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ABSTRACTS

PS003

One ring to rule them all – DNA segregation in bacteria

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The faithful distribution of hereditary information during cell division is critical for the generation of viable offspring. In most bacteria, newly replicated chromosomal DNA and low-copy number plasmids are segregated by the ParAB*S* DNA partitioning system. This widely conserved module is composed of the P-loop ATPase ParA, the DNA-binding protein ParB and short palindromic sequences (*parS*) that are clustered in the centromeric region of sister DNA molecules. The interaction of ParB with *parS* sites gives rise to large nucleoprotein complexes containing several hundred ParB molecules. These so-called partition complexes then act as docking points for the ATPase ParA, which interacts dynamically with ParB to drive the segregation process.
This talk will highlight recent findings that have provided fundamental new insights into the mechanism of partition complex assembly. Notably, it has emerged that ParB is a clamp-like protein that binds and hydrolyzes the ribonucleotide CTP. Its CTPase cycle enables it to embrace DNA in a ring-like fashion upon *parS* binding and to then slide away laterally from its initial binding site into the neighboring DNA regions. Repeated cycles of binding, diffusion and dissociation give rise a well-defined ParB diffusion gradient across the centromere region whose dynamics are critical for DNA segregation. ParB proteins thus emerge as a new class of CTP-dependent molecular switches that act in concert with ATPases and GTPases to control fundamental cellular functions.

PS009

The leaf microbiota: responses and impacts on plants

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Microorganisms occur in virtually all environments, are an integral part of complex multicellular organisms such as plants, animals and humans, and play a critical role in their health. We established reductionist approaches to unravel the inherent complexity of microbial interactions and plant–microbiota interactions *in situ* using the model plant Arabidopsis and gnotobiotic systems. We isolated bacterial strains that represent the majority of species living in the phyllosphere of Arabidopsis to serve as a basis for studying the structure and function of plant microbiomes. We conducted a systematic analysis of plant protection capabilities of the strain collection against the bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000. Protection against the pathogen varied, with about 10% of leaf microbiota strains providing full protection and 10% showing intermediate levels of protection. The most protective strains were distributed across different taxonomic groups. Synthetic community experiments revealed additive effects of strains but also that a single strain can confer full protection in a community context. We exemplify that multiple mechanisms contribute to plant protection and include both bacteria-bacteria interactions and indirect protection via the plant. By investigating the plant response to members of its microbiota, we identified a molecular response, which is induced by the presence of a majority of strains and include the most differentially regulated genes across treatments. Our results suggest that these genes are part of a defense adaptation strategy that is consistently elicited by diverse strains from various phyla and contributes to host protection.

PS013

Plastic pollution, a global challenge: How do we find the best plastic-eating bacteria in global metagenomes?

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Petroleum-based plastics are designed to be extremely stable and durable. Currently, about 360-450 million tons of synthetic polymers are produced annually and much of this is only meant for single use. Recycling concepts hardly exist and more than 90% of all synthetic and fossil-derived plastics are incinerated or end up either in landfills or directly in the environment. The main plastic litter we face is composed of single or mixed materials consisting of the polymers: polyurethane (PUR), polyethylene (PE), polyamide (PA), polyethylenterephthalate (PET), polystyrene (PS), polyvinylchloride (PVC), epoxy-based polymers (EP), polypropylene (PP) and tire rubber. Within this framework, microbial and enzyme-driven plastics degradation has only been studied since less than a decade and only for a fraction of the fossil-fuel-based polymers, functional enzymes are known. In fact, few enzymes and microorganisms are able to degrade PA oligomers, ester-based PUR, PET and natural rubber. However, no verified enzymes are known that degrade PE, PVC, PP, PS, EP and ether-based PUR. These polymers stand for the majority of all synthetic polymers. Thus, the identification of microbial enzymes and pathways acting on most of the current polymers is a major and urgent task. In my presentation, I will summarize our efforts to identify novel plastics-active enzymes. We established a full pipeline to identify plastic-active enzymes from Hidden-Markov-Model-based in silico screenings of global metagenome datasets to *in vitro* protein production and functional verification, characterization, and structural elucidation.
Of more than 30 novel and verified polymer-degrading enzymes, at least seven can degrade PET. They originate from diverse bacterial lineages. The first Bacteroidetes-derived PET-active enzymes and their potential role for PET degradation in nature will be presented here. Further, we will have an outlook on the enzymatic degradation of other types of plastics.

PS014

Focusing the ligand spectrum of a biosensor and application of this biosensor in high-throughput screenings

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Key Words: biosensor, protein engineering, molecular dynamics simulation, genome sequencing, *Corynebacterium glutamicum*

Cell factories converting bio-based precursors to chemicals present an attractive avenue to a sustainable economy, yet screening of genetically diverse strain libraries to identify the best performing whole-cell biocatalysts is a low-throughput endeavor. For this reason, transcriptional biosensors attract attention as they allow the screening of vast libraries when used in combination with fluorescence-activated cell sorting (FACS). However, broad ligand specificity of transcriptional regulators (TRs) often prohibits the development of such ultrahigh-throughput screens. Here, we solve the structure of the TR LysG of *Corynebacterium glutamicum*, which detects all three basic amino acids. Based on this information, we follow a semi-rational engineering approach using a FACS-based screening/counterscreening strategy to generate an L-lysine insensitive LysG-based biosensor. This biosensor was used to improve available L-histidine production strains by FACS, showing that TR engineering towards a more focused

ligand spectrum can expand the scope of application of such metabolite sensors.

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IL-SG001

Phylogenetics and database mining: complementary approaches towards novel enzyme catalysts

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Public sequence databases represent a rich and endless source for the identification of novel biocatalysts due to the ever-increasing number of sequence entries. However, the efficient identification of new enzymes, exhibiting also novel characteristics, within these databases requires smart approaches that go beyond simple homology searches. In this respect, detailed knowledge on the sequence-function relationship for an enzyme group of interest is highly beneficial.
We have previously used different database mining approaches based on sequence motifs to significantly increase the number of available halohydrin dehalogenases (HHDH) and β-etherases for biocatalytic application [1,2]. This has led, e.g., to the identification of the first halohydrin dehalogenase with activity on sterically more demanding substrates [3,4], as well as the discovery of the first non-bacterial HHDH enzyme. In a complementary approach making use of targeted phylogenetic analyses, we were now able to identify new members of the HHDH enzyme family that contain variations in otherwise conserved residues of the active site. This demonstrates that the HHDH family is more diverse than previously expected. Moreover, those enzymes with sequence motif variations exhibit again new functional characteristics.

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IL-SG005

Principles of high-specificity local and global c-di-GMP signaling

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The striking multiplicity, signal input diversity and output specificity of c-di-GMP signaling proteins in many bacteria has brought second messenger signaling back onto the agenda of contemporary microbiology. How can several signaling pathways act in parallel in a specific manner if all of them use the same diffusible second messenger present at a certain global cellular concentration? Recent research has now shown bacteria to achieve this by flexibly combining modes of local and global c-di-GMP signaling in complex signaling networks. Several criteria have to be met to define local c-di-GMP signaling: specific knockout phenotypes, direct interactions between proteins involved and actual cellular c-di-GMP levels remaining below effector K_d's and unchanged when the relevant DGC or PDE is mutated. These principles will be illustrated using examples from *E. coli* species. Adaptive changes in signaling network architecture can further enhance signaling flexibility.

IL-SG006

(p)ppGpp coordinates lipopolysaccharide biosynthesis in *Escherichia coli*

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The signaling molecules pppGpp and ppGpp, collectively referred to as (p)ppGpp, are formed in millimolar amounts as part of the stringent response. Triggers of the stringent response include nutrient starvation, membrane stress, and host defense mechanisms. Accumulation of (p)ppGpp leads to a well-documented global reprogramming of transcription to adapt the bacterial cell to deleterious environmental conditions. Lately, several publications have described new (p)ppGpp-binding proteins. In most cases, ppGpp-protein interaction resulted in reduced activity of the protein and, accordingly, downregulation of the respective pathway.

The Gram-negative cell envelope is composed of lipids, peptidoglycan, and lipopolysaccharides (LPS). The cell needs to balance these components to maintain its protective barrier. All three biosynthetic pathways share the same precursor molecules, and inhibitory effects of (p)ppGpp on lipid and peptidoglycan biosynthesis are known. We were drawn to the question, whether (p)ppGpp also inhibits LPS biosynthesis.

