction of respective deletion mutants. Differentially expressed gene copies of *pta*, *crt*, *adh*, *adhE*, and *eutE* are targeted by Green Biologics" CLEAVE™ technology. The enzymes encoded by these genes are responsible for acetic and butyric acid formation, as well as the conversion into corresponding alcohols. Growth experiments using the mutant strains were performed to evaluate the physiological effects of the respective gene knock-outs.

AMP038

Studies on the reaction mechanism of corrinoid-reducing metallo-ATPases

H. Dürichen[1], S. Postel[1], G. Diekert[1], S. Studenik*[1]
[1]*Friedrich-Schiller-Universität Jena, Jena, Germany*

Introduction & Objectives: Protein-bound corrinoid cofactors play an essential role as methyl group carriers in the C1-metabolism of anaerobes. To bind methyl groups, the corrinoid cofactor has to be in its super-reduced [Co^{I}]-state, which is highly sensitive to autoxidation. Studies on the corrinoid activation of *O*-demethylase enzyme systems of *Acetobacterium dehalogenans* led to the identification of a class of enzymes, which catalyze the ATP-dependent reduction of inadvertently oxidized corrinoid cofactors ([Co^{II}]-state). They were designated as RACEs, reductive activators of corrinoid-dependent enzymes, and were found in bacteria and archaea. Our studies aim to elucidate the reaction mechanism of RACEs.

Materials & Methods: Formate dehydrogenase, hydrogenase, and pyruvate:ferredoxin/flavodoxin oxdioreductase (PFOR) were tested as potential electron-delivering reactions for corrinoid reduction. The midpoint potential of protein-bound corrinoid cofactors was determined via redox titration with Eu(II) chloride as electron donor in the absence and presence of RACE, ATP, or AMP-PNP. Crystallization attempts were performed to elucidate the structure of the corrinoid protein in complex with its activator.

Results & Conclusion: Titanium(III) citrate that is typically used as artificial electron donor for corrinoid reduction can be replaced by reduced flavodoxin. Reduced flavodoxin was generated in course of oxidative decarboxylation of pyruvate to acetyl CoA and CO_2 by PFOR. Redox titrations revealed that in course of corrinoid reduction a potential difference of about 250 mV has to be overcome. The conditions for protein crystallization were optimized (resolution < 3 Å). Based on the new results, a tentative reaction scheme for ATP-dependent corrinoid reduction was proposed.

AMP039

Fermentation of Oxygen Containing Syngas

T. Mohr*[1], A. Infantes[1], L. Biebinger[1], A. Neumann[1]
[1]*Karlsruhe Institute of Technology, Institute of Process engineering in Life Science, Section II: Technical Biology, Karlsruhe, Germany*

Dwindling reserves and negative environmental effects associated with fossil fuels emissions underpin the necessity to develop novel, environmentally friendly and inexpensive means of producing value-added chemicals such as acetate, ethanol or 2,3-Butanediol from C1-sources. Most syngas fermenters, including *Clostridium ljungdahlii,* and *Moorella thermoacetica*, are strict anaerobes, which limits the use of industrial waste gases containing O_2. To use the anaerobic acetogenic microorganism for conversion of such gas mixtures, oxygen has to be removed first, a step which is cost intensive. On the other hand, most organisms used in biotechnology under aerobic conditions are sensitive to CO. However, the facultative anaerobic *Parageobacillus thermoglucosidasius* is capable of growing under high levels of CO and in the presence of oxygen and furthermore produces hydrogen via the water-gas shift (WGS) reaction (Mohr 2018). This metabolic versatility makes *P. thermoglucosidasius* a potential biological tool for the removal of O_2 from syngas mixtures to be utilized in subsequent syngas fermentation. In this study we performed a sequential cultivation of **(1)** *P. thermoglucosidasius* to remove oxygen and **(2)** either *C. ljungdahlii,* or *M. thermoacetica*. The initial gas atmosphere was 50% air and 50% CO. Growth and product formation were observed for both set ups. We therefore demonstrate the potential of using an O_2-depleting facultative anaerobe to facilitate the anaerobic production of value-added products from CO plus O_2 containing waste gases.

Mohr T, Aliyu H, Küchlin R, Polliack S, Zwick M, Neumann A, Cowan D, de Maayer P (2018) CO-dependent hydrogen production by the facultative anaerobe *Parageobacillus thermoglucosidasius*. Microb Cell Fact, **17**:108

BCP040

Biochemical basis for metal sequestration by antimicrobial S100 proteins from human and pig

A. Wartmann*[1], C. Toulouse[1], S. Knogl-Tritschler[1], G. Fritz[1], J. Steuber[1]
[1]*University Hohenheim, Cellular Microbiology 250b, Stuttgart, Germany*

Hetero- and homodimeric S100A8 and A9 are metal-binding proteins of the EF-hand calcium-binding S100 family [1]. They are released by neutrophils and act as molecular pattern by interacting with receptors of the innate immune system. Additionally, they possess antimicrobial properties by sequestration of nutrient transitions metals, such as Zn^{2+} and Mn^{2+} [2].

Metal withdrawing by dimeric S100 proteins plays an important role in the defense of bacterial infection. Porcine S100A9 exhibits an elongated histidine rich C-terminus compared to the human orthologue. Here, we addressed the question, if this tail contributes to metal sequestration and thus exhibits modified antimicrobial activity.

S100 proteins were recombinantly produced by using *E. coli* BL21(DE3) pLys as expression host. The cells were denatured in GnHCl and proteins were refolded and dimerized during dialysis. S100 dimers were further purified by immobilized metal affinity and size exclusion chromatography. Dissociation constants for transition metals were determined by complexometric titration.

Homo- and heterodimers of S100A8 and A9 contribute to transition metal sequestration and show high affinity for zinc. The dissociation constants were determined in the nM range. Interestingly, the Kds varied between human and porcine S100 variants. However, the elongated, porcine C-terminal domain did not contribute to enhanced zinc binding.

Literature:

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BCP041

Trapping and proteomic discovery of cellular substrates of the ClpXP protease involved in bacteriochlorophyll biosynthesis in *Dinoroseobacter shibae*

A. Rommerskirch*[1], D. Jahn[1], E. Härtig[1]
[1]*TU Braunschweig, Microbiology, Braunschweig, Germany*

Introduction: The marine bacterium *Dinoroseobacter shibae* is capable to perform aerobic anoxygenic photosynthesis. Screening of our transposon mutant library of *D. shibae* for loss of pigmentation and Bchl*a* absorbance identified Dshi_1387, encoding the ATP-dependent protease subunit ClpX of the ClpXP protease in *D. shibae*.

Objectives: Determination of cellular substrates of the ClpXP protease involved in Bchl*a* biosynthesis and pigmentation in *D. shibae*.

Materials & Methods: To identify substrates of the ClpXP protease, a proteolytic inactive form of ClpP ($ClpP^{trap}$) was expressed, that will retain but not degrade substrates translocated into its proteolytic chamber. Substrates captured inside the proteolytic barrel were co-purified along with the Strep-tagged ClpP complex and identified by mass spectrometry.

Results: In total, approximately 78 proteins were trapped in *D. shibae*. Among them well known interaction partners of ClpP like ClpX and ClpA are found. Moreover, the trapped proteins included proteins, which were previously shown to be substrates of ClpP in other bacteria, supporting the validity of the $ClpP^{trap}$. This group include the transcriptional regulators CtrA and LexA. Newly identified ClpP substrates include the proteins involved in energy metabolism (NuoB, NuoD, SdhB), flagellar biosynthesis (FlaF, FliH) and proteins essential for bacteriochlorophyll *a* biosynthesis: 5-aminolevulinate synthase (HemA1 and HemA2) and subunit I and H of the magnesium chelatase (BchI and BchH).

Conclusion: Our study underscores the central role of ClpXP-proteolysis in a number of pathways including the bacteriochlorophyll *a* biosynthesis.

BCP042

Glycogen serves as the main carbon source for PHB synthesis during nitrogen starvation in the unicellular cyanobacterium *Synechocystis* 6803

M. Koch*[1], S. Doello[1], K. Gutekunst[2], K. Forchhammer[1]
[1]*University Tübingen, Organismic Interaction, Tübingen, Germany*
[2]*Department of Biology, Botanical Institute, Department of Biology, Botanical Institute, Kiel, Germany*

Under nutrient starvation, certain cyanobacteria accumulate several storage polymers like Polyhydroxybutyrate (PHB) and glycogen (Klotz *et al.*, 2016). PHB is of high biotechnological relevance since it can be used as a source for bio-degradable plastics. Although the principle reactions of PHB synthesis in cyanobacteria have been analyzed, fundamental questions on PHB metabolism are still unanswered. As recently shown (Dutt & Srivastava, 2018), about 74 % of the carbon from PHB is coming from intracellular metabolites. The precise nature of intracellular carbon, however, remained unknown.

In the present study, we analyze the relation between glycogen and PHB metabolism in the model cyanobacterium *Synechocystis* PCC 6803. We demonstrate that the intracellular glycogen pool plays a pivotal role in providing the carbon metabolites for PHB synthesis. In the absence of glycogen degradation, as it is the case in a mutant of glycogen-phosphorylase *glgP1* or in the *glgP1/2* double mutant, PHB synthesis is abrogated. A mutant of the glycogen synthase *glgA1* showed a strong decrease in the overall PHB production, while the amount of glycogen, produced by GlgP2, remained constantly high. These results indicate a functional difference of GlgA1 and GlgA2-produced glycogen, similar to the observation from glycogen utilization during recovery from nitrogen starvation (Doello *et al.*, 2018). Of the various carbon catabolic pathways present in *Synechocystis* (the EMP, ED and OPP pathway), the EMP pathway, which seems dispensable for recovery form nitrogen starvation, plays the most important role in PHB production.

Together, this study clearly indicates that PHB in *Synechocystis* is produced from glycogen catabolism during nitrogen starvation periods.

BCP043

Effects of eukaryotic-type serine/threonine protein kinases (eSTPK)on Differentiation and Spore Wall Synthesis of *Streptomyces coelicolor*

B. Vollmer*[1], N. Ladwig[1], M. Franz-Wachtel[2], B. Macek[2], W. Wohlleben[1], G. Muth[1]
[1]*University of Tuebingen, Biotechnologie, Tübingen, Germany*
[2]*Proteome Center Tuebingen, Tübingen, Germany*

Streptomycetes are soil living bacteria of the Actinobacteria phylum. In contrast to most other bacteria which divide by binary fission, Streptomyces grows by apical tip extension forming a multiple branching mycelium. During morphological differentiation the aerial hyphae are transformed into spore chains by the simultaneous formation of dozens of sporulation septa. Septation and synthesis of the thickened spore wall involves the MreBCD proteins, which direct a rod-shaped morphology in other bacteria by positioning lateral wall synthesis.
Screening of a *S. coelicolor* genomic library by bacterial two-hybrid (B2H) analyses identified MreBCD interaction partners and led to the concept of the Streptomyces spore wall synthesizing complex (SSSC). Interestingly, the SSSC also included PkaI, a putative eukaryotic-type serine/threonine protein kinase, which is encoded within a cluster of five independently transcribed eSTPK genes (SCO4775-4779). The role of eSTPKs in bacteria and their influence on morphological differentiation and cell wall biosynthesis are not well characterized. However, the delay in sporulation and the presence of aberrant spores in NLΔpkaI and NLΔSCO4775–SCO4779 mutants suggests a regulation of the SSSC by protein phosphorylation. The specific phosphorylation of MreC and PBP2 by PkaI could be demonstrated in coexpression experiments.
In our project we want to elucidate the role of eSTPKs/phosphatases on the activity of SSSC proteins to understand whether protein phosphorylation is the key to control and coordinate differentiation in Streptomyces.

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BCP044

Microscopy-based screen for novel inhibitors of bacterial cell division

C. Matos de Opitz*[1], P. Sass[1]
[1]*University of Tuebingen, Microbial Bioactive Compounds, Tübingen, Germany*

Since the implementation of antibiotics as therapeutic agents, mortality related to microbial infections reduced significantly. However, the occurrence of pathogenic bacteria multi-resistant to many commercialized antimicrobial classes represents an ever-growing public health issue. A recent report from the world health organization (WHO) estimates that 700 000 deaths per year are attributable to antimicrobial resistance. Currently, multi-drug resistant bacteria are not only constricted to hospital settings, but found in the environment, e.g. waste water. Since the available options to treat life-threatening infections are narrowed, the search for novel antibiotics with promising modes of action is fundamental. Cell division is an essential process in bacteria and it has proven to be effective as an antimicrobial target, however, this target pathway has not been used by any of the clinically applied antibiotics to date. Cell-based assays allow to identify cells with perturbed cytokinesis, i.e. cells displaying a filamentous phenotype as a result of targeting the core cell division protein FtsZ with antimicrobial compounds. We here report a semi-automated microscopy method to detect such phenotypical changes as a result of perturbed bacterial cytokinesis in living bacteria. Indicator organisms (e.g. *Bacillus subtilis* or *Escherichia coli*) can be used in microtiter-based screens, and micrographs of bacteria are obtained via automated phase contrast microscopy and analyzed by means of bioinformatics tools. Potential hits are considered bacterial cells exhibiting a filamentous phenotype opposite to the physiological rod shape. By these means, the method allows to screen large synthetic and natural compound libraries to identify novel inhibitors of bacterial cell division.

BCP045

Function of a novel SPFH-domain-containing protein in *Bacillus subtilis*

A. Savietto*[1], M. Bramkamp[1]
[1]*LMU, Microbiology, Munich, Germany*

Compartmentalization of biochemical reactions is a prerequisite of life. Recent years have seen a remarkable advance in studies describing how proteins and lipids are organized in functional membrane domains. It is assumed that bacterial homologs of eukaryotic membrane proteins play a major role in establishment of these domains, in part because they seem to organize liquid ordered membrane domains. Flotillins harbor a Stomatin/Prohibitin/Flotillin/HflK-C (SPFH) domain of unknown function. We have recently identified a third *Bacillus subtilis* SPFH protein – YdjI – that, contrary to flotillins, is exclusively enriched in liquid disordered membrane regions, indicating that the presence of a SPFH domain alone is not sufficient to recruit the protein to liquid ordered membrane phases and, hence, SPFH domain proteins may not all be required for lipid domain formation.

Transcriptomic analysis of cells lacking *ydjI* gene revealed that the operons associated with stress response (*ytrABCDEF* and *yhcEFGHI)* are constitutively repressed. The data also show upregulation of the *σB* regulon (*rsbVW-sigB-rsbX).* In line with this, we identified a putative interaction of YdjI with RsbV, an anti-anti sigma factor *σB.* using a proteomic approach. We are currently analyzing the role of YdjI in bacterial stress response and its impact on cellular differentiation. Our data hint to a role of YdjI in bacterial stress response, rather than membrane compartmentalization.

BCP046

Characterization of a predicted SepF homolog in *Haloferax volcanii*

P. Nußbaum*[1], S. V. Albers[1]
[1]*Albert-Ludwigs-Universität Freiburg Institut für Biologie II - Mikrobiologie, Institut für Biologie II - Mikrobiologie , Molekulare Biologie der Archaeen, Freiburg i. Br., Germany*

Cell division is the most important process for a cell to generate progeny. While this process has been studied in great detail in eukaryotes and bacteria, cell division in archaea still remains poorly understood. Interestingly, different cell division mechanisms are found within the domain of Archaea. It is known that Crenarchaeota in most instances divide by homologs of the ESCRT (Endosomal Sorting Complex Required for Transport) system found in Eukarya. On the other hand, almost all members of the Euryarchaeota possess FtsZ homologs and therefore might divide in a manner more similar to bacteria.

To tether FtsZ filaments to the membrane it is predicted that Euryarchaeota use SepF since, with one exception (*Methanopyrus kandleri*), there is no archaeal genome encoding for an FtsA or other known FtsZ membrane anchors. A bioinformatical approach identified arCOG02263 (archaeal **C**luster of **O**rthologous **G**enes), containing archaeal orthologues of SepF (Makarova Kira S. et al. 2010).

In *Haloferax volcanii* a putative SepF homolog is encoded by the gene *hvo_0392*. This gene product is highly conserved within Haloarchaea. A C-terminal GFP fusion shows, that this protein localizes to mid-cell and moreover it co-localizes with FtsZ1. Several knock-out attempts remained without success, suggesting the essential role of *hvo_0392*. Furthermore, depletion of *hvo_0329* led to serious cell division defects.

Summarizing these results we provide first evidence that the gene *hvo_0392* encodes the FtsZ membrane anchor in *H. volcanii* and is therefore considered as SepF homolog.

BCP047

Polar localisation dynamics of the SRP-GTPase FlhF in *Shewanella putrefaciens*

J. C. Hook*[1], K. Thormann[1]
[1]*Institute for Molecular- and Microbiology, AG Thormann, Gießen, Germany*

The bacterial flagellum is a complex microstructure which, for a correct and functional assembly, requires strict regulation, on both a translational and posttranslational level. An important factor required for initiation of flagellar localisation and assembly in many γ-proteobacteria is the SRP-GTPase FlhF. In our model bacteria *Shewanella putrefaciens*, which possess both one lateral and one polar flagellar system, FlhF is only responsible for the polar flagellum synthesis and accordingly only localises polarly. The exact mechanism behind this strict localisation of FlhF is largely unknown, as are also the factor or factors, which are responsible and required for polar FlhF localisation. We observed, that FlhF still localises in a polarly manner, when both all the genes encoding motility factors of both the polar and lateral flagellar system and the polar landmark protein HubP were deleted. We therefore analysed the localisation behaviour of fluorophore-tagged FlhF in various *Shewanella putrefaciens* mutants carrying specific, flagellar assembly affecting deletions. The effect of these deletions on flagellar assembly was additionally evaluated via swimming assays. Thus, it is possible to conclude, that the polar localisation of FlhF in our model organism neither depends on motility factors of either

flagellar system nor does it depend on the polar landmark protein HubP.

BCP049

The *Bacillus subtilis*competence machinery contains a moonlighting enzyme

P. L. Graumann*[1], M. Burghard*[1], S. Altenburger[1]
[1]*Philipps-Universität Marburg, Biochemie, Marburg, Germany*

The DNA uptake machinery in Bacillus subtilislocalizes to a single cell pole at the onset of stationary phase. We show that the ComEB protein plays an important role in the recruitment of ATPase GomGA to the cell pole. ComEB localizes to the cell poles upon expression during exponential phase, indicating that it confers an anchor-function during competence. Interestingly, ComEB is also a deoxycytosine deaminase, for which a conserved cysteine residue is important. Cysteine-mutant ComEB is still active during transformation, whereas a comEBdeletion stain is highly impaired, indicating that ComEB is a true moonlighting enzyme. Single molecule tracking reveals that ComEB displays static and dynamic fractions, with 15% being bound at DNA uptake machinery. For ComGA, only 9% of the molecules are statically positioned at the cell pole, while two mobile fractions exist, one of which exchanges at the cell poles between bound and unbound in a time scale of 30 milliseconds. After addition of DNA to cells, ComGA turnover is considerably enhanced, while ComEB does not change its ratio of static and mobile fractions, revealing a highly dynamic role of ComGA during DNA uptake and an intricate interplay of DNA uptake and protein dynamics within this polar machinery.

BCP050

SMTracker: a tool for quantitative analysis, exploration and visualization of single-molecule tracking data reveals highly dynamic binding of *B. Subtilis* global repressor AbrB throughout the genome

P. L. Graumann[1], T. Rösch[1], L. Oviedo Bocanegra[1], G. Fritz[1], S. Dersch[1], B. Mayer*[1]
[1]*Philipps-Universität Marburg, Biochemie, Marburg, Germany*

Single-particle (molecule) tracking (SPT/SMT) is a powerful method to study dynamic processes in living cells at high spatial and temporal resolution. We developed SMTracker, a MATLAB-based graphical user interface (GUI) for automatically quantifying, visualizing and managing SMT data via five interactive panels, allowing the user to interactively explore tracking data from several conditions, movies and cells on a track-by-track basis. Diffusion constants are calculated a) by a Gaussian mixture model (GMM) panel, analyzing the distribution of positional displacements in x- and y-directionusing a two state diffusion model (e.g. DNA-bound vs. freely diffusing molecules), and inferring the diffusion constants and relative fraction of molecules in each state, or b) by square displacement analysis (SQD), using the cumulative probability distribution of square displacements to estimate the diffusion constants and relative fractions of up to three diffusive states, or c) through mean-squared displacement (MSD) analyses, allowing the discrimination between Brownian, sub- or superdiffusive behavior. A spatial distribution analysis (SDA) panel analyzes the subcellular localization of molecules, summarizing the localization of trajectories in 2D- heat maps.

Using SMTracker, we show that the global transcriptional repressor AbrB perfo highly dynamic binding throughout the Bacillus subtilisgenome, with short dwell times that indicate high on/off rates in vivo. While about a third of AbrB molecules are in a DNA-bound state, 40% diffuse through the chromosome, and the remaining molecules freely diffuse through the cells. AbrB also forms one or two regions of high intensity binding on the nucleoids, indicating that AbrB may also confer a structural function in genome organization.

BCP051

The Function of the Polyphosphate-associated CHAD Protein PptA of *Ralstonia eutropha*

H. Rosigkeit*[1], D. Jendrossek[1]
[1]*University of Stuttgart, Microbiology, Stuttgart, Germany*

Polyphosphate (polyP) is a linear chain of up to a few hundreds of phosphate residues linked by phosphoanhydrid bonds. PolyP was detected in all species that have been looked at. In bacteria, polyP is present in form of insoluble, globular inclusions of ≈50 to 200nm. It has the role of a reservoir for P and cations (Ca, Mg) but additional functions are assigned to polyP such as a stress mediator or source for nucleoside triphosphates (NTPs). However, the β-proteobacterium *Ralstonia eutropha* is able to survive in the absence of polyP under a variety of conditions [1]. Only little is known on the function in detail, the molecular structure and composition of polyP granules. We recently characterized polyP granules of *R. eutropha*. Remarkably*, R. eutropha* has two type 1 polyP kinases (PPK1a and PPK1b) and five type 2 polyP kinases (PPK2a-PPK2e). Four polyP kinases (PPK1a, PPK2c, PPK2d and PPK2e) and two additional proteins (PptA, PptB) were identified as being associated with polyP granules *in vivo* pointing to a complex macromolecular structure of polyP [2]. PptA also binds to glutamine synthetase A1 (GlnA1) *in vivo* [1], one of *R. eutropha"s* three annotated glutamine synthetases. The polyP-attached phosins PptA and PptB have a conserved histidine alpha helical domain (CHAD). In this study we started to address the function of PptA. We describe the properties of purified PptA and show the effect of *pptA* deletion. Furthermore we test the function of the three glutamine synthetases both *in vitro* and *in vivo* and investigate the role of PptA for GlnA1 activity.

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BCP052

Analysis of the wall teichoic acid composition in *Staphylococcus aureus* strains with intermediate susceptibility to vancomycin

M. Hort*[1], U. Bertsche[2], A. S. Schrötter[1], G. Bierbaum[1]
[1]*University of Bonn, Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany*
[2]*University of Hohenheim, Mass Spectrometry Unit, Stuttgart, Germany*

Vancomycin intermediate susceptible *S. aureus* (VISA) strains emerged during the treatment of *S. aureus* with glycopeptides. Common features of VISA strains include reduced autolysis, decreased virulence and a thickened cell wall with low peptidoglycan crosslinking. Here we focus on

the cell wall of a clinical VISA strain SA137/93A (MIC 8 µg/mL) and a laboratory VISA strain SA VC40 (MIC 64 µg/mL), which was generated by serial passage in the presence of vancomycin. The aim is to elucidate the role of the cell wall, especially wall teichoic acids (WTA), and autolysis in vancomycin non-susceptibility. UPLC analysis showed that both VISA strains have a thick cell wall with reduced crosslinking. This leads to an increase in false target sites for vancomycin. The strain SA VC40 also has a diminished negative cell wall charge measured by cytochrome C assay. A phosphate assay of isolated cell wall revealed a slight increase in the amount of WTA in VISA strains. Interestingly the WTA of SA VC40 were more D-alanylated compared to the controls. Teichoic acids are known to inhibit the activity of autolysins. Multiple lysis assays demonstrated a reduced lysis of *S. aureus* VC40, if WTA are present in the cell wall. Surprisingly, qRT-PCR and microarray analysis indicated an upregulation of multiple autolysin genes in *S. aureus* VC40. A zymogram of extracted autolysins suggested an alternate processing of AtlA, the major autolysin in *S. aureus*, reducing the active fraction. In conclusion, these results indicate that the decreased autolysis in VISA strains is caused by multiple interdependent phenomena, involving cell wall and wall teichoic acid composition.

BCP053

Two novel small proteins are important for cell elongation and lysis during induction of phage lambda in *Shewanella oneidensis* MR-1

V. Kreiling*[1], M. Kreienbaum[1], I. Oberheim[1], K. Thormann[1]
[1]JLU Giessen, Gießen, Germany

Phages have been known for over 100 years now, yet the exact interaction with its host and infection mechanism has not been completely resolved. Recently the interest in phages as a therapy method arouse again in part because of increasing antibiotic resistances in bacteria. Phages can roughly be distinguished into temperate phages and virulent phages. Contrary to the previous doctrine the dormant form of temperate phages, called prophages, has recently been shown to be beneficial to some bacterial populations, i.e. by increasing virulence and biofilm formation.

Our model organism *Shewanella oneidensis* MR-1 possesses four different prophages, λSo, MuSoI, MuSoII and CP4So. Even though phages are known to have comparatively small genomes most of them encode many small proteins with unknown function. The corresponding genes are often arranged in clusters. For instance, the genome of λSo contains three gene clusters of small undefined proteins, one of which was the focus of this work.

The goal of this study was to earn more information about the hitherto hardly known proteins. Therefore, we utilized different methods like bacteria and yeast two hybrid, time lapse fluorescence microscopy to investigate the roles of the proteins in the cluster.

We could show that the overexpression of one gene belonging to this cluster lead to significantly elongated cells even without the prophage present, but the additional expression of some other genes of the same cluster could prevent the elongated phenotype. Furthermore, the phenotype of the deletion mutant of another gene of the cluster indicates that this gene could be involved in cell lysis during the course of phage induction.

BCP055

BacStalk: a comprehensive and interactive image analysis software tool for bacterial cell biology

R. Hartmann[1], M. van Teeseling*[2], M. Thanbichler[2,1,3], K. Drescher[1,3,4]
[1]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
[2]Philipps-Universität Marburg, AG Thanbichler, Marburg, Germany
[3]LOEWE Center for Synthetic Microbiology, Marburg, Germany
[4]Philipps-Universität Marburg, Dept of Physics, Marburg, Germany

Prokaryotes display a remarkable spatiotemporal organization of processes within individual cells. Advanced image analysis software has revolutionized the cell-biological studies of established model organisms with largely symmetric rod-like cell shapes. However, algorithms suitable for analyzing features of morphologically more complex model species are lacking. We thus developed BacStalk, a simple, interactive, and easy-to-use MatLab-based software tool for quantitatively analyzing images of commonly and uncommonly shaped bacteria, including stalked (budding) bacteria. BacStalk automatically detects the separate parts of the cells as well as their connections, thereby allowing in-depth analyses of the organization of morphologically complex bacteria over time. BacStalk features the generation and visualization of concatenated fluorescence profiles along cells, stalks, appendages, and buds to trace the spatiotemporal dynamics of fluorescent markers. Cells are interactively linked to demographs, kymographs, and scatterplots, which enables intuitive and fast data exploration and, thus, significantly speeds up the image analysis process. Furthermore, BacStalk introduces a 2D representation of demo- and kymographs, enabling data representations in which the two spatial dimensions of the cell are preserved. The software was developed to handle large data sets and to generate publication-grade figures that can be easily edited. BacStalk therefore provides an advanced image analysis platform that extends the spectrum of model organisms for prokaryotic cell biology to bacteria with multiple morphologies and life cycles. Conference attendees will have the possibility to test the software on some of their microscopy images (phasecontrast or brightfield, and fluorescence).

BCP057

Investigation of PHB granules surface for an increased PHB yield in *Synechocystis* sp. PCC 6803

T. Orthwein*[1], M. Koch[1], K. Forchhammer [1]
[1]University Tübingen, Microbiology/Organismic Interactions, Tübingen, Germany

Polyhydroxybutyrate (PHB) is a promising alternative for conventional plastic due to its superior material properties as well as its biodegradability. The cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis)* is a natural producer of PHB and synthesizes the polymer under nutrient depletion especially during chlorosis (Klotz *et al*., 2016). PHB accumulates in membrane-free and protein-coated granules (Bresan *et al*., 2016). We could show that the cyanobacterial phasin (PHB coating protein) PhaP is associated on the surface of the granules and regulates its surface-to-volume ratio (Hauf *et al.*, 2015). It is known from other bacteria that PHB granules can harbor various kinds of phasins with regulating, anabolic or catabolic functions (Sznajder *et al*., 2016). Hence it is assumed that there are even more, yet undiscovered proteins involved in the PHB metabolism of *Synechocystis*.

Based on this knowledge we will characterize the PHB granules surface more intensively using different Omics technqiues. Transcriptome and Proteome approaches are used to display differences between vegetative-, chlorotic-

and recovering cells in transcript and protein content. Based on these results, genetical engineering of putative Pha proteins could provide deeper insight into the phsyiological function. Additionally, pulldown experiments will be used to verify interactions among the involved proteins.

One of the unknown proteins is the PHB depolymerase in *Synechocystis*. The detection and engineering of this enzyme can be exploited for increasing the intracellular PHB yield.

BCP058

A new protein crucial for microdomain stability

L. Kricks*[1], D. Lopez[1]
[1]*National Center for Biotechnology, Microbial Biotechnology, Madrid, Spain*

In bacteria, physiological processes like protein secretion, signal transduction and cytoskeleton rearrangement are confined into membrane microdomains. These Functional Membrane Microdomains (FMM) are structurally and functionally similar to lipid rafts in eukaryotes and their integrity depends on the presence of the scaffold protein flotillin. In prokaryotes the flotillin gene is co-transcribed with a gene encoding for a so-called NfeD protein. Members of the NfeD family only occur in prokaryotes and share a common domain resembling an OB-fold (Oligosaccharide/ Oligonucleotide binding) domain, but their function is yet unknown. Here we use the human pathogen *Staphylococcus aureus* to address the role of NfeD on FMM formation. Using a number of biochemical and cell biology approaches, we show that NfeD exclusively resides in FMM and physically interacts with the scaffold flotillin. The presence of NfeD is crucial for flotillin to localize into FMM and to oligomerize properly. Moreover, we found that NfeD and flotillin assemble into a large distinct heterooligomer *in vitro* and *in vivo*. A more detailed analysis of the NfeD protein reveals that the OB-fold is involved in the assembly into this heterooligomer. The importance of the formation of this heterooligomer on FMM formation and integrity is emphasized as the absence of NfeD leads to severe defects in FMM-associated cellular processes, including virulence.

BCP059

Functional characterization of the chlamydial Clp protease complex

S. Pan*[1], B. Henrichfreise[2], H. Brötz-Oesterhelt[1], P. Sass[1]
[1]*Institut für Mikrobiologie und Infektionsmedizin, Microbial Active Compounds, Tübingen, Germany*
[2]*Rheinische Friedrich-Wilhelms-Universität Bonn, Pharmazeutische Mikrobiologie, Bonn, Germany*

Clp proteases represent a conserved family of serine proteases with a distinctive chambered design made of 14 ClpP monomers and a stringent regulation of function by its associated Clp-ATPases. Clp proteases severely impact on virulence of pathogenic bacteria as they modulate expression of virulence factors or host-bacteria interactions [1,2].

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that is one of the most prevalent causes for sexually transmitted diseases and blinding trachoma [3]. Due to its obligate parasitic nature, *C. trachomatis* has evolved in a distinct manner adapted to exploit host cells, which is reflected by its specialized life cycle. Despite their complex life cycle, *Chlamydiae* have a severely reduced genome, implying that many of the retained genes may be crucial for fitness and viability. In this context, it is intriguing that *Chlamydiae*, like other pathogens such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, encode two *clpP* homologs, suggesting an important role of the Clp protease for the chlamydial lifestyle. Thus, Clp protease may constitute a potential new target for design and discovery of novel antibiotics to treat chlamydial infections.

To test this hypothesis, we set out to study the chlamydial Clp protease by means of purified proteins and established suitable *in vitro* assays to test and compare proteolytic activity to previously described Clp systems. We here report on the functioning of the Clp protease of *C. trachomatis* and its deregulation by antibiotic acyldepsipeptides, evaluating the potential of chlamydial Clp protease as an antibiotic target.

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BCP060

Deregulation of bacterial cytokinesis by antibiotic acyldepsipeptides

N. Silber*[1], C. Mayer[1], H. Brötz-Oesterhelt[1], P. Sass[1]
[1]*University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Microbial Bioactive Compounds, Tübingen, Germany*

FtsZ, the central pace maker protein of cell division in most bacteria, polymerizes into protofilaments to finally form the "Z-ring" at mid-cell, thereby leading bacterial cells into the division event. The Z-ring forms the scaffold which other cell division proteins adhere to, sequentially assembling the divisome that synthesizes the new cell membrane and cell wall (1). We have previously reported on the unprecedented finding that ADEP activates the bacterial peptidase ClpP to preferentially degrade the FtsZ protein, e.g. compared to other exemplary tested protein substrates including natural substrates of the Clp degradative complex, resulting in the inhibition of Z-ring assembly and cell division, and eventually cell death (2,3). We now study the reasons for this preference for FtsZ using an array of biochemical assays, and we further investigate the effects of ADEP treatment on the spatiotemporal organization of the divisome, the membrane, and the nucleoid. On the cellular level, at low inhibitory concentrations of ADEP, rod-shaped cells form long filaments and cocci develop into enlarged spheres. By studying the fate of FtsZ in *Bacillus subtilis* cells during ADEP treatment, time-lapse fluorescence microscopy revealed insights into the mechanism of FtsZ degradation and Z-ring formation. On the molecular level, we used ADEP/ClpP *in vitro* test systems for *B. subtilis* and *S. aureus* proteins, as well as a series of FtsZ mutant proteins, which revealed the molecular reasons for a preferred degradation of FtsZ, thereby allowing unprecedented insights into the molecular functioning of ClpP.

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BCP061

Adding a new function to the cyanobacterial inorganic carbon sensing protein SbtB: ATPase activity

K. Selim*[1,2], M. Hartmann[2], K. Forchhammer[1]
[1]*Tübingen University, IMIT/Organismic Interactions Dept, Tübingen, Germany*
[2]*Max Planck Institute for Developmental Biology, Protein Evolution Department, Tübingen, Germany*

The P_{II} superfamily consists of widespread signal transduction proteins found in all domains of life. In addition to canonical P_{II} proteins involved in C/N sensing, structurally similar P_{II}-like proteins evolved to fulfill diverse, yet poorly understood cellular roles[1]. Cyanobacteria evolved highly specialized carbon concentrating mechanism (CCM) to cope with limiting atmospheric CO_2 levels, augmenting intracellular inorganic carbon (C_i) levels to ensure efficient CO_2-fixation[2]. The sodium-dependent bicarbonate transporter SbtA is highly expressed under Ci limitation together with the conserved P_{II}-like SbtB protein[3]. SbtB can bind a variety of adenine nucleotides (ATP, ADP, AMP, and cAMP). The nucleotide-binding pocket was identified to be located between the subunit clefts of SbtB[3], perfectly matching the structure of canonical P_{II} proteins. Our previous results suggest that the novel P_{II}-like protein SbtB acts as C_i sensor protein via integrating the energy state of the cell and cAMP binding[3]. The evolutionary conserved role of cAMP/AMP as an indicator of cellular carbon status is well understood[3,4], but the function of ATP/ADP binding has remained unresolved. Here, we solved crystal structures of SbtB with ATP and ADP. Our structural and biochemical analysis revealed that SbtB has an ATPase activity. The slow ATPase activity of SbtB leads to a conformational change in the surface exposed T-loop of SbtB protein. We propose that the role of ATP/ADP binding is a molecular switch that drives a conformational change in the T-loops. We therefore suggest that the ATPase activity is likely to be a general property of most members of the PII superfamily[5]. However, the physiological role of the ATPase activity of SbtB remains obscure.

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BDP062

Application of endophytic actinobacteria to improve phytoremediation of cadmium polluted soils in the UAE

K. El-Tarabily*[1], S. AbuQamar[1], S. Al Raish[1], M. Alblooshi[1], A. Alblooshi[1]
[1]*UAE University, Department of Biology, College of Science, Al-Ain, United Arab Emirates*

Heavy metal contamination of soil causes a variety of environmental problems. Phytoremediation is an approach of removing contaminants from environment. Unfortunately, even hyper-accumulating plants that are tolerant to various environmental contaminants remain small in the presence of the contaminant. To remedy this situation, endophytic actinobacteria that facilitate the proliferation of plants under environmentally stressful conditions can be used. The aim of this study was to isolate from corn roots, cadmium-tolerant bacteria that produce the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase in order to reduce ethylene level and improve the growth of corn in contaminated soils. To achieve this, 65 endophytic actinobacterial isolates were selected for their ability to produce ACC deaminase as well as their abilities to tolerate different concentrations of cadmium. Out of these, only 25 were able to produce ACC deaminase. Under greenhouse conditions, the application of a mixture of five endophytic actinobacteria in cadmium nitrate contaminated soils significantly enhanced the growth of corn compared to control treatments. Soil inoculation with mixture of endophytic actinobacteria significantly enhanced cadmium uptake by corn from cadmium-contaminated soils compared to control treatments which included only corn grown in soil contaminated with cadmium nitrate without the application of endophytic bacteria. The results suggest that endophytic actinobacteria with ACC deaminase offer promise as inoculum for improvement of plant growth under unfavorable environmental conditions such as heavy metals contamination in soils. This is the first report to demonstrate the potential of endophytic actinobacteria to promote corn growth in soils contaminated with cadmium.

BDP063

Anaerobic degradation of polycyclic aromatic hydrocarbons (PAHs) by the sulfate-reducing bacterial enrichment culture TRIP

I. Kraiselburd*[1], N. Kotta[1], F. Kaschani[2], T. Brüls[3], M. Kaiser[2], R. U. Meckenstock[1]
[1]*Biofilm Centre, University Duisburg-Essen, Essen, Germany*
[2]*Centre of Medical Biotechnology, University of Duisburg-Essen, Essen, Germany*
[3]*Genoscope Institute of Genomics of the Atomic Energy and Alternative Energies Commission (CEA), Paris, France*

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed pollutants producing hazardous effects on human health. In PAH-contaminated sites, oxygen is rapidly depleted. Thus, microorganisms able to use these compounds as a carbon source in the absence of molecular oxygen are crucial for their consumption.

This work aims at elucidating the mechanisms for the anaerobic degradation of PAHs by a sulfate-reducing enrichment culture (TRIP) obtained from a natural asphalt lake.

The TRIP culture was grown anaerobically in freshwater mineral medium with different PAHs as carbon and electron sources and sulfate as electron acceptor. Bacterial growth was evaluated by cell counting (DAPI-staining) and sulfate consumption (ion chromatography). Bacterial composition was analyzed by 16S rRNA gene amplicon sequencing. The metaproteome of the TRIP culture was obtained in the different growth conditions by liquid chromatography-tandem mass spectroscopy.

The TRIP culture was able to anaerobically degrade the PAHs phenanthrene, acenaphthylene, 2-phenanthroic acid and 2-naphtoic acid. Shifts of the bacterial community were observed upon growth with each substrate, indicating that

different members of the enrichment culture are involved in the degradation of these compounds. Proteomic analysis in comparison to previously reconstructed genomes from the TRIP culture, revealed the dominant enzymes involved in the degradation process, as well as the organisms encoding such proteins. While degradation of phenanthrene involves a carboxylation step, followed by CoA ligation and a stepwise reduction, by enzymes encoded by a candidate *Desulfatiglans* bacterium; degradation of acenaphtylene appears to be performed by another bacterium of the same genus, and to involve a different mechanism.

BDP064

***beta beware:* Enantiomer discrimination in β-phenylalanine degradation by a newly isolated *Paraburkholderia* strain BS115 and type strain PsJN**

J. Rudat*[1], O. Buß[1]
[1]*Karlsruhe Institute of Technology (KIT), BLT_II: Technical Biology, Karlsruhe, Germany*

β-amino acids (β-aa) are degradation products of amine antibiotics, occur as part of natural compounds, and represent important building blocks for the synthesis of chiral pharmaceuticals. Soil and water microorganisms can be expected to be regularly affected with these molecules [1].

However, little is known about the metabolic fate of β-aa which is of importance in terms of *(i)* defense mechanisms of microorganisms affected with natural compounds containing β-aa *(ii)* environmental aspects referring to the persistence of β-aa in soil and water and *(iii)* pharmacokinetics of these natural compounds when used as drugs, e.g. cytostatics containing β-phenylalanine (β-Phe) derivatives.

We are investigating the biodegradation of β-aa using β-Phe as model compound. While former studies with *Paraburkholderia phytofirmans* PsJN (type strain) revealed strictly *(S)*-selective degradation of *rac*-β-Phe by a transaminase reaction [2], a closely related strain BS115 isolated in our lab additionally degrades the *(R)*-enantiomer by a so far unidentified pathway. This is the first report on degradation of both enantiomers of any β-aa by one single bacterial strain [3].

Analysis of the transaminase activities in BS115 revealed a broad substrate spectrum for several aromatic β-aa but rather strict *(S)*-selectivity. The *(R)*-degrading pathway appears to be induced during fermentation with *rac*-β-Phe as sole nitrogen source whereas the strain is not capable to grow with enantiopure *(R)*-β-Phe.

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BDP065

Study on the impact of common used rubber additives on the microbial and enzymatic degradation of poly(*cis*-1,4-isoprene)

A. L. Altenhoff*[1], J. de Witt[1], A. Steinbüchel[1]
[1]*Westfälische Wilhelms-Universität Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*

The increasing amount of rubber waste requires the development of an environmentally friendly disposal method. Therefore, research on biotechnical rubber degradation is essential. Regarding this topic, our study evaluates whether a microbial or an enzymatic process is more suitable for efficient biodegradation, due to less sensitivity towards rubber additives. We investigated the impact of 15 different frequently used rubber additives on the growth of the potent rubber degrader *G. polyisoprenivorans* VH2 and the enzyme Lcp1VH2. For this, cells were grown with poly(*cis*-1,4-isoprene) in presence of these rubber additives. Furthermore, the effect of those additives on the enzymatic cleavage of poly(*cis*-1,4-isoprene) by Lcp1VH2 was determined by *in vitro* studies. We found that accelerators, necessary to accelerate the rubber vulcanization process, are diminishing the growth of the microorganism, as well as the enzyme activity, regarding to their concentration. Accordingly, an increased concentration led to a higher toxicity. Sulfur prevents cell growth, but does not affect the *in vitro* degradation with Lcp1VH2. Additionally, we observed that *G. polyisoprenivorans* VH2 is able to metabolize stearic acid and paraffin wax. Plasticizers mainly prevented growth, but did not interfere with the enzyme. Antioxidants were identified as the most interfering group of additives for microbial and enzymatic rubber degradation. In summary, we found that the *in vitro* degradation by Lcp1VH2 is much more resistant and less sensitive towards the investigated rubber additives, when compared to the *in vivo* approach. We concluded that an enzymatic process might be a promising method to enhance rubber degradation.

BDP066

Characterization of LaoABCR as a novel system for the oxidation of mid- to long-chain alcohols in *Pseudomonas aeruginosa*

G. Panasia*[1], S. L. Drees[1], S. Oetermann[1], B. Philipp[1]
[1]*WWU Münster, IMMB, Münster, Germany*

The opportunistic pathogen *P. aeruginosa* is able to grow on a variety of compounds as growth substrates like long-chain alkanes or the toxic detergent sodium dodecyl sulfate (SDS). The degradation of both substrates includes the formation of the respective alcohol whose oxidation could be attributed to the recently discovered system LaoABC (long-chain alcohol/aldehyde oxidation) [1].

In order to analyze the new system in more detail, we purified the proteins and found LaoAB to form a FAD-dependent alcohol-dehydrogenase complex. This enzyme complex exhibited a broad substrate spectrum with a variety of hydrophobic alcohols demonstrated by photometric enzyme assays and in-gel activity stainings. Besides, LaoB is a known Tat substrate [2] and the Tat-dependent transport was shown to be an important factor for the functionality of the enzyme complex *in vivo*. Moreover, we identified a TetR-type repressor LaoR (PA0367) regulating the *lao* system and analyzed its binding site and putative ligand by electrophoretic mobility shift assays.

To summarize, LaoABC serves as a novel mid- to long-chain alcohol degradation system involved in the central metabolism. Detailed analysis of this system will give insight

in how *P. aeruginosa* is able to degrade uncommon and hydrophobic substrates and, thus, to survive and grow in hostile anthropogenic environments.

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BDP067

Isolation an ω-transaminase from Soil Microorganisms in Iran

N. Gord Noshahri*[1], J. Rudat[2], U. Engel[2], C. Syldatk[2], J. Fooladi[1]
[1]*Alzahra University, Biotechnology, Tehran, Iran*
[2]*KIT, Technical Biology, Karlsruhe, Germany*

ω–transaminases (ω-TAs) as a biocatalyst will be used to generate enantiomerically pure β-amino acids. β-amino acids constitute precursors for a number of important pharmaceutical drugs. ω-TAs have increasingly become an important due to such properties as expanded substrate spectrum, high enantioselectivity, high turnover number, and no need for regenerating external cofactor. However, product inhibition, low space-time-yields, and a lack of *(R)*-selective transaminases have been a major challenge to exploit the ω-TA reactions for industrial processes. Iran has a large amount of microbial storages due to variety in climate and can be used as a source for screening microorganism which are able to produce special biological products like enzymes for using pharmaceutical purposes. The purpose of this study is to investigate the novel ω-transaminase for the synthesis of enantiopure β-amino acids by screening soil microorganisms in Iran.

Microorganisms which produce ω-transaminase were screened based on growth on minimal media containing rac-α-Methylbenzylamine as sole Nitrogen source. The strains that showed high enzyme activity were selected in the colorimetric o-Xylylendiamine assay based on the reaction time in producing dark precipitations. The next step will be surveying the substrate spectrum, especially β-amino acids with aromatic and bulky side chains. The effect of different temperatures, pHs, organic solvents and discovering substrate specificity to determine the activity of the enzyme in different conditions will be investigated. The most promising strains will be identified by 16s rRNA analysis.

As a long-term objective, applications of the ω-TA in production of specific pharmaceuticals. will be tested.

BDP068

Isolation of thermostable alpha-Amylase from soil microorganisms

N. Gord Noshahri*[1], M. Zare Mehrjerdi[2], A. Sharifi Sharifabad[3], M. Shaban[1]
[1]*ACECR, Industrial Microorganism Biotechnology Research Department, Mashhad, Iran*
[2]*Shirvan University, Department of Plant Breeding and Biotechnology, Shirvan, Iran*
[3]*ACECR, Research Department of Ornamental Plants Biotechnology, Mashhad, Iran*

The aim of this study was to produce Amylase enzyme with acceptable amylolytic activity from local bacteria in Iran. Amylases are important industrial hydrolase enzymes which have been widely used since many decades and have approximately 25% of the enzyme market. Microorganisms produces a large variety of amylases which have major industrial importance.

Microorganisms were screened based on producing amylase in agar plate containing starch. The best one was selected based on clear white zone of hydrolysis around isolated colonies. The strain was then cultured in different media in order to optimize amylase production. Thermostability was analyzed at different temperature. The enzyme activity was quantified by Dinitrosalicylic acid (DNS) assay by reading the absorbance at 540 nm. The strain which could produce promising amounts of amylase enzyme was identified by 16s rRNA sequencing.

It was found that we came across *Bacillus subtilis* strain which was able to produce acceptable amounts of thermostable amylase enzyme which the activity was pretty considerable. Furthermore, enzyme production was constitutive.

BDP069

Isolation and identification of novel polyethylene-degrading bacteria from plastic-polluted sites in Iran

Z. Soleimani Dorcheh[1], G. Noshahri*[1], S. Gharavi[1], M. Soudi[2], Z. Moosavinejad[1], F. Hesami Zokai[1]
[1]*Alzahra University, Biotechnology, Tehran, Iran*
[2]*Alzahra University, Microbiology, Tehran, Iran*

Introduction: Synthetic polymer accumulation is the most important source of environmental pollutions. Polyethylene is the base material for plastic bags, water bottles, milk and food packaging that make up the largest volume of plastic pollutions. Polymers especially plastics are potential substrates for heterotrophic microorganisms. Low density polyethylene are used for plastic bags which make up a large part of the plastic waste.

Objectives: Isolation and identification of polyethylene-degrading bacteria for the biodegradation of polyethylene-based plastic pollutions.

Materials & methods: Soil samples were collected from different climatic zones in Iran where plastic pollutants accumulate. Soil is one of the most diverse microbial community niches in which polyethylene degrading bacteria can be found. Soil samples were enriched in specific media (polyethylene as carbon source) to increase the population of LDPE-degrading bacteria and incubated at 37℃ for 5-6 days on plate. The heterogeneous colonies (based on color, morphology and dominance) were isolated and identified using 16S rDNA gene sequencing. Various analysis (Weight loss, SEM, FTIR and Tensile test) for determination of the rate of polyethylene biodegradation is currently underway.

Results: Twenty-two bacteria have been isolated and identified which can use polyethylene as the sole carbon source. Most isolates were found to be *Actinobacteria*, comprising three genus of *Streptomyces, Nocardia* and *Rhodococcus*. as well as two *Bacillus* isolates. It seems *Streptomyces* species exhibit the highest degree of polyethylene biodegradation.

Identification of isolates and analysis of biodegradation of polyethylene show that *Actinobacteria* show high capability for biodegradation of polyethylene-based plastics.

BDP070

Evaluation of Bacterial Diversity from Metagenome Involved in The Biodegradation of Polyethylene-based Plastics

F. Hesami Zokai[1], G. Noshahri*[1], S. Gharavi[1], E. Asgarani[1], M. Soudi[2], M. Zarrabi[1], Z. Soleimani Dorcheh*[1]
[1]Alzahra University, Biotechnology, Tehran, Iran
[2]Alzahra University, Microbiology, Tehran, Iran

Introduction: One of the most ubiquitous and long-lasting plastic wastes is polyethylene (PE), posing a major threat to human health and the environment. Microbial degradation of these polymers has been implied as means for bioremediation strategies. However, more than 99% of the microbial species are unculturable and hence metagenomic mining for novel species and enzymes has been suggested as one of the potential tools at hand (1).

Object: The aim of this research is the study of bacterial populations from different niches which can consume and degrade PE as sole carbon source and their diversity.

Material & Methods: For this purpose, soil from two areas" with accumulation of plastic waste were sampled (2). Enrichment of the microbial cultures was carried out with low density polyethylene powder and hexadecane (model substrate) as sole carbon source (3).

Result: Soil DNA was extracted and the bacterial diversity of enriched samples were evaluated by NGS analysis of 16S rDNA PCR product sequences.

Conclusion: The results of the present work demonstrate the possibilty of a metagenomic approach for the study of microbial diversity in the search for potential PE biodegraders.

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BDP071

Enhanced biodegradation of low density polyethylene by thermostable fungi isolated from plastic polluted sites in Iran

Z. Soleimani Dorcheh[1], G. Noshahri*[1], S. Gharavi[1], M. Soudi[2], Z. Moosavinejad[1]
[1]Alzahra University, Biotechnology, Tehran, Iran
[2]Alzahra University, Microbiology, Tehran, Iran

Today plastics, due to their stable characteristics and cost, are used extensively, but these polymers accumulate in the environment and create a major threat to the ecology since it is not easily degradable and persist in the environment for extended periods of time. The ability of microorganisms to use polyethylene as a carbon source has only been recently established. Due to the accumulation of millions of tons of waste plastics every year, but also regarding the conservation of integrity for infra structures incorporating this plastic. In most studies, fungi were found to degrade LDPE (low density polyethylene) due to their ability to form hydrophobic proteins that can attach to polymer surfaces.

Isolation and identification of thermostable polyethylene-degrading fungal species for biodegradation of polyethylene-based plastics.

Soil samples were collected from different landfills in Iran, where plastic pollutants accumulate. Soil samples was enriched in polyethylene film media (polyethylene as carbon source) for 1 month following which they were incubated at 42℃ for 7 days on selective medium plates. The isolates were identified using sequencing of the ITS region. Different analytical approaches such as weight loss, SEM, FTIR and tensile test of plastic films to determine the rate of polyethylene biodegradation are underway.

Three fungal isolates have been isolated and identified which are capable of degrading polyethylene including two from the genus *Rhizomucor* and one *Aspergillus*. *Rhizomucor* species show the highest degree of LDPE degradation.

Rhizomucor genus is a suitable and robust thermostable fungi for the degradation of hydrophobic polymers. These fungal species are potentially useful for the bioremediation of plastic wastes in pollution sites.

BTP072

Metabolic flexibility of Pseudomonads – *Curse or blessing*?

S. Nies[1], B. E. Ebert*[1], L. M. Blank[1]
[1]RWTH Aachen University, Institute of Applied Microbiology, Aachen, Germany

Pseudomonas has been long proposed as superior biotechnological workhorse and with emerging synthetic biology tools is increasingly being domesticated and applied for biochemical production. Major arguments for the use of this microbe are its clean and versatile metabolism, tolerance of xenobiotics and solvents and ease of cultivation. We have previously shown, that *Pseudomonas* respond to harsh and highly energy demanding growth conditions with an up to 3-fold increased substrate uptake rate and a more than 6-fold increased redox cofactor regeneration rate.

This driven-by-demand response might be exploited for efficient synthesis of reduced products such as biofuels if it can be triggered under production conditions. The challenge, however, might be to deflect the reduced redox cofactors from oxidation in the respiratory chain of this obligate aerobic microbe to product synthesis.

Here we tested the proposed potential of *Pseudomonas taiwanensis* VLB120 by heavily constraining its NADH oxidation capabilities through deletion of genes encoding NADH consuming enzymes.

While a mutant without any NADH dehydrogenases of the respiratory chain seemed to be nonviable, single or double knockout strains showed none or only a marginal phenotype. An in-depth analysis of the metabolism of a variety of mutants by quantitative physiological experiments, transcript analysis, proteomics as well as enzyme activity assays showed that *Pseudomonas* has an incredible flexibility to cope with such genetic perturbations.

Yet, the apparent metabolic versatility poses challenges for an effective metabolic engineering of this species and calls

for further systems biological and physiological analyses to turn this promising microbe into a controllable, biotechnological workhorse.

BTP073

Electrocatalytic Biosynthesis using a Bucky Paper Functionalized by [Cp*Rh(bpy)Cl]$^+$ and a renewable Enzymatic Layer

L. Zhang[1], M. Etienne[1], N. Vilà[1], T. X. H. Le[1], G. W. Kohring*[2], A. Walcarius[1]
[1]*CNRS, Universitéde Lorraine, Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement, Villers-lès-Nancy, France*
[2]*Saarland University, Microbiology, Saarbrücken, Germany*

A bioelectrode for electroenzymatic synthesis was prepared, combining a layer for NADH regeneration and a renewable layer for enzymatic substrate reduction. The covalent immobilization of a rhodium complex mediator ([Cp*Rh(bpy)Cl]$^+$) on the surface of a bucky paper electrode was achieved by following an original protocol in two steps. A bipyridine ligand was first grafted on the electrode by electro-reduction of bipyridyl diazonium cations generated from 4-amino-2,2''-bipyridine, and the complex was then formed by reaction with $[RhCp^*Cl_2]_2$. A turnover frequency of 1.3 s^{-1} was estimated for the electrocatalytic regeneration of NADH by this immobilized complex, with a Faraday efficiency of 83%. The bucky paper electrode was then overcoated by a bio-doped porous layer made of glassy fibers with immobilized D-sorbitol dehydrogenase. This assembly allowed for the efficient separation of the enzyme and the rhodium catalyst, which is a prerequisite for effective bioelectrocatalysis with such bioelectrochemical system, while allowing effective mass transport of NAD+/NADH cofactor from one layer to the other. Thereby, it was possible to reuse the same mediator-functionalized bucky paper with three different enzyme layers. The bioelectrode was applied to the electroenzymatic reduction of D-fructose to D-sorbitol. A turnover frequency of 0.19 s^{-1} for the rhodium complex was observed in the presence of 3 mM D-fructose and a total turnover number higher than 12000 was estimated.

BTP074

Assessment of the flocculating potentials of *Alcaligenes faecalis* Isolated from the Estuary of Sodwana Bay

A. M. Ugbenyen*[1], A. K. Basson[2]
[1]*Edo University, Iyamho, Biochemistry, Auchi, Nigeria*
[2]*University of Zululand, Biochemistry and Microbiology, Empageni, South Africa*

Alcaligenes faecalis was previously isolated from Sodwana Bay, South Africa and was shown to be a bioflocculant producing microorganism. The bioflocculant production potential was further assessed through the optimization of the standardized culture media. The production and flocculating activity of the bioflocculant was assessed using different variables such as the inoculum size, carbon and nitrogen sources, time course and pH. Through optimization *A. faecalis* showed an improvement in the production and flocculating activity of its bioflocculant for the following factors: flocculating activity of 71% for an inoculum size of 1%. Maltose, as the most effective carbon source, showed flocculating activity of 91%, urea, as the most efficient nitrogen source, showed a flocculating activity of 97%, the optimum pH was 9. The time courses analysis between 60 and 72 hours showed the peak for bioflocculant production as shown by the flocculating activity. However, further studies on extraction, characterisation and application of the bioflocculants produced by this isolate is on-going to underscore the biotechnological importance of this microbe as producer of alternative substitute to the harmful chemical flocculants commonly used in water and wastewater treatment.

Keywords: Bioflocculant, *Alcaligene*, Flocculating Activity, Inoculum size.

BTP076

Effect of *Trichoderma reesei* degraded date pits on the growth performance and gut bacterial flora in broilers chickens

S. Alyileili[1], K. El-Tarabily*[2], W. Ibrahim [3]
[1]*UAE University, Department of Aridland Agriculture, College of Food and Agriculture, Al-Ain, United Arab Emirates*
[2]*UAE University, Department of Biology, College of Science , Al-Ain, United Arab Emirates*
[3]*UAE University, Department of Nutrition and Health, College of Food and Agriculture, Al-Ain, United Arab Emirates*

There is a demand for alternatives to antibiotic in poultry due to the risk of developing multiple antibiotic resistance in human pathogenic bacteria. To achieve that, a study was conducted to investigate the effect of degraded date pits on growth performance and gut bacterial flora in broilers chickens. Date pits were degraded using the fungus *Trichoderma reesei*. Broiler were distributed into 6 dietary treatments, treatment 1 (corn-soy diet control), treatment 2 (corn-soy with the antibiotic oxytetracycline (20%) as a growth promoter, whilst in treatments 3, 4 and 5 the broilers diet consisted of corn-soy with 10 % degraded date pits for 3, 4 and 5 weeks respectively. Treatment 6 consisted of corn-soy, non-degraded date pits and oxytetracycline. The feed of treatments 3 and 4 were withdrawn after 3 and 4 weeks respectively then the chicks were fed with corn-soy. Body weight, feed intake, feed conversion ratio and bacterial count in the gut of broilers were recorded. The time interval study showed that treatments 2, 5 and 6 had higher growth performance effect on broilers when compared to treatment 1, 3, however treatment 4 showed a moderate effect. The count of *Escherichia coli*, lactose fermenting enterobactericeae, non-lactose fermenting enterobactericeae, *Salmonella* and *Shigella* spp. were significantly higher in treatment 1 and 3 and significantly lower in treatment 2, 5, and 6. It can be concluded that degraded date pits diet (10 %) fed for 5 weeks and the antibiotic diet showed no significant difference on broiler growth performance and bacterial count. This suggests that degraded date pits, should be used for at least 5 weeks to replace antibiotics. Utilization of this by-product can reduce the risk of antibiotic resistance in human pathogenic bacteria.

BTP077

Fermentative production of *N*-methylglutamate from glycerol by recombinant *Pseudomonas putida*

T. Walter*[1], M. Mindt[1], J. M. Risse[2], V. F. Wendisch[1]
[1]*Bielefeld University, Genetics of Prokaryotes - Faculty of Biology & CeBiTec, Bielefeld, Germany*
[2]*Bielefeld University, Fermentation Technology - Technical Faculty & CeBiTec, Bielefeld, Germany*

Introduction: N-methylated amino acids are present in diverse biological molecules in all three domains of life. They have functions such as higher proteolytic stability and longer peptide half-lives, which are important for the design of new peptide-based drugs. Hence, there is an increasing interest

in this molecular class of alkylated amino acids by the pharmaceutical and chemical industries.

Objectives: Here, we describe *metabolic engineering* of *Pseudomonas putida* for the fermentative production of *N*-methylglutamate (NMeGlu) from simple carbon sources and monomethylamine (MMA). *P. putida* KT2440, which is generally recognized as safe and grows with the alternative feedstock glycerol as sole carbon and energy source, was engineered to produce NMeGlu.

Methods: For N-methylglutamate production, the operon *mgsABC-gmaS* of *M. extorquens* DM4 was heterologously expressed in a metabolically engineered *P. putida* strain. Fermentation was performed in shake flasks with minimal medium in the presence of 20 g L^{-1} glycerol as carbon source and 100 mM MMA as methyl group donor or in bioreactors operated in fed-batch mode.

Results: Deletion of transcriptional repressor gene *glpR* accelerated growth with glycerol. Concomitant chromosomal overexpression of the glutamate dehydrogenase gene *gdhA* improved provision of the glutamate. About 4 g L^{-1} NMeGlu accumulated in shake flask cultures. In fed-batch cultivation about 18 g L^{-1} NMeGlu were produced with a volumetric productivity of 0.13 g L^{-1} h^{-1}.

Conclusion: Taken together, expression of genes from *M. extorquens* in a *P. putida* strain KT2440 engineered for improved precursor supply and accelerated glycerol utilization enabled high yield NMeGlu production (Mindt M, Walter T, Risse JM and Wendisch VF (2018) Front. Bioeng. Biotechnol. **6:** 159).

BTP078

Optimizing the exploitation of unconventional secretion in *Ustilago maydis* by tackling proteolysis and establishing new carriers

M. Philipp*[1], M. Terfrüchte [1], K. Hußnätter[1], M. Reindl [1], M. Feldbrügge [1], K. Schipper [1]
[1]Heinrich-Heine University Düsseldorf, Institute for Microbiology, Düsseldorf, Germany

Introduction: There is an urgent demand for novel protein production platforms. We currently establish the corn smut fungus *Ustilago maydis* as a production platform utilizing the unconventional secretion pathway of the chitinase Cts1. This pathway circumvents the endoplasmic reticulum and enables the secretion of eukaryotic and bacterial proteins while avoiding potentially devastating *N*-glycosylation. Here we present several optimization steps to enhance protein secretion via this novel platform.

Objectives: In this work we aim to enhance secretory yields and apply this to newly identified target proteins.

Materials and Methods: We use genetic engineering, screening of media and buffer conditions, as well as UV mutagenesis and biochemical methods.

Results: Cultivation conditions were optimized and multiple protease deletion strains (up to 8-fold) were generated. Thereby yield and stability of target proteins could be increased. To further characterize the unconventional secretion pathway, we carried out a random UV mutagenesis screen to identify non-secreting mutants. All identified mutants expressed truncated versions of the protein Jps1 (jammed in protein secretion screen 1) which is also secreted unconventionally with about 3-times elevated levels compared to Cts1. Furthermore, we could show that Jps1 can be used for the export of heterologous proteins. In combination with its elevated secretion levels this makes it a promising candidate for further improvements of the secretion platform.

Conclusion: Several important steps have been taken to improve the unconventional secretion platform. Currently we are working on establishing Jps1 as a novel carrier protein, while simultaneously trying to produce new types of target proteins, such as anticalins.

BTP079

Synthesis of hydroxytriazenes by a chemo-enzymatic cascade

D. Tischler*[1], A. Gomez Baraibar[1], M. Mehnert[2], T. Heine[2]
[1]Ruhr-Universität Bochum, Faculty of Biology and Biotechnology, Microbial Biotechnology, Bochum, Germany
[2]TU Bergakademie Freiberg, Environmental Microbiology, Freiberg, Germany

Hydroxytriazenes are valuable compounds with promising uses as chelating agents, antibiotics or anti-inflammatory drugs[1]. They can be synthetized by coupling of diazene like molecules to hydroxylamine derivatives in a straightforward reaction. By combining enzymes from different metabolic pathways, a biocatalytic cascade was developed to produce a wide range of hydroxylamines from simple amino acids. Afterwards, with a final chemical step, several hydroxytriazenes can be easily obtained. The proposed enzymatic cascade consists of two enzymes: a decarboxylase and a monooxygenase.

The decarboxylation of amino acids is widespread reaction found among the tree of life. Using this huge variation, it is possible to find a specific decarboxylase for any type of amino acid, yielding a primary amine that can be further modified. Amino monooxygenases[2] are flavin dependent enzymes able to perform the hydroxylation of terminal amines. This reaction yields a number of N-hydroxy compounds which serve as a substrate in a chemcial diazene coupling at low pH.

By combining this hydroxylases with an amino acid decarboxylase with an overlapping substrate specificity, some highly interesting hydroxylamines can be produced and further transformed into hydroxytriazenes. Conventional cloning and protein production methods were employed. Products were isolated by solid and liquid extraction methods and analyzed by LC-MS. Thus a number of novel compounds were produced and verified.

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BTP080

Characterization of the ene-reductase FOYE-1 and upscaling reaction with (*R*)-carvone

E. Gädke*[1], D. Eggerichs[1], D. Tischler[1]
[1]Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany

The ene-reductase FOYE-1 from *Ferrovum* sp. JA12 belongs to the old yellow enzyme (OYE) family. These flavoenzymes reduce various α,β-unsaturated substrates at the expense of a nicotinamide cofactor, producing chiral molecules. In addition, some OYEs also accept artificial cofactors such as 1-benzyl-1,4-dihydronicotinamide (BNAH). Therefore, they may be of interest for biocatalysis. Due to the enhanced temperature stability of FOYE-1, it was characterized with respect to organic solvents stability and conversion of (*R*)-carvone with BNAH as electron donor.

Conventional cloning, expression and protein purification methods were employed. Enzyme assays were followed spectrophotometrically or by product extraction and subsequent determination via GC.

FOYE-1 shows a high specific activity on *N*-methylmaleimide with NADPH as cofactor. The synthetic BNAH is also accepted as an electron donor but has a significantly lower affinity with a K_M value of 600 µM. Nevertheless, the V_{MAX} value is quite high with 298 U mg^{-1} and *N*-methylmaleimide as substrate. FOYE-1 also shows a strong tolerance to organic solvents except for acetonitrile. The activity could be even increased with 20 vol-% of ethanol, acetone and isopropanol. It is also stable for up to 25 h in the before mentioned solvents, when stored on ice. An upscaling reaction with the crude extract and (*R*)-carvone as substrate and BNAH as cofactor resulted in a 60 % isolated yield of (2*R*, 5*R*)-dihydrocarvone. Of 751 mg (*R*)-carvone initially used, 450 mg of product could be determined.

In conclusion it can be stated that FOYE-1 has a high tolerance and stability to organic solvents. This is, together with the acceptance for the cost efficient BNAH, useful for biocatalysis, as many substrates are hardly water soluble.

BTP081

Astaxanthin production by *Corynebacterium glutamicum* and the establishment of a biosensor system

N. Henke*[1], P. Peters-Wendisch[1], V. F. Wendisch[1]
[1]*CeBiTec/ Universität Bielefeld, Genetics of Prokaryotes/ CeBiTec, Bielefeld, Germany*

Introduction & Objectives: *Corynebacterium glutamicum* is the workhorse of industrial amino acid production. Moreover, it is a natural producer of the yellow carotenoid decaprenoxanthin. Analysis of the carotenogenesis revealed that the repressor CrtR is controlling expression of the terminal carotenoid pathway in a metabolite-dependent manner with geranylgeranyl pyrophosphate (GGPP) as effector [1]. Astaxanthin is a red carotenoid that derives from GGPP as well. Metabolic engineering studies have shown that *C. glutamicum* is a suitable host for production of this high-value compound [2]. In this study, CrtR was validated as a biosensor for the terpenoid precursor GGPP.

Methods & Results: A series of *C. glutamicum* strains was engineered to accumulate GGPP to different concentrations within the cell. GGPP accumulation was enabled by deletion of *crtB* and improved by overexpression of *dxs* and *idi* encoding the limiting enzymes in the MEP pathway. In addition, overproduction of the GGPP synthase IdsA and the sigma factor A resulted in up to 4 mM of intracellular GGPP. Finally, a reporter system containing a transcriptional fusion of the carotenogenic *crtE* promoter and the reporter gene *gfpuv* was constructed. A correlation between the intracellular GGPP concentration and the reporter gene fluorescence was observed.

Conclusion: In this work, the first genetically encoded biosensor for the detection of a terpenoid precursor is described. Sensing of GGPP with CrtR allows applications in carotenoid (e.g. astaxanthin) production.

1 Henke et al., 2017, *Front Microbiol*, 8, 633.

2 Henke et al., 2016, *Marine Drugs*, 14, 7.

BTP082

Aspergillus ochraceus as a producer of proteases with protein C and factor X activator activity

A. Osmolovskiy[1], A. Orekhova*[1]
[1]*Lomonosov Moscow State University, Microbiology, Moscow, Russian Federation*

Micromycetes from *Aspergillus* genus are able to produce proteases capable to promote proteolysis of haemostasis proteins or, along with hydrolytic activity, to show the ability to convert proenzymes of this system activating them into an active form. In the same time, practical medicine needs in specific activators for quantitation of level of some plasma enzymes, especially protein C and factor X, the lack of which leads to the development of thromboembolic diseases

Aim of the research was study of production proteinases with protein C and factor X activating activity by *A. ochraceus* L-1.

A. ochraceus was cultivated under submerged conditions, protease activity detection was made using chromogenic peptide substrates specific for activated protein C (S-2366) and factor Xa (S-2765) after the addition of blood plasma (activator activity).

It was shown, that *A. ochraceus* can produce extracellular proteases with protein C and factor X activator activities. The maximum of accumulation of the proteases falls on the 4th day of cultivation. Using isoelectrofocusing was demonstrated that the activation of both proenzymes may proceed via limited proteolysis induced by proteases of *A. ochraceus*.

The activatory activity of *A. ochraceus* proteases toward essential hemostatic proenzymes, protein C and X factor, may be useful for practical needs. It is well known that similar enzymes, activators of protein C and X factor isolated from snake venom, South American copperhead *Agkistrodon contortrix contortrix* and Russell's viper *Daboia russelli*, respectively, are used for the *in vitro* diagnostics of the functional state of these proteins in blood plasma.

BTP083

Characterization of two novel glutathione S-transferases - styrene degradation in an actinobacterium

A. C. Lienkamp*[1], T. Heine[2], D. Tischler[1]
[1]*Ruhr-Universität Bochum, Microbial Biotechnology, Bochum, Germany*
[2]*TU Bergakademie Freiberg, Environmental Microbiology, Freiberg, Germany*

Styrene is a monoaromatic, toxic compound and produced in high amounts as product in industrial petrochemical and polymer processing. Hence, its degradation is of interest in bioremediation and pollution prevention. Among several unspecific degradation pathways, only a single styrene-specific pathway is known in bacteria. Herein, styrene is

degraded to phenylacetic acid (PAA) by three enzymes, namely styrene monooxygenase, styrene oxide isomerase (SOI) and phenylacetaldehyde dehydrogenase. *In vivo* the central metabolite PAA supplies the citric acid cycle, but it is also of industrial relevance as precursor for pharmaceuticals, flavors and fragrances.

The strain *Gordonia rubripertincta* CWB2 can utilize styrene as sole carbon source and was recently found to harbor a novel "hybrid" pathway lacking the SOI. This second enzymatic step seems to be circumvented via a glutathione S-transferase (GST) of which, though being rare in actinobacteria, two were found on a foreign plasmid in this strain (StyI, StyJ).

GST activity was detectable in the crude extract of styrene-grown wild type cells. Both GSTs were phylogenetically studied, cloned into pET expression vectors and successfully expressed in *E. coli*. Purification via affinity chromatography was achieved for StyI and activity of the purified enzyme was confirmed. StyJ was produced as inclusion bodies. Its production needs to be optimized. Both enzyme's activity properties including pH, solvent and temperature stability are currently under investigation. LC-MS analysis is used to identify products and elucidate plausible successive degradation steps. This study aims at resolving the anomalous styrene degradation pathway of CWB2, thus providing new ways for PAA production in industrial applications such as drug production.

BTP084

Development of an enzyme cascade process for the production of chiral β-amino acids

U. Engel*[1], C. Lohmann[1]
[1]*Karlsruher Institut für Technologie (KIT) , Institut für Bio- und Lebensmitteltechnik Bereich II: Technische Biologie, Karlsruhe, Germany*

Chiral β-amino acids are valuable building blocks for pharmaceuticals and fine chemicals (Rudat, Brucher, & Syldatk, 2012). Within this project chiral β-amino acids are to be produced applying a modified hydantoinase process using racemic dihydropyrimidines as educts. The process is to be based on two enzymes. A dihydropyrimidinase will be used for hydrolytic cleavage of the dihydropyrimidine ring followed by the reaction of a linear amidase able to decarbamoylate *N*-carbamoyl β-amino acids. Both enzymes are to be immobilized and applied in a microfluidic system.

While a screening for enzymes catalyzing the decarbamoylation step is ongoing it was already demonstrated in previous work that hydantoinases/dihydropyrimidinases can hydrolyze racemic 6-substituted dihydropyrimidines to the corresponding *N*-carbamoylated β-amino acids (Engel, Syldatk *et al.* 2012). Expression conditions for several recombinant dihydropyrimidinases were optimized to achieve soluble expression and acceptable amounts of enzyme. Additionally first immobilization experiments were conducted.

Next steps are the determination of kinetic parameters for the model substrate phenyldihydrouracil, the evaluation of optimal reaction conditions and immobilization of a dihydropyrimidinase in a microfluidic system.

Finally an enzyme cascade combining dihydropyrimidinase and decarbamoylating enzyme has to be established.

Rudat, J., Brucher, B.R. & Syldatk, C., *AMB Express*, **2**(1), (2012) p.11.

Engel, U., Syldatk, C. and Rudat, J., *Appl. Microbiol. Biotechnol.,* **94**(5), (2012), 1221-1231.

BTP085

Study of anti-inflammatory activity of tinocrisposide by inhibiting PGE2 production in lipopolysaccharides-stimulated raw 264.7 cells

A. Z. Adnan*[1], R. Ruslan[2], I. R. Suji[2], H. A. Ali[3]
[1]*Andalas University, Faculty of Pharmacy, Padang, Indonesia*
[2]*Andalas University, Pharmacy, Padang, Indonesia*
[3]*Andalas University, Padang, Indonesia*

Introduction: *Tinospora crispa* is a medicinal plant used in Indonesia to treat diabetes, inflammatory, and fever. From its stem extract has been isolated tinocrisposide, a diterpene glycoside (C27H36O11). Study of anti-inflammatory activity of the compound was previously conducted on LPS-stimulated RAW 264.7 cells. Test compound activity was quantified by measuring nitric oxide production. Tinocrisposide at concentrations of 1, 5, 25, 50, and 100 µM and 100 µM of dexamethasone (positive control) showed a lowering of NO level of the cells.

Objectives: The aims of the study were to investigate the anti-inflammatory activity of tinocrisposide by inhibition the PGE2 production.

Methods: Anti-inflammatory effect of the test compound was quantified spectrometrically at λ of 450 nm using PGE2 ELISA Kit (Elabscience) by measuring of PGE2 concentration in the supernatant of treated cells after it has been processed with various reagents according to official protocol.

Results: Tinocrisposide concentrations of 6.25, 12.5, 25, 50, 100 µg/ml and ibuprofen of 100 µg/ml (positive control) have been treated against the LPS-stimulated RAW 264.7 cells, and the results showed PGE2 level of 50.26, 46.89, 39.28, 32.13, 24.54 and 35.03 ρg/ml, respectively, and the inhibition level of 21, 26, 38, 49, 61, and 45 %, respectively. Tinocrisposide decreased the PGE2 production significantly ($p < 0.05$) in all the concentration tested.

Conclusion: It could be concluded that tinocrisposide has anti-inflammatory activity and furthermore its solution with concentrations of 100 µg/ml has higher anti-inflammatory activity than ibuprofen with the same of concentration.

Keywords: anti-inflammatory activity, PGE2, RAW 246.7 cell, Tinocrisposide, *Tinospora crispa*

BTP086

Self-immobilizing Biocatalysts for compartimentalised Reaction Cascades

P. Bitterwolf*[1], S. Gallus[1], T. Peschke[1], K. S. Rabe[1], C. M. Niemeyer[1]
[1]*Karlsruhe Institute of Technology, Institute for Biological Interfaces 1, Karlsruhe, Germany*

Biocatalytic flow processes are difficult to realize because the heterogeneous catalysis regime calls for effective surface immobilization techniques that are more demanding for enzymes than for conventional organo(metallic) catalysts.

Usual approaches use a biocatalyst which is non-specifically immobilized (e.g. physically adsorbed, chemically cross-linked or entrapped) and, thus, often decreased in its overall catalytic activity. Hence we have engineered biorthogonal self-immobilizing enzymes and cells, which enable the direct immobilization from crude mixtures while maintaining their catalytic activity.

As a proof of concept for our self-immobilizing enzymes, we recently described a one-pot enzymatic three-enzyme cascade that was adapted for the fluidic compartmentalised synthesis of *meso* diols. To this end, (*R)*- and (*S*)-specific ketoreductases were immobilized on superparamagnetic microparticles, which were loaded in a microfluidic packed bed reactor, thereby enabeling fluidic production of the *meso* diol from a prochiral CS-symmetric diketone. Furthermore, genetically encoded whole-cell immobilization *E. coli* strains were engineered, which display orthogonal immobilization tags on their surface while heterologously overexpressing stereoselective ketoreductases. We further expanded the scope of self-immoblilizing biocatalysts by establishing binary self-assembling biocatalytic all-enzyme hydrogels. Mounted in microfluidic reactors, the gels show excellent stereoselectivity with near quantitative conversion in the reduction of prochiral ketones along with high robustness under process and storage conditions. The gels function as a compartment that retains intermediates, thus enabling extraordinary high total turnover numbers of the expensive cofactor NADP(H).

BTP087

The impact of CO_2/HCO_3^- pool on glucose tolerance of PDHC-deficient *Corynebacterium glutamicum* strains

A. Krüger*[1], J. Wiechert[1], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

The pyruvate dehydrogenase complex (PDHC) catalyzes the decarboxylation of pyruvate to acetyl-CoA forming CO_2. Considering carboxylating and decarboxylating reactions which constitute the anaplerotic node, the intracellular CO_2/HCO_3^- pool has an important impact on flux distribution [1]. In this study, we assessed the impact of perturbations of this pool on growth of PDHC-deficient *Corynebacterium glutamicum* strains. We found that the deletion of the *pyc* gene, encoding the pyruvate carboxylase, caused a >15 h lag phase on medium containing glucose and acetate. Remarkably, increasing glucose concentrations led to even longer lag phases, while a deletion of *ptsG* eliminated this growth defect. We hypothesized that this defect is due to a lower intracellular CO_2/HCO_3^- pool in these strains impacting the glucose tolerance even in the presence of alternative carbon sources.

In line with this hypothesis, the addition of HCO_3^- complemented the observed growth defect, suggesting that an elevated CO_2/HCO_3^- pool can push metabolic flux over the PEP carboxylase. Remarkably, growth was also restored on substrates which are channeled into central carbon metabolism by an immediate decarboxylation, like gluconate or citrate, but not on ribose. Cultivation in media with higher pH (7.5 to 8) also eliminated the elongated lag phase, ascribed to the increased HCO_3^- solubility. Finally, an adaptive laboratory evolution experiment of Δ*aceE* Δ*pyc* on glucose and acetate emphasized the abolishment of glucose uptake as a predominant strategy of PDHC-deficient strains to allow for growth on acetate. Overall, these data highlight the importance of the intracellular CO_2/HCO_3^- pool for glucose tolerance of PDHC-deficient strains.

References:

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BTP088

Small phagic protein Cg1914 triggers SOS-dependent CGP3 induction in *Corynebacterium glutamicum*

L. Kever*[1], M. Hünnefeld[1], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1, Jülich, Germany*

Within recent years, small proteins (> 50 aa) were shown to participate in a wide range of biological processes and often feature regulatory functions including also the regulation of the lytic-lysogenic switch of bacteriophages [1]. A screening of diverse small proteins of *C. glutamicum* prophage CGP3 led to the identification of Cg1914, which caused prophage induction upon overproduction. Accordingly, this study focused on the characterization of the molecular mechanism of CGP3 induction via the small phagic protein Cg1914.

Remarkably, overexpression of cg1914 caused a severe growth defect and an elongated cell morphology. In the following, SOS-reporter strains (P_{recA}-*venus*) as well as an SOS-deficient prophage reporter strain (Δ*recA* P_{cg1974}-*venus*) were used to assess the correlation between CGP3 induction via Cg1914 and the host SOS-response with its key players RecA and LexA [2]. Overexpression of cg1914 in the SOS-reporter strain as well as in the SOS-deficient strain revealed SOS-dependent CGP3 induction. This is in line with a transcriptome analysis showing the upregulation of diverse LexA target genes upon overexpression of cg1914. The observed transcriptomic pattern showed striking similarities to the pattern obtained with the DNA-damaging agent Mitomycin C, which is a known trigger of the SOS response. Finally, a pulldown assay revealed co-purification of Cg1914 with several helicases. Altogether, these data suggest a function of Cg1914 as a DNA-interacting protein potentially involved in viral replication and/or recombination.

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BTP089

Production and characterization of 5-hydroxylated synthetic ectoine derivatives

J. Brauner*[1], E. A. Galinski[1], C. Ruprecht[2], C. Molitor[1], A. Meffert[1]
[1]*University of Bonn, Institut für Mikrobiologie und Biotechnologie, Bornheim, Germany*
[2]*Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften, Hamburg, Germany*

Introduction & Objectives: A widespread osmolyte utilized in the microbial world is the cyclic amino acid derivative ectoine (2-methyl-1,4,5,6-tetrahydropyrimidine 4-carboxylate). The formation of 5-hydroxyectoine is conducted by the ectoine hydroxylase EctD. hydroxylated ectoine has superior glass-forming characteristics which enable desiccation survival. Synthetic ectoine derivatives have been investigated for their special properties, homoectoine for example [1] is used as a PCR enhancer and improves the intestinal barrier in inflammatory bowel diseases [2]. Considering these and other confirmed advantageous properties we were interested

in producing and characterizing the 5-hydroxylated derivatives of the following artifical ectoine-type solutes: homoectoine, 2-amino-, 2-ethyl- and 2-propyl derivatives of ectoine.

Results & Conclusion: We used *E. coli* BL21 as a whole cell biotransformation system, which expressed a plasmid-encoded ectoine hydroxylase *ectD* from *Halomonas elongata*. HPLC-analysis revealed that *E. coli* was not only able to take up and hydroxylate the artifical ectoine-type derivatives but also to excrete the products into the medium. While the 6-membered OH-variants (2-amino-, 2-ethyl and 2-propylectoine) formed stable conformations, we observed that the 5-hydroxylated form of the 7-membered homoectoine was chemically instable and progressively degraded at neutral pH. The reaction products formed were subsequently analyzed using 2D-NMR

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[2] Castro-Ochoa KF *et al*, 2018: Homoectoine Protects Against Colitis by Preventing a Claudin Switch in Epithelial Tight Junctions. PubMed id: 30269272

BTP090

Novel penicillin G acylases from Gram-positive bacteria showing altered thermostability and mechanical properties

J. Mayer*[1], J. Pippel[2], G. Günther[1], M. Kubiak[3], C. Müller[1], T. Knuuti[1], C. Schilde[3], W. Blankenfeldt[2], R. Biedendieck[1]
[1]TU Braunschweig, Institute of Microbiology and BRICS, Braunschweig, Germany
[2]Helmholtz Centre for Infection Research , Structure and Function of Proteins, Braunschweig, Germany
[3]TU Braunschweig, Institute for Particle Technology, Braunschweig, Germany

Introduction: Penicillin G acylases (PGAs) are industrially relevant heterodimeric enzymes for the production of semi-synthetic ß-lactam antibiotics, as they catalyze the hydrolysis of penicillin G to 6-aminopenicillanic acid (6-APA) and the subsequent synthesis of penicillins using 6-APA as precursor.

Objectives: For industrial applications, the thermal and mechanical stability of enzymes should be improved e.g. by rational protein engineering. For this, the enzyme structure is of great interest to allow targeted modifications. Furthermore, enzymes can be stabilized by immobilization for a long-term use by formation of cross-linked enzyme crystals (CLECs).

Results: Two novel PGAs from *Bacillus* species were produced, characterized regarding activity and thermostability and the protein structure was elucidated, giving the first structure of PGAs from Gram-positives. Based on this, two types of mutants were generated: firstly, PGA mutants consisting of subunits from different species to examine the influence of the different subunits on the properties of the enzyme and secondly, PGA mutants to improve the thermostability by covalent binding of the subunits. These hybrid and single-chain PGAs were successfully produced and proven to be active catalysts. The thermostability of the single-chain PGA was raised, whereas the melting points of the hybrid PGAs were between those of the original variants. Furthermore, PGA crystals were cross-linked to stabilize the particles and mechanically characterized by indentation with an atomic force microscope showing a high hardness and Young´s Modulus.

Conclusion: By identifying novel PGA variants and rational design, the thermostability of PGAs has been improved. In addition, mechanical properties of PGA CLECs were determined.