Using a fluorescent *in vitro* assay, we demonstrated that (p)ppGpp inhibits the first step of LPS biosynthesis, catalyzed by LpxA. Conversely, overproduction of plasmid-encoded LpxA in the (p)ppGpp^0 mutant leads to prominent cell elongation. Furthermore, we detected elevated outer membrane vesicle (OMV) formation in the (p)ppGpp^0 mutant with LpxA overproduction. Both aspects suggest derepressed LPS biosynthesis in the absence of (p)ppGpp.

Besides (p)ppGpp, we introduce a second new player in the regulation of membrane biogenesis, the enzyme RnhB, which participates in replication through RNase H activity. We demonstrated that RnhB inhibits the first step of LPS biosynthesis *in vitro*. Furthermore, (p)ppGpp binds to RnhB and inhibits its RNase H activity. We are currently interested in exploring the regulatory mechanism and the role of (p)ppGpp in this process in more detail. LPS is essential in most Gram-negative bacteria, and (p)ppGpp is a key requirement for virulence. Therefore, unraveling the regulatory networks associated with membrane biogenesis is an important part of understanding this first line of defense.

All co-authors are located in Germany.

IL-SG007

A new facet of c-di-AMP mediated regulation in *Bacillus subtilis*

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In many bacteria, cyclic di-AMP is an essential second messenger that plays a major role in the control of potassium and osmotic homeostasis. In *Bacillus subtilis*, c-di-AMP binds to a variety of proteins, most of them involved in potassium homeostasis, as well as two two copies of a riboswitch. Strikingly, c-di-AMP also binds two regulatory proteins, DarA and DarB, that do not have a direct function, suggesting that they are required for signal transduction. We are interested in the identification of the function (s) of these proteins. For DarB, we have already shown that it binds the Rel protein to trigger a stringent response under condition of potassium starvation (1). Now, we have found that DarB also binds to pyruvate carboxylase, the enzyme that is responsible for replenishing the oxaloacetate pool in the citric acid cycle. Binding of DarB stimulates the activity of pyruvate carboxylase *in vivo* and *in vitro*. Both the interaction and the activation of enzyme activity require apo-DarB and are inhibited by c-di-AMP. Under conditions of potassium starvation and corresponding low c-di-AMP levels, the demand for citric acid cycle intermediates is increased. Apo-DarB helps to replenish the cycle by activating both pyruvate carboxylase gene expression and enzymatic activity via triggering the stringent response as a result of its interaction with the (p)ppGpp synthetase Rel and by direct interaction with the enzyme, respectively.

(1) Krüger et al., Nat. Commun. 12: 1210

IL-SG008

Cross-talk between cAMP and c-di-AMP signaling via carbon sensor protein SbtB: Linking CO_2 homeostasis with diurnal metabolic switch

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Cyanobacteria are phototrophic prokaryotes that evolved oxygenic photosynthesis ~2.7 billion y ago. For efficient CO_2 fixation at low ambient concentrations, they evolved highly specialized carbon concentrating mechanism that employs high affinity inorganic carbon (C_i) uptake systems, to augment intracellular C_i levels[1]. Due to this unique photosynthetic lifestyle, they evolved as well sophisticated regulatory mechanisms to adapt to oscillating day-night metabolic changes between autotrophic and heterotrophic metabolisms. Recently, we identified the PII-like protein SbtB as C_i sensing module[2]. Similar to nitrogen-regulatory PII proteins, SbtB is able to bind the adenine nucleotides ATP and ADP, but unlike these, it also binds AMP and moreover, it preferentially binds the second messengers cAMP[2] and c-di-AMP[3]. We showed that cAMP acts as carbon signal, whereas adenyl-nucleotide binding may link SbtB signalling to the energy state of the cells. In search for a function for SbtB as c-di-AMP receptor, we found that both of SbtB and c-di-AMP cyclase knockout mutants were impaired under diurnal growth. Moreover, SbtB was identified as controlling factor for glycogen metabolism through interaction with the glycogen branching enzyme GlgB. Thus, c-di-AMP signaling through SbtB turned out pivotal for day-night acclimation of cyanobacteria via regulation of glycogen metabolism. To our knowledge, this is the first signaling protein known to sense both cAMP and c-di-AMP. This highlights the central role of SbtB as a switch point in cyanobacterial cell physiology, integrating not only signals from the energy state and carbon supply through adenine-nucleotide and cAMP binding, respectively, but also from the diurnal state by binding to c-di-AMP.

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IL-SG009

Cyclic Nucleotide Signalling in virus:host conflict

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Prokaryotic cells utilise a diverse array of cyclic nucleotide second messengers to coordinate defence against invading mobile genetic elements (MGE). Type III CRISPR systems detect foreign RNA and generate cyclic oligoadenylate (cOA) second messengers that activate powerful degradative nucleases, while CBASS (Cyclic nucleotide based antiphage signalling systems) make a wide range of cyclic di- and tri-nucleotides that frequently activate abortive infection (cell death) pathways. This talk will focus on the mechanisms of cyclic-nucleotide signalling in anti-viral defence, focussing on two examples under study in our lab. The first is a prophage-encoded type III CRISPR system from *Vibrio cholerae* that generates a cyclic triadenylate (cA_3) signal, activating a powerful NucC DNA endonuclease effector. The second is a bacterial CBASS defence pathway that also functions via a cA_3 second messenger, which activates an effector with an enzymatic TIR domain for degradation of cellular NAD+. This CBASS defence involves the assembly of a highly unusual and visually striking effector complex structure. The two pathways can be "mixed and matched" and most likely both operate via abortive infection, highlighting the conserved paradigms for cyclic nucleotide based prokaryotic defence systems.

IL-SG015

Sequencing the Tree of Life

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Under the umbrella of the Earth Biogenome Project, many national and international projects have started to sample, sequence and assemble all eukaryotic life on earth to create the best possible reference genome assemblies. The Tree of Life (ToL) programme at the Wellcome Sanger Institute partners in the Darwin Tree of Life Project, sequencing all life on the British Isles, the Vertebrate Genomes Project (all vertebrates), the Aquatic Symbiosis Genomes Project (selected holobionts) and the European Genome Reference Atlas (all eukaryotes in Europe). We are also involved in the Human Pangenome Project generating the new human reference in graph format.
Producing assemblies at this scale, a process we call the genome Engine, puts immense demands on all lab and informatics workflows. Our methods are therefore under tight scrutiny and have to evolve constantly. I will describe the scope of the projects, the pipelines used and the achievements so far.

I will also give a brief overview of my own journey from a lab-based PhD into bioinformatics and eventually managing the ToL Genome Engine.

IL-SG017

Microbial methane metabolism in oxygen-limited ecosystems by novel Archaea

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During the evolutionary history of our planet, a set of microbial processes that evolved exclusively in the absence of oxygen changed the chemical speciation of all major elements. In the past 20 years we aimed to discover and elucidate the role of these anaerobic microbes in the methane (and nitrogen) cycles. Anaerobic oxidation of methane by *Methanoperedens* archaea at the expense of nitrate has recently been discovered. In addition to nitrate, these archaeal methanotrophs seem to use metal-oxides and metalloids as terminal electron acceptor, and are able to produce electricity in microbial fuel cells. *Methanoperedens* archaea are dependent on nitrite scavengers like *Methylomirabilis* or anammox bacteria to prevent toxicity, and together the microbes can be applied in sustainable, cost effective oxygen-limited wastewater treatment for the removal of methane and nitrogen compounds. Also, in coastal sediments, archaeal methanotrophs may play a crucial role as methane biofilter to prevent emissions of this notorious greenhouse gas, and are investigated in ERC Synergy MARIX 854088 and within the center of excellence in anaerobic microbiology (www.anaerobic-microbiology.eu) funded by the Netherlands Gravitation program 024.002.002 SIAM.

IL-SG018

A chronology of multicellularity evolution in cyanobacteria

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The transition fromunicellular to multicellularorganisms isoneof themost significant events in thehistory of life. Key to this process is the emergence of Darwinian individuality at the higher level: Groups must become single entities capable of reproduction for selection to shape their evolution. Evolutionary transitions in individuality are characterized by cooperation between the lower level entities and by division of labor. Theory suggests that division of labor may drive the transition to multicellularity by eliminating the trade off between two incompatible processes that cannot be performed simultaneously in one cell. We examined the evolution of the most ancient multicellular transition known today, that of cyanobacteria, where we reconstruct the sequence of ecological and phenotypic trait evolution. Our results show evidence that the prime driver of multicellularity in cyanobacteria was the expansion in metabolic capacity offered by nitrogen fixation, which was accompanied by the emergence of the filamentous morphology and succeeded by a reproductive life cycle. This was followed by the progression of multicellularity into higher complexity in the form of differentiated cells and patterned multicellularity. The chronology of the evolution of multicellularity in cyanobacteria shows that, once established, multicellular individuality opens new vistas of opportunities.

IL-SG019

Advanced monitoring and control of anaerobic digestion – Model predictive control and development of a microbial electrochemical sensor

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Anaerobic digestion (AD) is a widely distributed and well-established biotechnological process to generate biogas (a mixture of ~50 % CO_2 and 50 % CH_4) from, e.g., biogenic residues such as lignocellulosic biomass, cow manure or wastewater. Biogas is used to provide heat and electrical power or is upgraded to biomethane and injected in to the natural gas grid. From a microbiological point of view the process is divided in four steps, representing different stages of microbial degradation, i.e., hydrolysis, acetogenesis, acetogenesis and finally methanogenesis implemented by a wide range of fermentative bacteria as well as methanogenic archaea. Even if the process is already used at industrial scale there is a severe lack of process monitoring and control tools in commercial AD applications. This applies in particular for improved process control strategies, e.g., demand-oriented biogas production by varying the amount and frequency of substrate provision in order to realize a pre-defined biogas production schedule. Therefore, we developed and validated 1) a model predictive control (MPC) strategy for demand-oriented biogas production and 2) a microbial electrochemical sensor concept (see Figure below) for online monitoring of volatile fatty acids as important process intermediates. MPC uses simplified process models based on first-order kinetics to calculate the amount of substrate and temporal distribution feeding events in order to realize a pre-defined biogas production schedule. In full-scale application the MPC algorithm was able to dynamically operate the AD process, while achieving high revenues and reducing the required gas storage capacity. To improve online control of AD and guarantee stable process conditions at all time, we developed a biosensor concept that is based on electroactive bacteria of the genus *Geobacter* sp., forming a self-sustaining biofilm on a graphite electrode (i.e. anode). The bacteria oxidise acetate and other VFA as sole carbon source and use the graphite electrode as a terminal electron acceptor of their respiratory chain. Therefore, the amount of oxidised acetate correlates with the number of transferred electrons and, hence, the current signal. The sensor was successfully tested in a lab-scale biogas process using cow manure and maize silage as substrates. However, the receptor showed functional changes over time that may be related to interactions between bacteria and archaea.

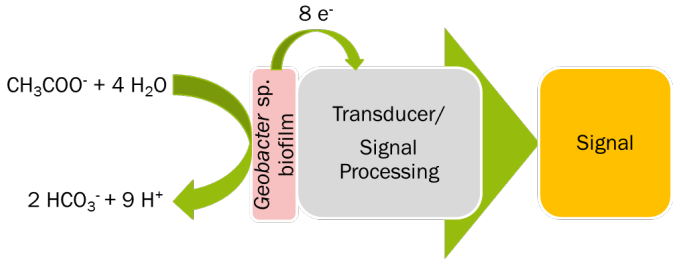

Figure 1: Basic principle of the microbial electrochemical sensor for AD process control

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IL-SG020

Automatic pattern analysis for the control of artificial and natural microbial communities in bioprocesses

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Microbial communities are omnipresent. They are in, on and around us and are therefore pivotal in medical diagnosis, health research and testing of foodstuffs and drinking water, but also act as biocatalysts in large scale industrial applications, such as the treatment of waste water, the production of biogas and the manufacturing of platform chemicals.
Information about a particular community's composition and the dynamics of its structure holds a key to understanding a wide variety of microbial processes. Since microbial species have different generation times, the composition of a microbial community can change rapidly over time. Moreover, mechanisms such as niche differentiation, competition and stochastic effects may cause rapid changes in a microbial community as well. These changes may influence the water quality of a lake, the yield of a production process or the health of a patient. In contrast to sequence-based approaches, flow cytometry provides the required temporal and single-cell resolution to act as a sensor for these community dynamics.
In order to not only recognize community changes retrospectively but monitor close to real time and eventually control communities and the processes they drive, fast detection and evaluation methods are required. These include automated sampling, sample-processing, flow cytometry and automated downstream statistical analysis. Within this pipeline it is crucial to find patterns – the subcommuities or cell-clusters - within the culture to quantify relevant cell types. Up to now this is achieved by time-consuming manual gating procedures that require extensive user-dependent input. Contemporary research provides automatic tools for identifing the cell clusters. In comparison to manual clustering this ensures replicability and objectiveness.
Given these cell numbers per subcommunity over time, the correlation with the abiotic parameters that are measured in parallel can act as control-parameters of the bioprocess via Process Analytical Technology (PAT). Moreover, correlations between the cell numbers per subcommunity can reveal interactions between the subcommunities, e.g. in metabolic pathways. Furthermore, tracking cell numbers per subcommunity over time may show the stability of the microbial community. A lack of stability can serve as a warning signal of underlying bioprocess failures.

IL-SG021
Glucose and lactate analysis in fermentation processes and development of new amino acids analytics by Allianz WiPro-Project
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Glucose is used as substrate for many microbial and eukaryotic cells. In cell cultivations and microbial fermentations the glucose concentration has to be monitored. Furthermore it is beneficial to control the glucose concentration on a certain level to reduce the formation of by-products.

TRACE C2 Control is an online analyzer for controlling the glucose concentration. The integrated PID controller is able to keep the glucose concentration on a low and constant level in fermentations. The installed feeding pump is suitable for bioreactors with a working volume up to 5L. Feeding in large bioreactors is also possible by direct connection of an external pump. Glucose can be controlled in a range between 0.1 g/L and 40 g/L. The parameter lactate can be additionally measured in a range between 0.05 g/L and 10 g/L.

Amino acids are very important in cell culture cultivations. Especially the amino acid glutamine is often used as a substrate. By online-monitoring of amino acid concentrations it is possible to optimize cultivations. A new method for online-measurements of amino acids was developed in the research project "Allianz WiPro". This online-method was used for measuring the total amino acid concentration in a cell culture process. The system was calibrated in a range between 0.1 g/L and 4 g/L leucine.

IL-SG022
Bioprocess analytical technology for online monitoring of biomass
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For the modern approval of biopharmaceuticals, the FDA's PAT initiative requires comprehensive control of industrial bioprocesses. Efficient processes can only be achieved through a deep understanding of the process, for which suitable sensors are needed. These sensors must record critical process variables reproducibly and reliably over the entire process runtime and concentration range. Biotechnological processes place high demands on sensors because they have a high biological variability in a very complex matrix and the biological component of the process often reacts very sensitively to changes.
Various spectroscopic methods have already been described and meet the requirements for monitoring and controlling bioprocesses. However, all these methods require multivariate data processing to extract the information from the spectral data. Different non-spectroscopic sensors for biomass monitoring are presented also. The limitations and possibilities are compared and their suitability for the automation of bioprocesses in the context of PAT is demonstrated.

IL-SG023
Marine N_2-fixing bacterium in seagrass roots echoes terrestrial symbioses
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Nitrogen (N_2)-fixing microorganisms are crucial to the supply of nitrogen in a variety of ecosystems. In the terrestrial environment, N_2-fixing symbioses between bacteria and plants are widespread and enable plants to grow in nitrogen-poor soils. Many of these symbioses are quite intimate and rely on a mutual exchange of nitrogen and carbon compounds. We recently discovered an N_2-fixing root endophyte in *Posidonia oceanica*, a contemporary seagrass whose ancestors evolved from terrestrial flowering plants about 100 million years ago. Similar to its terrestrial counterparts, the N_2-fixing bacterium, named *Candidatus* Celerinatantimonas neptuna, supplies ammonium and amino acids to its seagrass host, which supplies sugars in return. Genomic evidence indicates that the bacterial symbiont may also promote plant growth via the production of hormones, siderophores or antimicrobial compounds, traits commonly found in terrestrial endophytes. Yet, the novel symbiont is of marine origin and as such may have aided the migration of flowering plants into the sea. Relatives of *Ca*. C. neptuna are present in coastal ecosystems worldwide, where they may form similar symbioses with other seagrasses or salt marsh plants, allowing them to thrive in nutrient poor ecosystems.