BTP091

Recombinant production and purification of Envelope protein Domain III of Zika virus (EDIII) fused with the solubility tag Fh8

S. Carneiro Araujo*[1], L. Ramos Pereira[2], F. Grabarz[1], D. Borges de Figueiredo[1], L. Zane[1], L. C. Ferreira de Souza[2], V. Maimoni Gonçalvez[1]
[1]Instituto Buntantan, Laboratório Especial de Desenvolvimento de Vacinas, São Paulo, Brazil
[2]Instituto de Ciências Biomédicas/USP, Laboratório de desenvolvimento de vacinas, São Paulo, Brazil

Zika virus EDIII is an important target for inhibition of the cell-virus interaction and is the portion with fewer homology compared with others Flavivirus, thus, it is an important antigen to be used in vaccines and discriminatory serological assays. EDIII production in *E. coli* has been obtained as insoluble inclusion bodies, which demand extra steps to get the active conformation of the protein, leading to low recovery and high costs. An alternative is to modify culture parameters to avoid inclusion bodies formation. Some strategies are changing the culture medium, lowering the induction temperature and fusing the protein with solubility tags. We aimed to compare the production and purification of EDIII alone to EDIII fused with Fh8 solubility tag from soluble fraction of *E. coli*. Two *E. coli* BL21(DE3) carrying pET28a with Fh8EDIII and EDIII genes were grown in 1L of TB medium at 37°C until OD 600nm reaches 4. Induction was performed by reducing the temperature to 16°C and adding 0.4 mM IPTG. Cells (5g) were disrupted, the soluble portion was separated and applied to IMAC-Ni^{+2} for purification, since both proteins have His-tag. Proteins were quantified by Lowry assay. SDS-PAGE and band densitometry were used alongside the cultivation and purification to determine purity. The production of soluble EDIII and EDIIIFh8 in 1L culture was 1,03g and 1,96g, respectively, which represents a solubility increase of 91% with the tag. After chromatography, 95% of purity was achieved with a recovery of 38% and purification factor of 2.54 using Fh8EDIII. The purification of EDIII was not possible, since the protein precipitates when eluted, while the Fh8 tag allowed purification with high purity. The next steps are to remove the solubility tag and analyze the biological activity.

BTP092

Improving the biotechnological production of EMCP derived chiral compounds from methanol

L. Pöschel*[1], M. Buchhaupt[1]
[1]Dechema Forschungsinstitut, Industrielle Biotechnologie, Frankfurt a. M., Germany

The α-proteobacterium *Methylobacterium extorquens* is an extensively studied model organism for methylotrophy. It serves as promising production host for valuable chiral compounds directly from methanol. The ethylmalonyl-CoA pathway (EMCP) as part of its primary metabolism harbors several CoA-ester intermediates such as ethylmalonyl-, methylsuccinyl- or methylmalonyl-CoA. Those serve as potential precursors for promising chiral synthons. The release of a mixture of dicarboxylic (*S*)-methylsuccinic and mesaconic acid could be reached in the past by heterologous expression of an unspecific thioesterase. However, the selectivity of the hydrolyzing enzyme should be

increased for specific EMCP derived substrates in order to release ideally one certain product. Therefore, different amino acid exchanges in the binding pocket of the thioesterase were tested and several positions could be identified that affect the enzymatic reaction. Although none of the amino acid exchanges led to a shift in selectivity, one certain variant resulted in a 5-fold increased product concentration of (*S*)-methylsuccinic acid and mesaconic acid in production cultures. Furthermore, dicarboxylic acid re-import strongly limited the production efficiency in the past. By establishing new fermentation and feeding strategies the product re-uptake could be completely prevented. In summary we demonstrated different ways to increase the overall dicarboxylic acid production with *M. extorquens* from methanol by engineering the hydrolyzing enzyme and by optimization of the fermentation process.

BTP093

Analysis of global mRNA decay in *Gluconobacter oxydans* 621H

A. Kranz*[1], A. Steinmann[2], U. Degner[3], M. Bott[1], T. Polen[3]
[1]*Research Center Jülich GmbH, Metabolic regulation and engineering , Jülich, Germany*
[2]*Research Center Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*
[3]*Research Center Jülich GmbH, Genomics and Expression, Jülich, Germany*

Introduction: The strictly aerobic Gram-negative acetic acid bacterium *Gluconobacter oxydans* 621H is characterized by its ability to incompletely oxidize a broad variety of substrates in the periplasm and release of resulting products into the medium. Consequently, only a small fraction of the carbon source enters the cell, resulting in a low biomass yield. Glycolysis and the tricarboxylic acid (TCA) cycle are non-functional due to missing genes.

Objectives: Analysis of mRNA decay to identify short-lived mRNAs that could indicate possible bottlenecks in the metabolism of *G. oxydans*.

Methods: Bacterial cells were grown under standard conditions and harvested before and 2, 10, and 15 min after addition of the RNA polymerase inhibitor rifampicin. RNA was isolated and DNA microarray studies were performed to estimate mRNA half-lives. Furthermore, gene expression values determined by RNAseq in a previous study[1] were compared with the half-lives.

Results: mRNA half-lives for 2500 (95%) of the protein-coding genes of *G. oxydans* were estimated. Overall, they ranged from 3 to 25 min. In contrast to findings in other bacteria, transcripts of genes encoding the F-type H^+-ATP synthase involved in energy metabolism ranked among the least stable mRNAs. In addition, low expression values were determined for many genes of the incomplete TCA cycle and also the mRNA half-lives of some of those were below the global mean of 5.7 min.

Conclusion: We were able to identify possible bottlenecks in the energy and in the central carbon metabolism, which could be considered in future metabolic engineering approaches to improve the biomass yield of *G. oxydans*[2].

[1]Kranz et al. (2018) BMC Genomics 19, 24.
[2]Kranz et al. (2018) BMC Genomics 19, 753.

BTP094

Machine–assisted cultivation and analysis of biofilm in microfluidic bioreactors

M. Celikic*[1], E. Klein[2], S. Hansen[1], H. Horn[3], J. Gescher[2], C. M. Niemeyer[1]
[1]*Institute for Biological Interfaces, KIT, Eggenstein-Leopoldshafen, Germany*
[2]*Institut für Angewandte Biowissenschaften, KIT, Karlsruhe, Germany*
[3]*Engler-Bunte-Institut, Wasserchemie und Wassertechnologie, Karlsruhe, Germany*

Even though they are one of most abundant forms of life in the ecosystem, biofilms are far from being fully understood. This self-assembled mesh of different bacterial species and other microbial organisms forms a complex community, which is usually very robust and stress resistant. While on the one hand these properties makes them hard to handle and unwanted in field of medicine, on the other hand they can potentially be utilized in terms of biotechnology or environmental biology. Employing biofilms for the production of different pharmaceuticals or exploiting them for selective removal of undesirable substances would have a great impact. However, thorough investigation and better biological understanding of biofilm genesis and growth is the key to successful application of these complex communities.

Precise control and sampling can be enabled by cultivating biofilms in a microfluidic bioreactor. The latter, however, also requires a compatible analytical method. To this end a dedicated platform for automated cultivation and analysis of multi-species biofilm under flow has been developed. (Hansen et al. 2017, DOI: 10.1101/210583) The platform is designed to support multiple microfluidic flowcells of various geometries while keeping temperature, flow rate and environment settings constant. Flowcells, PDMS–glass hybrids, are compatible with analytical instrumentation such as automated light microscope, fluorescence reader or optical coherence tomography devices. The advantage of automated sampling lies in the ability to only remove very small amounts of sample volume while minimally invading the grown biofilm as well as in high spatiotemporal resolution in analysis of metabolites and biofilm composition.

BTP095

Metabolic engineering of *Corynebacterium glutamicum* for the production of Coenzyme Q_{10}

A. Burgardt*[1], P. Peters-Wendisch[1], J. H. Lee[2], V. F. Wendisch[1]
[1]*Bielefeld University, Genetics of Prokaryotes, Bielefeld, Germany*
[2]*Kyungsung University, Food Science & Biotechnology, Busan, South Korea*

Introduction: Ubiquinones are widely distributed compounds with various biological functions, e.g. in the electron transport chain. *Corynebacterium glutamicum* does not possess ubiquinone, but strains overproducing the Coenzyme Q_{10} (CoQ_{10}) precursor metabolites farnesyl pyrophosphate [1] and *para*-hydroxybenzoate [2] have been developed.

Objective: To enable CoQ_{10} production by *C. glutamicum*, decaprenyl pyrophosphate (DPP) synthesis by DPP synthase DdsA from *Paracoccus denitrificans* was chosen as first step, followed by prenylation of *para*-hydroxybenzoate and modification reactions leading to CoQ_{10} by proteins encoded by *ubi* genes from *E. coli*.

Materials & methods: Functional expression of heterologous genes was analyzed by metabolite analysis and by genetic complementation of *E. coli ubi* mutants.

Results: Activity of farnesyl pyrophosphate synthase IspA from *E. coli* could be shown in a *C. glutamicum* strain expressing amorpha-4,11-diene synthase. Expression of *ddsA* led to decreased carotenoid synthesis, an indirect hint to functionality. CoQ_{10} production was not detected, but plasmids with *ubi* genes complemented *E. coli ubi* mutants.

Conclusion: A possible bottleneck for CoQ_{10} production may be consumption of pHBA by hydroxylase PobA. If this and additional bottlenecks can be overcome, a proof-of-principle of ubiquinone synthesis in a non-ubiquinone containing organism is reached.

[1] Henke et al. (2018) *Genes* **9:**4.

[2] Syukur Purwanto et al. (2018) *J Biotechnol*. **282:**92-100.

BTP096

Production of acetone using recombinant *Acetobacterium woodii* strains

T. Schoch*[1], C. Erz[1], S. Hoffmeister[1], F. R. Bengelsdorf[1], P. Dürre[1]
[1]*Institut für Mikrobiologie und Biotechnologie, Ulm, Germany*

Acetone is an important platform chemical for industrial applications, and can be produced by gas fermentation using recombinant autotrophic acetogenic bacteria. One of these organisms is *Acetobacterium woodii*, which is capable of using CO_2 and H_2 as carbon and energy sources via the Wood-Ljungdahl pathway. However, *A. woodii* lacks an acetone production pathway. Therefore, the genes encoding thiolase (*thlA*), acetoacetyl-CoA: acetate/butyrate-CoA transferase subunits A and B (*ctfA/B*) and the acetoacetate decarboxylase (*adc*) from *Clostridium acetobutylicum* were assembled on the plasmid pJIR750_act, and the respective gene cluster was designated ASO. The constitutive thiolase promoter (P_{thlA}) from *C. acetobutylicum* was cloned upstream of the ASO. pJIR750_act was used to introduce recombinant acetone production in *A. woodii* (Hoffmeister et al., 2016).

The ASO was modified to further study the acetone production capabilities of the respective recombinant *A. woodii* strains.

Therefore, the genes *ctfA/ctfB* and *thlA* from *Clostridium scatologenes* were used to replace the genes from *C. acetobutylicum* in ASO. Since *C. scatologenes* habors two copies of corresponding genes, two plasmids designated pJIR750_ac3t3 and pJIR750_ac4t4 were constructed. The plasmid pJIR750_ac3t3 contains *thlA* as well as *ctfA/ctfB* (EG59DRAFT_00772-00774) and was used to obtain the strain *A. woodii* [pJIR750_ac3t3]. The other *thlA* gene copy and the other *ctfA/ctfB* copy (EG59DRAFT_01002-01004) were used to construct the plasmid pJIR750_ac4t4. This plasmid was used to generate the recombinant strain *A. woodii* [pJIR750_ac4t4]. The effect of the new ASOs regarding acetone production by the newly constructed recombinant *A. woodii* strains was examined by performing heterotrophic and autotrophic growth experiments.

BTP097

Production of the low-caloric sweetener 5-Ketofructose in *Gluconobacter species*

J. Hoffmann*[1], U. Deppenmeier[1]
[1]*Institute for Microbiology and Biotechnology , University of Bonn, Bonn, Germany*

The knowledge about the potential negative impact of dietary sugars on the consumer"s health has led to a growing demand for low-calorie, sugar-free foodstuff. A promising compound is 5-Ketofructose (5-KF), a low-calorie sweetener, that naturally occurs in various foods, for example in honey or white wines[1]. It can be produced from fructose by membrane-bound fructose dehydrogenases (Fdh) in some *Gluconobacter* strains[2]. Since 5-KF cannot be metabolized by the human digestive system and has the same natural taste and sweetness as fructose, we focused on the development of a microbial process that enables the efficient production of 5-KF. For a cost-efficient production, we improved the bacterial biotransformation by using the renewable substrate sucrose, which is the main storage substance of sugar beets and sugar cane, instead of the more expensive fructose.

A *Gluconobacter*-strain containing a genome-encoded fructose dehydrogenase complex (*fdhSLC*) was the starting point for the generation of a sucrose degrading mutant. To implement the ability to hydrolyze sucrose, a gene encoding a periplasmatic invertase from *Gluconobacter* sp. was cloned into a pBBR1MCS-based expression vector, which was transformed into *Gluconobacter fdhSLC*. HPLC analyses confirmed that 100 mM sucrose was completely hydrolyzed by the invertase within 30 hours. Fructose was successfully converted to about 60 mM 5-KF. To improve the production process, we intend to maximize the yield of 5-KF by additional genetic modifications of the investigated strain and optimizations of fermentation conditions.

[1]Siemen *et al*., 2018. *Appl Microbiol Biotechnol*. 102:1699-1710

[2]Kawai *et al*., 2013. *Appl Environ Microb* 79:1654–1660

BTP098

Halophilic Bacteria for Biocatalysis

C. Ohle*[1], G. Alfaro-Espinoza[1], H. J. Kunte[1]
[1]*Bundesanstalt für Materialforschung und -prüfung, Biologische Materialschädigung und Referenzorganismen, Berlin, Germany*

A sustainable bio-based economy enables the responsible use of natural resources and the generation of products that cannot be produced otherwise or can only be synthesized at high cost with chemical methods. Microorganisms as "cell factories" come into view as producers of biogenic products, as they can grow on different natural raw materials. In particular, halophilic bacteria are promising candidates for competitive production of biomolecules because they allow for a sustainable production from non-edible biomass waste (e. g. lignocellulose hydrolysates).

The halophilic bacteria *Halomonas elongata* is the industrial producer strain of the small organic compound ectoine, which is used in skin care and medical products for its protective effects. As part of this project, we have also modified *Halomonas elongata* for the stereospecific production of the amino acid derivative Nγ-acetyl-L-2,4-diaminobutyric acid (γ-NADA), which is the immediate synthetic precursor of ectoine. We significantly increased the production of both solutes by optimizing the translation initiation rate and by modifying the osmotically regulated σ38 promoter upstream of the ectoine synthesis genes. γ-NADA will have a dual function as high-value end-product similar to ectoine and as a precursor for biopolymer production. In summary, these described translational and transcriptional modifications can be applied to many other microbiological processes to increase productivity.

BTP099

Identifying phenotype-related genetic hot spots in chemically mutagenized cultures by automated high-throughput whole genome sequencing analysis

M. Dal Molin*[1], P. Baumann[1], S. Binder[2], J. Marienhagen[1]
[1]*Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany*
[2]*SenseUp, Jülich, Germany*

Microorganisms are increasingly exploited for the industrial production of amino acids, vitamins, antibiotics or biofuels. In wild-type organisms, metabolite formation is not optimized, but targeted or random engineering may result in an improved phenotype, i.e. increased metabolite production. Treatment with mutagens results in more than 200 random variations in the genome, which complicates the identification of beneficial mutations.

The aim of our project is to establish a high-throughput platform for the discovery of novel phenotype-genotype relationships in *Corynebacterium glutamicum*. For this, mutant libraries are generated via random chemical mutagenesis. Subsequently, biosensor-based, fluorescence-activated cell sorting (FACS) is conducted to isolate mutants with increased metabolite production. Finally, selected mutants are characterized by whole genome sequencing analysis. To circumvent the time-consuming and error-prone manual characterizing of mutations of several hundred genomes we developed a Linux-based automated genome analysis pipeline. It first identifies single nucleotide polymorphisms, insertions or deletions, and structural variants at a single cell level, and second tabulates all mutations to identify phenotype-related genetic hot spots, i.e. genes, gene regions or pathways.

In a preliminary proof of concept study, six mutants of *C. glutamicum* with increased intracellular L-lysine concentration were analysed. These selected mutants had no variations in previously known target genes. With our comparative analysis, we identified a mutational hot spot in *murE*; thereby reconfirming results of our previous work. Ultimately, this high-throughput analysis enables a fast and reliable identification of phenotype-genotype relationships.

BTP100

Functionalization of bacterial nanoparticles by genetic engineering and surface display of ligands and adapter molecules

M. Klein*[1], D. Schüler[1]
[1]*Universität Bayreuth, Lehrstuhl Mikrobiologie, Bayreuth, Germany*

Magnetotactic bacteria such as the alphaproteobacterium *Magnetospirillum gryphiswaldense* have the unique ability to synthesize membrane-enveloped magnetite nanocrystals. Their alignment into chains allows these so-called magnetosomes to function much like a compass needle, enabling the cell to sense the earth"s magnetic field. Due to their structural perfection and biocompatibility, combined with the ability to genetically engineer the surface proteins within the enveloping membrane, many applications have been proposed for these cuboctahedral crystals.

By displaying different proteins on the magnetosome surface, the properties of these biomaterials can be severely altered, giving them new functions in areas like biomedicine or biotechnology.[1] Highly abundant membrane proteins (MamC/A/F/G)[2] can serve as protein anchors and allows a controlled decoration of the particle surface and thus, flexibility regarding protein density.

As one possible application for magnetosomes we explored the expression of versatile coupling groups and receptor-activating ligands on the nanoparticle surface. By creating translational fusions to MamC, MamF, MamG and combinations of the three, different ligand densities were tested to adjust the rate at which the corresponding receptor is activated. Additionally, a two-component adapter system was implemented, with one component connected to a membrane anchor, while the other was fused to any other given (reporter) protein. Upon protein expression, both components formed a strong and irreversible interaction, turning the magnetosome surface into a modular platform for further functionalization.

[1] Raschdorf O., *et al.* (2018) *J. Proteom.* 172:89-99.

[2] Mickoleit F., Schüler D. (2018) *Bioinspir. Biomim. Nan.* doi:org/10.1680/jbibn.18.00005.

BTP101

Microbial biosynthesis of methane in a trickle bed reactor for power-to-gas applications

A. Böllmann*[1], R. Stöckl[1], L. Kalb[1], M. Thema[2], T. Weidlich[3], H. Huber[1], M. Sterner[2], A. Bellack[1]
[1]*University of Regensburg, Department of Microbiology and Archaeacenter, Regensburg, Germany*
[2]*Technical University of Applied Sciences, Regensburg, Germany*
[3]*University of Erlangen-Nuremberg, Erlangen-Nuremberg, Germany*

In terms of global warming and climate change, the Paris Agreement was signed to keep the global temperature rise well below 2°C above pre-industrial levels. A key provision of the agreement is to promote the mitigation of greenhouse gas emissions while fostering sustainable development including the transition to renewable energies.

To guarantee a secure and economical energy supply, power-to-gas is a promising innovative system solution. The idea of this concept is the conversion of electricity from renewable energy sources to hydrogen by electrolysis for direct usage or, in a second step, to methane and feeding it into the existing gas network for storage and distribution. The methanation can be done either chemically, known as Sabatier process, or biologically using methanogenic archaea. One mayor challenge of the latter method is the optimal and cost-efficient supply of methanogens with hydrogen and carbon dioxide since especially hydrogen dissolves only poorly in water.

The joint project "ORBIT" brings together process technicians, power engineers, microbiologists and field-leading industrial partners to develop new technological opportunities for the biological methanation. It aims to design and optimize a trickle-bed reactor by adjusting all operating parameters in simulations, lab and a field study at an existing power-to-gas site. Central part of the project is the interaction of methanogenic strains with the packing of the reactor to better understand the formation and long-time stability of biofilms.

The project will be presented by a poster and experiments affecting the optimal growth conditions will be displayed by the microbiological side of methanation.

BTP102

Engineering of transcriptional biosensors for accelerated strain development

P. Baumann*[1], M. Dal Molin[1], L. Flachbart[1], C. Sonntag[1], J. Marienhagen[1]
[1]Forschungszentrum Jülich GmbH, IBG-1, Jülich, Germany

Engineering of microorganisms for the biotechnological production of small molecules is a time-consuming, laborious, and expensive process. This can be mostly attributed to the fact that good producers cannot be readily obtained by high-throughput screening approaches since increased product formation usually does not confer a selectable or screenable phenotype to producing strain variants.

Recently, advances were made in the design and construction of genetically encoded biosensors for detecting small molecules at the single-cell level. In combination with fluorescent-activated cell sorting (FACS) we already demonstrated value and potential of these new tools for microbial strain development by screening large libraries of chemically mutagenized *Corynebacterium glutamicum* cells for L-lysine producers with the basic amino acid biosensor pSenLys. Here, whole genome sequencing of selected clones identified new mutagenic hot spots contributing to L-lysine-synthesis in the genome of this bacterium.

However, all attempts to also use this biosensor for engineering *C. glutamicum* towards overproducing the biotechnologically interesting amino acids L-histidine or L-arginine failed. Hence, we studied the transcriptional regulator LysG as recognition element of pSenLys in detail and semi-rationally engineered LysG towards no binding of L-lysine, while maintaining its L-histidine- and L-arginine binding capabilities. Subsequently, the engineered biosensor was successfully applied in a FACS-based screening campaign for isolating L-histidine producing *C. glutamicum* variants.

Currently, we continue engineering biosensors to alter their individual ligand specificities. Goal is the design of custom-made biosensors for the detection of compounds of biotechnological interest in single cells.

BTP103

Engineering of dioxygenase loops for enhanced oxyfunctionalization reactions

P. Heinemann*[1], J. Klenk*[1], A. Hunold[1], B. Hauer[1]
[1]Institute of Biochemistry and Technical Biochemistry , Department of Technical Biochemistry, Stuttgart, Germany

Introduction: The selective oxyfunctionalization of unactivated C-H-bonds is the focus of intense research. One group of enzymes, which can hydroxylate challenging substrates with energetically stable moieties like aromatic rings or with multiple functional groups are Rieske non-heme iron oxygenases (ROs). Besides mutagenesis of the active site, neighboring loops gained attention for enzyme engineering in recent years. Their importance is confirmed by heterologous expression of dioxygenases with different substrate scopes from *Phenylobacterium immobile* E, which differ mainly by one prominent loop above the active site.

Objectives: Deeper insights into the role of loops on activity, selectivity and substrate scope can have a major impact on biocatalyst performance. In our studies, we want to extend the potential of ROs for industrial applications.

Material & Methods: After heterologous expression in *E. coli*, the enzymes are characterized and further engineered to improve enzyme properties. Subsequently, enhanced enzyme variants are subject to process development.

Results: Substrate-specific oxygenases and thereby specific loops could be identified and characterized. Additionally, two corresponding active site loops of the cumene dioxygenase (CDO) from *Pseudomonas fluorescens* IP01 were selected based on sequence alignment with oxygenases of *P. immobile* E. These were subject of an alanine scan with subsequent rational and site-directed mutagenesis. Results reveal various previously undescribed positions that influence activity, selectivity and substrate scope.

Conclusion: Based on these results, we could show that besides traditional engineering of the active site, neighboring loops are promising candidates for enzyme engineering of this highly evolvable enzyme class.

BTP104

Inhibition of bacterial biofilm formation by natural compounds of entomopathogenic bacteria

A. Gazanis*[1,2], A. von Strempel[1,2], H. B. Bode[3], R. Heermann[1]
[1]Johannes-Gutenberg-Universität Mainz, Institut für Molekulare Physiologie, Mikrobiologie, Mainz, Germany
[2]Ludwig-Maximilians-Universität München, Biozentrum Mikrobiologie, Planegg-Martinsried, Germany
[3]Goethe-Universität Frankfurt, Fachbereich Biowissenschaften, Merck Stiftungsprofessur für Molekulare Biotechnologie, Frankfurt a. M., Germany

Bacterial biofilms not only affect health and environment, but also lead to a huge economic loss worldwide. Essential for biofilm formation is bacterial communication via small diffusible molecules, also referred to as quorum sensing (QS). Under high cell density, bacteria start to organize in biofilms by producing extracellular polymers to attach and grow on nearly every surface. Biofilms are problematic e.g. on ship hulls and buoys in the marine environment or within water pipes in the freshwater supply. Entomopathogenic bacteria of the genus *Photorhabdus* and *Xenorhabdus* produce a huge number of novel secondary metabolites. Here we show, that a large number of those natural compounds have a negative effect on biofilm formation without acting as simple bactericides. Therefore, we assume that these natural compounds might interfere with QS of the target bacteria to block their communication, a process that is referred to as quorum quenching (QQ). We observed, that bacteria of equal biofilm habitats exhibited similar susceptibility towards the same QQ-compounds. Interestingly, three of the tested compound classes had a severe effect on both single-species as well as multi-species marine and freshwater biofilms. To simulate more realistic native conditions for biofilm formation, we constructed specific microfluidic cells to observe and analyze bacterial attachment under constant flow. These flow chambers will be next used to test the effect of immobilized QQ-compounds on biofilm formation of the different bacterial communities. In summary, our data indicate that entomopathogenic bacteria represent an excellent reservoir of QQ-compounds that are putatively applicable as specific biofilm preventive agents.

BTP105

Characterization of two novel DyP-type peroxidases from *Streptomyces chartreusis*

N. Gibas*[1], A. Yayci[1], E. Hofmann[2], J. Bandow[1]
[1]*Ruhr University Bochum, Applied Microbiology, Bochum, Germany*
[2]*Ruhr University Bochum, Protein Crystallography, Bochum, Germany*

Enzymes as biocatalysts are becoming more prominent in modern industry, as they can provide some advantages compared to traditionally used chemical production processes. Peroxidases (EC.1.11.1.7) attract increasing interest in biotechnology because of their ability to perform several different regioselective and in some cases stereoselective reactions. Possible applications of these enzymes include synthesis of chiral building blocks and biodegradation of recalcitrant compounds, such as dyes and the plant polymer lignin. In contrast to fungal peroxidases, bacterial peroxidases are not well studied yet. However, sequence analyses reveal the presence of peroxidase genes in many Actinobacteria.[1] In this study, we characterized two novel peroxidases from *S. chartreusis*[2]. The dye-decolorizing peroxidases with N-terminal HIS_6-tags (SCDYP1 and SCDYP2) were successfully overexpressed in *E. coli* and subsequently purified. We found that both enzymes are complementary with regard to the conditions under which they are most active. SCDYP1 showed highest activity at pH 4, 10-20°C, and H_2O_2 concentrations around 400 µM. In contrast, SCDYP2 showed highest activity at pH 8, 30°C, and an H_2O_2 concentration of >50 mM. Given a sequence identity of ~44%, we compared the predicted structures of the enzymes *in silico* to identify structural differences that might explain the differences in the preferred operating conditions. Based on this analysis we will generate mutants to perform structure-activity assays.

[1] le Roes-Hill et al., Appl Biochem Biotechnol, 2011, *164*, 681-713.

[2] Senges et al., PNAS, 2018, *115*, 2490–2495.

BTP106

DNA extraction efficiencies of decreased sample material using the DNeasy PowerSoil Pro kit

S. Thiele*[1], D. O'Neill[1]
[1]*Qiagen, Microbiome Development, Hilden, Germany*

With the evergrowing capacity and accuracy of various sequencing techniques, DNA extraction becomes a vital part of studies addressing the microbial community in various environments. Since the discovery of the many influences of the gut microbiome onto human health, DNA extraction from human stool has become an integral part of many studies regarding the microbial community in the human gut system. Some difficulties in the extraction of DNA from stool are the often small amount of available sample material and the partially high amount of substances that inhibit PCRs and remain in the eluted DNA after cleaning. The DNeasy PowerSoil and the improved DNeasy PowerSoil Pro kit are usually done using the maximum amounts of samples recommended for the kit (250 mg). This can be difficult when sample material is sparse or high concentrations of inhibitory substances prevent further processing of the extract using PCR. Therefore, we hypothesized that lower sample amounts can be processed using the Qiagen PowerSoil Pro kit with similar results. Here we show that decreased sample amounts can still provide high yields, and is beneficial for some samples. We extracted DNA from 250 mg, 200 mg, 150 mg, 100 mg, 50 mg, 5 mg, and approx. 2 mg from 5 different stool sample and compared the yields using nanodrop. Furthermore, we compared the microbial community of the samples using Illumina MiSeq sequencing and subsequent processing of the sequences in the CLC Genomics Suite. DNA yields were highest from 150 mg samples and still considerable from 5 mg samples. Sequence analysis showed that alpha diversity, as well as taxonomic composition of the microbial community were comparable. This shows that the PowerSoil Pro kit can successfully be used for stool samples of amounts as low as 5 mg.

BTP107

DNAzymes as catalysts for L-tyrosine and amyloid beta oxidation

T. Köhler*[1], J. Thiele[1]
[1]*Leibniz Institute of Polymer Research, Nanostructured Materials, Dresden, Germany*

Question: Here, we present a new oxidation reaction for DNAzymes that are single-stranded DNA (ssDNA) having a catalytic activity when hemin is incorporated. We investigated the oxidation of L-tyrosine to dityrosine by hydrogenperoxide (H2O2) which can be catalyzed by DNAzymes. We screened for optimal stoichiometry of hemin in relation to ssDNA and investigated different sequences for their catalytic activity. These optimized parameters were used to apply this model reaction to the oxidation of amyloid beta (Aβ). That way, we can investigate a possible effect of DNAzymes in neurodegenerative diseases.

Methods: L-tyrosine oxidation was detected by fluorescence increase at 405 nm by using a plate reader. After optimizing the oxidation of L-tyrosine and its detection, we investigated the oxidation of Aβ by the same measurement setup. Furthermore, we investigated the influence of the oxidation of Aβ to its secondary structure by FTIR as well as CD spectroscopy.

Results: We found out that the fluorescence spectra of free L-tyrosine as well as the tyrosine within the peptide sequence of Aβ showed significant changes, when oxidized by free hemin as well as hemin being incorporated in DNAzymes. We observed an optimal stoichiometry of hemin-to-ssDNA of 1:10 to form a catalytically active DNAzyme. Further, we did not find an influence when adding only a single base to the DNAzyme sequence. Additionally, we could show that Aβ can by oxidized by hemin as well as synthetic or natural DNAzyme sequences.

Conclusions: We propose that DNAzymes can act as suitable catalyst for the synthesis of dityrosine by oxidizing L-tyrosine with H2O2. We further suggest a possible influence of DNAzymes in patho- and physiological processes that was exemplary shown by the oxidation of Aβ.

BTP108

Extremely thermoactive archaeal endoglucanase from a shallow marine hydrothermal vent from Vulcano Island

M. Suleiman[1], A. Krüger*[1], C. Schröder[1], B. Klippel[1], C. Schäfers[1], G. Antranikian[1]
[1]*Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany*

Introduction: Already characterized archaeal cellulases have proven to be highly useful for industrial processes, since they can withstand harsh industrial conditions with characteristics such as high thermo- and acid stability. These properties

provide promising features for the process of plant biomass degradation and biofuel generation [1].

Objectives: Since the number of known extremely thermoactive archaeal cellulases is low, the metagenome of the high-temperature enrichment culture was screened for the presence of novel endoglucanase-encoding genes of archaeal origin. In order to find new promising candidates, it is important to study not just the enzymes, but also the extreme habitats of hyperthermophiles, including their microbial diversity.

Materials & methods: In order to obtain new insights into community compositions of hyperthermophilic microorganisms, sediment and water samples were taken from two shallow marine hydrothermal vents (VsI and VsII) with temperatures of 100 °C at Vulcano Island, Italy. Metagenomic sequencing was used for microbial community analyses as well as sequence-based screening for thermoactive endoglucanases.

Results: The ORF vul_cel5A was detected, and the deduced protein was characterized as the most thermoactive endoglucanase described to date. Vul_Cel5A was identified as a thermoactive glycoside hydrolase family 5 endoglucanase, with the highest sequence identity (72–75%) to putative endoglucanases of archaeal genera. Vul_Cel5A showed the highest activity at 115 °C towards barley β-glucan (210.7 U/mg), and lichenan (209.9 U/mg), and further towards carboxymethyl cellulose (38.6 U/mg) and locust bean gum (83.0 U/mg). The endoglucanase exhibited a half-life time of 46 min at 100 °C and did not show any loss of activity after incubation at 75 °C for 48 h.

Conclusion: Due to the outstanding high thermoactivity and thermostability and tolerance to acidic conditions, Vul_Cel5A represents a promising novel archaeal endo-β-glucanase for application in biorefineries for an efficient biomass pretreatment.

[1] M Suleiman, C Schröder, B Klippel, C Schäfers, A Krüger, G Antranikian (2018) Extremely thermoactive archaeal endoglucanase from a shallow marine hydrothermal vent from Vulcano Island. *Applied microbiology and biotechnology*, 1-8

BTP109

Intracellular pHluorin to investigate activity of bacteriocins against *Listeria monocytogenes* – analysis on the single cell level and screening for novel producer strains

P. Crauwels[1], D. Weixler[1], L. Schäfer[1], K. Aßfalg[1], S. Goral[1], J. Weizenegger[1], G. M. Seibold[1], C. U. Riedel*[1]
[1]Ulm University, Institute of Microbiology and Biotechnology, Ulm, Germany

Listeria monocytogenes is a food-spoiling microorganism that frequently causes disease outbreaks around the world with fatal outcomes in at-risk individuals and is difficult to control in food production and processing environments and throughout food storage. Bacteriocins are antimicrobial peptides naturally produced by many bacteria and were shown to be effective against various pathogens including *L. monocytogenes*.

Recently, we constructed a derivative of *L. monocytogenes* EGD-e expressing the pH-sensitive fluorescent protein pHluorin as a sensor strain. This sensor strain was used to assess membrane damaging activity of commercial preparations of bacteriocins and in supernatants of producer strains. Here, we further investigate the potential of this strain for other applications. Sub-MIC concentrations of nisin and pediocin do not lead to complete depolarization of all bacteria. Microscopic analysis on the single cells level revealed that that is not caused by sublethal depolarization of the membrane of all cells of the population but rather the result of a mixed population of cells with either completely intact or completely depolarized membranes. Additionally, the sensor strain was used to implement a high-throughput screening approach to identify novel bacteriocin-producing strains. Screening a collection of newly isolated lactic acid bacteria identified a number of positive isolates. 16S rDNA sequencing suggests that these isolates are *Lactobacillus garvieae* strains potentially producing bacteriocins of the garvicin family.

Collectively these results suggest that intracellular expression of pHluorin in *L. monocytogenes* is a useful tool to identify and investigate membrane damaging compounds such as bacteriocins.

BTP110

Investigation of monoterpenoid resistance mechanisms in *Pseudomonas putida* GS1

F. M. Schempp*[1], K. Hofmann[1], J. Mi[1], F. Kirchner[1], H. Schewe[1], J. Schrader[1], M. Buchhaupt[1]
[1]DECHEMA-Forschungsinstitut, Industrial Biotechnology, Frankfurt a. M., Germany

Product toxicity is a common challenge for biotechnological production processes. Therefore, it is essential not only to increase the product formation rate by optimizing metabolic pathways, but also to enhance the strain"s tolerance towards the educts and products and thereby avoid limitations due to inhibitory effects. A high toxicity is known for many monoterpenoids, which are widely used in medicines, as flavor and fragrance compounds and in agriculture. The bacterium *Pseudomonas putida* shows an inherent extraordinarily high tolerance towards solvents including monoterpenoids. An understanding of the underlying factors can help to create suitable strains for monoterpenoids *de novo* production or conversion. In addition, knowledge about resistance mechanisms could allow a deeper insight into how bacteria can oppose monoterpenoid containing drugs, like tea tree oil.

To investigate the monoterpenoid resistance mechanisms of *P. putida* an approach, comprising the creation of a mutant library, the selection for monoterpenoid-hyperresistant mutants and further characterization via genome sequencing, deletion and complementation experiments and toxicity assays, was conducted.

Altered monoterpenoid tolerance was found to be mainly related to altered expression levels of efflux pumps. Surprisingly, while high efflux pump expression increased the tolerance against monoterpenoid alcohols, it led to reduced resistance towards monoterpenoid acids. However, an increase of monoterpenoid acid resistance could be gained by a mutation in an efflux pump component. It was also found that increased monoterpenoid tolerance can counteract efficient biotransformation ability, indicating the need for a fine-tuned and knowledge-based resistance improvement for production strain development.

BTP111

A biological treatment model inoculated with a novel sulfate-and arsenate-reducing bacterium treating artificial mining wastewater

H. Nguyen Thi[1], H. Dinh Thuy[1], P. Nguyen Minh*[2]
[1]*Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi, Viet Nam*
[2]*VNU University of Science, Vietnam National University, Environmental Technology , Hanoi, Viet Nam*

Treatment of mining wastewaters has been long time poorly concerned in Vietnam. The discharge of mining wastewaters into the receiving water bodies often causes detrimental effects without proper treatment on ecosystems and public health. The study investigated the capacity of a biological treatment model containing a neutralization tank, followed by a sulfate-reduction tank, aerobic and sand-filtration tank in treating artificial mining wastewater contaminated with sulfate (1152 mg L-1) and various metals (Fe, Zn, Cu, Pb, Ni, As) (the inflow rate was 2.88 L d-1). Fermented rice bran was used as an organic carbon source (COD concentration was 468 mg L-1). The sulfate-reduction tank was inoculated with the strain *Desulfovibrio oxamicus* S4, a novel sulfate and arsenate-reducing bacterium isolated from mining water in Tuyen Quang province, Northern Vietnam. In our previous experiment, the strain *D. oxamicus* S4 showed the notable capability of As5+ reduction, completely reduced 125.8 mg/L (1.68 mM) As5+ to As3+ within 7 days under completely anaerobic condition (initial arsenate concentration of 5 mM), with the respective arsenate reduction rate was 17,97 mg.L-1.d-1. The results showed that mean removal efficiencies of Fe, Zn, Cu, Pb, Ni, As by the treatment model with the inoculation after 2 experimental days were 100, 99.7, 99.9, 100, 99.8, 99.7%, respectively. In conclusion, the treatment model was efficient in decontamination of metal-rich mining water in the presence of strain *D. oxamicus* S4, the first bacterial strain reported in Vietnam for the ability of simultaneous reduction of sulfate and arsenate, contributing to the list of few strains with such capacity in the world.

BTP112

Systematic characterization of efficient low salt ectoine production in *E. coli*

L. Bethlehem*[1], K. D. Moritz[1], E. A. Galinski[1]
[1]*University of Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*

Introduction and Objectives: Despite increasing demands of the compatible solute ectoine in the health care sector [1, 2], the development of heterologous production systems stays behind expectations. This is even more surprising since natural producer strains seem to be optimised to their limits. One of the problems could lie in the fact that, foreign mostly extremophilic proteins of the ectoine biosynthetic pathway perform rather inefficient in their heterologous host systems. We could already show that the hydroxyectoine gene cluster of the non-halophilic *Acidiphilium cryptum* displayed optimal catalysis at low salt conditions, making this gene cluster an especially promising candidate for ectoine overproduction in *Escherichia coli*.

Results and Conclusion: Using tailored constructs and production conditions for *E. coli* we were able to achieve remarkable ectoine productivities of > 1g/gdcw, with ectoine being excreted into the medium at 0% NaCl and with glycerol as a cheap carbon source. A very high specific productivity of > 200 mg/(gdcw * h) was observed in the first eight hours of production, but could not be maintained for high cell density fermentation processes. To pinpoint the cause of this breakdown in productivity and enable further improvement at the high-cell density stage, the process was systematically characterized concerning plasmid copy number (qPCR), mRNA abundance (qPCR), protein abundance (MS) and composition of the metabolome (NMR).

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BTP113

Electrotrophic performance within the genus *Kyrpidia*

J. Lapp*[1], J. Reiner[1], T. Jung[1], J. Gescher[1]
[1]*Karlsruhe Institute of Technology, Institute of Applied Biosciences, Dept. Applied Biology, Karlsruhe, Germany*

Introduction: The ability of microbes to grow on cathodes as sole electron and energy source during microbial electrosynthesis allows several biotechnological processes independent of chemical electron donors and light. The crucial step in this process, the transport of cathode derived electrons into the cell remains at least partly enigmatic.

Objectives: The conversion of highly oxidized waste streams, such as CO2, into higher reduced valuable compounds remains a challenge for many chemical and biotechnological processes and is prevalently dependent on the usage of hydrogen as a reducing substrate. Microbial electrosynthesis represents a promising technology to decrease energy demand of biotechnological production chains and features a possible storage solution for excess electrical energy.

Methods & Results: During cultivation on a cathode poised at -500 mV vs. SHE, chronoamperometric data revealed the ability of *Kyrpidia spormannii* to take up electrons but surprisingly not of its next relative *K. tusciae*. Genomic comparison of both strains revealed prominent differences of several genes potentially involved in electron transfer, including a remarkable hydrogenase that seems to be unique in *K. spormannii*.

Conclusion: Differences in a limited number of genes seem to be responsible for the ability of cathodic electron uptake in *K. spormannii*. These differences indicate a pivotal role of the hydrogenase in electron uptake. Since different kinds of hydrogenases were previously shown to be involved in electrosynthesis, these results fortify the efforts of focussing on hydrogenases and hydrogenotrophic organisms for fundamental understanding of the process of electrotrophy.

BTP114

Polyphosphate analytics for *Saccharomyces cerevisiae* utilizing a new enzymatic assay

J. J. Christ*[1], L. M. Blank[1]
[1]*RWTH Aachen University, Institute for applied microbiology (iAMB), Aachen, Germany*

Question: Inorganic polyphosphate (polyP) can be found as a linear polymer of orthophosphate in almost all living organisms. To study polyP in *S. cerevisiae*, methods for analytical polyP extraction, and polyP quantification as well as length determination are required.

Objectives: The aims were to optimize an analytical polyP extraction for *S. cerevisiae*, and to develop an enzymatic assay for polyP quantification and length determination.

Materials & methods: PolyP extraction was done with an optimized phenol/chloroform extraction method. PolyP quantification and length determination was done by differential hydrolysis with two enzymes (*S. cerevisiae* exopolyphosphatase 1, *S. cerevisiae* inorganic pyrophosphatase 1) and subsequent colorimetric orthophosphate quantification.

Results: A new gold standard analytical polyP extraction from *S. cerevisiae* was developed [1]. The old gold standard takes 5.5 h and requires five reaction tubes. The new protocol extracts 40 % more polyP, takes 30 min, and requires one reaction tube. Furthermore, a novel enzymatic assay for comprehensive polyP quantification, and length determination of short chain polyP was developed [2]. Common biological impurities (DNA, RNA, ATP etc.) do not interfere.

Conclusion: The combination of the analytical polyP extraction and the enzymatic assay allows comprehensive and specific polyP analysis for *S. cerevisiae*.

References:

[1] Christ JJ, Blank LM (2018) Analytical polyphosphate extraction from *Saccharomyces cerevisiae*, *Analytical Biochemistry*, 563 (71-78).

[2] Christ JJ, Blank LM (2018) Enzymatic quantification and length determination of polyphosphate down to a chain length of two, *Analytical Biochemistry*, 548 (82-90).

BTP115

Recombinant pneumococcal fusion protein PspA-PdT: importance of the molecular linker for the stability and role of PspA for increasing PdT solubility

L. Zane[1,2], M. Cazelato Pires[1], M. Cabette Crivellenti[1], S. Kraschowetz[1,2], S. Carneiro Araujo[1,2], D. Borges de Figueiredo[1,2], V. Maimoni Gonçalvez*[1]
[1]*Instituto Butantan, Laboratório de Desenvolvimento de Vacinas, São Paulo, Brazil*
[2]*Universidade de Sao Paulo, Programa de Pos-Graducao em Biotecnologia, São Paulo, Brazil*

Streptococcus pneumoniae is an exclusively human pathogen responsible for respiratory and systemic diseases. Protein vaccines have been proposed as alternatives to the current polysaccharide based vaccines, which lead to the serotype replacement that mitigates the benefits of vaccination. Pneumococcal surface protein A (PspA) and genetically detoxified pneumolysin (PdT), promising vaccine candidates, were genetically fused and we aim to investigate if molecular linkers contribute to the stability of the hybrid and the effect of PspA in PdT solubility. PdT and fusion proteins were cloned into *Escherichia coli* BL21(DE3) using pET28a vector; 03 hybrids were obtained: without linker (PspA-0-PdT), with α-helix rigid (PspA-R-PdT) and glycine-rich flexible (PspA-F-PdT) linkers. PdT was produced as inclusion bodies (IB) in all culture media tested at 37°C. The solubility increased at lower temperature (25°C) using an auto-induction complex medium instead of chemically defined medium (CDM), but around half of PdT was still produced as IB. Fusion proteins were soluble in all media. As recombinant PspA is also soluble in *E. coli*, this solubility change can be attributed to PspA fusion. PspA-0-PdT was instable and broke down in the junction region during purification. The rigid linker solved this problem and the purified PspA-R-PdT was stable up to 14 month at -20°C. The presence of 08 glycines in the flexible linker together with several glycine rare codons in *pdt* gene led to truncated synthesis, which was solved by cloning into *Rosetta* DE3, a strain that has additional RNAt for rare codons. The purified PspA-F-PdT was stable at least for 3 month at -20°C. Besides making the PdT production feasible, fusion proteins have the advantage of producing 2 antigens in a single process.

BTP116

Genetically modified *G. oxydans* strains for the construction of specialized biocatalysts

S. Gruber*[1], W. Liebl[1], A. Ehrenreich[1]
[1]*Technical University of Munich, Chair of microbiology, Freising, Germany*

Acetic acid bacteria (AAB) like *Gluconobacter oxydans* are can incompletely oxidize a variety of different sugars, alcohols and polyols in a stereo- and regio- specific manner. Those oxidations are catalyzed by membrane-bound dehydrogenases (mDHs), which have an active site oriented to the periplasm and each having a broad, characteristic substrate spectrum. Therefore, *Gluconobacter* can be used to efficiently produce fine and bulk chemicals, which are not readily available by organic chemistry.

In this study strains of *G. oxydans* are developed, that are specifically tailored to be oxidative biocatalysts for the production of various industrially relevant products like L-erythrulose, 5-ketogluconate or tagatose. To this end we used the multideletion strain *G. oxydans* BP.9, where all its native mDHs were markerless deleted. This allows the expression of sole mDHs from various AABs relevant for specific product formation, thereby optimizing activity and circumventing undesired competing or follow-up reactions. The activity of mDHs was optimized by testing various promotors and plasmid versus chromosomal expression. Characterization of the substrate spectra of mDHs from different acetic acid bacteria strains, using a whole cell DCPIP activity assay, revealed great differences in the substrate oxidation spectra of mDHs, illustrating the vast reservoir of oxidations that can be achieved by AABs. This approach represents a general system for the construction of new oxidative catalysts.

BTP117

Genetic analysis of cellulose synthesis in acetic acid bacteria

M. Bimmer*[1], W. Liebl[1], A. Ehrenreich[1]
[1]*Technische Universität München, Chair of Microbiology, Freising, Germany*

Bacterial cellulose (BC) is a highly biocompatible material that is synthesized by several genera of bacteria. Organisms of the genus *Komagataeibacter* are able to synthesize biofilms, called mother of vinegar, which consist of pure microcrystalline cellulose. The genome of members of this group encodes three operons of cellulose synthases varying according to its complexity, containing the cellulose synthase (*acsAB*), a channel protein (*acsC*) and variable numbers of accessory proteins.

This study aims to determine the role of these operons in *K. hansenii* ATCC 23769. To this end, several clean-deletion strains were constructed to assess the components of the cellulose synthase operons for their effect on overall cellulose formation. To our best knowledge, this is the first genetic system available for a cellulose producing organism where specific chromosomal mutations can be constructed at

will. Deletions of the genes of interest were done using tri-parental conjugation, double homologous recombination and counter selection using 5-fluorocytosin and *codBA* markers. The markerless-deletion system is also adopted to *K. xylinus* ATCC 53582, an extraordinary high cellulose producing strain.

To characterize cellulose production colorimetric methods for cellulose quantification are established. The phenotype of the markerless deletion mutants reveal large differences in the significance of the respective cellulose biosynthesis operons for overall cellulose production. The phenotype of the different strains indicates for *K. hansenii* a dominant role of one *acsAB* operon (GXY_04277). This leads to the hypothesis that the other operons produce cellulose that has a more accessory role for the formation of the mother of vinegar.

BTP118

Unveiling the biotechnological potential of Recirculating Aquaculture Systems through metagenomics

P. Pérez-García*[1], J. Hüpeden[1], S. Güllert[1,2], E. Spieck[1], J. Chow[1], W. R. Streit[1]

[1]University of Hamburg, Microbiology and Biotechnology, Hamburg, Germany

[2]Christian-Albrechts-Universität zu Kiel, Institute of General Microbiology, Kiel, Germany

Aquaculture was conceived as a strategy to reduce overfishing of highly demanded fish species. Recirculating Aquaculture Systems (RAS) offer an ecological and efficient way of fish farming with minimal environmental impact. Unlike in early aquaculture, RAS recirculate the water between the culture and water treatment stages. Toxic ammonia and nitrite excreted by the fishes are removed by nitrifying chemolithoautotrophic microorganisms. This process takes place in biological filters (plastic biocarriers) in which ammonia and nitrite oxidizing bacteria form dense biofilms together with heterotrophic bacteria. These microbial communities harbor a huge amount of genes that could be of interest for biotechnological applications.

In this study, biofilters from three different RAS operated with freshwater (FR), brackish (BR) or marine (MA) water were investigated using a metagenomic approach in order to discover new biocatalysts.

Illumina NextSeq was used to sequence both the metagenome and metatranscriptome from the three samples. After running ORF prediction algorithms, several Hidden Markov Models (HMM) were applied to search for biotechnologically relevant enzymes. These included carboxyl esterases (CE), glycosyl hydrolases (GH), reductases and nitrilases. Gene expression was confirmed and quantified by mapping of the metatranscriptomic reads.

Proteobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia and Actinobacteria made up 80 % (FR) to 90 % (MA) of the bacterial communities. Almost 25,000 new, biotechnologically interesting, potential biocatalysts have been discovered using the described methods. CEs and GHs accounted for more than 75 % of them. 20 highly expressed genes (10 CEs and 10 GHs) were synthesized, expressed in *E. coli* and tested for activity.

BTP119

Improving 1-butanol production by pathway compartmentalization in yeast

J. F. Garcés Daza*[1], E. Boles[1]

[1]Göethe Universität Frankfurt am Main, Biowissenschaften, Frankfurt a. M., Germany

The alcohol 1-butanol highlights as a very promising biofuel. Compared to ethanol, the worldwide most produced biofuel, 1-butanol shows a higher energy content and a lower higroscopicity, making it more compatible with the current fuel infrastructure. Today 1-butanol is mostly produced via chemical synthesis, but its biological production has been known and mined for years. *Clostridium* species were the first discovered 1-butanol producers. Yet, their growth requirements, genetic recalcitrance and low carbon yield when producing 1-butanol impede their industrial application. Heterologous expression of the clostridial pathway has been attempted in commonly used industrial species like *E. coli* and *S. cerevisiae. E. coli* yielded higher 1-butanol titers than *S. cerevisiae*. However, the latter is more robust and exhibits a higher tolerance to 1-butanol, which is very relevant in such an industrial bioprocess. *Schadeweg* (2016) achieved the highest 1-butanol titer in *S. cerevisiae* (859 mg/L) by expressing a modified clostridial pathway in the cytosol. However, competing reactions or a suboptimal environment could be limiting the production yield. A promising solution to this could be the compartmentalization of the pathway in other organelles. This strategy was already successful for the production of other biofuels in yeast. Here, we study the effect of pathway compartmentalization in the production of 1-butanol. For this, novel signalling tags aiming those organelles were fused to enzymes involved in the pathway and the metabolism of *S. cerevisiae* was engineered to push the carbon flux towards these organelles. Although the butanol titer has not increased yet, the high occurrence of toxic pathway by-products suggests that there is still room for improvement.

BTP120

The panacea to food insecurity starts with rhizobia, mycorrhizal fungi and soybean in a controlled environment

N. O. Igiehon*[1], O. O. Babalola[1]

[1]North-West University, Microbiology Department, Mafikeng, South Africa

Food insecurity is a serious threat due to the increasing human population particularly in developing countries and may be remedy by the use of microbial inoculants. Therefore, in this study, the ability of rhizobial species and arbuscular mycorrhizal fungi (AMF) to enhance soybean (*Glycine max* L.) tolerance to drought stress in a controlled environment was investigated. A gradient of watering levels ranging from 100 to 40% of "soil retention capacity of water" was tested on non-inoculated soybean plants and plants inoculated with rhizobia and AMF in pot cultures. It was observed that the inoculated soybean plants especially soybean dually inoculated with *Rhizobium* sp. strain R1 and *Rhizobium cellulosilyticum* strain R3 (R1+R3) as well as *Rhizobium* sp. strain R1, *R. cellulosilyticum* strain R3 and mycorrhizal consortium (R1+R3MY) had significant impacts ($P<0.05$) on soybean leaf relative water content and electrolyte leakage respectively. The levels of accumulated soluble sugars and proline revealed that their concentrations increased mainly in microbially amended soybean plants exposed to drought stress (70 and 40% FC). Similar results were observed for chlorophyll content. Plants inoculated with R1+R3MY showed the highest number of spore and % mycorrhization in all the water regimes. At 40% FC, R1+R3MY treatment was found to promote soybean growth

since it had significant effects (P<0.05) on soybean shoot width, branch number, and root dry weight compared to the non-inoculated plants. These results revealed that co-inoculation of rhizobia and mycorrhizal fungi can be harnessed biotechnologically for field application to proffer solution to food insecurity intrinsic in developing countries especially in semi-arid regions.

BTP121

Whole cell catalysis for hydrogen storage and biohydrogen production using a thermophilic acetogen

F. M. Schwarz*[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: Recently a new group of enzymes were discovered in the two acetogenic bacteria *Acetobacterium woodii* and *Thermoanaerobacter kivui* which catalyze the direct hydrogenation of CO_2 to formic acid with exceptional high rates and in the absence of soluble cofactors, the hydrogen-dependent CO_2 reductases (HDCRs). These enzymes are promising biocatalysts for the storage of molecular hydrogen in form of liquid formic acid as well as for the production of molecular hydrogen from formic acid. To bypass the limitations of molecular hydrogen, the transient conversion of H_2 (and CO_2) into so-called liquid organic hydrogen carriers such as formic acid has already gained increased attention.

Objectives: To design a more efficient storage/production platform for molecular hydrogen and to take advantage of using whole cells from a thermophilic organism.

Materials & methods: Cells of *T. kivui* were grown in complex medium with pyruvate as substrate. In cell suspension experiments the formation of formate from H_2 and CO_2 as wells as the production of H_2 from formate was studied.

Results: The products formed from H_2 and CO_2 could be shifted from mainly acetate to formate by using ionophores/uncoupling agents or by simple pH change. Interestingly, the addition of $KHCO_3$ not only stimulated formate formation dramaticaly but it also completely abolished acetate formation. *T. kivui* reached highest specific formate production rates of wild-type strains in closed batch conditions ever reported (170 mmol g_{CDW}^{-1} h^{-1}). Formate was oxidized to H_2 with a maximal rate of 185 mmol g_{CDW}^{-1} h^{-1}.

Conclusion: The thermophilic acetogen *T. kivui* is a promising candidate for the biotechnological application in hydrogen storage and biohydrogen production.

BTP122

Multiplexed spectroscopic quantification of *P. aeruginosa* secondary metabolites

S. L. Drees*[1], S. Fetzner[1]
[1]*Westfälische Wilhelms-Universität Münster, Institute for Molecular Microbiology and Biotechnology, Münster, Germany*

Introduction: *Pseudomonas aeruginosa* is an opportunistic pathogen with high resistance against antibiotics. A characteristic of *P. aeruginosa* is its wealth of secondary metabolites, many of which are produced as virulence factors for sustaining infection. Levels of low molecular weight virulence factors are commonly assayed for strain phenotypization or evaluation of anti-virulence compounds. However, many established analytical procedures are either time consuming, technically challenging or only applicable for specific compounds.

Objectives: Development of a fast and precise analytical procedure for simultaneous quantification of low molecular weight secondary metabolites such as phenazines, alkyl quinolones and rhamnolipid.

Methods: Concentrations of pyocyanin, alkyl quinolones and rhamnolipid produced by *P. aeruginosa* grown under different conditions were determined using established procedures. In addition, UV/Vis and infrared (IR) spectra were recorded of the respective samples. Multivariate regression models were calculated from reference data to extract virulence factor concentrations out of spectroscopic information.

Results: Pyocyanin, rhamnolipid, alkyl quinolone and 2-alkyl-4-hydroxyquinoline-*N*-oxide concentrations could be determined from UV/Vis and IR spectroscopic data, using a support vector machine-based non-linear regression. Model accuracy is limited by the precision of reference data used for calibration. Sensitivity and sample consumption are comparable to established methods whereas hands-on time is drastically reduced.

Conclusion: Multiplexed spectroscopic quantification of low molecular compounds extracted out of bacterial cultures is a viable alternative to chromatographic techniques in exometabolome studies.

BTP123

Electrode-assisted fermentation of platform chemicals in *Escherichia coli* and *Shewanella oneidensis*

M. Edel*[1], L. A. Philipp*[1], T. Arinda[1], S. Beblawy[1], T. Kabbeck[2], J. Gescher[2,1]
[1]*Karlsruhe Institute of Technology, Institute for Applied Biosciences - Applied Biology, Karlsruhe, Germany*
[2]*Karlsruhe Institute of Technology, Institute for Biological Interfaces, Karlsruhe, Germany*

Introduction: Limitation of fossil resources demands a shift from industrial processes towards the use of sustainable raw materials. For this purpose, electrode assisted fermentations can provide platform chemicals from renewable carbon sources as well as a surplus of electrons that can be used in Bioelectrochemical systems (BESs). Using model organisms for extracellular electron transfer and microbial fermentations we seek to further improve these processes. Here we show strategies to enhance the current density in unbalanced fermentations for the anoxic production of acetoin via shuttle- and direct electron transfer based processes.

Objectives: In *S. oneidensis* the main limitation in BESs is the anode surface. To overcome this bottleneck, we aim to improve the biofilm formation and the electron flux towards the anode. In *E. coli* we seeked to enhance the current density by optimizing growth conditions and by selective adaption with regards to shuttle based electron transfer. Moreover, by dissecting the electron transfer pathway of *S. oneidensis* we try to establish a synthetic electron transfer chain to facilitate direct electron transfer in *E. coli*.

Methods & results: Using transcriptomic analyses we identified relevant genes for biofilm formation in *S. oneidensis* and established ways to induce biofilm formation synthetically. In *E. coli* we combined medium optimization, higher mediator concentrations, pH control and strain

development. This lead to a 6.5 fold improvement of the current density.

Conclusion: The results point out that electrode assisted fermentation of platform chemicals can be a suitable future biotechnological strategy. Further investigations will enable us to establish fermentation processes that have competitive yields compared to oxic production routines.

BTP124

Interplay between central metabolism and osmoprotection in *Halomonas elongata*

K. Hobmeier*[1], K. Pflüger-Grau[1], A. Kremling[1], A. Marin-Sanguino[1]
[1]*Technical University of Munich (TUM), Systems Biotechnology, Garching, Germany*

The halophilic bacterium *Halomonas elongata* is able to tolerate extremely saline environments by accumulating the compatible solute ectoine. This compatible solute is a valued ingredient in the cosmetics industry and has a high potential in medical applications. Understanding ectoine metabolism is therefore a mean to achieve two worthy goals: enhancing its biotechnological production and elucidating the underlying mechanisms of halotolerance in this organism. The synthesis pathway is encoded by the *ectABC* operon and its flux is strictly linked to central metabolism. Mathematical models and biochemical experiments (Kindzierski et al., 2017) have suggested that anaplerotic reactions and nitrogen assimilatory pathways have an impact. We could confirm the involvement of these central metabolic pathways by a genetic approach. The importance of nitrogen assimilation on ectoine production is not restricted to *H. elongata* but was also observed in the closely related but metabolically very different *Chromohalobacter salexigens* (Salar-Garcia et al., 2017). Therefore, we set out to study the role of central metabolism in *H. elongata* in respect to accumulation of ectoine in more detail. We will present our latest experimental results in an attempt to shed further light on the big picture of how the metabolic network of *H. elongata* achieves its efficient production of ectoine.

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BTP125

Hetero- and autotrophically driven C=C bond reduction in recombinant *C. necator*

L. Assil-Companioni*[1], S. Schmidt[1], P. Heidinger[2], H. Schwab[1], R. Kourist[1]
[1]*Institute for Molecular Biotechnology, TU Graz, Graz, Austria*
[2]*Austrian Centre of Industrial Biotechnology GmbH, Graz, Austria*

Cupriavidus necator, a hydrogen-oxidizing proteobacterium, is capable of growing under hetero- and autotrophic conditions. Its metabolic versatility along with the expansion of its genetic toolbox, make it a promising platform for the production of value-added products. The nicotinamide-dependent ene-reductase (YqjM) was thus introduced into the wild-type and a mutant incapable of polyhydroxybutyrate (PHB) production *via* a tailor-made *p*-cumate inducible vector.

The aim of this work was to investigate the catalytic potential of *C. necator* to drive C=C bond reductions powered by fructose or hydrogen. The establishment of hetero- and autotrophic conditions, allow for limitations and bottlenecks to be identified and improved upon. Thus, this work represents a building block towards an innovative and robust expression platform.

Fructose-driven conversions reached levels up to 100% with good enantiomeric excesses (*e.e*) of > 90%. Specific activities obtained under both hetero- and auto conditions ranged between 3 – 9 U/g and can be thought of as comparable to those reported using other autotrophic platforms such as the cyanobacterium *Synechocystis* PCC 6803. In addition, generated data suggests that PHB production and accumulation, may represent an alternate electron sink whose knock-out can increase electron availability and provide improved conversions and activities.

BTP126

A light-driven enzyme cascade reaction for the synthesis of lactones

H. Büchsenschütz*[1], V. Jurka[2], J. Spasic[2], S. Schmidt[1], R. Kourist[1]
[1]*University of Technology, Institute of Molecular Biotechnology, Graz, Austria*
[2]*University of Porto, Institute for molecular and cell biology, Porto, Portugal*

The application of cyanobacteria like *Synechocystis* sp. PCC 6803 as catalysts in sustainable bioprocesses is of great potential and the necessity for understanding the metabolic capabilities of these organisms strongly increases. NADPH, a main product of photosynthesis and cofactor of most oxidoreductases, can be directly used to drive enzyme-catalyzed reactions. However, living cells used as biocatalyst always bear the risk of side reactions due to native enzyme activities. In this particular case, saturated cyclic ketones which are substrates for the model enzyme CHMO from *Acinetobacter calcoaceticus* NCIMB 9871 are reduced to their corresponding alcohols in significant amounts. [1] Here, the ene-reductase YqjM from *Bacillus subtilis*[2] is combined with CHMO in an *in vivo* cascade reaction which reduces the hampering side reaction and different promoters are used to increase conversion rates. In the mixed cell approach, both enzymes are expressed in different cells before combined for biotransformation. This offers the possibility to compensate different expression levels and enzyme activities by precisely dosing both cells. On the other hand, co-expression of both enzymes would simplify the work progress and potentially also increase reaction rates because transport is reduced. By analyzing the effect of different promoters and expression systems we hope to establish a general strategy which can be used for future reaction cascades in cyanobacteria.

[1] Köninger, K.; Gómez Baraibar, A.; Mügge, C.; Paul, C. E.; Hollmann, F.; Nowaczyk, M. M.; Kourist, R. Angew. Chem. Int. Ed. **2016**, 55, 5582-5585.

[2] Böhmer, S.; Köninger, K.; Gómez Baraibar, A.; Bojarra, S.; Mügge, C.; Schmidt, S.; Nowaczyk, M. M.; Kourist, R. Catalysts **2017**, 7(8), 240.

BTP127

Expression strategies for cyanobacterial biotransformations

K. Bauer*[1], H. Büchsenschütz[1], G. Barone[1], N. Dyczmons-Nowaczyk[2], S. Schmidt[1], M. M. Nowaczyk[2], R. Kourist[1]
[1]*TU Graz, Graz, Austria*
[2]*Ruhr-Universität Bochum, Bochum, Germany*

Cyanobacteria, such as *Synechocystis* sp. PCC 6803, are becoming promising candidates for sustainable biocatalysis. Being phototrophic organisms, they are capable of converting light energy and CO_2 into chemical equivalents. As a product of photosynthesis, NADPH can be directly used to drive enzyme-catalysed reactions. The necessity of cofactor recycling limits the industrial application of enzymes, like the NADPH-dependent ene-reductase YqjM from *Bacillus subtilis* and therefore this system offers a promising alternative. In a previous study, YqjM was successfully integrated into the genome of *Synechocystis* via homologous recombination. [1] However, a solid replicative plasmid expression is particularly interesting as the genome integration is a time intensive process. The cyanobacterium *Synechococcus* sp. PCC 7002 is known to be more robust [2] than *Synechocystis* and might be a more suitable candidate for recombinant enzyme expression. In this study, different expression systems for YqjM are compared to identify optimal conditions for whole-cell-biotransformation.

[1] Köninger, K.; Gómez Baraibar, A.; Mügge, C.; Paul, C. E.; Hollmann, F.; Nowaczyk, M. M.; Kourist, R. Angew. Chem. Int. Ed. 2016, 55, 5582-5585.
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BTP128

Esterase profiling of new isolated yeasts in Morocco for prospective biotechnological applications

L. El Aamri*[1,2], F. Scordino[3], O. Romeo[2,3], M. Hafidi[1]
[1]*Faculty of Sciences. Moulay Ismail University, Biology, Meknes, Morocco*
[2]*University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Messina, Italy*
[3]*Scientific Institute for Research, Hospitalization and Health Care (IRCCS) - Centro Neurolesi "Bonino-Pulejo", Messina, Italy*

Microbial esterases are of substantial interest because of their prospective biotechnological and industrial application. In order to isolate new esterase fungal producers, one hundred and six fungal strains were isolated from different environmental samples (exhausted olive oil cake, fresh olive oil cake, black olive, rancid butter samples, Roquefort and rotten bread) taken from the region of Meknes (coordinates: 33°53′42″N 5°33′17″W) in Morocco. The isolated yeasts were tested for their ability to produce esterase by a qualitative method through Tween agar plates, the production was confirmed by a quantitative method through production in liquid medium, performed for 6 days at 28 °C with shaking.

ITS-sequencing method was used for phylogenetics studies and identification of the selected esterase producing yeasts. Preliminary results of ITS sequencing disclosed that three strains, belong to *Candida aaseri* species (LE.26, LE.27 and LE.31), another three strains to *Wickerhamomyces anomalus* species (LE.106, LE.112 and LE.115), however, the esterase activity revealed to be strain-dependent. While LE.153 was identified as *Metschnikowia rancensis*. LE.102 and LE.171 were identified as *Pichia* sp. and *Rhodotorula mucilaginosa*, respectively.

Further investigations are ongoing, in our laboratory, for additional characterization and for optimization of factors influencing the esterase production (temperature, pH) by liquid fermentation.

BTP129

Streamlined *Pseudomonas taiwanensis* VLB120 chassis strains with improved bioprocess features

B. Wynands*[1,2], L. M. Blank[2], N. Wierckx[1,2]
[1]*Forschungszentrum Jülich, IBG-1: Biotechnology, Jülich, Germany*
[2]*RWTH Aachen University, Institute of Applied Microbiology, Aachen, Germany*

Microbes have evolved under the selective pressure of their ecological niches. Hence, they harbor many traits that are dispensable or even unfavorable under industrial and laboratory settings. The elimination of such traits could improve the host"s efficiency, genetic stability, and robustness, thereby increasing the predictability and boosting its performance as a microbial cell factory.

In this study, we engineered solvent-tolerant *Pseudomonas taiwanensis* VLB120 to yield streamlined chassis strains with higher growth rates and biomass yields, enhanced solvent tolerance, and improved process performance. In total, the genome was reduced by up to 10% through the elimination of genes which enable the cell to swim and form biofilm. The megaplasmid pSTY and large proviral segments were also deleted. The resulting strain GRC1 had a 15% higher growth rate and biomass yield than the wildtype. However, this strain lacks the pSTY-encoded efflux pump TtgGHI, rendering it solvent-sensitive. Through re-integration of *ttgGHI* by chromosomal insertion without (GRC2) and with (GRC3) the corresponding regulator genes, the solvent-tolerant phenotype was enhanced, respectively, restored.

The generated *P. taiwanensis* GRC strains enlarge the repertoire of streamlined chassis with enhanced key performance indicators, making them attractive hosts for biotechnological applications. The different solvent tolerance levels of GRC1, GRC2, and GRC3 enable the selection of a fitting host platform in relation to the desired process requirements in a chassis *à la carte* principle.

BTP130

Studies on the Importance of Leader Sequence in the Expression and Secretion of Recombinant Uricase in Pichia pastoris

S. Tamimi[1], H. Moghimi[1], S. Asad[2], S. M. M. Dastgheib[3], K. Alishah*[2]
[1]*University of Tehran, College of Science, Department of Microbiology, Tehran, Iran*
[2]*University of Tehran,College of Science, Department of Biotechnology , Tehran, Iran*
[3] *Research Institute of Petroleum Industry, Microbiology and Biotechnology Research Group, Tehran, Iran*

Any patient with a rapid proliferating malignancy or following aggressive cancer therapy regimens that cause an increase in cell lysis is prone to hyperuricemia. Recombinant form of Aspergillus flavus urate oxidase (Rasburicase) is commercially produced in Saccharomyces cerevisiae and used for the treatment of hyperuricemia. However Pichia pastoris is considered as a promising host for the production of recombinant proteins owing to its favorable characteristics. P. pastoris secretes very low levels of native proteins, so secreted expression of recombinant proteins will simplify purification steps. The secretion signal sequence from the S. cerevisiae α-mating factor pre-sequence has been used successfully for secreted expression in P.pastoris. The Kex2

endoprotase favors the preliminary cleavage of the signal sequence, The Glu-Ala repeats are further cleaved by the STE13. Although the presence of Glu-Ala repeats after KEX2 cleavage site improves its performance and secretion yield, but there are some cases where Ste13 cleavage of Glu-Ala repeats is not efficient, and Glu-Ala repeats are left on the N-terminus of the expressed protein of interest. This is generally dependent on the protein of interest.

In this study, the codon optimized synthetic gene of A. flavus urate oxidase was cloned twice in pPICZαA expression vector as different constructs to examine the role of Glu-Ala repeat on enzyme production and secretion. Both vectors were transformed to P.pastoris GS115 strain. Multi-copy integrants were screened on zeocin gradients up to 2000 µg/ml. PCR was used to confirm the cassette insertion into the genome. SDS-PAGE, western blotting and enzymatic assay were performed to compare the enzyme expression and purification.

Keywords—Leader Sequence, Pichia pastoris, Secretion Efficiency, Uricase

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BTP131

Screening, Characterization and Biotechnological Use of Decarbamoylizing Enzymes

C. Lohmann*[1], U. Engel[1]
[1]*Karlsruher Institut für Technologie, Technische Biologie, Karlsruhe, Germany*

β-amino acids are interesting components for pharmaceutical drugs, peptidmimetica and fine chemicals (Rudat, Brucher, & Syldatk, 2012). They can be distinguished in β2-, β2,3- or β3-amino acids. The focus in this project is on β3-amino acids, which often can be found as part of natural products. One example is the cancer drug paclitaxel (Taxol) or antibacterial peptides (Wani et al., 1971; Spiteller 2009). Furthermore β-alanine and β-aminoisobutyric acid are end products of the pyrimidine catabolism.

The dihydropyrimidinase process for production of β-amino acids is based on the so called "Hydantoinase process", which has already been established in the industry for production of α-amino acids. The reaction of this enzyme cascade is shown in Figure 1. Theoretically it constitutes of two enzyme reactions. In the first step a dihydropyrimidinase cleaves the substrate, a substituted dihydropyrimidine, to the corresponding *N*-carbamoyl-β-amino acid. This project deals with the second part of this enzyme cascade, in which a decarbamoylizing enzyme should cleave an *N*-carbamoyl-β-amino acid to the corresponding β-amino acid. *N*-carbamoyl-β-phenylalanine will be used as a model substrate. Since decarbamoylizing enzymes occure naturally in the pyrimidine catabolism it is likely that several enzymes from different microorganisms can be found which catalyze this reaction.

Through microbial screening novel decarbamoylating enzymes have been discovered. It was shown that those enzymes degrade novel *N*-Carbamoyl-β-(Homo)-amino acids to the corresponding β-(Homo)-amino acid. Comparing those decarbamoylating enzymes with each other gives a novel insight into their function.

BTP132

Production of phenylalanine-derived aromatics utilizing a streamlined *Pseudomonas taiwanensis* chassis

M. Otto*[1,2], B. Wynands[1], L. M. Blank[2], N. Wierckx[1]
[1]*Forschungszentrum Jülich, IBG-1 Microbial Catalysis, Jülich, Germany*
[2]*RWTH Aachen University, iAMB, Aachen, Germany*

Bacteria of the genus *Pseudomonas* are receiving increasing attention as microbial cell factory for various biotechnological applications. We engineered the strain *Pseudomonas taiwanensis* VLB120 GRC3, a genome-reduced, streamlined chassis with enhanced bioprocess features to accumulate l-phenylalanine, a precursor for a variety of aromatic chemicals. Eight genes encoding enzymes involved in the degradation of aromatic intermediates were deleted. Additionally, the gene *pykA*, expressing a pyruvate kinase was removed to increase the precursor supply for the synthesis of aromatic amino acids. The flux through the shikimate pathway was further enhanced by genomic modifications of the genes encoding TrpE^{P290S}, AroF-1^{P148L}, and PheAT^{310I}, resulting in a strain able to accumulate up to 2.6 mM of l-phenylalanine from 20 mM glucose. The introduction of different heterologous genes into the engineered chassis resulted in the production of various aromatic model compounds. The expression of a gene encoding for phenylalanine ammonia-lyase (PAL) from *A. thaliana* enabled the conversion of l- phenylalanine to cinnamate, an organic chemical used in flavoring and pharmaceuticals. The strains are able to produce cinnamate with a Cmol-yield of up to 30% (12 mM cinnamate from 60 mM glucose) in shake flask cultivations. The introduction of a ferulic acid decarboxylase from *S. cerevisiae* in addition to PAL led to the production of styrene, the precursor to polystyrene. This work enabled efficient production of phenylalanine and its derivatives cinnamate and styrene, thereby demonstrating the versatility and high potential of *Pseudomonas* as hosts in bio-based production processes for the synthesis of a multitude of industrially relevant chemicals.

BTP133

Comparison of the Cytosolic and Secreted Expression of Recombinant Urate oxidase in Pichia pastoris

R. Khaleghi[1], S. Asad[1], K. Alishah*[1]
[1]*University of Tehran,College of Science, Department of Biotechnology , Tehran, Iran*

Urate oxidase (uricase, EC 1.7.3.4) is the crucial enzyme in the breakdown of uric acid, the end product of purine catabolism, into allantoin. However, humans lack this enzyme. Rasburicase is the recombinant form of the Aspergillus flavus uricase which is commercially produced in Saccharomyces cerevisiae and used for the treatment of hyperuricemia.The methylotrophic yeast Pichia pastoris is an advantageous microbial host for the production of biopharmaceuticals. Since P. pastoris secretes very low levels of native proteins, it is attractive for secreted expression of recombinant proteins for purification simplification purpose. The A. flavus uricase is made of four identical subunits (135 kDa) with a blocking acetyl group at the N-terminal and a potential glycosylation site; however, the enzyme is non-glycosylated and has no intra-or inter-disulfide bond. Passing through secretion pathway may cause unwanted post-translational modifications. The goal of the present study is the comparison between production yield and enzyme characteristics when produced in the P.pastoris cytosol or secreted to the culture media. For this purpose, the codon optimized synthetic gene of A. flavus uricase was cloned into pPICZA vector for cytosolic production and pPICZαA vector containing alpha-factor signal sequence for extracellular production. Both vectors were transformed to P.pastoris GS115 strain. Multi-copy integrants were screened on zeocin gradients up to 2000

µg/ml. PCR was used to confirm the cassette insertion into the genome. SDS-PAGE, western blotting and enzymatic assay were performed to compare the enzyme expression and purification. The purified enzymes from both constructs were checked for acetylation, unwanted glycosylation or disulfide bond formation.

KeywordsCytosoilc Production, Pichia pastoris, Secreted Production, Uricase.

BTP134

Enhanced itaconic acid production in *Ustilago cynodontis* with Metabolic and Morphologic Engineering

H. Hoseinpour Tehrani*[1], L. M. Blank[1], N. Wierckx[1,2]
[1]*RWTH Aachen University, Institute of Applied Microbiology, Aachen, Germany*
[2]*Forschungszentrum Jülich, Institute of Bio- and Geosciences IBG-1: Biotechnology, Jülich, Germany*

Besides Aspergillus terreus and Ustilago maydis, Ustilago cynodontis is known as a natural itaconate producing strain. Compared to the more established host U. maydis, U. cynodontis is more pH-tolerant, and it belongs to the best itaconate producing species in the family of the Ustilaginaceae. However, due its filamentous growth it was not the focus of attention thus far. This study aimed to change the strong filamentous growth of U. cynodontis with rational morphologic engineering to a yeast-like growth by deletion of genes in the PKA and MAPK pathway. Further goal was, to confirm the function of the putative itaconate cluster of U. cynodontis, and to engineer an Itaconic acid overproducing strain. Deletions of the genes ras2, fuz7 and ubc3 resulted in an altered morphology without filamentous growth, whereby fuz7 resulted in the best growth and Itaconic acid production. Further deletions of mtt1, itp1, ria1 and cyp3 resulted in strains, which showed a decrease up to a complete loss of itaconate, confirming the function of the putative itaconate gene cluster. Deletion of fuz7 and cyp3 in combination with an overexpression of mttA and the regulator ria1 resulted in 4.45-fold increased itaconate production compared to the wildtype. Finally with process optimization in a bioreactor a maximum titer of 83 g L-1, maximum a yield of 0.6 gITA gGLC-1 and a maximum productivity of 1.4 g L-1 h-1 for itaconate were achieved. This is a significant improvement compared to best published titer of 67 g L-1 itaconate reached with U. maydis and 160 g L-1 with A. terreus.

BTP135

Identification and extraction of L-asparaginases through large-scale screening of Caspian Sea metagenomics data

M. Sobat[1], M. Mehrshad[2], S. Asad[1], K. Alishah*[1]
[1]*University of Tehran,College of Science, Department of Biotechnology , Tehran, Iran*
[2]*Uppsala University, Department of Ecology and Genetics, Uppsala, Germany*

Background: Acute lymphoblastic leukemia (ALL) is a malignant progressive neoplasm of lymphoblasts that lead to 4% of all cancer deaths in U.S. L-asparaginase is an enzyme which has been used for ALL treatment for more than 30 years and various types of it has entered the market but due to their immunogenicity, short half-life, rapid clearance and L-glutaminase side activity, efforts continued to find new enzymes with more desirable properties. The aim of this study was to identify novel L-asparaginases from aquatic metagenomics data, which is both huge source of uncultured bacteria with novel genes and also since the salinity and osmolality of sea water is similar to blood, it is expected that proteins extracted from these sources have optimal activity in physiologic conditions.

Results: We analyzed metagenome assembled genomic material equitable to approximately 1000 bacterial genomes to identify novel L-asparginases. Based on efficient computational method we screened 3 million ORF (Open Reading Frame) of which 755 predicted to belong to asparaginase superfamily. Further elaborate functional exploration elucidate that 133 genes are asparaginase. The ability to hydrolyze L-asparagine was experimentally confirmed by cloning 3 representatives of these genes in E.coli. Enzymes specific activity, Km, Vmax and Kcat of the purified enzymes were measured and showed to be among the highest reported values of microbial L-asparaginases.

Conclusion: This study suggests that aquatic metagenomics data can be an advantageous candidate for finding novel proteins with therapeutic potential which are intended to be used intravascular and needs to sustain physiologic salinity of blood stream.

Key words: L-asparaginase, Acute lymphoblastic leukemia (ALL), metagenomics, Hidden Markov Model, Serum stability

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BTP136

Utilization of cyanobacterial TCA cycles for the production of *trans*-4-hydroxy-L-proline

F. Brandenburg*[1], E. Theodosiou[1], C. Köster[1], S. Klähn[1], A. Schmid[1], J. Krömer[1]
[1]*Helmholtz Centre for Environmental Research, Leipzig, Germany*

In contrast to heterotrophic prokaryotes, autotrophic cyanobacteria like *Synechocystis* sp. or *Synechococcus* sp. produce biomass from atmospheric CO_2 and do not require any additional carbon sources. Hence, cyanobacteria are of great interest for the biotechnological production of biofuels like ethanol or hydrogen and the synthesis of high value chemical compounds.

In our most recent work, we investigate the capacities of cyanobacterial tricarbolic acid (TCA) cycles for the sustainable and emission-free production of *trans*-4-hydroxy-L-proline (Hyp), a valuable chiral building block for the pharmaceutical and cosmetic industry. Due to their autotrophic nature, TCA cycles do not serve the same dominant role in provision of chemical energy in the form of ATP and reduction equivalents. In fact, cyanobacterial TCA cycles were considered incomplete until several bypasses were identified and characterized. For example, the conversion from α-ketoglutarate to succinyl-CoA is not taking place in cyanobacteria and instead is bypassed for example via succinic-semialdehyde in the so called cyanobacterial complete TCA cycle shunt.

In order to produce Hyp we added an additional bypass. The gene coding for α-ketoglutarate- dependent proline-4-hydroxylase (P4H) of *Dactylosporangium* sp. str. RH1 was fused with the copper-inducible *petE*-promoter. Both substrates, α-ketoglutarate and proline, are part or derivatives of the TCA cycle, respectively, and hence are provided by the host cells metabolism. Similarly, the co-product succinate is part of the TCA cycle and therefore further metabolized in the cell. In our latest results we are able to produce significant amounts of Hyp from CO_2 and show that it is possible to utilize the TCA cycle for the production of valuable chemical compounds.

BTP137

BioKat – Biocatalysts in bioreactorsFunctional microbial networks in semi-continuous operated biogas reactors

K. Willenbücher*[1], M. Conrady[2], D. Benndorf[3], B. Miltz[1], P. Ramm[2], A. Schlüter[4], M. Klocke[1]

[1]Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB), Department Bioengineering, Potsdam, Germany

[2]Institute of Agricultural and Urban Ecological Projects affiliated to Berlin Humboldt University (IASP), Department of Biogenic Raw Materials, Berlin, Germany

[3]Otto von Guericke University, Department Bioprocess Engineering, Magdeburg, Germany

[4]Bielefeld University, Center for Biotechnology (CeBiTec), Institute for Genome Research and Systems Biology, Bielefeld, Germany

The biogas production is a biological process in which organic substrates are fermented to biogas for the purpose of generate electricity or heat. These processes have not yet been completely researched. Our study investigates the microbial processes in semi-continuous biogas reactors during anaerobic digestion of lignocellulosic substrates. In our first experiment, we focus on the start-up phase. In this initial phase of a biogas process, a functional microbial community necessary for anaerobic fermentation is established.

The aim is to characterize the structure and functionality of the microbiome as well as its dynamics during the ongoing biogas processes. By a comprehensive analysis of the community at the metagenome, metratrascriptome and metaproteome level and a chemical analysis of the start-up phase, it is possible to identify key organisms as well as key characteristics that provide information about the major factors in the biogas process. A special interest lies in the key species responsible for the hydrolysis of lignocellulosic substrates.

An integrated *-omics* approach including subsequent data set analysis using bioinformatics tools is applied. The analysis of metagenome data sets allows determining both, the composition of the microbial communities involved in anaerobic digestion as well as their genetic potential for anaerobic biomass degradation and biomethanization. Pre-characterization is performed by monitoring the structure and development of microbial communities using DNA-based fingerprinting (terminal restriction fragment length polymorphism, TRFLP).

The results of this study will contribute to the understanding of the establishment of functional microbial networks in technical anaerobic digestion processes.

BTP138

Single Cell Oil Downstream Processing Optimization of a Newly Isolated Oleaginous Yeast *Saitozyma podzolica* DSM 27192

O. Gorte*[1], R. Hollenbach[1], K. Ochsenreither[1]

[1]Karlsruher Institut für Technologie (KIT) - Institut für Bio- und Lebensmitteltechnik 2 - Technische Biologie, Karlsruhe, Germany

A yeast isolate from peat bog soil was identified as *Saitozyma podzolica* DSM 27192 and characterized as a simultaneous intracellular single cell oil (SCO) and extracellular gluconic acid (GA) producer via a fermentative process on glucose, yielding 18 g/L SCO and 30 g/L GA. On xylose 11.1 g/L SCO was observed [1]. The ability of this oleaginous yeast to convert xylose to SCO makes this strain an interesting candidate for the maintenance of the carbon value chain by converting renewable waste material, e.g. hydrolyzed wood and straw, for worthwhile biodiesel production.

Since SCO is an intracellular product, downstream processing is one of the major obstacles to be solved for full economic efficiency of microbial lipids. For the extraction of lipids from biomass cell disruption is most important, because efficiency of cell disruption directly influences subsequent downstream operations and overall extraction efficiencies [2]. In this study we demonstrate a comparison of mechanical and chemical cell disruption methods combined with lipid extraction methods. Moreover, we have focused especially on lipid analytics via gas chromatography and HPLC-ELSD to be able to detect derivatized fatty acid methyl esters and free fatty acids, respectively. These accomplishments will also contribute to fundamental research of cell disruption of basidiomycetes in application for DNA and protein extraction.