IL-SG024

Evolution of Bacteroidetes bacteria - ancient symbionts across beetle families

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Nutritional symbionts have been a major factor driving the evolution of insects and probably one factor underlying their remarkable radiation. Convergently evolved associations of various Coleoptera with bacterial symbionts supplementing precursors for the synthesis of their cuticular exoskeleton might have driven the evolutionary success of this order of insects. Bacteroidetes bacteria include a distinguished, ancient lineage that is only found in association with insects, including the obligate symbionts of Blattodea and Auchenorrhyncha. However, we recently also identified Bacteroidetes bacteria in association with multiple beetle families, including the Bostrichidae, Silvanidae and Throscidae. Across these families, we find highly specialized symbionts contributing precursors for the synthesis aromatic amino acids, but also alternating strains able to recycle nitrogen as well as some that are reminiscent of recently acquired symbionts or reproductive manipulators like *Sodalis* or *Wolbachia*. These insights expand our understanding of the evolution of this particular clade of symbionts as well as its potential origin, but also their herbivore hosts.

IL-SG025

Unearthing the impact of climate change on soil viruses

J. Jansson
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Although viruses are the most abundant biological entity in soil ecosystems, their identities and responses to environmental shifts remain poorly understood. I will be discussing our research that investigates how climate change is impacting soil viruses. Specifically, I will describe how shifts in soil moisture, due to changes in precipitation patterns, impact soil DNA and RNA viruses and their activities. Of key interest is the functional capacity of soil viruses. Many viruses carry auxiliary metabolic genes (AMGs) that may contribute activities that influence ecosystem functioning. As an example, I will detail one of those AMGs – a chitosanase- that is closely related to a fungal enzyme but carried on a bacteriophage. The chitosanase enzyme was expressed and crystalized to obtain the protein structure. The structure is novel and provides a good example of novel functions carried on soil viruses that can participate in carbon turnover in soil. The second area of research I will discuss is how permafrost thaw is impacting soil DNA and RNA viruses and their activity. Most RNA viruses that emerge following thaw target eukaryotic hosts. By contrast, active DNA viruses in thawed permafrost are primarily bacteriophage. Together, these studies reveal that changes in environmental variables, such as are occurring with climate change, have dramatic impacts on soil viruses, which will in turn influence population dynamics of their hosts.

ST001

The Great Melting: Small Cells Change Global Processes.

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The rapid melting of the Greenland Ice Sheet and the associated sea level rise is controlled by changes in albedo, which is primarily a consequence of increased light absorbing particulates (LAP) on snow and ice surfaces. Among LAPs, and included in climate models is black carbon. However, other LAPs so far not included in climate models, because their role is poorly quantified, are mineral dust and pigmented algae. Such pigmented snow and glacier ice algae start growing in late spring and usually bloom during the summer. The snow algae form green to yellow and all the way to deep red snow coloured patches earlier in the season. We have shown that red snow changes albedo by up to 13 % [1] and that they create a positive feedback with melting. Once the snow has melted, blooms of deep purple glacier ice algae darken the surface of the bare ice dramatically. Such glacier ice algal blooms develop every year on glaciers and ice sheets worldwide. In Greenland, glacier ice algae expand in the fast growing "dark zone" mapped from satellites, where they reduce the albedo by between 13 and locally up to 26% [2]. In addition, we recently documented that a necessary interplay between geochemical, mineralogical and microbiological parameters fuels the annual glacial ice algal blooms in the dark zone, with mineral phosphorous playing a fundamental role in this process [3]. However, so far we still do not understand if it is the chemical, physical or microbiological triggers that initiate the snow or glacial ice algal blooms or how the interplay between these processes regulates blooms. I will show how in our current work, as part of the DeepPurple project, we ground truth and link a complementary suite of microbial ecological, inorganic and organic chemical, as well as physical surface property analyses with airborne and remote satellite data in our aim to quantify the role and consequences of water-mineral-microbe interface reactions in shaping large-scale processes across the Greenland Ice Sheet.

[1] Lutz et al (2016) Nat Com 7, 11968; [2] Cook et al (2020) The Cryosphere 14, 309-330. [3] McCutcheon et al (2021) Nat Com 12, 570

ST002

An attempt to identify the sources and hosts of antibiotic resistance genes in receiving water bodies

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Introduction: Antibiotic resistant bacteria (ARB) and the respective genes (ARG) represent biological water pollutants of increasing concern. ARB/ARG potentially originate from both point and non-point sources. While the role of wastewater effluents on the pollution of rivers with ARB/ARG has been demonstrated in a number of studies, the aspect of diffuse inputs to surface waters has gained less attention.
Objectives: To contribute to fill this gap of knowledge, the following three research questions were addressed:
(i) How does wastewater disposal shape the resistance profile of river bacterial communities? (ii) Is there evidence for a contribution of non-point sources? (iii) Is ARG abundance associated with the abundance of certain taxonomic groups?
Materials & Methods: The screening of the abundance and diversity of ARG as well as the community composition was performed in a small river basin close to Dresden, Saxony. Besides six wastewater treatment plants, the catchments hosts several big livestock facilities. Biofilm from the river bottom sediments was collected at 30 sites and the extracted DNA was shotgun sequenced. The metagenomic information was processed through a bioinformatics pipeline to obtain absolute and relative ARG abundances along with information on the composition of bacterial communities.

Results: We found a significant effect of treated wastewater for ARG families targeting aminoglycosides, macrolides and tetracycline. However, no unique effect was detected for

ARG encoding resistance against ß-lactams. Whereas the abundance of the carbapenemase genes blaIMP was significantly correlated with exposure to treated wastewater others were omnipresent in river biofilms. Type B trimethoprim resistance was only detected in river biofilms but not in wastewater effluents.
Whereas ARG families encoding for resistances against ß-lactams, macrolides and trimethoprim were positively associated with up to five bacterial orders, no correlation was found for ARG targeting aminoglycosides and tetracyclines.
Conclusion: Our analyses reveal groups of ARG which (i) are already spread and established in both compartments (human and river), (ii) are currently spread to the aquatic environment via treated wastewater and (iii) originate from the aquatic environment and may appear in pathogens in the future. Furthermore, our empirical correlation provide a starting point towards the identification of the main ARG hosts in receiving water bodies.

ST003
Microplastic Ingestion Affects the Gut Microbiome of Woodlice and Associated Production of Molecular Hydrogen
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Plastic pollution has become an environmental burden. Due to weathering and fragmentation, large plastic items are gradually transformed to slowly degrading microplastic particles (MP). MP may enter food webs via unintentional ingestion by the macrofauna, including woodlice (*Porcellio scaber*) in terrestrial ecosystems. For these organisms, an intact gut microbiome is important for their development, nutrition and immunity. Anaerobic conditions prevail inside the gut and therefore fermentative microbes producing molecular hydrogen (H_2) may play an important role. However, the consequences of MP ingestion on the host and its microbiome are not well known.

We hypothesise that biodegradable (polylactic acid, PLA) and non-biodegradable (polyethylene terephthalate, PET, and polystyrene, PS) MP have contrasting effects on *P. scaber* mediated by changes of the associated gut microbiome.

This was tested in MP-feeding experiments and subsequent analysis of the gut microbiome. Although a choice experiment revealed that woodlice avoided food containing PS, a 2.5% or 5% MP diet had no effect on the fitness of *P. scaber*. However, quantification of 16S rRNA genes and transcripts indicated significantly higher gut bacterial abundance when woodlice were fed with PLA-food, while the transcriptional activity of gut bacteria was stimulated when woodlice were fed with any MP-diet. Sequencing of 16S rRNA genes and transcripts revealed the microbiomes as highly diverse. However, more shared indicative taxa in the gut microbiome of woodlice fed with control and PLA-food were obtained than with control and PET- or PS-food. Amongst others species within *Opitutaceae, Saprospiraceae* and *Weeksellaceae* were indicative for the gut microbiome of PLA-fed woodlice. Potential pathogens such as *Mycobacterium* were found in guts of PET- or PS-fed woodlice. As an additional experiment, radial microsensor H_2 concentration profiles were recorded from guts of woodlice exposed to MP-containing or control food. Maximum H_2 concentrations reflected significantly more production in woodlice fed with PLA-food than without MP and lowest in PET- and PS-fed woodlice.

The results suggested a positive effect of biodegradable PLA on bacteria in the gut of *P. scaber* likely due to PLA hydrolysis and fermentation processes, while PET and PS had negative effects on fermentation activities. Woodlice were identified as significant mobile sources for H_2 that, along with the gut microbiome, was affected by MP.

ST004
Polluted marine habitats as prolific sources for polyester degrading enzymes
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The extensive use of plastics material has led to a dramatic littering problem in our oceans with patches of garbage as wide as 1.6 million square kilometers. Due to physical erosion, plastic breaks down to plastic particles of only ≤ 5 mm in size. Marine organisms mistaking it for food ingest this so-called micro-plastic as well as macro-plastic, which can lead to congestions and death. Ingested plastic particles may subsequently accumulate through the food web and ultimately end up in humans.

Novel polyester hydrolases may help to fight this urgent problem. As carbon sources are scarce in the oceans with plastic litter accumulating, it is reasonable to assume that bacteria have evolved mechanisms to utilize plastic litter as a carbon source. We therefore investigated the potential of marine bacteria for plastic degradation by using simple screening assays with artificial polyester substrates and UHPLC after recombinant expression of selected candidate enzymes.

We developed plate assays using emulsifiable artificial polyesters like Impranil® DLN and polycaprolactone diol[1]. These fast and easy assays allowed identifying bacteria of the *Pseudomonas pertucinogena* lineage as well as bacteria that we had sampled as putative polyester degraders from the ocean floor of the North Atlantic Garbage Patch during a scientific cruise. Ten polyester hydrolases from marine born strains were purified successfully and further analyzed and indeed degraded polyethylene terephthalate and diverse polyesters at 30 °C; however, further improvement of their hydrolytic efficiency is still necessary. We currently analyze the microbial community we have isolated from the garbage patch trying to uncover the complete polyester hydrolytic potential by using sequence- and activity-based metagenomics. In conclusion, we have already identified novel polyester hydrolases and currently characterize new species producing polyester hydrolases that may prove useful for industrial applications.

[1]Molitor *et al*. (2020) Microb Biotechnol. 13:274-284

ST005
Towards biorecycling of plastics: Strategies of *Pseudomonas capeferrum* TDA1 to grow on a polyurethane oligomer and monomers
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The continuing reports of plastic pollution in various ecosystems highlight the threat posed by the ever-increasing consumption of synthetic polymers. Recently, *Pseudomonas capeferrum* TDA1 was identified as the first bacterial strain capable of degrading 2,4-TDA, as well as a PU oligomer.

In order to understand different aspects of the biodegradation process, top notch techniques such as advanced genome annotation, RNA-seq transcriptomics and proteomics were applied to study the pathway of 2,4-toluene diamine (2,4-TDA) degradation as well as adaptive responses to environmental changes, stress conditions and cell interactions during growth on different carbon sources.

The previously reported degradation pathway for 2,4-TDA, a precursor and degradation intermediate of PU, could be confirmed by RNA-seq in this organism. Furthermore, growth of *P. capeferrum* TDA1 on a PU oligomer was examined and extracellular esterase activity was detected in different cell fractions. Strikingly, purified outer membrane vesicles (OMV) of *P. capeferrum* TDA1 grown on a PU oligomer showed higher esterase activity than cell pellets. Hydrolases in the OMV fraction possibly involved in extracellular PU degradation were identified by mass spectrometry. On this basis, we propose a model for extracellular degradation of polyester-based PUs by *P. capeferrum* TDA1 involving the role of OMVs in synthetic polymer degradation.

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Utomo et al. 2020. Defined microbial mixed culture for utilization of polyurethane monomers. ACS Sustain. Chem. Eng. 8:17466-17474.

Cardenas Espinosa et al. 2021. Screening and cultivating microbial strains able to grow on building blocks of polyurethane. in: Weber G., Bornscheuer U.T., Wei R. (Eds.) Enzymatic plastic degradation. Methods Enzymol. 648:423-434.

Cardenas Espinosa et al. 2021. An optimized method for RNA extraction from the polyurethane oligomer degrading strain Pseudomonas capeferrum TDA1 growing on aromatic substrates such as phenol and 2,4-diaminotoluene. PLoS ONE in press.

ST006

Inhibition of sulfate-reducing bacteria in a natural oil reservoir and in pure cultures

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Sulfate-reducing organisms produce H2S, which causes corrosion of pipelines and lowers oil quality, leading to massive financial losses in industry. Hence, there is a demand for practicable, cost-effective, and environmentally friendly methods for inhibiting sulfate-reducing microorganisms.

Here, we present field data from microbial communities in microliter-sized water droplets enclosed in heavy oil of the Pitch Lake, Trinidad and Tobago. Natural inhibition of sulfate-reducing bacteria was obvious from sulfate presence in the droplets. On this basis we tested and discussed the hypothesis if constant and high formate concentrations have the potential to inhibit microbial sulfate reduction *in situ*.

In 43 water droplets, we determined the ion composition and found surprisingly high sulfate concentrations up to 10.4 mM. In 21 water droplets, formate concentrations were around 2.37 ± 0.05 mM. The relative abundances of OTUs assigned to known sulfate-reducing organisms was < 0.2 % which together with the high sulfate concentrations indicated inhibition of sulfate reduction.

To assess the effect of formate on sulfate-reduction on a generic level, we incubated sulfate-reducing type stains with formate: *Desulfovibrio vulgaris*, *Desulfobacter curvatus*, and *Desulfococcus multivorans*, conducting different energy metabolisms *i.e.* incomplete lactate oxidation, citric acid cycle, and Wood-Ljungdahl pathway. The formate treatment lead to a strong delay in the onset and rate of sulfate reduction of *D. multivorans.* Furthermore, growth was completely inhibited as long as formate was added constantly. The growth rates of *D. vulgaris* and *D. curvatus* were slowed down around 1.5-fold but sulfate reduction rates or onsets of sulfate reduction and growth were unaffected.

We provide the first evidence that formate can delay and inhibit sulfate reduction and growth of specific sulfate-reducers, but the energy metabolism of the individual sulfate-reducing bacterium plays an important role for the response to formate treatment.

The experimental results support our hypothesis that sulfate-reducing microorganisms in the Pitch Lake water droplets are likely inhibited by high and constant *in situ* formate concentrations. Furthermore, the results propose that formate might be used for inhibiting sulfate reduction in oil production, thereby reducing corrosion and reducing financial losses. Since formate is a natural compound such treatment would largely exclude environmental hazards.

ST007

The current state of development of Exocube; an *in-situ* astrobiology exposure platform onboard the International Space Station.

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With many ongoing and planned space exploration missions (eg. ExoMars, Artemis, JUICE, Lunar Lander and Gateway), increasing our understanding of biological organisms and their composite biomolecules under space conditions is of critical importance. Given the remarkable ability of some microorganisms to prosper in extreme environments, several species have potential to survive under space conditions. This raises several interesting questions in regards to forward planetary contamination, life detection and life-support system technologies.

The space environment, in particular solar and cosmic radiation, is especially challenging to experimentally replicate. As such, Exocube (selected for implementation by the European Space Agency) will be mounted on the new Bartolomeo platform, outside of the International Space Station (ISS). Here, the survivability and cellular responses of microbial cultures exposed to conditions in low Earth orbit, will be monitored *in situ*.

Previous exposure experiments onboard the ISS have relied on pre-flight and post-flight comparisons, however, Exocube will be the first biological exposure platform to combine both the strengths of *in-situ* measurement and the capacity for sample return. To do so, Exocube utilizes a sophisticated microfluidics system, in combination with miniaturized absorbance and fluorescence measuring capabilities.

Exocube is currently in development phase C/D, and is expected to be mounted outside of the ISS in the 2023-2024 timeframe. Prototype hardware is currently being utilized for calibration, optimisation and biocompatibility tests, and an artificial solar UV light source is being used to examine radiation tolerances. Organisms of interest include halophilic and radiotolerant bacteria and archaea, photosynthetic microbes, and engineered biosensor organisms capable of monitoring cellular repair mechanisms in response to radiation-induced DNA damage. Finally, a variety of fluorescent markers are being assessed based on their function, long-term stability and degradation from radiation exposure.

The novel spectrofluorometric capabilities of Exocube will allow for the real-time *in-situ* monitoring of live microorganisms in the space environment. As the dramatically broader radiation levels beyond Earth's atmosphere present a particular challenge to overcome, Exocube aims to not only address fundamental questions of radiation biology, but also the sustainability of long-term space exploration.

ST008

Resistomes from 5943 metagenome-assembled genomes recovered from 165 active sludge and wastewater samples reveal almost 90% of species have antimicrobial resistance genes and that most of them may resist multiple drugs

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Antimicrobial resistance (AMR) has been recognized as a global challenge threatening human and animal health. However, we are far from understanding all consequences of AMR on human, animal, and ecosystem health.