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BTP139

Monitoring of Enrichments Cultures for Acetogens by 16S rRNA Gene Analyses

K. Funkner[1], D. Schneider[1], R. Daniel[1], A. Poehlein*[1]

[1]Institute of Microbiology and Genetics, Department of Genomic an Applied Microbiology & Goettingen Genomics Laboratory, Göttingen, Germany

Acetogens are microorganisms able to ferment CO2 or CO to acetyl-CoA, which can be used for the synthesis of valuable goods like ethanol, acetate, fuel and other chemicals. This offers the possibility to reduce greenhouse gas emissions from several industries, while producing further usable products. The group of acetogenic microorganisms is not monophyletic, widely dispersed throughout all bacterial lineages, and metabolically and ecologically diverse. Hence, they can thrive in a wide variety of environments like soil, the gut or extreme milieus. Optimal growth conditions for acetogens vary trough a wide spectrum: optimal temperatures lie between 20-70 °C and optimal pH was found between 5.4-9.8. Nevertheless, most acetogens are gram positive and belong to the phylum *Firmicutes*. Several acetogens have been isolated, including *Moorella thermoacetica*, isolated from horse faeces, *Clostridium formicoaceticum*, isolated from sewage sludge and *Clostridium scaltogenes*. Although acetogens are usually found in mixed clusters, some genera are monophyletic, like *Acetobacterium*, *Sporomusa* and *Moorella*.

In this study samples from a digestion tank from a sewage treatment plant and from horse faeces were grown with CO_2 and H_2 as carbon source in different media and incubated under different temperature ranging from 30 to 70 °C. Samples were taken every two days, 16S rRNA-PCRs were performed and sequenced with an Illumina MiSeq machine.

It has been shown that community composition varies over time and is influenced be reinoculation. Within this study, typical acetogens like *Sporomusa*, *Acetobacterium Moorella* or *Thermoanaerobacterium* were enriched in the different media and under different incubation temperatures.

BTP140

Can we optimize cellulase production by using not common fungal strains?

M. Schomber*[1]
[1]*Universität Leipzig, Fakultät für Lebenswissenschaften, Institut für Biologie, Abteilung Pflanzenphysiologie, Leipzig, Germany*

Bioethanol is produced in general by starch fermentation from corn or sugar beets. An alternative is the use of lignocellulose by digesting cellulose to glucose. This digestions is an enzymatic process in which endocellulases, cellobiohydrolses and β-glucosidase, named as cellulase complex, are interacting together. The most common used fungal strain for cellulase production, *Trichoderma reseei*, has been studied intensively and optimized in recent years. The metabolism of cellulase production has been described in different literatures for fungi but is not exactly understound so far. The problem of cellulase production by *Trichoderma reseei* is based on a not well balanced enzyme complex. There is a low production rate of beta-glucosidase in this fungi that leads to the addition of the minor enzyme in industrial scale fermentation.

Our studies focus on a cellulase production with *Penicillium verruculosum* mutants that form more balanced cellulase complexes. To improve the cellulase excretion rate a screening with ultraviolet light induction has been done to isolate new overproducing cellulase mutants. Furthermore a suicide screening has been done to select protease-less producing mutants, that leads to optimized fermentation results. On the other hand a screening for β-glucosidase overproducing mutants has been applied.

EMP141

Diversity of myxomycetes in typhoon prone areas: a case study in beach and inland forests of aurora and quezon province, philippines

E. Cabutaje*[1,2], M. Pecundo[3], T. E. dela Cruz[1,2]
[1]*University of Santo Tomas, UST Graduate School, Manila, Philippines*
[2]*University of Santo Tomas, Fungal Biodiversity, Ecogenomics and Systematics Group, Research Center for the Natural and Applied Sciences, Manila, Philippines*
[3]*De La Salle University - Manila, Biology, Manila, Philippines*

Typhoons alter the plant communities in a forest. Are microorganisms such as myxomycetes (slime molds) also affected by typhoons as observed with plants? In this study, the assemblages of myxomycetes were documented in the beach and inland forests of Aurora and Quezon Province, Philippines. Both are situated in coastline areas exposed frequently to tropical cyclones. Field specimens and substrates were collected seven months after a typhoon hit Aurora and a month after a typhoon hit Quezon. A total of 720 moist chambers were prepared from the collected leaf litter. Myxomycete records resulted in the identification of 63 species belonging to 21 genera. Five species, *Diachea megalospora* K.S. Thind & Manocha, *Didymium eximium* Peck, *Didymium listeria* Massee, *Physarum auriscalpium* Cooke, and *Stemonitis virginiensis* Rex, are new records for the Philippines, increasing the current record from 158 to 163. Comparing the two forest types, inland forest [Aurora: FAI=12.75, Quezon: FAI=18.27] had higher species diversity than beach forest [Aurora: FAI=8.78, Quezon: FAI=9.31]. A similar trend was noted with species richness (Hs, Hg) and evenness (E). For community analysis, a coefficient of community (CC) and a percentage similarity (PS) values of 0.66 were computed for both provinces indicating high similarity in their species composition. Relating the outputs with the impact of typhoons, a higher species diversity was observed for both forest types when field and substrate collections were conducted one month after a typhoon than when a longer period has already elapsed before field and substrate collections. Our results suggested that typhoon disturbance could alter the assemblages and even the diversity of myxomycetes in the typhoon-prone beach and inland forests.

EMP142

Grassland ecosystem in lahar-areas as habitat for slime molds: assessment of myxomycete diversity in mayon, pinatubo and taal volcanoes

V. J. Mapalo*[1], T. E. dela Cruz[2]
[1]*Philippines*
[2]*University of Santo Tomas, Graduate School - Microbiology, Manila, Philippines*

Natural disturbances made by volcanic eruptions and lahar flows have negative effect on the original vegetation of the affected areas. These may lead to the re-establishment of other vegetation, primarily of pantropical grasses. Recently, grassland ecosystems have been reported as habitat for myxomycetes in the Philippines, but none so far in areas affected by volcanic activities. In this study, the grasslands in areas covered by lahar from the previous volcanic eruptions of Mayon (11 years), Pinatubo (26 years) and Taal (40 years) served as study sites. A total of 1,080 moist chambers were set up from grass litter collected in these sites. From the 912 moist chambers positive for myxomycetes, a total of 40 species belonging to 11 genera were identified. Three species, *Diderma chondriodernum*, *Didymium difforme,* and *Physarum javanicum,* are new records for the Philippines. Among the three study areas, a high number of species (27) was recorded in Taal Volcano. Thirteen species were recorded common in all sites, with majority from the order Physarales. The assessment of species diversity showed that Taal had higher species diversity and richness than Mayon and Pinatubo. Comparing the myxomycetes assemblages in the three sites, a high Coefficient of Community (CC) value (71%) was computed between Taal and Mayon, indicating a high similarity in terms of species composition. Among the substrata, highest CC value (88%) was recorded between aerial litter (AL) and ground litter (GL) collected in Mayon Volcano. Our results showed that volcanic eruption can alter the vegetation of the area and in turn affects the diversity of grassland-inhabiting myxomycetes.

Keywords: volcanic eruptions, lahar, grasslands, slime molds, species diversity

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EMP143

The use of bacteriophages to control Lgionella *pneumophila* in environmental water samples in the UAE

N. Al-Kaabi [1], K. El-Tarabily*[1], A. Al Khajeh[1]
[1]*UAE University, Biology, College of Science , Al-Ain, United Arab Emirates*

The presence of *Legionella pneumophila* in cooling towers is a serious health hazard. Cooling towers have been linked to many outbreaks of Legionnaires" disease. Most *L. pneumophila* preventive measures for cooling towers are directed at either minimizing *L. pneumophila* counts in the tower or preventing *L. pneumophila* transmission from the tower to people. In an attempt to isolate lytic phages active against *L. pneumophila* in environmental water samples, three phages were isolated from untreated waste water samples. The optimum concentration of the three phages was found to be 108 plaques forming unit ml-1. The three

phages were isolated using *L. pneumophila* as a propagation host. Phages number 1 and 3 were found to lyse *Salmonella. enteritidis*, *Escherichia. coli, Pseudomonas aeruginosa,* and *Staphylococcus epidermidis*, whilst phage number 3 was found to only lyse *L. pneumophila*. Adsorption rate constant (K) of phage (Ø1), (Ø2) and (Ø3) were 1.22 X 10-6, 1.93 X 10-6, 1.78 X 10-6 ml min-1 respectively. Latent period values obtained for phage (Ø1), (Ø2) and (Ø3) were 40 minutes, 35 and 55 minutes, respectively. The rise periods of phage (Ø1), (Ø2) and (Ø3) were 30 minutes, 35 and 40 minutes, respectively. The average burst sizes of phage (Ø1), (Ø2) and (Ø3) were 21.5, 15.2, and 18.15 virions/cell, respectively. A mixture of these three phages was mixed with four environmental water samples artificially contaminated with *L. pneumophila*. The application of the phages mixture significantly reduced the number of *L. pneumophila* in the four environmental water samples. The phage cocktail obtained in the present study can be used as a safe and alternative technique to eradicate or reduce the population densities of *L. pneumophila* in cooling towers in the UAE.

EMP144

Biological efficiency of natural substances aqueous extract (*Cassia nigricans, Parkia biglobosa, Capsicum annum, Cleome viscosa*) against *Spodoptera frugiperda* on corn production and their effects on a ferruginous soil microorganisms, in Burkina Faso

K. Georges*[1]
[1]*INERA (Institute of Environmental and Agricultural Research), Crop production, Bobo-Dioulasso, Burkina Faso*

A study of four natural substances aqueous extract (Cassia nigricans, Parkia biglobosa, Capsicum annum, Cleome viscosa) biological efficiency against Spodoptera frugiperda which cause big damages to corn and their effect on soil microorganisms has been done at Kou Valley, in Burkina Faso. The experimental design was a randomized Fisherbloc of six treatments (unthreated control, Benzoate emamectin 0,5l/ha, *C. nigricans* 250l/ha, *P. biglobosa* 250l/ha,*C. annum* 250l/ha, *C. viscosa* 250l/ha) in four replications. The biological coefficient efficiencies of insecticides have been determined with the help of Afanasseva *and al.* (1983) formula and the counting of soil microorganisms has been done on specif media. The different extract have shown efficacy against *S. frugiperda* population as on their symptoms evolution on corn leaves. *C. nigricans* aqueous extract, the most efficient between them, allowed getting an average coefficient efficiency of 62.22% on S. frugiperda larva, leading to symptoms reduction of 12.98 to 13.57 in comparison with the untreated control. The synthetic insecticide (Benzoate emamectin) got an average efficiency coefficient of 70 leading to symptoms reduction of 66.98% to 71.22% during the same period. These factors allowed getting at *C. nigricans* and at Benzoate emamectin respectively yields increases of 20% and 23% in comparison with the untreated control. Indeed, the different products didn't affect the batural development of cellulolytic bacteria, microscopic fungi, ammonifying and nitrifying bacteria. C. nigricans aqueous extract can be recommended as biopesticide against S. frugiperda on corn production.

Key -words: Spodoptera frugiperda, natural substances, soil microorganisms, corn.

EMP145

Background bioaerosol investigations in rural environments

I. Laufer*[1], N. Heubach[1], D. Pöther[1], H. Kühne[2], U. Jäckel[1]
[1]*Federal Institute for Occupational Safety and Health Germany, 4.7 Biological Agents, Berlin, Germany*
[2]*Saxon state company for environment and agriculture, Radebeul, Germany*

Inhalation of bioaerosols can lead to adverse health effects, especially in some occupational settings with high concentrations of airborne particles. To put the bacterial load at occupational settings into the right context and to provide a reliable risk assessment for the workers" health appropriate background bioaerosol investigations are needed. So, the aim of this study is to investigate background bioaerosols for a period of one year at three different sampling sites in rural areas of Saxony, Germany.

In cooperation with the Saxon state company for environment and agriculture airborne particles are sampled with a high volume sampler on glass fiber filters for 24 h at 500 l/min every day over a time span of one year at three different rural spots: Brockau, Niesky, Radebeul-Wahnsdorf. The DNA is extracted from the filters with a newly established extraction protocol and bacteria are quantified via the 16S rRNA gene with Droplet Digital PCR. Endotoxin activity was measured by *Limulus* amebocyte lysate (LAL) assay. Additionally, meteorological data (temperature, humidity, barometric pressure, sun irradiance, wind direction and speed) and other air quality parameters (PM10, NO, NO2, O3) were measured.

First results from investigations of urban outdoor air revealed an increase of the bacterial load by a factor of 100 in summer compared to winter. Currently, daily routine assessments of outdoor bioaerosols from three different sampling sites in rural area over one year are used to find possible correlations between the bacterial load and meteorological parameters or other air quality parameters.

EMP146

Electronic waste (E-waste) dumpsites in Nigeria present a potential reservoir of metal tolerant and antibiotic resistant *Escherichia coli*

O. Ikhimiukor*[1,2,3], R. Adeleke[3], O. Adelowo[2], J. Müller [1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Department of Biotechnology (UBT), Leipzig, Germany*
[2]*University of Ibadan, Department of Microbiology, Ibadan, Nigeria*
[3]*Agricultural Research Council, Institute for Soil, Climate and Water (ARC-ISWC), Pretoria, South Africa, Department of Soil Science, Pretoria, South Africa*

Electronic waste (E-waste) are rich sources of heavy metals and are usually disposed in uncontrolled dumpsites. Heavy metals are known to aid co-selection of antibiotic resistance. Information on co-resistance of pathogenic bacteria to metals and antibiotics in E-waste dumpsites is poor. This study was designed to determine co-resistance to metals and antibiotics by *E. coli* strains from selected E-waste dumpsites and investigate genetic determinants mediating such co-resistance. *E. coli* strains were isolated and exposed to increasing concentrations of heavy metals (Cu2+, Pb2+ and Zn2+) to determine minimum inhibitory concentrations (MICs). Metal tolerant strains were tested against antibiotics (gentamicin, kanamycin, cefpodoxime, ceftazidime, ertapenem, imipenem, ciprofloxacin, sulphamethoxazole/trimethoprim, florfenicol and tetracycline). Eighty-four (84) *E. coli* strains were isolated. Exposure to Cu2+ and Zn2+ showed MICs from 400µg/ml and 600µg/ml respectively, whereas all strains tolerated up

to 1100µg/ml of Pb2+. Antibiotic susceptibility tests showed 46 distinct resistance phenotypes, with 94.04% of the strains multidrug resistant and 2.39% displaying resistance to all the test antibiotics. Polymerase chain reaction (PCR) gene amplification showed 88% and 67.86% of the strains were PCR positive for *blaTEM* and *CTX-M-9*, whereas it was 73.60%, 86.90% and 7.14% to *sul1*, *sul2* and *sul3* genes respectively. The *tetA*, *tetE* and *tetG* occurred in 67.90%, 11.90% and 3.60% of the strains. Copper resistance determinant *pco*, and Class 1 integrons, *IntI1*, were present in 83.30% and 92.86% of the strains respectively. Current results show E-waste dumpsites as a platform for the proliferation of metal tolerant and antibiotic resistant *E.coli*, thus posing a threat to public health.

EMP147

The power of power: Electric Field Effects on Bacterial Deposition and Transport in Porous Media

Y. Shan*[1], L. Y. Wick[1], H. Harms[1]
[1]*Helmholtz Center for Environmental Research-UFZ, Leipzig, Germany*

Transport and deposition of bacteria are fundamental processes in microbial ecology and biotechnology Often leading to the formation of biofilms. While the biofilms provide essential ecosystem services in natural and manmade systems, biofouling by contrast may give rise to unwanted corrosion of metals, clogging of filters/membranes or may even threaten human health. There is, hence, strong interest in measures to control microbial deposition to surfaces as the first step in the formation of biofilms.

We therefore tested whether electrokinetic forces (electroosmotic shear force (F_{EOF}), and electrophoretic drag force (F_{EP})) acting on bacteria may be used to control bacterial deposition during transport in laboratory percolation columns exposed to external direct current (DC) electric fields. For different bacteria, yet similar experimental conditions we observed that DC fields either enhanced or reduced bacterial deposition efficiencies (α) relative to DC-free controls. By calculating the DLVO force of colloidal interactions, F_{EOF}, F_{EP}, and the hydraulic shear forces acting on single cells at a collector surface we found that DC-induced changes of α correlated to $|F_{EOF}|$ to $|F_{EP}|$ ratios: If $|F_{EOF}| > |F_{EP}|$, α was clearly increased and if $|F_{EOF}| < |F_{EP}|$ α was clearly decreased. Our findings allow for better prediction of the forces acting on a bacterium at collector surface and, hence, the electrokinetic control of microbial deposition in natural and manmade ecosystems.

EMP148

Metagenomic analysis of microbial communities in fern *(Dicranopteris linearis)* and relations to nitrogen fixation

M. Nguyen Hong[1], H. Dinh Thuy[1], N. Mai Thi[2], M. Nguyen Ngoc[2], P. Nguyen Minh*[3]
[1]*Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi, Viet Nam*
[2]*VNU University of Science, Vietnam National University, Soil Resource & Environment, Hanoi, Viet Nam*
[3]*VNU University of Science, Vietnam National University, Environmental Technology , Hanoi, Viet Nam*

Dicranopteris linearis is a common fern growing in tropical regions. Ecological importances of *Dicranopteris* species in ecosystems (e.g., nutrient cycling, prevention of exotic species) have been well-documented and the role of *Dicranopteris* species in nitrogen uptake has been examined in several studies, however to date little is known about microbial communities in *D. linearis* and the relations to nitrogen fixation have not yet been revealed. The study aims to illucidate microbial communities involved in nitrogen fixation in *D. linearis*. Samples of *D. linearis* (leaves, stems and roots) and soils (0 - 20 cm depth) were taken from its natural habitat in Cao Bang province, Northern Vietnam. The Illumina sequencing technology was employed to identify microbial communities in fern samples with the 16S rRNA gene used as phylogenetic marker. Data was processed and visualized in R. The results interestingly revealed that the nitrogen-fixing bacteria *Rhizobium* was highly abundant in leaves and stems (accounting for 17 and 25% of total bacterial communities, respectively), while *Rhizobium* made up only about 3% of total bacterial communities in root and soil samples. Lesser *Rhizobium* found in the soil was likely because the soil has extremely low pH value (pH ~3). Notably, *Methylobacterium*, which has been known to be a leaf-residing nitrogen fixer, made up 10% of total bacterial communities in the leave samples. *Bradyrhizobium* was found to be dominant in root and soil samples (accounting for 7 and 6% of total bacterial communities, respectively). The findings help to improve our understanding of microbial nitrogen fixation in the non-legume plant - fern and highlight key bacterial nitrogen fixers in *D. linearis*, one of the most important pioneer species in ecological succession.

EMP149

Microscale 2D-Mapping of PH in the mycosphere

B. J. Xiong*[1], C. Dusny[2], K. Lindstaedt[2], L. Y. Wick[1]
[1]*Helmholtz-Center for Environmental Research UFZ, Environmental Microbiology, Leipzig, Germany*
[2]*Helmholtz-Center for Environmental Research UFZ, Solar Materials, Leipzig, Germany*

The mycosphere, defined as the microhabitat surrounding and affected by hyphae, has been proposed as a hotspot of multiple processes in the biogeosphere including the preferential dispersal of bacteria, the increased biotransformation of contaminants or the solubilisation of nutrients and carbon sources. Little is known, however, about the microscale environmental parameters such as pH, oxygen and carbon source concentration in the mycosphere. Towards a better understanding of the mycosphere as driving habitat of the functional stability of hyphae and their associated microorganisms, we here present a novel *in vivo* pH mapping approach that allows to 2D-mapping of pH in the hyphosphere. The system is based on a known cyanobacterial biosensor (*Synechocystis sp.* PCC6803_peripHlu) containing a pH-sensitive variant of the green fluorescent protein (pHluorin2). pHluorin2 displays a pH-dependent bimodal excitation spectrum with peaks at 395 nm and 475 nm and a maximum emission at 509 nm. pH values in the protein microenvironment can be quantitatively measured via ratiometric analyses of the fluorescence emission intensities. The hyphae of fungi and oomycetes were allowed to overgrow a monolayer of the biosensor and pH-sensitive changes were analysed by ratiometric epifluorescence microscopy. Our results clearly indicate the existence of microscale pH gradients in the mycosphere. Such µm-scale pH mapping enables us to better describe the mycosphere and its microbial interactions at the microscale.

EMP150

The lichen microbiome and its role in adaptation to climate change

M. Grimm*[1], J. Bernhardt[1], K. Riedel[1]
[1]*Univiersity of Greifswald, Institute of Microbiology, Greifswald, Germany*

Within the last decade, bacterial communities were identified as surprisingly abundant, stable, specific, and structurally integrated elements of the classical lichen symbiosis. There is strong evidence that the diverse microbiota contribute multiple aspects to the symbiotic system. In comparison to other lichens, *Lobaria pulmonaria* L. Hoffm. is less stress-resistant, only tolerates a quite narrow range of ecological conditions and prefers relatively cool and humid habitats with hardly any air pollution. This makes it an interesting model to investigate how genotypic and phenotypic plasticity enables adaption to changing environmental conditions. A recent metaproteome analysis of *L. pulmonaria* from Eymann *et al*. (2017), comparing two different sampling sites (Darß & Styria) indicated significant differences between the proteomes of the two lichen microbiomes in contrast to the rather stable fungal and algal protein profiles. Moreover, novel information about the partition of labor between the symbiotic partners has been gained. Our ongoing project aims on deciphering genotypic and phenotypic traits enabling this model lichen to adapt to changing climate conditions, such as temperature, light exposure or moisture. Based on the previous results, we hypothesize that especially changes in the composition and functionality of the lichen microbiome may enable the holobiont to cope with environmental changes and, thus, can be considered as an important ecological trait mediating lichen plasticity. Comprehensive state-of-the-art metaproteomic analyses of the protein profiles of all lichen constituents combined with phenotypic analyses of lichens sampled at different geographic sites or kept under varying conditions during transplantation experiments will proof this hypothesis.

EMP151

Accessing the uncultivated marine microbial biodiversity and its novel biomolecules

M. K. Peters*[1], I. Krohn-Molt[1], W. R. Streit[1]
[1]*Universität Hamburg, Microbiology and Biotechnology, Hamburg, Germany*

Background: The major goals of this project are to develop innovative tools and technologies to advance function-based searches in combination with sequence-based searches and to deliver valuable biomolecules of marine origin. Microbial biofilms affect many areas of our daily life, e.g. industry, health care and hospitals where they cause production failure and infections. In this case, the lack of knowledge and technology for inhibiting and/or eliminating microbial biofilms clearly has a strong impact on socioeconomic behaviour. As there are no general tools for biofilm prevention or removal available, our project will deliver novel bioactive molecules and technologies to remove biofilms, for the mining of marine non-cultivated bacteria and for the detection in the biomedical field. They offer alternatives to common antibiotics and even further have impact on a pressing the solution of societal challenges.

Material and Methods: Samples of five coral species and water of the shark tank were taken at the Hagenbeck Tropic Aquarium. We will use different strategies for the hunt of novel bioactive compounds. Enrichment cultures, 16S rRNA analysis, metagenome sequencing, binning approaches in combination with functional analyses will imply the high relevance and potential of marine habitats.

Results and outlook: Enrichment cultures were inoculated in Bacto Marine Broth at different temperatures. The supernatants were applied in activity tests with *Stenotrophomonas maltophilia* K279a in 96-well microtitre plates. First results showed that the culture with *Montipora foliosa* and the water from the shark tank contain biomolecules for biofilm prevention and degradation. DNA isolation, construction and screening of metagenome libraries will lead to a promising candidate.

EMP152

Characterization of *Rhodococcus qingshengii* and *Exiguobacterium* sp. in terms of plant growth promotion and healthy human nutrition

T. Kuhl*[1]
[1]*Helmholtz Center for Environmental Health Munich, Institute for Networkbiology, Neuherberg, Germany*

Plant beneficial microbes are frequently found in the rhizosphere and in the plant microbiome. The project aims to characterize the potentially endophytic bacteria *Rhodococcus qingshengii* (RL1) isolated from *Eruca sativa* and *Exiguobacterium* sp. (GK117) isolated from *Lactuca sativa* in terms of their plant growth promoting (PGP) activities. The genomes of both bacteria were sequenced, assembled with different assemblers, verified following stringent evaluation criteria and analyzed for their genetic potential. Sequence assembly of RL1 genome produced three contigs representing one chromosome and two plasmids. 6652 coding sequences were predicted containing several genes involved in quorum quenching, plant growth promotion and glucosinolate (GSL) metabolism. Additionally genes with predicted functions like heavy-metal resistance, nitrile degradation, antibiotic and osmoprotection were found.

Plant growth promoting activities were analyzed by plant inoculation with bacteria and colorimetric tests. Colonization patterns of the bacteria on their host plants were investigated with fluorescence *in situ* hybridization (FISH) and confocal laser scanning microscopy. Ongoing work focusses on the ability of *R. qingshengii* to convert glucosinolates with the enzyme myrosinase to potentially chemoprotective agents like isothiocyanates. Therefore, cultures of *R. qingshengii* grown with leaf extract or pure compounds (Glucoraphanin/Glucoerucin) were analyzed with LC-MS and GC-MS. Initial results point towards a biotransformation of Glucoraphanin to Glucoerucin by *R. qingshengii*. The ability of *R. qingshengii* for further degradation of glucosinolates will be analyzed. With this work we aim to demonstrate PGPB contribution to healthy plant nutrition.

EMP153

Discovery of lipid-degrading enzymes through functional metaproteomics

D. Sander*[1], Y. Yu[1], P. Sukul[1], S. Heinrich[2], F. Schulz[2], L. I. Leichert[1]
[1]*Ruhr-University Bochum, Institute of Biochemistry and Pathobiochemistry, Microbial Biochemistry, Bochum, Germany*
[2]*Ruhr-University Bochum, Institute of Organic Chemistry, Chemistry and Biochemistry of Natural Products, Bochum, Germany*

Lipolytic enzymes are important biocatalysts and are one of the few enzymes that are produced on a large-scale. They are widely used as industrial catalyst, e.g. in the detergent, food and leather industries. Lipases are generally resilient against harsh conditions due to their broad pH profile, temperature stability, halotolerance and activity in various organic solvents. They also show characteristics like enantioselectivity, which are an advantage in the production of certain fine chemicals. We have developed a functional metaproteomics approach combining the immediacy of an activity-based screening with the independence from lab-cultivability of "meta-omic" approaches to screen different soil samples for lipolytic enzymes (Sukul et al., 2017). We now want to adapt this methodology to directly discover

enzymes with desirable substrate specificities. In this work we tested 9 commercially available lipases for their chain-length specificity with fluorogenic substrates. We found that all tested enzymes had the highest activity with heptanoate (C7). We were also able to demonstrate in-gel activity with particularly long chain-length substrates (C16). Moreover, we could detect lipolytic enzymes in our panel, which showed a high activity for specific substrates with complex chemical structures, such as bulky side chains or aromatic properties.

EMP154

"Candidatus Thermonerobacter thiotrophicus," a non-phototrophic member of the _Bacteroidetes/Chlorobi_ with dissimilatory sulfur metabolism in hot spring mat communities

V. Thiel*[1,2], A. Garcia Costas[3,2], N. W. Fortney[4], J. N. Martinez[1,5], M. Tank[1,2], E. E. Roden[4], E. S. Boyd[6], D. M. Ward[7], S. Hanada[1], D. A. Bryant[2,8]

[1]*Tokyo Metropolitan University, Department of Biological Sciences, Hachioji, Japan*
[2]*Pennsylvania State University, Department of Biochemistry and Molecular Biology, Philadelphia, PA, United States*
[3]*Colorado State University, Department of Biology, Pueblo, CO, United States*
[4]*University of Wisconsin-Madison, Department of Geosciences, Madison, WI, United States*
[5]*University of St. La Salle, Department of Natural Sciences, Negros Occidental, Philippines*
[6]*Montana State University, Department of Microbiology and Immunology, Bozeman, MT, United States*
[7]*Montana State University, Department of Land Resources and Envrionmental Sciences, Bozeman, MT, United States*
[8]*Montana State University, Department of Chemistry and Biochemistry, Bozeman, MT, United States*

In this study we describe "*Candidatus* Thermonerobacter thiotrophicus", a novel, thermophilic *Bacteroidetes/Ignavibacteria/Chlorobi* member with dissimilatory sulfur metabolism with predicted sulfate-reducing directionality. Dissimilatory sulfate reduction (DSR) is an important and ancient metabolic process for energy conservation with global importance for geochemical sulfur and carbon cycling. Characterized sulfate-reducing microorganisms (SRM) are found in a limited number of bacterial and archaeal phyla. However, based upon highly diverse environmental *dsrAB* sequences, a variety of uncultivated and unidentified SRM still exist. The recent development of high-throughput sequencing methods allows the phylogenetic identification of some of these uncultured SRM. We identified a novel putative SRM inhabiting hot spring microbial mats that is a member of the OPB56 clade (*Ca*. Kapabacteria) within the *Bacteroidetes/Chlorobi* superphylum, tentatively named "*Ca.* Thermonerobacter thiotrophicus". Partial genomes for "*Ca.* Thermonerobacter spp." were retrieved from metagenomes from three different hot springs in Yellowstone National Park, USA, and Japan. Supporting the prediction of a sulfate-reducing metabolism for this organism during period of anoxia, diel metatranscriptomic analyses indicate highest relative transcript levels *in situ* for all DSR-related genes at night. The presence of terminal oxidases, which are transcribed during the day, further suggests these organisms might also perform aerobic respiration. The relative phylogenetic proximity to the sulfur-oxidizing, chlorophototrophic *Chlorobi* further raises new questions about the evolution of dissimilatory sulfur metabolism.

EMP155

Vanadium-dependent haloperoxidase of the marine γ-proteobacterium _Microbulbifer_ sp. HZ11 brominates antibiotic alkylquinolones

N. H. Ritzmann*[1], S. Ernst[1], S. L. Drees[1], U. Hennecke[2,3], S. Fetzner[1]

[1]*Westfälische Wilhelms-Universität, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*
[2]*Westfälische Wilhelms-Universität, Organisch-Chemisches Institut, Münster, Germany*
[3]*Vrije Universiteit, Chemistry Department, Brüssel, Belgium*

Introduction: Alkylquinolones (AQs) not only mediate quorum sensing and virulence factor regulation of *Pseudomonas aeruginosa* but can also be secreted by marine Gram-negative bacteria serving as antibacterials and algicidals. The marine gamma-proteobacterium *Microbulbifer* sp. HZ11 has the genetic potential to synthesize AQs, but also brominates exogenous AQs, leading to 3-bromo-AQ derivatives.

Objectives: Identification of the enzyme responsible for AQ bromination in order to illustrate its role for *Microbulbifer* sp. HZ11 physiology as well as its biotechnological potential.

Methods: For enzyme identification, we used an *in vivo* screening approach using a recombinant AQ producing *P. putida* strain. Growth inhibition of *Staphylococcus aureus* and marine isolates by brominated AQs was investigated. Eventually, catalytic properties and substrate preference of the brominating enzyme were elucidated.

Results: We identified a vanadium-dependent haloperoxidase (VHPO) capable of brominating AQs with high catalytic efficiency. The enzyme preferred substrates with longer alkyl chains (C5-C9) over other tested substrates. Brominated AQ-derivatives had decreased inhibitory properties towards HZ11. Interestingly, brominated 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) had increased antibiotic activity against *S. aureus* and tested marine isolates.

Conclusion: Whereas bromination of AQs and HQNO can be regarded a detoxification mechanism for strain HZ11, halogenated AQs and HQNO can have increased inhibitory activity against other bacteria. Preliminary studies on VHPO suggest the enzyme to be specific for 2-alkylated quinolones without the promiscuity typically encountered with vanadium halogenases.

EMP157

A sulfur oxygenase from the haloalkaliphilic bacterium _Thioalkalivibrio paradoxus_ with atypically low reductase activity

P. Rühl[1], F. Folgosa[2], M. Teixeira[2], A. Klingl[3], C. Frazão[4], A. Kletzin*[1]

[1]*Technische Universität Darmstadt, Schwefelbiochemie und mikrobielle Bioenergetik, Darmstadt, Germany*
[2]*ITQB NOVA, Metalloproteins and Bioenergetics, Oeiras, Portugal*
[3]*LMU München, Biozentrum, Dept. Biologie I - Botanik, München, Germany*
[4]*ITQB NOVA, Macromolecular Cr ystallography, Oeiras, Portugal*

Introduction: Iron-containing sulfur oxygenase reductases (SOR) catalyze the oxygen-dependent sulfur disproportionation with sulfite, thiosulfate and sulfide as products. The SOR of the meso- and alkaliphilic *T. paradoxus* (*Tp*SOR) branches deeply in a phylogenetic dendrogram of SORs so that the question arose whether the *Tp*SOR has SOR activity or whether the branching point indicates a paralogous subgroup.

Objectives: This study describes biochemical properties of the *Tp*SOR including enzyme activities, electron micrographs and *in vivo* gene expression. Preliminary X-ray crystallography results will be presented.

Methods: The sor gene was heterologously expressed in *E. coli*. Specific enzyme activities were determined both by colorimetric assays and HPLC. Thermal unfolding was recorded by nanoDSF. Gene expression was examined by qRT PCR.

Results: Electron micrographs and the crystal structure of the *Tp*SOR show a hollow, ball-shaped homo-oligomeric protein complex similar to other SORs but with altered active site pocket. The reductase activity is significantly reduced compared to other SORs and can only be quantified by HPLC analysis. The ratio of oxidized to reduced products was 30:1 whereas other SORs had ratios of 1.3:1. The optimal activity and melting points were recorded at 80 and 75˚C, respectively. Although the protein has its temperature optimum at 80°C, the gene is expressed in *T. paradoxus* cells at 30°C during growth with thiosulfate.

Conclusion: The results of this study show (i) that the SOR is a thermozyme produced by mesophiles, (ii) that structural changes of the *Tp*SOR could cause the shift in the product stoichiometry and (iii) that the enzyme is a SOR with reduced reductase activity.

Rühl et al. 2017; J Bacteriol: 199:e00675-16.

EMP158
The functional relevance of microbe-plant-insect interaction in a cereal crop system
S. P. Klink*[1], M. Rothballer[1]
[1]Helmholtz Center of Environmental Health, Institute of Network Biology, Neuherberg, Germany

Beneficial rhizobacteria bear a high potential to improve the plant's resistance against biotic stress. However, it is not well understood how microbial signaling influences the plant's response against economically important pests. *N*-acyl homoserine lactones (AHLs) may play a key role in priming plants against pest attack. *Acidovorax radicis N35* is a known AHL-producing rhizobacterium. Inoculation of barley (*Hordeum vulgare)* seedlings with this rhizobacterium clearly influenced the growth rate of aphids (*Sitobion avenae)*. Greenhouse experiments revealed a strong positive effect on cv. Grace and cv. Chevalier but a negative effect on cv. Scarlett. Moreover, *A. radicis* and its AHL-negative mutant stimulated different immune responses in barley. While *A. radicis N35* wild type showed only a very weak induction of early defense responses in plant RNA expression analysis, the *araI* AHL-negative mutant caused increased expression of flavonoid biosynthesis genes. Thus, the synthesis of AHLs by *A. radicis* has implications on the perception by the host plant and contributes to the function of the bacteria-plant interaction.
Ongoing experiments aim to interpret these findings in the context of a microbe-plant-aphid interaction. In axenic and soil systems, the biocontrol effect of *A. radicis* is studied on four barley cultivars exposed to aphids. Changes in AHL signaling are measured by Enzyme-linked Immunosorbant Assay and qPCR analysis. Pure AHL substances are applied to distinguish between cell effects and actual signaling. AHL uptake and degradation in the plant are monitored with High Performance Liquid Chromatography. Understanding inter-kingdom communication represents a promising basis to improve agricultural systems by enhancing crop resistance against herbivorous insects.

EMP159
Microbial-mediated soil formation in maritime Antarctica under simulated environmental conditions
P. Krauze*[1], L. A. Meier[2], T. Scholten[2], P. Kühn[2], C. W. Mueller[3], D. Wagner[1,4]
[1]GFZ German Research Centre for Geosciences, Geomicrobiology, Potsdam, Germany
[2]University of Tübingen, Institute of Geography, Chair of Soil Science and Geomorphology, Tübingen, Germany
[3]TU München, Lehrstuhl für Bodenkunde, Freising, Germany
[4]University of Potsdam, Institute of Earth and Environmental Science, Potsdam, Germany

Ice-free oases of Antarctica offer an exceptional opportunity to improve our understanding of the impact of microbial processes on soil formation and its feedback on microbial community composition. Here, the development of microbial habitats and soil formation are largely controlled by temperature, soil moisture, soil organic carbon accumulation, and soil structure formation. A laboratory simulation experiment was conducted in order to understand the role of microorganisms and biotic processes for pedogenesis, and the impact of these microbial-mediated pedogenic processes and climate change effects on microbial communities. To clarify the biotic impact on early soil formation and its feedback processes, this experiment was performed with untreated, *in-situ* samples including the indigenous microbial communities from James Ross Island, Antarctica, as well as gamma-radiated, abiotic control samples. These soils were exposed to repeated freeze/thaw cycling. Different treatments were applied to simulate the effects of climate change on Antarctic soils, such as different temperature ranges (-5 °C to 5 °C vs. -10 °C to 10 °C), increased soil moisture (40% vs. 80% water holding capacity), and the addition of organic matter. The soil samples were analysed after 100, 250, and 500 freeze/thaw cycles through an interdisciplinary approach combining pedochemical and microbiological methods. Microbial communities were characterized using qPCR and 16S rRNA gene HiSeq sequencing. This study gives insight in the interrelations between soil forming and microbial processes in juvenile soils of maritime Antarctica, and provides a better understanding of the future development of Antarctic soil environments and the response of microbial communities to changing environmental conditions.

EMP160
Deciphering bacterial and fungal endophyte communities in leaves of two maple trees with green islands
F. Wemheuer*[1,2], B. Wemheuer[3], R. Daniel[4], S. Vidal[2]
[1]The University of New South Wales, Evolution and Ecology Research Centre, Sydney, Australia
[2]Department of Crop Sciences, Göttingen, Germany
[3]The University of New South Wales, Centre for Marine Bio-Innovation, Sydney, Australia
[4]Institute of Microbiology and Genetics, Göttingen, Germany

A phenomenon, typically referred to as "green island" of plant tissues, is frequently observed in plant-pathogen interactions. To date, our knowledge on bacterial-fungal interactions in these diseased plant tissues remains incomplete because previous studies have not simultaneously investigated fungi and bacteria colonizing the infected tissue. In this study, we assessed bacterial and fungal endophyte communities in leaves of field maple (*Acer*

campestre L.) and the temperate Norway maple (*A. platanoides* L.) without green islands and in infected leaves with green island areas and the surrounding yellow leaf areas using large-scale metabarcoding. Green island areas of *A. campestre* and *A. platanoides* were dominated by *Sawadea polyfida* and *S. bicornis*, respectively, suggesting that these fungi are responsible for the green islands. Fungal and bacterial richness were significantly lower in infected than in control leaves of *A. campestre*. Moreover, the infection significantly altered the composition of fungal endophyte communities of both *Acer* species. This was mainly related to differences in fungal community composition between control leaves and leaves with green island areas and between green and yellow tissues of infected leaves. In contrast, fungal infection altered the bacterial community composition in leaves of *A. campestre* but not in those of *A. platanoides*. Overall, we observed *Acer* species-specific responses of endophyte communities towards infection. This might be attributed to several fungi and bacteria specifically associated with one *Acer* species. Our results further suggest that the two *Sawadea* species might manipulate bacterial and fungal endophyte communities in different ways.

EMP161
Groundwater cable bacteria conserve energy by sulphur disproportionation

H. Müller *[1], S. Marozava[2], A. J. Probst[1], R. U. Meckenstock [1]
[1]*University of Duisburg-Essen , Biofilm centre, Essen, Germany*
[2]*Helmholtz Center Munich, München, Germany*

Cable bacteria of the family *Desulfobulbaceae* couple spatially-separated sulphide oxidation and oxygen or nitrate reduction by a long-distance electron transfer over centimeter distances. However, the types of energy metabolism of cable bacteria are currently unknown. Here, we report on the enrichment of cable bacteria from a 1-methylnaphthalene-degrading enrichment culture (1MN) and on the elucidation of the energy conservation.

We grew culture 1MN in the presence of elemental sulphur and thiosulphate with nitrate as electron acceptor. The microbial community composition was evaluated by T-RFLP and metagenome sequencing. Cable bacteria were identified by fluorescence in situ hybridization (FISH) and atomic force microscopy (AFM).

FISH staining revealed the presence of *Desulfobulbaceae* as filaments of several hundred micrometres length, but also as single cells or filaments shorter than 50 µm. The filaments showed the characteristic cable bacteria morphology with the continuous ridge pattern over the whole length. An abundance of more than 90% and metagenomic analysis provided strong evidence that the *Desulfobulbaceae* are involved in sulphur cycling in culture 1MN. The *Desulfobulbaceae* did not only grow with nitrate as electron acceptor and elemental sulfur and thiosulphate as electron donor but also by sulphur disproportionation.

Our results suggest that cable bacteria can conserve energy by substrate-level phosphorylation during sulphur disproportionation. The proposed energy metabolism also explains the energy source of cells that are in the middle of the cable bacteria filaments where neither sulphide nor oxygen is present. These cells probably thrive on sulphur disproportionation in a cryptic sulfur cycle where sulphide never reaches detectable concentrations.

EMP162
Metaproteomic analyses of Pacific Ocean deep sea sediments

L. Wöhlbrand*[1], J. Kalvelage[1], V. Schnaars[1], R. Rabus[1]
[1]*Institute for Chemistry and Biology of the Marine Environment (ICBM), General and Molecular Microbiology, Oldenburg, Germany*

Introduction: Marine sediments represent a large and diverse habitat in which global, microbially-mediated processes occur. Since previous studies focused on continental margins, data from open ocean sites are scarce. Deep sea sediment composition, substrate and nutrient availability strongly depend on the productivity and size of the overlying water column and diffusion-driven supply from underlying sediment layers. Although geochemical processes (metabolic cycles) and composition of microbial communities have been studied, proteins involved in these processes and maintaining metabolism of the very slow growing microbial community in this low energy habitat have not been studied so far.

Objectives: Insights into the protein complement formed by the microbial community that drive the biogeochemical processes and sustain live under the extreme conditions.

Methods: Proteins were extracted from Pacific Ocean deep sea sediment, separated by SDS-PAGE, and identified using nanoLC ESI-Iontrap MS/MS.

Results & Conclusion: Surface sediment from the oligotrophic North Pacific Subtropical Gyre (NPTG) and the nutrient rich Bering Sea (BS) differed markedly with respect to cell numbers (2.3 × 109 *vs* 1.3 × 1010 cells/cm³) and load of organic carbon (0.7% *vs* 1.3%). Correspondingly, the amount of extractable protein was higher for BS sediment (54 *vs* 4 µg/g sediment). According to GO annotations, proteins involved in DNA metabolism constitute a high share of the detected metaproteome. Apparently, transmembrane transport proteins constitute a larger share at the NPTG, resembling the situation reported for oligotrophic, pelagic communities. In contrast, proteins involved in cell wall synthesis revealed a higher share in the BS sediment community, possibly reflecting a higher growth.

EMP163
Mycelium-like networks allow bacteria to overcome zones of elevated toxicity

C. Papendorf*[1], T. Mainka[1], A. Worrich[1], T. Banitz[1], L. Y. Wick[1]
[1]*Helmholtz-Centre of Environmental Research, Ecological Modelling, Leipzig, Germany*

Bacteria living inside the heterogeneous soil matrix are commonly confronted with zones of elevated toxicity. The functional impacts of such spatially distinct zones with adverse environmental conditions are not well understood. Theoretical studies have highlighted the importance of bacterial dispersal for overcoming periods and areas of adverse environmental conditions. Little however is known on the effect of preferential bacterial dispersal along fungal hyphae and hypha-like networks on the bacterial ability to cross areas of elevated toxicity. We here developed a model soil ecosystem with spatially distinct toxic zones (given by increased concentration of silver ions) and quantified the dispersal of flagellated and non-flagellated soil bacteria *Pseudomonas putida* KT2440 in presence and absence of mycelium-like glass fiber networks. Our results show that motility and dispersal of strain KT2440 significantly decrease in presence of silver ions. Mycelium-like dispersal networks, however, promote bacterial dispersal, reduce bacterial exposure to toxic conditions and increase their functional

performance (assessed by the degradation of benzoate as a representative polar aromatic contaminant). From our preliminary data we conclude that the presence of fungal mycelia may allow bacteria to cross spatially distinct toxic zones and, hence, improve soil functional stability (e.g. in degrading anthropogenic chemicals).

EMP164

The spectacles microbiota - Cultivation-independent analyses using 16S rRNA gene sequencing

B. Fritz*[1], S. Weis[1], S. Wahl[2], F. Ziemssen[3], M. Egert[1]
[1]Furtwangen University, Institute of Precision Medicine, Villingen-Schwenningen, Germany
[2]Eberhard Karls University, ZEISS Vision Science Lab, Tübingen, Germany
[3]Eberhard Karls University, Centre for Ophtalmology, Tübingen, Germany

Background: Surfaces with contact to the human body are usually contaminated with microorganisms and might be considered as fomites. The same applies for spectacles, but only little is known about their microbial contamination. Aerobic cultivation analyses from our group [Fritz et al., 2018] revealed a bacterial load strongly dominated by staphylococci. To better account for aerotolerant anaerobes, slow growing and yet-uncultivated bacteria, we performed a 16S rRNA gene sequencing approach.

Methods & Results: 35 spectacles from university staff and students were swab-sampled at three sampling sites (nosepads, glasses and earclips). We sequenced the V3 – V4 region of the 16S rRNA gene on the Illumina MiSeq platform using an optimized library preparation protocol. 28 earclips, 23 nosepads and 27 glasses yielded good quality sequences, which were used for downstream analyses. After rarefication 22 phyla and 622 genera of bacteria were detected. *Actinobacteria* (42%), *Firmicutes* (39%) and *Proteobacteria* (14%) were the dominant phyla. At genus level, *Staphylococcus* (28%), *Propionibacterium* (21%), *Corynebacterium* (11%), *Lawsonella* and *Streptococcus* (4%, each) were the most abundant taxa. Statistical analyses revealed a significantly higher bacterial diversity on the glasses compared to nosepads and earclips.

Conclusions: Here, we report the first cultivation-independent composition of the bacteriota on worn spectacles. Spectacles are contaminated with bacteria of mostly human skin origin, including potentially pathogenic ones. They may play a role as fomites in clinical environments and contribute to eye infections.

Fritz et al. (2018), *PLoS One*, in press, doi: 10.1371/journal.pone.0207238

EMP165

Effects of bacterial dispersal on biomass distribution and activity in spatially disturbed synthetic ecosystems

T. Mainka*[1], A. Worrich[1], T. Banitz[1], L. Y. Wick[1]
[1]Helmholtz-Center for Environmental Research, UMB, Leipzig, Germany

Microbial habitats such as soil are often exposed to disturbances of various frequencies and intensities. However, the factors controlling the functional stability (resistance, recovery) of microbial systems are only partly understood. In particular, little is known on how spatial disturbance characteristics influence the functional stability of microbial turnover of anthropogenic chemicals. Previous *in silico* studies by our group (König et al, 2018) indicate (i) increased functional stability of ecosystems exposed to spatially fragmented rather than clumped disturbances and (ii) an important role of bacterial motility for both the speed and extent of recovery. To challenge these hypotheses, we developed an agar-based synthetic microbial ecosystem ("microbial landscape") using fluorescently labeled *Pseudomonas putida* KT2440-gfp and its non-flagellated mutant KT2440-Δflim dsRed. UV-light was used to apply disturbances varying in spatial configuration (clumped vs. fragmented). We assessed the bacteria"s spatial organization and recolonization dynamics of disturbed areas by cell counting and microscopy tools. The degradation of benzoate was used as a measure of bacterial functional performance. Preliminary data show an effect of the disturbances spatial configuration on benzoate degradation. In accordance with our hypotheses, recolonization of the disturbed areas was faster when the disturbance occurred in a spatially fragmented pattern. Moreover, benzoate degradation was accelerated for the motile KT2440 compared to the non-motile KT2440-Δflim strain. These findings underpin the critical role of both spatial characteristics of disturbances and bacterial motility for the functional stability of heterogeneous soil microbial ecosystems.

EMP166

The impact of bacterial activity on decay and fossilization of arthropods

K. Janssen*[1], B. Mähler[2], G. Bierbaum[1], J. Rust[2]
[1]Institut für medizinische Mikrobiologie, Immunologie und Parasitologie, Bonn, Germany
[2]Institut für Geowissenschaften und Meteorologie, Bonn, Germany

The fossilization process in aquatic environments is influenced by abiotic and biotic factors. Especially the impact of bacterial activity remains an unexplored territory. In addition to the decay of organisms, bacteria can contribute to the formation of fossils by synthesizing biofilms. There is nothing known about the species that mediate these processes and if they are intrinsic or extrinsic microorganisms. Preliminary experiments yielded first insights into the microbial diversity and changes in the composition of bacterial community.

To observe microbial shifts, the decay of eight crayfish specimens of *Cambarellus chapalanus* in different types of water was tested. All samples were stored at 30 °C for 14 days. For 16S amplicon analysis, DNA from water and crayfish samples was sequenced. All reads with 97 % identity were sorted into 4510 different OTUs which were then compared to a curated database of 16S rRNA sequences.

937 genera could be detected in the samples. The water samples showed a higher diversity than the tissue samples. Shannon Indices of the samples revealed a decrease in diversity over time. Additionally, the amount of anaerobic bacteria increased from day 7 to day 14 which demonstrated an adaptation of the microflora to the lack of oxygen at this time point. Bacteria found on crayfish decomposed in lake water mostly derived from soil or gut. The dead specimens stored in tank water mainly harboured water and soil bacteria.

The goal of this project is to improve the understanding of fossilization processes connected to the microbial activity. Besides the observation of physical disarticulation, influence of chemical, physical and biological parameters will be tested

to identify the best conditions for mineralization and fossilization of arthropods.

EMP167
Quantifying Interactions of Phages with Non-Host Bacteria
X. You*[1], A. Chatzinotas[1], H. Harms[1], L. Y. Wick[1]
[1]Helmholtz-Centre for Environmental Research GmbH – UFZ, Department of Environmental Microbiology, Leipzig, Germany

Bacteriophages (phages) are viruses that infect specific bacteria (also called hosts). Having a typical size in the nanometer range they are the most abundant biological entities on Earth. In nature, phages specifically infect their hosts through an irreversible host-receptor associated interaction, which leads to an infection event. However, the random collision of phages and bacteria in a natural environment can also lead to non-specific interactions. Such interactions have been neglected yet may contribute to better explain initial viral sorption mechanisms, transport-mediated virulence or viral diversity in natural ecosystems. To explore non-specific phage-bacteria interactions, we used hydrophobic *Enterobacterio* phage T4 (T4) and hydrophilic *Pseudoalteromonas* phage HS2 (HS2) in combination with the two non-host bacteria (*Pseudomonas putida* KT2440, *Pseudomonas fluorescens* LP6a) of clearly distinct physico-chemical surface hydrophobicity. The bacteria were co-incubated with either of the phages for 1 hour. Phages in the supernatant were separated from phages attached to non-host bacteria by centrifugation. Numbers of phages in the supernatant were then quantified by plaque forming units and compared to numbers of phages added to the bacteria. We found significant interactions of the nano-colloidal phages with bacteria and maintenance of virulence of attached phages. Magnitude of phage attachment depended on the hydrophobicity of the phage and –to lesser extent- on the hydrophobicity of the bacteria as was also predicted by the extended DLVO (XDLVO) theory of colloidal stability. Our study suggests that even non-specific phage-bacterial interactions may play an important role in viral transport,transport-mediated virulence and viral diversity in natural ecosystems.

EMP168
The *Hells Bells* from Yucatán – unique speleothems that grow through microbially induced calcite precipitation?
K. Leberecht*[1,2], S. Ritter[3], L. Klose[3], W. Stinnesbeck[3], M. Isenbeck-Schröter[3], J. Gescher[1,2]
[1]Karlsruhe Institute of Technology (KIT), Institute for Applied Biology (IAB), Department of Applied Biology, Karlsruhe, Germany
[2]Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces, Eggenstein-Leopoldshafen, Germany
[3]University of Heidelberg, Institute for Earth Sciences, Heidelberg, Germany

Introduction: Extraordinary, bell-shaped calcite formations were discovered in the subaquatic system of the cenote El Zapote on the Yucatán Peninsula, Mexico, nine years ago. The so-called "Hells Bells" show a complex, unique morphology and cover the cave in the water depth of 28-38 m reaching a size of more than 2 m. So far, little research has been devoted to the development of these speleothems. However, previous studies of our cooperation partners indicated, that the Hells Bells did not develop by stalactite-like genesis but grew underwater and potentially through precipitation that results from microbial activities.

Objectives: To elucidate if these speleothems develop through the known phenomena of microbially induced or influenced mineralization, we focused on the analysis of the microbiome of every sub-biotope of the cenote (fresh water, halocline, salt water, biofilm on the bells in the three water zones), the identification of the microbial key players and their diverse metabolic activity.

Methods: The functional diversity of the different sub-biotopes was determined via 16S rRNA amplicon sequencing and connected to a metagenomic and –transcriptomic profiling of the microbial community in the water column and the biofilm on the Hells Bells.

Results & Conclusion: Phylogenetic analysis of the microbiome revealed highly diverse microbial communities in the water column and biofilm on the Hells Bells in the different water zones. With the focus on the halocline, where the Hells Bells still grow, we found a dominant fraction of bacteria and archaea involved in the nitrogen and sulphur cycle, some of which could potentially drive organomineralization by metabolically creating alkaline microenvironments.

EMP169
Wind-driven emission of *Enterococcus faecium* from agricultural soil fertilized with poultry manure
N. Thiel*[1], V. Junker[1], S. Münch[2], P. Siller[3], O. Biniasch[4], M. Faust[5], K. Schepanski[5], T. Amon[3,4], U. Rösler[3], R. Funk[2], U. Nübel[1]
[1]Leibniz Institute DSMZ, Braunschweig, Germany
[2]Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany
[3]Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany
[4]Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany
[5]Leibniz Institute for Tropospheric Research (TROPOS), Leipzig, Germany

Application of livestock manure as fertilizer on agricultural fields results in environmental pollution with fecal bacteria. So far only little is known about the spread of dust-associated bacteria from arable land. We investigated especially the dispersal of *Enterococcus faecium* from chicken manure during fertilizer application and subsequent tillage operations. Emission through wind erosion in the weeks after fertilization was simulated in wind tunnel experiments.

Unexpectedly, we found that the average monthly dust emission caused by wind erosion exceeded the dispersion during tillage operations by far. Our measurements indicate that wind erosion from the tested soil can be initiated at a threshold wind speed of 7.8 m/s. Although the dust release increases with increasing wind speed we have evidence that manure particles are preferably released at wind speeds close to the threshold. Based on 16S rRNA gene sequencing we found that in samples released at low wind speeds manure-derived bacteria are enriched. Therefore even the regular wind load can result in a constant emission of manure-derived bacteria and loss of fertile soil components from agricultural areas.

For the first time whole genome sequencing was applied to trace airborne bacteria. *Enterococcus faecium* isolates from manure, soil and dust samples were collected and could be traced back to their origin in the chicken housings. Even 4 weeks after fertilization isolates with similar genomes were found in soil samples. The fate of dust particles and associated bacteria that are dispersed during tillage

operations were simulated suggesting that depending on the atmospheric conditions, emitted dust particles can reach heights of 2.5 km and travel over distances of more than 350 km.

EMP170

Preliminary analysis of the microbiota of regularly microwave-sanitized and non-sanitized kitchen sponges using metagenome shotgun sequencing

S. Jacksch*[1], J. Thota[1], M. Egert[1]
[1]*Institute of Precision Medicine / Furtwangen University, Villingen-Schwenningen, Germany*

Background: Kitchen sponges belong to the biggest microbial reservoirs in domestic environments. They collect and spread microorganisms over domestic surfaces [1]. Although sanitation measures cause a short-term reduction of the microbial load [2], their effects on microbial community composition and its pathogenic potential are unknown. We analyzed the impact of sponge sanitation on microbial composition using a controlled experimental setup.

Methods & Results: 20 polyurethan kitchen sponges were issued to the participants of the study and used under domestic kitchen conditions over a period of ~4 weeks. Ten sponges were regularly sanitized by microwaving, while the others were not sanitized. Subsequently, sponge samples were analyzed by shotgun sequencing using Illumina MiSeq technology. The obtained sequences were uploaded to the MG-RAST platform for bioinformatic analyses. Sponges were mainly colonized by bacteria (97.0% of all sequences). However, in addition, viral (2.7%), eukaryotic (0.2%) and archaeal (0.006%) sequences were found. *Proteobacteria* (85.6%), *Bacteroidetes* (7.4%) and *Actinobacteria* (3.6%) were the dominant bacterial phyla, followed by unclassified viral sequences (2.7%). At genus level, *Acinetobacter*, *Enhydrobacter*, *Agrobacterium* and *Pseudomonas* were relatively most abundant. Statistical analyses revealed a tendency towards a lower microbial diversity in sanitized sponges compared to sponges without cleaning.

Conclusion: Our study gives first insight into the microbial colonization of kitchen sponges, considering all domains of life, and will unravel the effect of microwave sanitation on microbial community composition and its health relevance.

[1] Cardinale et al. (2017) Sci Rep 7(1): 5791, [2] Sharma et al. (2009) Food Control 20(3): 310–313

EMP171

DNA-Stable Isotope Probing (DNA-SIP) to identify key polycyclic aromatic hydrocarbon (PAH) degraders in a sulfate-reducing enrichment culture

S. Suresh*[1], N. Abu Laban[1], H. Müller[1], R. U. Meckenstock[1]
[1]*University of Duisburg-Essen, Aquatic microbiology, Essen, Germany*

Until now, anaerobic degradation of PAHs has mostly been studied with the 2-ring compounds like naphthalene. Very little is known about the anaerobic degradation pathways of larger PAHs with 3 or more rings. We enriched an anaerobic, sulfate-reducing and phenanthrene-degrading culture (TRIP) from the "Pitch lake" in Trinidad and Tobago, a natural asphalt reservoir nourished from the oil reservoir below (Meckenstock et al., 2014).

Our study aimed to identify the key-degraders of the PAH, phenanthrene (C14H10) in the TRIP culture. Stable isotope probing with DNA as the biomarker was employed to identify the active members of the enrichment culture involved in the assimilation of 13C14H10. Mineralisation of labelled phenanthrene was continuously monitored by measuring the stable isotopic composition of CO2.

Microcosms grown with 13C14H10 exhibited exponentially increasing 13CO2 emission with time compared to the controls. 16S rRNA gene amplicon sequencing analysis of the heavy fractions obtained from DNA-SIP indicated high abundancy of members of the genus *Desulfatiglans* in contrast to the light fractions obtained from the control microcosms.

Hence using DNA-SIP, it was possible to identify the members of the genus *Desulfatiglans* as key phenanthrene degraders within the complex enrichment culture.

EMP172

Genetic analysis of biofilm formation of *Sphingomonas* sp. strain S2M10 on different plastic surfaces

M. Czieborowski*[1], A. Hübenthal[1], B. Philipp[1]
[1]*WWU Münster, Institut für molekulare Mikrobiologie und Biotechnologie, Münster, Germany*

Bacteria of the genus *Sphingomonas* are well-known for biofilm formation in water-distribution systems. Some sphingomonads produce viscous exopolysaccharides called sphingans. As biofilm of sphingomonads contribute to deterioration of technical water systems such as reverse osmosis membranes, a mechanistic understanding of their biofilm formation is useful for the developing antifouling coatings.

The goal of this study was to analyze biofilm formation of the tap-water isolate *Sphingomonas* sp. strain S2M10 on different plastic surfaces by transposon mutagenesis. For this, 1300 transposon mutants were screened for altered biofilm formation in polystyrene microtiter plates. Mutants forming reduced or increased biofilm were genetically and phenotypically characterized for colonization of PET, PTFE, PE PP and PVC surfaces.

A mutant defect in sphingan formation (*spnB*) and two mutants (*fliF*, *flhA*) with defects in flagella synthesis showed reduced biofilm formation on all materials with the exception of PTFE. A further mutant of a gene encoding a hypothetical protein within the flagella gene cluster showed reduced biofilm formation and less sphingan production. One mutant with reduced biofilm formation had a defect in a gene encoding a putative peptidoglycan-associated protein. Finally, one mutant with defect in a putative fatty acid reductase showed enhanced autoaggregation as well as altered and partly increased biofilm formation on some plastic surfaces.

These results show that biofilm formation of strain S2M10 is not only influenced by known surface structures but also by so-far unknown factors that may even lead to enhanced biofilm formation. The impact of these different factors may also vary among different plastic materials.

EMP173

Large scale isolation of bacteriophages infecting marine *Rhodobacteraceae*

V. Bischoff*[1], B. Heyerhoff*[1], A. Bögeholz[1], F. Zucker[1], A. Poehlein[2], M. Simon[1], C. Moraru[1]
[1]*University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany*
[2]*Georg-August-University Göttingen, Institute of Microbiology and Genetics, Department of 12 Genomic and Applied Microbiology & Göttingen Genomics Laboratory, Göttingen, Germany*

Bacteriophages are highly abundant in the marine environment and play a major role in controlling their host populations and in biogeochemical cycles. One important bacterial group in the marine environment is the *Roseobacter* Group. Roseobacters are heterotrophs with a high metabolic versatility, present in the water column, sediments and on surfaces. They are known to increase in abundance in algal blooms, for example reaching as high as 30% from the total bacterial community. Although roseobacters are highly diverse, to date only few roseophages, that is phages infecting roseobacters, have been isolated. We have performed a large scale roseophage isolation experiment, in which more than 400 roseobacter strains were challenged with five different phage samples. The phage samples were obtained by concentrating cell free seawater originating from the North Sea coast. We picked and further purified 420 phage plaques. Screening using randomly amplified polymorphic DNA (RAPD) PCR resulted in more than 100 individual roseophages. Most of them infected the genus *Sulfitobacter*, but also *Lentibacter, Octadecabacter, Roseovarius* and other roseobacters. Genome sequencing of the roseophage isolates is in progress. Analysis of the new roseophage collection will provide new insights into the genetic and taxonomic diversity of roseophages.

EMP174

Comparative proteome analysis of glucose degradation in *Bacillus stamsii* – axenic culture versus methanogenic co-culture

F. Gräßle*[1], D. Schleheck[1], B. Schink[1], N. Müller[1]
[1]*University of Konstanz, Biology, Konstanz, Germany*

In sediments of freshwater lakes, methanogenic communities are proven to utilize a broad spectrum of organic substrates. In Upper Lake Constance, *Bacillus stamsii* was isolated via agar shakes in the presence of the methanogenic archaeon *Methanospirillum hungatei* and found to be an abundant, obligately syntrophic glucose degrader. Later, it was further found to becapable of axenic growth when supplied with amino acids and pyruvate in addition to glucose. Whilst in co-culture with *M. hungatei, B. stamsii* excretes both hydrogen and formate for interspecies electron transfer. Thereby, the levels of theses metabolites are maintained at a sufficiently low and thermodynamically favorable standing. Furthermore, the genomic DNA of *B. stamsii* was sequenced and annotated. The sequencing results yielded a genome size of 3 441 423 bp and 3 980 protein coding genes. The axenic cultures of *B. stamsii* and its co-cultures with *M. hungatei* were each grown with glucose at a concentration of 2 mM or 5 mM. Analysis of both the cytoplasmic and membrane proteome revealed, that all glycolysis genes are expressed under all growth conditions. In contrast, enzymes involved in hydrogen and formate formation were specifically upregulated in the co-culture. Even though *B. stamsii* does not grow anaerobically in axenic culture, it would be able to ferment glucose to lactate, as lactate dehydrogenase is constitutively expressed. It is therefore questionable, why *B. stamsii* obligately depends on either syntrophic cooperation or pyruvate as growth stimulating agent during anaerobic, fermentative growth. The possible reasons are discussed based on proteome analysis.

EMP175

Characterization of *Acinetobacter* spp. isolated from input (livestock manure) and output samples of German biogas plants

D. Pulami*[1], T. Schauss[1], S. Mishra[1], J. Bender[2], J. Blom[3], A. Goesmann[3], P. Kämpfer[1], G. Wilharm[2], S. P. Glaeser[1]
[1]*Justus Liebig University Giessen, Institute of applied microbiology, Gießen, Germany*
[2]*Robert Koch Institute, Wernigerode, Germany*
[3]*Justus Liebig Univeristy Gießen, Institute for Bioinformatics and Systems Biology, Gießen, Germany*

The clinical relevance of multidrug resistant (MDR) *Acinetobacter* has globally increased in current era of antibiotic resistance. Livestock is one source of resistant bacteria including *Acinetobacter* species. Spread of *Acinetobacter* species from livestock via manure into the environment was not studied in detail yet. The digestion of manure in biogas plants is considered as biotechnological barrier to prevent the release of manure-associated bacteria, and may also alter the release of *Acinetobacter*. The presence of *Acinetobacter* species was studied by an *Acinetobacter* specific 16S rRNA gene sequence targeting approach in input and output material of 15 German biogas plants. The concentration was in the range of 10^6 to 10^8 in input and 10^5 to 10^6 16S rRNA gene targets g^{-1} × FW output materials. During a non-targeted cultivation-based study *Acinetobacter* isolates were culture from input and output source, including six strains of *A. baumannii*. Here we give a detailed phylogeny, antibiotic resistance and MLST profiling of those strains. Distinct differences were obtained between input and output strains. Furthermore, the epidemiological and ecological relevance of the *A. baumannii* strains was studied by a comparative MLST and the genome wide analysis approaches. These strains represented three previously described and three new ST-types were identified which did not represent any known ST-types in MLST database of *A. baumannii*, which showed the huge diversity among this pathogen. The bla_{OXA-51} typing showed all *A. baumannii* strains in our study harboured bla_{OXA-51} like gene, which is an intrinsic class D beta-lactamase. This study gives a first detailed study and evidence of *A. baumannii* strains released via manure and biogas digestate into the environment.

EMP176

Tree holes as model systems to study bacterial community assembly and biogeography

F. Bajerski*[1], J. Pascual[1,2], A. Methner[1], A. Roberts[3], M. M. Gossner[3], J. S. Petermann[4], J. Overmann[1]
[1]*Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Department Microbial Ecology and Diversity, Braunschweig, Germany*
[2]*Darwin Bioprospecting Excellence S.L, Paterna, Spain*
[3]*Swiss Federal Research Institute WSL, Forest Entomology, Birmensdorf, Switzerland*
[4]*University of Salzburg, Department of Biosciences, Salzburg, Austria*

Microbial biogeography touches one of the most discussed paradigms in microbial ecology: Everything is everywhere, the environment selects (Becking, 1934).Tree holes, small aquatic ecosystems with island-like characteristics, are suitable habitats to study the effects of dispersal, environmental selection, and stochastic processes on microbial community assembly.

Little is known how deterministic and stochastic factors shape biogeographic patterns of bacterial communities. Here we show that aquatic bacteria colonizing *Fagus* tree holes respond to different deterministic and neutral factors along a geographical scale.

In the framework of the "Biodiversity exploratories", microbial communities of 29 *Fagus* tree holes from 2 different geographic regions in Germany were analysed using Illumina amplicon sequencing of the V3 region of 16S rRNA genes. The effects of local and regional distances and environmental parameters on community assembly were analyzed.

At local scale between tree holes within sites, bacterial communities were driven by environmental factors independently of the geographical distances of the tree holes. At regional scale between sites, bacterial communities were driven by both dispersal limitation and environmental factors. A linear distance-decay pattern was observed for the bacterial community assemblage at a regional scale, suggesting that geographic distances imposed strong constraints on community composition.

Due to the geographical isolation of the study sites, ecological drift (variation in the bacterial assemblage due to random survival) in combination with deterministic processes during bacterial colonization of tree holes (transport by insects, tree leaves) cause a high variance of bacterial communities that develop in tree holes.

EMP177

Root-derived organic carbon fuels diverse fermentative processes in a methane-emitting fen

A. Meier[1], S. Hunger[1], H. L. Drake[1], O. Schmidt*[1]
[1]*University of Bayreuth, Ecological Microbiology, Bayreuth, Germany*

Fens are methane-emitting mires that are overgrown by grasses and sedges. Roots of these fen plants deposit organic carbon into the fen soil. Fermenters attached to roots or located in close proximity to roots are conceptualized to convert root-derived organic carbon to (in)direct methanogenic substrates and are consequently linked to methane production in fens. In this study anoxic incubations with roots or soil from the fen plant *Carex rostrata* (bottle sedge) were conducted to study root-dependent fermentative processes, and Illumina 16S rRNA transcript analysis was performed to identify active fermenters either associated to the roots or located in the soil. Ethanol, acetate, propionate, H2, and CO2 accumulated to millimolar concentrations in incubations with washed roots in which 16S rRNA transcripts related to mixed acid fermenters of the *Enterobacteriaceae* and propionate fermenters of the *Veillonellaceae* were most abundant. Butyrate concentrations and transcript abundances of *Clostridiaceae* (typical butyrate fermenters) were higher in incubations with unwashed compared to washed roots. In contrast to root incubations only traces of acetate and CO2 were detected in incubations with sieved soil (largely devoid of roots) and the transcript abundances of the aforementioned fermentative taxa were at least tenfold lower. However, soil fermenters (especially *Clostridiaceae*) could be activated by mixing sieved soil with washed roots in a 1:1 ratio. The collective results suggest that root-derived organic carbon stimulates metabolically and phylogenetically diverse fermenters that produce direct or indirect methanogenic substrates and are either tightly associated to roots or are present predominately in the soil surrounding the roots of the fen plant *Carex rostrata.*

EMP178

Aurantimycin resistance genes contribute to survival of *Listeria monocytogenes* during life in the environment

S. Hauf*[1], J. Herrmann[2], M. Miethke[2], J. Gibhardt[3], F. M. Commichau[3], R. Müller[2], S. Fuchs[1], S. Halbedel[1]
[1]*Robert Koch Institut, Division of Enteropathogenic Bacteria and Legionella (FG11), Wernigerode, Germany*
[2]*Helmholtz-Institute für Pharmazeutische Forschung Saarland, Saarbrücken, Germany*
[3]*Georg-August-Universität Göttingen, Göttingen, Germany*

Background: Transcriptional regulators of the PadR family contribute to resistance against antibiotics and small toxic compounds in many bacteria. LftR, a PadR-homologue of the human pathogen *Listeria monocytogenes* was previously shown to affect ethidium bromide resistance and virulence. To better understand the role of LftR in the physiology of *L. monocytogenes*, we studied phenotypes of *lftR* mutants.

Material and methods: The influence of LftR on global gene expression was studied using RNA sequencing. LftR-dependent genes were confirmed using promoter-*lacZ* fusions. Agents inducing the LftR response were identified using screening strains expressing LftR-dependent β-galactosidase.

Results: RNA sequencing showed that LftR tightly represses its own operon and that of the LieAB drug efflux pump. The LacZ reporter strains revealed that both operons are induced in the presence of the streptomycete antibiotic aurantimycin A. Aurantimycin A is a potent antibiotic disrupting the cell membrane of gram-positive bacteria. The expression of the LieAB efflux pump markedly increases listerial resistance to aurantimycin by preventing cell lysis. The underlying mechanism seems to be the energy dependent extrusion of aurantimycin from the cell membrane.

Conclusion: We discovered the first resistance mechanism against the depsipeptide antibiotic aurantimycin A. The resistance is mediated by the LieAB efflux pump whose expression is controlled by the PadR-like regulator LftR. Genes homologous to *lftR* and *lieAB* are widespread in the phylum *Firmicutes* to which *Listeria* belongs. As *Firmicutes* and *Streptomyces* are both ubiquitous soil inhabiting bacteria, it is likely that this resistance mechanism is of importance for their interaction and survival in the natural environment.

EMP179

Novel H_2-uptake active enzymes from deep-sea hydrothermal vents: evidence for the need of functional metagenomics

N. Adam*[1], M. Perner[1]
[1]*GEOMAR Helmholtz Centre for Ocean Research Kiel, Geomicrobiology, Kiel, Germany*

Hydrogenase enzymes catalyze the interconversion of molecular hydrogen to protons and electrons and are widespread among Bacteria and Archaea. In deep-sea hydrothermal vent systems hydrogen oxidation can be one of the most important energy sources, fueling microbial biomass production. Hydrogenases can also serve to recycle reducing equivalents through hydrogen evolution. Until recently, culture-dependent or sequence-based metagenomic approaches represented the only means for the identification of new hydrogenases. Both techniques proved to be successful for discovering hydrogen-converting enzymes but they are biased against hydrogenases from uncultured microbes that do not resemble known enzymes deposited in the public databases.

In order to circumvent these drawbacks, we recently developed an activity-based screen for identifying H_2-uptake active enzymes in metagenomic libraries. By applying this screen to 14,400 clones of three different deep-sea hydrothermal vent metagenomes, we identified four H_2-uptake active clones. We here describe the biochemical properties and sequence information of the metagenome-derived enzymes. The open reading frames encoded on the metagenomic inserts of three of the H_2-uptake active clones do not exhibit homologies to known hydrogenases or in some cases to any sequences (available in the public databases) at all. Hence, our activity-based screen greatly improves the toolbox for the recovery of (novel) hydrogenases from the environment, as our metagenomic clones would not have been identified with the so far available culture- and sequence-based techniques.

EMP180

Induction of the viable but non-culturable state (VBNC) in bacterial pathogens and tolerance testing against antimicrobials based on de-novo ATP production

C. Robben*[1], A. Witte[1], B. Stessl[2], D. Schoder[2], P. Rossmanith[1], P. J. Mester[1]
[1]*Vetmeduni Vienna, CD-Momico, Vienna, Austria*
[2]*Vetmeduni Vienna, Institute for milk hygiene, Vienna, Austria*

Bacteria are exposed to environments with constantly changing conditions. To cope with those environmental stresses, many bacteria enter a VBNC state in which they can resist unfavorable conditions, antimicrobials or antibiotics. Due to their non-culturability, it is experimentally challenging to reliably determine and investigate their tolerance.

Objectives: In this work we set up a protocol for MIC and MBC screening of VBNC cells by using the BacTiter-GloTM Microbial Cell Viability Assay.

Materials and Methods: For the induction of the VBNC state in four *L. monocytogenes*, three *E. coli*, three *P. aeruginosa* and two *B. cereus* strains, a previously published protocol based on salt/detergent combinations was used. VBNC induction and the MIC and MBC screening of nine stress conditions was determined with plating, viable cell count (*Bac*Light), ATP generation and metabolic activity testing.

Results: For each strain, a reliable VBNC induction condition was identified and optimized for subsequent MIC and MBC testing. The results show that the new method is suitable for a quick, reliable and cost efficient determination of the tolerance of VBNC cells. An initial screening performed with nine different stress conditions showed that cells in the VBNC state are more tolerant against antimicrobials, disinfectants and antibiotics compared to culturable cells.

Conclusion: This study suggest that adaptation of routine diagnostics and including more viability detection techniques is essential for correct estimation of the health risk and significance of VBNC pathogens, especially regarding their survival of disinfection measures.

EMP181

The Alphaproteobacterium *Paracoccus denitrificans* employs the glyoxylate cycle in addition to the ethylmalonyl-CoA pathway to speed up C2-assimilation

K. Kremer*[1], L. Schada von Borzyskowski[1], M. van Teeseling[2], M. Thanbichler[1,2], T. J. Erb[1,2]
[1]*Max-Planck-Institute for terrestrial Microbiology, Biochemistry & Synthetic Metabolism, Marburg, Germany*
[2]*Philipps-Universität, Biology, Marburg, Germany*

For optimal growth, bacteria have to balance their metabolic fluxes between catabolism and anabolism. Acetyl-CoA resembles a control point for this. It either enters the tricarboxylic acid (TCA) cycle for the conservation of energy or an anaplerotic pathway for the production of biomass. By the formation of C4-compounds from C2-units the latter enables bacteria to grow on small carbon compounds, such as acetate. Two key anaplerotic pathways function in bacteria: the glyoxylate cycle (GC) and the ethylmalonyl-CoA pathway (EMCP).

While the majority of bacteria possess only one of these pathways, the alphaproteobacterium *P. denitrificans* holds genes encoding both of them.

In this study, we show that *P. denitrificans* employs both the EMCP and the GC for the assimilation of C2-compounds. Enzyme assays in cell-free extracts and the analysis of fluorescence reporter strains show that the EMCP is constitutively used by the cells while the GC is specifically activated following upregulation of the EMCP in response to growth on acetate. However, knock-out studies highlight that the presence of only one pathway is sufficient to sustain growth under these conditions. While the growth defect of cells lacking the EMCP results in increased lag phases that can be overcome by metabolic adaptation of the cell to growth on acetate, the lack of the GC significantly slows down the growth of *P. denitrificans* on this carbon source.

Together, our data suggest that *P. denitrificans* uses the EMCP in order to rapidly adapt to a changing environment and specifically employs the GC to speed up C2-assimilation.

Our future studies aim at understanding the regulation of the expression and activity of the individual pathways on single cell level and on identifying the regulatory elements involved.

EMP182

Elevated atmospheric CO_2 effects diversity and abundance of heterotrophic and methylotrophic bacteria cultivated by dilution-to-extinction cultivation from the phyllosphere of *Arrhenatherum elatius* and *Galium album* plants

E. L. Aydogan[1], O. Budich*[1], M. Hardt[2], G. Moser[3], C. Müller[3,4], P. Kämpfer[1,3,4], S. P. Glaeser[1]
[1]*Justus-Liebig-Universität Giessen, Institut für Angewandte Mikrobiologie, Gießen, Germany*
[2]*Justus-Liebig-University Giessen, Biomedical Research Center Seltersberg - Imaging Unit, Gießen, Germany*
[3]*Justus-Liebig-Universität Giessen, Institut für Pflanzenökologie (IFZ), Gießen, Germany*
[4]*University College Dublin, School of Biology and Environmental Science, Dublin, Ireland*

Global climate changes increase atmospheric CO_2 concentrations. This affects plants, bacterial communities colonizing the aerial part of plants (phyllosphere) and the interactions of plants and phyllosphere bacteria.

Aim of this study was to determine the effect of elevated CO_2 on the concentration, diversity and abundance of heterotrophic and methylotrophic phyllosphere bacterial communities.

Leaves of two abundant grassland plant species of the Giessen Free Air Carbon Dioxide Enrichment system (Gi-FACE) in Linden (Germany) were collected in August 2015 from three areas of three control (CC) and three rings exposed to +20% elevated CO_2 (CE). Leaf associated heterotrophs and methylotrophs were enriched by dilution-to-extinction. The concentration of cultivated bacteria was determined by the most probably numbers method (MPN). Bacterial assemblages enriched in the three highest positive dilutions were compared by community fingerprinting using 16S rRNA gene targeting PCR- DGGE analysis. Bacteria from highest positive dilutions were furthermore isolated and identified by 16S rRNA gene sequencing.

Concentrations of cultured heterotrophs and methylotrophs were not affected by elevated CO_2, but non-metric multidimensional scaling (NMDS) of community patterns showed significant differences of the phylogenetic compositions of cultivated bacteria. *Pseudomonas* was the most isolated heterotrophs *and Methylobacterium* was the most isolated methylotrophs grown on both plant species.

This study indicates an adaptation of specific phylotypes of the common isolated genera (*Pseudomonas, Methylobacterium)* to elevated CO_2 conditions and establishes specifically adapted ecotypes.

EMP183

Atypically high nitrogen fixation rates in the chemocline of the meromictic Lake Cadagno, Switzerland

M. Philippi*[1]
[1]*MPI Marine Microbiology, Biogeochemistry, Bremen, Germany*

The new input of nitrogen (N) by dinitrogen (N_2) fixation in meromictic lakes is often relatively low in comparison to N loss processes and N turnover. Low rates and the presence of N_2-fixing microorganisms (diazotrophs), however, have stimulated research on N_2 fixation in these ecosystems. Lake Cadagno is a permanently stratified lake in the Swiss Alps with a high abundance of phototrophic sulfur bacteria and sulfate reducing bacteria at the chemocline. Previous studies have reported extremely low N_2 fixation rates in Lake Cadagno. Moreover, *in situ* activity has only been shown for the rare green sulfur bacterium *Chlorobium phaeobacteroides* despite a diverse diazotrophic community. In August 2018, we performed stable isotope incubations with $^{15}N_2$ gas in order to measure *in situ* N_2 fixation rates and to identify the major contributors in and below the chemocline of Lake Cadagno. Water column profiling showed a much deeper chemocline than in previous studies, with exceptionally high N_2 fixation rates in the turbidity maximum. Below the chemocline N_2 fixation rates were undetectable. Single-cell analyses using nanometer-scale secondary ion mass spectrometry (nanoSIMS) measurements revealed *in situ* N_2 fixation activity by the highly abundant purple sulfur bacteria *Chromatium* spp. and *Thiodictyon* spp. which so far have not been shown to be fixing N_2 in this environment. Currently it is unclear what the underlying causes are for the observed differences in the diazotrophic community and the variability in the biogeochemistry. Our study, though, highlights the potential importance of N_2 fixation in these ecosystems, which have been used as ancient ocean analogs.

EMP184

Proteomic insights into ulvan degradation by *Formosa agariphila* KMM 3901T

M. K. Zühlke*[1,2], L. Reisky[1,2,3], J. H. Hehemann[2,4], S. Markert[1,2], M. Bäumgen[3], F. Unfried[1,2], A. Trautwein-Schult[5], D. Becher[5], R. Amann[4], U. T. Bornscheuer[3], T. Schweder[1,2]
[1]*Universität Greifswald, Institute of Pharmacy,Greifswald, Germany*
[2]*Institute of Marine Biotechnology, Greifswald, Germany*
[3]*University of Greifswald, Department of Biotechnology & Enzyme Catalysis, Institute of Biochemistry, Greifswald, Germany*
[4]*Max Planck Institute for Marine Microbiology, Bremen, Germany*
[5]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*

Massive proliferation of algal biomass has detrimental impacts on coastal ecosystems and poses diverse economical threats. At the same time, these algal blooms represent an emerging resource for rare algal components like sulphated polysaccharides. Strategies to harness these compounds are thus urgently required. Macroalgae of the genus Ulva recurrently grow into extensive blooms (1). The formed biomass harbours the sulphated, structurally complex polysaccharide ulvan, which makes up to 29 % of the algal dry weight (2) and contains valuable sugars as building blocks, such as rhamnose. The marine flavobacterium Formosa agariphila KMM 3901T is able to use ulvan as a growth substrate. Relevant enzymes of this strain are therefore of high interest to promote the establishment of productive refinery concepts. In F. agariphila, almost 40 genes encoding ulvan-utilizing proteins are genomically co-located in a so-called polysaccharide utilization locus (PUL) (3).

A combined approach of metabolic labeling and subcellular protein fractionation revealed that the expression of most of the ulvan PUL-encoded proteins was specifically up-regulated in ulvan-cultivated cells. Ulvan depolymerization by the bacterial strain releases various monosaccharides. Proteome analyses pointed to enzymes, which might catalyze the corresponding monosaccharide metabolization. Altogether, these results support our understanding of the complex degradation pathway of ulvan by F. agariphila (4).

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EMP185

The microbiome of desert plants: hints for an increasingly warmer and drier planet

L. P. Partida-Martínez*[1]
[1]*Centro de Investigación y de Estudios Avanzados (CINVESTAV), Departamento de Ingeniería Genética, Guanajuato, Mexico*

Arid and semi-arid ecosystems represent approx. 41% of the Earth"s surface. Plants living in these regions are subjected to several types of abiotic stress, including extreme temperatures, high UV radiation, nutrient-deprived soils and long periods of drought. Noteworthy two diverse and taxonomic unrelated plant lineages native to the American continent have flourished under these harsh environmental

conditions: the genus *Agave* and the family Cactaceae. Despite their ecological relevance, an understanding of the relationships these plants establish with microorganisms and their functions was lacking.

Phylogenetic profiling of the bacterial, archaeal and fungal communities associated with the soils, rhizosphere, roots, leaves and phyllosphere of five wild and cultivated *Agave* and cacti species of North and Central America revealed an unexpected high microbial diversity. The plant compartment and the biogeography of the plant species played the most prominent roles in the assembly of the plant-associated prokaryotic and fungal communities, respectively1-3.

Taxonomic, metagenomic and network analyses support the presence of a *core microbiome* in these desert plants3,4, which functions, including the production of microbial volatile organic compounds (mVOCs), improve growth and development of model and host plants5. Altogether our studies highlight the potential of using microbiome-based knowledge to enhance the productivity and sustainability of agriculture in arid areas of an increasingly warmer and drier planet.

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FPP186

Localization of the germinal centre kinase SmKIN3 is dependent on the conserved STRIPAK signaling pathway

V. Stein*[1], R. Märker[1], D. Radchenko[1], B. Blank-Landeshammer[2], A. Sickmann[2], U. Kück[1]

[1]Ruhr Universität Bochum, Allgemeine und Molekulare Botanik, Bochum, Germany

[2]Leibniz-Institut für Analytische Wissenschaften-ISAS-e.V, Dortmund, Germany

The striatin interacting phosphatase and kinase (STRIPAK) complex is a highly conserved protein complex, found in eukaryotes from yeast to human. In humans, STRIPAK regulates cell division, differentiation and migration. In the filamentous model fungus *Sordaria macrospora* however, the complex controls multicellular differentiation, hyphal fusion and vegetative growth. The core of STRIPAK consists of striatin, protein phosphatase PP2A, striatin interacting proteins 1 and 2, *sarcolemmal membrane associated protein,* phocein/Mob3 and two germinal centre kinases (GCKs), SmKin3 and SmKin24. However, the role of these kinases is not yet fully understood. SmKin3 interacts physically with PRO11, the striatin homolog in *S. macrospora.* Moreover, a deletion mutant of *SmKin3* is lacking septa in vegetative hyphae. In humans, GCKs connect the STRIPAK complex with the Hippo pathway, the homolog of the *S. macrospora* septation initiation network (SIN), which regulates septation. Recent data indicates a crosstalk between STRIPAK and SIN. However, this signalling network has to be elucidated in more detail. Using fluorescence microscopy, we localized SmKin3 in different STRIPAK deletion mutants. In strains lacking PRO11 and PP2A catalytic subunit, SmKIN3 localizes to the first septum in the hyphal tip, while it localizes prevalently to the third septum in the wild type. We propose that STRIPAK-dependent phosphorylation state of SmKIN3 affects its affinity to septal proteins and thus its localization. Expanding knowledge of the connection between SIN and STRIPAK will provide detailed insight into the fungal development, which might be extrapolated to higher eukaryotes (Kück et al. 2016).

FPP187

Structural studies of the novel MLLE-PAM2L interaction involved in endosome-mediated mRNA transport in plant pathogenic fungus *Ustilago maydis*

S. Devan*[1], S. Schott-Verdugo[2], T. Pohlmann[1], M. Feldbrügge[1]

[1]Institute for Microbiology, Heinrich Heine University, Düsseldorf, Germany

[2]Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine university, Düsseldorf, Germany

The transport of mRNAs coupled with local translation ensures a spatiotemporal regulation of gene expression. Active transport along the cytoskeleton is a common mechanism of mRNA localization in polar growing cells. This transport can be dependent on the actin or microtubule cytoskeleton and requires the involvement of specific RNA binding proteins, molecular motors, and adaptors that connect those proteins. In the phytopathogenic fungus, *U. maydis* the RNA binding protein Rrm4 is a key component of long-distance transport of mRNAs. Loss of Rrm4 leads to disturbed hyphal growth and reduced pathogenicity.

Rrm4 contains three RNA-recognition motifs (RRMs) for cargo binding and two MLLE domains for protein interaction. Rrm4-containing messenger ribonucleoprotein (mRNPs) complexes are transported along the microtubules by hitchhiking on Rab5a-positive endosomes. Rrm4 itself does not have a membrane-binding domain, but it is recruited to the organelle surface by interacting with the endosomal protein Upa1. Upa1 contains a C-terminal FYVE domain for attachment to the endosomal membrane and two short regions for interaction with the Rrm4 MLLE domains. These short regions resemble the well-studied PAM2 motif and were therefore termed PAM2- like motif (PAM2L). In addition, Upa1 has one PAM2 motif which can interact with the MLLE domain in the poly(A)-binding protein Pab1, which is an additional component of transport mRNPs. $MLLE^{Rrm4}$-PAM2L and the $MLLE^{Pab1}$-PAM2 interaction are specific.

To further understand the structural basis of these interactions and specificity we are investigating the 3D structure of these protein complexes by using a combined approach of biochemical and biophysical assays, molecular modeling, X-ray crystallography and functional analysis in *U. maydis.*

GRP188

Strategies of insect pathogenic *Photorhabdus luminescens* 2° cells for adaptation to the rhizosphere environment

N. Dominelli*[1], S. Eckstein[1,2], R. Heermann[1]

[1]Johannes-Gutenberg-Universität Mainz, Institut für Molekulare Physiologie, Mikrobiologie, Mainz, Germany

[2]Ludwig-Maximilians-Universität München, Biozentrum, Bereich Mikrobiologie, Martinsried, Germany

Photorhabdus luminescens is a bioluminescent, Gram-negative entomopathogenic bacterium of the family of *Enterobacteriaceae*. Its dualistic life cycle involves mutualistic symbiosis with *Heterorhabditis bacteriophora*

nematodes and a pathogenic part towards a wide range of insect hosts. *P. luminescens* exists in two phenotypically different cell variants: primary (1°) and secondary (2°) cells. 2° cells lack several 1° specific features like bioluminescence, pigmentation and antibiotic production. Furthermore, in contrast to 1° cells, they are not able to live in symbiosis with the nematodes anymore and are therefore left in soil when all nutrients of the insect cadaver are depleted. This leads to the assumption that 2° cells are better adapted to a free life in soil, which might include interaction or attraction towards plants in the rhizosphere. Here we show that *P. luminescens* 2° cells, in contrast to 1° cells, are specifically attracted towards living plant roots. Indeed, 2° cells were not only highly motile after sensing the roots, they also massively accumulated around plant root hairs. Additionally, a higher chemotaxis motility towards different additives and plant root exudate for 2° cells could be observed.

Furthermore, our data underline the hypothesis for the ability of 2° cells to utilize alternative nutrients, which might also be plant derived. In summary, the fate of *P. luminescens*2° cells in the rhizosphere gives first insights into the life phase of bacterial pathogens outside of their infection cycle.

GRP189
Regulation of the fructosyllysine metabolism in *Escherichia coli*

J. Lassak*[1], B. F. C. Graf von Armansperg[1], F. Koller[1]
[1]Ludwig-Maximilians Universität München, Biologie I/Mikrobiologie, Planegg-Martinsried, Germany

Nε-Fructosyllysine (FrK) is a Maillard sugar produced by the non-enzymatic reaction of glucose and lysine. As a post-translational protein modification it plays a role in diabetes mellitus as well as aging processes in the human body. *Escherichia coli* can grow on FrK as a sole carbon source. The corresponding metabolic proteins are encoded in an operon *frlABCD*. Upon uptake of FrK by the permease FrlA, the amino sugar is decomposed into lysine and glucose-6-phosphate by the concerted enzyme activities of the kinase FrlD and the deglycase FrlB. In contrast to the decomposition, the genetic regulation is not yet understood. Bioinformatic analyses and studies on the *Bacillus subtilis* FrK degradation pathway suggest that the putative transcription factor FrlR plays a pivotal role here. Employing $P_{frlABCD}$-Lux reporter fusions of varying length we succeeded in unmasking FrlR as a road block repressor and identifying the corresponding operator sequence. Using microscale thermophoresis and surface plasmon resonance spectroscopy, we confirmed our finding *in vitro* and also determined DNA binding affinity. A structural model identifies FrlR as a GntR-like transcription factor consisting of an UTRA substrate binding domain and a Helix-Turn-Helix-DNA binding domain. In summary, our data lead to a regulatory model in which dimeric FrlR blocks the transcription of the *frlABCD* operon. After the addition of FrK, FrlR dissociates into its monomers and liberates the operator. As additional levels of regulation we found catabolite repression by the cAMP-responsive transcription activator CRP and heat stress-induced expression by the alternative sigma factor σ^{32}. This complexity enables optimal adaptation to intestinal growth where FrK is present as a component of human food.

GRP190
Towards a regulatory network – light-dependent biosynthesis of bacteriochlorophyll *a* in *Dinoroseobacter shibae*

M. Becker *[1], D. Jahn[1], E. Härtig[1]
[1]Technische Universität Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany

Introduction: The marine bacterium *Dinoroseobacter shibae* possesses a gene cluster, encoding all necessary components to perform aerobic anoxygenic photosynthesis. Transcriptome analyses revealed a light dependent expression of this gene cluster. Screening of the transposon mutant library of *D. shibae* for negative pigmentation and bacteriochlorophyll (Bchl *a*) phenotypes identified the gene loci Dshi_1135, encoding a potential blue light-dependent LOV (light, oxygen, voltage) histidine kinase and PpsR, coding for a putative repressor of Bchl*a* biosynthesis.

Objectives: Determination of the roles of Dshi_1135 and PpsR in light-dependent regulation of Bchl *a* biosynthesis in *D. shibae*.

Materials & Methods: The *D. shibae* Dshi_1135 protein was heterologously produced and purified under red light conditions. UV/Vis spectroscopy and autophosphorylation assays were used for biochemical studies under different light conditions. Regulatory effects of Dshi_1135 and PpsR on Bchl *a* biosynthesis genes were analyzed *via* promoter-*lacZ* gene fusions.

Results: UV/Vis measurements of purified Dshi_1135 showed the typical maxima for a FMN cofactor at 380 nm and 450 nm. Exposure of the protein to blue light led to quenching of the absorbance. Absorbance could be restored by returning the sample back into the dark. In contrast, red light had no effect. β-galactosidase activity assays indicated that Dshi_1135 is an essential regulator for Bchl *a* biosynthesis. Furthermore it was confirmed that PpsR acts as a strong repressor in Bchl *a* synthesis.

Conclusion: Dshi_1135 undergoes a LOV-protein typical reversible blue light driven photocycle. Together with PpsR it is a major regulator in light-dependent Bchl *a* biosynthesis.

GRP191
Regulation of the β-hydroxyaspartate pathway in *Ruegeria pomeroyi* DSS-3 by an IclR-type regulator acting both as an repressor and activator

L. Hermann*[1], L. Schada von Borzyskowski[2], T. J. Erb[2], E. Bremer[1]
[1]Philipps University Marburg, Microbiology, Marburg, Germany
[2]Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany

Primary production of biomass is one of the most important processes on earth and is mainly achieved via the Calvin-Benson-Bassham (CBB) cycle whose central enzyme RuBisCo produces the toxic compound 2-phosphoglycolate in a wasteful side reaction. 2-Phosphoglycolate is detoxified in energetically expensive photorespiration processes, releasing CO2 and the waste products glycolate and glyoxylate. Both C2-compounds are excreted by algae and cyanobacteria on a large scale, can be detected in the oceans in µM concentrations and get recycled by bacteria as carbon and energy sources via photorespiration. The most efficient natural route for this photorespiration is the β-hydroxyaspartate pathway (BHAP) in which two C2 molecules are converted into one C4 molecule by consuming only one reducing equivalent. The question we are addressing is how the genetic regulation of the BHAP is achieved. In the metabolically versatile and ecologically

important *Roseobacter* clade, the genes employed in the BHAP are widespread and organized in an operon (*bhaABCD*) positioned adjacent to an IclR-type regulator (*bhaR*). In *Ruegeria pomeroyi* DSS-3, expression of *bhaABCD* as well as *bhaR* are induced in cells grown on glycolate and glyoxylate. Deletion mutants of *bhaR* in combination with *lacZ* reporter-assays show that BhaR acts both as an activator of the *bhaABCD* operon as well as a repressor of its own gene. However induction of *bhaABCD* and *bhaR* by glycolate and glyoxylate is not abolished *per se* in a *bhaR*-deletion strain, therefore some other, still unknown regulator(s) have to take part in this regulatory circuit. The widespread phylogenetic distribution of the BHAP make an understanding of its genetic regulation a key factor in understanding the marine global carbon cycle.

GRP192

Elucidating cyanobacterial phytochrome 1-mediated signaling pathways in the cyanobacterium *Synechocystis* sp. PCC 6803

N. Priyadarshini*[1,2], A. Wilde[1]
[1]*University of Freiburg, Molecular Genetics, Freiburg i. Br., Germany*
[2]*Spemann Graduate School of Biology and Medicine, Freiburg i. Br., Germany*

Phytochromes are red, far-red photoreceptors that act as light sensitive switches to regulate diverse cellular mechanisms. Earlier they were believed to be present only in plants however, whole genome sequencing analysis of the cyanobacterium *Synechocystis* sp. strain PCC 6803 revealed the presence of a conserved cyanobacterial phytochrome gene sequence called cyanobacterial phytochrome 1 (*cph1*). The gene product, Cph1, has an N-terminal sensory module regulated by light and a C-terminal histidine kinase module, which is typical for bacterial two-component sensory kinases. Cph1 has its own cognate response regulator, Rcp1. Since the discovery of Cph1 and Rcp1 over almost two decades ago, not much is known about their interacting partners nor about the intracellular pathways they regulate. Here, we show our strategy and first results to identify possible interacting partners of this two-component signaling system in order to elucidate cellular functions, that might be influenced by this system.

GRP193

Regulation of polar flagellar assembly in *Shewanella putrefaciens*

M. Schwan*[1], D. Mrusek[2], A. Khaledi[3], T. Glatter[4], S. Häußler[3], G. Bange[2], K. Thormann[1]
[1]*Justus-Liebig Universität Gießen, Institut für Mikro- und Molekularbiologie, Gießen, Germany*
[2]*Philipps Universität Marburg, LOEWE Zentrum für Synthetische Mikrobiologie, Marburg, Germany*
[3]*Helmholtz-Zentrum für Infektionsforschung, Molekulare Bakteriologie, Braunschweig, Germany*
[4]*Max Planck Institute for Terrestrial Microbiology, Massenspektrometrie und Proteomics, Marburg, Germany*

Flagella are organelles of locomotion and multiprotein complexes whose assembly and positioning requires complex spatiotemporal control. Bacteria have a transcriptional regulatory mechanism for flagella assembly. However, among all species, regulation of flagellar genes is only known for mRNA, but not at protein level. Therefore, we have studied the regulation of polar flagellar genes by the regulators FlrA, RpoN (σ54) and FliA (σ28) in *Shewanella putrefacie*ns CN-32.

The major regulators, FlrA and RpoN, activate the transcription of the basal body building blocks, the flagellar export gate and the further regulator FliA. However, only a few of these early flagella proteins are regulated while the others are present at normal levels in the absence of the master regulators. The sequential expression ensures that the subsequent transcription of genes activated by FliA and coding for the main filament subunit Flagellin is only transcribed after completion of the hook arrangement.

In addition, we investigated the regulation of the MinD-like ATPase FlhG, which influences the number of flagella. A negative feedback of the FlrA-dependent gene expression is triggered by the interaction of FlhG with the HTH-domain of FlrA. These findings provide new insights into the transcriptional regulation and assembly of polar bacterial flagella.

GRP194

Functional analysis and cofactor determination of the iron response regulator IscR of *Dinoroseobacter shibae*

L. Plötzky*[1], M. Behringer[1], K. E. Rennhack[1], D. Jahn[1], E. Härtig[1]
[1]*TU Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany*

Introduction: *Dinorosebacter shibae* is a member of the *Roseobacter* group of marine bacteria. According to the highly oxygenated sea water at pH 7, iron in the soluble ferrous form is limited. Next to the iron-dependent regulators Fur, Irr and RirA, *D. shibae* also possesses a gene, encoding a regulator of the Rrf2-family of transcription factors with 41.9 % identity to the iron sulfur cluster regulator IscR of *E. coli*. Interestingly, in the amino acid sequence of *D. shibae* IscR the cysteines involved in Fe-S cluster coordination are missing.

Objectives: Determination of the role of *D. shibae* IscR for iron-dependent adaption and characterization of its cofactor.

Material & Methods: IscR fused to a StrepII-tag was recombinantly produced and purified under anaerobic conditions. UV/Vis and EPR spectroscopy were used for identification of the cofactor. DNA binding of anoxic IscR wildtype protein was analyzed, using electro mobility shift assays (EMSA). Gene regulatory effects of IscR regarding to iron availability were analyzed *via* promoter-*lacZ* gene fusions and transcriptome analyses.

Results: Transcriptome data revealed that IscR has an impact on iron-dependent gene regulation. β-galactosidase activity assays indicated that IscR acts as a repressor of *hemB2* expression. Furthermore, EMSA analyses revealed binding of the IscR protein to the *hemB2* promoter DNA sequence. UV/ Vis spectroscopy of purified IscR showed the characteristic absorption maximum of heme at 420 nm. EPR studies confirmed the presence of a heme cofactor.

Conclusion: The iron sulfur cluster regulator IscR of *D. shibae* is involved in iron-dependent gene regulation and is able to measure iron availability by binding of a heme cofactor.

GRP195

Mechanisms of gene regulation by the attenuator sRNA rnTrpL and the leader peptide peTrpL

S. Li*[1], H. Melior[1], E. Evguenieva-Hackenberg [1]
[1]*Institute for Microbiology and Molecular Biology, Justus Liebig University, Giessen, Gießen, Germany*

Sinorhizobium meliloti (*S. meliloti*) is living in soil and belongs to the alpha-proteobacteria. It exists either in symbiosis with its leguminose host plants or in a free-living state. In *S. meliloti*, the tryptophane biosynthesis genes are organized in the three operons *trpLE(G)*, *trpDC* and *trpFBA*, but only the *trpLE(G)* operon is regulated by transcription attenuation. We found that the leader peptide peTrpL and the liberated attenuator sRNA rnTrpL have regulatory functions in *trans*. To analyze their mechanisms, we constructed suitable deletion mutants of strain *S. meliloti* 2011 and plamids allowing ectopic expression of the peptide, the sRNA and of their mutated derivatives. Bioinformatic predictions and changes in mRNA levels upon induced ectopic expression of rnTrpL in strain 2011*ΔtrpL* suggested primary targets of the sRNA. Several targets were validated by induced co-expresison with *egfp* reporters fusions, using mutations in the sRNA and compensatory mutations in the targets. In addition, we found that the leader peptide peTrpL mediates multiresistance. Alanine scanning mutagenesis of the 14 aa peptide revelaed that Thr4, Ser8, and Trp12 are essential for this function, suggesting that an alpha helix conformation is adopted upon binding to an interaction partner. Altogether, our data suggest that rnTrpL is a versatile riboregulator using several modules to interact with multiple mRNA targets in an extended network responding to tryptophan availability. In addition, it is a dual-function sRNA encoding the first described leader peptide with an own physiological role in bacteria. Our results suggests that its function to increase multiresistance is exterted at the posttranscriptional level with the help of additional factors.

GRP196

Posttranscriptional regulation of SAM and m6A modification of RNA in the alpha-proteobacterium *Sinorhizobium meliloti*

R. Scheuer*[1], H. Melior[1], O. Rossbach[2], J. Bathke[3], A. Goesmann[3], E. Evguenieva-Hackenberg[1]
[1]*Institute of Microbiology and Molecular Biology, Gießen, Germany*
[2]*Institute of Biochemistry, Gießen, Germany*
[3]*Institute of Bioinformatics & Systems Biology, Gießen, Germany*

N-6-methyladeonsine (m6A) is the most abundant mRNA modification in eukaryotes where it plays important regulatory roles [1]. Bacterial mRNAs and sRNAs also contain m6A, but nothing is known about the physiological relevance of this modification and the responsible methylase is also unknown [2]. We have shown that in the nitrogen fixing plant symbiont *Sinorhizobium meliloti,* RNases E and J are needed for homeostasis of the main methyl donor S-adenosylmethionine (SAM) [3]. In the *S. meliloti* 2011 mutants with mini-Tn5 insertions in the corresponding genes *rne* and *rnj* the SAM level was increased. This leads to the assumption that RNase E and RNAse J have common sRNA or mRNA targets responsible for SAM regulation. Moreover both mutants show higher m6A- and N-acyl homoserine lactone (AHL)-levels, which can be related to the increased SAM-level. We performed RNase E or J depletion to address the post-transcriptional mechanisms leading to increased SAM concentration. For the RNase depletions we used mutant strains that are complemented by IPTG-induced ectopic expression of the corresponding RNase. Indeed we were able to identify secondary effects of RNase depletion like changes in the amount of AHLs and in the m6A-level were observed 15 and 25 min after IPTG removal, respectively. The last time point was used for iCLIP to investigate "hot spots" of m6A. Most recent results will be presented. Taken together we aim to reveal new mechanisms for post-transcriptional regulation of gene expression in bacteria.

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GRP197

Small proteins in the soybean symbiont *Bradyrhizobium japonicum*

S. Azarderakhsh*[1], E. Evguenieva-Hackenberg[1]
[1]*Institut für Mikro- und Molekularbiologie, Gießen, Germany*

A longtime underestimated feature of bacterial genomes are genes for so called µ-proteins – proteins smaller than 50 amino acids (aa). A major reason for this is the fact, that current annotation algorithms do not consider open reading frames (ORF) smaller than 30-50 aa and biochemical detection is exceedingly challenging [1]. *Bradyrhizobium japonicum* USDA 110 belongs to Alphaproteobacteria and can fix atmospheric nitrogen in symbiosis with the soybean plant. Recently a dRNA-seq analysis was used to map transcription start sites (TSSs) and predicted promoters in its 9.1 Mb large genome. In that analysis, the genome was re-annotated adding 1391 ORFs with lengths between 30 and 80 aa [2,3]. For detection of smaller ORFs, we inspected small novel transcripts with mapped TSSs and promoters. One of them is a small mRNA that shows a high conservation within the family of *Bradyrhizobiaceae*. This mRNA has 17 nt perfect complementarity to the 3''-end of the 16S rRNA, binds strongly to the ribosomal subunit 30S, and the encoded 14 aa peptide RreB is poorly translated [4]. The second transcript is the 5'' transcription attenuator of the *trpE* gene, which encodes the 14 aa leader peptide Bj-peTrpL. Overproduction of Bj-peTrpL resulted into increased resistance to tetracycline. Currently we are investigating the interaction partners of both µ-proteins. Most recent results will be presented.

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GRP198

Synthetic counter-silencing as an approach to study evolutionary network expansion

J. Wiechert*[1], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences IBG-1: Biotechnology, Jülich, Germany*

Horizontal gene transfer is a major driver of bacterial evolution but requires stringent regulation of foreign gene expression. Previously, CgpS was identified as a xenogeneic silencer (XS) of *Corynebacterium glutamicum* prophages (1). Binding of specific TFs to silenced promoter regions may oppose silencer activity leading to counter-silencing of the phage promoter, thereby providing access to horizontally acquired DNA.

In this study, a modular synthetic promoter design was used to provide insights into the evolution of TF-mediated xenogeneic counter-silencing. Synthetic CgpS target promoters with and without additional TF operator sites were fused to reporter genes (e.g. *eyfp*). The insertion of TF operator sites within various CgpS target promoters resulted in an increased promoter activity upon binding of the effector-responsive TF acting as counter-silencer. Binding site composition as well as its position showed a significant impact on the dynamic range and maximal promoter output suggesting how bacteria can evolve for modulated expression of horizontally acquired genes. Binding of TFs and CgpS to synthetic promoter constructs was confirmed *in vivo* and *in vitro* by surface plasmon resonance and EMSAs.

Based on the strong inducibility and low background activity of counter-silencers, suitable constructs were implemented in a metabolic toggle, which was successfully applied to control L-valine production in *C. glutamicum*.

We propose that the presented synthetic counter-silencing approach will enhance our understanding of evolutionary network expansion in bacteria and that the mechanism of xenogeneic counter-silencing bears a great potential for the modular design of synthetic regulatory circuits.

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GRP199

The two nitrogenase systems in *Rhodobacter capsulatus* mutually control each other

L. Demtröder*[1], B. Masepohl[1]
[1]*Ruhr-Universität Bochum, Microbial Biology, Bochum, Germany*

Biological nitrogen fixation, the reduction of inert dinitrogen (N2) from air to bioavailable ammonia (NH3), is catalyzed by nitrogenases exclusively synthesized by diazotrophic bacteria and archaea. The photosynthetic alphaproteobacterium *Rhodobacter capsulatus* possesses two nitrogenases, a molybdenum (Mo)-nitrogenase (*nifHDK*-encoded) and a Mo-free Fe-nitrogenase (*anfHDGK*-encoded). Since N2 fixation is highly energy-demanding, expression of N2 fixation genes is strictly repressed by ammonium. Under ammonium-limiting conditions, NtrC activates transcription of the *nifA* and *anfA* genes, which code for the transcriptional activators of *nifHDK* and *anfHDGK* genes, respectively. In contrast to *nifA*, transcription of *anfA* is repressed by molybdate, thus limiting synthesis of Fe-nitrogenase, which is less active than Mo-nitrogenase, to Mo-limiting conditions.

Our recent studies revealed that disruption of *anfA* abolished *anfHDGK* transcription, well in line with earlier studies. Surprisingly, *anfHDGK* expression was also greatly reduced in a Δ*nifA* mutant indicating that NifA is required for maximal expression of Fe-nitrogenase genes. Correspondingly, Fe-nitrogenase-dependent growth is abolished in the Δ*nifA* strain indicating that NifA is essential for Fe-nitrogenase activity. On the other hand, *nifHDK* transcription was enhanced in a Δ*anfA* strain suggesting that AnfA represses Mo-nitrogenase production. In line with our expression studies, both NifA and AnfA were shown to bind the *nifH* and the *anfH* promoters *in vitro*. These and other findings suggest that the two nitrogenase systems mutually control each other by direct binding of NifA and AnfA to distinct *cis*-regulatory regions.

GRP200

The role of the C_4-Dicarboxylate-transporter DcuA and the aspartate ammonia-lyase AspA in nitrogen assimilation by *Escherichia coli*

C. Schubert*[1], A. Strecker[1], G. Unden[1]
[1]*Johannes Gutenberg-University, Institute of Molecular Physiology, Mainz, Germany*

DcuA of *Escherichia coli* is known as an alternative C_4-dicarboxylate transporter, while AspA catalyzes the reversible conversion of L-aspartate to fumarate and ammonia. *dcuA* is expressed constitutively under aerobic and anaerobic conditions and DcuA was suggested to serve as a backup transporter, or for the anabolic uptake of C_4-dicarboxylates [1]. In this work, it is shown that DcuA is required for aerobic growth with L-aspartate as a nitrogen source. When L-aspartate was supplied as the sole nitrogen source together with glycerol as the carbon source, L-aspartate was taken up by the bacteria and fumarate was excreted in equimolar amounts. L-Aspartate was taken up in amounts required for nitrogen but not for carbon metabolism. Therefore, DcuA catalyzes an L-aspartate/C_4-dicarboxylate antiport serving as a nitrogen shuttle for nitrogen supply without net carbon supply [2]. Data suggest a more complex transcriptional and post-translational regulation of AspA, to meet the role of DcuA and AspA in N-assimilation.

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GRP201

Two novel XRE-transcriptional regulators play a major role in regulation of phenotypic heterogeneity in the insect pathogen *Photorhabdus luminescens*

S. Eckstein*[1], M. Seidel[1], R. Heermann[1,2]
[1]*Ludwig-Maximilians-Universität München, Biozentrum, Bereich Microbiologie, Martinsried, München, Germany*
[2]*Johannes-Gutenberg-Universität Mainz, Institut für Molekulare Physiologie, Mikrobiologie, Mainz, Germany*

Photorhabdus luminescens is a Gram-negative bacterium that lives in symbiosis with soil nematodes and is simultaneously highly pathogenic towards insects. The bacteria exist in two phenotypically different forms, designated as primary (1°) and secondary (2°) cells that differ in various phenotypical and morphological traits. Yet unknown environmental stimuli as well as global stress conditions induce phenotypic switching of up to 50% of 1° to 2° cells. RNA-Seq analysis demonstrated that *plu4294* was highly up-regulated in 2° cells, whereas *plu4299* was down-regulated in 2° cells. Both genes belong to the XRE superfamily, which represents the 2nd most frequent transcriptional regulator family in bacteria. Inserting additional copies of *plu4294* or deleting *plu4299* in 1° cells, respectively, induced the 2° phenotype. In contrast, deletion of *plu4294* or insertion of extra copies of *plu4299* in 2° cells induced the 1° phenotype. MST and SPR binding kinetics revealed binding of Plu4294 to its own promoter $P_{plu4294}$ and to the promoter of the bacterial luciferase operon (P_{luxC}).

Furthermore, binding of Plu4294 to $P_{plu4295/4296}$ was observed. Plu4295 and Plu4296 are homologs to the CcdA/CcdB toxin-antitoxin system (TAS) and are also up-regulated in 2° cells. Since TAS are known to be involved in the process of persister cell formation in other bacteria, it is likely that the TAS Plu4295/Plu4296 is also involved in phenotypic switching of *P. luminescens*. The environmental signals as well as the molecular mechanism how the two XRE-like transcriptional regulators Plu4294 and Plu4299 are involved in the process of phenotypic switching remains to be elucidated.

GRP202

GabR activates the γ-aminobutyrate (GABA) catabolic pathway in *Corynebacterium glutamicum*

L. Zhu*[1], M. Baumgart[1], M. Bott[1]
[1]*IBG-1: Biotechnology, Institute of Bio- and Geosciences, Forschungszentrum Jülich, Jülich, Germany*

γ-Aminobutyric acid (GABA) is a non-proteinogenic amino acid. In this study, we demonstrated that GABA can serve as sole carbon and nitrogen source for growth of the Gram-positive cell factory *Corynebacterium glutamicum*. Remarkably, the presence of ammonia partially inhibited growth on GABA and evidence is provided that this effect is due to a negative effect of ammonium on GABA catabolic enzymes, which are encoded in the *gabTDP* operon for GABA aminotransferase, succinate semialdehyde dehydrogenase, and a GABA transporter. DNA microarray analysis revealed that the transcription of *gabTDP* was strongly increased in *C. glutamicum* wild type grown with GABA as sole carbon and nitrogen source compared to the same strain cultivated with glucose as carbon source and ammonia plus urea as nitrogen source.

The gene *gabR* is located divergently to *gabTDP* and was annotated as a potential transcriptional regulator of this operon. We could show that a *gabR* deletion mutant failed to grow on GABA, but showed no growth defect on glucose. Growth of the Δ*gabR* mutant on GABA was restored by plasmid-based expression of *gabR*. A reporter plasmid was constructed in which the promoter of *gabTDP* controlled expression of the *eyfp* gene for a fluorescent protein. *C. glutamicum* wild-type cells carrying this plasmid showed a strong fluorescence during growth with GABA, but not during growth with glucose. Glucose in the presence of GABA had a negative effect on the expression of the reporter gene, suggesting glucose repression. In the Δ*gabR* mutant transformed with the reporter plasmid, GABA (in the presence of glucose) did not trigger expression of the reporter gene. These data indicate that GabR activates *gabTDP* transcription in the presence of GABA.

GRP203

Regulation of the the latex clearing proteins in *Gordonia polyisoprenivorans* VH2 by $LcpR_{VH2}$

S. Oetermann*[1], R. Jongsma[1], A. Steinbüchel[1,2]
[1]*Westfälische Wilhelms-Universität Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*
[2]*King Abdulaziz University, Department of Environmental Sciences, Jeddah, Saudi Arabia*

Introduction: Bacterial degradation of poly(*cis*-1,4-isoprene), the main component of rubber, was studied in the last years. The actinomycete *Gordonia polyisoprenivorans* VH2 harbors two genes coding for latex clearing proteins (Lcps), which cleave poly(*cis*-1,4-isoprene) into oligoisoprenes. Transcriptional studies detected an increased *lcp* expression when cells were grown in presence of poly(*cis*-1,4-isoprene). Nevertheless, nothing is known about the regulation of *lcp* expression. A gene coding for a putative regulator of the TetR-family (*lcpR*$_{VH2}$) was detected 131 bp upstream of *lcp1*$_{VH2}$.

Objective: It was our aim to purify and characterize the putative regulator $LcpR_{VH2}$ and to evaluate its importance for expression of *lcp1*$_{VH2}$ and *lcp2*$_{VH2}$.

Materials & methods: $LcpR_{VH2}$ was purified as a His_6-tagged protein from a recombinant *E. coli* strain. Its oligomeric state was analyzed by size exclusion chromatography, its DNA-binding activity evaluated by electrophoretic mobility shift assays (EMSAs) and the exact binding sequence identified by DNase I footprinting assay.

Results & Conclusion: $LcpR_{VH2}$ forms a dimer in its native state with a size of 52.7 kDa. The shift assays with the purified protein revealed a binding to the intergenic region between *lcpR*$_{VH2}$ and *lcp1*$_{VH2}$. Within this region, an inverted repeat was identified *in silico*, likely being the binding site of $LcpR_{VH2}$. This binding sequence was confirmed with a DNase I footprinting assay. Interestingly, no regulator was detected upstream of the second *lcp* (*lcp2*$_{VH2}$). Therefore, we performed EMSA studies with $LcpR_{VH2}$ and the putative operator region upstream of *lcp2*$_{VH2}$, and we identified another binding site upstream of *lcp2*$_{VH2}$. Hence, we concluded that $LcpR_{VH2}$ is a regulator of both *lcps* in *G. polyisoprenivorans* VH2.

GRP204

Analysis of iCLIP data to identify targets of ribonucleases in Synechocystis sp. PCC 6803

U. Hoffmann*[1], K. Voigt[1], O. Siadat[1], J. Behler[2], A. Wilde[1]
[1]*Institute for Biology III, Molecular Genetics, Freiburg i. Br., Germany*
[2]*Institute for Biology III, Genetics and Experimental Bioinformatics, Freiburg i. Br., Germany*

Specific degradation of RNA molecules helps bacteria to adapt quickly to changing environmental conditions. The endoribonuclease RNase E plays a central role in RNA degradation and maturation in *E. coli* [1]. It might also have a similar function in the cyanobacterial model organism *Synechocystis* sp. PCC 6803 (*Synechocystis*). Individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) is used to investigate the target specificity of RNA binding proteins (RBPs), such as RNase E. Furthermore, iCLIP enables the localisation of RBP binding sites with a single nucleotide resolution.

iCLIP data sets for RNase E and RNase III were analysed to elucidate their binding behaviours. The similarity of the iCLIP sites to *in vitro* verified targets of RNase E after comparison proved the adequacy of the method. New potential targets of RNase E and RNase III were identified among which, genes involved in photosynthesis were functionally enriched. These findings suggest a potential role of both RNases in the regulation of photosynthesis. This is in concordance with prior analysis for RNase E targets [2]. RNase III was observed to have a preference for binding to 3' and 5' untranslated regions and intergenic regions.

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GRP205

The phosphotransferase protein EIIA^{Ntr} regulates AcnB aconitase activity in a phosphorylation-independent manner in *Escherichia coli*

F. Sikora*[1], M. Mörk-Mörkenstein[1], B. Görke[1]
[1]*Max F. Perutz Laboratories, Vienna, Austria*

Bacteria constantly adapt their metabolism to the fluctuating availability of carbon and nitrogen sources. A central system for carbohydrate utilization is the canonical PEP-dependent phosphotransferase system (PTS). In parallel, many *Proteobacteria* possess a nitrogen related PTS, which exclusively exerts regulatory functions and consists of the three proteins EI^{Ntr}, NPr and EIIA^{Ntr} (encoded by *ptsN*). EIIA^{Ntr} regulates various cellular processes depending on its phosphorylation state **[1]**. However, not all phenotypes of *E. coli* lacking *ptsN* can be explained by the known targets of EIIA^{Ntr}. For instance, flux through the tricarboxylic acid (TCA) cycle was reduced in a Δ*ptsN* but not in Δ*ptsP* or Δ*ptsO* strains, in which EIIA^{Ntr} is mainly non-phosphorylated **[2]**. These findings suggest that flux through the TCA cycle is regulated by EIIA^{Ntr} levels rather than its phosphorylation state.

In this study, the aconitase AcnB was identified as a novel interaction partner of EIIA^{Ntr}. This interaction is independent of the EIIA^{Ntr} phosphorylation state. Furthermore, higher aconitase activity was measured in a Δ*ptsN*, but not in a Δ*ptsP* strain, suggesting regulation of AcnB depending on EIIA^{Ntr} levels, but independent of its phosphorylation state. We show that EIIA^{Ntr} amounts are regulated in an Hfq-dependent manner. In addition, we carry out a transposon mutagenesis screen to find other potential factors involved in regulating *ptsN* expression. These findings will allow to draw further conclusions about the conditions, under which EIIA^{Ntr} regulates AcnB and thereby flux through the TCA cycle.

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GRP206

The GntR family transcriptional regulator PMM1637 regulates the highly conserved cyanobacterial sRNA Yfr2 in marine picocyanobacteria

C. Steglich*[1], J. Lambrecht[1], M. Wahlig[1]
[1]*University of Freiburg, Genetics, Freiburg i. Br., Germany*

Prochlorococcus is a marine picocyanobacterium with a streamlined genome that is adapted to different ecological niches in the oligotrophic oceans. There are currently more than 20 regulatory small RNAs (sRNAs) that have been identified in the model strain *Prochlorococcus* MED4. While most of these sRNAs are ecotype-specific, sRNA homologs of Yfr1 and of the Yfr2 family are widely found throughout the cyanobacterial phylum. Although they were identified 13 years ago, the functions of Yfr1 and Yfr2 have remained unknown. We observed a strong induction of two Yfr2 sRNA homologs of *Prochlorococcus* MED4 during high light stress and nitrogen starvation. Several *Prochlorococcus* and marine *Synechococcus yfr2* promoter regions contain a conserved motif we named CGRE1 (cyanobacterial GntR family transcriptional regulator responsive element 1). Using the conserved promoter region as bait in a DNA affinity pull-down assay we identified the GntR family transcriptional regulator PMM1637 as a binding partner. Similar to Yfr2, homologs of PMM1637 are universally and exclusively found in cyanobacteria. We suggest that PMM1637 governs the induction of gene expression of Yfr2 homologs containing CGRE1 in their promoters under nitrogen-depleted and high-light stress conditions.

GRP207

Complex activation of quorum sensing target genes in a *B. subtilis* isolate

B. Steinfeld*[1], S. Trauth[1], I. Bischofs-Pfeifer[1]
[1]*MPI for Terrestrial Microbiology, Marburg, Germany*

Quorum sensing (QS) by diffusible signalling molecules is commonly used to coordinate the behaviour of bacterial populations to orchestrate gene expression in response to changes in cell density. It is often employed to globally synchronize changes in gene expression across the population. However, it may also function in the phenotypic diversification of isogenic populations. In principle, both regulatory modes may also coexist since a QS system typically regulates several target genes. Here we use microscopic investigations with fluorescent promoter fusions to study QS gene expression in a natural isolate of *B. subtilis*. We find that transcription of two QS-regulated targets, pectate lyase and the surfactin operon, is limited to a morphologically defined subpopulation of cells. Interestingly, under the same conditions another target gene, *rapA*, is uniformly transcribed across the entire population. Both uniform and bimodal gene expression can also be obtained with synthetic promoters by varying the promoter architecture. We therefore suggest that complex QS gene expression patterns could emerge within a population by utilizing different promoter designs.

GRP208

The analysis of two antisense located within genes *gumB* and *gumD* RNAs of *Xanthomonas campestris* pv. campestris B100

A. F. Lopez Hernandez *[1,2], V. Ortseifen[1,2], K. Niehaus[1], A. Pühler[2]
[1]*Bielefeld University, Proteome and Metabolome Research, Bielefeld, Germany*
[2]*CeBitec/Bielefeld University, Genome Research of industrial microorganisms, Bielefeld, Germany*

The gram-negative plant pathogen *Xanthomonas campestris* pv. campestris (*Xc*c) B100 is the producer of the polysaccharide Xanthan that has multiple industrial applications. Based on its stable thickening properties and pH and temperature resilience it gets used as bonding agent for example in food, cosmetics and oil industries [1,2]. *Xc*c B100 can be analyzed on the genomic level. Recent transcriptomic studies revealed several intragenic antisense RNAs (asRNA) within the *gum* gene cluster that is responsible for the xanthan production [3,4]. The aim of the study is to get a deeper understanding of the possible regulation mechanism mediated by the asRNAs.

To investigate the function of the asRNAs within *gumB* and *gumD,* the promotors of these two asRNAs have been mutated to abolish or reduce antisense transcription. The exchange of nucleotides within the antisense promotors has been performed without destroying the function of the respective genes. The mutants AS1P and AS2P were characterized through growth studies, qRT- and xanthan quality and quantity analysis. The growth behaviour in the mutants showed no significant change in comparison to the wildtype.

Currently, it is planned to test three common asRNAs mechanism models - 1) the change in RNA stability of sense RNA, 2) the modulation of translation and 3) the transcriptional interference with sense RNA [5]. The qRT-PCR data of the promotor deficient mutant suggest the exclusion of the 3rd model (transcriptional interference). Whether the 1st model (modifying RNA stability), the 2nd model (interference on the translational level) or a more complex mechanism are responsible for a regulatory effect has to be tested.

GRP209
sRNA scr5239 – an important regulator of the C-metabolism in *Streptomycetes*
F. Engel*[1], M. Vockenhuber[1], B. Süß[1]
[1]*TU Darmstadt, Synthetic Genetic Circuits, Darmstadt, Germany*

We are interested in the identification and characterization of small non-coding RNAs (sRNAs) in *Streptomyces coelicolor*. Using RNAseq we identified (amongst several 100s others) a 159 nt transcript called scr5239. It has a high degree of sequence and structure conservation and is constitutively expressed under most conditions.

To identify targets of this sRNA we performed an iTRAQ analysis comparing the wild type to sRNA overexpression and deletion strains. 32 of ~1800 identified proteins showed a significant sRNA dependent change in protein expression. Among them were transcriptional regulators, enzymes of central metabolism but also hypothetical proteins of unknown function. Analysis of the mRNA level of all 32 proteins showed that only six of them show the same behaviour of mRNA and protein implying cleavage and digestion of the mRNA upon sRNA binding or sRNA dependent changes of transcriptional regulation of these genes.

Four of the identified proteins are associated with the central carbon metabolism: The acetyl-CoA acetyltransferase ThiL, the malate-oxireductase MOR, the phosphoenolpyruvate-carboxykinase PEPC (TCA draining reactions), and the glucosamine-6-phosphate deaminase NagB (PTS, glycolysis). Overexpression or deletion of scr5239 leads to repression or induction of protein expression respectively. We used the native promotors and a synthetic promotor to show posttranscriptional regulation. After the investigation of the interaction between the sRNA and the ThiL mRNA we are currently characterizing the regulation of MOR and PEPC by the sRNA in detail using bioinformatic methods and gel shift assays in combination with mutational analyses.

This will help us understand how scr5239 affects the expression of these enzymes of the central carbon metabolism.

GRP210
Proteome analysis of *Streptococcus suis* under stress conditions and in host-pathogen interaction
D. Dittmar*[1], C. Weiße[2], P. Hildebrandt[1], U. Mäder[1], M. Gesell Salazar[1], S. Michalik[1], C. Baums[2], U. Völker[1]
[1]*University Medicine Greifswald, Interfaculty Institute for Genetics and Functional Genomics, Department of Functional Genomics, Greifswald, Germany*
[2]*University of Leipzig, Institute of Bacteriology and Mycology, College of Veterinary Medicine, Leipzig, Germany*

Introduction: *Streptococcus suis* is a commensal of pigs, but it can also cause invasive infections. As an emerging zoonotic agent *S. suis* is able to induce meningitis, arthritis and septicemia in humans. As a pathogen, it is responsible for high economic losses in swine farming worldwide. The species has been divided into 35 serotypes. Serotype 2 is the most prevalent serotype worldwide [1], followed by serotypes 9, 7 and 3 in Europe [2]. A better understanding of the mechanisms involved in the specific adaptation of *S. suis* as a commensal and as an invasive pathogen will improve our knowledge on potential virulence factors and enable design of new vaccines.

Objectives: Using comparison of *ex vivo* (blood plasma and cerebrospinal fluid (CSF) of healthy swine) and *in vitro* (nutrient-rich medium) conditions to explore the adaptation to host niches, the study is focused on the identification of potential virulence and fitness factors, which may constitute potential components of new multi-component vaccines.

Materials & methods: We analyzed the proteome of *S. suis* (serotype 2, 9, 7) from different growth phases using Q Exactive™ Plus and data-independent acquisition (DIA) mass spectrometry workflow.

Results: For DIA data analysis an in-house spectral library was generated. Proteome analysis revealed several differentially abundant proteins between CSF or blood plasma, respectively, and nutrient-rich medium, thereby identifying potential new virulence and fitness factors as well as cellular pathways involved in adaptation to the host environment.

Conclusion: The identified proteins provide a promising basis to evaluate immunogenic and conserved antigens for multicomponent vaccines.

[1] Rungelrath V *et al*., Virulence 2018

[2] Fittipaldi N *et al*., Future Microbiol. 2012

ARP211
Investigations of the *gvpACNO*-gene cluster
A. Jost*[1], F. Pfeifer[1]
[1]*Technische Universität Darmstadt, Microbiology & Archaea, Darmstadt, Germany*

The major structural proteins GvpA and GvpC are encoded in the *gvpACNO* cluster. GvpA is essential for the gas vesicle (GV) assembly and constitutes almost entirely the GV wall, GvpC stabilizes this wall on the outer surface as scaffolding protein. Little is known about the other two proteins encoded by this gene cluster, GvpN and GvpO. It is proposed that GvpN plays an important role in GV assembly since a deletion of *gvpN* results in mini gas vesicles. GvpO is essential for GV formation, but its function is unknown[1].

Question: The importance of the amino acids (aa) in the calculated ATP-binding motif was investigated by point mutations and their influence on GV formation.

Methods: Mutations were introduced in *gvpN* by mutagenesis PCR and the resulting plasmids were used to transform *Hfx. volcanii* WR340 carrying the ΔN construct. ΔN contains all 13 *gvp* genes except for *gvpN*. Transformants were inspected by electron microscopy for the presence of GVs.

Results: Any alteration of essential aa of the Walker A motif (GPTGCGKT) and in the ATP-binding motif resulted in mini GVs with a length smaller than 100 nm. These results were

similar to the results of the ΔN transformant and imply that the ATP-binding motif of GvpN is important for GV growth. In contrast, mutations of other aa outside of this motif had no effect. Such $\Delta N+N_{mut}$ transformants contained spheric GVs of 300-600 nm in length, just as the control transformant $\Delta N+N_{wt}$.

Conclusion: The ATP-binding motif of GvpN was shown to be important for the function of GvpN in the extension of GVs. GvpN might bind and hydrolase ATP, yielding energy for the GV assembly. The mini GVs are not enlarged to the normal size, *i.e.* due to the lack of GvpA (and GvpC) incorporation in the GV wall.

[1] Offner S, Hofacker A, Wanner G, Pfeifer F (2000). J. Bacteriol. 182, 4328-4336

ARP213

SaUSP1, an ATP-binding universal stress protein, enhances the adaptation of *Sulfolobus acidocaldarius* cells to nutrient starvation and high salinity stress

X. Ye*[1], C. van der Does[1], S. V. Albers[1]
[1]*University of Freiburg, Insititute of Biology, Freiburg i. Br., Germany*

In *Sulfolobus acidocaldarius*, the protein phosphatase Saci-PP2A plays important regulatory roles in many cellular processes, including cell growth, cell shape and archaellum synthesis. A conserved prokaryote protein, designated as SaUSP1, was identified as an interaction partner of Saci-PP2A, which was confirmed in *in vitro* protein interaction assays. SaUSP1 belongs to the universal stress protein (Usp) superfamily that widely exists in bacteria, archaea, plants and invertebrates. Biochemical analysis showed that SaUSP1 is an ATP-binding protein, which forms a homo-dimer in solution. The substitution of the conserved residue glycine 97 with an alanine significantly reduced the ATP binding capacity. Interestingly, the binding of ATP enhanced SaUSP1's interaction with Saci-PP2A. In contrast to bacterial *Usp* genes, environmental stress conditions including stationary phase, starvation stress, high salinity stress and UV stress did not stimulate the transcripts of *SaUSP1*. Deletion of *SaUSP1* lead to premature production of the archaellin FlaB in *S. acidocaldarius* although motility was not affected. Δ*SaUSP1* showed a significant growth defect in high salinity stress. Compared with the wild type strain, its growth or survival was not affected under UV stress and heavy metal stress. Taken together, we identified an Usp homolog SaUSP1 from *S. acidocaldarius*, and investigated its regulatory roles in archaellin expression and high salinity stress response.

ARP214

Regulation of the *mtsDFH*-genes from *Methanosarcina acetivorans* as a paradigm of archaeal signal transduction

L. Blasius*[1], N. Frankenberg-Dinkel[1]
[1]*Technische Universität Kaiserslautern, Microbiologie, Kaiserslautern, Germany*

Methanosarcina acetivorans is an obligate anaerobic archaeon, which is capable to use a wide range of substrates for methanogenesis. In order to metabolise methyl sulfides, the three corrinoid/methyltransferase fusion proteins MtsD, MtsF and MtsH are important. These are transcriptionally and post-transcriptionally regulated by the availability of dimethyl sulfide. The regulators MsrC, MsrF and MsrG are involved in this transcriptional regulation. Genetic studies indicate the regulators" function as activators [1]. Beside the mentioned regulators, the sensor kinase MsmS is involved in the regulation of the methyl transferase MtsF. MsmS is a multi-domain protein which shows autophosphorylation activity in dependence of the protein`s redox state. The genome of *M. acetivorans* codes for two additional sensor kinases, which are all encoded in a genomic region close to the Msr regulators and the Mts methyl transferases. Therefore, it has been suggested that the sensor kinases and the regulators are part of a two-component regulatory system.

In order to investigate whether MsmS and MsrG belong to one signal transduction pathway, protein interaction studies were performed. First results suggest that MsmS and MsrG interact with each other. Performed transphosphorylation assays indicate no phosphotransfer from MsmS to MsrG. However, a strong autophosphorylation signal of MsrG was observed. Using electrophoretic mobility shift assays we were able to show that MsrG specifically binds to two potential sites in the promoter region of *mts*H. Apart from the identification of a specific MsrG binding site, future studies aim at exploring the signal transduction within this system to get a better understanding of archaeal signal transduction.

[1] **Bose A. et al. (2009)** Microbiol. 1.

ARP215

Heavy metal stress on *Halobacterium salinarum*: Effects on EPS composition and the proteome of biofilm cells

S. Völkel*[1], F. Pfeifer[1]
[1]*TU Darmstadt, Mikrobiologie und Archaea, Darmstadt, Germany*

Introduction & aim: *Hbt. salinarum* R1 is an extreme halophilic archaeon forming characteristic tower-like biofilm structures. The addition of metal ions to mature biofilms results in significant alterations of the biofilm architecture when treated with copper or nickel. A quantitative proteome analysis comparing metal- and non-treated biofilms yielded significant metal-specific differences. This study focuses on the quantitative analysis of the extracellular polymeric substances (EPS) in metal-treated biofilms and the generation and characterization of gene deletion mutants based on the proteomic data.

Methods: Mature biofilms were exposed to copper and nickel ions for 24 h, before EPS was isolated and the amount of proteins, carbohydrates and uronic acids was quantified by colorimetric assays. Metal quantification was done by atomic absorption spectrometry. Gene deletion mutants were generated based on a double-homologous recombination and characterized by microscopy, adhesion- and metal-sensitivity assays.

Results: Quantitative analysis of the EPS composition of metal-treated biofilms revealed differences in protein content without affecting the amount of carbohydrates and uronic acids. Quantification of metals in isolated EPS showed that the majority of metals remain in EPS; 65% of the copper ions and 85% of the nickel ions, respectively. Deletions of single genes potentially involved in heavy metal metabolism resulted in an altered biofilm phenotype after heavy metal treatment compared to the wild type.

Conclusion: Metal treatment leads to specific effects on the biofilm architecture and its EPS composition, indicating an important role of the EPS in metal resistance. Gene deletions suggest that a number of gene products participate in heavy metal resistance.

ARP216

Translation initiation factor network in *Haloferax volcanii*

F. Schramm*[1], U. Linne[2], J. Soppa[1]
[1]*Goethe University Frankfurt, Institut für Molekulare Biowissenschaften, Frankfurt a. M., Germany*
[2]*Philipps-University Marburg, Marburg, Germany*

In all three domains of life initiation is the rate-limiting step of translation and thus initiation determines the rate of protein synthesis. The efficiency of translation initiation and its differential regulation depends on translation initiation factors (IFs). Bacteria contain only three IFs, whereas the number is significantly higher for Archaea (aIFs) and Eukaryotes (eIFs), e.g. *H. volcanii* contains 14 genes that encode (subunits of) aIFs. A deletion analysis revealed that five aIFs are essential, while nine deletion mutants were viable.

To get insight into the interaction network of aIFs, his-tagged versions were overexpressed in the respective deletion mutant, if possible, or in the wildtype, for essential aIFs. Co-affinity purification was used to isolate the aIFs and their binding partners, which were then identified by peptide mass fingerprinting (PMF).

Initial work concentrated on the heterotrimeric aIF2, which binds the initiator tRNA and is thus of central importance for preinitiation complex formation. All three subunits of aIF2, as bait, led to the co-isolation of the other two subunits. Additional interaction partners included further aIFs, translation elongation factors, and ribosomal proteins. Unexpectedly, also RNA-polymerase subunits were co-isolated, indicating a tight coupling between transcription and translation in haloarchaea. This strategy was extended to further aIFs, and currently co-affinity isolation has been performed for a total of 10 (subunits of) aIFs. The results enabled the reconstruction of a very extended protein-protein interaction network involved in translation initiation in haloarchaea.

ARP217

Influence of *Methanosarcina spherical virus* (MetSV) on hosts transcription

F. O. Petersen*[1], K. Weidenbach[1], R. A. Schmitz[1]
[1]*Institut für Allg. Mikrobiologie, Kiel, Germany*

Introduction: Recently a novel archaeal lytic virus targeting species of the genus *Methanosarcina*was isolated. Because of its spherical morphology and its limited host range of exclusively *Methanosarcina* species, the virus was called *Methanosarcina spherical virus*(MetSV). We showed that the virus contained a double stranded linear DNA genome of 10.5 kbp and predicted 22 open reading frames (ORFs), which are all in the same direction of transcription. Although potential functions for some of these ORFs like DNA polymerase or structural proteins were predicted using bioinformatics tools, functions of the majority including all predicted small ORFs are still unknown.

Objectives: Our studies aim was to characterize the virus-host relationship regarding its potential as a model system for methanoarchaea.

Material & Methods: For functional analysis *M. mazei* cultures were infected. On transcriptional level, the virus host interaction was analyzed by microarrays, qRT-PCR and RNA sequencing. RNA was isolated from infected and uninfected cultures after defined periods of time after infection (30 and 180 min).

Results: During infection remarkable changes in transcription were observed e.g. *replication factor A1*was upregulated more than 300 fold. Further significant reduction of transcription levels in transport was shown. qRT-PCRs for *cas*-genes showed an increase in regulation of some genes (e.g. Cas4), whereas changes in regulation of potential key regulators (e.g. Csa3) were not detectable.

Conclusion: The virus-hostsystem will help to light up unknown interaction mechanisms and it will help to develop molecular tools.

ARP218

Characterization of the transcriptome of *Haloferax volcanii* with mixed RNA-Seq

A. Borst*[1], S. Laass[1], K. U. Förstner[2], J. Kliemt[1], M. Hammelmann[1], F. Pfeiffer[3], J. Soppa[1]
[1]*Goethe-Universität Frankfurt, Institut für molekulare Biowissenschaften, Frankfurt a. M., Germany*
[2]*Universität Würzburg, IMIB, Würzburg, Germany*
[3]*Max-Planck-Institut, Computatioal Biology Group, Martinsried, Germany*

Recently, a differential RNA-Seq (dRNA-Seq) study was used to analyze the primary transcriptome of *Haloferax volcanii*, a well-established model organism for haloarchaea. As this approach solely yields information about the 5'-ends of unprocessed RNAs and does not contain any length information, a RNA-Seq study was initiated to further characterize the transcriptome. Therefore, RNA was isolated from *H. volcanii* grown under four different culture-conditions, mixed, and RNA-Seq (mixed RNA-Seq) was performed to screen for transcripts that were present under at least one of these four conditions.

This resulted in 50 million paired end reads of 150nt length and detected 1923 protein-coding RNAs (cdRNAs), 1229 cis-antisense RNAs (asRNAs), 214 internal-sense RNAs (isRNAs), and 172 intergenic RNAs (igRNAs). 915 cdRNAs were not detected in the dRNA-Seq study and are therefore specific for one or more of the three additional conditions. It was shown that 2/3 of coding transcripts are monocistronic and 1/3 is polycistronic, while most non-coding RNAs are rather short with a median length of about 150nt. Moreover, Northern blot analyses revealed in-detail information about many characteristic features of the *H. volcanii* transcriptome. These include e.g. 1) the transcript patterns of gene clusters vary in complexity, 2) some genes are transcribed into RNA isoforms of different length that can also be differentially regulated, 3) transcripts with very long 5'-UTRs/3'-UTRs exist, 4) about 30% of all cdRNAs have overlapping 3'-ends, and 5) most, if not all transcripts have differential levels under the four analyzed conditions. Based on these results, a multitude of future investigations can be performed which aim to elucidate RNA-mediated gene-regulation in haloarchaea.

ARP219

Interaction of Haloarchaeal Gas Vesicle Proteins Determined by Split-GFP

K. Völkner*[1], J. Born[1], F. Pfeifer[1]
[1]*Technische Universität Darmstadt, Microbiology and Archaea, Darmstadt, Germany*

Introduction: Split-GFP is a common method to investigate protein-protein interactions (PPI) in bacteria and yeast, but not in Haloarchaea. The method is based on the principle that the green fluorescent protein (GFP) is split into 2

fragments which are fused to the proteins to be investigated. In case of an interaction, the NGFP and CGFP can assemble a functional GFP. We adapted the split-GFP method to high salt to analyze PPI of gas vesicle (GV) proteins in *Haloferax volcanii* to investigate the formation of a putative nucleation complex in early stages of GV formation[1].

Objectives: We aimed to detect interaction partners of GvpM and to confine their interaction sites.

Methods: The salt-adapted mGFP2 was split between residues 157 and 158 and the resulting NGFP and CGFP were fused N- or C-terminally to the proteins tested. The respective *Hfx. volcanii* transformants were examined for fluorescence.

Results: We verified the interaction of GvpM with GvpH, GvpJ and GvpL *in vivo*. In addition, GvpF was identified as a novel interaction partner of GvpM. Fragmentation of GvpM into the peptides M(25N) (first 25 aa) and M(25C) (last 25 aa) showed that GvpL interacted predominantly with the N-terminus of GvpM, whereas GvpH, GvpJ and GvpF interacted with the C-terminus of GvpM. The results were supported by deletion variants of GvpM.

Conclusion: The use of split-GFP is a powerful method for the detection of *in vivo* PPI under high salt conditions. Now we are able to unravel interactions on the formation of a putative nucleation complex at the beginning of GV formation. For that, the remaining GvpG, I and K are examined.

[1] Winter K., Born J. and Pfeifer F. (2018). Front. Microbiol. 9:1897. doi: 10.3389/fmicb.2018.01897

ARP220

Establishing Genetic Tools for the Thermophilic Methanogen *Methanothermobacter thermautotrophicus* ΔH

C. Fink*[1], L. T. Angenent[1], B. Molitor[1]
[1]*University of Tuebingen, Center for applied Geosciences, Environmental Biotechnology, Tübingen, Germany*

The thermophilic methanogen *Methanothermobacter thermautotrophicus* is already used in a commercially available biomethanation process as an energy storage and carbon recycling system. This microbe is suitable for such a process because of the possibility of intermittent gas feeding without compromising the biocatalyst and high volumetric methane production rates. For the optimization of the biomethanation process different strategies can be used. First, the reactor design, process parameters, and media compositions can be optimized. Second, the microbe itself can be optimized through laboratory evolution or genetic modification. So far, no techniques for the genetic modification of *M. thermautotrophicus* were published in the literature. Few reports on approaches with low efficiency are available for the closely related species *Methanothermobacter marburgensis*, such as transduction using the specific phage ΨM2, and transformation by genomic DNA of a 5-Fluoruracil-resistant strain using natural competence. Together with modern seamless cloning techniques and the existing genetic systems for mesophilic methanogens, we will establish genetic tools for *M. thermautotrophicus*.

To accomplish this, we first developed self-integrating DNA constructs that carry a puromycin resistance cassette. Additionally, we are constructing shuttle vectors for *E. coli* and *M. thermautotrophicus* in a modular fashion that will make it easy to test various origins of replications and resistant markers. The self-integrating constructs and the shuttle vectors enable us to test DNA-transfer systems to generate gene deletion mutants and express foreign genes in *M. thermautotrophicus*. Afterwards, the genetic toolbox can be expanded to include regulated promoters, marker genes, and CRISPR-based methods.

ARP221

Single-domain zinc finger µ-proteins in *Haloferax volcanii*

S. Zahn*[1], A. Machulla[1], C. Nagel[1], J. Soppa[1]
[1]*Goethe Universität Frankfurt a. M., Institut für Molekulare Biowissenschaften, Frankfurt a. M., Germany*

Very small proteins (µ-proteins) have hardly been studied for a long time, but recently became an emerging field of research. The archaeon *Haloferax volcanii* is annotated to encode 282 µ-proteins with a length of less than 70 amino acids, only 24 of which have an assigned function. 43 of these µ-proteins contain at least two CPXCG motifs and are, therefore, most probably single-domain zinc finger µ-proteins.

Until now 18 of the respective genes have been deleted, and 11 deletion mutants differed from the wildtype under at least one condition, e.g. growth capabilities with different carbon/phosphate/nitrogen sources, resistance against UV-/pH-/oxidative or osmotic stress, biofilm formation, or motility/chemotaxis. Four deletion mutants showed the same pleiotropic phenotype, i.e. swarming motility was considerably decreased, while biofilm formation, resistance to UV irradiation as well as resistance to osmotic stress were increased.

HVO_2753 encodes the only µ-protein containing four CPXCG motifs and thus two zinc fingers. Its deletion led to a loss of swarming and decrease in biofilm formation. Swarming could be restored by the expression of the native *HVO_2753* gene. In contrast, single amino acid mutants in each CPXCG motif did not enhance swarming, proving that all four motifs are essential. A fluorescence assay experimentally confirmed that HVO_2753 binds zinc. *In vivo* cross-linking revealed that HVO_2753 forms a complex with other proteins, and the identification of binding partners is currently under way. Taken together, one domain zinc finger µ-proteins fulfill important functions in *H. volcanii*.

ARP222

Genome-scale Investigations of *Methanothermobacter thermautotrophicus*

I. Casini[1], C. Fink[1], D. Evseeva[2], A. Dräger[2], D. Huson[2], L. T. Angenent[1], B. Molitor*[1]
[1]*Universität Tübingen, Zentrum für Angewandte Geowissenschaften, Tübingen, Germany*
[2]*Universität Tübingen, Wilhelm Schickard Institute for Computer Science, Tübingen, Germany*

Renewable energy sources are well-established parts of the electricity mix for many countries. However, an imbalance between production and consumption makes storage of electric power necessary. Pure cultures of acclimated, but non-genetically modified *Methanothermobacter* species are utilized in Power-to-Gas processes for storage of renewable electric power. In this bioprocess, hydrogen (from electrolysis of water) and carbon dioxide (from biogas or industrial off

gases) are converted to methane with high process stability and at high conversion rates. The resulting biomethane can be introduced into the existing natural gas grid for storage and distribution.

The utilization of pure culture fermentation for Power-to-Gas processes is already progressed from a process engineering point of view. However, the detailed understanding of the underlying biological processes is necessary to harness the full potential of this promising biotechnology and to implement chemical production routes besides methane. Therefore, differences in metabolic versatility in various *Methanothermobacter* strains are being investigated by comparative genomics. Together with genome-scale metabolic modeling, this will provide a platform for the analysis of systems biological data sets. Genetic tools will enable to test hypotheses resulting from metabolic modeling, fermentation, and systems biology.

ARP223

Enzymes of the 3-hydroxypropionate/4-hydroxybutyrate cycle in *Nitrosopumilus maritimus*

L. Liu*[1,2], M. Könneke[3], D. Schubert[2], P. Brown[2], I. A. Berg[1,2]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*
[2]*University of Freiburg, Department of Microbiology, Freiburg i. Br., Germany*
[3]*University of Bremen, MARUM - Center for Marine Environmental Sciences& Department of Geosciences, Bremen, Germany*

Ammonia-oxidizing archaea of the philum *Thaumarchaeota* is an abundant microbial group that contributes significantly to the global N and C cycles. They are capable to grow at extremely low ammonia concentrations using the 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle for autotrophic CO_2 fixation [1], which was originally discovered in an autotrophic thermophilic crenarchaeon *Metallosphaera sedula* [2]. Most of the enzymes of the thaumarchaeal HP/HB cycle are not homologous to the corresponding *M. sedula* enzymes, suggesting a convergent evolution of the cycle in *Cren-* and *Thaumarchaeota.* Here we present comparative analysis of the enzymes involved in the conversions of 3-hydroxypropionyl-CoA into acryloyl-CoA and of crotonyl-CoA into acetoacetyl-CoA in the HP/HB cycle in a thaumarchaeon *Nitrosopumilus maritimus* and a crenarchaeon *M. sedula*. While a specific 3-hydroxyproponyl-CoA hydratase and a bifunctional crotonyl-CoA hydratase/3-hydroxybutyryl-CoA dehydrogenase catalyze these reactions in *M. sedula*, *N. maritimus* uses a promiscuous 3-hydroxypropionyl-CoA/3-hydroxybutyryl-CoA dehydratase and a monofunctional 3-hydroxybutyryl-CoA dehydrogenase. Phylogenetic analysis suggests independent evolution of these enzymes in *N. maritimus* and *M. sedula*, and biochemical analysis shows a high efficiency of both variants. Interestingly, our results indicate participation of several additional (iso)enzymes in the conversions in *M. sedula*, although the reactions are catalyzed by single enzymes in *N. maritimus*. It highlights genome streamlining as an adaptation of *N. maritimus* to constantly low energy supply.

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[2] I. A. Berg *et al.*, *Science* **318**, 1782-1786 (2007).

ARP224

An unusual type of glycerol-3-phosphate dehydrogenase is involved in glycerol degradation in Sulfolobus acidocaldarius

C. Schmerling*[1], X. Zhou[1], C. Bräsen[1], B. Siebers[1]
[1]*Universität Duisburg Essen, Biofilm Centre, Essen, Germany*

Sulfolobus acidocaldarius is an aerobic thermoacidophilic crenarchaeon known for its metabolic versatility, which enables the growth on various carbon sources such as different sugars and peptides. Recently, *S. acidocaldarius* was also shown to hydrolyze lipids [1]. However, so far the further degradation of glycerol as a major building block and cleavage product of lipids remains elusive in *S. acidocaldarius*. In this study, we showed that *S. acidocaldarius* is able to grow with glycerol as sole carbon and energy source. Transcriptomic analyses and crude extract measurements indicated that glycerol is first phosphorylated to glycerol 3-phosphate (G3P) by glycerol kinase (GK) and then oxidized by means of G3P dehydrogenase (G3PDH) to dihydroxyacetonphosphate (DHAP), which is channeled into the common lower shunt of the Embden-Meyerhof-Parnas (EMP) pathway.

The genes Saci_2032 and Saci_2033 encoding G3PDH and GK, respectively, were overexpressed and the recombinant proteins were purified and characterized. According to oligomeric structure, sequence identity, and kinetic parameters, Saci_2033 is highly similar to bacterial and haloarchaeal GKs. In contrast, G3PDH shows significant sequence and structural differences to known G3PDH homologs from bacteria and haloarchaea, lacking key subunits for membrane binding and electron transfer. The recombinant expressed protein was found to be a homodimer with a non-covalently bound flavin cofactor and with high specificity for G3P (K_m 34.2 µM and k_{cat} 43.6 s^{-1}). Thus, the pathway for glycerol degradation in *S. acidocaldarius* was unrevealed, involving an unusual type of G3PDH.

[1] Zweerink S *et al.* (2017) Nat Commun 8:15352

ARP225

Fatty acid degradation in *Sulfolobus acidocaldarius*

X. Zhou*[1], C. Schmerling[1], D. Sybers[2], K. Wang[3], E. Peeters[2], A. C. Lindås[3], C. Bräsen[1], B. Siebers[1]
[1]*FB Chemie - Biofilm Centre, Universität Duisburg-Essen, Molekulare Enzymtechnologie und Biochemie, Essen, Germany*
[2]*Vrije Universiteit Brussel, Bioengineering Sciences Department, Brüssel, Belgium*
[3]*Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, Sweden*

One of the unique properties of Archaea is that they lack fatty acids (FAs) as major building blocks of their lipid membranes, which are instead composed of isoprenoid side chains ether linked to *sn*-glycerol 1-phosphate. However, it has been shown that several archaeal representatives habour genes with high homology to bacterial β oxidation suggesting the ability to metabolize FAs [1]. Furthermore, the aerobic thermoacidophilic Crenarchaeon *S. acidocaldarius* (78°C, pH 2-3) growing heterotrophically on a variety of organic substrates has recently been reported to cleave short chain fatty acid based lipids like tributyrin by means of esterases. The esterases are encoded in a distinct gene cluster together with β oxidation homologs [2]. However, the function of this β oxidation related genes and the degradation of FAs has not been analysed in detail in any archaeon so far. Here, the growth of *S. acidocaldarius* on butyrate and hexanoate was studied. Moreover, a full set of β oxidation enzymes encoded within the above mentioned gene cluster

was recombinantly produced, including an acyl-CoA synthetase, an acyl-CoA dehydrogenase, a fused hydroxyacyl-CoA dehydrogenase/enoyl-CoA hydratase as well as a ketothiolase. The recombinant enzymes were characterized in detail with respect to molecular and kinetic parameters. The results indicate that *S. acidocaldarius* utilizes FAs as sole carbon and energy source and contains a functional β oxidation pathway for FAs degradation. This is the first detailed physiological and biochemical study on FA degradation via β oxidation in the domain of archaea.

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[2] Zweerink S et al. (2017) Nat Commun 8:15352

ARP226

Archaeal biofilms - Composition of extracellular polymeric substances, exopolysaccharide synthesis and transport in *Sulfolobus acidocaldarius*

L. Kuschmierz*[1], M. Meyer[2], B. Meyer[1], J. Wingender[1], C. Bräsen[1], S. V. Albers[3], O. Schmitz[2], B. Siebers[1]
[1]*Universität Duisburg-Essen, Biofilm Centre, Essen, Germany*
[2]*Universität Duisburg-Essen, Applied Analytical Chemistry, Essen, Germany*
[3]*Universität Freiburg, Molecular Biology of Archaea, Freiburg i. Br., Germany*

Biofilms are defined as microbial communities embedded in a hydrated, self-produced matrix of extracellular polymeric substances (EPS). Although Archaea are ubiquitous and believed to also exist predominantly in the biofilm mode, knowledge about archaeal biofilm formation, structure, EPS composition and synthesis is limited [1].

Sulfolobus acidocaldarius is a thermoacidophilic, aerobic Crenarchaeon (78°C and pH 2-3) known to form biofilms, which can also be cultivated under laboratory conditions [2].

In this study, we further investigate *Sulfolobus* biofilms with a special focus on synthesis and transport of exopolysaccharides (PS). PS constitute the major EPS component, suggesting an important role in *Sulfolobus* biofilms. A gene cluster encoding several glycosyltransferases (GTs) as well as membrane proteins (MPs), likely involved in the exopolysaccharide synthesis, was identified in *S. acidocaldarius*. In order to analyse the function of this gene cluster, a new cultivation method was established, growing archaeal biofilms on membranes, floating on liquid medium. Furthermore, several deletion mutants have been constructed lacking certain GT and MP encoding genes from the PS gene cluster. A combination of methods like: (i) isolation and quantification of EPS components, (ii) visualisation of biofilm and PS via confocal laser scanning microscopy (CLSM, using DNA staining and labelled lectins) and (iii) detailed PS analysis via MS analytics is applied comparing wildtype and mutant strains and also including molecular biology and biochemical analyses.

[1] M. van Wolferen et al. (2018). *Nature Rev. Microbiol.* 16(11), 699-713.

[2] S. Jachlewski et al. (2015). *Front. Bioeng. Biotechnol*. 3, 123.

ARP227

Uptake of xylose and arabinose in *Haloferax volcanii* is mediated by an ABC-Transporter

U. Johnsen[1], M. Ortjohann*[1], J. M. Sutter[1], P. Schönheit[1]
[1]*Christian-Albrechts-Universität zu Kiel, Institut für Allgemeine Mikrobiologie, Kiel, Germany*

The halophilic archaeon *H. volcanii* has been shown to degrade D-xylose and L-arabinose via an oxidative pathway to α-ketoglutarate rather than via the typical bacterial phosphorylated pathways forming xylulose-5-phosphate. All enzymes of this pathway are encoded by the *xac* gene cluster (xylose and arabinose catabolism) which also contains *xacR*, encoding XacR, the transcriptional activator of all *xac* genes (1, 2). So far, transport of xylose and arabinose in *H. volcanii* has not been analyzed. Here we report analyses of xylose and arabinose uptake and its transcriptional regulation in *H. volcanii*. Transcriptional studies, growth experiments with knock-out mutants and amino acid sequence analyses were performed. It is shown that the *xac* gene cluster comprises five genes, *xacGHIJK*, encoding all components of an ABC transporter, i.e. a substrate binding protein, two transmembrane domains and two nucleotide binding domains. These genes were transcriptionally induced by xylose and arabinose forming a cotranscript. Further, it is shown that pentose specific regulation of *xacGHIJK* is mediated by the transcriptional activator XacR. Sequence analyses attribute the pentose specific ABC transporter to the CUT1 (carbohydrate uptake transporter) subfamily of ABC transporters (3).

(1) Johnsen U *et al.* (2009) J Biol Chem. 284(40): 27290-27303
(2) Johnsen U *et al.* (2015) Environ Microbiol. 17(5): 1663-1676
(3) Schneider (2001) Res Microbiol. 152(3-4): 303-310

ARP228

RhcR – a transcriptional activator of L-rhamnose catabolism in *Haloferax volcanii*

A. Reinhardt*[1], U. Johnsen[1], P. Schönheit[1]
[1]*Christian Albrechts Universität zu Kiel, Institut für Allgemeine Mikrobiologie, Kiel, Germany*

L-rhamnose (6-deoxy-L-mannose) is an abundant deoxy-sugar in nature and serves as carbon and energy source for microorganisms. In most bacteria, e. g. *Escherichia coli*, L-rhamnose is degraded via a phosphorylated pathway yielding dihydroxyacetonephosphate and L-lactaldehyde. So far, rhamnose degradation in the domain of archaea has not been studied.

Here we report a comprehensive analyses of rhamnose catabolism and its transcriptional regulation in haloarchaeon *H. volcanii*. The studies include (I) transcript analyses of candidate genes by Northern blot analyses or RT-PCR experiments, (II) characterization of recombinant enzymes, (III) growth studies of deletion mutants of the encoding genes and (IV) identification of a transcriptional regulator and its inducer molecule.

Together, the data indicate that in *H. volcanii* rhamnose is taken up by an ABC-transporter and is degraded to pyruvate and L-lactate via the oxidative diketo-hydrolase pathway rather than the phosphorylated pathway known from bacteria. All genes encoding the ABC transporter and the diketo-hydrolase pathway form a rhamnose catabolism gene cluster (*rhc* gene cluster) which includes the transcriptional regulator gene *rhcR*. It is shown that RhcR is an activator of all *rhc* genes. 2-Keto-3-deoxyrhamnonate, an intermediate of

the rhamnose degradation pathway, was identified as inducer molecule for RhcR. This is the first comprehensive analysis of rhamnose catabolism in the domain of archaea.

ARP229

How do archaea assimilate nitrogen? Structural insights of the archaeal glutamate synthase reveal a "primitive" multi-component machinery fueled with a new electron donor

T. Wagner*[1], S. Shima[2]
[1]*Max Planck Institute for Marine Microbiology, Microbial Metabolism, Bremen, Germany*
[2]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

The glutamate synthase (GOGAT) orchestrates ammonia (NH_3) assimilation via a sophisticated process. NH_3 is extracted from glutamine and transferred through an internal channel to the next active site where it is branched on oxoglutarate. The intermediate is reduced and generates glutamate (a nitrogen carrier). GOGAT from plants and bacteria are single polypeptides constituted of four domains (glutaminase, central, glutamate synthase and β-helicoidal domains) and uses NADH, NADPH or reduced ferredoxin as electron providers*. The central domain, similar to the glutamate synthase domain, organizes the NH_3-channel. The enzyme from archaea is mysterious; each domain is encoded by different genes and the central domain is missing, raising the question of how this GOGAT would maintain its physiological function.

We investigated the archaeal GOGAT by purifying and crystallizing it natively from a methanogen.

Its crystal structure has been obtained at 1.95-Å and shows a 650-kDa tetramer of heterotetramers containing a total of 24-[4Fe-4S] clusters. It is composed of a F_{420}-oxidase electronically connected to a GOGAT core formed by the three domains. The NH_3-channel is generated at the dimeric interface of two glutamate synthase domains (in the tetramer). The $F_{420}H_2$-dependent glutamate synthase"s activity was confirmed *in vitro* and should be very effective *in vivo* by the immediate reduction of the F_{420} by a hydrogenase.

Our discovery retraces the GOGAT evolution: after duplication of the glutamate synthase domain, the copy evolved to a central domain and the four subunits fused together to generate the stand-alone GOGAT found in plants and bacteria. These duplication and fusion events in the GOGAT operon are found across archaeal lineages.

*Vanoni and Curti. *iUBMB Life* 2008

BIP230

Characterization of a multidomain hybridkinase and its role in signal transduction in *Methanosarcina acetivorans*

A. Sexauer*[1], N. Frankenberg-Dinkel[1]
[1]*TU Kaiserslautern, Microbiology, Kaiserslautern, Germany*

Methanogenesis is a metabolic pathway which methanogenic archaea use to acquire energy by producing methane out of hydrogen, carbon monoxide, acetate and one-carbon compounds. *Methanosarcina acetivorans* is capable of metabolizing different substrates by the carboxythrophic-, acetoclastic and the methylotrophic pathway to conduct methanogenesis.

The ability to perceive different nutrients is important to any organism living under limited conditions. Therefore, prokaryotic microorganisms have evolved well-organized signal transduction systems to respond optimally to environmental changes. The hybridkinase MA4377 is part of such a system that is likely involved in regulating the utilization of alternative substrates for the methylotrophic part of methanogenesis. MA4377 is a multidomain protein consisting of three sensor domains, directly connected to two receiver domains: A membrane-bound CHASE4 domain followed by a HAMP domain and PAS domain.

In order to understand the function of MA4377, the protein and truncated variants were recombinantly produced in *Escherichia coli* and purified using affinity chromatography. To investigate if the redox state has an influence on the kinase activity of MA4377, autophosphorylation assays were performed. Interestingly, the protein displayed high autophosphorylation activity in the oxidized state, which is significantly attenuated under reducing conditions. In the next step, we will investigate whether cysteine residues are involved in both, the dimerization and redox control. From a long term perspective we will investigate the role of MA4377 in the control of a set of specific corrinoid-methyltransferase fusion proteins involved in the utilization of one-carbon compounds in the methylotrophic pathway.

BIP231

Interactions of cyanobacteria and predators

A. Bauer*[1], E. Kemen[2], K. Forchhammer[1]
[1]*Universität Tübingen, Organismic Interactions, Tübingen, Germany*
[2]*Universität Tübingen, Microbial Interactions, Tübingen, Germany*

Cyanobacteria live as primary producers in ecosystems together with many predators. Their successful propagation in these environments implies efficient defense mechanisms against predation by amoeba, fungi or predatory bacteria. The current project aims to investigate the defense mechanisms of filamentous cyanobacteria. This includes the recognition of the predator as well as the preparation and execution of the actual defense mechanism. Since the intercellular communication in filamentous cyanobacteria is not limited to adjacent cells but extends to the whole filament, the response of the entire filament during an attack is a main interest of the project. To establish a predator-prey model, freshwater lakes will be screened for microorganisms predating on filamentous cyanobacteria of the genus *Anabaena*. In order to visualize the interactions between cyanobacteria and predators, various microscopic methods will be employed, including fluorescence microscopy, FRAP experiments and electron microscopy. Moreover, the effect of an attack will be investigated at the molecular basis, using proteome and transcriptome analysis.

BIP232

Esca disease of grapevine: the role of secondary metabolites in plant pathogen interaction

J. Fischer*[1], B. Berkelmann-Löhnertz[1], T. Opatz[1], A. Schüffler[1], E. Thines[1]
[1]*Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Kaiserslautern, Germany*

Esca is known as a destructive trunk disease of grapevine caused by a multiplicity of fungal pathogens. Those can

occur as mutualistic endophytes, inducing no obvious symptoms or cause the Esca disease and various other grapevine trunk diseases (GTDs). Two of the fungi involved are *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium minimum* (Pmi). All along it was suggested that Pch and Pmi secrete phytotoxins, which are translocated and cause the symptom development, since no fungal growth is detectable in symptomatic leaves. Several toxins produced by Esca-associated fungi have been reported, e.g. scytalone and isosclerone. Those compounds are phytotoxic but do not induce the typical "tiger-strip pattern" in necrotic leaf tissue.

Therefore, in order to characterize phytotoxic metabolites produced by the mentioned fungi, the organisms were grown in *Vitis* leaf medium. We were able to identify kaempferol (KAE), as well as kaempferol-3-glucoside (K3G). The latter was detectable in significantly higher concentrations in the medium inoculated with Pch compared to the sterile medium. To unravel this finding, we started to investigate the kaempferol-glycosylation in artificial medium. KAE itself inhibited the growth of Pch at concentrations higher than 20 mg/L.

We were able show that Pch is able to alter and thereby "detoxify" KAE to K3G by adding UDP-glucose to medium supplemented with 10 mg/L KAE. We monitored the glycosylation of KAE to K3G using HPLC-MS analysis.

In addition, we injected K3G into *Vitis vinifera* cv. `Riesling` greenhouse plants and observed Esca-like leaf symptom formation. Therefore, we propose, that the Pch-induced change of the *in planta* KAE/K3G ratio endorses Esca leaf symptoms supplementary to other, previously published phytotoxins.

BIP233

Searching for beneficial organisms to mitigate apple replant disease: a metagenomic approach

V. Radl*[1], J. B. Winkler[1], S. Kublik[1], G. Vestergaard[1], P. Schröder[1], F. Buegger[1], T. Winkelmann[2], M. Schloter[1]
[1]Helmholtz Zentrum München, Oberschleißheim, Germany
[2]Universität Hannover, Hannover, Germany

Apple replant disease (ARD) is a phenomenon that occurs in areas where apple plants or closely related species have been cultivated for many years. Even though ARD is a well-known phenomenon, which has been observed in different regions and soil types, the causes behind its emergence remain unclear. In the present work we tested the hypothesis that ARD is caused by changes on assembly of the soil microbial community, particularly a reduction of beneficial organisms, such as mycorrhizal fungi and plant growth promoters. To test this hypothesis, we performed pot experiments where apple plants were grown in a virgin (CO) or apple fatigued (ARD) soil from an experimental nursery area, localized in Ellerhoop (Schleswig-Holstein, Germany). Microbial communities were assessed using 16S rRNA amplicon and shotgun metagenome. Utilization of plant derived carbon by soil microbes was investigated using $_{13}CO_2$ labeling experiments performed in climate chambers. Ten weeks after apple plantlets were transferred to soils we could detected lower plant biomass in ARD compared to control pots. We observed larger differences in composition of bacterial communities of ARD and CO after 12 weeks. Shotgun metagenome showed a clear differentiation between the rhizosphere and bulk soil compartments. In general, CO soils have a higher abundance of genes related to carbon metabolism, including those related to the degradation of aromatic compounds, whereas for the ARD soil we found more genes related to defense and host-microbe interactions, e.g. type secretion systems IV and VI. This is line with the ^{13}C-labeling experiments, which showed a lower incorporation of ^{13}C in the microbial biomass from ARD soils.

BIP234

Exploring the freshwater sponge – bacteria interspecies interaction

M. Jogler*[1], N. Kallscheuer[2], T. Kohn[2], F. Brümmer[3], R. W. Müller[3], S. Wiegand[2], M. Rohde[4], C. Jogler[2]
[1]Leibniz Institute DSMZ, Braunschweig, Germany
[2]Radboud University, Nijmegen, Netherlands
[3]University of Stuttgart, IBBS, Stuttgart, Germany
[4]Helmholtz Centre for Infection Research, Braunschweig, Germany

Marine sponges are known as rich source of novel small molecules, which are mainly produced by their microbiota. In contrast, sponges from freshwater habitats are barely studied and little is known about their associated bacteria. Many studies even doubt that a specific freshwater sponge / bacterial interaction exists. Furthermore, little is known about bioactive molecules produced by freshwater sponges.

In this proof of concept study, we applied cultivation independent 16S rRNA amplicon sequencing and metagenomics along with two different cultivation approaches from freshwater sponges sampled at different German lakes.

Amplicon- and metagenomic analysis point towards a conserved association of distinct bacterial phylogenetic groups within freshwater sponges. This indicates that, as their marine relatives, freshwater sponges contain a specific microbiome.

During our cultivation experiments, we isolated the novel planctomycetal strain spb1 from *Ephydatia fluviatilis* (targeted approach) and 11 strains belonging to a novel genus of the rarely cultivated phylum Gemmatimonadetes obtained from *Spongilla lacustris* (unbiased cultivation). The genome sequence of spb1 revealed the potential to produce small molecules while its cell biology points towards the utilization of complex carbon substrates through a novel fiber mediated uptake mechanism. The novel genus from the barely studied Gemmatimonadetes show an unusual cell morphology, with more than 10 µm length and divide asymmetrically. Furthermore, spherical cells tubular connected to the rod-shaped cells were observed. Whether these strains are symbiotic, commensal or even pathogenic remains enigmatic.

BIP235

Fungal Secondary Metabolites in Environmental Dynamics: Impact of Antagonistic and Mutualistic Interactions associated with Tree Canker

L. Heck*[1], L. Antelo[2], K. Andresen[1], E. Thines[2,1]
[1]Institute of Molecular Physiology, Johannes Gutenberg-University, Microbiology and Wine Research, Mainz, Germany
[2]Institute of Biotechnology and drug research [IBWF] gGmBH, Kaiserslautern, Biotechnology, Kaiserslautern, Germany

The causal agent of tree canker, *Neonectria ditissima*, affects a wide range of hardwood plant hosts like *Fagus*, *Malus* and *Pyrus*. To date, there is no effective method to eliminate the disease and little is known about potential virulence factors.

In this study, we were interested in secondary metabolites as determinants of interaction between the pathogen *N. ditissima* and fungal endophytes within the plant environment, since chemical communication might provide new approaches for integrated plant protection strategies. Our research based on the antagonistic and mutualistic balance of the pathogen *N. ditissima* and the endophytic consortia in different host trees. For that reason, a broad variety of organisms were isolated from healthy plant material and were tested for antagonistic activity against the pathogen. Several compounds impairing vegetative growth of the pathogen were identified from submerged cultures of various endophytes.

Investigations into tree canker inducing metabolites, led to the identification phytotoxins secreted by *N. ditissima*. Extracts of axenic cultures of the phytopathogen grown in diverse cultural conditions were tested in an apple assay. Citrinin and p-hydroxybenzoic acid were detected among other substances.

Furthermore, the genome of *N. ditissima* has been sequenced and potential gene clusters responsible for the secondary metabolite biosynthesis were determined. Moreover, based on transcriptome analysis, we will be able to narrow our search of corresponding genes related to phytotoxicity. To characterize the function of proteins involved in biosynthesis of secondary metabolites, we implemented two techniques: first "loss of function" mutagenesis and second heterologous expression in fungal model organisms.