In this study, we aimed to assess the presence of antimicrobial resistance genes (AMR) in publicly available active sludge and wastewater metagenomes to provide a comprehensive analysis of AMR genes compositions and abundance.

We retrieved 165 public metagenomes from various active sludge and wastewater treatment plants covering 14 different countries. Subsequently, metagenome-assembled genomes (MAGs) were recovered using MetaWRAP. We assigned as MAGs the bins recovered by MetaWRAP with quality above 50. The following equation determines quality: % Completeness – (5 x % contamination). Completeness and contamination are calculated by measuring the presence and absence of 122 known to be single-copy genes. MAG taxonomy was assigned using GTDB-tk and AMR genes were predicted using DeepARG. The MAGs were divided into genome operational taxonomic units (gOTUs) using 0.95 average nucleotide index as cutoff (a proxy for species level). We defined resitome as the collection of AMR genes present in a specific MAG.

We recovered a total of 5943 MAGs. These were divided into 1204 gOTUs covering 63 different phyla. Taxonomy analysis of our gOTUs showed *Proteobacteria, Actinobacteriota, Bacteriodota* and *Firmicutes* as part of the dominant groups in the studied samples. We found resistomes in 1076 (89.4%) gOTUs and a total of 5435 AMR genes. These AMR genes were dereplicated into 323 unique AMR genes and distributed in 26 AMR classes. The most dominant AMR genes were multidrug (1519), glycopeptides (889), bacitracin (362), tetracyclines (332), and macrolide-lincosamide-streptogramin (276). A total of 282 AMR genes predicted by DeepARG were unclassified and may encompass novel classes of antibiotics. Further, our data revealed multidrug (37.3%) and glycopeptides (25.0 %) as the most prevalent AMR classes within our data. Our analysis also demonstrated that most of the gOTUs (94.1%) containing resitomes had multiple AMR genes.

Our study provided a comprehensive overview of AMR genes in different active sludge and wastewater treatment plants, highlighting the prevalence of AMR-carrying species. The outcome of this research may help to design measures to diminish AMR transmission and evolution.

ST009

Design of a synthetic enzyme cascade for the *in vitro* fixation of a C_1 carbon source to a functional C_4 sugar

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The transformation toward a sustainable global society will also require a more consistent use of our waste streams, such as the valorization of the greenhouse gas carbon dioxide into value-added products. Therefore, biocatalytic processes have to be developed that allow the selective production of complex molecules from simple one-carbon compounds, as this is usually a green and sustainable technology. In this work, we addressed the utilization of formaldehyde, which can be sustainably derived from other C_1 feedstocks, as a C_1 carbon source for enzymatic C-C bond formation. In this context, we developed a synthetic enzyme cascade for the production of the functional C_4 sugar erythrulose. The enzyme formolase, which has been optimized for the fixation of formaldehyde, was redesigned for this purpose by a semi-rational approach from a three-carbon producer (dihydroxyacetone) to sets of variants with enhanced two-carbon (glycolaldehyde) or four-carbon (erythrulose) activity. This was accomplished by developing a high-throughput combinatorial screening to evaluate the glycolaldehyde, dihydroxyacetone, and erythrulose activity of each variant. With the application of the two most promising variants in a cell-free synthetic enzyme cascade, we were able to extend the oligomerization of the C_1 carbon source by formolase and produce the functional C_4 sugar rather than the C_3 ketose. Moreover, we demonstrated in a fully atom-economical biocatalytic process that one of our optimized formolase variants was able to convert 25.0 g L^{-1} glycolaldehyde into 24.6 g L^{-1} erythrulose (98% theoretical yield). This is the highest concentration of erythrulose achieved *in vitro* to date.

ST010

Tailoring *Clostridium ljungdahlii* for Improved Ethanol Production by Genetic Engineering and Adaptive Evolution

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Syngas fermentation is a promising platform for efficient recycling of carbon and energy from syngas and gasified wastes to bio-based fuels and chemicals. During this anaerobic biological process, acetogenic bacteria convert syngas (a mixture of carbon monoxide [CO], carbon dioxide [CO2], and hydrogen [H2]) to fermentation products, such as acetate and ethanol, *via* the Wood-Ljungdahl pathway.

Clostridium ljungdahlii is a model microbe for syngas fermentation. The bacterium grows under strictly anaerobic conditions with H2/CO2 or syngas as its sole carbon and energy source. Recent results show that the aldehyde:ferredoxin oxidoreductase (AOR) is a key enzyme for ethanol production by *C. ljungdahlii*. The focus of this work is to investigate the role of the two isoforms of this key enzyme in the metabolism of *C. ljungdahlii* and their engineering potential for biotechnology.

To this end, an adapted CRISPR-Cas9 system was applied to delete both AOR genes from the genome individually, as well as both genes in one strain. The AOR deletion mutants were characterized phenotypically to gain a thorough understanding of the role of the AOR in *C. ljungdahlii*. The best performing strain will be chosen for an adaptive laboratory evolution approach to further improve productivity. Whole genome sequencing and reverse engineering will be conducted to gain insights into beneficial mutations on the genome.

ST011

Isolation and characterization of novel acetogens for syngas fermentation

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Introduction: A strategy for the reduction of greenhouse gas emissions is the transformation of waste gases into valuable bulk chemicals and fuels by chemical or biological catalysts. Synthesis gas (syngas) is a characteristic industrial waste gas comprised of varying amounts of H_2, CO_2 and CO. Acetogenic bacteria possess the outstanding trait to metabolize syngas during autotrophic growth using the Wood-Ljungdahl pathway. Thus, they are promising biocatalysts for the fermentation of waste gases into biocommodities. Biocatalysts offer several advantages over competing chemical catalysts, but the commercialization of syngas fermentation is currently limited by low growth rates and product yields of the available acetogens.

Objectives: The project goal is the enrichment and isolation of novel efficient acetogens from environmental samples and the examination of their applicability as a biocatalyst for the fermentation of syngas into biocommodities by physiological and genomic characterization.

Materials & Methods: Acetogens were enriched at mesophilic and thermophilic temperatures by autotrophic growth on CO or a mixture of H_2 and CO_2. The anaerobic enrichment cultures were inoculated with a variety of environmental samples such as hot spring sediment, bioreactor sludge, river sediment and horse feces. The bacterial composition of enrichment cultures was monitored by 16S rRNA gene-based community analysis. Enriched acetogens were isolated and subsequently genome-sequenced with a combination of Illumina MiSeq™ and Oxford Nanopore™. Salt-, temperature- and pH-optima, as well as substrate utilization patterns were determined for selected isolates.

Results: The bacterial community analysis of enrichment cultures showed cultivation of acetogenic and syntrophic bacteria. Within the mesophilic cultures novel bacterial acetogens were enriched belonging to the genera *Clostridium sensu stricto 12* and *Terrisporobacter*. Thermophilic enrichments lead to the growth of novel strains belonging to the known acetogenic species *Moorella thermoacetica* and *Moorella humiferrea*. The obtained isolates were genome-sequenced and the physiological characterizations showed distinct differences in growth optima and substrate utilization.

Conclusion: Acetogens have been successfully obtained from environmental samples, genome-sequenced and physiologically characterized.

ST012

HyPerFerment II – Microbial Process Development and Evaluation of a Fermentative Hydrogen Production Pilot Plan

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Despite the declared aim to reduce CO_2, the consumption of fossil fuels is still increasing, especially in transportation. In order to achieve its defossilisation, hydrogen will play a major role. The project HyPerFerment II aims at the generation of H_2 as a pre-stage of already existing biogas plants, thus utilising established infrastructure.

We are currently constructing a 10 m³ pilot plant which is planned to be installed in spring 2022 as part of a local biogas plant. In said pilot we will use specific microbial cultures which are able to form H_2 from maize silage in a continuous process. Following the hydrogen production, the outflow will be further used as substrate in a conventional biogas production plant. The produced hydrogen is planned to be purified to such an extent as to be usable as fuel.

In, still ongoing, preliminary studies we surveyed several suitable cultures of which we selected one as the designated "pilot plant culture". This culture is able to produce hydrogen reliably from various substrates, including the "standard substrate" maize silage, but also waste products such as wheat bran, whey, or used coffee grounds. In several continuous fermentations those substrates were tested for several months in stirred tank reactors of up to 30 L. During these experiments an average concentration of approximately 50 to 55 % H_2 in gas yield was achieved. Besides the generally high robustness of the used culture, it could withstand several severe shifts in temperature and pH. A restart and fresh inoculation has never been necessary.