BIP236

FISH protocol optimization for an anaerobic, biofilm-forming consortium from Costa Rica

L. Dengler*[1], A. Bellack[1], R. Rachel[1], D. Grohmann[1], H. Huber[1]
[1]*Universität Regensburg, Regensburg, Germany*

Microbial biofilms are widespread in nature and understanding of the organismic composition, the biofilm formation pathway but also the spatial distribution of the organisms in the biofilm is of high interest. FISH (fluorescence in situ hybridization) presents a well-established method to visualize and differentiate cells of different organisms using fluorescently labeled oligonucleotides. In this study, we employed FISH to a biofilm originally isolated from an ancient oil well in Cahuita, Costa Rica. The biofilm consists of a methanogenic Archaeum of the genus *Methanobacterium* and a sulfate-reducing bacterium (SRB) of the genus *Desulfomicrobium*. Both microbes are stable in pure culture, as well as in an artificial co-culture. The sulfate-reducer acts as initiator of biofilm-formation, while the methanogen secondarily attaches to the matrix. Initial analyses showed that the probes unspecifically interact with the matrix surrounding the organisms, causing severe background signals. In order to reduce the unspecific signal, we worked towards an improved FISH protocol to be able locate the individual cells within the biofilm by CLSM (confocal laser scanning microscopy). Hybridization was performed using the fluorescently labeled universal archaeal probe ARCH915, as well as SRB385 and DSM213 targeting SRB. The adaption of SDS and formamide concentrations, tests with heating steps and washing time, as well as the analysis of organic and inorganic substances within the biofilm was crucial for protocol design. In summary, the versatile approach eliminated some problems and led to an improved FISH protocol.

BIP237

Insight into smut fungal infection strategies: the genome and the transcriptome of the Brassicaceae smut fungus *Thecaphora thlaspeos* reveal conserved and novel effectors

K. Courville[1], L. Fratzeskakis[1], S. Gul[1], R. Kellner[1,2], B. Day[3], B. Usadel[4], P. van Esse[5], A. Brachmann[6], M. Feldbrügge[1], V. Göhre*[1]
[1]*Heinrich-Heine University Düsseldorf, Institute for Microbiology, Düsseldorf, Germany*
[2]*Max Planck Institute for Plant Breeding Research, Cologne, Germany*
[3]*Michigan State University, Department of Plant, Soil, and Microbial Sciences, East Lansing, MI, United States*
[4]*RWTH Aachen University, Institute for Biology I, Aachen, Germany*
[5]*The Sainsbury Laboratory, Norwich, United Kingdom*
[6]*Ludwig-Maximilians-Universität München, Genetics, München, Germany*

Smut fungi are globally distributed plant pathogens that infect agriculturally important crop plants such as maize or potato. During the major part of their life cycle, these Basidiomycetes grow as endophytes inside the host without causing disease symptoms. Only at late stages of infection they interfere with seed development by producing large amounts of fungal spores in different plant organs. To date, molecular studies on host species of smut fungi are difficult due to lack of genetically tractable model host plants. Therefore we set out to investigate the only known smut fungus of Brassicaceae hosts: the *Arabis*-specific smut fungus *Thecaphora thlaspeos*.

Here, we present a genomic and transcriptomic analysis of *Thecaphora thlaspeos*. The *T. thlaspeos* genome was assembled to the chromosome level. Our analysis revealed that it is a typical smut genome in terms of size and genome characteristics. With regard to the infection strategy, *in silico* prediction of candidate effector genes revealed common smut effector proteins and unique members. For three candidates, we have functionally demonstrated effector activity. The well-described peroxidase inhibitor Pep1 is functionaly conserved also in *T. thlaspeos*. One of the unique effectors, *Tt*Tue1, suggests a potential link to cold acclimation. On the plant side, studying transcriptional responses of *Ar. hirsuta* towards *T. thlaspeos* infection, we obtained evidence for a typical immune response as it is present in other infection systems, despite the absence of any macroscopic symptoms.

Our findings suggest that *T. thlaspeos* distinctly balances its virulence during biotrophic growth ultimately allowing for long-lived infection of its perennial hosts.

BIP238

Changes of the bacterial communities and the antimicrobial and disinfectant resistance gene abundance in the gut microbiota of *Hermetia illucens* larvae and the residual of the employed substrate during a rearing process

Y. A. Cifuentes Triana*[1], S. P. Glaeser[1], J. Mvie[1], H. Gutzeit[2], A. Vilcinskas[1,3], P. Kämpfer[1]
[1]*Justus-Liebig-Universität Gießen, Gießen, Germany*
[2]*Technische Universität Dresden, Dresden, Germany*
[3]*Fraunhofer Institute for Molecular Biology and Applied Ecology, Gießen, Germany*

Hermetia illucens, commonly known as black soldier fly (BSF), is one of the most promising insects for future application to recycle organic waste in combination with the production of high quality proteins as feed for agricultural animals. The gut microbiota plays a key role in the digestion efficiency and the deactivation of potential pathogenic

bacteria of the organic waste used as substrate. A first feeding experiment was performed to learn more about the dynamics of the gut microbiota and their effect on the microbiota of the residue of the employed feed substrate. Both were investigated at different growth stages by 16S rRNA gene amplicon based Illumina MiSeq sequencing. Non-metric multidimensional scaling of bacterial community patterns indicated that the gut microbiota and the substrate associated bacterial communities were clearly different among each other. Both changed over time. Significant changes of the bacterial diversity and community composition however were only observed in the substrate residue. Many bacterial genera containing potential pathogens, as *Staphylococcus* spp. or *Acinetobacter* spp., strongly decreased in their relative abundance in the residue. In parallel to the community profile, the abundance of antibiotic resistance and disinfectant resistance genes (*qacE*) were determined by real-time PCR. Neither antibiotic nor disinfectant resistance genes accumulated in larvae but the abundance of *qacE* genes significantly increased in the residue of the employed substrate. This is the first study which indicates the relevance of disinfectant resistance genes in insect rearing which is of practical importance and will be studied in more detail in further experiments.

BIP239

Development of photoautotrophic biofilm communities on microplastic particles in freshwater environments

R. Jongsma*[1], B. Philipp[1]
[1]*WWU Münster, IMMB, Münster, Germany*

The constant increase of plastic production and its improper disposal has led to accumulation of plastic waste in the environment. This includes particles

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CCP240

Cell-to-cell-signaling in microbial communities of pyrite-oxidizing acidophiles

S. Bellenberg*[1], M. Vera[2], M. Dopson[1]
[1]*Linnaeus University, Centre for Ecology and Evolution in Microbial Model Systems, Kalmar, Sweden*
[2]*Pontificia Universidad Católica de Chile, Institute for Biological and Medical Engineering. Schools of Engineering, Medicine & Biological Sciences. Department of Hydraulic & Environmental Engineering, Santiago, Chile*

Microbial communities are complex, multivariate systems that can consist of many species interacting with each other and respond to various environmental influences. The importance of cell-to-cell communication for succession of natural and biotechnical microbial consortia is poorly understood and the influence of biochemical signaling on microbial consortia in acid mine drainage ecosystems has been studied. This ecosystem is particularly suitable as it has lower diversity than most pH neutral environments. We found quorum sensing pathways in several acidophiles and some intra- and interspecies effects of signal compounds have been demonstrated. Specifically, production of extracellular polymeric substances, biofilm formation on pyrite or its dispersal, as well as inhibition of iron(II)-oxidation is differentially effected. Concentrations of signal molecules will be analyzed in axenic cultures, laboratory mock communities, and environmental samples. Succession of microbial communities and activities will be elucidated using 16S rRNA tag sequencing and meta-transcriptomics. The manipulability of the microbial ecosystems will be demonstrated by the addition of selected signaling molecules to mock communities. Finally, we may contribute to opening new avenues for acid mine drainage remediation and fostering biomining of copper and gold from low-grade ores. In addition, the development of methods for the targeted manipulation of consortia in the environment or in medical or biotechnological applications could be facilitated with an improved understanding of cell-to-cell signaling in microbial communities.

CCP241

The role of microbiome in chronic systemic diseases

T. Koal*[1], B. Wolf[1]
[1]*BIOCRATES Life Sciences AG, Reserach and Development, Innsbruck, Austria*

Introduction: Over the past years, microbiome research has dramatically reshaped our understanding of human biology and how microbes mediate digestion and disease processes. The symbiotic relationship of microbiome and host, including homeostatic balance, is getting more attention in the medical biomarker research and fundamental disease research. The hype about metabolomics in microbiome research is based on the need for new answers regarding the impact of microbiome composition on pathophysiology and chronic systemic diseases.

Objectives: The best tool to identify and analyze functional host-microbiome interaction is metabolomics allowing the investigation of functional microbial metabolic activities. This presentation will provide an overview of the physiological role of the microbiome and the relation to metabolites produced and/or biochemically modified by microbes.

Materials & methods: 10% of metabolites in blood are derived from the gut microbiota. New metabolomics mass spectrometry-based approaches and strategies will be discussed for multiplexed analysis of 500+ central host- and gut bacteria-derived metabolites in blood and feces by mass spectrometry.

Results: The focus will be placed on an overview about the variety of target metabolites (driven by choline metabolism, bile acids, branched-chain amino acids, and tryptophan metabolism) in microbiome research, and their connection to pathophysiological processes and diseases including microbiome related drug interaction.

Conclusion: The microbiome-host interaction is a key aspect developing new insights and understanding of commonalities in chronic systemic diseases: diabetes, liver, oncology, nephrology, neurological, autoimmune and cardiovascular disorders.

CCP242

Impact of loss of quorum sensing and tropodithietic acid production on the *Phaeobacter inhibens* exometabolome

S. Srinivas*[1], J. Niggemann[2], T. Brinkhoff[1,3]
[1]*ICBM, University of Oldenburg, Biology of Geological Processes, ICBM, Oldenburg, Germany*
[2]*ICBM, University of Oldenburg, ICBM-MPI Bridging Group for Marine Geochemistry, Oldenburg, Germany*
[3]*ICBM, University of Oldenburg, Biology of Geological processes, Oldenburg, Germany*

Phaeobacter inhibens, a model organism of the marine *Roseobacter* group, is known for *N*-acyl homoserine lactone (AHL)-mediated quorum sensing (QS) and production of tropodithietic acid (TDA), a multifunctional secondary

metabolite. Both AHL and TDA influence global gene expression and thus the lifestyle of *P. inhibens* as biofilm former and host-associated bacterium. We examined the impact of QS and TDA production on the exometabolome of *P. inhibens* DSM 17395 by comparing the wild type (WT) strain with a QS- (*pgaR*-) and a TDA-deficient mutant (*tdaE*-) via Fourier transform ion cyclotron resonance mass spectrometry. Exometabolomes of WT and both mutants differed markedly from each other and at various growth stages. For *pgaR*- mutant, number of detected masses was nearly twice as high as for *tdaE*- and 10% higher than WT. About 85% of the masses were strain-specific and all strains showed an increase in number of masses from lag to stationary phase. Molecular formulae were assigned to 738, 1070 and 635 masses for WT, *pgaR*- and *tdaE*-, respectively. We annotated a small fraction of these molecules using metabolite databases, revealing several masses analogue to known bioactive compounds and precursors. Our findings indicate that QS and TDA production greatly impact the amount and composition of released metabolites, thereby affecting the interactive relationships of *P. inhibens*.

CCP243

Antibacterial effect of TiO2 and ZnO nanoparticles on some Pathogenic bacterial strains

A. Selma*[1], T. Mostefaoui[1]
[1]*Université de Bejaia, Biologie Physico chimique, Bejaia, Algeria*

In the past century, the widespread strategy to prevent microbial contamination and to kill microorganisms, consisted in adding antibiotics. However, the emergence of bacterial resistance to antibiotics has prompted researchers to turn to alternative solutions. One of these solutions uses reaction chemistry that produces antimicrobial coatings on desired surfaces with significant benefits. Nanotechnology has become one of the most practical technologies, because of unique physical and chemical properties of nanomaterials. The antibacterial activities of some nanoparticles make them attractive candidates as new agents against pathogenic bacteria. In the present work, antibacterial effects of nanoparticles are measured with cylinder diffusion technique, liquid and agar nutrient medium are used for bacterial culture. Antibacterial effects with different concentration of TiO2 and ZnO nanoparticles are used in the three analyzed bacterial strains were known for their resistant to a lot of antibiotics (E.coli, Salmonella sp and Staphylococcus aureus). Inhibition zone measurement showed an interesting result. Based on this study, we have measured the antibacterial effects of TiO2 nanoparticles and it was found for the first time that ZnO nanoparticles show a high activity against staphylococcus and there by can be used as an antibacterial agent for different purposes.

LPP244

Comparative Analysis of the *N*-Terminal Domain Protein Fusion of the Hydrogenase Large Subunit HycE from the Formate Hydrogenlyase Complex and NuoCD of the Respiratory Complex I

P. Skorupa*[1], C. Blumenscheit[1], U. Lindenstrauß[1], S. Burschel[2], T. Friedrich[2], C. Pinske[1]
[1]*Martin-Luther-University Halle-Wittenberg, Institute of Biology/ Microbiology, Halle a. d. S., Germany*
[2]*Albert-Ludwigs-University Freiburg, Institute of Biochemistry, Freiburg i. Br., Germany*

The membrane-associated formate hydrogenlyase (FHL) complex of *Escherichia coli* comprises seven subunits and produces molecular hydrogen under fermentative growth conditions. The hydrogenase 3 large subunit HycE uses electrons derived from formate oxidation to generate H_2 from two protons[1]. Early work established a phylogenetic relationship between the proteins of the FHL complex and complex I of the respiratory chain (NADH:ubiquinone oxidoreductase)[2]. Like HycE, NuoCD of *E. coli* complex I is a fusion protein of a hydrogenase-like *C*-terminal domain with a cofactor-free *N*-terminal domain. Nevertheless, some organisms still synthesize distinct NuoC and NuoD proteins. With future biotechnological H_2 production from FHL in mind, it is important to understand the evolutionary advantage of creating a protein fusion. Using chromosomal deletion variants of the *N*-terminal domain and genetically de-fused proteins of HycE or NuoCD, we analysed the influence on the protein composition and activity of the respective protein complexes. A deletion of the *N*-terminal domain resulted in a complete absence of both FHL and complex I activities due to an inherent instability of the complexes. When the respective *N*-terminal domain was provided either *in trans* or *in cis*, then the activity of the FHL complex was reduced to 24 or 73% of the parental, respectively, while complex I did not regain its function. Although the *N*-terminal domains do not harbour their own cofactors and are not involved in the catalytic reaction of either complex I or FHL, they are absolutely required for activity because they appear to have an important structural role.

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LPP245

Characterization of cystargolide A and B as inhibitors of the caseinolytic protease P

A. Illigmann*[1], F. Wolf[2], L. Kaysser[2], H. Brötz-Oesterhelt[1]
[1]*University of Tübingen, Microbial Bioactive Compounds, Tübingen, Germany*
[2]*University of Tübingen, Pharmaceutical Biology, Tübingen, Germany*

Fighting the development of antibiotic resistance is a challenging task. Therefore, innovative antibacterial agents urgently need to be discovered and especially microbial natural products represent promising lead-structures for that purpose.

The natural β-lactones cystargolide A and B are produced by *Kitasatospora cystarginea* and exhibit proteasome-inhibitory activity[1]. Interestingly, synthetic β-lactones were identified to inhibit *Staphylococcus aureus* ClpP (SaClpP)[2]. This motivated to analyze, whether cystargolide A and B can also inhibit ClpP. ClpP, the proteolytic core of the bacterial serine protease Clp, is involved in intracellular protein turnover and regulatory proteolysis. ClpP forms a tetradecameric core-enzyme that is able to degrade small peptides. In concert with cognate Clp-ATPases, the protease complex is formed, allowing specific and tightly regulated protein degradation[3].

The potency of ClpP inhibition by the cystargolides was quantitatively assessed by determination of $k_{observed}/I$ values in a SaClpP activity assay. In contrast to the commonly determined IC_{50}, k_{obs}/I values for covalent inhibitors are determined independently of incubation time and enzyme concentration[4]. To compare the mechanism of inhibition with synthetic β-lactones that either retain or destabilize tetradecameric SaClpP[4], we examined the state of SaClpP oligomerization upon cystargolide A binding via size exclusion chromatography. Our findings allow to conclude,

that cystargolide A and B represent structurally new inhibitors of ClpP.

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LPP246

HypD and its Role in Diatomic Ligand Biosynthesis of NiFe-Hydrogenases: A Resonance Raman Study of the HybG-HypD Complex

K. Nutschan*[1], C. Kulka[2], I. Zebger[2], G. Sawers[1]
[1]*Martin-Luther-Universität Halle-Wittenberg, Institute of Biology/ Microbiology, Halle a. d. S., Germany*
[2]*Technical University of Berlin, Institute of Chemistry/ Biophysical Chemistry, Berlin, Germany*

[NiFe]-hydrogenases have an unusual bimetallic active-site cofactor, whereby the iron ion carries a CO and two CN-groups as diatomic ligands. While the CN- ligands are generated from carbamoylphosphate, the biosynthesis of the CO ligand remains unresolved. Biosynthesis of the Fe(CN)2CO group occurs on a Hyp protein scaffold complex and includes the HypC, HypD, HypE and HypF proteins. Notably, only the iron-sulphur (FeS) cluster-containing HypD protein is redox-active. We hypothesize that HypD likely plays a key role in CO ligand generation, as well as being involved in transfer of the CN- ligands from the HypE protein to the iron ion. Preliminary evidence suggests that the CO ligand might be generated from endogenous CO2 already bound to an iron ion associated with the HypD-HybG (HybG is a HypC paralogue in *E. coli*) sub-complex. The reduction of CO2 to CO, however, requires a redox potential of approximately -535 mV, but the redox potential of the FeS-Cluster of HypD (E0´= -260 mV) is too positive to be able to reduce CO2 directly. Theoretically, therefore, this step could be achieved if coupled to ATP hydrolysis; however, HypD has no obvious ATP binding site. We have adopted two approaches to study the activity of HypD in the isolated HypD-HybG sub-complex: in one of these we developed an HPLC-based assay to analyze ATP hydrolysis by the sub-complex and the influence of HypE on this process. In the second approach, we used Resonance Raman (RR) spectroscopy to study whether either ferredoxin (Fdx) or flavodoxin (Fld) can provide the electrons to reduce the FeS cluster of HypD. We have shown that the HypD-HybG sub-complex indeed has a weak ATPase activity but we rule out that Fdx acts as an effective electron donor to reduce the FeS cluster of HypD.

LPP247

Protein-protein interactions involved in the Stickland fermentation in *Clostridioides difficile*

K. E. Rennhack*[1], J. M. Borrero de Acuña[1], J. Wissing[2], D. Jahn[1]
[1]*TU Braunschweig, Institut für Mikrobiologie , Braunschweig, Germany*
[2]*HZI, Zelluläre Proteomforschung, Braunschweig, Germany*

The anaerobic, Gram-positive bacterium *Clostridioides difficile* is causing a severe diarrhoea which leads to several thousand deaths per year in Germany. Stickland fermentation of amino acids represents the most prominent energetic generation process for *C. difficile* growth. Importantly, it has been unequivocally correlated to toxin production. This metabolic pathway employs pairs of amino acids as electron acceptor-donor systems for generating ATP. The proton motive force and its consequent ATP synthesis appears on the proton-translocating ferredoxin:NAD+ oxidoreductase complex (Rnf), whose proton pump functioning is coupled to the performance of reductases. Hence, our group strives toward the elucidation of protein-protein-interactions involved in the Stickland pathway. For that, interactomic techniques encompassing affinity co-purification of bait-prey protein complexes and proteomics-based strategies for the identification of interaction partners were chosen as scientific approach. At first the cytoplasmic protein subunits PrdA and PrdB of the D-proline reductase will be subjected to interactomic studies. Their Strep-II tagged versions will be employed as baits to capture the potential preys stabilizing the transient protein-interactions by *in vivo* cross-linking. The production and purification of both were confirmed by western blot analysis through Strep-tagII detection. In conclusion preliminary experiments on the purification of the fusion protein PrdA-Strep and PrdB-Strep have been successfully conducted. Subsequently, *in vivo* cross-linking experiments captured many different protein interaction partners, amongst others subunits of the Rnf complex, that are involved in energy generation.

LPP248

A 1-MDa metalloenzyme complex in *Geobacter metallireducens* catalyzes benzene ring reduction possibly by two flavin-based electron bifurcations

S. E. L. Anselmann*[1], S. G. Huwiler[1], C. Löffler[1], H. J. Stärk[2], M. von Bergen[3,4], J. Flechsler[5], R. Rachel[5], M. Boll[1]
[1]*Albert Ludwigs Universität Freiburg, Freiburg i. Br., Germany*
[2]*Helmholtz Zentrum für Umweltforschung, Department of Analytic Chemistry, Leipzig, Germany*
[3]*Helmholtz Zentrum für Umweltforschung, Department of Molecular Systems Biology, Leipzig, Germany*
[4]*Universität Leipzig, Leipzig, Germany*
[5]*Universität Regensburg, Regensburg, Germany*

Aromatic compounds are ubiquitous in nature and are used as carbon source by aerobic and anaerobic microorganisms. In anaerobic bacteria the central intermediate benzoyl-CoA is dearomatized to cyclohexa-1,5-diene-1-carbonyl-CoA (1,5-dienoyl-CoA) by different classes of benzoyl-CoA reductases (BCRs). Class I BCRs couple aromatic ring dearomatization to a stoichiometric ATP hydrolysis, whereas class II BCRs are ATP independent. The structurally characterized BamBC subunits of class II BCR accomplish benzene ring reduction at an active site tungsten cofactor, however the components and mechanism involved in the energetic coupling of endergonic benzene ring reduction have remained hypothetical. We present a 1-MDa, membrane-associated, Bam[(BC)2DEFGHI]2 complex from the anaerobic bacterium *Geobacter metallireducens* harboring 4 tungsten, 4 zinc, 2 selenocysteines, 6 FAD, and >50 FeS cofactors. The results suggest that class II BCRs catalyze electron transfer to the aromatic ring yielding a cyclic 1,5-dienoyl-CoA via two flavin-based electron bifurcation events.

LPP249

Co-evolution of curli components CsgA and CsgB

J. Chilimoniuk*[1], P. Mackiewicz[1], M. Burdukiewicz[2]
[1]*University of Wrocław, Department of Bioinformatics and Genomics, Wrocław, Poland*
[2]*Warsaw University of Technology, Faculty of Mathematics and Information Science , Warsaw, Poland*

Introduction: CsgA, also known as a major curli component, is ubiquitous in biofilms of gram-negative bacteria. Thanks to its ability to self-assemble in the amyloid-like manner into durable fibers, CsgA is a dominant proteinaceous scaffold of biofilms. The presence of pre-formed amyloid fibers can accelerate aggregation of other amyloids in the process known as cross-seeding. It is extremely sequence specific and can be restricted by a single amino acid difference. CsgA is in vivo cross-seeded by its nucleator protein, self-aggregating CsgB.

It was recently proven that CsgA proteins can, probably through cross-seeding, induce the aggregation of alpha-synuclein accelerating development of Parkinson"s disease.

Objectives: We investigate co-evolution of CgsA and CsgB proteins to shed more light on the process of cross-seeding.

Materials and methods: We combined sequences of around 500 CsgA-CsgB pairs from E. coli strains. To further investigate similarity of proteins, we used a simplified alphabet of amino acid optimal for amyloid-like aggregation.

Results: Both CsgA and CsgB are characterized by a regional structure of five repeated motifs. We found out that the general motif (S-X5-Q-X-G-X2-N-X-A-X3-Q) (the serin in absent in the case of CsgB) is faithfully preserved among different variants of CsgA and CsgB. The residual variability in motifs of one protein does not affect the sequence of other protein.

Conclusion: We have not identified any simultaneous mutations between CsgA and CsgB. A single mutation in single repeated region is not enough to change the protein function and to cause mutations in another protein. That implies that all CsgA variants from E. coli can cross-seed alpha-synuclein.

LPP250

Molecular basis of the FliC-DnaK-NirS complex in the periplasm of *Pseudomonas aeruginosa*

K. Müller*[1], J. M. Borrero de Acuña[1], G. Molinari[2], M. Rohde[2], M. Jahn[1], D. Jahn[1]
[1]*TU Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany*
[2]*HZI, Zentrale Einheit für Mikroskopie, Braunschweig, Germany*

Energy generation of Gram negative bacteria is accomplished by electron transport chains which transfer electrons via redox reactions across the inner membrane. In *Pseudomonas aeruginosa* anaerobic energy generation during denitrification has been shown to involve a protein supracomplex (Borrero-de Acuña JM, *et al*. 2017; PMID: 28857512). During the corresponding interactomics studies, an unexpected triple complex of the periplasmic nitrite reductase NirS, the cytoplasmic chaperone DnaK and the extracellular flagella filament protein FliC was identified and initially characterized (Borrero-de Acuña JM, *et al*. 2015; PMID: 26170416).

Double immunogold labelling and succeeding TEM analysis showed that DnaK and FliC are co-localized in the periplasm (Borrero-de Acuña JM, *et al*. 2016; PMID: 26903416), but also at the flagellum. Generation of a *dnaK* conditional mutant was used to evaluate the impact of DnaK abundance decay on the flagella assembly and export. A drastically higher intracellular location of FliC was observed by immunofluorescence when *dnaK* expression was knocked down revealing that this chaperone is playing crucial role in FliC export. To determine the molecular basis for the interaction between DnaK and FliC, a spot membrane assay was performed. Thus, the active site of DnaK (a.a. 535-552) and a-helices of the region D1 (a.a. 46-60 and 421-435) of FliC were identified as interacting domains. Due to the known interaction of FliC with NirS, a molecular model for the NirS-FliC-DnaK triple complex can be proposed.

LPP251

Structure of the Nitrite Oxidoreductase (Nxr) from the Anammox Bacterium *Kuenenia stuttgartiensis*

T. Moreno-Chicano*[1], A. Dietl[1], A. Mohd[1], N. de Almeida[2], W. J. Maalcke[2], J. T. Keltjens[2], B. Kartal[2], M. S. M. Jetten[2,3], T. R. M. Barends[1]
[1]*Max Planck Institute for Medical Research, Biomolecular mechanisms, Heidelberg, Germany*
[2]*Radboud University , Department of Microbiology, Nijmegen, Netherlands*
[3]*Delft University of Technology, Department of Biotechnology, Delft, Netherlands*

Anaerobic ammonium-oxidizing (anammox) bacteria play a crucial role in the nitrogen cycle. They catalyse the oxidation of ammonium (NH_4^+) with nitrite (NO_2^-) to yield nitrogen gas (N_2) and water, involving the uncommon and toxic intermediates nitric oxide and hydrazine [1]. The reactions of the anammox metabolism take place in a dedicated cellular compartment known as the "anammoxosome". As chemolithoautotrophs, anammox bacteria fix carbon dioxide through the Wood-Ljundahl pathway which needs a supply of electrons. These are putatively supplied by a nitrite:nitrate oxidoreductase (Nxr) which oxidizes NO_2^- to NO_3^- (E'_0 = +0.42 V). Interestingly, Nxr has been found to be present in fibre-like assemblies within the anammoxosome [2].

We determined the crystal structure of Nxr from *Kuenenia stuttgartiensis* at 3.0 Å resolution, which consists of a heterotrimer forming an octamer of trimers in the asymmetric unit with a coil-like arrangement. The largest subunit of the heterotrimer (NxrA) harbours the active site formed by a bis-molybdopterin cofactor. Five iron-sulfur clusters within the NxrA and NxrB subunits establish a path for electrons through the whole multiprotein complex towards a heme B cofactor in the NxrC subunit.

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LPP252

Investigation of the kinetochore structure by aberration-free and quantitative multi-color single-molecule localization microscopy imaging

I. Vojnovic*[1], D. Virant[1], J. Winkelmeier[1], M. Rigl[1], U. Endesfelder[1]
[1]*Max-Planck Institute for terrestrial Microbiology, Department of Systems and Synthetic Microbiology, Marburg, Germany*

A key element for proper DNA segregation during mitosis is the kinetochore, a multi-protein complex that links the centromeric chromatin to the microtubules attached to the spindle pole body [1]. Our work aims at constructing a detailed map of the human-like regional kinetochore of

Schizosaccharomyces pombe by single-molecule localization microscopy (SMLM).

For data acquisition, we set each kinetochore protein of interest (POI) into relation to two reference proteins (cnp1 at the centromere and sad1 at the spindle pole). This triple-color arrangement allows to triangulate the position of each POI and in time, to build a kinetochore model; assisted by visual analytics tools and resolved over the cell cycle at a nanometer resolution.

We rely on our recently developed mEos3.2-A69T [2,3] fluorescent protein variant, as it can be photoconverted by a novel mechanism using blue and infrared light, termed primed conversion (PC). This enables us to perform aberration-free multi-color imaging by combining mEos3.2-A69T with the UV-photoactivating protein PAmCherry to image two targets in the same spectral detection channel using the orthogonal PC and UV-activation modes [3]. The orientations of the complexes are determined by the conventional FP mScarlet-I, marking the spindle poles.

This way we determine protein stoichiometry, elucidate patterns in protein deposition and turnover, measure protein-protein distances. With this protein map we can visualize the assembly and regulation of the *in situ* kinetochore architecture for the very first time.

[1] Yamagishi et al. FEMS Microbiol Rev 2014 38(2)

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[3] Virant et al. Int J Mol Sci 2017 18(7)

LPP253

Creating a three-color fluorescent protein fusion tag library of kinetochore proteins to investigate chromosome segregation in *Schizosaccharomyces pombe*.

M. Rigl*[1], J. Winkelmeier[1], I. Vojnovic[1], D. Virant[1], U. Endesfelder[1]
[1]*Max Planck Institute for terrestrial Microbiology, Systems and Synthetic Microbiology, Marburg, Germany*

The kinetochore is a multi-protein complex consisting of more than 50 different structural and regulatory proteins involved in the process of segregating the replicated chromosomes during cell division [1].

We use Photoactivated Localization Microscopy (PALM) [2] to build a structural map of the kinetochore architecture of *S. pombe*. Our strategy is based on building a library of genetically modified strains where the different kinetochore proteins of interest (POI) are tagged by fluorescent proteins (FPs). Here, by always tagging three proteins, the POI and two reference proteins (sad1 at the spindle pole body (SPB) and cnp1 at the centromere), the whole kinetochore can be accessed. To make the construction of our three-color strain library fast and efficient, we adapted a cloning strategy based on Hayashi et al [3]. Here, we use Overlap Extension PCR [4] to generate POI-FP fusion DNA fragments. Then, we transform our constructs into *S. pombe*, where it is integrated at the native gene locus in the genome by homologous recombination. We first created a dual color reference strain (*S. pombe* sad1-mScarlet-I PAmCherry1-cnp1) to serve as template for the library. By tagging all POIs with our recently developed mEos3.2-A69T FP [5] using the template strain, we generated a library of currently 20 three-color *S. pombe* strains.

In this poster, we present the details of our library construction work as well as give insight into some first super-resolved imaging results of the kinetochore proteins.

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LPP254

Structural adaptations of photosynthetic complex I enable ferredoxin-dependent electron transfer

J. M. Schuller[1], J. A. Birrell[2], H. Tanaka [3,4], T. Konuma[5], H. Wulfhorst*[6,7], N. Cox[2,8], S. K. Schuller[9], J. Thiemann[6], W. Lubitz[2], P. Sétif[10], T. Ikegami[5], B. D. Engel[11], G. Kurisu[3,4], M. M. Nowaczyk[6]
[1]*Max Planck Institute of Biochemistry, Department of Structural Cell Biology, Martinsried, Germany*
[2]*Max Planck Institute for Chemical Energy Conversion, Mülheim a. d. Ruhr, Germany*
[3]*Osaka University, Institute for Protein Research, Suita, Japan*
[4]*Osaka University, Department of Macromolecular Science, Graduate School of Science, Toyonaka, Japan*
[5]*Yokohama City University, Graduate School of Medical Life Science, Tsurumi-ku, Japan*
[6]*Ruhr University Bochum, Plant Biochemistry, Faculty of Biology and Biotechnology, Bochum, Germany*
[7]*Daiichi Sankyo Deutschland GmbH, München, Germany*
[8]*Australian National University, Research School of Chemistry, Canberra, Australia*
[9]*Ludwig-Maximilians-Universität, Gene Center and Department of Biochemistry, München, Germany*
[10]*Université. Paris-Saclay, Institut de Biologie Intégrative de la Cellule (I2BC), IBITECS, CEA, CNRS, Gif-sur-Yvette*
[11]*Max Planck Institute of Biochemistry, Department of Molecular Structural Biology, Martinsried, Germany*

Photosynthetic complex I enables cyclic electron flow around photosystem I, a regulatory mechanism for photosynthetic energy conversion. We report a 3.3-Å resolution cryo-EM structure of photosynthetic complex I from the cyanobacterium *Thermosynechococcus elongatus*. The model reveals structural adaptations that facilitate binding and electron transfer from the photosynthetic electron carrier ferredoxin. By mimicking cyclic electron flow with isolated components in vitro, we demonstrate that ferredoxin directly mediates electron transfer between photosystem I and complex I, instead of using intermediates such as NADPH. A large rate constant for association of ferredoxin to complex I indicates efficient recognition, with the protein subunit NdhS being the key component in this process.

MTP255

Biogenesis of Iron assembly cofactors of*Vibrio cholerae* and its unique sodium pump (Na^+-NQR)

S. Agarwal*[1], M. Bernt[1], G. Fritz[1], J. Steuber[1]
[1]*University of Hohenheim, Microbiology (250b), Stuttgart, Germany*

Iron–sulfur clusters (Fe–S), the ancient, ubiquitous and versatile classes of metal cofactors are evolutionarily conserved in nature. Iron/sulfur centers are key cofactors of proteins with varied functions such as gene expression, DNA

repair, RNA modification, central metabolism and respiration [1]. Thus, themechanisms of biosynthesis of Fe/S centres and insertion into polypeptides have gained much importance in prokaryotes and eukaryotes [2]. Despite the essentiality of this process, the molecular mechanisms of assembly of these inorganic cofactors remains elusive yet in *Vibrio cholerae*. Our current study delineates the presence of different proteins involved in the assembly of Fe assembly cofactors using an extensive proteomic analysis in *Vibrio cholerae.* Analysis of the complete bacterial genome has revealed a putative gene, nqrM that is present only in Na^+-NQR-containing bacteria. Na-translocating NADH:quinone oxidoreductase (Na^+-NQR) catalyzes electron transfer from NADH to ubiquinone in *Vibrio cholerae*, coupled with Na^+ translocation across the membrane [3]. NqrM is hypothesised to deliver the iron-sulfur cluster in NQR [4]. Growth studies on the wild type and the nqr deletion mutant in iron deficient media were performed to analyse the expression of known Fe assembly factors by a proteomic approach and of NqrM by RT-PCR. Even though *nqrM* is on the same operon as that of the other *nqr (A-F)* genes, nqr deletion mutant showed 2.5 fold more expression of *nqrM* than that of wildtype strain. This indicates towards the fact that NqrM maybe invloved in the delivery of FeS clusters to proteins other than Na^+-NQR.

1.C Wachnowsky *et al Metallomics* **2018**

2.B Roche *et al Biophysica Acta* **2013**

3.J Steuber *et al Nature* **2014**

4.VA Kostyrko *et al Journal of Bacteriology* **2016**

MTP256

Outer membrane vesicles in an algae-bacteria coculture

J. Mansky*[1], H. Wang[1], S. Sanchez Garcia[1], N. Beier[1,2], C. Boedeker[3], M. Neumann-Schaal[4,5], J. Overmann[1,3], S. Engelmann[1,2], I. Wagner-Döbler[1]
[1]Technische Universität Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany
[2]Helmholtz-Center for Infection Biology (HZI), Proteomics, Braunschweig, Germany
[3]DSMZ, Department of Microbial Ecology and Diversity Research, Braunschweig, Germany
[4]DSMZ, Bacterial Metabolomic, Braunschweig, Germany
[5]TU Braunschweig, Department of Bioinformatics and Biochemistry, Braunschweig Integrated Centre of Systems Biology (BRICS), Braunschweig, Germany

Microorganism communicate to respond to environmental changes and coordinate responses. One possible method of communication is the production of outer membrane vesicles (OMV). These vesicles can contain DNA, signaling molecules, lipids or proteins and allow cell-cell communication. This mechanism is particularly important in dilute environments like the ocean. The marine alphaproteobacterium *Dinoroseobacter shibae* and the dinoflagellate *Prorocentrum minimum* form a so-called Jekyll and Hyde interaction, consisting of a mutualistic and a pathogenic phase. We were able to isolate OMVs from both organisms and attempted to clarify the composition and physiological role of these OMVs. Size and abundance of OMVs was assayedanalysed by electron microscopy and nanoparticle tracking analysis. Fluorescence microscopy revealed the presence of DNA in the vesicles, DNA was extracted and sequenced. *P. minimum* OMVs were analyzed with respect to their fatty acid composition. The proteome of *D. shibae* OMVs was determined, revealing an enrichment of specific proteins.

MTP257

Substrate-dependent cluster density dynamics in bacterial phosphotransferase system permeases

G. Benevides Martins*[1], G. Giacomelli[1], M. Bramkamp[1]
[1]Ludwig-Maximilians Universität München, Mikrobiologie, München, Germany

Many bacteria take up carbohydrates by membrane-integral sugar specific phosphoenolpyruvate-dependent carbohydrate:phosphotransferase systems (PTS). Although the PTS is centrally involved in regulation of carbon metabolism, little is known about localization and putative oligomerization of the permease subunits (EII) of PTS. Here, we analyzed localization of the fructose specific PtsF and the glucose specific PtsG transporters from *Corynebacterium glutamicum* using widefield and single molecule localization microscopy. PtsG and PtsF form membrane embedded clusters that localize in a punctate pattern. Size, number and fluorescence of the observed clusters change upon presence or absence of the transported substrate. In presence of the transport substrate, clusters significantly increased in size. Photo-activated localization microscopy (PALM) data revealed that, in presence of different carbon sources, the number of EII proteins per cluster remains the same, however the density of these clusters reduces. Our work reveals a simple mechanism for efficient membrane occupancy regulation. Clusters of PTS EII transporters are densely packed in absence of a suitable substrate. In presence of a transported substrate, the EII proteins in individual clusters occupy larger membrane areas. This mechanism allows for efficient use of the limited membrane space under varying growth conditions without need of protein removal and re-synthesis.

MTP258

Functional role of a small protein released via outer membrane vesicles from the plant pathogen *Agrobacterium tumefaciens*

L. R. Knoke*[1], S. U. Abad Hererra[2], B. H. Justesen[2], T. Günther-Pomorski[2], S. Schäkermann[3], J. Bandow[3], M. Aktas[1]
[1]Ruhr University Bochum, Microbial Biology, Bochum, Germany
[2]Ruhr University Bochum, Molecular Biochemistry, Bochum, Germany
[3]Ruhr University Bochum, Applied Microbiology, Bochum, Germany

Bacterial outer membrane vesicles (OMVs) are considered as novel players in intra- and interspecies communication and pathogenicity. They are shed from the outer membrane of Gram-negative bacteria containing proteins, peptides, genetic material and secondary metabolites. Although much is known about their content, the mechanism of content uptake by surrounding bacteria is not fully understood.

Using electron microscopy, we recently demonstrated that the phytopathogen *Agrobacterium tumefaciens* releases OMVs into the growth medium. Isolation and MS-based proteomic study of the OMVs revealed about 60 proteins with various functions. Among these, the small putative lipoprotein At8019 was identified, which is predicted to act as membrane-active toxin. The present study aims to investigate the biological role of the OMV-associated Atu8019 protein by combining genetic and biochemical approaches.

Deletion of *atu8019* did not influence general growth, biofilm formation, motility, virulence or susceptibility against various stress factors. Strikingly, overproduction of Atu8019 in *A. tumefaciens* triggered extensive cell aggregation and decreased cell membrane integrity as indicated by live/dead staining. We purified His-tagged Atu8019 and reconstituted it

into DOPC-liposomes to investigate its membrane-active properties *in vitro*. Atu8019-containig liposomes attached to giant unilamellar vesicles (GUVs) and to the cell envelope of some selected Gram-negative bacteria. However, Atu8019-liposomes did not attach to Gram-positive bacteria indicating that envelope interaction is limited to specific Gram-negative bacteria. In conclusion, our data suggest that Atu8019 might be involved in adhesion and fusion of *Agrobacterium* OMVs to surrounding Gram-negative bacteria.

MTP259
Unravelling Formate Translocation by FocA in *Escherichia coli*
M. Kammel*[1], D. Hunger[1], G. Sawers[1]
[1]*Martin Luther University Halle-Wittenberg, Microbiology, Halle a. d. S., Germany*

Formate is a key intermediate in mixed-acid fermentation of glucose in enterobacteria. The *E. coli* FocA channel translocates formate to the periplasm, where it can be oxidized to CO2 by formate dehydrogenases. This pentameric membrane protein belongs of the family of formate-nitrate transporters (FNTs), which share a common fold, properties and translocation mechanism. FNTs possess a conserved histidine residue in the translocation pore that controls anion transport through the hydrophobic channel via transient protonation. Regulation of bidirectional transport of formate by FocA appears to be dependent on the bacterium"s metabolic state. It has been suggested that besides passive export, FocA can respond to acidification of the environment by switching to active import of formic acid. This complex, pH-constrained translocation raises further questions regarding the details of the translocation mechanism. Here we report on how formate transport is influenced by specific amino acid residues and structural regions within FocA. Formate translocation with various FocA derivatives, including single amino acid exchange variants, N- and C- terminal truncated proteins and homologues (e.g. FocB), was investigated using an *in vivo* reporter system based on a formate-inducible promoter. Formate import was examined using cells lacking the formate-producing enzyme PflB, therefore keeping the intracellular formate level low. The data points out that the N-terminal region of FocA is essential for both formate import and export. Moreover, FocA was shown to be the major translocator of formate, since FocB could not compete with formate transport rates. Our results reveal essential structural features of transporters determining small molecule translocation in enterobacteria.

MTP260
Characterization of a bacterial phospholipid *N-methyltransferase* with membrane deformation activity
J. Kleetz*[1], L. Danne[1], M. Aktas[1], R. Erdmann[2], F. Narberhaus[1]
[1]*Ruhr-University Bochum, Microbial Biology, Bochum, Germany*
[2]*Ruhr-University Bochum, Institute of Biochemistry and Pathobiochemistry, Bochum, Germany*

While in eukaryotes the phospholipid phosphatidylcholine (PC) is very abundant, it is only rarely a component of bacterial membranes [1]. Intriguingly, a remarkable proportion of bacteria with PC-containing membranes are species that interact with eukaryotic hosts. One example is the plant pathogen *Agrobacterium tumefaciens*, which is unable to infect plants in the absence of PC [2]. In *A. tumefaciens,* the phospholipid *N*-methyltransferase (PmtA) catalyzes the *S*-adenosyl methionine (SAM)-dependent 3-fold methylation of the phosphatidylethanolamine (PE) headgroup to yield PC [3]. PmtA is a cytosolic protein, which binds reversibly to membranes via electrostatic interactions of two amphipathic alpha-helical regions with anionic lipids [4]. Membrane dissociation is induced by increasing PC concentrations. In addition to its enzymatic activity, PmtA deforms membranes containing the anionic phospholipid cardiolipin (CL) [5].

In the present study, we aim to unveil the catalytic mechanism of the methyltransferase and the membrane-deformation activity. A combination of biochemical, microscopic and spectroscopic approaches are applied to analyze the lipid substrate binding properties, protein accumulation and localization in *A. tumefaciens* and biological function of the membrane-deformation activity.

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MTP261
Structural and functional analysis of bacterial phospholipid biosynthesis enzymes from *Xanthomonas campestris*.
G. Vasilopoulos*[1], R. Moser[1], C. Simon[1], M. Aktas[1], F. Narberhaus[1]
[1]*Ruhr-University Bochum, Microbial Biology, Bochum, Germany*

Recent studies revealed that membrane composition and biosynthetic pathways in plant-interacting bacteria are different than in *Escherichia coli*. Bacterial membranes usually consist of phospatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL) [1]. In this study, phospholipid biosynthesis in the plant pathogen *Xanthomonas campestris* was investigated. Besides common bacterial lipids, this bacterium synthesizes phosphatidylcholine (PC), an important lipid for host-microbe interactions [2]. In *X. campestris*, PC production is independent of the two well-known bacterial pathways; the Pmt-catalysed PE methylation and the choline-metabolizing pathway, suggesting a novel route for PC synthesis in this bacterium. Previous studies showed that glycerophosphocholine (GPC) is acylated by an unknown acyltransferase (AT) to lyso-PC and in a second step to PC via the two putative AT Xc_0188 and Xc_0238 [3]. In order to biochemically characterize those enzymes, the respective genes were heterologously expressed in *E.coli*, and a *X. campestris* reporter strain expressing Flag tagged fusion-proteins was constructed. Assays for PC formation, localization, solubilization and purification under different conditions were conducted. Our findings will contribute to the expanding knowledge of bacterial lipid diversity.

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MTP262
Magnesium transport in cyanobacteria
A. C. Pohland*[1], D. Schneider[1]
[1]*JGU Mainz, Biochemie, Mainz, Germany*

Magnesium is the most abundant divalent cation in living cells. It serves as a cofactor in many enzymatic reactions and is required for various intracellular functions. CorA, MgtE and MgtA/B are the three super-families of Mg-transporters in prokaryotes. Solved crystal structures (CorA and MgtE) and diverse analytical experiments examined the sensing, the uptake, and the cell homoeostasis of magnesium. Cyanobacteria are prokaryotes, having distinct compartments and are the ancestors of modern chloroplasts. Our focus lies on the magnesium transport in the model cyanobacterium *Synechocystis sp. PCC6803* with a particular focus on the translocation across thylakoid membranes. We have designed *homologous genes knockout* mutants of the predicted CorA and MgtE channels, to investigate differences in their magnesium homeostasis. Furthermore, the MgtE channel has been fused to an m-turquoise2 fluorescent protein for its localization in living cells. Additionally, we have cloned the genes for overexpression and purification in *Escherichia coli* to analyze the channel properties in vitro.

MTP263
The UDP-galactose 4-epimerase HgdA and the ABC transporter components HgdB and HgdC are important for heterocyst function in *Anabaena* sp. PCC 7120
D. Shvarev*[1], I. Maldener[1]
[1]*University of Tuebingen, Tübingen, Germany*

Anabaena sp. PCC 7120 is a filamentous fresh-water cyanobacterium. It develops specialized cells, called heterocysts, when combined nitrogen is lacking in the environment. The heterocysts fix atmospheric nitrogen by the nitrogenase enzyme complex and provide it to vegetative cells. To protect the highly oxygen sensitive nitrogenase from oxygen, heterocysts follow several strategies, one of which is the building of a specific gas-impermeable envelope, consisting of an outermost polysaccharide layer and an underlying glycolipid layer [1]. Components of the glycolipid layer are exported by an efflux pump composed of the ABC transporter DevBCA connected to the outer membrane protein HgdD [2]. There are different ABC transporter systems, including several DevBCA homologues, present in the genome of *Anabaena* [3]. In the present study, we are focusing on one of these homologues, HgdBC [4, 5]. Curiously, its gene cluster is missing an ATPase subunit gene, however it has a gene coding for the epimerase HgdA instead. We will present phenotypes of created mutants in the genes *hgdB* and *hgdA*, which are not able to grow diazotrophically and have considerable defects in the heterocyst glycolipid layer composition. Additionally, we will show data on biochemical characterization of the HgdA enzyme and discuss possible roles of the gene cluster *hgdBCA* in the heterocyst maturation of *Anabaena*.

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MTP264
Minor porins as alternative antibiotic translocation routes in *E. coli*
D. Hörömpöli*[1], A. Berscheid[1], H. Brötz-Oesterhelt[1]
[1]*University of Tübingen, IMIT, Microbial Bioactive Compounds, Tübingen, Germany*

The outer membrane of Gram-negative bacteria is an effective barrier against a variety of harmful agents including antibiotics. Porins, water-filled channels embedded within the outer membrane, enable diffusion of small, hydrophilic molecules into the periplasm. *E. coli* expresses multiple different porins and especially the major porins OmpF and OmpC have been studied intensively as uptake opportunities for antibiotics. The regulation of expression of these porins is strongly dependent on growth conditions, allowing rapid adaptation to different environments. This ability is used by pathogenic bacteria as a mechanism to counter antibiotic pressure. In multiple clinical isolates the reduction of porin expression or even the total loss of specific porins was shown to result in higher antibiotic resistance (1).

Lower porin expression leads to reduced nutrient uptake and therefore fitness deficits. *E. coli* can compensate this handicap by producing minor porins, which are less expressed and more constricted in substance specificity than major porins (2). This backup strategy allows *E. coli* to survive in the presence of antibiotics as nutrient uptake is still ensured, even though at limited rates.

We investigated the role of minor porins in the context of antibiotic translocation by generating multiple porin deletion strains, susceptibility testing different mutants and visualising the amounts of different porins in outer membrane fractions.

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MTP265
Mixing *Mycobacterium tuberculosis* and *Corynebacterium glutamicum* Tat systems
E. J. Schniete*[1], L. Ostermann[1], R. Freudl[2], T. Brüser[1]
[1]*Leibniz University Hannover, Institute of Microbiology, Hannover, Germany*
[2]*Forschungszentrum Jülich GmbH, Institut für Bio- und Geowissenschaften 1, IBG1, Jülich, Germany*

In prokaryotes, Tat systems have the very unusual task to transport folded, usually globular and often even oligomeric proteins across the cytoplasmic membrane. These Tat systems can play important roles in respiratory pathways, as many Tat substrates are redox proteins that have to be transported after a cytoplasmic cofactor assembly. Some obligate respiratory organisms, such as *Mycobacterium tuberculosis*, even require functional Tat systems for survival.

To establish a system that permits an analysis of the *M. tuberculosis* Tat system in a non-pathogenic, genetically and biochemically better amenable high-GC Gram-positive bacterium, we attempted the functional transfer of the *M. tuberculosis* Tat components into *Corynebacterium glutamicum*. *C. glutamicum* can grow slowly without *tat* genes, although it requires a Rieske protein for efficient growth under oxic conditions [1].

Re-establishment of normal growth by introduction of *tat* genes in a Tat-deficient strain thus is a reliable tool to monitor Tat functionality. At first, we established a functional complementation system for *C. glutamicum tatA*, *tatB*, and *tatC* genes in a strain deleted in the chromosomal copies of that genes. Thereafter, we exchanged the coding regions of the *C. glutamicum tat* genes by those of *M. tuberculosis*. While a complete exchange of the system was not successful, the exchange of the TatC components resulted in an active mixed translocon, indicating that the systems must share remarkable similarity and most likely a high degree of functional independency of the individual components.

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MTP266

A second mode of energy conservation in acetogenic bacteria: Ech instead of Rnf as respiratory enzyme

M. C. Schoelmerich*[1,2], V. Müller[2]
[1]*University of Hamburg, Microbiology & Biotechnology, Hamburg, Germany*
[2]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: Acetogenic bacteria use the energy-neutral reductive acetyl-CoA pathway to fix CO_2 to acetate. During a chemolithoautotrophic lifestyle, energy conservation relies solely on the chemiosmotic mechanism. The gradient-establishing bioenergetic coupling site in some acetogens is the Rnf complex, but genomic data indicate that in others, it must be an energy converting hydrogenase (Ech) instead.

Objectives: The objective of this work was to biochemically identify and characterize a hitherto purely hypothetical Ech complex as bioenergetic coupling site in acetogenic bacteria.

Materials & methods: The bioenergetics of CO metabolism in the CO-grown thermophilic acetogen *Thermoanaerobacter kivui* were dissected. Furthermore, inverted membrane vesicles were prepared, to assess and characterize the energy converting hydrogenase and investigate its respiratory function.

Results: Resting cells catalyzed H_2 and ATP formation from CO, which was coupled to the chemiosmotic mechanism. Inverted membrane vesicles exhibited ferredoxin-dependent H_2 formation activity from CO. This activity was stimulated in an abolished electrical field. Furthermore, ferredoxin-dependent H_2 formation from CO led to the transport of $^{22}Na^+$ and a ΔpH formation. ATP synthase activity also led to a ΔpH formation.

Conclusion: In conclusion, this work demonstrated that (i) there is a second mode of energy conservation in acetogenic bacteria that relies on Ech rather than Rnf, (ii) a respiratory hydrogenase and an ATP synthase are a sufficient respiratory chain to facilitate microbial life depending on the chemiosmotic mechanism and (iii) a respiratory hydrogenase is a functional ion-translocating coupling site in a bacterium.

MTP267

Transport of citrate is mediated by a tripartite tricarboxylate transport system in *Advenella mimigardefordensis* DPN7T

L. Schäfer*[1]
[1]*WWU Münster, IMMB, Münster, Germany*

In this study, a tripartite tricarboxylate transport system (TTT) system in the biotechnologically interesting Gram-negative betaproteobacterium *Advenella mimigardefordensis* DPN7T was identified and investigated. To date, TTT systems of bacteria are only poorly characterized. In general, TTT systems are composed of a binding-protein-dependent three-component (TctA, TctB and TctC) transport system, associated with two regulatory components designated as TctD and TctE. The interaction between the periplasmic binding-protein TctC1 and ligands like citrate were investigated.

For this, marker-free deletion mutants of *A. mimigardefordensis* DPN7T were generated. The genes *tctABCDE1* (MIM_c39170-39210), *tctABDE2* (MIM_c22990-23030) and *tctDE3* (MIM_c17190-17200) were deleted. Additionally, the substrate-binding protein TctC1 (MIM_c39190) was purified after heterologous expression of *tctC1* in *Escherichia coli* C41 using the pET23a vector. Thermal shift assays were performed using different ligand concentrations (2.5 µM to 2000 µM) and 1 µM TctC1.

Deletions of genes encoding the TTT transport system (TctABCDE1, MIM_c39170-MIM_c39210) led to inhibition of growth of *Advenella mimigardefordensis* strain DPN7T with citrate indicating that TctABCDE1 is the transport system for the uptake of citrate. A dissociation constant *Kd* for citrate of 41.7 µM was determined. The triple deletion mutant *Advenella mimigardefordensis* ΔMIM_c39170-39210 ΔMIM_c22990-23030 ΔMIM_c17190-17200 showed leaky growth in comparison to the wildtype using α-ketoglutarate as the sole carbon source.

MTP268

Extracytoplasmic loop-loop interactions activate an ECF-type Co^{2+} transporter

S. N. Pecina*[1], M. Gäde[1], F. Finkenwirth[1], T. Eitinger[1]
[1]*Humboldt-Universität zu Berlin, AG Mikrobiologie, Berlin, Germany*

Energy-coupling-factor (ECF) transporters form a subclass of prokaryotic ABC importers for uptake of micronutrients including vitamins and the transition metal ions Co^{2+} and Ni^{2+}. Consistent with the common architecture of ECF importers, the Co^{2+} transporter $CbiMNQO_2$ comprises a membrane-bound substrate-binding unit (CbiM), two cytoplasmic ATPases ($CbiO_2$), and a coupling membrane protein (CbiQ). A special feature of metal-specific ECF transporters is their dependency on an additional membrane protein (here: CbiN) harboring an extensive extracytoplasmic loop. The 103 amino acid residues-containing CbiN includes two transmembrane helices connected by a 37 residues-long negatively charged loop. CbiN is essential for activity of the holotransporter, and, in the absence of $CbiQO_2$, activates CbiM to result in the minimal functional unit capable of Co^{2+} transport. Nevertheless, neither $CbiMQO_2$ nor CbiM form stable complexes with CbiN. $^{57}Co^{2+}$ uptake assays with recombinant *E. coli* cells identified the exact length rather

than the exact amino acid sequence of the CbiN loop as essential, since single replacements even of strongly conserved residues were tolerated whereas any deletions were not. An in silico 3D model predicted distinct interactions among segments of the CbiN loop and individual extracytoplasmic loops of CbiM. Those interactions were verified by site-specific crosslinking upon generation of CbiM/CbiN pairs with individual Cys residues. Our findings suggest that a network of transient CbiM-CbiN loop-loop interactions is instrumental in loading of Co^{2+} into the metal-binding pocket of CbiM.

MTP269

Identification and functional analysis of the building block of homooligomeric IM30 rings

B. Junglas*[1], R. Orru[2], A. Axt[3], S. Weber[3], H. J. Butt[3], E. Wolf[2], D. Schneider[1]
[1]*Johannes Gutenberg-University Mainz, Institute of Pharmacy and Biochemistry, Mainz, Germany*
[2]*Johannes Gutenberg-University Mainz, Institute of Molecular Biology, Mainz, Germany*
[3]*Max Planck Institute for Polymer Research, Mainz, Germany*

Cyanobacteria, as well as other photosynthetic organisms, which use oxygen as an energy source, express the *inner membrane-associated protein of 30 kDa* (IM30), also known as the *vesicle-inducing protein in plastids* (Vipp1). IM30 is localized at thylakoid membranes as well as at the cyanobacterial cytoplasmic membrane, and it is most likely involved in thylakoid membrane biogenesis and/or maintenance. IM30 is a member of the PspA/IM30 family, and one of the most remarkable features of this protein family is the tendency to form large homooligomeric rings of at least 1 MDa. In case of IM30, rings with masses >2 MDa and a variable rotational symmetry have been described. These rings are supposed to be formed by tetrameric building blocks, although the exact structure of the building blocks and the corresponding monomers remaines enigmatic.

We used SAXS analyses to determine the shape of a dimeric IM30 variant that allowed us to identify and model the building block of IM30 rings.To elaborate on the functionality of the tetrameric building block, we first looked at the membrane binding properties of a number of IM30 variants. We found, that IM30 variants with a decreased tendency to form higher-ordered oligomers have an increased membrane binding capacity. In line with this observation, the ability of IM30 variants to rescue damaged membranes also increased with a lowered oligomerization tendency.In in-liquid AFM analyses of IM30 on solid supported DOPG bilayers we found that the olgimerization state of the IM30 variants correlate to how efficient the proteins cover the bilayer with a "carpet-like" structure.

Thus, we conclude that solely the building block of IM30 rings is needed to exert the membrane protecting function, but not a complete ring structure.

MTP270

The mechanism behind bacterial lipoprotein release: Phenol-soluble modulins mediate TLR2 activation via extracellular vesicle release from *Staphylococcus aureus*

K. Schlatterer*[1], C. Beck[1], D. Hanzelmann[1], B. Fehrenbacher[2], M. Schaller[2], P. Ebner[1], M. Nega[1], D. Kretschmer[1], A. Peschel[1]
[1]*Universität Tübingen, Tübingen, Germany*
[2]*University Hospital, Tübingen, Germany*

The innate immune system uses Toll-like receptor (TLR) 2 to detect conserved bacterial lipoproteins of invading pathogens. The lipid anchor attaches lipoproteins to the cytoplasmic membrane and prevents their release from the bacterial cell envelope. How bacteria release lipoproteins and how these molecules reach TLR2 remains unknown. *Staphylococcus aureus* has been described to liberate membrane vesicles. The composition, mode of release, and relevance for microbe-host interaction of such membrane vesicles has remained ambiguous. We recently reported that *S. aureus* can only releases lipoproteins when surfactant-like small peptides, the phenol-soluble modulins (PSMs), are expressed. Herein we demonstrate that PSM peptides promote the release of membrane vesicles from the cytoplasmic membrane of *S. aureus* via an increase in membrane fluidity and we provide evidence that the bacterial turgor is the driving force for vesicle budding under hypotonic osmotic conditions. Intriguingly, the majority of lipoproteins is released by *S. aureus* as components of membrane vesicles and this process depends on surfactant-like molecules such as PSMs. Vesicle disruption at high detergent concentrations promotes the capacity of lipoproteins to activate TLR2. These results reveal that vesicle release by bacterial-derived surfactants is required for TLR2-mediated inflammation.Our study highlights the roles of surfactant-like molecules in bacterial inflammation with important implications for the prevention and therapy of inflammatory disorders. It describes a potential pathway for the transfer of hydrophobic bacterial lipoproteins, the major TLR2 agonists, from the cytoplasmic membrane of gram-positive bacteria to the TLR2 receptor at the surface of host cells.

MTP271

Cell membranes and the diversity of hydrophobic compounds that play a central role in its functioning and integrity

B. J. Tindall*[1]
[1]*Leibniz-Institut DSMZ, Braunschweig, Germany*

The cell membrane is the most important part of the cell, with as much as half of the genome encoding for products that are either integrated into it or directly associated with it. Although typically defined as a semi-permeable barrier it is becoming increasingly clear that the transport of material in and out of the cell is carried out in a regulated fashion. While proteins constitute a significant part of the cell membrane a key aspect of the structural components are lipids. The detailed study of lipids and their importance in the integrity and functioning of the cell membrane remains a fringe area and one where the full diversity remains to be fully appreciated. Given the fact that chemical and physical parameters play a central role in determining the functioning of lipids in the cell membrane, rather than being limited to a small number of compounds across all prokaryotes the full diversity of lipids remains to be documented. Documenting that diversity is the first step in beginning to appreciate the discontinuous distribution of different lipids in different evolutionary groups and the significance these differences have in membrane functioning/integrity.

MTP272

Recovery of the Peptidoglycan Turnover Product of the Atl Autolysin in *Staphylococcus aureus* involves the Phosphotransferase System Transporter MurP and the Unique 6-phospho-*N*-acetylmuramidase MupG

R. Kluj*[1]
[1]*Universität Tübingen, Tübingen, Germany*

The peptidoglycan of the bacterial cell wall undergoes a permanent turnover during cell growth and differentiation. In the Gram-positive pathogen *Staphylococcus aureus*, Atl is the major peptidoglycan hydrolase, involved in cell division, daughter cell separation and autolysis. Atl is a bifunctional *N*-acetylmuramoyl-L-alanine amidase/endo-β-*N*-acetylglucosaminidase that releases peptides and the disaccharide *N*-acetylmuramic acid-β-1,4-*N*-acetylglucosamine (MurNAc-GlcNAc) from the peptidoglycan. Here we revealed the recycling pathway of the cell wall turnover product MurNAc-GlcNAc in *S. aureus*. The disaccharide is internalized and phosphorylated by the phosphotransferase system (PTS) transporter MurP, which had been implicated previously in the uptake and phosphorylation of MurNAc. We show that MurP mutant cells accumulate MurNAc-GlcNAc and not MurNAc in the culture medium during growth. We further characterized a unique 6-phospho-*N*-acetylmuramidase, named MupG, which intracellularly hydrolyses MurNAc 6-phosphate-GlcNAc, the product of MurP-uptake and phosphorylation, yielding MurNAc 6-phosphate and GlcNAc. The corresponding gene *mupG* of *S. aureus* strain USA300 is the first gene within a putative operon, together with genes encoding the MurNAc 6-phosphate etherase MurQ, MurP, and a putative transcriptional regulator MurR. Using mass spectrometry, we observed cytoplasmic accumulation of MurNAc 6-phosphate-GlcNAc in Δ*mupG* and Δ*mupGmurQ* markerless non-polar deletion mutants, but not in wild type strain or the single mutant complemented with a MupG-expressing plasmid. MurNAc 6-phosphate-GlcNAc accumulation in *S. aureus* was increased in transition to stationary phase, in accordance with previous observations that recycling in *S. aureus* occurs primarily within stationary phase.

MTP273

An archaeal ATP synthase with a *c*-subunit consisting of four transmembrane helices with only one ion-binding site is able to synthesize ATP

D. Litty*[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: The ATP synthase of archaea (A_1A_O ATP synthase) is a chimeric enzyme complex that combines features of the V_1V_O ATPase from eukarya and the F_1F_O synthase from bacteria. A key feature of A_1A_O ATP synthase is the high diversity of *c*-subunits in the rotor domain. Analysis of the genome sequence of the acetogenic bacterium *Eubacterium limosum* revealed the presence of a gene cluster coding for an A_1A_O ATP synthase with a *c*-subunit consisting of four transmembrane helices (TMH"s) with only one Na^+-binding site.

Objectives: So far, it was assumed that an enzyme having a *c*-subunit with only one ion-binding site in four TMH"s lost its function as an ATP synthase. Since the only ATP synthase encoded in *E. limosum* is of the A_1A_O-type with only one Na^+-binding site in a *c*-subunit with four TMH"s, our goal was to purify and characterize the enzyme to check whether this enzyme acts as ATP synthase despite its unusual *c*-subunit.

Material & methods: The ATP synthase was purified to apparent homogeneity by chromatography and reconstituted into proteoliposomes in order to measure ATP synthesis.

Results: The 9-subunit A_1A_O ATP synthase was purified without loss of subunits. ATP hydrolysis was Na^+-dependent. After reconstitution of the enzyme into liposomes, ATP synthesis could be driven by an electrochemical Na^+ potential. ATP synthesis was inhibited by DCCD.

Conclusion: This is the first experimentally proof that an ATP synthase with a *c*-subunit having four transmembrane helices and only one ion binding site is capable of ATP synthesis.

MTP274

Gain-of-function mutations in the phospholipid flippase MprF confer specific daptomycin resistance

C. Ernst[1], C. Slavetinsky[2,3], S. Kuhn[2], J. Hauser*[2], M. Nega[2], N. Mishra[4], A. Bayer[4], A. Peschel[2]
[1]*Department of Molecular Biology and Center for Computational and Integrative Biology, Massachusetts General Hospital, Boston; Department of Genetics, Harvard Medical School, Boston; The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States of America, Boston, MA, United States*
[2]*Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen, Germany, Tübingen, Germany*
[3]*Department of General Pediatrics, Oncology/Hematology, University Children's Hospital Tübingen, Germany, Tübingen, Germany*
[4]*Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, United States*

Daptomycin is a calcium-dependent lipopeptide antibiotic of last resort against methicillin-resistant *Staphylococcus aureus* whose mode of action is not fully understood. Spontaneously daptomycin-resistant *S. aureus* mutants emerge during therapy, which often possess single nucleotide polymorphisms (SNP) in the *mprF* gene. MprF is a bifunctional bacterial resistance factor that synthetizes the positively charged lipid lysyl phosphatidylglycerol (LysPG) and translocates it subsequently from inner to outer membrane leaflet. This process leads to increased *S. aureus* surface charge and reduces the susceptibility to cationic antimicrobial peptides and antibiotics.

We proofed and characterized the most frequently occurring daptomycin resistance-associated MprF mutations in a defined genetic background.

Variants of *mprF* bearing reported SNPs were generated and analyzed in the *mprF* deletion mutant of SA113. We compared those MprF SNPs in MIC for daptomycin and other antimicrobials, production and translocation of LysPG, surface charge and intramolecular protein interactions.

We found that only some MprF mutations can reproducibly cause daptomycin resistance. Those SNPs did not alter LysPG synthesis, translocation or *S. aureus* cell surface charge. MprF-mediated resistance relied on a functional flippase domain and was restricted to daptomycin and the related cyclic lipopeptide antibiotic friulimicin B. Notably, those SNPs led to relaxed intramolecular domain interactions of MprF suggesting a novel resistance mechanism that alters the substrate range of the MprF flippase to directly translocate lipopeptide antibiotics or other membrane components with crucial roles in the activity of these antimicrobials.

MTP275

Comparison of the bacterial phospholipase PlaF and its human homolog ABHD6

C. H. Strunk*[1], F. Bleffert[1], D. Paffen[1], S. Schott-Verdugo[2], H. Gohlke[2,3,4], F. Kovacic[1], K. E. Jaeger[1,5]
[1]*Heinrich Heine University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Heinrich Heine University Düsseldorf, Institute of Pharmaceutical and Medicinal Chemistry, Düsseldorf, Germany*

[3]*Forschungszentrum Jülich, Institute of Complex Systems, ICS-6: Structural Biochemistry, Jülich, Germany*
[4]*Forschungszentrum Jülich, John-von-Neumann.Institute for Computing (NIC) & Jülich Supercomputing Centre (JSC), Jülich, Germany*
[5]*Forschungszentrum Jülich, Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany*

Enzymes of the α/β-hydrolase superfamily are found in virtually all organisms and have functional implications that are important to human health (Lord *et al.*, 2013) and bacterial pathogenesis (Flores-Diaz *et al.*, 2016). PlaF, a recently identified phospholipase A, has been shown to be a relevant virulence factor of *P. aeruginosa* with functions for swimming motility and biofilm formation. We identified a human α/β-hydrolase domain 6 esterase named ABHD6 which shares 49% sequence similarity with PlaF. Interestingly, PlaF and ABHD6 are both anchored to the plasma membrane *via* a transmembrane helix and may act on membrane bound lipid substrates.

So far, ABHD6 has only scarcely been studied biochemically; therefore, we have constructed an expression plasmid for the heterologous production of ABHD6 in *E. coli* BL21(DE3). The protein was purified after solubilization with detergents. *In vitro* activity measurements revealed that PlaF can readily hydrolyze the physiological lipid substrates of ABHD6, 2-arachidonylglycerol (2-AG) and bis(monoacylglycero)phosphate (BMP), although these lipids were not found in *P. aeruginosa*. Furthermore, we could show that ABHD6 hydrolyses various phospholipids and lysophospholipids, the natural substrates of PlaF. The similar substrate profiles of PlaF and ABHD6 are in agreement with the finding that a homology modeled structure of ABHD6 and the X-ray structure of PlaF differ only slightly (RMSDCα = 1.05 Å).

Interestingly, we observed that ABHD6 can at least partly take over the function of PlaF. Expression of the *abhd6* gene in a *P. aeruginosa* Δ*plaF*strain restored the swimming motility of this strain indicating that the function of these two enzymes retained preserved during evolution.

MTP276
PlaB, a novel intracellular phospholipase A of *Pseudomonas aeruginosa* with activity towards endogenous phospholipids

A. J. Weiler*[1], C. H. Strunk[1], J. Berger[1], K. E. Jaeger[1,2], F. Kovacic[1]
[1]*Heinrich-Heine-University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Forschungszentrum Jülich, Institute of Bio- and Geosciences IBG-1, Biotechnology, Jülich, Germany*

Pseudomonas aeruginosa is a serious threat for immunocompromised individuals including cystic fibrosis, AIDS and cancer patients, due to its pathogenicity and multiresistance against antibiotics (Gellatly *et al*.,2013). This Gram-negative bacterium produces and secretes various hydrolytic enzymes that mediate infections, e.g. through damage of host cell components (Jaeger *et al.,*2014). Several *P. aeruginosa* phospholipases were previously described as virulence factors facilitating the hydrolysis of the host membrane phospholipids what leads to cell lysis or modulation of host lipid signaling pathways (Flores-Diaz *et al*.,2016). Searching for novel phospholipases of *P. aeruginosa* PA01 we have identified gene *pa2927* encoding a protein (PaPlaB) with sequence homology to LpPlaB, a major intracellular phospholipase A (PLA) of *Legionella pneumophila*. PaPlaB purified from detergent solubilized membranes of *E. coli* C43(DE3) showed comparable PLA and lysoPLA activities. Mutation of the putative catalytic triad residues of PaPlaB, namely Ser79, Asp196 and His244, to alanine confirmed that all three residues are essential for phospholipid hydrolysis. *In vitro* substrate profiling revealed specificity of PaPlaB for phospholipids with long chain fatty acids while no preference for the phospholipid head group was observed. Disruption of gene *pa2927* in *P. aeruginosa* leds to the nearly complete loss of intracellular PLA activity indicating a function of PaPlaB related to the hydrolysis of endogenous phospholipids. To validate this hypothesis we have isolated phospholipids from the membranes of *P. aeruginosa* and could demonstrate their rapid *in vitro* degradation by PaPlaB. The role of this novel intracellular PLA for virulence of *P. aeruginosa* still needs to be elucidated.

MTP277
Structural and functional investigation of the novel ectoine uptake system EctI

Y. Hellmich*[1], L. Czech[2], I. Tascon[1], E. Bremer[2], I. Hänelt[1]
[1]*Goethe University , Biochemistry, Frankfurt a. M., Germany*
[2]*University, Marburg, Germany*

Transport proteins belonging to the Sodium Solute Symporter (SSS) family are secondary active transporters, which facilitate the uptake of substrates by the symport of sodium ions. They are involved in important processes such as osmoadaptation, uptake of nutrients and signaling molecules in many organisms. EctI, as a member of the SSS transporter family, mediates the uptake of the compatible solutes ectoine, hydroxyectoine and glycine betaine. EctI is therefore an important factor in the adaption to hyperosmotic stress of its marine host organism *Hyphomonas neptunium*.

The project aim is to elucidate the substrate specificity of EctI and its underlying transport mechanism. To achieve these goals, in vitro assays are performed investigating substrate binding and the coupling stoichiometry of transport. The structural basis of compatible solute transport is determined by structural investigations including LILBID-MS and X-ray crystallography.

Here we will present the purification of EctI using styrene-maleic acid copolymers and detergents, respectively. The function of His-tagged EctI is demonstrated both by *in vivo* osmostress protection growth assays and *in vitro* transport studies. LILBID-MS measurements demonstrate the monomeric state of EctI both in detergent and the lipid environment. Workflows using vapor diffusion and lipidic cubic phase crystallization will be depicted, which aim at solving a first structure of EctI.

MTP278
Analysis of intracellular pH and c-di-AMP levels in *Corynebacterium glutamicum* strains altered in ion homeostasis

D. Weixler*[1], T. Lhkaasuren[1], S. Reich[1], O. Goldbeck[1], G. M. Seibold[1]
[1]*Ulm University, Microbiology and Biotechnology, Ulm, Germany*

The cyclic dinucleotide cyclic diadenosine monophosphate (c-di-AMP) was recently discovered as a novel second messenger in bacteria, which besides others functions in the control of K^+ uptake and export in various Gram-positive bacteria. The monovalent ions K^+ and Na^+ play important roles for bacterial cells. The differential distribution of these ions underlies various physiological processes: The Na^+ gradient across the cell membrane enables transport via

Na^+/solute transport and pH-homeostasis under alkaline conditions via cation/proton antiporters. For pH-homeostasis under acidic conditions a K^+ uptake is required. In *C. glutamicum* Na+ ions are mainly taken up via Na^+/solute symport. K^+ is taken up in this bacterium only via the channel CglK. The export of Na^+ and K^+ is brought about by a set of 4 cation/proton antiporters: ChaA, NhaP, Mrp1 and Mrp2. Depending on culture conditions intracellular cation concentrations in *C. glutamicum* 4ΔCpa lacking all four antiporters and *C. glutamicum* Δ*cglK* are drastically altered compared to *C. glutamicum* WT. However, intracellular pH and c-di-AMP levels have not been analysed in these strains. By the use of the genetically encoded sensor protein pHlourin we here show that recovery of the intracellular pH after acidic shocks depends on the K^+ uptake via CglK and that intracellular pH is strongly affected by the lack of the cation-proton-antiporters. For analysis of intracellular c-di-AMP levels a sensor based on the *ydaO*-riboswitch from *Bacillus subtilis* was constructed genetically. Analysis in *C. glutamicum* 4ΔCpa and *C. glutamicum* Δ*cglK* revealed that c-di-AMP levels are low in cells requiring K^+ uptake. These results further illustrate the interplay of cation and pH-homeostasis with c-di-AMP signalling in *C. glutamicum*.

MTP279

Assembly of the unique potassium uptake system KtrAB

J. Stautz*[1], M. Schrecker [1], J. Vonck[2], H. Strahl[3], I. Hänelt[1]
[1]*Goethe University Frankfurt, Institute of Biochemistry, Frankfurt a. M., Germany*
[2]*Max-Planck-Institute of Biophysics, Frankfurt a. M., Germany*
[3]*Newcastle University, Centre for Bacterial Cell Biology, Newcastle upon Tyne, United Kingdom*

Upon a hyperosmotic shock bacterial cells are able to counteract the loss of water by a rapid uptake of potassium ions. In many bacteria this initial uptake is accomplished by the K^+ uptake system KtrAB. The system consists of two subunits: The K^+-translocating channel subunit KtrB, which forms a dimer in the membrane, and the regulatory cytosolic subunit KtrA, which associates to KtrB as an octameric ring. Thus, the architecture of the KtrAB complex strongly differs from the majority of prokaryotic ligand-gated K^+ channels, in which covalently tethered cytoplasmic domains regulate a single tetrameric pore. This raises the questions why nature has evolved this alternative system and whether the interaction and assembly of the two subunits KtrA and KtrB is a dynamic procedure allowing the adaptation to for example changing osmotic conditions.
Here, we present first insights into the dynamics of KtrAB. Assembly of the complex *in vivo* was investigated by fluorescence microscopy, using GFP-fused KtrAB variants in living *Bacillus subtilis* mutants, lacking endogenous K^+ uptake systems. Complementary, pull-out assays in combination with size-exclusion chromatography were performed to visualise and analyse complex formation *in vitro*. The kinetics of the complex assembly were determined using ITC. In addition, we present a new cryo-EM structure of KtrAB in its intact assembly, revealing the structural features which are essential for the complex formation and its regulation.

MTP280

The function of outer membrane vesicles of a soil-bound Acidobacterium

C. Boedeker*[1], H. Wang[2], P. Henke[1], M. Rohde[3], I. Wagner-Döbler[2], M. Neumann-Schaal[1], J. Overmann[1]
[1]*DSMZ, Braunschweig, Germany*
[2]*TU Braunschweig, Microbiology, Braunschweig, Germany*
[3]*HZI, Braunschweig, Germany*

The formation of outer membrane vesicles (OMV) is an important trait of many living cells. Their content can comprise e.g. biomolecules, genetic material or proteins that can be used for intra- and inter-species communication. Here we analyzed the function of secreted OMV of the Acidobacterium *Blastocatella fastidiosa,* isolated from semiarid savanna soil. Acidobacteria are ubiquitous, highly diverse but represent an under-sampled phylum. Their ecological functions range from the aerobic degradation of complex substrates within the carbon cycle to an anaerobic dissimilatory sulfur metabolism. However, the function of the production of OMV within this phylum is completely unknown.

By using time-lapse microscopy the formation and movement of OMV on the cell surface could be monitored. Culture conditions were improved to extract secreted outer membrane vesicles by tangential flow filtration and ultracentrifugation. To analyze the size and quantity of secreted OMV, SEM and the NanoSight system were used. By using DAPI staining, we localized DNA within the OMV. To understand its function, OMV-DNA was sequenced after a PMA (Propidium monoazide) and MDA (Multidisplacement Amplification) treatment, to elucidate its role. In addition, a metabolic profile of the OMV was analyzed, revealing new insights in the composition of outer membrane vesicles of Acidobacteria.

MTP281

The chloramphenicol/H^+ antiporter CraA of *Acinetobacter baumannii* reveals a broad substrate specificity

W. E. Foong*[1], H. K. Tam[1], J. J. Crames[1], B. Averhoff[2], K. M. Pos[1]
[1]*Goethe University Frankfurt, Institute of Biochemistry, Frankfurt a. M., Germany*
[2]*Goethe University Frankfurt, Department of Molecular Microbiology and Bioenergetics, Institute of Molecular Biosciences, Frankfurt a. M., Germany*

Acinetobacter baumannii is a worldwide nosocomial pathogen responsible for opportunistic infections. It belongs to the ESKAPE pathogens, emphasizing their capability to "escape" from common antibacterial treatments. Multidrug efflux pumps play a prominent role in the multidrug phenotype of *A. baumannii*. CraA, a drug/H^+ antiport member of Major Facilitator Superfamily from *A. baumannii*, was reported to be present and upregulated in clinical isolates. It was identified as the homolog of the multidrug efflux transporter MdfA in *E. coli,* but reported to confer resistance in *A. baumannii* to chloramphenicol only. We determined the substrate specificity of CraA in an *E. coli* background and identified residues involved in the binding of substrate and/or H^+. CraA was heterologously overproduced in *E. coli* and its substrate specificity was determined by drug susceptibility assays and whole cell fluorescent dye uptake experiments using ethidium and dequalinium as substrates. We used ACMA quenching in everted CraA variants containing membrane vesicles to characterize H^+/drug antiport process. The function of CraA in *A. baumannii* was determined by drug susceptibility assay using *A. baumannii* ATCC 19606 Δ*craA*. Our results showed that *craA* expression in *E. coli* resulted in multidrug resistance towards phenicols (chloramphenicol, thiamphenicol, and florfenicol), monovalent cationic compounds (ethidium, and TPP^+), antiseptics (chlorhexidine and dequalinium), a biocide (benzalkonium), and a chemotherapeutic agent (mitomycin C). In *A. baumannii*, however, CraA could only be shown to confer resistance towards phenicols (chloramphenicol, thiamphenicol, and florfenicol). We conclude that *craA* encodes a broad-spectrum efflux pump rather than a specific chloramphenicol transporter.

MTP282

L-Malate transporter DcuE of *Actinobacillus succinogenes* catalyses reversible exchange of C_4-dicarboxylates

M. N. Rhie[1], Y. Lee[1], Y. B. Cho[1], O. B. Kim*[1]
[1]*Ewha Womans University, Department of Life Science and Interdisciplinary Program of EcoCreative, Seoul, South Korea*

Rumen bacteria *Actinobacillus succinogenes* naturally produces a large amount of succinate, which is the result of fumarate respiration. Succinate production from anaerobic growth with C_4-dicarboxylates requires transporters catalysing uptake and efflux of C_4-dicarboxylates. Transporter DcuE (Asuc_1999) found in *A. succinogenes* belongs to the Dcu family and was considered the main transporter for fumarate respiration. The deletion of *dcuE* affected L-malate uptake of *A. succinogenes* rather than fumarate uptake. Time-dependent uptake and substrate competition assays demonstrated that L-malate is the most preferred substrate for uptake by DcuE. The Vmax of DcuE for L-malate was 20 µmol/gDW·min with Km of 57 µM. The Km for L-malate was 8 times lower than that for fumarate, and the catalytic efficiency of DcuE for L-malate was 7-fold higher than that for fumarate, indicating high affinity and high efficiency for L-malate. Furthermore, DcuE catalysed the reversible exchange of three C_4-dicarboxylates—L-malate, fumarate, and succinate. Under physiological conditions, the C_4-dicarboxylates were reduced to succinate. Therefore, DcuE is proposed as the L-malate/succinate antiporter in *A. succinogenes*. In addition, DcuE showed a differentiated membrane topology compared to DcuB and DcuA.

MTP283

Influence of bacterial lipopolysaccharide modifications on the efficacy of antimicrobial ionic liquids

T. Gundolf*[1], B. Rauch[1], R. Kalb[2], P. Rossmanith[1], P. J. Mester[1]
[1]*Institut for Milk Hygene, Milk Technology and Food Science, CD-MOMICO, Vienna, Austria*
[2]*Proionic Production of Ionic Substances GmbH, Grambach, Austria*

Introduction: Bacterial resistance to antimicrobials is a huge problem in food and clinical environments. The lipopolysaccharide (LPS) layer in Gram-negative bacteria provides an effective permeability barrier to antimicrobials. LPS mutations can drastically influence resistance to these substances. Novel antimicrobials, like ionic liquids (ILs), could therefore also be affected by the LPS.

Objectives: This study investigates the influence of *E. coli*LPS length modifications on the susceptibility to ILs as well as common antimicrobials.

Material&Methods: Susceptibility of three *E. coli* LPS gene-deletion mutants and corresponding wild-type to five QACS and ten ILs with varying number and length of alkyl chains was investigated applying a minimal inhibitory concentration test. The strains were also subjected to a disc diffusion test with 13 different antibiotics and 9 API- ILs based on the intracellularly active antibiotic nalidixic acid.

Results: Decreasing LPS length in *E. coli*resulted in an increased susceptibility to ILs with one alkyl side chain that was comparable to QACs. An increasing number of IL-side chains led to a decreased susceptibility, especially for the IL [C10,10mim]Cl no MIC difference between wild-type and LPS mutants were detected. Incorporating nalidixic acid into an IL did not increase the overall toxicity of the antibiotic, neither could this API-IL overcome the LPS barrier.

Conclusion: While known structure-activity relationships, like the side- chain effect, were confirmed for all strains, decreasing LPS lengths resulted in increased susceptibility to ILs and API-ILs. However, ILs with two and three alkyl side chains proved to be a promising alternative to address this resistance mechanism and are thus of interest for further study.

MTP284

Uncovering the principles of type IV secretion of membrane proteins in *Legionella pneumophila*

S. Malmsheimer*[1], I. Grin[1], L. Krampen[1], S. Wagner[1]
[1]*Eberhard Karls University Tübingen, Germany & German Center for Infection Research, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Section Cellular and Molecular Microbiology, Tübingen, Germany*

Section of Cellular and Molecular Microbiology, Interfaculty Institute of Microbiology and Infection Medicine, Eberhard Karls University Tübingen, Germany & German Center for Infection Research, Partner Site Tübingen

During the course of infection, *L. pneumophila* translocates a large number of effector proteins in a type IV secretion system (T4SS)-dependent manner into the host cell to promote intracellular survival and colonization. Among these effector proteins are integral membrane proteins that find their final destination in one of the host cell''s membranes. Transmembrane segments (TMSs) within these substrates can be recognised by the Signal Recognition Particle (SRP) during translation and promote inner membrane (IM) targeting and insertion. Since the T4SS has the ability to accept substrates from three different cellular compartments, the cytoplasm, the IM, and the periplasm [1], transmembrane domain (TMD) effectors might be translocated in a two-step secretion, with integration into the bacterial inner membrane as a first step and their extraction and secretion in a second step.

We aim to understand the targeting principles of TMD-effectors inside bacteria. Towards this end, we studied the membrane targeting and integration potential of 260 T4SS effectors of *L. pneumophila subsp. pneumophila Philadelphia 1.* We noticed that some proteins avoid inner membrane insertion by a fine-tuned lower hydrophobicity of their TMSs, which is similar to what we observed for T3SS substrates in *Salmonella Typhimurium* [2]. Other T4SS substrates, however, show a markedly high hydrophobicity of their TMSs. While these effectors have been reported to be *bona fide* T4SS substrates, we observe that their TMSs are in principle able to promote inner membrane insertion. Membrane fractionation of *Legionella pneumophila* and subsequent mass spectrometry support our notion that many TMD-substrates follow a two-step secretion pathway via an inner membrane intermediate.

[1] Nagai et al. 2001

[2] Krampen et al., Nat Commun. (2018)

MEP285

Synergistic effects of antimicrobial substances from some bacteria against human pathogens and mycotoxigenic fungi

H. G. Song*[1], D. S. Lee[1]
[1]*Kangwon National University, Biological Sciences, Chuncheon, South Korea*

Introduction: Propagation of microorganisms in cosmetics and foodstuffs not only deteriorates the quality of the products but also causes various diseases in human and therefore it should be prevented.

Objectives: Aim of this study is to evaluate the activity of antimicrobial substances produced by isolated bacteria against some human pathogens and mycotoxigenic fungi.

Materials & methods: Bacterial strains showing antimicrobial activity were isolated by agar well diffusion test. Their antimicrobial substances were extracted and analyzed, and their antimicrobial activity and synergistic effects between them were examined.

Results: Strains DS381, DS518, DS620 and DS1515 showed 15.3 to 35.3 mm inhibition zone diameter against most bacteria and yeast, and inhibited mycelial growth (~66.7%) and sporulation (~85.7%) of target fungi. The purified antimicrobial substances (lipopeptide, chitinase, siderophore, protease and anthracyclic antibiotics, etc.) from isolated bacteria exhibited low minimum inhibitory concentrations (0.0078-10,000 mg/ml) on target organisms. When the synergistic effect of antimicrobial substances was investigated, combinations of antimicrobial substance displayed synergistic effects against each different target organism (0 < fractional inhibitory concentration index < 0.75). In time-kill assays, most combinations reduced more than 105 in colony count of bacteria and yeast during 24 h. Also, combination of antimicrobial substances showed spore degradation (28.2~91.6%) and spore germination inhibition (97.2~100%).

Conclusion: These results suggest that isolated bacteria and antimicrobial substances may be utilized as an environment-friendly preservative and biocontrol agent against human pathogens and mycotoxigenic fungi.

MEP286

Biodegradation of mycotoxins and growth inhibition against mycotoxigenic fungi by *Streptomyces* spp.

H. G. Song*[1], J. S. Hwang[1]
[1]*Kangwon National University, Biological Sciences, Chuncheon, South Korea*

Introduction: Mycotoxins are common contaminants of stored grains and pose health hazards to human and livestock and economic loss in agricultural industry.

Objective: This study aims to explore degradation of aflatoxin B1 (AFB1) and fumonisin B1 (FB1) and antifungal activity against mycotoxigenic fungi by isolated bacteria.

Materials & methods: Mycotoxin degrading bacteria were isolated using coumarin medium, and their antifungal activity (inhibition of mycelia growth, sporulation, spore germination) and degrading capability of AFB1 and FB1 were measured.

Results: *Streptomyces sporoverrucosus* JS383 and *S. lavendulae* JS669 degraded AFB1 (0.1 mg/L) by 93.7 and 96.8%, respectively in nutrient broth (72 h, 30℃). They also degraded FB1 (0.1 mg/L) by 91.2 and 95.9%, respectively on same conditions. JS383 and JS669 showed excellent thermostability in AFB1 degradation (up to 121℃), and JS383 also displayed broad temperature range (4~75℃) for FB1 degradation. The antifungal activity of JS383 and JS669 was evaluated by co-culture with 3 strains of aflatoxigenic *A. flavus* (KACC44986, 45068 and 45146) and 4 strains of fumonisigenic *Fusarium* spp. (*F. fugikuroi* KACC46888 and 48352, *F. verticillioides* KACC48354 and *F. proliferatum* KACC48356). JS383 and JS669 effectively inhibited mycelial growth of target mycotoxigenic fungi (68.4~90.2%) and suppressed sporulation of target organisms up to 97.3 and 97.2%, respectively. They also displayed inhibition of spore germination by 99.0 and 97.3%, respectively. Ethyl acetate extracts of bacterial cultures showed low minimum inhibitory concentrations (1.25~5 mg/ml) on target mycotoxigenic fungi.

Conclusion: JS383 and JS669 can be used for mycotoxin biodegradation and control of mycotoxigenic fungi in food and feed industry.

MEP287

Deducing human impact on the environment via sedimentary DNA information from lake Tiefer See NE Germany

E. Nwosu*[1], D. Wagner[1], A. Brauer[1], S. Liebner[1]
[1]*German Center for Geosciences, Potsdam, Geomicrobiology, Potsdam, Germany*

Human impacts on biodiversity via climate warming and eutrophication are well known. These impacts advance the spread of harmful cyanobacteria in lakes around the world, which affects water quality and destroys the aquatic food chain. What remains largely unknown is how cyanobacteria dynamics have changed within the Holocene with increasing anthropogenic influence. Due to the preservation of seasonally laminated (varved) sediments, lake "Tiefer See" located in northeast Germany is an excellent climate archive for the Holocene and has been the focus of several paleolimnological studies. The DNA information of this archive, however, has been neglected so far. To investigate the suitability of sedimentary DNA as a paleo-ecological archive and to deduce if it can resolve natural and anthropogenic disturbances in the past, Illumina paired-end sequencing (NGS) and quantitative PCR of 16S rRNA fragments specific to Bacteria and Cyanobacteria was used to reconstruct microbial community dynamics from lake Tiefer See sediments spanning the last 355 years. The results show declining gDNA concentration with increasing depth (age). Community analysis revealed a high abundance of the genera *Prochlorococcus* which has not been previously described in lakes and Proteobacteria as the most important phylum with substantial preservation even in sediments dating back 1935 – 1676. Canonical correlation analysis (CCA) of microbial community composition and environmental parameters such as total nitrogen, đ13C, đ15N and reconstructed vegetation openness explained 42% of the compositional variation. These findings highlight the importance of amplicon sequencing in investigating the dynamics of past microbial communities in lake sediments and paves the way for Holocene cyanobacteria exploration.

MEP288

Microbial Isolation Source Ontology: #Tagging the origin of microbial strains

L. Reimer*[1], J. Sardà[1], S. Huang[1], A. Yurkov[1], S. Gronow[1], J. Overmann[1]
[1]*Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany*

Associated with almost every ever isolated microbial strain, the information where a strain was found is usually provided in databases as isolation source. For microbiologists the source of isolation is of utmost value as it can be used to

infer a possible ecological role of a species. The problem is that this information can be provided in any format ranging from a single word like "soil" to a very detailed description that contains every detail recorded during the sampling. This makes even simple queries like "all microorganisms found in the marine environment" for a large data set almost impossible.

To overcome this problem we developed a hierarchical vocabulary to supplement original isolation source entries with a set of tags, similar to hashtags used in social media. The vocabulary used for these tags is not limited to environmental parameters, but also includes host relations, biotechnological applications, and medical observations. As the result, each isolation source can be associated with multiple tags, all of which give the desired flexibility in describing even complex cases.

A total of 380 tags comprising the vocabulary were applied to annotate 25,000 isolation sources available in the database Bac*Dive*. To query this data a new online tool was developed. It allows to combine several tags at the same time and additionally to include geographic and taxonomic information. Queries can be visualized by displaying all associated tags for a taxonomic group in a krona plot. This gives an overview of the decisive factors and can be viewed as an ecological fingerprint for the selected group.

Applied to large data sets the microbial isolation source ontology enables new ways to analyze the relationship between taxonomy and ecological habitat.

MEP289

Evolutionary history of symbiotic digestion: Gut flagellates of termites and their bacterial symbionts

T. Kropp*[1], K. Meuser[1], S. Altinay[2], R. Radek[2], A. Brune[1]
[1]Max-Planck-Institut für terrestrische Mikrobiologie, RG Insect gut microbiology and symbiosis, Marburg, Germany
[2]Free University of Berlin, Institut für Biologie/Zoologie, Berlin, Germany

The digestion of lignocellulose in termites has been attributed to the acquisition of cellulolytic flagellates by an ancestral cockroach. Although it is well established that all lower termites are colonized by host-specific flagellate assemblages comprising both Parabasalia and Oxymonadida, the evolutionary history of this symbiosis is entirely in the dark. The analysis is hampered by a lack of phylogenetic information, particularly on the flagellate species colonizing the most basal host lineages. To date, there are no reports on the presence of oxymonads in termites of the family Stolotermitidae, and also the information on the phylogenetic relationships of their parabasalian symbionts is sparse. Here, we analyzed the diversity of parabasalian and oxymonadid flagellates in damp-wood termites of the genus *Porotermes.* Based on a phylogenetic analysis of their SSU rRNA genes and their morphological and ultrastructural features, we describe three novel, deep-branching lineages of Oxymonadia (*Termitimonas*, *Oxynympha,* and *Oxymonoides* gen. nov.). A detailed analysis of the bacterial symbionts associated with flagellates of the genus *Trichonympha* corroborated that different host lineages independently acquired their bacterial symbionts (Candidatus *Endomicrobium* and Candidatus *Adiutrix*). These findings significantly advance our understanding of the diversity of parabasalid and oxymonadid flagellates and provide important clues that will help in reconstructing the evolutionary history of symbiotic digestion in termite guts.

MEP290

Marine fungal community dynamics through a spring algal bloom

T. Priest*[1], M. Reich[2], R. Amann[1], B. Fuchs[1]
[1]Max Planck Institute for Marine Microbiology, Molecular Ecology, Bremen, Germany
[2]University of Bremen, Molecular Ecology, Bremen, Germany

Fungi contribute substantially to biogeochemical cycles in terrestrial and freshwater ecosystems, and are involved in, or mediate, many ecological interactions. It is known that freshwater fungi exist as parasites of microalgae, driving mass mortality of algal blooms, and as saprotrophs, contributing significantly to organic matter degradation. Whether fungi exhibit these same ecosystem roles in marine algal bloom dynamics is yet inconclusive. Here, fungal community dynamics throughout a coastal spring algal bloom is examined. Surface seawater samples, representing the different stages of an algal bloom, were collected from the research station "Kabeltonne" on Helgoland, Germany, in 2017. 18S rRNA tag sequencing was coupled to fluorescence *in situ* hybridisation (FISH) to elucidate community members and their abundance. The application of FISH on marine fungi is still in its infancy and as such, specific oligonucleotide probes are lacking for most major phylogenetic groups. Using a manually curated 18S ribosomal RNA database for fungi, ten specific oligonucleotide probes were designed targeting the major phyla and sub-phyla of the fungal phylogenetic tree. These probes are being tested and optimized on fungal cultures before application on plankton samples from Helgoland. The data obtained will then be subject to multivariate statistical analysis with co-occurring bacterioplankton, phytoplankton and environmental variables to ascertain driving factors or ecological interactions.

MEP291

A Complex and Diverse Community of Chlorophototrophic Bacteria Thrive in Hot Spring Microbial Mats

M. Tank*[1], Y. Shirotori[1], S. Yoshida[1], D. M. Ward[2], S. Hanada[1], D. A. Bryant[3]
[1]Tokyo Metropolitan University, Hachioji, Tokyo, Japan
[2]Montana State University, Bozeman, MT, United States
[3]The Pennsylvania State University, Philadelphia, PA, United States

Hot spring microbial mats harboring chlorophototrophic bacteria develop at 40 to ≤75º C, and contain heterogeneous microenvironments displaying strong vertical and horizontal physico-chemical gradients, overlaid with diel community activity patterns. These communities are typically dominated by *Cyanobacteria* and/or phototrophic *Chloroflexi* (*Chloroflexus* and *Roseiflexus*).

Our studies of slightly alkaline hot springs in Yellowstone National Park, using NGS sequencing methods and cultivation approaches revealed a highly complex and diverse community of chlorophototrophic bacteria in these mats. 17 different chlorophototrophic taxa with many ecotypes representing diverse members of 6 out of the 7 known chlorophototrophic bacteria containing phyla have been identified. Numerous novel phototrophs, e.g., *Chloracidobacterium thermophilum* (*Acidobacteria*), "*Ca*. Thermochlorobacter aerophilum" (*Chlorobi*), and "*Ca.* Chloranaerofilum corporosus" as well as "*Ca*. Roseilinea gracile" (both *Chloroflexi*), "*Ca*. Elioraea thermophila" (AAPB), and a novel genus of thermophilic purple sulfur bacteria (PSB) are in culture for in depth characterization and co-cultivation experiments. We also obtained cultures of novel *Chloracidobacterium* species, "*Ca.* Thermochlorobacter sp." and another novel genus of a

thermophilic PSB from similar hot springs in Bulgaria and Japan.

Our studies demonstrate hot spring microbial mats as treasure chests for the discovery and isolation of novel phototrophs; and multi-method approaches as a powerful tool. Omics analyses facilitate the discovery of unusual bacteria and disclose their metabolic potentials, leading to successful cultivation. Detailed analysis of the cultures will deepen our knowledge of their physiology and ecology, and strengthen reference databases.

MEP292

Establishment of a robust metaproteomics pipeline to investigate structure and function of the gastrointestinal tract microbiome

L. Gierse*[1], A. Meene[2], C. Wünsche[3], N. Siemens[4], D. Zühlke[1], D. Becher[3], T. Urich[2], K. Riedel[1]
[1]University of Greifswald, Microbial Physiology and Molecular Biology, Greifswald, Germany
[2]University of Greifswald, Bacterial Physiology, Greifswald, Germany
[3]University of Greifswald, Microbial Proteomics, Greifswald, Germany
[4]University of Greifswald, Molecular Genetics and Infection Biology, Greifswald, Germany

Over the past years, microbiome research became more and more important as numerous diseases, eg.g. IBD or *C. difficile* infections, have been found to correlate with microbiome dysbiosis. In contrast to most studies focusing on the composition of the communities by using 16S rRNA gene sequencing, we aim to elucidate the structure and function of the metabolically active gastrointestinal tract microbiome employing a metaproteomics approach.

To this end, we established a highly standardized and robust metaproteomic pipeline. First, feces sampled during a time period of 31 days were frozen and mechanically homogenized. Second, the resulting feces powder was subjected to a TRIzol based extraction protocol. Subsequently, the extracted proteins were separated via 1D SDS PAGE and peptides, derived by trypsin- digestion, were analyzed by state-of-the-art mass-spectrometry.

A preliminary database search using the UniRef100 Database identified about 4000 protein groups for each sample. Most of these protein groups belong to the phyla *Bacteroidetes* and *Firmicutes*. The three genera with most identified protein groups were *Prevotella*, *Lactobacillus* and *Clostridium*. While 53% of the identified protein groups are of unknown function, the other 47% could be assigned to functions involved in protein synthesis (32%), energy metabolism (6%) and protein fate (3%). In a next step we will employ a specifically designed database containing exclusively proteins from the phyla present in feces samples of our model organisms, which will result in a better metaproteome coverage.

The established metaproteomics pipeline will now be used to decipher in the metaproteome of healthy and influenza A infected animals aiming on identification of molecular infection markers on the proteome level.

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Unraveling the drivers of microbial communities in mangrove ecosystems

O. Jeske*[1], V. Helfer[2], K. Grunewald[3], R. U. Syrbe[3], C. Ulpinnis[4], B. Fedder [2], H. Reuter[2], S. Neumann[4], M. Zimmer[2], J. Overmann[1]
[1]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
[2]Leibniz Centre for Tropical Marine Research, Bremen, Germany
[3]Leibniz Institute of Ecological Urban and Regional Development, Dresden, Germany
[4]Leibniz Institute of Plant Biochemistry, Halle a. d. S., Germany

Mangroves represent economically important, yet from a microbiological perspective largely uncharted ecosystems which are characterized by the rapid turnover of organic carbon, particularly by high rates of remineralization in root associated sediment. This process is driven by bacteria and involves tight cross-domain interaction with floral and faunal communities and physicochemical parameters in the sediments. Using a combination of cutting-edge technologies in biology, chemistry and socio-ecology, we aim to unravel how these parameters together with human resource-use govern microbial processes in mangrove ecosystems.

In order to study principal differences in mangrove ecosystems on a global scale, we investigate six distinct regions: Singapore, Brazil, Colombia, South Africa, Oman and Australia. In each region, roots and rhizosphere from the major mangrove species and bulk sediments are examined. For the bacterial community, corresponding DNA and RNA extracts are analyzed using 16S rRNA sequencing to compare the abundant versus the active community.

The analysis of 455 samples revealed a high diversity of mangrove associated bacteria. Notably, up to 55% of the identified bacterial sequences cannot be resolved below kingdom level. In addition, the most abundant taxa are not reflected in the most active. Aside from a clear difference in root and bulk sediment communities, we detected significant differences between the bacterial root communities of individual mangrove species.

This integration of microbial community data together with land use, environmental parameters and social aspects provides a basis for a better understanding of specific ecosystem processes for knowledge-driven conservation planning not solely in mangroves, but also for other coastal wetlands.

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Detoxification spectrum of various toxic substances by yeast-like symbiont *Symbiotaphrina* strains

J. Lee*[1], T. Engl[1], M. Kaltenpoth[1]
[1]Johannes Gutenberg University, Evolutionary Ecology Department, Mainz, Germany

It is questionable how the cigarette beetle, *Lasioderma serricorne*, survive from the attack of tobacco plant-derived toxin factors. Here, we hypothesize that tobacco-feeding insects are resistant to plant-derived toxic substances via the gut microbiome or specialized symbionts. To elucidate the detoxifying reactions provided by intracellular yeast-like endosymbionts *Symbiotaphrina kochii* and *Symbiotaphrina buchneri* of tobacco-feeding beetles, various plant-derived toxic substances were tested to culture of *Symbiotaphrina* strains. Firstly, *Symbiotaphrina* strains increased their susceptibility to higher tannic acid flavone concentration in CFU assay, but were found to grow when OD800nm was measured after 1 day. However, *Symbiotaphrina* strains exhibited more susceptibility to sorbic acid than other yeast

strains. Moreover, When *Symbiotaphrina* cells were incubated with tannic acid in vitro condition, we confirmed the degradation of tannic acid and decreased level of total phenolic compounds. These results suggested that *Symbiotaphrina* cells may utilize toxins and contribute to the survival of their host by detoxifying toxins.

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***Phaeobacter inhibens* DSM 17395 - Conquest of new ecological niches via horizontal gene transfer**

L. Birmes*[1], H. Brinkmann[1], S. Verbarg[1], S. Hahnke[1], S. Spring[1], J. Petersen[1]

[1]Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Background: Acquisition of plasmids via conjugation seems to be the most important strategy of *Rhodobacteraceae* to quickly adapt to varying ecological conditions. In this project the model organism *P. inhibens* DSM 17395 was used as the recipient to plasmid conjugation of genes such as the nitrate reductase from the denitrification pathway. Our aim was the conjugational transfer of two missing genes from distally related species. Generated transconjugants were expected to have acquired the capacity to reduce nitrate to nitrite, which was subsequently tested.

Methods: We labelled our plasmids of interest with resistant genes via undirected mutagenesis. Conjugation between recipients and donor strains was initiated in the presence of the respective antibiotic, which allowed us to screen for successful gene transfer. Multi-gene BLAST analyses were performed to determine the distribution of denitrification genes within *Rhodobacteraceae*.

Results: Phylogenetic analyses have shown that denitrification genes have a patchy distribution in the *Rhodobacteraceae* and are frequently located on extrachromosomal replicons (ECR). Two plasmid-located denitrification genes (*nar*, *nos*) could be successfully transferred by conjugations from *Marinovum algicola* DG898 and *P. inhibens* P72 into *P. inhibens* DSM 17395. In contrast to the wild type the newly generated transconjugant is able to reduce nitrate. Anaerobic growth should also be possible and is currently tested.

Conclusion: The newly generated transconjugant should be able to grow anaerobically and to obtain its energy from the denitrification pathway. The study proved the transfer of ECRs beyond the species border and thus demonstrated the bacterial potential of natural evolutionary adaptation to new habitats.

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Micrococcin P1 expression by a nasal *Staphylococcus aureus* isolate

S. Krauss*[1], B. Krismer[1], A. Peschel[1]

[1]Eberhard-Karls Universität Tübingen, Interfaculty Institute of Microbiology and Infection Medicine Tübingen - Infection Biology, Tübingen, Germany

The increasing number of resistant bacteria is causing an urgent need for novel antibiotics to combat the ongoing antibiotic resistance crisis. Bacteria from the human nasal microbiota have emerged as a promising source for antimicrobials. The steady competition for nutrients promotes the evolution of strategies to surpass competitors, for instance the production of antimicrobials. A collection of nasal isolates was screened for antimicrobial activity against epidemic *S. aureus* USA300, resulting in the identification of the USA300-inhibiting *S. aureus* isolate D4-19. The strain also exhibited strong antimicrobial activity against other clinical relevant bacterial pathogens. Genes required for the antimicrobial effect were identified by transposon mutagenesis. A putative bacteriocin-biosynthetic gene cluster located on plasmid pD4-19 with low similarity to biosynthesis genes of micrococcin P1 of *Macrococcus caseolyticus* plasmid pBAC115 was inactivated in the mutant. Mass spectrometry analysis confirmed that *S. aureus* isolate D4-19 but not the transposon mutant produces a compound with the mass of micrococcin P1. Despite the differences in the modification enzymes, *S. aureus* D4-19 and *M. caseolyticus* 115 produce identical MP1. This is the first report of micrococcin P1 production in *S. aureus* suggesting that nasal *Staphylococcus* strains can take up bacteriocin plasmids by horizontal gene transfer. Transformation of *S. aureus* RN4220 with pD4-19, which encodes an intrinsic penicillin resistance gene, enabled the ectopic production of micrococcin P1 in RN4220. These results support the notation that the human nose is a rich source for new antimicrobials of high diversity and interchangeability, which evolve continuously by horizontal exchange and recombination.

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Ecological role of plasmids in Siberian permafrost-affected soils

A. Gorecki[1], S. Holm*[2], M. Dziurzynski[1], M. Winkel[2], S. Liebner[2,3], D. Wagner[2,4], L. Dziewit[1], F. Horn[2]

[1]University of Warsaw, Institute of Microbiology, Warsaw, Poland
[2]German Research Centre for Geosciences, Section 5.3, Potsdam, Germany
[3]Universität Potsdam, Institute of Biochemistry and Biology , Potsdam, Germany
[4]Universität Potsdam , Institute of Earth and Environmental Science, Potsdam, Germany

Permafrost-affected soil is characterized by a low biomass, spatial heterogeneity and strong environmental fluctuations. The thawing of high Arctic permafrost environments cause a release of nutrients leading to higher microbial activity. These environmental conditions have been suggested to create a "hot spot" for plasmid exchange. Plasmids have the potential to rapidly transfer genetic traits between bacterial communities. Plasmid diversity and the plasmid-mediated plasticity of soil bacteria are studied by metagenomic sequencing of environmental DNA, pure isolates and transformed bacterial cultures. Our knowledge about the role of plasmids in the process of adaptation of microbial communities to changing environmental conditions in the Arctic is still limited. In this study, we applied structural and functional annotations of sequenced metaplasmidomes from two sites of the polygonal tundra of Samoylov Island, Northeast Siberia. This was combined with 16S rRNA gene sequencing, quantification of functional genes by qPCR, and PCR replicon typing for the identification of plasmid incompatibility groups. The metaplasmidome analysis shows that the original hosts of the plasmids are bacteria of the genera *Acinetobacter*, *Pseudomonas*, *Serratia* and *Janthinobacterium*. The taxonomic diversity of potential plasmid hosts does not resemble the bacterial diversity of the full environment. Sequenced plasmid genes show potential for mobilization (the presence of *tra* genes), microbial stress tolerance (multidrug efflux systems [SMR, RND]), heavy metal resistance genes (e.g.*Czc*D, *Ter*C), and systems for UV resistance. These traits suggest an important role of plasmids in the adaptation of certain bacterial taxonomic groups to harsh changing environmental conditions in the Arctic.

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Surviving the ferruginous Archean ocean – Assessing the potential toxicity of Fe^{2+} on basal Cyanobacteria in anaerobic conditions

A. Herrmann*[1], M. Gehringer[1]
[1]*Technische Universität Kaiserslautern, Microbiology, Kaiserslautern, Germany*

The oxygenation of early Earth"s atmosphere ~2.4 Ga ago, known as the great Oxygenation Event (GOE), was presumably caused by oxygenic photosynthesis by (proto-)Cyanobacteria. Prior, the Archean oceans were anoxic with high levels of Fe^{2+} (40-120 µM Fe^{2+}). However recent studies suggest that Fe^{2+} concentrations of >100 µM are toxic to modern, marine Cyanobacteria. (Swanner ED, Mloszewska AM, Cirpka OA, Schoenberg R, Konhauser KO & Kappler A, *Nat.Geosci*. **Vol. 8**(2015):126). This would have modulated their expansion in the ferruginous Archean oceans, necessary for the GOE. Studies to date have focused on closed systems with elevated CO_2, allowing the build-up of O_2. This study compairs the potential toxicity of Fe^{2+} on two basal strains of cyanobacteria in an atmosphere representing the Archean in a closed and open culture system.

Pseudanabaena PCC7367 & *Synechococcus* PCC7336, were acclimatised to an anoxic, elevated CO_2 atmosphere in buffered ASNIII Media with Fe^{2+}. Cultures were split between the two culture systems, exposed to increasing Fe^{2+} concentrations (15 µM, 120 µM & 600 µM) and monitored for chlorophyll and Fe^{2+}/ Fe^{3+} content for 21 days. Mid and late logarithmic cultures were assessed for respiration (CTC), viability (SYTOX Green) and Fe^{3+} precipitation using fluorescence microscopy. Media was assessed for dissolved oxygen, nitrate and phosphate content.

While the closed system controls indicated a similar Fe^{2+} toxicity response to that seen in the literature, the cultures grown in an open system showed interesting deviations in Fe^{3+} accumulation and growth morphologies.

This study emphasises the influence of experimental design and importance of using different strains in investigating ecological trends, especially during the period leading up to the GOE.

MEP299

The Good, the Bad and the Smelly – developing a representative model for the human axillary microbiome

S. Nordzieke*[1], B. Diesing[1], M. Singer[2], R. Wittlake[3], I. Lanfermann[4], S. Winkler[4], G. Schmaus[1], M. Pesaro[1], C. Koch[1]
[1]*Symrise AG, Global Innovation Cosmetic Ingredients, Microbiology, Holzminden, Germany*
[2]*Symrise AG, Global Innovation Scent&Care, Sensory Performance, Holzminden, Germany*
[3]*Symrise AG, Global Innovation Research&Technology, Analytical Research Center, Holzminden, Germany*
[4]*Symrise AG, Global Innovation Scent & Care, Fragrance Analytics, Holzminden, Germany*

Developing new deodorizing actives to fight human sweat malodor is challenging. High throughput laboratory screenings, performed with selected pure cultures, often turn out inefficient *in vivo*. This is due to the fact that these screenings ignore the natural diversity of the human skin microbiome. Thus, understanding the activity of the axillary microbiome in regard to malodor formation is crucial for the development of new deodorant actives.

We established an *ex-vivo* human sweat model that combines the advantages of *in vitro* and *in vivo* studies covering sensory performance, chemical, and microbiological analyses.

The experimental setup was validated with the natural development of fresh human sweat. We were able to show the representative conversation of non-odorous precursors towards odorous products, production of malodourous volatiles from amino- or fatty acid degradation, and a general increase of odor intensity based on a shift in microbiome composition and abundance.

Consistently, we applied Triclosan, a well-known antimicrobial agent frequently used in personal hygiene applications. Sensory analysis revealed an inhibition of odor formation, confirmed by chemical and microbiological analyses. Despite this, microbiome analysis also revealed a potential drawback: Triclosan treated samples showed an enrichment of the genus *Pseudomonas*, which may have negative effects on human health.

In conclusion, the developed model represents the human axillary microbiome regarding its species composition and malodor formation. Therefore, its application will lead to a better understanding of the microbial ecology of the axillary microbiome, providing a shortcut towards the development of new deodorizing actives.

MEP300

Shifts in soil management regime affect bacteria which influence soil structure formation in a mine reclamation site

M. Vuko*[1,2], B. Cania[1], C. Vogel[2,3], M. Schloter[1,2], S. Schulz[1]
[1]*Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Research Unit Comparative Microbiome Analysis, Neuherberg, Germany*
[2]*Technical University of Munich, Freising, Germany*
[3]*TU Dresden, Dresden, Germany*

A major concern of post-mining agricultural reclamation is the development of soil structure due to its effect on erosion, crop growth, and nutrient leaching. Here, we examined the bacterial potential to influence soil structure formation via the production of exo- (EPS) and lipopolysaccharides (LPS) as soil gluing agents during two stages of reclamation: (i) the initial cultivation of alfalfa and (ii) the agricultural management phase. As the production of these substances is primarily triggered by environmental changes, we hypothesize that the relative abundance of the investigated genes will be higher in the second phase due to annual disturbance by tillage, and higher nutrient availability owing to fertilization. To test this, we used shotgun metagenomic sequencing to investigate bacteria potentially involved in the production of EPS (e.g. alginate, colanic acid, levan), and LPS, at sites representing a chronosequence of an open-cast mine reclamation. The potential to produce EPS and LPS increased in the first year of alfalfa cultivation, and peaked directly after the transition from the initial to the agricultural management phase. At this time point, *Sphingomonadaceae* showed the highest EPS and LPS production potential. This family is known for EPS production and characteristic sphingolipids on the cell membrane. After twenty years of agricultural practice, the potential to produce EPS and LPS decreased, and the genes were largely harboured by the *Nitrospiraceae* family. These results indicate that the potential of bacteria to influence soil structure formation increases as a response to changes in the reclamation process, and decreases as the community adapts in each stage. As a second step, metagenomic data will be supported by EPS content measurement.

MEP302

Fungal networks in subtropical Chinese forests

C. Weißbecker*[1], A. Heintz-Buschart[1,2], T. Wubet[1,2], H. Bruelheide[2,3], F. Buscot[1,2]
[1]*Helmholtz-Centre for Environmental Research UFZ, Soil Ecology, Halle a. d. S., Germany*
[2]*German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany*
[3]*Martin Luther University Halle Wittenberg, Institute of Biology, Halle a. d. S., Germany*

Soil fungi are a highly diverse group of organisms, providing many ecosystem services, such as organic matter decomposition, element cycling, plant nutrition and plant protection. The subtropics harbor a high diversity of evergreen and deciduous tree species, enabling the investigation of a broad range of fungal functional groups. In 2009, the forest **b**iodiversity–**e**cosystem **f**unctioning experiment BEF China was established to study the effects of tree diversity on ecosystem functioning. Recently, we reported functional group-dependent effects of tree diversity on soil fungi from these forests. Yet, environmental conditions and plant composition explained only a fraction of fungal community assembly.

Microbial interactions could be another important facet of fungal community assembly and functioning. We investigate fungal interactions by means of fungal co-occurrence networks and correlation analyses. We hypothesize that the fungal communities present on low tree diversity plots (1 or 2 tree species) will comprise many specialist fungi while forests comprising a higher tree diversity of 4, 8 and 16 tree species will harbor more generalist fungi. We investigate fungal taxonomic and functional versatility of identified network clusters. Fungal network topology will show a higher degree and less modularity for the high tree diversity forests. We investigate whether there are fungal generalist taxa that are only able to grow within high tree diversity forests or specialist fungal species that are unique to monocultures or low tree diversity forests. This will yield a more detailed picture on the effect of tree species loss on soil fungal communities.

MEP303

Finding ssDNA prophages in bacterial genomes

D. Lücking*[1], F. Zucker[1], C. Moraru[1]
[1]*ICBM Oldenburg, Oldenburg, Germany*

Bacteriophages are thought to be the most abundant entities on earth and to impact biogeochemical cycles through viral lysis, horizontal gene transfer and bacterial metabolism manipulation. More than a third of the environmental bacteria could harbour temperate phages in their genomes. Most of the known prophages belong to the order *Caudovirales,* which contains exclusively dsDNA phages. From the two ssDNA virus families infecting *Bacteria*, only the *Inoviridae* are known to integrate into their host genome. The *Microviridae* contains to date only lytic isolates. Microvirus-like prophages have been identified bioinformatically only in a few bacterial genomes, belonging to *Bacteroidetes* and *Sphingomonadales*. We evaluated the existent tools for prophage prediction and found that they perform poorly in the identification of ssDNA prophages. This raised questions regarding the true extent of ssDNA prophage distribution and diversity in bacterial genomes. Therefore, we have developed a prophage identification pipeline specialized in the discovery of ssDNA prophages. The pipeline works in three steps. In a first step, all proteins from ssDNA viruses are clustered. In a second step, a protein database is built for all bacterial genomes of interest. In the last step, the protein clusters from step 1 are used as bait for prophage identification in all bacterial genomes from step 2. Initial testing of the pipeline on marine *Rhodobacteraceae* confirmed the presence of ssDNA prophages in several genomes. We are currently applying the pipeline to all bacterial genomes publically available in the NCBI database, with the further aim to characterize the genomic and taxonomic diversity of the predicted ssDNA prophages.

MEP304

Microbial ecology of hydrothermally active Kermadec vent field

B. Dede*[1], R. Zitoun[2,3], S. Sander[2,4], J. Thal[5], A. Türke[5], M. Walter[6], W. Bach[5], R. Amann[1], A. Meyerdierks[1]
[1]*Max Planck Institute for Marine Microbiology, Molecular Ecology, Bremen, Germany*
[2]*University of Otaga, Chemistry, Dunedin, New Zealand*
[3]*National Institute for Water and Atmospheric Research, Oceonography, Dunedin, New Zealand*
[4]*International Atomic Energy Agency, Nuclear science and applications, Monaco, Monaco*
[5]*University of Bremen, Geosciences, Bremen, Germany*
[6]*Institute of Environmental Physics, Bremen, Germany*

Hydrothermal vent fields are fascinating and highly dynamic habitats for microorganisms. They are gaining more and more importance since each year a considerable number of vents are newly discovered, suggesting that they contribute significantly to marine biogeochemical cycling. The microbial communities at these sites are poorly studied, although they are among the factors controlling emission of vent derived compounds into the far field environment. Therefore, we here characterized microbial communities and their metabolic potential across geochemical gradients in hydrothermal fluids and plumes of the Kermadec Arc region in the South Pacific. 16S rRNA tag analysis of fluid communities sampled at the submarine volcanoes Brothers and Macauley suggested a dominance of *Epsilonbacteraeota* and *Alcanivorax.* In contrast, clades SAR11 (*Alphaproteobacteria*), SAR324 (*Deltaproteobacteria*), SAR202 (*Chloroflexi*) and SUP05 (*Gammaproteobacteria*) were dominant in the hydrothermal plume. The high abundance of potentially sulfur-oxidizing SUP05 cells in hydrothermal plumes was additionally confirmed by fluorescence in situ hybridization. Analysis of genomic bins reconstructed from plume metagenomes provided insights into the metabolic potential of some of the most abundant clades in the plume ecosystems. Statistical analysis including geochemical data indicated a correlation of SUP05 clade abundance with total iron concentrations. However, a metabolic potential for iron oxidation could not be confirmed by the analysis of the genomic bins. Future analysis such as transcriptomics and enrichment cultures are planned to shed light on the expressed metabolic pathways and the ecophysiological niche of the microorganisms dwelling in this extreme ecosystem.

MEP305

Investigation of the role of the β-hydroxyaspartate cycle in a diatom-bacteria co-culture system

F. Severi*[1], L. Schada von Borzyskowski[1], P. Claus[1], T. J. Erb[1]
[1]*Max-Planck-Institut für terrestrische Mikrobiologie, AG Erb, Marburg, Germany*

Photosynthetic algae in the oceans are responsible for up to 50% of global carbon fixation, annually converting gigatons of carbon dioxide into biomass. In this context, photorespiration becomes a relevant process in the global carbon cycle. While higher plants salvage the toxic 2-phosphoglycolate through several energetically expensive reactions, many unicellular algae simply excrete formed

glycolate into the ocean water. This compound can then be utilized by heterotrophic bacteria.

Here we investigated the hypothesis that glycolate released by phytoplankton is assimilated by heterotrophic bacteria via the β-hydroxyaspartate cycle, whose recently discovered genes are widespread in marine Proteobacteria.

We addressed this question using a co-culture system based on the diatom *Thalassiosira pseudonana* and the heterotrophic marine α-proteobacterium *Ruegeria pomeroyi*. First, growth of the bacterial strain on glycolate was studied in detail. In the co-culture system growth was monitored *via* flow cytometry; glycolate concentrations in supernatants from axenic algal cultures and co-cultures were determined by liquid chromatography coupled to mass spectrometry.

We could show that *R. pomeroyi* is able to grow at µM concentrations of glycolate as sole carbon source. In co-culture, while being able to utilize a wealth of substrates excreted by *T. pseudonana*, the bacterium also feeds on glycolate produced by the alga. The involvement of the β-hydroxyaspartate cycle in this process is currently being investigated using reporter assays and omics approaches.

In conclusion, the β-hydroxyaspartate cycle might be an important route for the assimilation of glycolate produced by phytoplankton, thus representing a crucial and previously overlooked link in the global marine carbon cycle.

MEP306

Metagenomic approaches towards low abundance microbiomes: analyzing bacterial communities colonizing the human lung

H. Ghosh*[1], W. Behrens[2], A. Methner[2], F. Klann[2], C. Herzmann[3], S. Krauss-Etschmann[4,1], S. L. M. .[3,5,2], J. Sikorski[2], B. Bunk[2], J. Overmann[5,2]
[1]*Institute for Experimental Medicine,Christian-Albrechts-Universität zu Kiel, Kiel, Germany*
[2]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Department of Microbial Ecology and Diversity Research, Braunschweig, Germany*
[3]*Center for Clinical Studies, Research center Borstel, Borstel, Germany*
[4]*Leibniz Lung Center, Member of the Airway Research Center North (ARCN), Division of Early Life Origins of Chronic Lung Diseases, Research Center Borstel, Borstel, Germany*
[5]*Microbiology, Braunschweig University of Technology, Braunschweig, Germany*

The human lung represents a previously unrecognized microbial habitat which is of relevance for multiple aspects of human health and disease. These communities are accessible through bronchoalveolar lavage(BAL). However, one of the major obstacle in shotgun metagenome sequencing of low abundance microbiomes such as that of the human lung is the high abundance of human DNA, despite the much larger bacterial cell counts. This renders shotgun metagenomic sequencing highly inefficient and unpredictable. We developed and optimized a novel method for enriching microbial reads for shotgun metagenome sequencing and compared our method with available techniques for the depletion of human DNA (immuno capture or DNA-crosslinking). Our improved method comprises filtration to remove intact human cells and a subsequent modified DNase treatment to digest the remaining free human DNA. Applying the newly developed methodology, we assessed the beta-diversity of the lung microbiome across different probands and detected significant changes associated with different life styles.

Our metagenomic analyses revealed that our novel approach significantly improved the bacteria to human DNA ratio a 98.22±1.765% in untreated Vs 51.35±5.13% in BAL samples.

We propose that our novel approach could be a cost effective, easily applicable method to remove host-derived sequencing reads from human BAL fluid for the shotgun metagenomic sequencing.

MEP307

Abiotic factors shape single-cell metabolic heterogeneity in monoclonal microbial populations

F. Calabrese*[1], H. Stryhanyuk[1], F. Musat [1], H. H. Richnow[1], M. Thullner[2], L. Y. Wick[2], N. Musat[1]
[1]*Helmholtz-Zentrum für Umweltforschung GmbH - UFZ, Isotope Biogeochemistry Department , Leipzig, Germany*
[2]*Helmholtz-Zentrum für Umweltforschung GmbH - UFZ, Environmental Microbiology, Leipzig, Germany*

Populations of genetically identical microorganisms residing in the same environment can display marked variability in their metabolic traits. The relevance of such heterogeneity in natural habitats and the main factors that trigger metabolic heterogeneity are largely unknown. This is mostly because measurements of a statistically relevant number of single-cell metabolic activities of the same species in complex microbial communities are technically difficult and were not even possible until few years ago.

In this study we aim to understand if abiotic factors i.e. electron donors also used as carbon sources can cause metabolic heterogeneity in putative monoclonal bacterial populations and if such heterogeneity shapes the species degradation capabilities.

As model organisms, we used *Pseudomonas putida KT2440*, *Pseudomonas stutzeri* and *Thauera aromatica*, ubiquitous in terrestrial environments and known for their capability to degrade aromatic compounds. Batch cultures were grown with various concentrations of 13C-labelled acetate or benzoate under aerobic and anaerobic conditions. The combination of stable isotope probing and NanoSIMS allowed for the investigation of single cells 13C uptake during the exponential growth phase.

Our preliminary data show that intra-population heterogeneity is species dependent and is strongly influenced by electron donor concentration. These results have implications in understanding the ecological and functional role of metabolic heterogeneity and its potential impact on pollutant´s biodegradation in the environment.

MEP308

Manipulation from within – how does functional niche occupancy impact a next generation probiotic?

A. Ramirez Garcia[1], J. Zhang[1], E. Wortmann[1], M. Wandres[1], S. Sturla[1], C. Lacroix[1], C. Schwab*[1]
[1]*ETH Zurich, Zürich, Switzerland*

Next generation probiotics with defined function such as short chain fatty acid (SCFA) formation have been suggested to address microbial dysbiosis observed in gastrointestinal disorders. The gut microbe *Eubacterium hallii* produces butyrate from hexoses, or from lactate and acetate and forms propionate from 1,2-propanediol (1,2-PD). The key enzyme is a glycerol/diol dehydratase (GDH), which also uses

glycerol as substrate yielding the multicomponent system reuterin. Reuterin exhibits antimicrobial activity and transforms carcinogenic heterocyclic amines. Due to its functional versatility, *E. hallii* is considered a candidate next generation probiotic. However, little studies have been conducted on the role of functional niche occupancy if a microbe is added to complex microbiota.

We spiked *E. hallii* (10^7 cells/mL) to 8 different colonic microbiota in the absence and presence of glycerol or 1,2-PD. Using a combined molecular and bioanalytical approach, we determined the abundance of *gdh* (quantitative PCR), SCFA concentrations (HPLC-RI) and reuterin formation (nanoLC-MS/MS).

Spiking of *E. hallii* had little impact on SCFA profile. Supplement of glycerol decreased total SCFA production, whereas addition of 1,2-PD increased propionate levels in microbiota >log 4 *gdh*/mL. In low-*gdh* microbiota (<log 4 genes/mL), combined glycerol and *E. hallii* addition decreased acetate levels, increased butyrate production, and enhanced reuterin formation. Similarly, propionate levels increased after addition of 1,2-PD and *E. hallii* if the microbiota harboured >log 4 *gdh*/mL.

Our results indicate the possibility of selective manipulation of complex microbiota with a GDH positive microbe if the prevalence of microbes with the target function is low.

MEP309

Host-Gut Microbiota Interaction in the Drosophila model of Dextran Sodium Sulfate-induced Colitis

M. Hegab*[1], T. Roeder[1]
[1]*Christian-Albrechts University, Department of Molecular Physiology, Institute of Zoology, Kiel, Germany*

Inflammatory Bowel Diseases, are typical lifestyle diseases characterized by host-microbiota interaction in the gut milieu to modulate disease incidence and progression. Functional studies in animal models were intensively conducted to get a better understanding of the cross-talks between genetic factor and microbial dysbiosis. Interestingly, the fruit fly *Drosophila melanogaster* has become an invaluable model in translational research. Here, we present a Dextran Sodium Sulfate (DSS)- induced colitis model in the fruit fly system as a step towards recapitulating and better understanding major aspects of the disease. The induced phenotypes were very similar to those observed in the corresponding murine. Most impressive were induced leakiness of the gut (Septate junction genes) and an inflammatory response (JAK-STAT pathway) and dysplasia. We adopted an integrative platform to study the role of intestinal microbiome in established phenotype. Investigating germ-free animals together with targeted metagenomics (16s rRNA profiling) and microbiota transplantation supported the notion that bacterial dysbiosis is a cardinal pathomechanism. Decreased diversity indices and abundance of *Bacteriodetes, Actinobacteria* and *Proteobacteria* in the midgut were evident after DSS treatment. Transplantation of dysbiotic gut microbiota to germ-free animals has induced the phenotype without DSS indicating that dysbiosis plays a causal role in the disease induction.

MSP310

Alterations in gene expression of *Bacillus subtilis* caused by high salinity and the compatible solute glycine betaine

H. Rath[1], A. Reder[1], L. Steil[1], P. K. Sappa[1], T. Hoffmann[2], E. Bremer[2], U. Völker[1], U. Mäder*[1]
[1]*University Medicine Greifswald, Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Germany*
[2]*Philipps University Marburg, Laboratory for Microbiology, Marburg, Germany*

In its soil habitat, *B. subtilis* is frequently exposed to high salinity. By accumulating compatible solutes, bacteria are able to maintain turgor and to proliferate under hyperosmotic conditions. Compatible solutes are known to exert additional cellular effects, particularly through stabilization of proteins and nucleic acids. Previous studies of *B. subtilis* exposed to high salinity revealed profound alterations in gene expression, affecting multiple physiological processes: uptake/synthesis of osmoprotectants, cell wall metabolism and cell division, iron metabolism, endospore formation, and motility. In order to reveal additional facets of adaptation of *B. subtilis* to high salinity, we performed combined whole-transcriptome analysis using strand-specific tiling arrays and mass spectrometry-based proteome analysis. Moreover, we analyzed the effects of glycine betaine, one of the most widely distributed and effective osmoprotectants, on gene expression under hyperosmotic and standard cultivation conditions.

Continuous propagation of a prototrophic derivative of *B. subtilis* 168 with 1.2 M NaCl affected the expression of more than one-fourth of the protein-coding genes as well as numerous non-coding RNAs. Among the novel findings, strong downregulation of the two operons required for biofilm matrix formation expands our understanding of the impact of high salinity on the developmental pathways of *B. subtilis*, primarily mediated by the Spo0A and DegSU regulatory systems. A regulon-based analysis of the tiling array data indicated that additional factors might influence gene expression under conditions of high salinity. We could identify one such factor by showing that the Fur-mediated response to high-salinity-induced iron limitation is modulated by changes in DNA topology.

MSP311

Sodorifen emission in *Serratia* spp. – A reaction to biotic stress

N. Magnus*[1], B. Piechulla[1]
[1]*University of Rostock/ Institute of Biological Sciences, Biochemistry, Rostock, Germany*

Sodorifen is a novel volatile sesquiterpene, exclusively emitted by certain *Serratia plymuthica* strains. Its unique structure, resembling a polymethylated, bicyclic carbohydrate, is produced by a unique biosynthetic pathway, involving a SAM-dependent methyltransferase which induces simultaneous cyclization of the FPP substrate. [1] To date, no function could be assigned to the emission of sodorifen.

Only recently, promoter analysis revealed that transcription of the sodorifen biosynthetic gene cluster is strongly σs-dependent. Accordingly, the assumption was obvious that sodorifen emission is triggered as a reaction to stress. Using a highly sensitive VOC collection system and Northern blots, the effects of several abiotic (pH, NaCl, heat shock) and biotic stress conditions (fungal co-cultivation) on sodorifen emission as well as cluster transcription were assessed.

As a result, only biotic stress, i.e. growth in the presence of fungal volatiles, lead to increased sodorifen production. Interestingly, *Serratia plymuthica* reacted differently towards distinct fungal isolates ranging from sodorifen inhibition to high induction. Furthermore, co-cultivation with *Bacillus subtilis* also enhances sodorifen emission. [2]

Taken together, these results suggest a crucial role of this unique sesquiterpene in stress adaptation during microbial interactions, but may also act as a communication signal.

[1] von Reuß et al., JACS, 2018

[2] Kai, Piechulla, FEMS, 2018

MSP312

Genetic and metabolic plasticity recruits arginine metabolism for osmostress adaptation in *Bacillus subtilis*

D. Stecker[1], T. Hoffmann[1], H. Link[2], F. M. Commichau[3], E. Bremer*[1]
[1]*Philipps-Universität Marburg, Molekulare Mikrobiologie, Fachbereich Biologie, Marburg, Germany*
[2]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[3]*Georg August University Göttingen, Institute of Microbiology and Genetics, Department of General Microbiology, Göttingen, Germany*

Introduction: *Bacillus subtilis* synthesizes large amounts of the compatible solute proline (up to 0.6 M) when it is exposed to high osmolarity in order to balance the environmental and cytoplasmic differences in osmolarity and maintain turgor and cellular crowding within physiological boundaries. Because the anabolic proline biosynthesis route (ProB-ProA-ProI) is tied to the anabolic needs of the cell, *B. subtilis* possesses a specialized osmotically-controlled proline biosynthetic route (ProH-ProA-ProJ) (1).

Objectives: The ProA protein interconnects the two proline biosynthetic routes and disruption of *proA* interrupts both. Analysis of proline prototrophic Pro^+ suppressors revealed mutations in genes involved in arginine metabolism, that allow a RocD-dependent bypass of the ProB-ProA pathway (2).

Methods: Because all Pro^+ suppressors were proline prototrophs but failed to adapt to high osmolarity due to limited proline synthesis, we isolated a second generation of Pro^+suppressors, through selection for growth under high osmolarity and analyzed the resulting strains by genome sequencing and metabolomic studies.

Results: All second-generation Pro^+ suppressors carried single point mutations, which affect AhrC-dependent transcriptional regulation of the arginine metabolic genes. These cells produce two- to tenfold increased intracellular levels of proline and/or intermediates of the arginine pathway.

Conclusion: Transcriptional de-regulation of the arginine biosynthetic pathway redirects the arginine metabolism to synthesize large amounts of proline and/or intermediates of the arginine route and thereby restored the osmoadaptation properties of the mutants.

(1) Brill *et al.*(2011) JBacteriol. 193:5335-5346.

(2) Zaprasis *et al.*(2014) EnvironMicrobiol. 16:701-717.

MSP313

The AGXX® antimicrobial coating causes a thiol-specific oxidative stress response and protein *S*-bacillithiolation in *Staphylococcus aureus*

V. L. Vu*[1], T. Busche[1,2], T. Preuß[1], J. Kalinowski[2], J. Bernhardt[1,3], H. Antelmann[1]
[1]*Freie Universität Berlin, Institut für Biologie-Mikrobiologie, Berlin, Germany*
[2]*Bielefeld University, Center for Biotechnology, Bielefeld, Germany*
[3]*University of Greifswald, Germany, Institute for Microbiology, Greifswald, Germany*

Introduction: The antimicrobial surface coating AGXX® is a novel broad-spectrum antimicrobial composed of two transition metals, silver and ruthenium that can be electroplated on various surfaces, such as medical devices and implants. AGXX® has been shown to kill pathogens by production of reactive oxygen species (ROS). Since treatment options for methicillin-resistant *Staphylococcus aureus* infections are limited, ROS-producing agents are attractive alternatives to combat multi-resistant strains.

Question: Here, we were interested to investigate the effect of AGXX® on the changes of the redox homeostasis in *S. aureus* USA300.

Methods: We used RNAseq transcriptomics, redox biosensor measurements and phenotype analyses to study the mode of action of AGXX® particles in *S. aureus* USA300.

Results: In the RNAseq transcriptome, AgXX® caused a strong thiol-specific oxidative stress response and protein damage as revealed by the induction of the PerR, HypR, QsrR, MhqR, CstR, CtsR and HrcA regulons. The derepression of the Fur, Zur and CsoR regulons indicates that AGXX® also interferes with the metal ion homeostasis inducing Fe2+- and Zn2+-starvation responses as well as export systems for toxic Ag+ ions. The induction of the SigB and GraRS regulons reveals also cell wall and general stress responses. AGXX® stress was further shown to cause protein *S*-bacillithiolation, protein aggregation and an oxidative shift in the bacillithiol (BSH) redox potential. In phenotype assays, BSH and the HypR-controlled disulfide reductase MerA were required for protection against ROS produced under AGXX® stress in *S. aureus*.

Conclusion: Our study revealed a strong thiol-reactive mode of action of AgXX® in *S. aureus* USA300 resulting in an increased BSH redox potential and protein *S*-bacillithiolation

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MSP314

The impact of the bacilliredoxin pathway under oxidative stress and infection conditions in *Staphylococcus aureus*

N. Linzner*[1], V. V. Loi[1], S. Stenzel[1], M. Wirtz[2], R. Hell[2], K. Tedin[3], M. Fulde[3], H. Antelmann[1]
[1]*Institute for Biology-Microbiology, Berlin, Germany*
[2]*Centre for Organismal Studies, Department IV Molecular Biology Plants, Heidelberg, Germany*
[3]*Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany*

Introduction: *Staphylococcus aureus* is a major human pathogen, which can cause life-threatening diseases. During infections, *S. aureus* is exposed to reactive oxygen and chlorine species (ROS, RCS). Bacillithiol (BSH) functions as protection mechanism against ROS to maintain the reduced redox state. Under HOCl stress, BSH forms mixed disulfides with proteins, termed as *S*-bacillithiolations, which are reduced by the bacilliredoxins BrxA/B. The NADPH-

dependent pyridine nucleotide disulfide reductase YpdA is postulated to function as a BSSB reductase, but experimental evidence is missing.

Objectives: In this study, we investigated the impact of the bacilliredoxin (BrxA/B/YpdA) pathway in *S. aureus* under oxidative stress and infection conditions.

Materials & Methods: We used growth and survival assays, HPLC thiol metabolomics, redox biosensor measurements and infection assays with murine macrophages.

Results: The *ypdA* mutant showed a growth defect under sub-lethal NaOCl stress and the *brxAB* mutant was impaired in survival under lethal NaOCl stress. Both BrxAB and YpdA were required also for survival of *S. aureus* inside macrophages during phagocytosis assays. Quantification of the BSH and BSSB levels using HPLC thiol metabolomics revealed an increased BSSB level and a decreased BSH/BSSB ratio in the *S. aureus ypdA* mutant. Furthermore, we measured changes in the BSH redox potential using Brx-roGFP2 biosensor. The results showed that YpdA is required for the regeneration of the reduced state of the BSH redox potential after recovery from oxidative stress.

Conclusion: Our results indicate an important role of the Brx/YpdA pathway in the redox homeostasis under NaOCl stress and infection conditions in *S. aureus*.

MSP315

Identifying the players in the oxidative stress response of *Clostridioides difficile*

D. Troitzsch*[1], P. Wehmeier[1], S. Dittmann[1], K. Riedel[1], S. Sievers[1]
[1]*University Greifswald, Institute of Microbiology, Greifswald, Germany*

Introduction: *C. difficile* is considered to be a strictly anaerobic bacterium, therefore the presence of oxygen and reactive oxygen species should hamper its growth. However, previous studies have shown a high oxygen tolerance of the pathogen.

Objectives: To understand the high tolerance of *C. difficile* to oxidative conditions we will identify and describe candidates in the genome of *C. difficile* which are related to the oxidative stress response.

Materials & Methods: According to the annotations, we looked for potential players in oxidative stress reponse. A special focus was set on *perR* and its operon. We used CRISPR-Cas genome editing to generate knock-out mutants of the genes encoded in the *perR* operon. Moreover, we screened DNA 100 bp upstream of all oxidative stress genes for further PerR-Boxes.

Results: Screening of *C. difficile* genome revealed candidates putatively involved in the oxidative stress response and described in other bacteria to mediate tolerance against oxidative stress. In particular, a ruberythrin, encoded by *rbr1*, is highly abundant in *C. difficile* 630 Δ*erm*. The gene is located in an operon with the genes encoding the transcriptional repressor PerR; a desulfoferrodoxin and a glutamate dehydrogenase with a rubredoxin like structure at the N-terminus, proteins which are also highly abundant in the late exponential phase of growth in *C. difficile* 630 Δ*erm*.

Conclusion: *C. difficile* has a broad variety of poorly characterized players potentially involved in the oxidative stress response. This study sheds light on the function and interplay of proteins involved in detoxification of oxygen and reactive oxygen species and is therefore a starting point for the abrogation of oxidative adaptation mechanisms.

MSP316

Stable integration of the Mrx1-roGFP2 biosensor to monitor dynamic changes of the mycothiol redox potential in *Corynebacterium glutamicum*

N. T. Quach*[1], V. L. Vu[1], T. Busche[2,1], A. Nerlich[3], M. Mieth[3], J. Milse[2], J. Kalinowski[2], A. C. Hocke[3], H. Antelmann[1]
[1]*Freie Universität Berlin, Institut für Biologie-Mikrobiologie, Berlin, Germany*
[2]*Universität Bielefeld, Centrum fuer Biotechnologie (CeBiTec), Bielefeld, Germany*
[3]*Charité -Universitätsmedizin Berlin, Department of Internal Medicine/Infectious Diseases and Respiratory Medicine, Berlin, Germany*

Introduction: Mycothiol (MSH) functions as major low molecular weight thiol in the industrially important *C. glutamicum*. The redox-regulation of S-mycothiolated proteins is controlled by mycoredoxin 1 (Mrx1) which acts in concert with MSH and the MSH disulfide reductase to reduce *S*-mycothiolated proteins.

Question: Application of Mrx1-roGFP2 provided novel insights into redox changes of *M. tuberculosis* however Mrx1-roGFP2 has not been applied in the industrial platform *C. glutamicum*.

Methods: We genomically integrated an Mrx1-roGFP2 in *C. glutamicum* to measure dynamic changes of the E_{MSH} during the growth and under oxidative stress.

Results: *C. glutamicum* maintains a highly reducing E_{MSH} throughout the growth curve with basal E_{MSH} levels of ~-296 mV. Consistent with H_2O_2 resistant phenotype, *C. glutamicum* responds weakly to 40 mM H_2O_2, but is rapidly oxidized by low doses of NaOCl. We monitored basal E_{MSH} changes and the H_2O_2 response in various mutants which are compromised in redox-signaling of ROS (OxyR, SigH) and in the antioxidant defense (MSH, Mtr, KatA, Mpx, Tpx). While the probe was constitutively oxidized in the *mshC* and *mtr* mutants, a smaller oxidative shift in basal E_{MSH} was observed in the *sigH* mutant. The catalase was confirmed as major H_2O_2 detoxification enzyme required for fast biosensor re-equilibration upon return to non-stress conditions. In contrast, the peroxiredoxins Mpx and Tpx had only little impact on E_{MSH} and H_2O_2 detoxification. Further live imaging experiments using confocal laser scanning microscopy revealed the stable biosensor expression and fluorescence at the single cell level.

Conclusion: Mrx1-roGFP2 biosensor was successfully applied to monitor dynamic E_{MSH} changes during the growth, under oxidative stress and in different mutants.

MSP317

Making the stress-protectant Ectoine: Structural and Biochemical Insights into the Aminotransferase EctB from *Paenibacillus lautus*

A. A. Richter*[1], C. N. Mais[2,3], L. Czech[1], S. H. J. Smits[4], G. Bange[2,3], E. Bremer[1,3]
[1]*Philipps-Universität Marburg, Microbiology, Marburg, Germany*
[2]*Philipps-Universität Marburg, Chemistry, Marburg, Germany*
[3]*Philipps-Universität Marburg, LOEWE- Center for Synthetic Microbiology, Marburg, Germany*

[4]*Heinrich-Heine-Universität Düsseldorf, Biochemistry, Düsseldorf, Germany*

Introduction: Ectoine is a compatible solute widely synthesized by microorganisms as an osmoprotectant.[1] Due to its stabilizing effect on macromolecules, it is also addressed as a chemical chaperone and used for medical and biotechnological applications.[1,2] The L-2,4-diaminobutyrate aminotransferase (EctB) catalyzes the first step of the ectoine synthesis pathway, the amino-transfer from glutamate to the precursor L-aspartate-β-semialdehyde with L-2,4-diaminobutyric acid (DAB) and 2-oxoglutarate as products.[1,2]

Objectives: Here we focus on the biochemical characteristics and bioinformatic studies of EctB from the thermo-tolerant bacterium *Paenibacillus lautus*. To understand the biocatalytic mechanism of EctB, crystal structures and the biochemical characteristics of the enzyme are of interest.

Materials & Methods: A codon optimized *ectB* gene from *P. lautus* was cloned into expression vectors for heterologous expression in *E. coli*. The produced protein was purified and used for enzymatic assays or crystallization trials. The activity of EctB was assessed by HPLC-based quantification of glutamate.

Results & Outlook: Preliminary biochemical characterization of EctB showed an optimal working condition of the enzyme at 45 °C and pH 7.0. An *in silico* enzyme model reveals residue Lys274 as putative binding partner for the essential cofactor pyrodoxal phosphate (PLP). Site-directed mutagenesis studies will be performed to assess the influence of Lys274 on the enzyme activity. Further biochemical characterization of EctB, bioinformatics of *ect* gene clusters in *Paenibacilli*, and crystallization studies, are in progress.

[1] L. Czech, *et al*. (2018), *Genes. 9*, 177.
[2] J. M. Pastor, *et al*. (2010), *Biotechnol. Adv. 28*, 782–801.

MSP318

Comparison of the ROS detoxification capacities between methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* isolates

A. Sommer*[1], O. Krupper[1], V. V. Loi[1], B. Walther[2], M. Wirtz[3], R. Hell[3], H. Antelmann[1]
[1]*Freie Universität Berlin, Institute for Biology-Microbiology, Berlin, Germany*
[2]*Robert Koch Institute, Advanced Light and Electron Microscopy, Berlin, Germany*
[3]*Centre for Organismal Studies, Department IV Molecular Biology Plants, Heidelberg, Germany*

Introduction: *Staphylococcus aureus* is a commensal bacterium, but can cause serious life-threatening infections in humans and animals. In the case of methicillin-resistant *S. aureus* (MRSA) the treatment options are limited. Antibiotic resistance can lead to a fitness burden and redox changes, resulting in increased susceptibility to reactive oxygen species (ROS) under infection conditions, as shown for other pathogens.

Objectives: Here, we studied the impact of methicillin resistance on the changes in the bacillithiol (BSH) level, BSH redox potential and the ROS detoxification capacities of *S. aureus* isolates.

Materials & Methods: The Brx-roGFP2 biosensor was applied to measure the changes in the BSH redox potentials during the growth of various *S. aureus* isolates [1]. The intracellular BSH levels were measured by HPLC-thiol-metabolomics analysis. The BSH levels and BSH redox potentials were compared with the levels of ROS resistance as revealed by growth and survival assays under sodium hypochlorite (NaOCl) stress.

Results: The currently analyzed MSSA-isolates were generally more resistant to NaOCl stress compared to the MRSA-isolates. In addition, we could measure differences in the BSH redox potentials between few MRSA and MSSA isolates, but further experiments are required to draw conclusions about a link between the BSH redox potential and antibiotic resistance.

Conclusion: Our current data indicate that MRSA-isolates show lower ROS-resistance compared to MSSA-isolates and differences in the BSH redox potentials.

MSP319

A novel antimicrobial coating inhibits biofilm formation and reduces pathogenesis of MRSA

A. Vaishampayan*[1], A. de Jong[2], D. Wight[3], J. Kok[2], C. Meyer[4], U. Landau[4], E. Grohmann[1]
[1]*Beuth Hochschule fuer Technik Berlin, Berlin, Germany*
[2]*Univeristy of Groningen, Groningen, Netherlands*
[3]*Free University of Berlin, Berlin, Germany*
[4]*Largentec GmbH, Berlin, Germany*

Multi-drug resistant and biofilm forming pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA) are a major problem in health-care due to the challenges in treating detrimental infections caused by them. Hence, novel antimicrobials need to be developedurgently. AGXX® is a long lasting, self-renewable, novel antimicrobial surface coating consisting of micro-galvanic elements of silver and ruthenium. We studied the effect of AGXX® on biofilm formation in a clinical strain of MRSA (*S.aureus* 04-02981) using standard crystal violet assay as well as confocal microscopy. AGXX® strongly inhibited biofilm formation in this MRSA strain (1). We also tested the effect of AGXX® on the transcriptome of MRSA by total RNA sequencing. RNA sequencing data were analyzed via RNA-seq analysis pipeline T-REx, focusing on known biofilm, and virulence-associated genes.Many of these genes, namely, *agrC*(quorum-sensing regulator), *sdrC* (surface adhesin), *cap5A*(capsular polysaccharide synthesis), *lukE* (leukotoxin), and the enterotoxin gene, were down-regulated in presence of AGXX®.Based on our data, we conclude that AGXX® is an effective antimicrobial and a biofilm inhibitor. In addition, we are also studying the application of AGXX® in a two-component filter system where component one is functionalized graphene oxide covalently attached to a polymer (GOX) and component two is AGXX®. In the filter, GOX binds the free bacteria and AGXX® kills these bound bacteria thus resulting in successful filtration. This effective filter system can be used for a long term due to self-renewal properties of AGXX®.

1.Vaishampayan,A., de Jong,A., Wight, D.J., Kok,J., and Grohmann,E.2018.A novel antimicrobial coating represses biofilm and virulence-related genes in methicillin-resistant *Staphylococcus aureus*. *FrontMicrobiol.*9, 1–14.

MSP320

CO_2/HCO_3^- and PCA induced effects in the iron homeostasis of *Corynebacterium glutamicum*

F. Müller*[1,2], J. Rapp[2], A. L. Hacker[2], A. Feith[2], R. Takors[2], B. Blombach[1,2]
[1]*Technical University of Munich, Microbial Biotechnology, TUM Campus Straubing for Biotechnology and Sustainability, Straubing, Germany*
[2]*University of Stuttgart, Institute of Biochemical Engineering, Stuttgart, Germany*

Our previous work disclosed a regulatory link between the CO_2/HCO_3^- and iron induced regulatory response in *Corynebacterium glutamicum*. At high CO_2 levels an activation of DtxR was observed (Blombach et al., 2013), which is described as the master regulator of iron homeostasis in *C. glutamicum* (Wennerhold & Bott, 2006).

The goal of this study is to elucidate the interaction between CO_2/HCO_3^- and iron homeostasis in *C. glutamicum*.

We constructed a reporter strain which enabled the activation state of DtxR upon binding Fe^{2+} to be monitored *in vivo* by the means of eGFP production. Quantification of intracellular iron, reduction assays and LC-MS/MS analytics further supported our findings.

In accordance with previous experiments, increased DtxR activation was observed in the *C. glutamicum* reporter strain when it was cultivated at elevated levels of CO_2. Thus, retarded growth at standard CO_2 levels was linked to the lower intracellular Fe^{2+} availability. Supplementation of protocatechuic acid (PCA) - an iron chelator that is routinely added to cultivation media (Keilhauer et al., 1993) in some labs - also improved growth and eGFP production in the reporter strain was several fold higher compared to conditions without PCA. It turned out to our surprise, that the greater intracellular Fe^{2+} availability was not the result of enhanced iron uptake. Instead, Fe^{3+} was reduced to Fe^{2+} at the expense of PCA. The initial reduction rate was greatly enhanced in the presence of HCO_3^-. We hypothesize that HCO_3^- catalyzed Fe^{3+} reduction by functionalized aromatics might be widespread in nature.

MSP321

Proteomic profiles of *Vibrio vulnificus* grown in infection-related conditions

M. Conway*[1], L. Cutugno[2], A. Boyd[2], J. Pané-Farré[1]
[1]*Institute for Microbiology, Greifswald University , Microbial Physiology and Molecular Biology, Greifswald, Germany*
[2]*NUI Galway, Galway, Ireland*

Introduction: *Vibrio vulnificus* is a Gram-negative, marine dwelling halophile that causes infection from consumption of contaminated seafood or contact with contaminated water ranging from milder forms of gastroenteritis to more serious conditions such as septicaemia and necrotising fasciitis

Oxygen and iron depleted conditions are frequently encountered by the bacterium and successful adaptation to these conditions is necessary in order to survive in the environment or cause infection. A cluster of genes, *vvuR-vvuS-vvuT*, encoding the stressosome in *V. vulnificus* is associated with sensing and responding to stress in the Gram-positive model organism *Bacillus subtilis*. However, little is known on the function of this signal-sensing and integration complex in *Vibrio* species.

Objective: Comparing proteomic signatures between the wild type and stressosome mutant strains under different growth conditions aids in the identification of differentially expressed proteins whose expression may be influenced by the activation of the stressosome.

Methods: *Vibrio vulnificus* CMCP6 wild type and Vibrio vulnificus CMCP6 str. ΔRSTX were grown in chemically defined media under high and low iron conditions and prepared for global proteomic characterization.

Conclusion: In this study, proteomic profiles of *Vibrio vulnificus* CMCP6 and its isogenic stressosome mutant were generated and proteins that show strong regulation under iron limited conditions and during anaerobiosis were identified.

MSP322

Investigation of plasma resistance-mediating genes in *Escherichia coli*

T. Dirks*[1], M. Krewing[1], K. Weisgerber[1], F. Jarzina[1], J. Lackmann[1], J. Benedikt[2], J. E. Bandow[1]
[1]*Ruhr-Universität Bochum, Applied Microbiology, Bochum, Germany*
[2]*Christian-Albrechts Universität, Experimental Plasma Physics, Kiel, Germany*

There is a special interest in finding alternatives to antibiotics because of increasing antibiotic resistance. For some application cold atmospheric pressure plasmas can present such an alternative since they inactivate even multiresistent bacteria [1].

To investigate potential intrinsic mechanisms of plasma resistance, we screened a single-gene knockout library of *E. coli* for strains exhibiting increased plasma sensitivity. The collection constructed at KEIO university includes about 4000 strains, each missing one non-essential gene [2]. 87 mutants with increased plasma sensitivity were identified, indicating a potential function of these genes in mediating plasma resistance. The increased plasma sensitivity of four strains (Δ*iscS,* Δ*mntH,* Δ*rep,* Δ*cysB*) was verified with an independent quantitative CFU-based assay and complementation experiments. Overexpression of *iscS*, *rep,* and *cysB* led to survival rates higher than those of the wild type, indicating that plasma resistance can increase resulting in less sensitive strains. Of the 87 initial hits, 17 strains lacked a gene related to iron, sulfur, or [Fe-S]-cluster metabolism. *In vitro* analyses of [Fe-S]-proteins revealed that [Fe-S]-enzymes are more susceptible to plasma-induced inactivation than cluster-free enzymes. The overexpression of *sodA* and *katE* completely protected the [Fe-S]-enzymes from plasma-induced damage. Overall, our findings suggest that [Fe-S]-clusters limit survival of plasma stress and protective mechanisms mediate (low level) plasma resistance.

[1] **Lackmann J.W. & Bandow J.E.** (2014) *Appl Microbiol Biotechnol.* 98: 6205-6213

[2] **Baba T.** *et al.* (2006) *Mol Syst Biol.* 2: 2006-2008

MSP323

Physiological characteristics of biochemical processes of mycobacterial cells in dormancy and in reactivation

V. Nikitushkin*[1], G. Demina[1], M. Shleeva[1], A. Kaprelyants[1]
[1]*Russische Akademie der Wissenschaften, Föderales Forschungszentrum "Biotechnologie" ; A.N. Bach Institut für Biochemie, Moskau, Russian Federation*

Tuberculosis (TB) caused by the bacterial pathogen *Mycobacterium tuberculosis* is the leading cause of death from infectious diseases. According to the WHO, 1/3 of the world"s population is latently infected with TB, more than 8 Mio. people fall ill with active TB yearly. Mycobacteria have evolved various strategies to overcome the host"s immune response, e.g. transition into the state of non-culturability, or dormancy. Latent TB is associated with dormant state. One of the serious obstacles in development of new anti-TB drugs aimed at eradication of dormant forms is a lack of understanding of the mechanisms of reactivation – reverse transition from quiescent into active state. The main objective of the current study was the study of the most important physiological parameters such as changes in activities of key enzymes, respiration rates and values of TM potential ($\triangle\Psi$) of mycobacteria in dormant state and in reactivation. Analysis of the changes in the value of TM potential, allows qualitatively to establish the functionality of biochemical processes, and also to determine the moment when activation of metabolism happens. Application of the methods of flow cytometry and fluorescent microscopy allowed us to track the changes in values of the TM potential in reactivation of dormant *M. smegmatis* cells: the maximum level of TM potential can be achieved in 8 hours after the beginning of reactivation and remains on the same level till the beginning of bacterial division. The experimental data demonstrate the unique ability of mycobacteria to adapt to adverse environmental conditions: starvation, hypoxia, specific (TMC207) and non-specific (ciprofloxacin, kanamycin, cyanide) antibiotics. **Acknowledgement:** This current research has been supported by the RFBR grant 17-04-00564

MSP324

Identification and characterization of secondary active betaine, choline and carnitine transporters in *A. baumannii*

J. Breisch*[1], B. Averhoff[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: The nosocomial pathogen *Acinetobacter baumannii* is an emerging threat due to the increase in antibiotic resistant strains. An extraordinary trait of *A. baumannii* is its high desiccation and osmostress resistance, which is based on strong osmostress response mechanisms. An excellent osmostress protection mechanism is the uptake of solutes by betaine/choline/carnitine (BCC) transporters. *A. baumannii* has a whole set of BCC transporters indicating a role in adaptation to the clinical environment and the human host.

Objectives: We aimed to elucidate the role of six different BCC transporters in osmostress protection and in persistence of *A. baumannii* in the human host.

Materials & methods: Overexpression of BCC transporters in an *E. coli* strain lacking all compatible solute transporters, uptake studies with radioactively labelled compatible solutes and mutant studies.

Results: We characterized the unique BCC transporter, Aci01347, which transports choline and carnitine osmostress independent. Since carnitine was used by *A. baumannii* as sole carbon and energy source, Aci01347 plays an important role in metabolic adaptation. Furthermore, we identified two BCC transporters essential for choline uptake and three for betaine uptake. Interestingly, three of the six transporters are osmostress independent. Surprisingly, the betaine transporter BetTY, which is an osmostress dependent BCC transporter, has a short C-terminus, which questions the suggested role of the C-terminus of BCC transporters in regulation of osmostress dependent activity.

Conclusions: Our findings suggest that multiple BCC transporters play distinct roles in osmostress protection of *A. baumannii* but are also important for adaptation to clinical environments and the persistence in host cells.

MSP325

Adaptation of *Clostridioides difficile* to osmotic stress

A. M. Michel*[1], T. Hoffmann[2], E. Bremer[2], D. Jahn[1]
[1]*TU Braunschweig , Institute of Microbiology and Braunschweig Integrated Centre of Systems Biology (BRICS), Braunschweig, Germany*
[2]*Philipps-Universität Marburg, Department of Biology, Laboratory for Microbiology, Marburg, Germany*

Infections by the strict anaerobic, Gram-positive bacterium *Clostridioides difficile* are causing severe diarrheas which lead to several hundreds if not thousands deaths per year in Germany. Little is known about the gene regulatory, protein interaction and metabolic networks underlying the host associated life cycle of *C. difficile.* Here we are investigating the response of the bacterium to osmotic stress. Several osmoprotectant uptake (opu) transporters including proline transporter (put) play an important role in the protection of *Bacillus subtilis* against changing osmotic conditions [1]. The synthesis and uptake of proline is one of the first responses to osmotic stress in *B. subtilis* [2]. Blast analyses identified gene homologues of *B. subtilis opuC* and *putP* in *C. difficile*. To determine their functional role mutants deficient in the production of functional clostridial OpuC (CDIF630erm_01021) and PutP (CDIF630erm_03896) were generated and grown in minimal medium with increasing salt and sugar concentrations. Different osmolytes (e.g. glycine betaine, carnitine, choline) will be tested for their contribution to osmoprotection. The for *C. difficile* essential amino acid proline will be also studied as osmoprotectant. To obtain a holistic view on the osmotic protection process a systems biology approach encompassing RNAseq, proteomics and metabolomics experiments will be performed.

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MSP326

Mechanisms of maturation of bacterial sRNA-mRNA-cotranscripts

D. T. Spanka*[1], G. Klug[1]
[1]*Justus-Liebig Universität Gießen, Institut für Mikrobiologie und Molekularbiologie, Gießen, Germany*

During the past decades different types of non-coding RNAs (ncRNAs) were detected in prokaryotes. Within this class the small RNAs (sRNAs) soon became a main focus of interest. These sRNAs play a major role during gene regulation on a post-transcriptional level and can act in different ways to either facilitate or to impede translation of the targeted mRNAs.

RNA-Seq data of the model organism *Rhodobacter sphaeroides* indicated the presence of several mRNA-sRNA-cotranscripts. They consist of the coding sequence itself and an additional sRNA which is located in the 5' untranslated region (UTR) or in the 3'UTR. We are elucidating the mechanisms of maturation and aim to identify the RNases involved in this processing. The involvement of RNase E in the maturation of several sRNAs was already demonstrated (Förstner et al., 2018), but the role of other RNases needs further investigation. As a facultative photosynthetic alpha-proteobacterium *Rhodobacter sphaeroides* needs a tight regulation of photosynthesis genes to avoid photooxidative stress. We are investigating the regulation of processing during cellular stress, i.e. in response to reactive oxygen species (ROS). Moreover the aim is to analyze the processing of sRNAs during different growth phases as well as the regulation of the amount and activity required RNases.

Konrad U Förstner, Carina M Reuscher, Kerstin Haberzettl, Lennart Weber, Gabriele Klug. RNase E and the transcriptome of *R. sphaeroides.* Life Science Alliance Aug 2018, 1 (4) e201800080; DOI: 10.26508/lsa.201800080

MSP327

High-throughput proteomics of TisB-dependent persister cells in *Escherichia coli*

D. T. Spanka[1], A. Konzer[2], D. Edelmann[1], B. Berghoff*[1]
[1]University of Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany
[2]Max-Planck-Institute for Heart and Lung Research, Biomolecular Mass Spectrometry, Bad Nauheim, Germany

Bacterial populations produce phenotypic variants called persisters to survive harmful conditions. Persisters are highly tolerant to antibiotics and repopulate environments after the stress has vanished. During a persister life cycle, cells first enter the persistent state and then eventually recover in order to resume growth. High-throughput proteomics by mass spectrometry is a valuable tool to assess persister physiology during any stage of the persister life cycle, and is expected to considerably contribute to our understanding of persister cells. Here, an *Escherichia coli* strain, that overproduces the membrane-depolarizing toxin TisB, was established as a model for persistence by the use of high-throughput proteomics. Labeling of TisB persisters with stable isotope-containing amino acids (pulsed-SILAC) revealed an active translational response to ampicillin, including several RpoS-dependent proteins. Subsequent investigation of the persister proteome during postantibiotic recovery by label-free quantitative proteomics identified AhpF, a component of alkyl hydroperoxide reductase, and the outer membrane porin OmpF to be upregulated. Gene deletions of *ahpF* and *ompF* caused a delay in growth resumption, indicating that both gene products are important for the recovery process. Subsequent experiments showed that functions needed for the recovery process have to match the specific physiological state of a persister cell. Our study provides important insights into persister physiology and the processes behind recovery of depolarized cells.

MSP328

The oxidative stress regulator SoxS counteracts TisB toxicity in *Escherichia coli*

D. Edelmann*[1], B. Berghoff[1]
[1]University of Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany

Persister cells are phenotypic variants within isogenic bacterial populations. Due to their high tolerance toward stress, persister cells contribute to the survival of populations under harmful conditions by a bet-hedging strategy. The persistent state is characterized by a particularly low metabolic turnover, and is probably controlled by toxin-antitoxin (TA) systems. In *Escherichia coli*, the *tisB/istR-1* TA system was shown to drive persister formation upon DNA damage. Toxin TisB is a small hydrophobic protein that localizes to the inner membrane. Transcription of *tisB* is induced upon treatment with DNA-damaging agents. Translation of *tisB* mRNA is inhibited by two regulatory RNA elements: (i) an intrinsic secondary structure in the 5' UTR of *tisB* mRNA and (ii) the RNA antitoxin IstR-1. Upon enduring stress, some cells may, however, produce enough TisB to cause breakdown of the proton motive force (i.e. depolarization). Subsequent ATP depletion is believed to increase the probability of individual cells to enter the persistent state. Here we present evidence that depolarization is accompanied by an increased level of reactive oxygen species (ROS). Furthermore, we show that mRNA levels of the oxidative stress regulator SoxS are induced upon TisB over-expression, and that SoxS contributes to survival. Finally, simultaneous deletions of the *tisB* regulatory RNA elements and *soxS* cause a synthetic decrease in cell viability. We hypothesize that the capacity of a TisB-expressing cell to clear itself from ROS probably determines whether it may endure as a persister or is eradicated during the persister formation process.

MSP329

A multi-omics approach to evaluate natural products for *Clostridioides difficile* therapy

M. Brauer*[1,2], S. Wagner[3], J. Herrmann[4], C. Wolff[1], S. Sievers[1], R. Müller[4], T. M. Fuchs[3], K. Riedel[1,2]
[1]University of Greifswald, Institute of Microbiology, Greifswald, Germany
[2]Institute of Marine Biotechnology, Greifswald, Germany
[3]Federal Research Institute for Animal Health, Institute of Molecular Pathogenesis, Jena, Germany
[4]Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology, Helmholtz Institute for Pharmaceutical Research Saarland, Department Microbial Natural Products, Saarbrücken, Germany

Introduction: Antibiotic-induced infections with the anaerobic pathogen *Clostridioides difficile* (CDI) have become a major problem in healthcare settings. Therefore, new antibiotics for CDI that mostly spare the intestinal microbiota, and thereby, in contrast to many standard antibiotics, do not promote CDI, are urgently needed.

Objectives: Our multi-omics approach aims at deciphering the mode-of-action of the natural antibiotics Chlorotonil A/B (macrolides) and Chelocardin (atypical tetracycline) and at evaluating their potential as therapeutic agents to either treat or prevent CDI.

Materials & methods: Exponentially growing *C. difficile* cultures were exposed to sublethal concentrations of either Chlorotonil A, a Chelocardin derivative or the reference antibiotics Tylosin and Doxycycline. Samples of stressed and unstressed cells were taken 30 and 90 minutes after stress onset and the soluble protein fraction was analyzed by gel-free LC-IMSE. Furthermore, we currently analyze the impact of Chlorotonil A and Tylosin on the composition and functional capacity of the intestinal microbiota of piglets using a multi-omics approach.

Results: Chelocardin and, especially, Chlorotonil A/B show significant activity against human and

porcine *C. difficile* isolates (MIC ranges: 2-4 and 0.1-6.4 µg/ml) and show an individual substance-specific proteome signature. Moreover, Chlorotonil A appeared to be less harmful to the intestinal microbiota than Tylosin in our animal study.

Outlook: By integrating (meta-)transcriptome and (meta-)proteome data from our lab experiments and our animal study we will extensively characterize the mode-of-action and the activity spectrum of Chlorotonil A/B and Chelocardin to determine their clinical applicability in the context of CDI.

MSP330

Reduction of *N*-chlorinated amino acids: a new role for thioredoxin

M. Varatnitskaya*[1], K. Xie[1], N. Lupilov[1], L. I. Leichert[1]
[1]*Ruhr-University Bochum, Institute of Biochemistry and Pathobiochemistry, Microbial Biochemistry, Bochum, Germany*

HOCl, generated in the phagolysosome, can react with the biomolecules of bacteria that have been phagocytized by neutrophils. One of the main targets of HOCl are proteins. Reactions of HOCl with proteins lead to a variety of different protein modifications that are mostly deleterious, but sometimes activate proteins. Recently, it has been shown that HOCl-induced *N*-chlorination of positively charged amino acids activates the chaperone function of RidA from *E. coli*. The chaperone activity of *N*-chlorinated RidA ($RidA_{HOCl}$) could be reversed by the thiol-disulfide oxidoreductase TrxA, indicating a novel thioredoxin-induced reduction mechanism of *N*-chlorinated proteins. The aim of this work was to investigate the nature of the RidA-TrxA interaction. TrxA uses the attacking cysteine C32 to form mixed disulfides with its client proteins, which are then resolved by C35. We generated the mutant $TrxA_{C35S}$, which lacks the resolving cysteine and thus is not able to resolve mixed intermediates. Using catalytic amounts of $TrxA_{WT}$ and $TrxA_{C35S}$, we demonstrated that the resolving cysteine C35 is required for the inactivation of $RidA_{HOCl}$. $TrxA_{C35S}$ was, however, able to reduce $RidA_{HOCl}$, when used in stoichiometric excess, although, a higher excess was needed than for $TrxA_{WT}$. Based on the detection of a covalently linked RidA-$TrxA_{C35S}$ intermediate, we propose a model of N-Cl reduction by thioredoxin, based on intermolecular sulfenamide formation.

MSP331

Insights into the cell membrane protection module of *Bacillus subtilis*

L. Bewersdorf[1], L. Großmann[1], M. Reineck[2], K. Stetter*[1], M. Bramkamp[2], D. Wolf[1]
[1]*TU Dresden, Institute for Microbiology, Dresden, Germany*
[2]*LMU München, Department I, Microbiology, München, Germany*

In its natural habitat the soil, *Bacillus subtilis* has to adapt to environmental changes rapidly. The cell envelope of a bacterial cell represents the first and major line of defence, given that it is essential for bacterial survival. Therefore, the integrity of the bacterial cell envelope is monitored carefully by a network of cell envelope stress response (CESR) orchestrating signal transduction pathways. The phage-shock protein (psp) homolog LiaH together with the small membrane protein LiaI are the main targets of the LiaRS two-component system. However, the genome of *B. subtilis* also encodes a second Psp-like protein in the *pspA-ydjGHI* operon. Expression of *pspA* is controlled by the extracytoplasmic function sigma factor σ^W, which represents an additional part of the CESR network. The localisation of LiaH and PspA on the cell membrane under stress conditions as well as the dynamics of both proteins within the cell support the assumption that these proteins are playing essential roles in maintaining the integrity of the cell membrane of *B. subtilis*. Similar behaviour of homologous Psp-proteins in other organisms (*e.g.* PspA in *Escherichia coli*) also strongly support our hypothesis. Furthermore, recent findings revealed a third operon (*yvlABCD*) relevant for cell membrane protection. The *yvl* operon is also regulated by σ^W. Here, the cytoplasmic protein YvlB seems to serve as "adapter" that interacts with LiaH and PspA and consequentially connects the two psp-like responses. In summary, our data suggest that proteins of all three gene loci seem to collaborate in a complex interaction module counteracting cell membrane stress.

MSP332

Sulfolipids as trophical marker in a lacustrine freshwater ecosystem

B. Frommeyer*[1], J. Käppel[1], A. Lapointe[1], D. Martin-Creuzburg[2], D. Schleheck[1]
[1]*Universität Konstanz, Biology, Konstanz, Germany*
[2]*Universität Konstanz, Limnological Institute, Konstanz, Germany*

Sulfolipids (sulfoquinovosyl diacylglycerol, SQDG) are found almost exclusively in photosynthetic organisms [1], with the known exception of plant associated Rhizobiaceae [2]. SQDGs are known to act as surrogate for phospholipids in the marine environment under phosphate-limited growth conditions [3] and are vital for photosystem II function in the cyanobacterium *Thermosynechococcus elongatus* [4]. Using LC-MS, we demonstrate the presence of SQDGs in phytoplankton samples taken from oligotrophic Lake Constance. The lipid composition and quantity is compared to individual cyanobacteria, green algae and diatom species cultivated in the lab under phosphate-rich (eutrophic) and phosphate-limited (oligotrophic) growth conditions. We aim to establish the SQDGs as a marker for phototrophs and their nutrient status in the Lake Constance water column and, if present, in Lake Constance sediments. A sedimentary deposition of SQDGs, or of any of its degradation intermediates [e.g.,5], would allow also for retrospective assumptions on the trophic state of this lake, given its past eutrophication and re-oligotrophication events.