In an experimental set-up comparable to the aimed process of preceding hydrogen production and consecutive biogas generation we were able to show an overall energy win over the whole process. Furthermore, the biogas generation rate

of already fermented substrate has shown an increase while maintaining a similar or even higher methane yield.

With the shown experiments we were successfully able to lay the groundwork for hydrogen production in a 10 m³ pilot plant.

ST013

High versatility of IPP and DMAPP methyltransferases enables synthesis of C_6, C_7 and C_8 terpenoid building blocks

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The natural substance class of terpenoids covers an extremely wide range of different structures, although their building block repertoire is strongly limited. Nearly all terpenes discovered so far are made up of the two terpene precursors DMAPP and IPP, both having five carbon atoms. Recently, S-adenosylmethionine-dependent IPP MTases (methyltransferases) were described, which can transfer one or two methyl groups to this C_5 prenyl pyrophosphate. Our recent study aimed at the characterization of novel MTases that modify terpene precursors and the demonstration of their suitability for biotechnological purposes. After selecting putative candidate genes from different bacterial operon contexts, the substrate and product spectra of four so far undescribed and three known MTases were analyzed and compared. All seven enzymes accepted IPP as substrate and altogether five C_6 compounds and six C_7 compounds were formed within the reactions. A high deprotonation site selectivity as well as high stereoselectivity could be observed for most of the biocatalysts. Only one enzyme also accepted DMAPP as substrate, converting it into (*R*)-2-methyl-IPP *in vitro*. To test the applicability of the MTases *in vivo*, respective *E. coli* strains were investigated with regard to the release of corresponding terpene alcohols. Within these experiments one strain with a specific IPP-MTase turned out to produce different C_8 terpenoids, which were further investigated with isotopic labelling studies. They revealed the occurrence of a hydride shift step within the MTase-catalyzed reaction and enabled structure clarification for this compound. Our study demonstrates the occurrence of C_5 prenyl pyrophosphate MTases with very different catalytic properties in bacteria, which provide biosynthetic access to many novel terpene-derived structures.

ST014

Machine learning guided optimization of the new-to-nature CO_2-fixation cycle CETCH

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Since CO2 molecules are very stable, the conversion into multi-carbon-compounds is energy demanding and mainly done by organisms which use light as an energy source. Therefore, the main workhorses for capturing CO2 from the atmosphere are plants and algae, which incorporate the carbon for the production of biomass. In these organisms the Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) is responsible for the carboxylation. The dilemma of RuBisCO is its evolutionary past since it derives from a time where no free oxygen was present in the atmosphere. This is thought to be the reason why RuBisCO incorporates in approximately every fourth reaction oxygen instead of CO2. This leads to a toxic metabolite and a wasteful detoxification process. Hence, photosynthesis is a rather slow and ineffective way of aerobic CO2 fixation and attempts to engineer RuBisCO to be more specific and faster have failed so far. In 2016, Schwander et al. published a completely new-to-nature pathway to fix CO2 using the Crotonyl-CoA carboxylase/reductase (Ccr), one of the fastest carboxylases known to date. This pathway, named the crotonyl-coenzyme A (CoA) /ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle, is more efficient than natural aerobic CO2-fixation pathways and harbors therefore the possibility to play an important role in the reduction of atmospheric CO2 levels.

To optimize this pathway we established a workflow to test hundreds of CETCH assays in parallel *in vitro*, allowing us to screen more than a thousand conditions. Prerequisite was the implementation of an acoustic liquid handler with a minimal pipetting volume of 25 nl. This enabled a high throughput by two factors: Pipetting speed and volume. While downscaling to 10 µl assays was crucial in terms of resources, e.g. purified enzymes, pipetting speed was essential since each round of 125 assays in triplicates consists of >10.000 pipetting steps. The generated data was used to iteratively train an XGBoost based machine-learning algorithm to optimize the CETCH cycle to produce up to 2870 µM glycolate starting from 100 µM propionyl-CoA, which is more than ten times more efficient than the original version published in 2016. To democratize machine-learning based optimization of biological systems, this algorithm was built to be used as easy as possible and is freely available.

ST015

A protein in search of a function: The c-di-AMP-binding protein DarA of *Bacillus subtilis*

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The nucleotide c-di-AMP is the only known essential second messenger. Several functions have been linked to this nucleotide, including osmoregulation, cell wall biosynthesis and DNA integrity. The conserved c-di-AMP receptor DarA of *Bacillus subtilis* is a PII-like protein with a yet unknown function (1). PII and PII-like proteins are the largest family of signal transduction proteins and interact with a plethora of targets such as transporters, enzymes, and transcription factors (2). Despite extensive research, the function of DarA has not been elucidated.

To get a glimpse of the function of DarA, we profiled the phenotypes of a *darA* mutant and performed suppressor screens under conditions where DarA is crucial for the growth of *B. subtilis*. The analyses of the *darA* mutant suggest a functional link between potassium availability and amino acid biosynthesis. A strain lacking both high-affinity potassium importers is unable to grow at low potassium concentrations unless it acquires suppressor mutations that facilitate the synthesis of positively charged amino acids. Interestingly, *darA* is essential in these suppressor mutants and cells lacking *darA* acquire mutations that affect the synthesis of positively charged amino acids. The role of DarA in these mutants and their suppressors was investigated via metabolome analyses and revealed perturbed concentrations of intermediates of arginine biosynthesis. It is tempting to speculate that DarA integrates a signal to

counteract extreme potassium limitation by elevating levels of positively charged amino acids.

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ST016

Characterization of the FNR-type regulator GoxR in the obligatory aerobic acetic acid bacterium *Gluconobacter oxydans*

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Introduction: *Gluconobacter oxydans* is a strictly aerobic Gram-negative acetic acid bacterium that serves as a cell factory for oxidative biotransformation and as a model organism for elucidating the metabolism of acetic acid bacteria.

Objectives: Although *G. oxydans* is not cable of anaerobic growth by respiratory or fermentative pathways, it responds to a shift from oxygen excess to oxygen limitation by changes in the expression of about 500 genes[1]. In our study, we characterized a transcriptional regulator of the FNR family in *G oxydans* (GoxR) and its role during the response to oxygen limitation.

Methods: A Δ*goxR* mutant strain was used to compare the global gene expression changes after the switch from oxygen access to oxygen limitation. In addition, we used ChAP-Seq analysis for the genome-wide identification of *in vivo* GoxR binding sites.

Results: Comparison of global gene expression in the Δ*goxR* mutant with that in the parental strain by RNA-Seq showed two genes with a ≥2-fold increased expression in the Δ*goxR* mutant: *cioA* and *cioB*, encoding the two subunits of the bd-type terminal oxidase CIO. Among the genes with a ≥2-fold decreased expression in the Δ*goxR* mutant were those of the *pnt* operon. Furthermore, we could confirm with ChAP-Seq that GoxR binds *in vivo* to the GoxR sequence motif (TTGAT-N_4-ATCAA) that was determined by bioinformatic means for the target genes identified by RNA-Seq analysis. The evidence for the presence of an iron-sulfur cluster in GoxR, which is characteristic for FNR-type regulators, was given by a small peak at 420 nm in the UV-visible spectrum for the protein purified under anaerobic conditions.

Conclusion: With this study we were able to identify target genes of GoxR such as the *cioAB* genes encoding a cytochrome bd oxidase with low O2 affinity, which were repressed by GoxR, and the *pnt* operon, which was activated by GoxR. The *pnt* operon encodes a transhydrogenase, an NADH-dependent oxidoreductase, and another oxidoreductase. Evidence was obtained for GoxR being active despite a high dissolved oxygen concentration in the medium. We suggest a model in which the very high respiration rates of *G. oxydans* due to periplasmic oxidations cause an oxygen-limited cytoplasm and insufficient reoxidation of NAD(P)H in the respiratory chain, which might be compensated by the activity of GoxR[2].

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ST017

The on-switchable phosphodiesterase PdeB creates heterogeneity

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Motility of bacterial cells is strongly influenced by their c-di-GMP level. The second messenger c-di-GMP is synthesized by diguanylate cyclases and degraded by phosphodiesterases. Many bacteria harbor a repertoire of plenty of enzymes that regulate the level of c-di-GMP. The regulation is often entangled in complex networks. In *Shewanella putrefaciens*, a single polar localized phosphodiesterase, namely PdeB, appears to be the major mediator between sessility and motility.