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MSP333

A c-di-AMP-dependent riboswitch, the three component system EsrISR, and the two component system MtrAB contribute to control of the *nlpC*-operon in *Corynebacterium glutamicum*

T. Weiß[1], T. Lkhaasuren*[1], S. Reich[1], G. M. Seibold[1]
[1]*Ulm University, Institute of Microbiology and Biotechnology, Ulm, Germany*

The Gram-positive *Corynebacterium glutamicum* is employed for the industrial production of amino acids and widely used as model organism for osmoregulation and cell wall synthesis. The *nlpC*-operon of *C. glutamicum* encodes two NlpC-like endopeptidases involved in cell separation and growth. Within the *nlpC* promoter region, binding sites for the

response regulators EsrR and MtrA are located upstream of the transcriptional start site and a putative c-di-AMP dependent riboswitch (RS). The nucleotide second messenger c-di-AMP is involved in potassium uptake, resuscitation, osmoregulation, and cell wall homeostasis in Gram-positive bacteria. In *C. glutamicum*, c-di-AMP inhibits potassium uptake by binding to the potassium channel CglK. The response of the *ydaO*-type *nlpC*-RS to c-di-AMP was analyzed in an *E. coli* test system: Expression of a reporter gene under control of the RS was inhibited in presence of c-di-AMP. Moreover, expression of *nlpC* was reduced in strains with increased internal c-di-AMP levels. The two-component system MtrAB controls genes for cell wall modification negatively and genes for uptake of compatible solutes positively. Analysis of transcript amounts and reporter assays in MtrAB deficient strains revealed only a minor influence of MtrAB on transcription of the *nlpC*-operon. However, application of a hyperosmotic shock lead to a transient MtrAB-dependent inhibition of *nlpC*-transcription. EsrISR represents a novel type of stress-responsive system, which is activated e.g. by antibiotics inhibiting the lipid II cycle. Transcription of the *nlpC* operon was also affected by the lack of EsrISR. Taken together, these results indicate that the c-di-AMP RS probably acts as gatekeeper to mediate the influence of EsrISR and MtrAB on the *nlpC* operon in *C. glutamicum*.

MSP334

Comprehensive characterization of the structure of the general stress "modulon" of *Bacillus subtilis*

A. Reder*[1], A. Arnab Bandyopadhyay[2], U. Mäder[1], U. Gerth[3], M. Hecker[1,3], L. Kaderali[2], U. Völker[1]
[1]*University Medicine Greifswald, Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Germany*
[2]*University of Greifswald, Institute for Bioinformatics, Greifswald, Germany*
[3]*University of Greifswald, Institute for Microbiology, Greifswald, Germany*

A general stress response is inherent to almost all living organisms and is usually induced by and protects against a broad range of otherwise lethal environmental stresses. In *B. subtilis* the general stress response is under primary control of the alternative sigma factor SigB that has been shown to function as a global activator of stress gene expression. Although SigB activation is the compelling prerequisite for general stress gene expression this is not sufficient for an effective expression of most general stress regulon members or the development of their protective function. Here, we show that the SigB regulon is much larger and far more complex than previously described. SigB dependent induction of secondary regulators allows the introduction of additional signals into the cascade to modulate gene expression levels and creating stress specific sub-regulons. Besides collaborating with SigB at SigB-type promoters, these regulators also drive activation from SigA dependent promoters adding a new level of complexity to the network. Furthermore, we could assign a completely new role as a global negative regulator to the alternative sigma factor SigB that shuts off the expression of hundreds of genes in the face of harsh environmental stress. Two major examples for this are the inactivation of sporulation (Spo0A) as well as competence development (ComK). Here, we use modeling to reveal hierarchically ordered regulatory pathways leading to a highly complex general stress modulon consisting of a "core" SigB regulon, several stress specific sub-regulons including SigA-type regulation as well as a huge negatively regulated group of genes where SigB acts as an "emergency brake system" to silence alternative developmental programs under severe stress conditions.

MSP335

Effect of NaCl stress on the biofilm formation of *Clostridium acetobutylicum*

M. Lehmann*[1], K. D. Fuchs[1], A. Poehlein[2], H. Bahl[1], R. J. Fischer[1]
[1]*University of Rostock, Microbiology, Rostock, Germany*
[2] *Georg-August University of Göttingen, Genomic and Applied Microbiology, Göttingen, Germany*

The Gram-positive bacterium *Clostridium acetobutylicum* is well known for its ability to produce solvents like butanol, a next-generation biofuel. As a soil-dwelling microorganism, this strict anaerobic bacterium is constantly exposed to osmotic changes caused by e.g. drying up of lakes or ponds. Therefore, it could be expected that a salt-specific stress response is crucial for its survival. However, little is known about the salt and osmotic stress response of *C. acetobutylicum*. In general, the protection against abiotic stressors like NaCl can also be achieved through the formation of biofilms, in which cells are embedded in a matrix of extracellular polymeric substances (1).

We started to analyse the salt-specific stress response of *C. acetobutylicum*. One approach are RNA-Seq experiments of cells growing in batch culture in synthetic medium with and without NaCl. First results revealed an upregulation of polysaccharide biosynthesis genes as well as a downregulation of motility genes of *C. acetobutylicum* in NaCl-containing media. Furthermore, effects of NaCl stress on the biofilm formation are under investigation. Here, visualization and quantification of biofilm assays will be presented.

The results gained in this study leads to the conclusion that NaCl stress seems to be an important environmental factor triggering biofilm formation of *C. acetobutylicum*.

(1) Wingender J., Neu T.R., Flemming HC. (1999) "What are Bacterial Extracellular Polymeric Substances?". In: Wingender J., Neu T.R., Flemming H.C. (eds) Microbial Extracellular Polymeric Substances. Springer, Berlin, Heidelberg

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MSP336

Metabolic requirements for successful spore revival in *B. subtilis*

C. Kaspar*[1,2], K. Nagler[1,2], A. Mutlu[1,2], I. Bischofs-Pfeifer[1,2]
[1]*Max Planck Institute for Terrestrial Microbiology, MPG Research Group CATs (Complex Adaptive Traits), Marburg, Germany*
[2]*BioQuant Center of the University of Heidelberg, Heidelberg, Germany*

In response to starvation *B. subtilis* and related bacteria differentiate into metabolically dormant endospores. These spores return back to life, when conditions become favorable again. Revival is initiated by germination which is triggered by specific nutrients and leads to the rehydration of the spore. The conditions and molecular processes that are required for the subsequent onset of metabolism and successful outgrowth of the germinated spore are less well studied. Here we use microscopic spore revival studies in combination with molecular level analysis based on fluorescent reporter assays and spore proteomics, respectively to identify the requirements for spore revival. We show that certain amino acids together with the germinant L-alanine are sufficient to enable the completion of spore revival. Enzymes for metabolizing these amino acids are present in spores and their abundance correlates with the capacity for successful spore outgrowth. This indicates that amino acid metabolism plays a key role for spore outgrowth in minimal media. We furthermore provide

evidence that these enzymes contribute to the phenotypic memory of spores and to population heterogeneity during spore revival.

OTP337

Unlike scientists opinions stem cells can be seen in unicellular organisms as well

R. Amirmardfar*[1]
[1]*SabzAndishan, Tabriz, Iran*

This paper is about a new theory on appearance of the first stem cells on earth. This theory explains that how and when the first animal and plant cells have arisen on the earth. This theory also says (unlike scientists opinions)stem cells can be seen in unicellular organisms as well. According to this theory the course of appearance of the first stem cells is similar to endosymbiosis. The difference is that genes of two symbiotic cells have been merged. Nevertheless both cells can separate from each other but after separation genes of two symbiotic cells remain with them as safekeeping, thus they are able to generate the cell itself and the symbiotic cell too. Scientists call " distinction " the generation of the first stem cells from the cell concluded by symbiotic. Scientists are able to force cells to generate its symbiotic cell by tissue culture, but they don't know the nature and origin of the issue and can't answer the question the first stem cells how and when and where have come to exist.

OTP338

Polarization of ancestor relations reveals the order of traits in the evolution of cyanobacterial multicellularity

K. Hammerschmidt*[1], G. Landan[1], F. Tria[1,2], T. Dagan[1]
[1]*CAU Kiel, Kiel, Germany*
[2]*Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany*

Understanding the events during the evolution from unicellular organisms to multicellularity is a long-standing challenge. The first known transition to multicellularity occurred more than 3 billion years ago in cyanobacteria. This is a monophyletic phylum that nowadays still includes unicellular and filamentous genera, some of which represent the peak of prokaryotic complexity. Previous research on the evolution of multicellularity in this phylum has been hampered by the lack of knowledge on the phylogenetic relations among unicellular and filamentous genera. We present a novel approach that does not depend on previous knowledge of phylogenetic relationships but infers ancestor-descendent relations of ancestral nodes in phylogenetic trees. Combining the method with the assignment of phenotypic traits to ancestral nodes enable the inference of pairwise relative priority, without the need to postulate a species tree. Applying our approach to 199 cyanobacterial genomes yields a temporal sorting of traits. Our results reveal, for example, that the formation of filaments co-occurred with the ability to fix nitrogen before higher complexity evolved. This finding is in line with theory that predicts the trade-off between oxygen-producing photosynthesis and oxygen-sensitive nitrogen fixation to be the main driving force for multicellularity in this phylum.

OTP339

On the taxonomy of the genus *Corynebacterium.*

B. J. Tindall*[1]
[1]*Leibniz-Institut DSMZ, Braunschweig, Germany*

Two recent papers on the taxonomy of the genus *Corynebacterium* are based on genomic data. These papers will become the standards by which the taxonomy of the genus will develop further. Closer examination of one of the papers and consultation of the earlier relevant literature however presents a rather different picture. As in many cases the genus *Corynebacterium* has developed from the initial proposal of the name by Lehmann and Neumann to a rapid increase in the number of species post 1990. Key methods have changed from morphological, biochemical and physiological criteria to essentially a single gene, the 16SrRNA gene sequence. The result is a poorly defined taxon (genus) where the nomenclature, for better or for worse is the starting point for the evaluation of the genomic data. Consequently the inclusion of *Turicella otidis* within the radiation of the genus *Corynebacterium* has been taken to show unambiguously that this taxon should be a member of the genus *Corynebacterium* together with strong evidence for the fact that features such as the presence / absence of mycolic acids, menaquinone, degree and localisation of positions of unsaturation are unreliable markers in the genus *Corynebacterium*. Closer examination of papers advocating the genome based approach show evidence of circular logic, an over emphasis on the role of the existing nomenclature and the inability to go back to grass roots and question how the genus *Corynebacterium* is classified. There are significant errors in the first publication that strongly influence the interpretation that is carried over into the second paper. Alternatively, as currently defined the genus *Corynebacterium* should be divided into a number of genera and that further work is needed to properly define these taxa.

OTP340

Species identification by analysis of eDNA through molecular biological techniques & MALDI-TOF-MS

P. Holm*[1], A. Findeisen*[1], R. Pabst*[1], C. Cordes[1]
[1]*Hochschule Anhalt, Bernburg, Germany*

Having knowledge of the distribution, presence, and absence of certain animal and plant species is essential for various fields such as nature conservation, biodiversity, and ecosystem management. In addition, and to retain biodiversity, the European Union passed the Fauna-Flora-Habitats Directive (FFH directive, EU 92/43/EWG) stipulating a regular monitoring of various species. However, detecting specific species can be difficult not least because of rough terrain, small species numbers or inconspicuous developmental stages of several species.

Solving the difficulty, modern and time-saving analyses are used to investigate environmental samples with the objective of developing validated analytics to detect aquatic and terrestrial organisms.

The verification relies on the noninvasive investigation of environmental DNA (eDNA) through molecular biological techniques and MALDI-TOF-MS (matrix-assisted laser desorption/ionization time of flight mass spectrometry). eDNA originating from faunal or plantal cells is extracted from environmental specimens. Applying species-specific primers, the extracted DNA is examined with diagnostic PCR (polymerase chain reaction) methods. Conducting the analysis by MALDI-TOF-MS, the animal and plant samples are directly tested after preparation.

To date, the extraction of eDNA from water samples is successfully developed and specific FFH relevant amphibians (e.g. the great crested newt *Triturus cristatus*) can be precisely detected. Analysis with MALDI-TOF-MS concerning the environmental sector is in the state of development.

In the light of protecting and preserving our environment, the advancement of analyzing biological molecules will be extended to facilitate precise and reliable detection of especially endangered and invasive species.

OTP341

Comparative secretome analyses of human and zoonotic *Staphylococcus aureus* isolates of CC8, CC22 and CC398

T. Busche[1,2], M. Hillion[1], V. V. Loi[1], D. Berg[1], B. Walther[3], T. Semmler[3], B. Strommenger[4], W. Witte[4], C. Cuny[4], A. Mellmann[5], M. A. Holmes[6], J. Kalinowski[2], L. Adrian[7], J. Bernhardt[8], H. Antelmann*[1]
[1]*Freie Universität Berlin, Institute for Biology-Microbiology, Berlin, Germany*
[2]*CeBiTec Bielefeld, Bielefeld, Germany*
[3]*Robert Koch Institute, Advanced Light and Electron Microscopy, Berlin, Germany*
[4]*Robert Koch Institute, Wernigerode Branch, Wernigerode, Germany*
[5]*University Hospital Münster, Institute of Hygiene, Münster, Germany*
[6]*University of Cambridge, Department of Veterinary Medicine, Cambridge, United Kingdom*
[7]*Helmholtz Centre for Environmental Research-UFZ, Department Isotope Biogeochemistry, Leipzig, Germany*
[8]*University of Greifswald, Institute for Microbiology, Greifswald, Germany*

Question: The spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in the community, hospitals and in livestock is mediated by highly diverse virulence factors that include secreted toxins, superantigens, enzymes and surface-associated adhesins allowing host adaptation and colonization.

Methods: Here, we combined proteogenomics, secretome and phenotype analyses to compare the secreted virulence factors in selected *S. aureus* isolates of the dominant human and livestock-associated genetic lineages CC8, CC22 and CC398.

Results: The proteogenomic comparison revealed 2181 core genes and 1306 accessory genes in 18 *S. aureus* isolates reflecting the high genome diversity. Using secretome analysis, we identified 869 secreted proteins with 538 commons in eight isolates of CC8, CC22 and CC398. These include 64 predicted extracellular and 37 cell surface proteins that account for 82.4% of total secretome abundance. Among the top 10 most abundantly secreted virulence factors are the major autolysins (Atl, IsaA, Sle1, SAUPAN006375000), lipases and lipoteichoic acid hydrolases (Lip, Geh, LtaS), cytolytic toxins (Hla, Hlb, PSMß1) and proteases (SspB). The CC398 isolates showed lower secretion of cell wall proteins, but higher secretion of a- and ß-hemolysins (Hla, Hlb) which correlated with an increased Agr activity and strong hemolysis. CC398 strains were further characterized by lower biofilm formation and staphyloxanthin levels due to decreased SigB activity.

Conclusion: Overall, comparative secretome analyses revealed CC8 and CC22-specific enterotoxin and Spl protease secretion as well as Agr- and SigB-controlled differences in exotoxin and surface protein secretion between human-specific and zoonotic lineages of *S. aureus*.

OTP342

de.NBI–The German Network for Bioinformatics Infrastructure

R. Alkhateeb*[1], T. Dammann-Kalinowski[1], D. Jording[1], A. Tauch[1], D. Wibberg[1], M. Wittchen[1], A. Pühler[1]
[1]*German Network for Bioinformatics Infrastructure (de.NBI), Bielefeld, Germany*

In recent years, the ever-growing applications of omics techniques have revolutionized life science and simultaneously created the need to store, process, and analyze large amounts of data. Handling Big Data can be a challenging task for life science researchers. The German Network for Bioinformatics Infrastructure (de.NBI) offers researchers the support they need in the processing and the analysis of their data. The key characteristics of de.NBI are:

Services and Tools: de.NBI provides over 100 services and tools ranging from databases and standalone tools to complete workflows for the processing and analysis of omics data.

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Compute Resources: de.NBI offers large computing power and storage capacity through a free cloud environment that allows researchers to process and analyze their own data.

de.NBI is a distributed bioinformatics infrastructure funded by the German Ministry of Research and Education (BMBF). The network consists of eight Service Centers covering 39 project partners throughout Germany. Each Service Center is specialized in different omics field assuring excellent services and high level of epertise in their respective field.

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OTP343

Detection of protein rhamnosylation in bacteria using a set of novel amino sugar specific antibodies

F. Koller*[1], D. Gast[2,3], S. Wunder[2,3], R. Krafczyk[1], A. Hoffmann-Röder[2,3], J. Lassak[1]
[1]*Ludwig-Maximilians Universität München, Biologie I/Mikrobiologie, Planegg-Martinsried, Germany*
[2]*Ludwig-Maximilians-Universität München, Chemie, München, Germany*
[3]*Ludwig-Maximilians-Universität München, Center for Integrated Protein Science Munich, München, Germany*

The previously discovered posttranslational arginine mono rhamnosylation is essential for elongation factor P (EF-P) dependent rescue of polyproline stalled ribosomes in clinically relevant species such as Pseudomonas aeruginosa or Neisseria meningitidis and represents the first example of protein rhamnosylation in bacteria. Considering that glycosylation is a universal strategy to alter structural and functional properties of proteins, one can assume that both N- and O-rhamnosylation might be more widespread in nature. In this regard, and based on new chemicals synthesis routes towards α- and β-rhamnosyl asparagine/ serine and threonine glycopeptide haptens a set of anti-rhamnosyl antibodies was generated. This in turn allows the detection of protein rhamnosylation in diverse species.

OTP344

GTP-Cyclohydrolase I: The second member of the Zur-regulated *cobW1*-gene cluster

V. Schulz*[1], M. Herzberg[1], D. H. Nies[1]
[1]*Martin-Luther-University of Halle-Wittenberg, Microbiology, Halle a. d. S., Germany*

Zinc is an important trace element and about 5% of all proteins in prokaryotes are zinc-binding proteins, even though zinc is toxic in high concentration. The ß-proteobacterium *Cupriavidus metalliduran*scan cope with both - zinc excess and starvation conditions. To understand the mechanism of zinc homeostasis, we characterized an operon-like clusters that are regulated by Zn^{2+}- dependent repressor Zur. The Zur-dependent *cobW1*gene cluster contains six genes, which are strongly expressed under zinc starvation conditions and contains two Zur-boxes in the operator region. The first gene of this cluster is *cobW1*, which encodes a zinc chaperone of the G3E family of P-loop GTPases. The genes downstream of *cobW1*encode paralogs of zinc-dependent proteins. Rmet_1099 or *folE1B2* is the gene directly downstream of *cobW1*. This gene product is a putative class I GTP-cyclohydrolase (GCHY I) converting GTP into 7,8-dihydroptyrine, which subsequently enters different metabolic pathways, e.g. leading to tetrahydrofolate or modified tRNA nucleosides such as queuosine and archaeosine. *C. metalliduran*scontains three GTP-cyclohydrolases I, the type IB proteins $FolE_{1B2}$(Rmet_1099) and $FolE_{1B1}$ (Rmet_2614), and the type IA protein $FolE_{1A}$(Rmet_3990). The IA-type enzymes contain usually Zn and are constitutively expressed, this was verified in the case of $FolE_{1A}$by ICP-MS and reporter-gene studies. The proteins of the type IB may use different transition metals as a cambialistic enzymes. A main aim is to investigate the interplay of GCHY-I-paralogues in the various deletion strains and under different conditions, the role of CobW1for the cofactor delivery and the activity of those three proteins with regard to the different metal cations cofactors.

OTP345

Analysis of biofilm phenotypes of 300 clinical and environmental *Stenotrophomonas maltophilia* reveals high levels of phenotypic and structural heterogeneity

I. Alio[1], M. Gudzuhn*[1], S. Bielfeld[1], U. Mamat[2], U. Schaible[2,3], S. Niemann[4,3], T. Kohl[4], J. Steinmann[5], W. R. Streit[1]
[1]*University Hamburg, Department of Microbiology and Biotechnology, Hamburg, Germany*
[2]*Research Center Borstel, Cellular Microbiology, Borstel, Germany*
[3]*German Center for Infection Research, TTU-TB, Borstel, Germany*
[4]*Research Center Borstel, Molecular and Experimental Mycobacteriology, Borstel, Germany*
[5]*Universitätsinstitut der Paracelsus Medizinischen Privatuniversität , Institut für Klinikhygiene, Medizinische Mikrobiologie und Klinische Infektiologie, Nürnberg, Germany*

The multidrug resistant opportunistic pathogen *Stenotrophomonas maltophilia* is a biofilm forming and gram-negative bacterium. It contributes to disease progression in cystic fibrosis patients and is found in wounds and on catheter surfaces. To identify the processes and genes involved in its biofilm formation, we sequenced the genomes of over 350 clinical and 40 environmental isolates. In parallel, we investigated the biofilm profile of these isolates. The genome data together with the biofilm analysis and other phenotypic and metabolic data will generate the largest data set of *S. maltophilia* and its biofilm formation on a genus and pangenome-wide level.

Microtiter plate analyses of 300 clinical and environmental *S. maltophilia* isolates revealed a strong variation in biofilm forming ability. 2.33 % of all isolates formed very strong biofilms, while 6 % formed strong, 82.67 % formed moderate and 9 % formed weak biofilms. Clinical and environmental isolates do not differ in their biofilm forming abilities.

Analyses of biofilm 3D-structures of 30 isolates grown in flow cells identified high heterogeneity in the biofilm matrix and appearance independent of the strains and their phylogenetic position in the genus. Thus, we speculate that under *in vivo* conditions *S. maltophlilia* also displays varying biofilm architectures on a strain-specific level, which might be another strategy to escape antibiotic treatment. However, no correlations between biofilm formation abilities, 3D-structure and resistance to the antibiotic colistin were observed.

Transcriptome data for selected isolates are underway to estimate the biofilm formation on a global level and to analyze if the high variation in biofilm architecture correlate with *S. maltophilia* strain specific expression patterns.

OTP346

The small DUF1127 RSP_6037 protein from *Rhodobacter sphaeroides* is a putative RNA-binding protein

J. Grützner*[1], G. Klug[1]
[1]*Institute of Microbiology and Molecular Biology, University of Giessen, Gießen, Germany*

The facultative phototrophic bacterium *Rhodobacter sphaeroides* is able to adapt its life style to changing environmental conditions. In the past our group elucidated in *R. sphaeroides* the role of protein regulators and small, regulatory RNAs (sRNAs) in the stress responses, particularly to oxidative stress. The four homologouse sRNAs CcsR1-4 (conserved CCUCCUCCC motif stress-induced RNA), which are expressed by the alternative sigma-factors RpoHI and RpoHII, modulate the C1 metabolism under various stress conditions (Berghoff *et al*. 2009). These sRNAs are derived from the 3´ UTR of the RSP_6037 mRNA. The RSP_6037 gene encodes a small protein of 70 amino acids and the amino acids 23-62 constitute a domain of unkown function (DUF1127), which is structcurally related to eukaryotic RNA binding proteins (Billenkamp *et al*. 2015). Small proteins with the DUF1127 domain are widely distributed among Alpha- and Gammaproteobacteria and more than 5000 bacterial sequences with DUF1127 proteins are listed in InterPro.

Our previous data show that the small protein RSP_6037 influences the amount of the CcsR sRNAs and that the uncharacterized DUF1127 domain is sufficient for the function of this small protein. By a Co-immunoprecipitation we could show binding between the sRNA CcsR1 and the DUF1127 protein. We test the hypothesis that binding of the DUF1127 domain of RSP_6037 influances the stability and processing of RNA transcripts and may play an important role in RNA maturation by dRNASeq and Northern Blot analysis.

OTP347

Biosorption of rare earth elements by bacteria, fungi and algae

A. Breuker*[1], S. Ritter[1], A. Schippers[1]
[1]*Bundesanstalt für Geowissenschaften und Rohstoffe Hannover (BGR), Hannover, Germany*

Question: Rare earth elements (REE) are economically critical raw elements (Orveillon et al., 2018) and important metals in catalysts, magnets, metallurgical alloys, phosphors,

ceramics, powder production and medical applications. Biosorption has been shown predominantly for individual REEs by algae, fungi and bacteria. Aim of this study was to develop an economically feasible and ecologically friendly method to recover selective multiple REEs from low concentrated solutions from REE leaching processes.

Methods: Biosorption of REE was tested for strains of algae, bacteria, fungi (including yeasts) by measuring changing REE concentrations in solution under various conditions.

Results: *Bacillus subtilis* showed pH dependence and quick biosorption preferring higher REEs at low pH but overall better biosorption at pH > 3. One yeast strain and the fungus *Fusarium oxysporum* needed longer time for biosorption. *F. oxysporum* exhibited also high biosorption capacity at pH < 4. Like *B. subtilis*, another yeast showed preference for higher REEs at low pH. Biosorption by *B. subtilis* and the fungal strains showed up to 60-100% efficiency. In contrast, published results on REE biosorption by two strains of algae could not be reproduced in similar experiments.

Conclusion: *Bacillus subtilis* and fungi showed efficient REE biosorption. The tendency to element selective biosorption by *B. subtilis* and yeast with a more efficient biosorption of higher REEs offers an approach to concentrate REEs in further experiments.

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OTP348

Structural and spectroscopic characterization of a HdrA-like subunit from *Hyphomicrobium denitrificans*

T. Koch[1], K. Kayastha[2], C. Ernst*[1], S. Venceslau[3], I. Cardoso Pereira[3], U. Demmer[2], U. Ermler[2], C. Dahl[1]
[1]*Universität Bonn, Institut für Mikrobiologie & Biotechnologie, Bonn, Germany*
[2]*MPI für Biophysik, Frankfurt a. M., Germany*
[3]*Universidade de Lisboa, Lisboa, Portugal*

Many Bacteria and Archaea employ a novel pathway of sulfur oxidation involving a heterodisulfide reductase (Hdr)-like enzyme complex resembling HdrABC of methanogens. In the Alphaproteobacterium *Hyphomicrobium denitrificans* functional *hdrB1C1A-hyp-hdrC2B2* genes are indispensable for the oxidation of thiosulfate to sulfate [1].

Methanogenic HdrA binds an electron-bifurcating FAD that constitutes the core for the HdrABC-[NiFe]-hydrogenase catalyzed reduction of ferredoxin and the coenzyme M-B heterodisulfide with H2 [2]. As a first step in the biochemical characterization of Hdr-like proteins from sulfur oxidizers (sHdr), we determined the structure of the recombinant hyphomicrobial sHdrA-like protein at 1.4 Å resolution. Each monomer of the homodimeric sHdrA adopts a thioredoxin reductase fold and carries one FAD and one [4Fe-4S] cluster but not the additional Fe/S center binding domains of methanogenic HdrA. The edge to edge distance between FAD and the [4Fe-4S] cluster bound by cysteines of a CX12CX3CC motif is 9.4 Å allowing physiologically relevant electron transfer rates between them. The midpoint redox potential of the Fe-S center was determined to be -194 mV by redox titration followed by EPR spectroscopy. Recombinant sHdrA was isolated with FAD in the semiquinone state as observed by visible as well as by EPR spectroscopy. Titration of fully oxidized protein at pH 6.0 also revealed a stable semiquinone intermediate. An instable semiquinone state has been proposed to represent a hallmark of bifurcating flavins [2]. Thus, a bifurcating function for the HdrA protein from sulfur oxidizers appears unlikely.

[1] Koch T and Dahl C 2018 ISME J 12, 2479

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OTP349

Towards the lytic system of prophages of *Bacillus subtilis* 168

S. Freitag-Pohl[1], H. Watzlawick*[2], E. Pohl[1], A. Jasiliones[3], M. Plotka[4], M. Hakansson[5], A. Svensson[5], R. Kovacic[5], L. Wang[2], M. Welin[5], B. Walse[5], J. Altenbuchner[2], E. Nordberg-Karlsson[3], A. K. Kaczorowska[4], T. Kaczorowski[4], S. Al-Karadaghi[5], A. Aevarson[6]
[1]*Durham University, Department of Chemistry, Durham, United Kingdom*
[2]*Institut für Industrielle Genetik, Universität Stuttgart, Stuttgart, Germany*
[3]*Lund University , Department of Biotechnology, Lund, Sweden*
[4]*Gdansk University, Gdansk, Poland*
[5]*SARomics-Biostructures-AB, Lund, Sweden*
[6]*MATIS ltd., Reykjavik, Iceland*

The EU funded H2020 project Virus-X1 (http://virus-x.eu) explores the virosphere of selected microbial ecosystems of extreme environments such as hot springs and deep sea vents. It aims to identify and characterize novel proteins and enzymes from viruses, bacteriophages and archaephages.

As a starting point the host cell lysis system of two defective prophages PBSX and SPβ from *Bacillus subtilis* 168 were investigated. The putative genes of the lytic proteins XepA, XlyA, XlyB from PBSX and YomS from SPβ were cloned and expressed in *E.coli* and their gene products were characterized. Whereas purified XepA, XlyA and XlyB showed lytic activity for different Gram+ bacteria, YomS exhibits no lytic activity. YomS displayed high sequence similarity with the C-terminal part of XepA. In order to unravel the molecular basis of XepA and YomS, the crystal structure of these proteins were solved. XepA forms two pentameric discs connected by a linker tunnel. The structure of YomS is a pentamer of β-sandwich monomers and resembles in its overall fold the XepA pentameric lower disc and lacking the upper disc and tunnel section of XepA.

OTP350

RNA thermometers in transcripts of type 3 secretion system components in *Yersinia pseudotuberculosis*

S. Pienkoß*[1], F. Narberhaus[1]
[1]*Ruhr University Bochum, Microbial Biology, Bochum, Germany*

Complex networks of regulatory non-coding RNAs, such as RNA thermometers (RNATs), riboswitches, and small regulatory RNAs facilitate the rapid adaptation of bacteria to changing environmental conditions. RNATs are typically located in the 5'-untranslated or in the intercistronic region within an mRNA and regulate the expression of downstream-located genes via structural rearrangements. The conformational shift of RNATs occurs in response to an increasing temperature, resulting in the liberation of the ribosome binding site. Subsequently, translation is initiated by the binding of the ribosome to the Shine-Dalgarno sequence.

We focused on the gram-negative foodborne pathogen *Yersinia pseudotuberculosis* and its temperature-dependent RNA elements under environmental (25 °C) and infection-relevant conditions (37°C). Based on PARS profiling (1), three putative RNATs were identified, which were found in the transcripts of the type 3 secretion system (T3SS) components YscJ, YscT and YopN (2).

The putative RNATs were translationally fused to the thermostable beta-galactosidase BgaB and confirmed via beta-galactosidase assay at 25 °C in comparison to 37 °C. Furthermore, the RNA secondary structure and the ability of ribosome binding of the RNATs were investigated via enzymatic structure probing and primer extension inhibition (toeprint) at 25, 37 and 42 °C. In conclusion, RNAT-mediated temperature sensing represents an efficient and energy-saving strategy for bacterial pathogens to regulate the expression of virulence-associated genes.

References:

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[2] Dewoody *et al*., (2013) *Front Cell Infect Microbiol* 3:4.

OTP351

Assembly of phycoerythrin III in *Prochlorococcus marinus* SS120

B. Gu*[1], N. Frankenberg-Dinkel[1]
[1]*Technische Universität Kaiserslautern, Mikrobiologie, Kaiserslautern, Germany*

Prochlorococcus lacks phycobilisomes (PBS) but uses divinyl chlorophyll complexes as major light harvesting structures. However, *Prochlorococcus sp.* retained remnants of the PBS in form of single phycoerythrin (PE). In *P. marinus* SS120 it is called PE III and consists of an α- and β-subunit. These subunits carry covalently attached linear tetrapyrrole chromophores termed phycobilins. While the genome of *P. marinus* SS120 possesses genes for the biosynthesis of the phycobilins phycocyanobilin (PCB) and phycoerythrobilin (PEB), only PEB and phycourobilin (PUB) have been observed *in vivo*. The final step in PE III biosynthesis is the post-translational addition of the phycobilin to the α- and β- subunits. *In vivo*, the correct covalent attachment of most chromophores is catalyzed by specific phycobiliprotein lyases. *P. marinus* SS120 possesses five putative lyase genes and additionally the open reading frame Pro1634. Within this project, we wish to explore the composition of PE III and consequently, how PUB is made. In addition, we are keen to understand the function of the PCB biosynthetic enzyme.

In order to investigate the composition of PE III, we will reconstitute PE III in *Escherichia coli*. Therefore, we will establish the pDuet coexpression system. First, all genes potentially involved in PE III synthesis will be individually cloned in a specific pDuet expression vectors. Using this system, we will be able to co-express single or multiple PBP lyase genes together with the α- or β-subunit and phycobilin biosynthesis genes. First data demonstrated a specific transfer of PEB by the phycobiliprotein lyase CpeS to the β-subunit. This was visible by a pink color pink color and a strong fluorescence emission at 564 nm (λ_{ex}=550 nm) indicating that the approach is feasible.

OTP352

Comparison of different methods to detect and quantify living and dead yeast cells in wine

C. Baier[1], V. Yovkova[1], C. von Wallbrunn[2], S. Loos-Theisen*[1]
[1]*Hochschule Geisenheim University, Institute for Food Safety, Geisenheim, Germany*
[2]*Hochschule Geisenheim University, Institute of Microbiology and Biochemistry, Geisenheim, Germany*

Detection and differentiation of living and dead yeast cells of *Saccharomyces cerevisiae* EC1118 could be performed by various methods. The aim of this project was to compare four different quantification methods for living and dead cell counts. For this purpose, membrane filtration, fluorescence microscopy using propidium iodide, microscopy with methylene blue staining and real-time PCR were carried out to detect and quantify living and dead yeast cells, respectively. In order to perform the experiments to compare these methods, wine samples were spiked with known quantities of yeast cells. It is possible to detect small amounts of living cells by membrane filtration. Fluorescence microscopy using propidium iodide and microscopy with methylene blue staining, on the other hand, need higher concentrations of yeast cells to determine the proportion of living and dead cells. Since no results were obtained at concentrations of a few cells per milliliter, higher concentrations of yeast cells are also required for measurements with real-time PCR.

OTP353

New functions of hypochlorous acid-modified human plasma proteins in the defense against pathogens

A. Ulfig*[1], A. Schulz[1], L. I. Leichert[1]
[1]*Ruhr-Universität Bochum, Biochemie&Pathobiochemie, Bochum, Germany*

Neutrophils are at the front line in our defense against microbial intruders. When activated, neutrophils produce the strong oxidant and bactericidal agent hypochlorous acid (HOCl). To prevent tissue damage at sites of inflammation, excess HOCl is scavenged by serum albumin and other plasma proteins. Here we show that plasma proteins are not only an effective sink for HOCl, but that HOCl-induced N-chlorination converts complete plasma, as well as any individual plasma fraction tested, into protective chaperones. Chaperone-like conversion could be abrogated by antioxidants and methylation of basic amino acids. Furthermore, exposure to higher concentrations of HOCl, as those present at sites of chronic inflammation, converted the majority of plasma proteins into efficient activators of neutrophil-like cells. This activation resulted in increased generation of reactive oxygen species (ROS), providing a positive feedback loop that could play a role in the effective clearance of microbial intruders but also in chronic inflammation.

OTP354

Immobilization of laccase for multiple use in textile industry

D. Köpke*[1], C. Marschelke[2], K. Wantz[1], A. Matura[1], A. Synytska[2]
[1]*Technische Universität, Technische Biochemie, Dresden, Germany*
[2]*Institut für Polymerforschung Dresden, Functional Particles and Interfaces Group, Dresden, Germany*

Laccase is described as an ideally green biocatalyst as the reaction simply requires molecular oxygen and produces water as the only by-product. Today, laccase is mainly used in pharmacy and food and textile industry. Since most of the

textile dyes are light resistant and robust against chemical substances, the produced sewage cannot be fed into the local hydrological circle. Instead of using harsh chemicals, laccase may be used to degrade phenolic compounds. The practical application is hampered due to low stability and no reusability of free laccase itself. An approach to solve these problems is to immobilize the enzyme on a surface. Immobilization is an efficient way to increase stability, resistance and the reusability of enzymes.

In this work we investigated the immobilization of laccase from *Trametes versicolor* on polymer brush-modified core-shell particles with controlled design and properties. A successful method for immobilization was developed and immobilization yields on carboxy- (PAA (polyacrylic acid)) and amino-functionalized (PDMAEMA (poly (2-dimethylaminoethyl methacrylate)) colloids were compared. Form and size of the particles, as well as the grafting density of the polymer brushes were varied to obtain the highest possible yield. A particle formulation was found which led to up to 80 % immobilization yield. Furthermore, temperature, pH and long-term stability were improved or stayed comparable to those of free laccase. Cibacron Blue P-3R and Remazol Bordo B, two commonly used textile dyes, were successfully bleached by free and immobilized laccase. Furthermore, multiple use of immobilized laccase was proven to be successful.

OTP355

Biosynthesis of cell wall glycopolymers in *streptomyces coelicolor* A3(2)

N. Steblau*[1], S. Sigle[1], W. Wohlleben[1], G. Muth[1]
[1]University of Tübingen, Microbiology/ Biotechnology, Tübingen, Germany

The Gram–positive soil bacterium *Streptomyces coelicolor* A3(2) undergoes a complex life cycle with differentiation in substrate mycelium, aerial mycelium and spores that are protected by a thick spore envelope. Sporulation septation and synthesis of the thickened spore wall are directed by the *Streptomyces* Spore Wall Synthesizing Complex, a multi-protein complex including proteins involved in synthesis of PG and cell wall glycopolymers (CWG).

In this study, we aim to elucidate the role of CWGs in the life cycle of *S. coelicolor* and to characterize their biosynthesis. Two distinct CWGs, known as the Kdn-containing teichulosonic acid and polydiglycosylphosphate (PDP) have been detected in *S. coelicolor* [1]. The S. *coelicolor* genome encodes six putative CWG-polymerases containing a TagF-like glycerophosphotransferase domain and eleven putative CWG-transferases of the LytR-CpsA-Psr (LCP) family[2]. To identify their function, we started to delete all genes, characterized the mutant phenotypes and analyzed the cell wall composition of vegetative mycelium and spores.

Thus we could show that SCO2578 (PdtA) is a PDP-transferase, which is crucial for proper PG-incorporation at the hyphal tips under stress conditions and required to ensure the integrity of the spore envelope[2].

SCO2997 was identified as a spore wall specific PDP polymerase for the synthesis of elongated PDPs, which are involved in the proper assembly of the hydrophobic rodlet layer on the spore surfaces.

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OTP356

Marburg Collection: A highly flexible cloning toolbox for *Vibrio natriegens*

D. Stukenberg*[1], T. Hensel[2], J. Hoff[1], R. Inckemann[3], G. Fritz[1]
[1]LOEWE Center for Synthetic Microbiology, Marburg, Germany
[2]Philipps-University, Chemistry, Marburg, Germany
[3]Max-Planck-Instituts für terrestrische Mikrobiologie, Marburg, Germany

*Vibrio natriegens*is known as the world"s fastest growing organism with a doubling time of less than 10 minutes. This incredible growth speed proposes *V. natriegens*as a chassis for molecular biology and for replacing *E. coli*in many applications, including cloning, protein expression and as a new prokaryotic model organism. Many applications require the construction of large numbers of different plasmids to build synthetic circuits, metabolic pathways or devices for the characterization of genetic parts. Hierarchical golden-gate assembly, first described by Weber et al. (2011), provides a method enabling the fast and flexible cloning of new plasmids from a set of reusable basic parts. Here we present the Marburg Collection, the most flexible golden-gate based cloning toolbox for prokaryotes. Started in the project of iGEM Marburg 2018, we designed and constructed a toolbox for complete *de novo*assembly of plasmids, inversion of individual transcription units and a theoretically infinite cloning capacity. Thorough characterization of the components of our toolbox greatly expands the repertoire of molecular tools in V. natriegens, and paves the way towards rational design of more complex synthetic circuits in this organism. Among others, we provide the first experimental data about the strength of a set of constitutive and inducible promoters, ribosome binding sites and establish several codon-optimized reporter genes. Moreover, we investigated the ability of our newly designed connectors to block transcriptional readthrough. In summary, the Marburg Collection represents a novel and highly flexible golden-gate based toolbox with parts characterized for their behavior in the newly emerging chassis *V. natriegens*.

OTP357

Natural products produced by *Photorhabdus luminescens* are essential for nematode development

H. Vural*[1], H. B. Bode[1]
[1]Goethe Universität, Molekulare Biotechnologie, Frankfurt a. M., Germany

Heterorhabditis bacteriophora is an insect-parasitic nematode used commercially in agriculture as a biocontrol agent. The nematode lives in an obligate symbiosis with the entomopathogenic bacterium, *Photorhabdus luminescens*. The non-feeding stage, also called infective juveniles (IJs), of *H. bacteriophora* penetrate insect larvae living in the soil. After releasing *Photorhabdus* from the nematode gut, the bacteria proliferate and produce toxins to kill the insect. The IJs develop into adult, self-fertilizing hermaphrodites while feeding on cadaver and bacterial biomass. *H. bacteriophora* proceed to breed until nutrients are exhausted. Then, new IJs develop and leave the cadaver to search for another insect prey. In addition to killing of the insect, *P. luminescens* also support nematode development throughout the life cycle by providing a wide variety of natural products (NPs) such as

those derived from polyketide synthases and non-ribosomal peptide synthetases. However, little is known about the NPs that are particularly crucial for supporting the development process. For this study, we used a nematode bioassay as a tool to investigate the impact that these NPs play in the development of *H. bacteriophora*. By using several *P. luminescens* deletion mutants defective in the production of selected NPs, we demonstrate that the absence of some metabolites attenuate nematode development, while the bacteria is still able to kill insect larvae.

OTP358

Clostridia citrullinate proteins by putative peptidyl arginine deiminase activity

J. Fischer*[1], H. Bahl[1], K. Kriebel[1]
[1]*Universität Rostock/ Institut für Biowissenschaften, Mikrobiologie, Rostock, Germany*

Posttranslational modifications (PM) lead to an expanded and more complex proteom. PM are achieved by the addition of various groups to the side chain of the amino acid residue backbone of proteins. One type of PM is citrullination, which is a physiological process in humans, catalyzed by peptidyl arginine deiminases (PAD). *Porphyromonas gingivalis* is the only bacterium descripted so far which is also able to citrullinate proteins by the activity of a porphyromonas peptidyl arginine deiminase (PPAD). It is known that excessive citrullination can lead to inflammation processes such as in periodontitis or rheumatoid arthritis. We asked wether also clostridia are able to increase the level of citrullinated proteins by the activity of a putative peptidyl arginine deiminase (pPAD).

We performed *in silico* analyses of different pathogenic and nonpathogenic clostridia and found a close relationship between the structures of PPAD and pPAD, with *Clostridium difficile* having an identical catalytic centre. A PAD assay (Knipp *et al* 2000; mod.) was used to confirm these findings. Therefore, clostridial strains were cultured anaerobically for 24 h, the cells were adjusted to an optical density of 1 and harvested. The resulting pellet was resuspended in assay buffer. A colourimetric assay, using the substrate BAEE, was used to measure citrulline, giving information on pPAD activity. As a control, the PPAD activity was also measured. We found that enzyme activity is strain specific. From all clostridia analyzed, *C. difficile* showed highest enzyme activity with differences in citrullination level between different *C. difficile* isolates.
We conclude that clostridia are able to citrullinate proteins. Thus, they may have an impact on inflammation processes in the human body.

OTP359

Development of a high-throughput *in vitro* screen for the identification of *Staphylococcus aureus* PBP4 inhibitors

N. Jelden*[1], J. S. Puls[2], K. Ludwig[2], J. Deisinger[2,3], T. Schneider[2,3], F. Grein[2,3]
[1]*Institute for Pharmaceutical Microbiology, Bonn, Germany*
[2]*University of Bonn, Institute for Pharmaceutical Microbiology, Bonn, Germany*
[3]*German Center for Infection Research, partner site Bonn-Cologne, Bonn, Germany*

Due to the ability of *Staphylococcus aureus* to develop resistance towards modern antibiotics, the organism is a big challenge for medicine and public health highlighting the urgent need for new antibiotic drugs. Prominent targets for potent antibiotics are enzymes involved in the bacterial cell wall biosynthesis such as the penicillin binding proteins (PBPs). Besides transglycosylation of the cell wall precursor lipid II PBPs catalyse crosslinking of peptidoglycan (PG) via transpeptidation in a two-step reaction mechanism. In the first step an acetyl-enzyme complex is formed by PBPs binding to PG stem peptide thereby releasing the terminal D-alanine. In the second step PG stem peptides can be crosslinked via pentaglycine bridges. *S. aureus* PBP4 is classified as a low-molecular-weight PBP with carboxy- and transpeptidase activity and is essential for ß-lactam resistance in community-acquired methicillin-resistant *S. aureus*. We adapted an *in vitro* assay previously established (Gutheil et al. Anal Biochem 287: 196-202 DOI: 10.1006/abio.2000.4835) to assess the activity of PBP4 by measuring D-alanine release. D-alanine is processed by a D-amino-acid-oxidase to yield pyruvate, ammonium and H2O2. The latter is quantified using commercially available, fast and sensitive fluorescent or bioluminescent assays. The assay was adapted to a 384-well microtiter format allowing high-throughput screenings without loss of sensitivity and validated using several ß-lactam antibiotics. Herein we found that ß-lactams of different classes inhibit PBP4 with different efficiency. Exploiting the peptidase activity of PBP4 this *in vitro* assay allows to identify novel anti-infectives against *S. aureus.*

OTP360

Bacterial cell wall biosynthesis as target for a novel antibiotic isolated from *Streptomyces spec*.

K. Ludwig*[1], A. Müller[1,2], T. Schneider[1,2]
[1]*University of Bonn, Institute for Pharmaceutical Microbiology, Bonn, Germany*
[2]*German Center for Infection Research (DZIF), partner site Bonn-Cologne, Bonn, Germany*

The rapid emergence of infections caused by antibiotic-resistant pathogens represents a serious health threat, especially as only a few antibiotics have entered the market throughout the last decades. The bacterial cell wall biosynthetic network is historically known to be an effective target for antibiotics, and numerous natural product inhibitors have been discovered, though becoming harder to find. Here we report on a novel cell wall biosynthesis inhibitor isolated from *Streptomyces spec* with potent activity against Gram-positive bacteria including multi-drug resistant pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE). This study was aimed at identifying the molecular target and the detailed mode of action of the compound, as well as its resistance determinants. Using whole cell assays and selected *in vitro* test systems, it was shown that the compound targets the cell wall precursor lipid II. Cross-resistance was observed against cell wall active antibiotics, e.g. vancomycin and nisin, which also bind to lipid II.

OTP361

Genomic Analysis of the Recent Viral Isolate vB_BthP-Goe4 Reveals Increased Diversity of φ29-like Phages

T. Schilling[1], M. Hoppert[2], R. Hertel*[1]
[1]*Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology, Göttingen, Germany*
[2]*Institute of Microbiology and Genetics, Deptment of General Microbiology, Göttingen, Germany*

We present the recently isolated virus vB_BthP-Goe4 infecting *Bacillus thuringiensis* HD1. Morphological investigation via transmission electron microscopy revealed key characteristics of the genus *Phi29virus,* but with an

elongated head resulting in larger virion particles of approximately 50 nm width and 120 nm height. Genome sequencing and analysis resulted in a linear phage chromosome of approximately 26 kb, harbouring 43 protein-encoding genes and a packaging RNA. Sequence comparison confirmed the relation to the *Phi29virus* genus and genomes of other related strains. A global average nucleotide identity analysis of all identified φ29-like viruses revealed the formation of several new groups previously not observed. The largest group includes Goe4 and may significantly expand the genus *Phi29virus* (*Salasvirus*) or the *Picovirinae* subfamily.

OTP362

Providencia entomophila sp. nov., a new bacterial pathogen of the olive fly, _Bactrocera oleae_

C. Schuster*[1], I. Ksentini[2], H. Gharsallah[2], M. Sahnoun[2], S. Hamli Amri[2], R. Gargouri[2], M. Ali Triki[2], M. Ksantini[2], A. Leclerque[1]
[1]*Hochschule Geisenheim University, Geisenheim, Germany*
[2]*Olive Tree Institute, Sfax, Tunisia*

Bioprospection for potential microbial biocontrol agents associated with three major insect pests of economic relevance for olive cultivation in the Mediterranean area, namely the olive fly, *Bactrocera oleae*, the olive moth, *Prays oleae*, and the olive psyllid, *Euphyllura olivina*, led to the isolation of several strains of readily cultivable Gram-negative, rod-shaped bacteria from Tunisian olive orchards.

Laboratory bioassays demonstrated the pathogenicity of these new isolates for adult olive flies. Determination of 16S ribosomal RNA encoding sequences identified the bacteria as members of the taxonomic genus *Providencia* (*Enterobacteriales; Morganellaceae*). A more detailed molecular taxonomic analysis based on a previously established set of protein-encoding marker genes led to the conclusion that the new isolates should be organized in a new species within this genus.

With reference to their original insect association and demonstrated entomopathogenic potential, the designation "*Providencia entomophila*" has been proposed for this new taxon.

OTP363

Isolation and molecular identification of _Erwinia amylovora_ bacteria from Central Asia

C. Schuster*[1], S. Bobushova[2], Z. Umiralieva[3], M. Konurbaeva[2], A. A. Dzhajmurzina[3], B. Kopzhasarov[3], A. M. Uspanov[3], B. A. Duisembekov[3], T. Doolotkeldieva[2], A. Leclerque[1]
[1]*Hochschule Geisenheim University, Geisenheim, Germany*
[2]*Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan*
[3]*Kazakh Scientific-Research Institute for Plant Protection and Quarantine (KRIPPQ), Almaty, Kazakhstan*

The bacterial phytopathogen *Erwinia amylovora* (*Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae*) causes Fire Blight, an economically relevant disease of pear, apple and quince. Fire Blight originates from North America and has reached Western Europe half a century ago, spreading since to Central and Eastern European countries. In 2011 and 2012, symptoms of Fire Blight have for the first time been reported from several Central Asian countries.

It has been an objective of this study to isolate and characterize bacteria associated with Fire Blight symptoms from apple and pear orchards in Kyrgyzstan and Kazakhstan.

Bacteria were isolated from symptomatic and asymptomatic samples by sequential subcultivation on Miller-Schroth agar, copper agar, and levan agar. Clonal cultures were identified by diagnostic PCR with *E. amylovora* specific primer pairs. Assignment to the taxonomic species *E. amylovora* was corroborated using phylogenetic reconstruction based on an MLSA scheme comprising genes *atpD, infB,* and *rpoB*.

Bacterial isolates from Kazakhstan and Kyrgyzstan displayed the expected growth phenotype on isolation media, were positive in diagnostic PCR, and in the reconstructed phylogenetic trees were located in well bootstrap supported clades together with reference strains representing the taxonomic species *Erwinia amylovora*.

The study contributes to document at a molecular diagnostic level the spread of the Fire Blight pathogen to Central Asia.

OTP364

Comparative genomics of _Clostridioides difficile_ isolated from different geographic locations

T. Riedel*[1,2], J. Wittmann[1], H. Junca[1], I. Schober[1], B. Bunk[1], C. Spröer[1], J. Meier-Kolthoff[1], M. Göker[1], J. Sikorski[1], M. Steglich[1,2], O. Zimmermann[3], H. Liesegang[4], U. Groß[3], R. Daniel[4], U. Nübel[1,2,5], J. Overmann[1,2,5]
[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[2]*German Center for Infection Research (DZIF), Partner site Hannover-Braunschweig, Braunschweig, Germany*
[3]*Institute of Medical Microbiology, University Medical Centre, Göttingen, Germany*
[4]*Department of Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Georg-August-University, Göttingen, Germany*
[5]*Braunschweig Integrated Center for Systems Biology (BRICS), Technical University Braunschweig, Braunschweig, Germany*

Clostridioides difficile is a nosocomial pathogen which has emerged as a global health threat. Infections can range from antibiotic-associated diarrhea to pseudomembranous colitis or toxic megacolon. The pathogen has mostly been studied using clinical isolates from Europe, North America and Australia. In contrast, only little is known about the spread and the genomic characteristics of *C. difficile* in other geographic regions.

Within this study, we sequenced the complete genomes of several *C. difficile* isolates recovered from comparable patient cohorts in rural Germany (Europe), Ghana (Africa) and Indonesia (Asia). The genomes were sequenced by a combination of single-molecule real-time (SMRT) and Illumina sequencing technology.

Our comparative genomics approach that included over 100 complete genome sequences (including several reference genomes) revealed a high conservation of the chromosomal sequences but also a high variability of acquired and mobile genetic elements. Analysis of the toxin genes revealed several clade-specific and different patterns. A considerable number and variety of extrachromosomal elements, including different types of plasmids as well as new phage types, was detected.

This largest set of closed high-quality genomes presently available for *C. difficile* will improve the knowledge about the species gene pool, the adaptive potential, the genome dynamics and the evolution of this emerging pathogen.

OTP365

Characterization of *Pseudomonas aeruginosa* genes of unknown function

N. Babic*[1], K. E. Jaeger[1], F. Kovacic[1]
[1]*Institute for Molecular Enzymtechnology at Forschungszentrum Jülich, Heinrich Heine University Düsseldorf, Jülich, Germany*

Pseudomonas aeruginosa is an opportunistic pathogen characterized by its high antibiotic resistance which causes life-threatening infections in immunocompromised patients. Therefore, the WHO recently classified *P. aeruginosa* as a critical pathogen for research and development of new antibiotics. However, the knowledge about *P. aeruginosa* is still limited as nearly 40 % of its genes are still genes of unknown function (GUFs). We aim to experimentally characterize all these GUFs to identify novel putative virulence factors of *P. aeruginosa*.

In a first step, 2214 *P. aeruginosa* GUFs were synthesized by optimized PCR in 96-well microtiter plates with an estimated success rate of more than 90 %. In order to generate an expression plasmid library, suitable pGUF plasmid for heterologous expression in *E. coli* and homologous expression in *P. aeruginosa* were constructed by modification of the broad host vector pBBR1mcs3. The so constructed vector will allow the screening of bacterial clones carrying an expression plasmid with a correctly cloned gene of interest using the agar plate based "blue/white" selection system. As well, a fusion of a cleavable affinity tag to the C-terminus of the target protein will allow for the analysis of protein expression by ELISA and protein purification. The functionality of the pGUF expression vector and the feasibility of the cloning strategy were tested by cloning gene *pa2949* of *P. aeruginosa* encoding the known phospholipase PlaF. *E. coli* and *P. aeruginosa* carrying the pGUF-*plaF* plasmid showed increased phospholipase activity compared with the empty vector strain, thus indicating full functionality of the newly constructed plasmid. The automated construction of an expression vector library harboring all GUFs is ongoing.

OTP366

Biocontrol agents against *Eutypa lata* – candidates and compounds

F. Schmidt[1], Y. N. Shi[2], H. Laatsch[3], O. Sterner[4], H. B. Bode[2], E. Thines[1,5], A. Schüffler*[1]
[1]*IBWF, Kaiserslautern, Germany*
[2]*Goethe Universität Frankfurt, Molekulare Biotechnologie, Fachbereich Biowissenschaften, Frankfurt a. M., Germany*
[3]*Universität Göttingen, Institut für Organische und Biomolekulare Chemie, Göttingen, Germany*
[4]*Lund University, Center for Analysis and Synthesis, Department of Chemistry, Lund, Sweden*
[5]*Johannes Gutenberg-Universität in Mainz, Mikrobiologie und Weinforschung am Institut für Molekulare Physiologie, Mainz, Germany*

Eutypa lata is a phytopathogenic ascomycete and the causal agent of Eutypa dieback, also known as eutyposis or "dying arm disease". It is a progressive perennial canker disease reducing the productive life of mature grapevines tremendously. A suitable protection against the devastating grapevine disease Eutypa dieback is provided through wound treatment with fungicides and biological control agents. However, the approval of several broadband fungicides has been withdrawn and effective compounds are needed more than ever. Especially since the invasive spread of the pathogen cannot be controlled and the progression of the disease inevitably leads to the death of the plant, which implies a considerable economic loss. In recent years biological control agents became more and more attractive for preventive treatment but to date only few fungi were evaluated. Thus the objective of this research is to detect further potential antagonists. Fungal isolates known to colonize plants were examined in artificial confrontation assays and several candidates were able to restrict the growth of *Eutypa lata*. In a further approach the secondary metabolites of the antagonistic fungi were examined whether they are responsible for the bioactivity. These studies resulted in the isolation of a couple of natural products whereof six (E11375-3, 1-acetyl-β-carboline, climacocyclin, fusidienol A, cercosporamide, asperfuran) were able to inhibit the growth of *Eutypa lata.* In addition, the corresponding antagonists were tested for their ability to prevent a colonization with *Eutypa lata* in a *Vitis*-shoot assay.

OTP367

Vibri*-Clone – a novel engineered strain for cloning in *Vibrio natriegens

J. Hoff*[1], B. Daniel[1], D. Stukenberg[1], M. Muratoglu[2], F. Müller[2], J. Jung [2], G. Fritz [1]
[1]*LOEWE Center for Synthetic Microbiology, Marburg, Germany*
[2]*Max-Planck-Institute for terrestrial Microbiology, Marburg, Germany*

Vibrio natriegensis an extremely rapidly growing gram-negative bacterium, which might substitute Escherichia colias the workhorse for synthetic biological and microbiological applications. Especially for molecular cloning, the V. natriegensdoubling time of less than 10 min could tremendously reduce cultivation times and speed up routine work. Here we show that several commonly used cloning methods, like Golden Gate- and Gibson cloning can be performed in V. natriegenswild type. Strikingly, we demonstrate that a complete cloning cycle can be performed in one working day – with plasmids being harvested in less than 12 hours. In order to optimize cloning methods in this organism, we establish genome editing approaches, demonstrating the functionality of the Flp/frtsystem, multiplexed Genome Editing and the ability to activate natural competence by expression of the competence master regulator Tfox. To date there exists only one available genetically modified V. natriegensstrain, which is specifically designed for protein expression and is under commercial use. Thus, our new, freely available Vibri-Clone strain will provide an important step towards accelerating molecular cloning in the scientific community.

OTP368

The role of *Myroides* mVOCs during infection

D. Hiller*[1], S. Barthels[1], D. Jahn[1], M. Jahn[1]
[1]*TU Braunschweig, Institute of Microbiology, Braunschweig, Germany*

The rod shaped gram negative opportunistic pathogen *Myroides spp*. belongs to the family of Flavobacteriaceae which carries some unusual features: next to being highly resistant to most classes of antibiotics, it emits unique microbial volatile organic compounds (mVOCs) as infochemicals implementing detrimental effects on other organisms. Out of 55 esters isolated from *Myroides* one of these mVOCs, a mere furfurylester, causes *P. aeruginosa* to lose its green colouring suggesting that this ester might impair the pyoverdin biosynthesis. In addition, these esters attenuated the swarming motility of *Proteus mirabilis*. Moreover, applying the esters on cultures of other $Gram^+$ or $Gram^-$ bacteria, such as *Staphylococcus aureus*, *Streptococcus pneunomiae, Enterobacter cloacae* or *Clostridium difficile*, the bacteria die. However, neither their biosynthesis nor their role during infection of other

microorganisms or even human cells have been investigated.

We aim to infer the role of mVOCs biochemical production as well during the infection process and for studying the virulence of Myroides.

We used double immunflourescence microscopy, Omics (metabolomic, transcriptomic, proteomic, volatilomic), BIOLOG Phenotype MicroArrays ™

Preliminary results revealed that co-culture experiments of a T24 cells, a bladder carcinoma cellline, with our *Myroides* isolate revealed an invasive character. Current BIOLOG array experiments will give fast information about different used carbon and nitrogen sources to te us establish a metabolomic map about Myroides as wll as about the sensitivity against different antibiotics in a high throughput manner. Glucose was found not to be the best carbon source for respiration of our isolate.

Myroides A21 has the ability to adhere and infect human cells.

OTP369
Investigations of microbial contamination of cocoa beans
S. Busch[1], S. Loos-Theisen*[1]
[1]Hochschule Geisenheim University, Institute for Food Safety, Geisenheim, Germany

Cocoa beans are the most important raw material for cocoa and chocolate products in the food industry. Due to various post-harvest treatments of the cocoa beans in the form of fermentation and uncontrolled outdoor drying, there is a risk of microbiological contamination. In the worst case, mycotoxins or pathogenic organisms may occur. Filamentous fungi of the genera *Aspergillus* and *Fusarium* infect cocoa beans and produce mycotoxins, e.g. ochratoxin A (OTA) or deoxynivalenol (DON) (1, 2). In this study cocoa beans from West Africa and South America were examined before and after roasting. The presence of OTA and DON was investigated. The concentrations of OTA and DON were determined by ELISA (enzyme-linked immunosorbent assay) and lateral flow assays, respectively. The mycotoxin OTA was not detectable in any of the cocoa beans analysed. In addition, cocoa beans are frequently contaminated with the pathogenic bacterial genus *Salmonella*. *Salmonella* infections were the cause of foodborne outbreaks caused by chocolate consumption. In this study, raw and roasted cocoa beans were incubated with *Salmonella enterica* subsp. *enterica* DSM 11320 for several weeks of persistence studies. The results showed that the growth or survival of *Salmonella* depends on when inoculation takes place.

(1) Ardhana and Fleet (2003); (2) Copetti et al. (2010)

OTP370
Efficacy of vancomycin with fosfomycin against VRE using agar dilution method
G. Aktas*[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Medical Microbiology, Istanbul, Turkey

Objective: *Enterococcus faecalis* and *Enterococcus faecium* can cause community-acquired and nosocomial infections. Combination antibiotherapies have a distinct advantage over monotherapies in terms of their synergistic effect.

In the study, it was aimed to investigate in vitro activity of vancomycin combined with fosfomycin against VRE strains.

Methods: A total of 30 clinical VRE strains were included in the study. Bacterial identifications of the strains were undertaken using conventional routine methods. The resistance to vancomycin was investigated by using microbroth dilution method. Glucose-6-phosphate (25 mcg/mL) for fosfomycin were used in all experiments.

The MIC was performed by agar dilution method, and the results interpreted in accordance with the CLSI guidelines. The activity of antibiotics in combination was assessed using a microbroth checkerboard. The fractional inhibitory concentration (FIC) indexes (FICI) were interpreted as follows: synergism, FICI≤0.5.

Time-kill testing: Two strains were also studied to determine the time-kill curves to verify the synergistic results. Both antibiotics were studied alone and in combination at 1xMIC. The synergy was defined as a ≥ 2 log10 cfu/mL reduction or more in colony count at 24 and 36 h with the combination, compared with the most active agent alone.

Results: All strains were resistant to vancomycin. Susceptibility rate to fosfomycin was found as 16.6 % (5/30). The MIC50,90 and MICrange values of antimicrobials were 512, 512 and 512 - 1024 mcg/mL for vancomycin, and 128, 128**,** and 64 - 256 mcg/mL for fosfomycin. The rate of synergism was found as 100 %.

Conclusion: The result shows that synergistic combination of vancomycin with fosfomycin could be useful in treatment of infections caused by VRE.

OTP371
Improving the Accuracy and Reproducibility of Microbiome Measurements Across Labs
R. Kemp[1], S. Tang[1], J. Kappel*[2]
[1]Zymo Research Corporation, Irvine, CA, United States
[2]Zymo Research Europe GmbH, Freiburg i. Br., Germany

The field of microbiomics has developed rapidly in the past several years. However, there are concerns due to poor data reproducibility across labs. To objectively assess the performance of different microbiomics workflows, it is essential to have accessible, well-defined, and accurately characterized mock microbial community standards to serve as reference materials for optimization, validation and controls of microbiomic workflows. Acknowledging this deficit, we have created well-characterized standards to be used as reference material for microbiome measurements. Using these, we assessed the performance of several of the most cited DNA extraction protocols used in the Microbiomics field and the effect of various library preparation techniques for 16S and shotgun sequencing. We found that the most commonly used protocols in this field for DNA extraction, including the HMP fecal DNA extraction protocol, are significantly biased. Most protocols over-represent the abundance of easy-to-lyse organisms, such as *Bacteroidetes*. Using the standards, we were also able to assess bias in the library preparation steps, such as GC bias in shotgun sequencing and PCR chimera in 16S sequencing. Thus, improving all steps involved from sample collection and DNA extraction to sequencing and bioinformatics will

harmonize the data generated in this very fast expanding field of research.

OTP372

Optimization and evaluation of the qPCR-based pooling strategy DEP-pooling in dairy production

P. J. Mester*[1], A. Witte[1], C. Robben[1], E. Streit[1], S. Fister[1], D. Schoder[2], P. Rossmanith[1,2]
[1]*Vetmeduni Vienna, CD-Momico, Vienna, Austria*
[2]*Vetmeduni Vienna, Institute for milk hygiene, Vienna, Austria*

Introduction: Diagnostic strategies to detect foodborne pathogens such as *L. monocytogenes* in food processing environments are cost and time consuming necessities to ensure safe food products. While two-step pooling diagnostic strategies incorporating PCR have been successfully introduced in recent years, such strategies to date have not been employed for food and hygiene monitoring.

Objectives: The objective of this study was to develop and evaluate the applicability of a cost-effective PCR pooling approach called direct enrichment PCR (DEP)-pooling based on an existing *L. monocytogenes* monitoring setup.

Materials and Methods: The proposed pooling strategy is based on a first enrichment step, subsequent PCR-pooling and re-evaluation of PCR-positive pools with the VIDAS DUO.

Results: Overall, more than 3,000 individual routine samples from an European cheese production facility were tested with the proposed DEP-pooling and compared in parallel with existing *L. monocytogenes* monitoring. In this approach equivalent results were obtained. Proposed DEP-pooling utilizes the advantages of both microbiological enrichment and PCR to identify negative samples faster in a cost efficient way with an overall cost reduction of ~ 60% based on 1% positive samples.

Conclusion: We proved the applicability of a microbiological enrichment method in combination with the PCR-based pooling strategy DEP-pooling to significantly reduce costs without impaired safety.

These results should encourage researchers, biomedical companies and industrial customers alike to implement such new cost-effective methods to ensure a safe food production environment.

OTP373

A novel method for sampling and long-term monitoring of microbes using stickers of plain paper

M. Bobal*[1], A. K. Witte[1], P. J. Mester[1], S. Fister[1], D. Schoder[2], P. Rossmanith[1]
[1]*Vetmeduni Vienna, Christian Doppler Laboratory for Monitoring of Microbial Contaminants, Department of Veterinary Public Health and Food Science, Vienna, Austria*
[2]*Vetmeduni Vienna, Institute of Milk Hygiene, Milk Technology and Food Science, Department of Veterinary Public Health and Food Science, Vienna, Austria*

Introduction: Detection of pathogens is crucial in food production areas. While being well established, swabbing as a state-of-the-art sampling method offers several drawbacks in respect of yield, standardization, overall handling and long-term monitoring.

Objectives: We aimed to develop and evaluate a method that is easier to use at a lower cost and that should be at least as sensitive as the conventional swabbing system.

Materials & methods: (Artificially contaminated) Stickers of plain paper were analyzed using qPCR specific for *Listeria monocytogenes* and *Escherichia Coli*.

Results: After evaluating promising sundry materials, we tested paper stickers for their suitability to uptake and release *Listeria monocytogenes* using quantitative PCR over a 14 day time period. The recovery rate was similar to previous studies using conventional swabs, and we also confirmed the feasibility of pooling besides resilience to cleansing and disinfection. In an experiment under real conditions that sampled various door handles, the occurrences of *L. monocytogenes* and *Escherichia coli* were determined. Results suggest that the presented sticker system might offer a promising cost effective alternative sampling system with improved handling characteristics.

Conclusion: As a ubiquitous bacterium, *Listeria monocytogenes* has a propensity to enter food production areas via fomites such as door handles and switches. While it might not be in direct contact with the food products, knowing the microbial status of the surroundings is essential for risk assessment. Our investigation for a novel qPCR-based sampling system with the highest sensitivity and ability to monitor over long periods of time, yet based on paper, proved to be cost-effective and reasonably convenient to handle.

OTP374

Tracking the establishment of metabolic heterogeneity in single cell eukaryotic communities over time

S. Zilkenat*[1], O. Zabaco[2], M. Ralser[1,3]
[1]*The Francis Crick Institute, Molecular Biology of Metabolism, London, United Kingdom*
[2]*The Francis Crick Institute, Making Science and Technology Platform, London, United Kingdom*
[3]*Charité, Institute of Biochemistry, Berlin, Germany*

Metabolite exchange between cells occurs within intra- and multi-species populations. While Saccharomyces cerevisiae, a unicellular eukaryote, is known to participate in multi-species communities, their degree of metabolic intra-species cooperation is less known, as they usually maintain prototrophic genomes, meaning that each cell has the capability to produce all necessary metabolites.

When co-culturing two in theory complementing auxotrophic strains they are unable to compensate for each others metabolic deficiencies. However, it was shown in previously that metabolic exchange in yeast can be generated in auxotrophic strains by progressive loss of plasmids carrying the complementing metabolic markers (Self-establishing metabolic cooperating communities, SeMeCos). These SeMeCo demonstrated growth efficiency and biomass formation as well as extracellular metabolite concentrations akin to the wild type under nutrient limited conditions. This indicates that similar cooperation can also occurs naturally.

We adapted a microfluidic device intended for the visualization of cell monolayers. This enables us to track the budding time on a single cell level under different nutrient availabilities and to study how metabolic heterogeneity develops in a highly controlled environment. Adjustment of the flow rate of the chamber can simulate sharing and non-sharing conditions and allows for rapid switching between

different conditions. Alongside auxotrophic and prototrophic strains, combining the SeMeCo model with fluorescent labelling allows us to track cells of known metabotypes in this system. The study of metabotype heterogeneity in single-cellular eukaryotes like S. cerevisiae can help advance the understanding of the high variability of cellular metabolism.

OTP375

Predictability of Ionic Liquid´s Virucidal Potential in Biological Test Systems

J. Sommer*[1], S. Fister[1], T. Gundolf[1], B. Bromberger [1], P. J. Mester [1], A. K. Witte [1], R. Kalb [2], D. Schoder[1,3], P. Rossmanith [1,3]
[1]University of Veterinary Medicine, Christian Doppler-Laboratory for Monitoring of Microbial Contaminants, Institute of Milk Hygiene, Milk Technology and Food Science, Department for Farm Animal and Public Veterinary Health, Vienna, Austria
[2]Proionic Production of Ionic Substances GmbH, Grambach, Austria
[3]University of Veterinary Medicine, Institute of Milk Hygiene, Milk Technology and Food Science, Department for Farm Animal and Public Veterinary Health, Vienna, Austria

Introduction: Through globalization, even of the food industry, the threat of virus infections is increasing and highlights the necessity to develop new virucidal disinfectants. Over the last decades, especially ionic liquids (ILs) have come to the fore as a new class of pharmaceuticals and antimicrobials. Although more and more is known about the antimicrobial effects of ILs, their virucidal potential and the mechanisms of action of this new class of substances is less investigated so far.

Objectives: A systematic analysis of specific structural motifs of 27 ILs on three different biological test systems was performed to gain information whether effects of these structure parts are present and if their virucidal potential is predictable.

Material & methods: The virucidal concentrations of different viruses (P100, MS2 and Phi6), the minimum inhibitory concentration of the corresponding bacterial host strains and enzyme inhibition potential via qPCR assay, of 27 different ILs weredetermined. Comparison of all data was insightful in respect of predicting the toxicity of ILs on viruses.

Results: No clear SARs for non-enveloped viruses P100 and MS2 could be identified. Slight effects of the IL cations on virus P100 were recorded,in contrast to virus MS2 were no toxicity associated with IL cationic compartments could be observed. Apart of the outcome of the non-enveloped viruses, a correlation has been demonstrated between the effects of IL cations on enveloped viruses, bacteria and enzyme, while there was no observed effect of IL anion chaotropicity.

Conclusions: While virucidal effects of ILs on non-enveloped viruses occurred, previously described SARs are not applicable and future research must focus on identifying their actual mode(s) of action.

OTP376

Evaluation of the detection efficiency of MRSA in manure

A. Belitz*[1], S. Glaeser[1], P. Kämpfer[1]
[1]Justus-Liebig-Universität Gießen, Applied Microbiology, Gießen, Germany

The transmission of manure-borne antimicrobial resistant (AMR) bacteria into the environment and finally to human populations is a well-known issue of the ONE Health problematic. One group of potentially human pathogenic AMRs derived from livestock husbandry are methicillin resistant *Staphylococcus aureus* (MRSA), which are wide spread among the livestock animal population and well known to cause severe problems in hospitals. MRSA are mainly colonising skin and mucosa of animals but *mecA* genes and *mecA* carrying *Staphylococcus* spp. were frequently detected in manure samples (Glaeser *et al*. 2016). Based on these findings manure as a source for MRSA transmission cannot be excluded. So far culturable MRSA were not detected in manure sample analyzed in our previous studies (Glaeser *et al.* 2016). It cannot be excluded that applied cultivation methods are ineffective to cultivate inactive (dormant) MRSA cells present in manure.

We performed a spiking experiment to evaluate the detection efficiency of published MRSA cultivation technologies including a direct plating strategy and four different one to two step enrichment technologies always combined with three MRSA specific cultivation media. Initial data indicate strong differences in the detection efficiency of different concentrations of spiked cells among the applied pre-enrichment methods and among the applied diagnostic agar media.

This study clearly illustrates, that detection of AMR bacteria is always dependent on the applied detection methods and a detailed evaluation of the applied methods is strongly recommended.

OTP377

The public health implications of *enterobacteriaceae* associated with toilet door handles within University of Benin, Nigeria

J. M. Ogbonnaya[1], E. E. Imarhiagbe[2], B. I. Idemudia[1], F. Ekhaise*[1]
[1]University of Benin, Department of Microbiology, Benin, Nigeria
[2]University of Benin, Department of Environmental Management and Toxicology, Benin, Nigeria

The location and process of toilets are of utmost importance for the maintenance of good health and the safety of the general public. Considering the role of toilets as convenience centers for human excretion, door handles which are used to access toilets are well positioned and as such, serve as fomite which aids the transfer and exchange of bacteria amongst individuals. The study investigated the presence and frequency of occurrence of this insidious threat of *Enterobacteriaceae* to public health on door handles of public toilets in the University of Benin premises. A total of 300 samples were collected using sterile cotton swab sticks from the door handles of the sampled locations for a period of six months. Total *Enterobacteriaceae* Counts of Public Toilet Door Handles within University of Benin, revealed that the least Enterobacteriaceae counts (0.49 ± 0.17 x 102 cfu/cm^2) was recorded in the University Library, while, the highest counts (32.51 ± 5.49 x 102 cfu/cm^2) was recorded in the female hall of residence. Molecular characterizations revealed the presence of *Escherichia coli* strain 14EC020, *Enterobacter cloacae* strain UM-11, *Klebsiella pneumoniae* strain NR5632, *Proteus mirabilis* strain IITRM5, *Salmonella bongori* strain GH3Rp, *Serratia marcescens* strain SM2616, *Shigella flexneri* strain GH24 and *Serretia marcescens* strain IARI-CRK. The MAR indexes obtained from this study are above the Clinical and Laboratory Standards Institute (CLSI) value of 0.2; hence the isolates obtained from this study are of public health significance. This study further confirmed that door handles of public toilets are possible sources

through which potential pathogenic microbes such as *Enterobacteriaceae* are introduced into the University community. Hand washing which is a first line of defense against disease spread is ignored by many people especially after using the rest room while some do it without proper attention.

OTP378

Bacteria associated with door handles and staircase handrails in the central administration buildings, University of Benin, Nigeria

I. O. Osaretin[1], F. Ekhaise*[1]
[1]*University of Benin, Department of Microbiology, Benin, Nigeria*

Fomites consist of both porous and non-porous surfaces or objects that can become contaminated with pathogenic microorganisms thereby serving as vehicles for the transmission and spread of infections. This study investigates the proliferation of microorganisms and their level of contamination on fomites associated with door handles and staircase handrails in the Central Administration Building, University of Benin, Benin City. A total of 144 samples were collected from 120 door handles and 24 staircase handrails using sterile swab sticks. The samples were analyzed using the basic microbiological techniques and further characterization was carried out by the molecular biology techniques. The total viable bacterial counts on door handles sampled ranged from 0.3 ± 0.1 × 103 cfu/cm^2 to 3.0 ± 0.6 × 103 cfu/cm^2 in the Vice Chancellor and Registry buildings. The total viable bacterial on staircase handrails sampled ranged from 2.3 ± 0.6 × 103 cfu/cm^2 to 5.1 ± 1.1 × 103 cfu/cm^2 in the Vice Chancellor and Registry buildings. Eight bacterial isolates were characterised and identified and they include *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Klebsiella oxytoca, Pseudomonas aeruginosa, Micrococcus luteus* and *Staphylococcus epidermidis. Staphylococcus aureus* (32.6%) recorded the highest percentage frequency of occurrence while the least was recorded for *Bacillus subtilis* and *Micrococcus luteus* (5.8%). The plasmid profile analyses revealed detectable plasmid fragments in *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella oxytoca* and *Micrococcus luteus*. There was no plasmid extracted from *Staphylococcus aureus* and their resistance to Augmentin, a beta-lactam antibiotic was observed before and after curing. It is therefore imperative that surface contamination of fomites thus plays a significant role in the transmission and proliferation of microorganisms of public health importance in the environment.

SeSiP379

A cyclic-di-GMP dependent lateral flagellar brake

A. Pecina*[1], T. Leonhard[1], P. Klüber[1], K. Thormann[1]
[1]*Justus-Liebig-Universität Gießen, Institut für Mikro- und Molekularbiologie, Gießen, Germany*

Shewanella putrefaciens CN-32 possesses two distinct flagellar systems encoded by two separate gene clusters enabling us to study the specificity of flagellar proteins. The polar flagellum is responsible for the main propulsion of the cell and responds to the chemotaxis system while the lateral flagellar system improves spreading efficiency by increasing directional persistence and establishes only in a subpopulation of cells during planktonic growth in complex media. This enables *S. putrefaciens* to employ a bet-hedging strategy with respect to motility: while a subpopulation of cells saves the energy required for synthesis and maintenance of the additional flagellum, another subpopulation is motile by use of both flagellar systems to occupy new environments. However, the mechanisms regulating the formation and operation of the lateral flagellum are unknown. We were able to show that the lateral system assembles and functions independently of the polar system and its chemotaxis system. We were able to identify and characterize several proteins involved in cyclic-di-GMP signaling, which affect lateral flagellar gene expression and function. FlgZ, an YcgR-like PilZ-domain protein, functions as a specific brake for the lateral flagellum. FlgZ reduces spreading ability on soft agar plates and slows down swimming speed in its c-di-GMP bound state. The second messenger molecule c-di-GMP thereby affects swimming motility of *S. putrefaciens* at the level of transcriptional control as well as posttranslational on motor function.

SeSiP380

***Escherichia coli* K-12 mutants with defective acetate production hint to a function of acetyl-P in controlling metabolic fluxes**

K. Bettenbrock*[1], A. Schütze[1], D. Benndorf[2]
[1]*Max-Planck Institut für Dynamik komplexer technischer Systeme, Experimental Systems Biology, Magdeburg, Germany*
[2]*OvGU Magdeburg, Bioprozesstechnik, Magdeburg, Germany*

Acetate is a typical by-product of *E. coli* K-12. While the reasons for the aerobic acetate production are still unclear, anaerobic acetate production is important for ATP synthesis. Acetate is produced by a pathway consisting of phosphate acetyltransferase (Pta), producing acetyl-phosphate from acetyl-coenzyme A, and acetate kinase (AckA), producing acetate from acetyl-phosphate, coupled to the production of ATP.

We investigated different mutants in the acetate pathway under aerobic and fermentative conditions. All mutants lack the possibility of ATP synthesis via the acetate pathway but differ in the potential to produce acetyl-phosphate. We determined growth rate as well as by-product synthesis under aerobic and anaerobic conditions. We analyzed expression of selected genes as well as protein abundance and acetylation.

Aerobically, the mutants showed no obvious phenotype. Under anaerobic conditions though, the mutants showed severe reduction in growth rate, coupled to changes in the by-product pattern. Differences in the expression and abundance of PFL, formate hydrogenlyase and lactate dehydrogenase were detected.

The most severe growth defect was observed for the AckA$^-$ mutant. This mutant shows significantly increased protein acetylation. Acetylation was increased for many enzymes of central metabolic pathways, like enolase and phosphoglycerate kinase. Also, in this mutant the *ato* operon involved in acetoacetate metabolism was upregulated.

The data show that production and accumulation of acetyl-phosphate has a strong impact on anaerobic growth of *E. coli*. They hint to an important function of protein acetylation in the control of metabolic fluxes. Protein acetylation might be a general means to fine tune fluxes in central metabolism of *E. coli*.

SeSiP381

The nucleotide second messenger cyclic di-AMP controls potassium homeostasis and cell wall remodeling in *Corynebacterium glutamicum*

S. Reich*[1], T. Weiß[1], G. M. Seibold[1]
[1]*Ulm University, Institute of Microbiology and Biotechnology, Ulm, Germany*

The Gram-positive *C. glutamicum* is widely known for its application in amino acid production and as a model organism for osmoregulation and cell wall biosynthesis. The nucleotide second messenger c-di-AMP has been shown to be involved in the control of e.g. resuscitation, potassium uptake and cell wall homeostasis in Gram-positive bacteria. Analysis of c-di-AMP levels in *C. glutamicum* revealed its involvement in the control of potassium homeostasis and cell wall metabolism. C-di-AMP hereby negatively affects potassium uptake by binding to the regulatory domains of the potassium channel CglK and controls the *nlpC* operon for cell wall remodeling via an *ydaO*-type riboswitch. In difference to most other bacteria, *C. glutamicum* possesses exclusively the diadenylate cyclase (DAC) DisA for synthesis and the phosphodiesterase PdeA for degradation of c-di-AMP. We analysed how c-di-AMP levels in *C. glutamicum* are controlled in response to potassium limitation and cell wall stress by these two proteins. DisA has been reported to bind DNA and was described as DNA integrity scanning protein. To assess control of c-di-AMP synthesis, we characterized DisA and show that presence of DNA slightly reduces DAC activity. It was described for other bacteria, that also the DNA damage related protein RadA, whose gene is located in an operon with *disA*, interacts with DisA and reduces its DAC activity. Contrary to this, RadA from *C. glutamicum* had no effect on c-di-AMP formation *in vitro* and *in vivo* upon co-expression of both genes in *E. coli*. Moreover, we analysed effects of potassium concentrations on the activity and abundance of DisA and PdeA of these proteins in different cultivation conditions. Based on these data the intertwined mechanisms for control of c-di-AMP levels will be discussed.

SeSiP382

Positive and negative control of the phosphorylation state of the response regulator NreC of *S. carnosus*

R. Klein*[1], A. K. Kretzschmar[1], G. Unden[1]
[1]*Johannes Gutenberg-University Mainz, Institute of Molecular Physiology, Mainz, Germany*

For maximal induction of the nitrate reductase (*narGHJI* genes) in Staphylococci anaerobic conditions, the presence of nitrate and the NreABC regulatory system are required[1]. The sensor kinase NreB represents the oxygen sensor of the NreBC two-component system with NreC as the response regulator. NreA acts as a nitrate receptor. NreA interacts with NreB and controls NreB phosphorylation in a nitrate dependent manner. NreA/NreB form a nitrate/oxygen co-sensing unit.

NreB contains like NarX, a putative phosphatase site (DxxxQ motive) adjacent to the conserved histidine residue. Mutants of the DxxxQ motive show reduced autophosphorylation of NreB and phosphoryl transfer to NreC. NreB was able to dephosphorylate NreC-phosphate, but only when NreA was present in the nitrate deficient form. We conclude, that NreB is a bifunctional sensor histidine kinase, which controls the phosphorylation state of NreC in complex with NreA.

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SeSiP383

Phosphorylation and DNA-binding of the two component DcuS-DcuR complex

S. Gencheva*[1], G. Unden[1]
[1]*Johannes Gutenberg-University Mainz, Institute for Molecular Physiology, Mainz, Germany*

The C_4-dicarboxylate responsive two component system DcuS-DcuR of *E. coli*, controls expression of genes for aerobic and anaerobic C_4-dicarboxylate metabolism including the transporter DctA (*dctA*) and DcuB (*dcuB*) [1]. It is composed of the membrane-bound histidine kinase DcuS and the cytoplasmic response regulator DcuR. Under aerobic conditions, DcuS forms a DcuS/DctA sensor complex. DcuS, DcuR and DctA co-localize close to the cell pole of *E. coli* forming a tripartite DctA/DcuS/DcuR sensor/regulator complex [2]. DcuS-DcuR complex and its response to phosphorylation and DNA-binding was studied *in vitro* with purified components, suggesting a non-expected pre-activation of DcuR by non-phosphorylated DcuS.

SeSiP384

α-helical axis dynamics in DcuS sensorkinase inter-domain signal transmission

M. Stopp*[1], A. Strecker[1], W. Steinchen[2], G. Bange[2], G. Unden[1]
[1]*Johannes Gutenberg-Universität, Institute of Molecular Physiology, Mainz, Germany*
[2]*Philipps-Universität, LOEWE-Center for Synthetic Microbiology, Marburg, Germany*

The DcuS/DcuR two component system regulates the C_4-dicarboxylate (C_4DC) metabolism in *E. coli* [1]. The perception of periplasmic C_4DC by the membrane-integral sensor histidinekinase DcuS results in cytoplasmic autophosphorylation [2]. The physical separation of signal input and output (phosphorylation) on different sides of the membrane requires a signal transfer mechanism. Signal transduction across the membrane is achieved by a piston type displacement of transmembrane helix 2 [3], but the complete mechanism is unknown.

Transmembrane signal and transfer to the cytoplasmic PAS_c domain has been investigated. A dimeric interface of DcuS has been characterized by *in vivo* cysteine crosslinking between TM2 and an extension of TM2 into PAS_c. Structural data indicate that TM2, a linker region and subsequent PAS_c α-helix 1 form a continuous α-helical axis. The dynamics in signal transduction has been analyzed by time resolved crosslinking and hydrogen/deuterium exchange mass spectrometry (HDX-MS) [4]. The data show static parts and dynamic rearrangement in the TM2-linker-PAS_cα1 of DcuS during fumarate induced signal transduction. This reveals different dynamics in the mechanism of connecting periplasmic signal input and cytoplasmic signal processing.

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SeSiP385

Acclimation of *Synechocystis* 6803 to a day-night cycle using canonical clock proteins

N. Scheurer*[1], C. Köbler[1], A. Wilde[1]
[1]*Albert-Ludwigs-Universität Freiburg, Biologie 3, Freiburg i. Br., Germany*

Cyanobacteria exhibit rhythmic gene expression with a period length of 24 hours to adapt to daily environmental changes. In the model organism *Synechococcus elongatus* PCC 7942 the central oscillator consists of the three proteins KaiA, KaiB and KaiC and utilizes the histidine kinase SasA and its response regulator RpaA as output-signaling pathway. In this organism, which is an obligate photoautotroph, the circadian clock system is rather well understood, whereas circadian networks of other cyanobacteria can be very diverse and are less exhaustively investigated. The cyanobacterial model strain *Synechocystis* sp. PCC 6803 contains in addition to the canonical *kaiAB1C1* gene cluster two further homologs of the *kaiB* and *kaiC* genes. In addition, the clock output pathway consisting of the SasA-RpaA two-component system is conserved. However, evidence of true circadian oscillation in *Synechocystis* sp. PCC 6803 is still inconclusive. Chemoheterotrophic growth of a Δ*rpaA* strain in the dark was abolished completely. Transcriptomic data revealed that RpaA is mainly involved in the regulation of genes related to CO2-acclimation in the light and to carbon metabolism in the dark. Specifically, genes involved in the metabolism of the storage compound polyhydroxybutyrate are upregulated in the dark in the wild type but not in the Δ*rpaA* strain. In summary, we show a wide-ranging involvement of RpaA in the regulation of the metabolism of *Synechocystis* sp. PCC 6803 in a diurnal light-dark rhythm. We suggest that *Synechocystis* sp. PCC 6803 employs homologs of the known canonical central and output clock components to control the switch between autotrophic (in the light) and chemotrophic (in the dark) metabolic modes.

SeSiP386

Biochemical and functional characterization of the bacterial phytochrome *Pa*BphP of *Pseudomonas aeruginosa*

C. Huber*[1], J. Pielage*[1], N. Frankenberg-Dinkel[1]
[1]*Technische Universität Kaiserslautern, Institut für Mikrobiologie, Kaiserslautern, Germany*

Through the ability to sense and respond to different environmental conditions bacteria can adapt rapidly to their surroundings. This allows bacterial pathogens to persist successfully in human and animal hosts. Sequence analysis revealed that the opportunistic pathogen *Pseudomonas aeruginosa* codes for a red and far-red light sensing bacteriophytochrome (*Pa*BphP). Phytochromes constitute a major photoreceptor family found in plants, algae, fungi, and bacteria. These photochromic biliproteins have been shown to play a central role in vital processes such as seed germination in plants and have also been linked to virulence in plant pathogens, but the role of the bacteriophytochrome in *P. aeruginosa* is still unclear.

Previous studies established that the genes encoding the two necessary bacteriophytochrome components of *P. aeruginosa* BphO, a chromophore-producing heme oxygenase, and BphP, the apo-phytochrome, are co-transcribed in a bicistronic operon. Transcription was shown to be induced in the stationary phase in a cell-density-dependent manner and dependent on the alternative sigma factor RpoS.

In order to elucidate the biological function of *Pa*BphP we constructed a markerless deletion mutant of *bphP* (PA14Δ*bphP*) which we will employ for phenotypical characterizations in red and far red illumination. We furthermore demonstrated autophosphorylation of *Pa*BphP, confirming its role as a sensor kinase and a postulated sensor of a two-component regulatory system, but the corresponding response regulator is still unknown. By applying homologously overexpressed BphP for cross-linking experiments and proteomics we hope to gain insight into the downstream signaling pathway of *Pa*BphP and advance our understanding of the role of a light sensor in *P. aeruginosa*.

SeSiP387

The diversity of PQS cleaving dioxygenases

S. Wullich*[1], A. Arranz San Martin*[1], S. Thierbach[1], S. Fetzner[1]
[1]*WWU Münster, Institute for Molecular Microbiology and Biotechnology, Münster, Germany*

Introduction: *Pseudomonas aeruginosa*, one of the leading nosocomial pathogens, uses the Pseudomonas quinolone signal (PQS; 2-heptyl-3-hydroxy-4(1H)-quinolone) besides N-acylhomoserine lactones to regulate its virulence. So far only a few 2,4-dioxygenases of the α/β-hydrolase-fold superfamily (e.g., AqdC from *Mycobacteroides abscessus* and HodC from *Arthrobacter* sp. Rue61a) are known to cleave PQS, thereby interfering with the virulence of P. aeruginosa. To examine the properties of PQS cleaving dioxygenases, homologous enzymes from different organisms were characterized regarding PQS cleaving ability, stability, and hydrolase-like properties.

Experiments: Putative PQS dioxygenases, identified by BLAST analysis, were expressed and purified, and their catalytic properties were determined. The thermal stability and the stability against *P. aeruginosa* exoproteases were tested by thermal shift assays and by specific protease assays, respectively.

Results: All tested enzymes had the ability to cleave PQS. The dioxygenases of *M. abscessus* and *Nocardia farcinica* showed the highest specific activity (> 60 U/mg) and k_{cat} values. Based on amino acid alignments, the complete catalytic triad of the α/β-hydrolase-fold superfamily is conserved in three of the seven examined dioxygenases, while in the others the nucleophilic residue is replaced by an alanine. Dioxygenases lacking the nucleophilic serine show up to 6-times higher k_{cat} values resulting in higher catalytic efficiency. We suggest that the presence of a nucleophilic residue reduces the reactivity of the substrate or later intermediates.

SeSiP388

Analysis of the sensor-phosphodiesterase NbdA of *Pseudomonas aeruginosa*

S. Zehner*[1], K. Heieck[1], K. Gerbracht[1], M. Rüger[1], N. Frankenberg-Dinkel[1]
[1]*Technische Universität Kaiserslautern, Mikrobiologie, Kaiserslautern, Germany*

The opportunistic human pathogen *Pseudomonas aeruginosa* is able to form biofilms, resulting in hardly treatable bacterial infections. The switch between the sessile and the planktonic lifestyle is controlled by the second messenger cyclic di-GMP (bis-(3"-5")-cyclic dimeric guanosine monophosphate). Several environmental cues have been identified to trigger biofilm dispersal. One of these

cues are non-lethal concentrations of nitric oxide (NO) [1]. The activation of the dispersal process involves the degradation of the second messenger c-di-GMP. In deletion and complementation assays, the protein NbdA (NO-induced biofilm dispersion locus A) was found to play a major role in the NO-induced biofilm dispersal response of *P. aeruginosa* [2]. NbdA consists of three conserved domains: MHYT-AGDEF-EAL. The MHYT domain contains 6 transmembrane helices and was postulated to be directly involved in NO sensing [3]. Functional complementation of an *E. coli* phosphodiesterase deficient strain with a truncated NbdA variant confirmed PDE activity *in vivo*. In order to study the impact of NO sensing on the enzymatic activity, full-length protein NbdA was recombinantly produced and purified via affinity chromatography. Enzymatic activity was tested for recombinant full length protein and cytoplasmic domains.The obtained data will be presented and discussed with respect to the current model of NO-induced biofilm dispersal in *P. aeruginosa.*

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SeSiP389

Identification of candidate genes associated with dimorphic switch and thermoregulation in *Zymoseptoria tritici* by genome-wide RNA-Seq time-course analysis

A. Yemelin*[1], S. Jacob[1,2], L. Antelo[1,2], K. Andresen[2], J. Sema[1], L. Heck[2], E. Thines*[1,2]
[1]*Institut für Biotechnologie und Wirkstoff-Forschung gGmbH (IBWF), Kaiserslautern, Germany*
[2]*Johannes Gutenberg-University Mainz, Mikrobiologie und Weinforschung am Institut für Molekulare Physiologie, Mainz, Germany*

Septoria tritici blotch disease, caused by *Zymoseptoria tritici* is one of the most devastating diseases affecting wheat worldwide. The fungus exhibits an environmentally triggered yeast-to-hyphal transition representing an essential prerequisite for pathogenesis. Disruption of this early event in infection would be therefore an effective means to prevent the disease. However, there is currently a limited understanding of the genetic mechanisms governing the dimorphic transition. In order to explore the genetic determinants and to elucidate the molecular basis of the regulatory mechanisms of the dimorphic switch process in *Z. tritici* a time course transcriptome analysis with the wildtype strain IPO323 was performed. We have used the next-generation RNA-Seq technique to analyze transcriptional changes upon nutrient deprivation and elevated temperature stress responses. The subsequent gene set enrichment and gene co-expression network analysis revealed a set of significantly regulated genes associated with the dimorphic switch. Among them, one candidate gene designated as *MYCO25* was validated by target gene inactivation. Analysis of the resultant mutant demonstrated that *MYCO25* is essential for the dimorphic transition since the mutant strain failed to undergo the dimorphic transition under inducing conditions and exhibited drastically reduced virulence. In addition, strains lacking this gene were found to be thermo-sensitive due to their inability to switch towards filamentous growth upon increased temperatures, making it a promising candidate for the elucidation of the genetic basis underlying the thermoregulation. With this study, we present several novel and functionally characterized genes unveiling new insights into the dimorphism related processes in *Z. tritici*.

SeSiP390

Fungicide resistance towards fludioxonil conferred by overexpression of the phosphatase gene *MoPTP2* in *Magnaporthe oryzae*

S. Bohnert[1], L. Heck[2], C. Gruber[1], H. Neumann[1], U. Distler[3], S. Tenzer[3], A. Yemelin[1], E. Thines[1,4], S. Jacob*[2]
[1]*Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Kaiserslautern, Germany*
[2]*Johannes Gutenberg-Universität (JGU) Mainz, Institut für Molekulare Physiologie, Mainz, Germany*
[3]*University Medical Center of Mainz, Institute for Immunology, Mainz, Germany*
[4]*Johannes Gutenberg-Universität (JGU) Mainz, Institut für Molekulare Physiologie, FB10, Mainz, Germany*

The fungicide fludioxonil causes hyperactivation of the Hog1p MAPK within the high osmolarity glycerol signaling pathway essential for osmoregulation in pathogenic fungi. The molecular regulation of MoHog1p phosphorylation is not completely understood in pathogenic fungi. Thus, we identified and characterized the putative MoHog1p-interacting phosphatase gene *MoPTP2* in the filamentous rice pathogen *Magnaporthe oryzae*. We found overexpression of *MoPTP2* conferred fludioxonil resistance in *M. oryzae*, whereas the "loss of function" mutant *ΔMoptp2* was more susceptible towards the fungicide. Additionally, quantitative phosphoproteome profiling of MoHog1p-phosphorylation revealed lower phosphorylation levels of MoHog1p in the MoPtp2p overexpression mutant compared to the wildtype strain, whereas MoHog1p phosphorylation increased in the *ΔMoptp2* mutant. Furthermore, we identified a set of MoHog1p-dependent genes regulated by the MoPtp2p expression level. Our results indicate that the phosphatase MoPtp2p is involved in the regulation of MoHog1p phosphorylation and that overexpression of the gene *MoPTP2* is a novel molecular mechanism of fungicide resistance.

SMP391

Biosynthesis of Sphingofungins in *Aspergillus fumigatus*

J. Rautschek*[1], A. Bissell[1], J. E. Kufs[1], D. J. Mattern[2], S. Hoefgen[1], Y. Huang[1], A. A. Brakhage[2], V. Valiante[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Leibniz Research Group - Biobricks of Microbial Natural Product Syntheses, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department of Molecular and Applied Microbiology, Jena, Germany*

Filamentous fungi are a treasure trove for biologically active secondary metabolites that can potentially be used as new therapeutics. They comprise a class of compounds that inhibit the biosynthesis of sphingolipids in eukaryotes. Since imbalanced sphingolipid homeostasis was found in numerous human diseases, such as diabetes and Alzheimer"s disease, sphingolipid inhibitors (SIs) are considered promising candidates for new therapeutics.

Analysis of genome databases suggests that there are more than three hundred putative fungal gene clusters encoding for SIs. Among the reported SIs are the fumonisins, identified in *Fusarium* and *Aspergillus*, and the structurally related sphingofungins from *Aspergillus fumigatus*. While the fumonisin biosynthesis was already studied, it remains

elusive whether analogous processes take place in other SI biosyntheses.

In order to elucidate SI biosynthesis, we identified the sphingofungin biosynthetic gene cluster in *A. fumigatus*, and created an overexpression mutant which produces higher amounts of sphingofungins upon cultivation. Furthermore, we prepared deletion mutants for all genes composing the biosynthetic genes cluster. With means of optimized large scale fermentation, we work on the isolation of intermediates and final products.

By gaining knowledge about the sphingofungin biosynthetic genes and intermediates, we can conclude general aspects of SI biosynthesis and will eventually be able to combine genes from different organisms in order to generate novel active compounds.

SMP392

Antibacterial activities of endolichenic fungi associated with tropical spiecies of *Parmotrema* and *Usnea*

J. A. Paguirigan*[1], M. A. Alcantara[1], S. M. Castillo[1], P. L. Co[1], E. Dumo[1], A. J. Espino[1], F. S. Flores[1], F. Lina[1], K. Pua[1], T. E. dela Cruz[1]
[1]*University of Santo Tomas, Biological Sciences, Sampaloc, Manila, Philippines*

Lichen-associated fuungi are gaining attention as sources of biologically active secondary metabolites. In this study, lichen thalli of *Parmotrema tinctorum, P. mesotropum, Usnea longissima, U. barbata* and *U. bismoliuscula* collected from Central Philippines were surface-sterilized for the isolation of endolichenic fungi (ELF). Fourteen of these ELF were mass-produced in solid-fermentation set-ups, extracted with ethyl-acetate and tested against *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa* and *Pantaoea agglomerans*. *Choniothyrium sp.* (US6-A) and *Oidendron sp.* (US5-G), both from *Usnea*, exhibited inhibitory activities against *Pantoea agglomerans* (16mm zone of inhibition, ZOI) and *Klebsiella pneumoniae* (30mm ZOI). *Syncephalastrum sp.* (F034D) isolated from *Parmotrema sp.* showed a weak inhibitory activity against *Staphylococcus aureus* (14mm ZOI). A weak ZOI (≤10mm ZOI) was observed for all crude culture extracts at a concentration of 30µg/µL. Culture media also influenced the antibacterial activity with endolichenic fungi cultivated on brown and white rice media exhibiting inhibitory activities up to 19mm ZOI. Endolichenic fungi are seen here as ideal candidates for drug discovery programs.

SMP393

Engineering of the filamentous fungus *Acremonium chrysogenum* for improved 7-aminocephalosporanic acid biosynthesis

X. Lin *[1], T. Dahlmann[1], U. Kück[1]
[1]*Ruhr-Universität Bochum, General and Molecular Botany, Bochum, Germany*

Introduction: Cephalosporins are a class of β-lactam antibiotics with a broad spectrum against both, Gram-positive and Gram-negative bacteria, and they act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. Most of the highly effective semi-synthetic cephalosporins are produced by modifying the side chains of 7-aminocephalosporanic acid (7-ACA), which is derived from cephalosporin C (CephC) by deacylation. CephC is exclusively produced by the filamentous fungus *Acremonium chrysogenum*, and in industrial applications CephC is converted to 7-ACA in vitro using two- or three-step enzymatic methods. Due to the high cost of complex enzymatic approach, scientists have exploited bacterial CephC acylases for one-step deacylation of CephC, but the efficiency in vitro was not significant for an industrial scale.

Objective: The goal is to express the CephC acylase gene in *A. chrysogenum* for efficient conversion of CephC into 7-ACA.

Methods: Two different classes of CephC acylase from *Pseudomonas* are available. Using mutated variants, both acylases were optimized by improvement on the substrate specificity towards CephC. DNA-mediated transformation was done by PEG-mediated protoplast transformation, followed by western blot and HPLC-MS analysis.

Results: After codon optimization, both CephC acylase genes were synthesized, and vector construction was done for optimal expression of the bacterial gene in the fungal host. Transformants have been selected, showing optimal CephC acylase activity. Further analysis includes enzyme measurements and cellular localization.

Conclusion: The preferred method to generate the precursor of semi-synthetic cephalosporins is an in vivo one-step bioconversion from CephC to 7-ACA in the filamentous fungus *A. chrysogenum.*

SMP394

Unraveling the enzymatic basis of pseudochelin biosynthesis

L. Winand*[1], J. Korp[1,2], A. Sester[1,2], M. Nett[1]
[1]*TU Dortmund University, Department of Biochemical and Chemical Engineering, Laboratory of Technical Biology, Dortmund, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany*

A wide range of soil inhabiting bacteria produce and secrete siderophores to complex insoluble ferric iron in the environment. Among these, the catecholates myxochelin A and B are widely distributed siderophores. Recently, the marine bacterium *Pseudoalteromonas piscicida* S2040 was found to produce a novel myxochelin derivative named pseudochelin A along with myxochelin A and B. It was predicted that pseudochelin A is formed by an intramolecular condensation in myxochelin B generating the characteristic 4,5-dihydroimidazole moiety of pseudochelin A.
To identify the key enzyme of this conversion, we compared the myxochelin regulons of two myxobacterial strains that produce solely myxochelin A and B with those of *P. piscicida* S2040. This approach revealed a gene exclusive to the myxochelin regulon in *P. piscicida* S2040, coding for an enzyme of the amidohydrolase superfamily. To prove that this enzyme is indeed responsible for the postulated conversion, the reaction was reconstituted *in vitro* using a hexahistidine-tagged recombinant protein expressed in *Escherichia coli*, with myxochelin B as the substrate. This reaction resulted in the production of a new compound, which was identified as pseudochelin A. With these results, the postulated biosynthetic pathway for pseudochelin A production in *P. piscicida* S2040 was validated. We identified a cofactor-independent enzyme belonging to the amidohydrolase family that is responsible for the imidazole ring formation in myxochelin B.

SMP395

Synthesis of calcimycin and its analogs in *Streptomyces chartreusis*

K. Arend*[1], J. Bandow[1]
[1]*Ruhr-Universität Bochum, Applied Microbiology, Bochum, Germany*

The genus *Streptomyces* is known for producing many biologically active substances in order to compete with other microorganisms in the natural environment. One of these substances is the polyether ionophore calcimycin produced by *Streptomyces chartreusis*, which transports divalent cations like Fe^{2+}, Mn^{2+}, and Ca^{2+} across membranes. [1] Besides calcimycin, *S. chartreusis* produces precursors and analogs of calcimycin, originating from the same biosynthetic pathway. [2] In previous studies it was shown that the synthesis of calcimycin and its analogs depends on the growth conditions. [3] We analyzed the production of calcimycin and its analogs at different temperatures and at different nutrient and precursor availability. The metabolites were extracted from the mycelia and culture supernatants, and analyzed by liquid chromatography–coupled tandem mass spectrometry. Based on the fragmentation pattern, calcimycin and known analogs were identified. Unknown analogs with similar fragmentation patterns, could be identified by molecular networking. The production of calcimycin and analogs was compared across growth conditions. We speculate that *S. chartreusis* synthesizes specific analogs depending on the ion and nutrient availability. A characterization of the ion transportation capabilities of the analogs is planned to shed light on their function.

[1] N. Raatschen *et al*., *Proteomics* **2013**, *13*, 1358-1370.

[2] Q. Wu *et al.*, *Antimicrobial Agents and Chemotherapy* **2011**, *55*, 974-982.

[3] C. H. R. Senges *et al.*, *Proceedings of the National Academy of Sciences of the United States of America* **2018**, *115*, 2490-2495.

SMP396

Purification of polyether ionophores of *Streptomyces chartreusis*

L. Sagurna *[1], C. Senges[1], J. Bandow[1]
[1]*Ruhr-Universität Bochum, Applied Microbiology , Bochum, Germany*

Soil bacteria of the genus *Streptomyces* are a source for numerous bioactive compounds that are widely used in medicine and industry. Current research focuses on the discovery of new secondary metabolites by combining genomics with untargeted data-dependent tandem MS and molecular networking. By using this approach >1000 distinct secreted metabolites were detected in culture supernatants of *S. chartreusis*[1]. For functional analysis the novel metabolites need to be purified.

Here we present a workflow for the purification of secondary metabolites produced by *S. chartreusis*[1] by isolating the polyether ionophore calcimycin. Calcimycin transports divalent cations like Ca^{2+}, Fe^{2+}, and Mn^{2+} across membranes and is therefore commonly used as biochemical tool to study calcium-dependent processes[2].To determine optimal production conditions, we analyzed the dependency of the calcimycin production on the duration of the cultivation, nutrient and ion availability. Highest amounts of calcimycin were measured after cultivation for 14 d in complex medium. Ca^{2+} supplementation led to an inhibition of calcimycin production while Mg^{2+} supplementation increased the production of the precursor cezomycin. The purification was performed by Flash chromatography. The purified samples were identified by tandem MS and structurally characterized by NMR spectroscopy.

In conclusion we optimized production conditions and established a workflow for the purification of calcimycin. This workflow can be adapted to purify and characterize detectable but so far uncharacterized secondary metabolites.

[1] Senges *et al.*, PNAS 2018, *115* 2490-2495

[2] Kozian *et al.*, BMC genomics 2005, *6* 146-158

SMP397

(Opto)genetic control of microbial cell factories for an efficient biosynthesis of valuable secondary metabolites

F. Hilgers*[1], F. Hogenkamp[2], D. Binder[1], A. Loeschcke[1], J. Pietruszka[2,3], K. E. Jaeger[1,3], T. Drepper[1]
[1]*Institute of Molecular Enzyme Technology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany*
[2]*Institute of Bioorganic Chemistry, Heinrich Heine University Düsseldorf, Forschungszentrum Jülich , Jülich, Germany*
[3]*Institute of Bio- and Geosciences IBG-1, Forschungszentrum Jülich, Jülich, Germany*

Secondary metabolites produced by plants and microorganisms represent a valuable source of compounds with pharmaceutical or biotechnological potential. Their efficient biosynthesis in a heterologous host is often hampered by certain bottlenecks, e.g. inefficient metabolic fluxes towards the aimed compound, or accumulation of unwanted or toxic intermediates. Such effects can be abolished by applying modular systems allowing for a strict control over the expression of key enzymes within recombinant biosynthetic cascades.

This project therefore aims to achieve a precise and straightforward control over complex gene cluster-encoded pathways by the application of light-triggered optogenetic switches such as photocaged compounds. As a proof of concept, one-step cleavable photocaged arabinose was synthesised and applied as a novel light-responsive inducer for heterologous gene expression in *E. coli*. Exposure of caged arabinose to UV-A light resulted in a rapid induction of protein production. The applicability of this phototrigger in a biotechnological context was demonstrated by successful expression of the violacein biosynthetic pathway from *Chromobacterium violaceum* and its gradual induction in *E. coli* by increasing light-intensities.

Next, the photoswitch principle will be transferred to the alternative production hosts *Rhodobacter capsulatus* and *Pseudomonas putida*, in order to optimize the synthesis of novel sesquiterpenes as well as prodiginines by light-triggered fine-tuning of the respective metabolite fluxes. For this purpose, expression studies with β-caryophyllene synthases were performed in *R. capsulatus* under specifically adapted conditions, resulting in a substantial production of the heterologous sesquiterpene with final yields of approx. 150 mg/L culture.

SMP398

Natural product and precursor production by effective TREX-mediated gene cluster expression in *Pseudomonas putida*

R. Weihmann*[1], S. Kubicki[1], S. Thies[1], A. Domröse[1], H. U. C. Braß[2], A. S. Klein[2], J. Pietruszka[2,3], T. Drepper[1], K. E. Jaeger[1,3], A. Loeschcke[1]

[1]Institute of Molecular Enzymtechnology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

[2]Institute of Bioorganic Chemistry, Heinrich-Heine University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

[3]Institute of Bio- and Geosciences IBG-1: Biotechnology, Forschungszentrum Jülich GmbH, Jülich, Germany

Background: High-value compounds can be produced efficiently by recombinant biosynthesis in microbial hosts like *Pseudomonas putida*. However, the transfer of entire metabolic pathways is typically hampered by difficulties in cloning and expression of large DNA fragments.

Methods: We developed molecular genetic tools named TREX (transfer and expression)[1] that consist of gene cassettes enabling i) the straightforward cloning of gene clusters *via* ligase independent cloning, ii) the transfer of a TREX-labeled gene cluster into a range of bacterial hosts and iii) its integration into the host chromosome *via* transposition. iv) Finally, expression of the gene cluster can be realized by random integration downstream of a chromosomal promoter or by employment of convergent T7 RNA polymerase-mediated expression.

Results: Using these tools, we could integrate several gene clusters up to 21 kb into the *P. putida* chromosome, enabling production of bioactive compounds including prodigiosin, violacein and a phenazine[1]. Furthermore, we could assemble distinct parts of different gene clusters and achieved the production of precursor molecules like prodeoxyviolacein of the violacein pathway. Likewise, strains producing the bipyrrole precursor MBC of the prodigiosin pathway were established that can be used for mutasynthesis approaches to create new-to-nature prodiginine compounds[2]. Moreover, random genomic gene cluster integration enabled the identification of naturally highly transcribed chromosomal regions suitable for gene cluster expression.

Conclusion: Our approach enables the rapid generation of bacterial production strains for the synthesis of valuable compounds.

[1]Domröse *et al.* 2017, doi:10.1016/j.synbio.2017.11.001

[2]Klein *et al*. 2018, doi:10.1002/cbic.201800154

SMP399

Bacterial bodyguards? – The defensive role of termite-associated actinomycetes

J. Schwitalla*[1], E. Seibel*[1], R. Benndorf[1], H. Guo[1], M. Poulsen[2], W. Z. de Beer[3], C. Beemelmanns[1]

[1]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Chemical Biology of Microbe-Host Interactions, Jena, Germany

[2]University of Copenhagen, Department of Biology, Section for Ecology and Evolution, Copenhagen, Denmark

[3]University of Pretoria, Forestry and Agricultural Biotechnology Institute (FABI), Pretoria, South Africa

Introduction: *Macrotermitinae* cultivate a mutualistic food fungus for nourishment in so called "fungus gardens". Symbiotic bacteria presumably defend the a nutrient-rich fungus garden by secretion of antimicrobials, which prevent growth of the insects' microbial competitors and pathogens. Bioassay-guided metabolomics analyses of two gut-associated actinomycetes led to the characterization of novel natural products with promising biological activities.

Objectives: By combining genomic and metabolomic approaches, we will explore the biosynthetic potential of two termite-associated Actinobacteria to assess their importance as defensive symbionts.

Methods: We isolated bacteria from the termite gut and fungus garden and assessed their bioactivities against fungal competitors and entomopathogenic fungi in co-cultivation studies. Bacterial strains with antifungal activity were chemically analyzed and the produced secondary metabolites were structurally characterized. Genome mining revealed their putative biosynthetic pathways. To assess the importance of these metabolites for the termite fungiculture, deletion mutants were generated.

Results: Two gut-associated actinomycetes with antimicrobial activity were isolated and their genome sequenced. *Amycolatopsis* sp. M39 produced the novel macrolactam macrotermycins and *Actinomadura* sp. RB29 the PKS-derived rubterolones, both showing promising antimicrobial or anti-inflammatory properties. Genome mining revealed their putative biosynthetic pathways and current studies are directed towards the heterologous expression and genetic manipulation of these biosynthetic pathways to reveal mechanistic insights into their biosynthesis.

Conclusion: The chemical analysis of symbiotic systems represents a rich source of new microbial species and novel natural products with unique chemical scaffolds.

SMP400

Discovery of natural products from soil metagenomes at the Schönbuch Forest

T. Negri*[1], F. Höhn[1], H. Sales-Ortells[1], N. Ziemert[1]

[1], Applied Natural Products Genome Mining, Tübingen, Germany

Metagenomic studies of bacterial 16S DNA sequences have shown that cultivable bacteria represent only a small fraction of the thousands of unique bacterial species present in the environment, and particularly in soil. Bacteria, and specifically soil bacteria, are one of the richest sources of antibiotics and other natural products. Although metagenomic analyses have previously led to the discovery of new molecules and shown that there is more diversity of secondary metabolites than has been previously cultivated, not much is known about the biosynthetic capacity and variability of secondary metabolites in different soil types and how to connect this diversity directly to molecules.

To more easily identify environments with the highest potential for natural product discovery we used a screening method that uses specific domains (Ketosynthase domains) as small sequence tags for the rapid detection and analysis of secondary metabolite genes. Using this method we screened three different soil types and their horizons from the Schönbuch Forest. We were able to identify "Cambisol" as one of the most promising types of soils analyzed and constructed a metagenomic fosmid library consisting of approx. 18000 clones and inserts of 20 kb on average that we screened for secondary metabolite gene clusters. Here we show first results about the diversity of secondary metabolites in soil and how this strategy can lead to the discovery of new secondary metabolite natural products with therapeutic application such as new antibiotics.

SMP401

Biosynthesis and biological function of a new and biologically active deoxy-sugar from *Synechococcus elongatus*

J. Rapp*[1], B. Klaus[1], R. Pascal[1], G. Stephanie[1], F. Karl[1]
[1]*Universität Tübingen, Tübingen, Germany*

Cyanobacteria are known as producers of a wide range of bioactive compounds. Most of them were isolated from filamentous species. Only little is known about bioactive compounds which are formed by unicellular cyanobacteria. In a previous work (Brilisauer et al. 2018), a bioactive compound from the unicellular cyanobacterium *Synechococcus elongatus* PCC 7942 (*S. elongatus*) could be isolated and characterized. The compound showed bioactivity against *Anabaena variabilis* ATTC 29413 (*A. variabilis*) and could be determined as a C7 sugar with a terminal deoxy-group. Further, other cyanobacteria as well as the producer strain are growth inhibited by the addition of this sugar. The *in vivo* biosynthesis and the physiological function of this deoxy-sugar are still unknown. First results have shown that the compound is not specifically produced by *S. elongatus* but also by other cyanobacteria. This leads to the hypothesis that it is not a specifically formed secondary metabolite but rather a side product of the primary metabolism. This is also underlined by the fact that the compound could be chemoenzymatically synthesized *in vitro* via a transketolase based reaction. The intracellular target of the compound is the shikimate pathway. Sensitivity towards the compound is dependent on an active uptake of the compound. In the highly sensitive *A. variabilis* strain, it could be shown that the deoxy-sugar is taken up via the fructose transporter.

References:

Brilisauer, K.; Rapp, J.; Rath, P.; Schöllhorn, A.; Bleul, L.; Weiß, E.; Stahl, M.; Grond, S.; Forchhammer, K. (2018): Under revision.

SMP402

Identification and ecological role of secondary metabolites produced by *Termitomyces*- the fungal mutualist of fungus growing termites

N. Kreuzenbeck*[1], I. Burkhardt[2], E. Seibel[1], H. Hu[3], M. Poulsen[3], J. S. Dickschat[2], C. Beemelmanns[1]
[1]*Hans Knöll Institut für Naturstoffforschung und Infektionsbiologie, Chemische Biologie der Mikroben Wirt Interaktionen, Jena, Germany*
[2]*Kekulé Institut für Organische Chemie und Biochemie, Bonn, Germany*
[3]*University of Copenhagen, Department of Biology, Copenhagen, Denmark*

Introduction: *Macrotermitinae* cultivate the mutualistic food fungus *Termitomyces* in so called "fungus gardens", a nutrient-rich environment prone to exploitation by alien species. Symbiotic bacteria and the mutualistic fungus presumably defend the garden by secretion of antimicrobials, which prevent growth of garden parasites. The close interspecies relationship requires recognition and interaction that is most likely of chemical nature and might require the production of volatile compounds.

Objectives: We hypothesize that secondary metabolites produced by *Termitomyces* sp. contribute to the protection of the symbiosis and influence termite behaviour.

Methods: Based on available genome data of *Termitomyces* sp., we identified several putative terpene cyclases. Additional RNAseq data revealed the actively transcribed genes encoding terpene cyclases in the fungus comb. To match the respective metabolites to genes, we heterologously expressed candidate genes and analyzed the produced compound by GC-MS and NMR. Now, we are investigating the ecological function of these metabolites by monitoring gene expression of the respective natural product biosynthetic genes using different co-culture set-ups and by performing insect antifeedant assays.

Results: We identified about 30 genes encoding for terpene cyclases in the *Termitomyces* sp. P5 genome, half of which were actively transcribed in the fungus comb and under lab conditions. Three terpenes were identified from heterologously expressed enzymes. In addition, terpene derivatives were found and structurally characterized from *Termitomyces* sp. culture extracts.

Conclusion: We propose that *Termitomyces* sp. actively orchestrates its interaction with the insect by secretion of bioactive small molecules.

SMP403

Characterization of secondary metabolite gene clusters from the human-pathogenic fungus *Aspergillus calidoustus*

J. E. Kufs*[1], J. Lin[1], J. Rautschek[1], S. Hitzler[1], S. Hoefgen[1], V. Valiante[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Leibniz Research Group Biobricks of Microbial Natural Product Syntheses, Jena, Germany*

The fungus *Aspergillus calidoustus* is an opportunistic human pathogen harboring a huge potential for the production of natural products. Whole genome sequencing and genome mining revealed the presence of 53 potential secondary metabolite gene clusters. However, only few compounds have been identified so far. This suggests that most of the gene clusters remain still silent under laboratory conditions.

In order to circumvent this issue we started to express putative secondary metabolic gene clusters from *A. calidoustus* heterologously. A computationally identified gene cluster encoding a polyketide synthase and five tailoring enzymes was expressed in the well-studied fungus *Aspergillus nidulans* using a polycistronic expression system. We performed comparative metabolomics using High-Resolution Mass Spectrometry which led to the discovery of new metabolites that are currently under investigation. By assembling the PKS gene with different combinations of the tailoring enzymes, we work on determining the underlying biosynthetic pathways.

SMP404

Biochemical and physiological characterization of the FAD-dependent monooxygenase HmqL from *Burkholderia thailandensis*

A. Mährlein*[1], S. Ernst[1], N. H. Ritzmann[1], S. L. Drees[1], S. Fetzner[1]
[1]*WWU Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*

Introduction: 2-Alkyl-4-hydroxyquinoline N-oxides (AQNOs) are antibiotic compounds produced by several species of the *Burkholderia* genus. Their biosynthesis involves the key enzyme HmqL, a flavin dependent monooxygenase catalyzing the hydroxylation of a primary aromatic amine. The isofunctional *Pseudomonas aeruginosa* homolog PqsL

possesses unique features such as elevated generation of ROS and utilization of reduced flavin as co-substrate. According to preliminary data, HmqL shows major differences as regards the reductive half reaction.

Objectives: Our aim was to study the co-substrate specifity of HmqL and the kinetics of individual reaction steps. Additionally, specific phenotypes that result from biochemical differences between PqsL and HmqL were investigated.

Methods: FAD binding and the kinetics of the oxidative and reductive half reaction of HmqL were analyzed using various spectroscopic methods. For physiological investigations, *P. aeruginosa ΔpqsL* was complemented with *hmqL* and growth, biofilm formation and AQNO production under various conditions were determined.

Results: HmqL resembles PqsL in structure and catalytic activity. Reduced flavin and, to a low extent, NADH are used as electron donors, whereas NADPH is not. The rate constant of the oxidative half reaction is virtually identical to that of PqsL. In contrast to PqsL, HmqL is able to stabilize reduced flavin and thereby reduces uncoupling. The impact of biochemical differences between PqsL and HmqL on *P. aeruginosa* physiology was demonstrated by genetic complementation.

Conclusion: Although HmqL and PqsL catalyze the same reaction and are involved in the same biosynthetic pathway, both enzymes show remarkable differences in their biochemical properties, which are also reflected in phenotypic differences.

SMP405

Introduction and characterization of ECF σ factors in the emerging model organism *Vibrio natriegens*

B. Daniel*[1], P. Schoenberg[1], G. Fritz[1]
[1]*Synmikro, Computational Biology, Marburg, Germany*

Extracytoplasmic function σ factors (ECFs) are increasingly important in the construction and assembly of orthogonal synthetic circuits. A wide variety of ECFs havebeen shown to work host independently and orthogonally, enabling complex circuits with minimal impact on chassis performance [Pinto et al., 2018].

Recently, *V. natriegens* has gained attention as an alternative, faster host for synthetic biology. Its up to three-fold higher doubling rate could reduce development time and costs. By showing for the first time that ECFs work reliably and predictably in this new host, and by establishing a rational characterization methodology, we aim at significantly shortening the build-test design cycle for novel synthetic regulatory networks.

To this end we incorporated a diverse set of ECFs into the Marburg Collection – a Golden Gate-based toolbox for *V. natriegens* and benchmarked their activity with a codon optimized luciferase cassette. Initial results show that ECFs feature similar orthogonality to what was previously observed in *E. coli*.

Hence, by establishing ECF σ factors as orthogonal regulators in the emerging chassis *V. natriegens,* the goal of quickly building and optimizing synthetic circuits seems within reach.

SMP406

Investigation of the Δ^3,Δ^2-enoyl-CoA isomerase PaaG of the phenylacetic acid catabolic pathway

M. Spieker*[1], R. Saleem Batcha[1], R. Teufel[1]
[1]*University Freiburg, Biology, Freiburg i. Br., Germany*

Aromatic compounds are the second most abundant class of natural growth substances comprising, e.g., aromatic amino acids, raw oil components and environmental pollutants. Phenylacetic acid (paa) is a key intermediate in the aerobic degradation of many aromatic compounds and is further catabolized via CoA-bound intermediates (1). After initial epoxidation of the aromatic ring, the Δ^3,Δ^2-enoyl-CoA isomerase PaaG catalyzes an unusual isomerization step that converts epoxyphenylacetyl-CoA into oxepin-CoA. We set out to characterize PaaG in detail on a biochemical and structural level. We acquired co-crystals of PaaG with the native substrates, which allowed us to further investigate the catalytic mechanism via site-directed mutagenesis. Notably, after hydrolytic ring-cleavage of oxepin-CoA, the open-chain intermediates are then processed via beta-oxidation like steps. Here, PaaG may catalyze a second isomerization step that converts cis-3,4-didehydroadipyl-CoA into cis-2,3-didehydroadipyl-CoA, which is currently under investigation. Our data contributes to our knowledge how inert aromatic compounds are degraded in nature.

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SMP407

Formation of an Inorganic-Organic Polymer

J. Hildenbrand*[1], D. Jendrossek[1], S. Reinhardt[1]
[1]*Universität Stuttgart, Institut für Mikrobiologie, Stuttgart, Germany*

Polyphosphate (polyP) is an inorganic polymer that can be produced by all living organisms. PolyP is synthesized from ATP by the action of polyphosphate kinases (PPKs). Poly(3-hydroxybutyrate) (PHB) is an organic biopolymer that constitutes a reserve material for carbon and energy in many prokaryotic species. PHB is synthesized by the PHB synthase (PhaC)-catalyzed polymerization of the monomeric precursor 3-hydroxybutyryl coenzyme A (3HB-CoA). The beta-proteobacterium ***Ralstonia eutropha*** is able to produce separately localized polyP and PHB granules if cultivated under imbalanced growth conditions. Here, we constructed a gene fusion of the PHB synthase gene ***phaC*** with the polyP kinase gene ***ppk2c*** and expressed the ***ppk2c-phaC*** fusion gene in a PHB-polyP negative background in which the PHB synthase gene and all (seven) known polyP kinase genes had been deleted. Expression of the ***ppk2c-phaC*** fusion restored the ability to synthesize polyP and PHB granules. Remarkably, the polyP and PHB granules produced by ***ppk2c-phaC*** expressing cells were physically attached to each other and often formed chains of alternating polyP granules and PHB granules ***in vivo***. Physically attached polyP and PHB granules were also synthesized ***in vitro*** using the purified PPK2c-PhaC fusion protein to which the monomeric substrates of the PPK2c and PhaC domain had been added (ATP and 3HB-CoA).

SMP408

Photocaged IPTG derivatives for light-controlled gene expression in *Rhodobacter capsulatus*

O. Klaus*[1], A. Lappe[1], F. Hilgers[1], F. Hogenkamp[2], A. Loeschcke[1], J. Pietruszka[2,3], K. E. Jaeger[1,3], T. Drepper[1]

[1]Institute of Molecular Enzyme Technology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

[2]Institute of Bioorganic Chemistry, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

[3]Institute of Bio- and Geosciences IBG-1, Forschungszentrum Jülich, Jülich, Germany

Optogenetic tools allow the precise regulation of cellular processes like gene expression in a non-invasive fashion. Besides photoreceptors, photocaged compounds, which release bioactive molecules after short illumination, are highly valuable tools for light-triggered engineering of biotechnological processes in bacteria. For example, multi-step syntheses of valuable secondary metabolites such as terpenoids are often hampered by ineffective metabolite fluxes. This limitation and the modular architecture of these secondary metabolite pathways suggest that they are well suited for the concept of light control. A bacterium engineered for light-triggered adjustment of metabolic fluxes toward the central isoprenoid precursors could thus be used as a highly adaptive platform host applicable for the synthesis of a broad range of valuable terpenoids.

In our recent studies we could demonstrate that the phototrophic bacterium *Rhodobacter capsulatus* offers unique physiological properties that are favorable for the biosynthesis of hydrophobic terpenes. In order to evaluate if the phototrophic lifestyle of *R. capsulatus* can be combined with optogenetic engineering, we evaluated various photocaged IPTG derivatives for light-triggered control of heterologous gene expression. The new caged inducer molecules differ in the hydrophobicity of the respective protecting group resulting in differential solubility and membrane permeability. By using YFP as a reporter protein we could demonstrate that the cIPTG derivatives can be used for UV-A-light-mediated control of P_{tac} promoter activity under phototrophic and non-phototrophic conditions. In a next step, the optimal inducer/strain combination will be applied for the optogenetic engineering of sesquiterpenoid production in *R. capsulatus*.

SMP409

The iGEM competition at the Center for Biotechnology, Bielefeld UniversityInsights of ten years iGEM Bielefeld

N. C. Lübke*[1,2], J. Droste[1], B. Pucker[3], C. Rückert[1], J. Kalinowski[1]

[1]Bielefeld University / Center for Biotechnology, Microbial Genomics and Biotechnology , Bielefeld, Germany

[2]Bielefeld University / Center for Biotechnology, teutolab biotechnology, Bielefeld, Germany

[3]Bielefeld University / Center for Biotechnology, Genetics and Genomics of Plants , Bielefeld, Germany

The international Genetically Engineered Machine (iGEM) competition gathered in 2018 roughly 3000 enthusiastic synthetic biologists from all around the globe at its final the Giant Jamboree, in Boston, USA. Since 2004, the competition offers students the possibility to realize own projects in the field of synthetic biology using standardized DNA parts, called BioBricks. Further, the competition fosters the participants" engagement in assessing their projects beyond the lab bench by engaging various industrial and social stakeholders.

The poster will present the ten years from 2010-2019 of Bielefeld University"s Center for Biotechnology"s (CeBiTec) participation at the iGEM competition. The organizational framework of starting, establishing, and maintaining an iGEM collegiate team and the new High School team will be illustrated. In addition, benefits for participants and universities were elaborated at the example of the Bielefeldian teams.

The foci of the projects span from waste water treatment and biosensors to microbial fuel cells, *in vivo* direct evolution of binding proteins, or the incorporation of unnatural base pairs and non-canonical amino acids. Some of these results led to published papers regarding iGEM"s human practice efforts, a comprehensive biosafety review, and the transcriptome analysis of *Croton tiglium*.

The poster will use the successful 10 years iGEM Bielefeld story - over 100 actively participating people, 9 gold medals, and different special prizes ranging from track prizes to European Regional Winners" - to illustrate the possibilities the competition can offer for attendees and participating universities. The iGEM projects harbor additional experience for the training of molecular biologists.

SMP410

Studies on the formation of the indolopyrrole moiety during pyrroindomycin biosynthesis

S. Bernhardt*[1], K. H. van Pée[1]

[1]Technische Universität Dresden, Technische Biochemie, Dresden, Germany

Pyrroindomycin B is a chlorinated secondary metabolite produced by *Streptomyces rugosporus* which shows *in vivo* activity against Gram-positive bacteria and a good *in vitro* activity against vancomycin-resistant Enterococci and methicillin-resistant *Staphylococcus aureus* (MRSA).

In 2005, Zehner *et al*. characterised PyrH as a halogenase responsible for chlorination of L-tryptophan in position 5 to give the intermediate 5-chlorotryptophan. In the next step, the second pyrrole ring is formed to generate the pyrroloindolic acid. In the biosynthesis gene cluster of pyrroindomycins, the gene *pyrK1* was identified, which shows sequence homology to the family of tryptophan dioxygenases. The amino acid sequence of the enzyme shows an identity of 35% and a homology of 51% to PrnB, a haem-dependent tryptophan dioxygenase from pyrrolnitrin biosynthesis. PrnB catalyses the rearrangement of 7-chlorotryptophan to monodechloroaminopyrrolnitrin via an intermediate, which is suggested to be also formed by PyrK1. Therefore, we assume that PyrK1 catalyses the cyclization of tryptophan to the indolopyrrole derivative. In the gene cluster, near to *pyrK1,* the genes *pyrI3*, *pyrK2* and *pyrK3* were found, which encode for an acyl-CoA synthetase (AMP-forming)/ AMP-acid ligase, a non-ribosomal peptide synthetase and a ketoacyl-ACP synthase. These enzymes might be also involved in the formation of the indolopyrrole moiety and the transfer to the deoxytrisaccharide.

To analyse, whether PyrK1, PyrI3, PyrK2 and PyrK3 are actually involved in the biosynthesis of pyrroindomycin, we are performing several different investigations: 1) deletion and complementation of the genes in *S. rugosporus* and 2) overexpression and purification of the enzymes to show *in vitro* activity in order to clarify the reaction mechanism.

SMP411
Siderophore Biosynthesis in Esca-Associated Fungi
I. Büttel*[1], L. Antelo[2], A. L. Hiller[1], K. Andresen[1], E. Thines[2,1]
[1]*Johannes Gutenberg-University Mainz, Institute of Molecular Physiology, Microbiology and Wine Research, Mainz, Germany*
[2]*Institute of Biotechnology and Drug Research (IBWF), Kaiserslautern, Germany*

Iron is essential for almost all living organisms, but its bioavailability is limited. Therefore, many fungi secrete siderophores for iron acquisition. Siderophores are high-affinity iron-chelating compounds. However, in several plant-pathogen interactions these molecules were described as virulence factors.

Esca is one of the major grapevine trunk diseases that causes devastating damage and decay of grapevines worldwide. It is considered to be a disease complex comprising several fungi; e.g. *Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium minimum* (*Pmi*), *Fomitiporia mediterranea* (*Fmed*) and *Eutypa lata* (*Ela*). The disease trigger has not yet been identified and effective methods to control esca are still needed. To date the role of siderophores involved in esca disease has not been addressed to large detail.

Studies in our group led to the identification of the secreted siderophores of the esca-associated fungi in axenic culture.

Putative gene clusters containing essential genes needed for the biosynthesis of siderophores (non-ribosomal peptide synthetase-, L-ornithine-N5-monooxygenase- and transacetylase-encoding gene) have been identified. Corresponding gene inactivation mutants were generated for *Pch* and *Pmi* by *Agrobacterium tumefaciens*-mediated transformation system and confirmed by Southern Blot analysis. Furthermore, the phenotypes were analyzed with respect to iron-depending siderophore biosynthesis. Since no efficient transformation system for gene inactivation in *Ela* and *Fmed* have been reported to date, the function of the predicted genes of *Ela* and *Fmed* are investigated using a heterologous transformation system in *Magnaporthe oryzae*.

SMP412
Investigation of natural product biosynthesis in entomopathogenic bacteria
M. Westphalen*[1], S. Wenski[1], Y. M. Shi[1], H. B. Bode[1]
[1]*Goethe Universität Frankfurt am Main, Institut for Molekulare Biowissenschaften, Frankfurt a. M., Germany*

Entomopathogenic bacteria from the genera *Photorhabdus* and *Xenorhabdus* live in mutualistic symbiosis with their nematode host and together infect and kill insect larvae. Within this complex relationship the bacteria produce natural products that might for example contribute to killing the insect prey and to the defence of food competitors for the insect cadaver. The bacteria"s challenging lifestyle requires adaptation to the changing conditions and hence several layers of regulation are assumed and only a few of them have been discovered so far.

Natural products with a biological activity represent a potential for the development of drugs. Hence, we analyse natural product biosynthesis gene clusters for their natural product production. We generated arabinose-inducible P_{BAD} promoter exchanges in *X. hominickii* in front of the respective gene clusters and tested different cultivation conditions and media for optimal production conditions, followed by compound purification for structure elucidation. Furthermore, we investigated the natural product"s role in the bacteria"s life as they can not only be responsible for killing of the insect or repelling competing bacteria but might also function as signalling molecules within the bacteria or inter-species. Promoter exchange and/or deletion mutants for natural product biosynthesis gene clusters were constructed and analysed using HPLC-MS. Comparison between mutant and wild type strain regarding phenotype, MS profile and growth behaviour can help elucidate the natural product"s biological function. Furthermore, analysis of the biosynthesis mechanism and regulation of involved genes and proteins, e.g. transcriptional regulators, as well as their transcription profile are powerful tools to answer this question.

SMP413
Identification of the biosynthetic gene cluster for antibiotic acyldepsipeptides in *Streptomyces hawaiiensis* NRRL 15010
D. Thomy*[1], E. Culp[2,3], M. Adamek[4], Y. Q. Cheng[5], N. Ziemert[4], G. D. Wright[2,3], P. Sass[1], H. Brötz-Oesterhelt[1]
[1]*University of Tübingen, Department of Microbial Bioactive Compounds, Tübingen, Germany*
[2]*McMaster University, Department of Biochemistry and Biomedical Sciences, Hamilton, Canada*
[3]*McMaster University, M. G. DeGroote Institute for Infectious Disease Research, Hamilton, Canada*
[4]*University of Tübingen, Department of Applied Natural Products Genome Mining, Tübingen, Germany*
[5]*University of North Texas Health Science Center, UNT System College of Pharmacy, Fort Worth, TX, United States*

Streptomyces hawaiiensis NRRL 15010 is the producer strain of a set of acyldepsipeptides (ADEPs), one of which, ADEP1, possesses promising activity against Gram-positive bacteria. It is composed of a hexadepsipeptide core, comprising a non-canonical (2*S*,4*R*)-4-methylproline (MePro), and a C8-triene side-chain, and is the precursor for a variety of synthetic derivatives.[1,2]

ADEPs target the proteolytic core ClpP of the bacterial caseinolytic protease, an ATP-dependent serine protease with an important role in protein homeostasis and quality control. By binding to ClpP, ADEPs outcompete associated Clp-ATPases, thus inhibiting all natural functions of the complex, and subsequently induce a conformational shift that widens the ClpP entrance pores, leading to entry and degradation of essential proteins, which is fatal to the bacterial cell.[2]

Despite extensive investigations on the mechanism of action and antibiotic potency against various organisms, the biosynthesis of ADEP1 by *S. hawaiiensis* remains to be elucidated. We have identified a putative ADEP biosynthetic gene cluster in *S. hawaiiensis* utilizing a combination of bioinformatic and phylogenetic approaches, and applied a substrate specificity assay for isolated adenylation domains of nonribosomal peptide synthetases (NRPSs) for biochemical characterization.[3] Heterologous expression of the region of interest, which includes genes encoding two NRPSs, a type II PKS, and genes for the biosynthesis of MePro, led to successful ADEP production. Thus, we could confirm the identity of the gene cluster responsible for ADEP biosynthesis in *S. hawaiiensis.*

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[2] Brötz-Oesterhelt *et al.* 2005, *Nat Med* 11:1082-1087

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SMP414

Unexpected Bacterial Origin of the Antibiotic Icosalide: Two-Tailed Depsipeptide Assembly in Multifarious Burkholderia Symbiont

S. Niehs*[1], B. Dose[1], K. Scherlach[1], L. Flórez[2], M. Kaltenpoth[2], C. Hertweck[1]
[1]*Hans-Knöll-Institut Jena, BMC, Jena, Germany*
[2]*Institute of Organismic and Molecular Evolution, Department for Evolutionary Ecology, Mainz, Germany*

Burkholderiaceae represent one of the most diverse bacterial families known, with members being distributed in varied niches all over the world. The frequent association of *Burkholderia* spp. with higher organisms, such as the South American pest beetle *Lagria villosa*, has garnered significant interest regarding the mutualistic interactions of both symbiotic partners. In this symbiosis in particular, the survival of the insect eggs is supported through the antibacterial and antifungal properties of *Burkholderia*-derived natural products. From extracts of endosymbiotic *Burkholderia gladioli* we recently detected an unusual two-tailed lipocyclopeptide, icosalide, that was previously isolated from the fungus *Aureobasidium* sp. Icosalide biosynthesis in the bacterial symbionts involves a non-canonical NRPS with two starter condensation domains for β-hydroxy fatty acid incorporation in two different modules. *In silico* analyses revealed that this unusual NRPS assembly line is widespread among *Burkholderia* species, perhaps indicating a role for icosalide in symbiotic interactions. Bioactivity assays showed the inhibitory effect of icosalide towards potential environmental competitors. Generation of a null mutant confirmed the responsible biosynthetic gene cluster and, unexpectedly, revealed an anti-swarming activity of icosalide. Our results not only demonstrate that icosalide is a bacterial metabolite but also reveal another weapon in the protective chemical arsenal of *Burkholderia* symbionts of *Lagria* beetles. Furthermore, the non-canonical NRPS module architecture we identified might inspire synthetic biology approaches to generate therapeutically useful two-tailed lipopeptides.

SMP415

Model-based pathway engineering towards optimized heterologous triterpene production in *Synechocystis sp.* PCC 6803

A. Germann*[1], S. Triesch*[1], M. Dietsch[1], R. Steuer[2], I. M. Axmann[1]
[1]*Heinrich-Heine-University, Synthetic Microbiology, Düsseldorf, Germany*
[2]*Humboldt-University, Institute for Theoretical Biology, Berlin, Germany*

Triterpenes and triterpenoids build a large class of almost ubiquitous natural products with a broad structural diversity, hence gaining increased importance in pharmacology as well as in plant sciences and molecular biology, to name but a few.

Since the synthesis of those structures is a chemically highly sophisticated task, it is far from any industrial application. Therefore, the use of genetically modified microorganisms provides a sustainable alternative for large scale plant triterpenoid synthesis. One effort to overcome initially low yields of heterologous expression is constraint-based flux analysis, a mathematical approach to identify target reactions for modification to enhance product formation.

The current study is focused on the cyanobacterial MEP-pathway, and the production of cyclized oxidosqualene metabolites. To evidently review the modification targets suggested by modeling, key reactions in the MEP-pathway were enhanced by overexpression. Afterwards, triterpene-directed metabolite flux was compared with the model prediction.
Preliminary results show enhanced triterpene yield when IDI, an enzyme catalyzing a key reaction in the MEP pathway, is synthesized above native rates.

Nonetheless, the genome-scale reconstructions, needed for computational analysis, are often incomplete and need to be supplemented with experimental data. For that purpose different metabolite fluxes were quantified, analyzed and fed into the metabolic model, to make the predictions more precise.

SMP416

Lentinulin A and B: structure and bioactivity of new RiPPs from *Lentinula edodes* CBS 454.69

J. Kühlborn[1], T. Opatz[1], E. Thines[2,3], K. Andresen*[2]
[1]*Johannes Gutenberg-University Mainz, Institute of Organic Chemistry, Mainz, Germany*
[2]*Johannes Gutenberg-University Mainz, Microbiology and Wine Research, Institute of Molecular Physiology, Mainz, Germany*
[3]*Institut für Biotechnologie und Wirkstoff-Forschung gGmbH (IBWF), Kaiserslautern, Germany*

The nematicidal substance omphalotin A, a cyclic peptide with backbone N-methylation, was long thought to be synthetized by a NRPS until the genome of *Omphalotus olearius* was first published. Since then it was obvious that the genome contains no such large NRPS necessary for a non ribosomal synthesis. In 2017 van der Velden *et al.* published that omphalotin A belongs to the group of ribosomally synthesized and posttranslationally modified peptides (RiPPs) and proposed the name "borosins" for this new family of RiPPs. The gene (OmpA) bearing the nucleotide sequence that determines the omphalotin A peptide at its 3´end is also encoding the enzyme for the N-methylation and is part of a gene cluster that can as well be found in other basidiomycetes.

Our findings show the arrangement of a homologous gene cluster in *Lentinula edodes*. This fungus is also known as shiitake and is a very important edible mushroom in East Asia. Two new cyclic peptides of the borosin RiPP type, lentinulin A and B were isolated from the culture extract of a fungal strain *L. edodes* CBS 454.69, by silica gel, gel filtration and HPLC purification. The chemical structures of lentinulin A and B were elucidated as new N-methylatad cyclic dodecapeptides by NMR and mass spectrometry. Lentinulin A and B both exhibited specific nematicidal activity towards second-stage juveniles larvae (J2) of the plant pathogenic root-knot nematode *Meloidogyne incognita*. In this experiment, the compounds were even more active than the commercially available nematicide ivermectin. Both compounds showed no activity towards the saprophytic nematode *Caenorhabditis elegans*, were not active in phytotoxic, antibacterial or antifungal screens and had no cytotoxic activity.

SMP417

Heterologous expression of NRPS-like coding genes in *Magnaporthe oryzae*

H. Neumann*[1], L. Antelo[2], A. Schüffler[2], K. Andresen[1], E. Thines[1,2]
[1]*Institute of Molecular Physiology (IMP), Mikrobiologie und Weinforschung, Mainz, Germany*
[2]*Institute of Biotechnology and Drug Research (IBWF) , Kaiserslautern, Germany*

Non-ribosomal peptide synthases (NRPS) are modular enzyme complexes, which are able to synthesize short-chain oligopeptides independent of Ribosomes. NRPS synthesize peptides by the incorporation of amino acids as components, which can be non proteinogenic or chemically modified, resulting in a high structural diversity. In contrast to NRPS, NRPS-like consist of only one module which can be divided into adenylation, thiolation and thioesterase domains, but lacking a condensation domain.

Guignardia bidwellii is the causal phytophatogenic fungus of black rot in vines posing a massive threat to organic viticulture. The phytotoxic dioxolanones phenguignardic acid and guignardic acid have been identified as potential virulence factors. *Allantophompsis lycopodina* on the other hand is the causal agents of black rot in cranberries. In Contrast to this, Allantofuranone, a furanone, shows fungistatic activity against *P. variotii* and *Penicillium* species.

To date a genetic manipulation of *these fungi* has not been successfully established in order to generate "*loss-of-function*"- or "*over-expression*"-mutants and investigate the importance of secondary metabolites for the biotic interaction in more detail.

The genomes have been sequenced and two putative NRPS-like genes of both microorganismen have been identified and introduced into *Magnaporthe oryzae* via *Agrobacterium tumefaciens*-mediated transformation in order to express these NRPS-like enzymes in a heterologous manner under the control of a constitutive promoter. After expression of the NRPS-like 1 from *G. bidwellii* in *M. oryzae* phenguignardic acid could be detected. Furthermore, expression of the NRPS-like 2 from *A. lycopodina* in *M. oryzae* led to the accumulation of a new compound, which structure elucidation is ongoing.

SMP418

Actinobacteria are neglected alternative sources to plants and fungi for the discovery of complex sesqui and diterpenes

E. J. N. Helfrich*[1], G. M. Lin[2], C. Moore[3], C. A. Voigt[2], J. Clardy[1]
[1]*Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology , Boston, MA, United States*
[2]*Massachusetts Institute of Technology, Department of Biological Engineering, Cambridge, MA, United States*
[3]*Novartis, Novartis Institute for BioMedical Research, Basel, Switzerland*

Terpenes are generally considered plant or fungal metabolites with more than 50,000 terpenes known to date. Prokaryotes, on the other hand, are not considered prolific terpene producers although several odoriferous terpene metabolites have been known for decades. Many terpenes serve important ecological functions. Others are of high pharmaceutical value. Examples include the anti-cancer agent taxol and the anti-malaria drug artemisinin. It is believed that complex bioactive sesqui- (C15) and diterpenes (C20) constitute ideal drug candidates as they fulfill all physico-chemical requirements for a druglike compound. Terpenes are formally synthesized from isoprene units, that first undergo polymerization, followed by complex cyclization reactions. Additional structural diversity is introduced through a variety of tailoring reactions, many of which are carried out by P450 enzymes. Surprisingly, genome sequencing efforts have revealed putatively talented actinobacterial producers of highly modified sesqui and diterpenes. Yet, despite their simple building blocks, structural predictions for bacteria-derived terpenes based on genome sequence information are not possible to date. We therefore heterologously expressed entire terpene biosynthetic pathways in a genome-optimized *Streptomyces* host. The isolation of highly modified terpenes from bacterial sources falsifies earlier believes that bacteria do not produce complex terpenes and paves the way towards the rational prediction of terpene scaffolds from genome sequences.

SMP419

The methoxylated, highly conjugated C_{40} carotenoids, spirilloxanthin and anhydrorhodovibrin, can be separated using high performance liquid chromatography with safe and environmentally friendly solvents

C. Autenrieth*[1], R. Ghosh[1]
[1]*University of Stuttgart, Institute of Biomaterials and Biomolecular Systems, Department of Bioenergetics, Stuttgart, Germany*

High performance liquid chromatography (HPLC) is one of the most frequently used techniques in carotenoid research. So far, however, very little attention has been paid to the fact that a majority of the organic solvents used in HPLC, impose a significant threat to both human (especially women) health and our environment. In our studies, we have developed new HPLC solvent systems for separating the C_{40}, methoxylated carotenoid spirilloxanthin, present in the purple bacterium *Rhodospirillum rubrum*. Spirilloxanthin is one of the most hydrophobic carotenoids known, and usually requires very aggressive solvent systems for HPLC. Our new HPLC solvent systems are not only capable of separating all the precursors of the carotenoid biosynthesis pathway, beginning with phytoene, but are also environmentally friendly and suitable for use in a typical biological laboratory.

SMP421

Application of NanoBit for monitoring the interaction of signal transduction protein PII with PII -interacting protein X, *in vitro*

R. Rozbeh*[1], K. Forchhammer[1]
[1]*university of Tuebingen, organismic interactions, Tübingen, Germany*

Protein-protein interactions play key roles in regulation of many biological processes. The interactions are affected by a complicated interplay of structural conformations and interference of effector molecules. To investigate the complexity of protein-protein interactions, analytical methods that do not disrupt the native cellular context are required. To this purpose, split luciferase proteins have recently been applied to develop a novel complementation reporter called NanoLuc Binary Technology (NanoBit), based on the smallest luciferase enzyme termed NanoLuc. The NanoLuc luciferase is split in two subunits, a Large Bit (18 kDa polypeptide) and Small Bit (1.3 kDa peptide), which only weakly associate, so that their assembly, which restores luciferase activity, depends on the interaction characteristics of target proteins onto which they are fused. In this study, we started to develop a new NanoBit sensor based on the interaction of signal transduction protein PII from *Synechococcus elongatus* PCC 7942 with the PII-interacting protein X (PipX). Preliminary result demonstrated that the complex of PII and PipX proteins shows luminescence when Larg Bit is attached at the C-terminus of PII protein and Small Bit is attached at the C-terminus of PipX protein. Therefore, the NanoBit system is functional for use and simplifies interaction studies of protein complexes and metabolite effects.

SMP422
Generation of a synthetic endosymbiont
N. T. Odermatt*[1], P. D. Gerlinger[1], T. J. Erb[1]
[1]*MPI für terrestrische Mikrobiologie, Biochemistry and Synthetic Metabolism, Marburg, Germany*

The uptake of a bacterium by an archaeal host cell around 2 billion years ago changed the direction of all life on earth; the new endosymbionts provided their hosts with novel capabilities such as aerobic respiration or autotrophic carbon fixation. Although a variety of organelles evolved, no such engulfment of one microbe by another was observed in recent life history. Understanding how host as well as endosymbiont adapted to this relationship is key to exploit the potential hidden in a stable internal co-culture. Artificial endosymbionts might be used as producers of precursor in biosynthesis of natural products, or to expand the metabolic capabilities of a desired host strain.

Here, we show how to implant an *Escherichia coli* cell into the host *Saccharomyces cerevisiae*, two of the most widely used microorganisms in biosynthetic production of high-value biomaterials. The uptake was mediated by a Polyethylene glycol mediated transformation followed by removal of remaining extracellular bacteria. Positive events were investigated by confocal microscopy and intact cells were found up to 16 hours post-implantation. These results demonstrate the feasibility of implanting bacteria into a non-natural host cell.

SMP423
A novel bacterial chassis system for the production of secondary metabolites
H. Kage*[1]
[1]*TU Dortmund University, Biochemical and Chemical Engineering, Dortmund, Germany*

Heterologous production of secondary metabolites is a powerful method for obtaining secondary metabolites from cryptic biosynthetic gene clusters. Despite the apparent simplicity, there are challenges in the heterologous reconstitution of secondary metabolite biosynthesis, such as (1) the diversity of codon usage patterns between donor and host organisms, (2) limitation of precursor supply and (3) the lack of broadly applicable host systems. To address these problems, the present study attempts to generate a novel chassis system, which derives from the model bacterium *Agrobacterium tumefaciens* C58. Prominent studies on this unique bacterium have revealed several useful traits such as 1) fast growth, 2) compatibility with standard recombineering tools and 3) the ability to produce both endogenous and exogenous secondary metabolites. [1-4] Based on the genome streamlining concept,[5] we currently modify a genome of the candidate bacterium by targeting non-essential genes for its survival, namely megaplasmids. The generation of a new bacterial chassis system will expand a selection of chassis systems, promoting both the discovery and the production of secondary metabolites.

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SMP424
Discovery of novel coenzyme F_{420} derivatives from *Paraburkholderia rhizoxinica*
D. Braga[1], I. Richter[1], G. Lackner*[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Synthetic Microbiology, Jena, Germany*

Conversely to ubiquitous cofactors, coenzyme F_{420} has a rather restricted phylogenetic distribution and is involved in biochemical reactions belonging to specialised metabolic pathways, *e.g.* methanogenesis in archaea or the support of *Mycobacterium tuberculosis* persistence during infection. Nonetheless, the potential to observe structural nuances and to unearth interesting F_{420}-dependent enzymes is immense. Especially in Gram-negative bacteria, which have sporadically acquired F_{420} biosynthesis genes by horizontal gene transfer, the role of F_{420} is completely elusive to date. Here, we report the biosynthetic gene cluster (BGC) for the production of coenzyme F_{420} from the proteobacterium *Paraburkholderia rhizoxinica*, an endosymbiont of the phytopathogenic fungus *Rhizopus microsporus.* HRMS/MS and fluorimetric measurements of *P. rhizoxinica* extracts evidenced the presence of the chromophore moiety of coenzyme F_{420}. However, mass spectral molecular networking revealed the presence of an unknown group of molecules in the extracts that shared the fragmentation pattern with coenzyme F_{420} suggesting them to be hydroxylated derivatives. Heterologous expression in *Escherichia coli* yielded the same molecules. Interestingly, this seems to be a complete metabolic shift in favour of hydroxyl-F_{420}, since canonical species of the coenzyme were never detected. To this point, this is the first report hydroxyl-F_{420} and its biosynthesis genes. Further studies are warranted to confirm the cofactor function of hydroxyl-F_{420} and to investigate F_{420}-dependent processes in the producing organisms.

SMP425
Establishing a synthetic photorespiratory bypass in *Synechococcus elongatus* PCC7942
P. Pfister*[1], J. Zarzycki[1], T. J. Erb[1]
[1]*Max-Planck-Institute for terrestrial Microbiology, Biochemistry and Synthetic Metabolism, Marburg, Germany*

The Calvin-Benson-Bassham cycle is a wide spread CO2 assimilation pathway, present in cyanobacteria, algae and plants. Its key enzyme RuBisCO (Ribulose-1-5-bisphosphate carboxylase/oxygenase) is known to perform a deleterious side reaction with O_2, leading to the toxic intermediate 2-phosphoglycolate (2PG)[1]. The metabolic process recycling 2PG to central carbon metabolites is called photorespiration. The photorespiratory reactions lead to the release of CO_2 and ammonia. It has been estimated that in C_3-plants up to 30% of the carbon is lost due to this process. Photorespiration as well as the refixation of the lost carbon requires additional input of ATP and reducing equivalents. Previously engineered photorespiratory bypasses were only able to circumvent the loss of nitrogen, but still resulted in (even higher) CO_2 release. In contrast, our approach aims at direct CO_2 fixation via an additional carboxylase reaction. We proposed a cyclic bypass[2] that is based on the 3-

Hydroxypropionate Bi-Cycle, an alternative CO2 fixation pathway found in the thermophilic phototroph *Chloroflexus aurantiacus*[3]. All enzymes required for the bypass could be produced in cyanobacteria and showed activity in cell extracts. The major bottle-neck was found to be acetyl-CoA carboxylation. In order to increase production and activity of acetyl-CoA carboxylase (ACC) we investigated the posttranslational biotinylation of ACC isoenzymes from different source organisms and their respective biotin ligases

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SMP426

Exploiting termite nest microbiomes for antibiotic discovery by using an ultra-high throughput Microfluidics/FACS driven pipeline combined with a microtiter plate based cultivation strategy

M. Spohn*[1], M. Oberpaul[1], M. Marner[1], P. Hammann[2], A. Vilcinskas[1], J. Glaeser[1]
[1]IME-Fraunhofer, Bioresources BR, Gießen, Germany
[2]Sanofi-Aventis Deutschland GmbH, R&D TSU Infectious Disease, Frankfurt a. M., Germany

Termite nests harbor suitable, highly diverse microbiomes in which bacterial taxa are present that are known for their genetic potential to produce natural compounds.

In a first step, the diversity of *Coptotermes* species nest microbiomes was assessed carefully by using 16S rDNA amplicon sequencing and nest material was selected to obtain viable cells by using Nycodenz density gradient centrifugation. In order to analyze the diversity of the culturable termite nest microbiome, bacterial cells were either distributed in 384-well plates (approach 1) or encapsulated in small spheric agarose beads by an high throughput microfluidics technique (approach 2) upfront. After incubation of cells in agarose beads grown microcolonies were sorted by using fluorescence associated cell sorting (FACS) and distributed in 384-well plates. Cultures obtained from approach 1 and 2 were reformatted using automated liquid handling stations and scaled-up in 96-well Duetz-systems for characterization of diversity by genetic finger prints and 16S rRNA gene sequencing and for rapid bioactivity screening. After elimination of redundancy, we perform a fast scale-up of prioritized cultures from approach 1 and 2 in 24-well Duetz-systems to validate the activity and to identify compounds underlying the observed bioactivity using UPLC-high resolution MS in combination with an in-house database.

Implementation of this pipeline allows us to prioritize hundreds of antibiotics producing strains from a bio-resource where antibiotics producing bacteria are naturally enriched by screening in an ultra-high throughput fashion and by cultivation of a broad biodiversity in our plate based approach.

SMP427

New Tools for Targeted Cloning and Over Expression of Biosynthetic Gene Clusters

M. Liles*[1], D. Mead[1], D. Johnson[1], J. MacDonald[1], P. Brumm[1], R. Stankey[1]
[1]Varigen Biosciences Corporation, Madison, WI, United States

Introduction: The genomics revolution and corresponding development of biosynthetic gene cluster (BGC) prediction and analysis tools have resulted in a wealth of new biosynthetic potential for further examination. Once a BGC of interest is identified, isolating a physical DNA clone for expression, refactoring, and other analyses can be slow and expensive.

Objective: Here we describe a rapid technique to directly clone large BGCs from genomic DNA without using gels or agarose plugs.

Materials and Methods: Using CRISPR-Cas9 on intact genomic DNA, we targeted cuts to regions flanking BGCs of interest. Linearized *Streptomyces* BAC shuttle vectors with overlaps matching the BGC cut sites were prepared using PCR, and the vector and restricted DNA were assembled and transformed.

Results: 22 BGCs from *Streptomyces* and fungal strains were successfully captured, ranging from 21-95 Kb. To improve the chances of heterologous expression a new BGC expression vector was developed that uniquely includes *two* inducible promoter elements, one flanking each side of the cloning site. The blue and red colored antibiotic compounds from *Streptomyces coelicolor* ACT (21 kb) and RED (33 kb) BGCs were cloned in both orientations of the pDual vector and integrated into *S. lividans* ΔredΔact. Qualitative inducible production of the blue and red pigments was demonstrated in the inducible promoter system but not from the native promoters in this heterologous expression experiment.

Conclusion: These results indicate that any sequenced biosynthetic gene cluster can be cloned intact from complex genomes and heterologously expressed to produce secondary metabolites, thereby expanding our available resources for natural product discovery.

SMP428

Genetically encoded photosensitizers as a versatile tool to regulate bacterial processes by light

N. L. Bitzenhofer*[1], Y. S. Ackermann[1], F. Hilgers[1], T. Gensch[2], S. Nonell[3], K. E. Jaeger[1,4], T. Drepper[1]
[1]Institute of Molecular Enzyme Technology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany
[2]Institute of Complex Systems, ICS-4: Cellular Biophysics, Forschungszentrum Jülich, Jülich, Germany
[3]Institut Químic de Sarrià, Universitat Ramon LIull, Barcelona, Spain
[4]Institute of Bio- and Geosciences IBG-1, Forschungszentrum Jülich, Jülich, Germany

Genetically encoded photosensitizers (PS) produce reactive oxygen species (ROS) upon illumination and due to this feature they are suitable optogenetic tools for a multitude of *in vivo* applications. However, to specifically regulate cell viability by light, a precise adjustability of phototoxicity is required. For this purpose, we comparatively analyzed various PSs encompassing GFP-based and flavin-binding fluorescence proteins (FbFPs) to establish a versatile optogenetic toolbox.
First, we determined ROS production of the chosen PSs via spectroscopic and enzymatic *in vitro* assays. In addition, we analyzed differences in phototoxicity in an *in vivo* approach

using the Gram-negative bacterium *E. coli*. To demonstrate the applicability of these PSs for antimicrobial photodynamic inactivation (aPDI), we further expanded our phototoxicity studies to various Gram-positive and -negative bacteria including e.g. the human pathogen *Pseudomonas aeruginosa*. Based on the *in vitro* studies we could show that all of the tested PSs were able to produce ROS but exhibited remarkable differences in selectivity and yield. The *in vivo* studies further demonstrated that particularly FbFPs are potent PSs, which can be used for aPDI. Especially SOPP3 exhibited a high phototoxicity allowing to efficiently kill bacterial cells already after few seconds of light exposure. In contrast, GFP-based and some flavin-binding PSs showed only low phototoxic properties enabling a more gradual regulation of cell viability by light. Consequently, the here characterized PSs constitute a versatile light-responsive biobrick system with adjustable phototoxicities which can be applied for future optogenetic and biomedical applications including chromophore-assisted light inactivation of target enzymes (CALI).

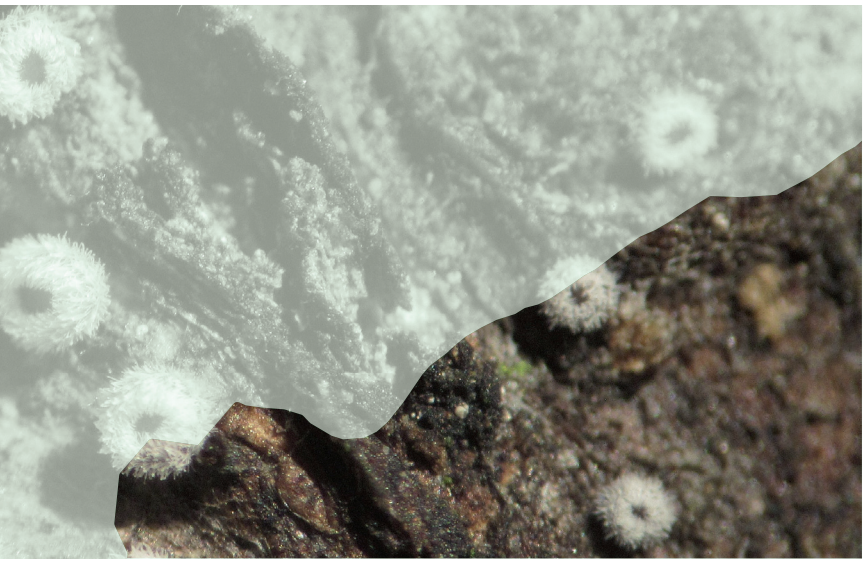
SMP429

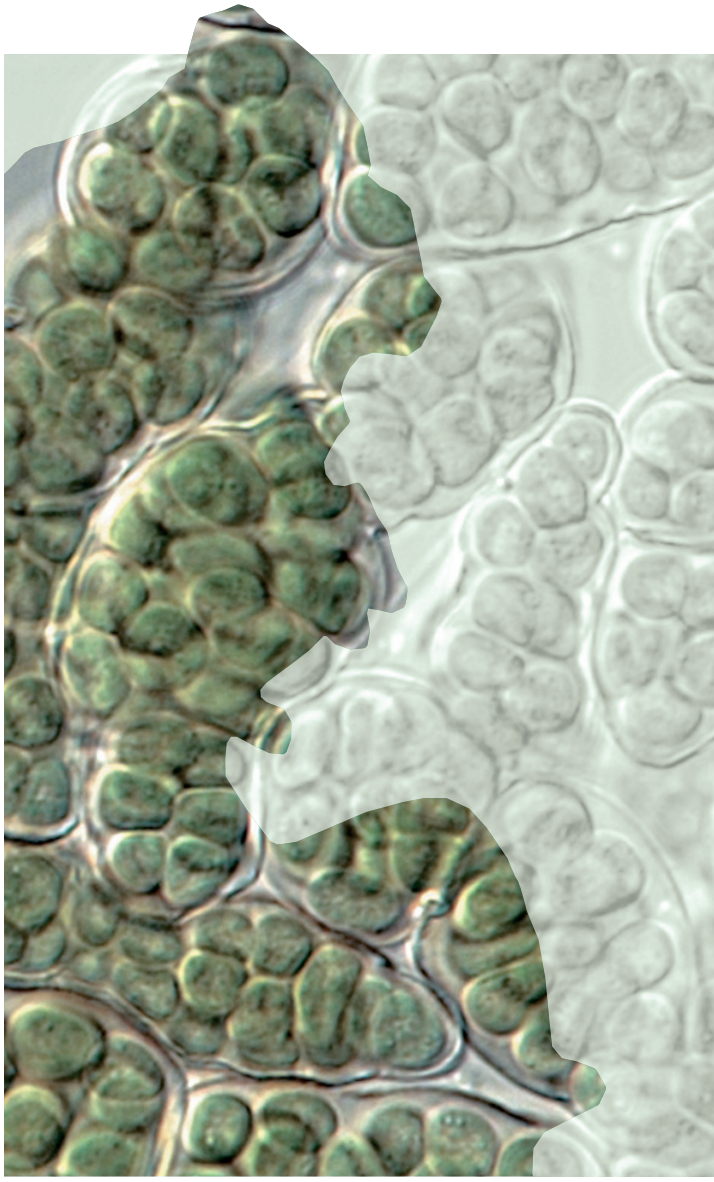
Rapid purification and characterization of recombinant proteins and antibodies: Capturem high-capacity membranes

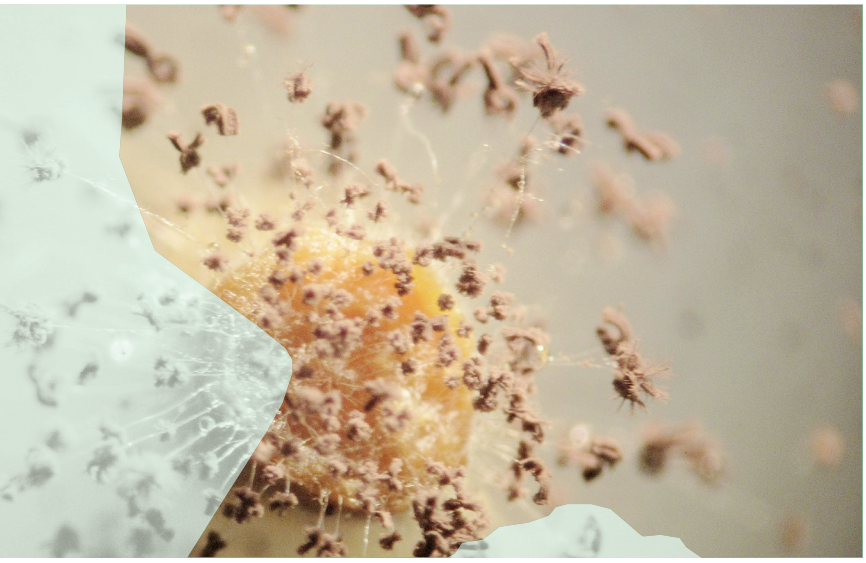
G. Jokhadze*[1], C. Hoppmann[1], M. T. Vierra[1], B. Levitan[1], M. Li[1], T. Larson[1], A. A. Farmer[1], M. Raman[2]

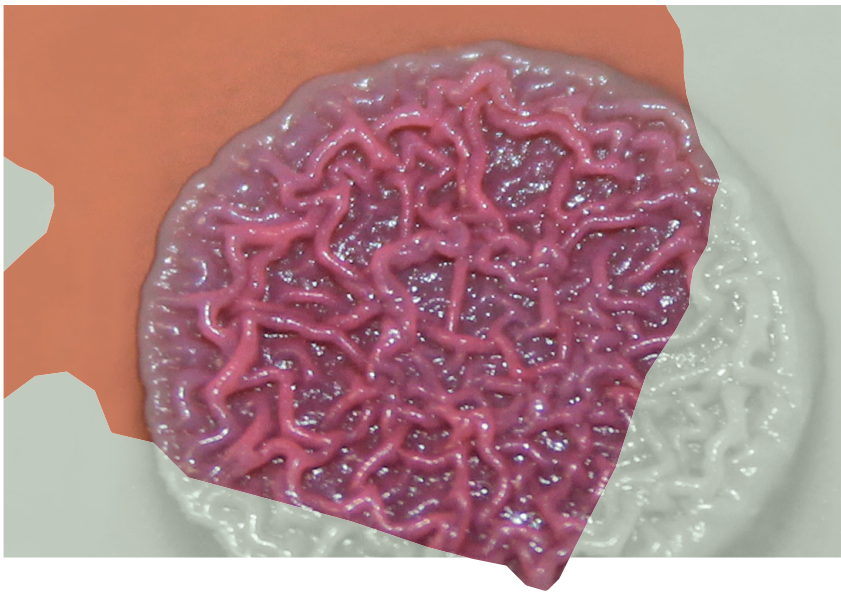
[1]Takara Bio USA, Inc., Mountain View, CA 94043, USA
[2]Takara Bio Europe SAS, 78100 Saint-Germain-en-Laye, France

Recombinant protein production is immensely important in many research settings, including academic research institutions, biopharmaceutical organizations, and enzyme and agricultural industries. Fusion tags are widely used to improve yields and enable purification and characterization of protein structure and function. Polyhistidine tags, which incorporate 6-10 histidines at either terminus of the target proteins, are the most popular tag used for purification. The affinity of the histidines to immobilized metal ions such as Co^{2+} and Ni^{2+} is utilized to selectively bind the tagged construct to the matrix, while washing away unwanted materials, before eluting the target with low-pH or imidazole-containing buffers. Typical purification methods using immobilized metal affinity chromatography (IMAC) columns take several hours to complete due to long column equilibration/binding times and slow diffusion of large macromolecules through the resin bed. The long times increase the risk of proteolytic degradation and activity loss due to unfolding or denaturation. Membrane-based affinity systems have rapid flow-induced mass transport and short residence times; however, they have been plagued with low capacity due to small internal surface areas. Here, we describe a novel, nylon-membrane-based IMAC system with a chemically enhanced surface area of the pores that allow protein binding capacities comparable to, or better than, resins at 75 mg or more per cm^3 of membrane. Unlike traditional resin-based systems, the entire purification process—from loading the lysate to eluting pure protein—can be completed at room temperature in less than five minutes. We have assembled these membranes into spin columns and filtration devices and demonstrated their ability to purify his-tagged proteins produced in bacterial and mammalian cells. The millisecond residence time of the proteins on the membrane during binding minimizes the possibility of degradation. These membranes function perfectly in the presence of additives such as ethylenediaminetetraacetic acid (EDTA), reducing agents such as dithiothreitol (DTT), and under denaturing conditions (in the presence of urea and guanidium hydrochloride). We have extended the high-capacity membrane technology to immobilize Protein A and G, enabling extremely fast purification of antibodies from various matrices based on the affinity of these proteins for the fragment crystallizable region (Fc) region of antibodies. Antibody purification can be accomplished in less than 10 minutes, with capacities of up to 75mg/ml or more, far exceeding the capacity of resin-based columns. More recently, we have immobilized trypsin and pepsin enzymes on these membranes to carry out proteolysis of proteins for their characterization, identification, and quantitation through mass spectrometry analysis. In contrast to the long incubation period (6-24 hours) of conventional in-solution digestions, the proteolytic membranes generate peptides suitable for downstream analysis, with the same or improved sequence coverage, in less than a minute, for downstream analysis. Additionally, we are expanding the membrane technology with immobilized streptavidin suitable for enrichment of target proteins, antibodies, and oligos. These novel membrane-based spinnable affinity columns and filtration devices will be useful for purifying a variety of recombinant proteins and antibodies and their proteomics characterization in academic and industrial settings.









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