Notably, the activity of PdeB is dependent on the interaction with the so-called landmark protein HubP. Precisely, the interaction between an enzymatically inactive GGDEF domain of PdeB and the FimV domain of polar localized HubP allows phosphodiesterase activity.

At least two factors that are important in the attachment to surfaces are regulated by PdeB. In particular PdeB regulates the activity of the type IV MSHA pilus and the expression and translation of the *bpf* operon, encoding a surface adhesin that promotes biofilm formation.

Even though PdeB does not influence the synthesis of the polar flagellum, it seems to affect the chemotaxis. This effect is potentially achieved by indirectly altering the methylation state of the chemotaxis receptors.

Additionally, the appearance of PdeB at the cell pole typically occurs long after cell fission and the copy number of the protein differs notably. Both circumstances promote an asymmetric cell division with mother cells harbouring a PdeB cluster and with daughter cells without one. That creates a population of cells with a high degree of heterogeneity to benefit the proliferation of the bacterial population.

ST018

Comparative analysis of redox-controlled activity of the 5 CSS domain c-di-GMP phosphodiesterases of *Escherichia coli*

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In Escherichia coli a dynamic balance of phosphodiesterases (PDEs) and diaguanylatecyclases (DGCs) controls the levels of the second messenger c-di-GMP and further the promotion of bacterial biofilm formation. Many of these enzymes are membrane bound with N-terminal sensory input domains that are responding to intra- or extracellular signals. Out of the 13 known PDEs in E. coli, five exhibit a common domain architecture including a periplasmic CSS domain, with two highly conserved cysteines, flanked by two transmembrane (TM) domains which is completed by a

catalytic EAL domain in the cytoplasm. With this shared "CSS domain" structure, PdeB, PdeC, PdeD, PdeG and PdeN describe a novel class of c-di-GMP degrading enzymes. A mechanism for activation was recently shown for PdeC, where DsbA/DsbB promoted disulfide bond (DSB) formation in the CSS domain reduced PDE activity while the free-thiol form revealed high enzymatic action with the TM2 region dimerizing. Further analysis indicated that this form is processed by DegP and DegQ forming a highly active TM2+EAL fragment that is processed by further proteolysis. In this work we demonstrate a comparison of all five CSS-PDEs, which besides their conserved periplasmic cysteines and cytosolic EAL domain, show high variability in their amino acid (AA) sequence, pointing towards a different mechanism in activation and function. Like PdeC, biofilm macrocolony matrix production was reduced for PdeB and PdeG when lacking one or both conserved cysteines, whereas PdeN and PdeD were highly active in their oxidized form. Although DSB formation is again facilitated by DsbA in PdeB/D/G, DSBs in PdeN are formed independent of this oxidoreductase. In order to investigate the inverse redox control of the PDEs the AA sequence of PdeB and PdeN was studied more intensively, reporting charged AA upstream of TM2 (box II) in both proteins. In contrast to PdeB, revealing positively charged AA in box II, PdeN shows a negatively charged pattern. Exchanging box II of PdeB and PdeN leads to an inverse phenotype, showing reduction of biofilm matrix with the reduced form of PdeB while the DSB form of PdeN suddenly lacks its enzymatic activity. This opposite behavior for both PDEs indicates that the activity is not simply explained via the ability of DSB formation but includes specific AA patterns that may influence the orientation of domains in order to get a fully functional enzyme.

ST019

The DUF1127 protein CcaF1 from *R. sphaeroides* is a novel RNA-binding protein involved in sRNA maturation and RNA turnover

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The facultative phototrophic bacterium *Rhodobacter sphaeroides* can 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 stress responses, particularly the response to oxidative stress. The four homologous sRNAs CcsR1-4 (conserved CCUCCUCCC motif stress-induced RNA) are expressed under various stress conditions and modulate the C1 metabolism [1]. These sRNAs are derived from the 3´ UTR of the *ccaF1* (conserved CcsR associated factor) mRNA. The *ccaF1* gene encodes a small protein of 71 amino acids and the residues 23-62 constitute a domain of unkown function (DUF1127). This functional unknown protein domain is structurally related to eukaryotic RNA binding proteins [2]. Small proteins with the DUF1127 domain are widely distributed among α- and γ-proteobacteria and more than 17,000 bacterial sequences with DUF1127 proteins are listed in InterPro.

By Norther Blot analysis and electrophoretic mobility shift assays we could show that the DUF1127 domain of CcaF1 is a novel RNA-binding domain in *R. sphaeroides*. The studies also show that the DUF1127 protein CcaF1 has an influence in the processing, maturation and degradation of the sRNAs CcsR1-4 and other coding and non-coding RNAs. Thereby different physiological processes, like the growth behavior and stress resistance will be influenced in *R. sphaeroides* [3].

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ST020

DNA interference states of the hypercompact CRISPR–CasΦ effector from huge bacteriophages

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CRISPR–CasΦ, a small RNA-guided enzyme found uniquely in bacteriophages, achieves programmable DNA cutting as well as genome editing. To investigate how the hypercompact enzyme recognizes and cleaves double-stranded DNA, we determined cryo-EM structures of CasΦ (Cas12j) in pre- and post-DNA-binding states. The structures reveal a streamlined protein architecture that tightly encircles the CRISPR RNA and DNA target to capture, unwind and cleave DNA. Comparison of the pre- and post-DNA-binding states reveals how the protein rearranges for DNA cleavage upon target recognition. On the basis of these structures, we created and tested mutant forms of CasΦ that cut DNA up to 20-fold faster relative to wild type, showing how this system may be naturally attenuated to improve the fidelity of DNA interference.

ST021

Identification and characterization of RNA-binding proteins in the cyanobacterial model *Synechocystis* sp. PCC 6803

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Cyanobacteria are in the focus of science as they can fix atmospheric CO2 and convert it to organic carbon molecules, making them promising subjects for research on the production of carbon neutral alternatives to fossil fuels or as nutrition supplements (1–3).

In order to make use of cyanobacteria for industrially relevant metabolic engineering, it is crucial to understand their metabolism, biochemistry and regulatory pathways.

A big part of their regulation depends on RNA molecules. One part of RNA based regulation is carried out by small noncoding RNAs (sRNA) by modulating the translation of their target mRNA via complementary base pairing (4). Furthermore, it was shown that the localization of the mRNA in the cell also plays an important role for the protein expression and function of these proteins (5). Both of these mechanisms require assistance by RNA-binding proteins

(RBPs). RBPs regulate a variety of processes in the bacterial cell, ranging from the regulation of transcription termination and translation initiation to the regulation of RNA decay (6). While RBPs are well studied in bacteria like *E.coli* or *Salmonella*, there is almost no existing literature about their functions in cyanobacteria. Recently, our group investigated potential RBPs in *Synechocystis* sp. PCC 6803 and proposed a number of promising candidates (7). Furthermore, a conserved family of cyanobacterial cold-shock proteins seems to be involved in the transport of photosystem mRNAs to the thylakoid membrane (8) . Therefore, the in-depth characterization of these RBPs as well as the identification of new RBPs in our model is being addressed in this project.

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ST022

RNAylation of proteins – a new post-translational protein modification mediated by NAD-RNAs and a T4 phage ADP-ribosyltransferase

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The mechanisms by which viruses hijack their host"s genetic machinery are of enormous current interest. One mechanism is adenosine diphosphate (ADP) ribosylation, where ADP-ribosyltransferases (ARTs) transfer an ADP-ribose fragment from the ubiquitous coenzyme nicotinamide adenine dinucleotide (NAD) to acceptor proteins. Here, we report that bacteriophage T4 ARTs surprisingly accept not only NAD but also NAD-capped-RNA (1) as substrate, thereby covalently linking entire RNA chains to acceptor proteins *in vitro* and *in vivo* (2). We term this reaction an "RNAylation".

This new post-translational protein modification (PTM) changes the properties of the target protein, such as charge and size. In *in vitro* experiments, we were able to show that the RNAylation of the ribosomal protein S1 by ModB using radiolabeled NAD-capped RNA dramatically increases the protein size. Western blot experiments showed that the RNAylation also occurs *in vivo* – in *E. coli* during T4 phage infection. Removal of ADP-ribosylation and RNAylation, during T4 phage infection dramatically decelerates lysis, thereby underlining the significance of these PTMs for T4 phage infection.

Our findings challenge the established views of phage infection and exemplify that the structural and functional boundaries between different classes of biopolymers become increasingly blurry. We suggest that RNAylation by ARTs could play so far undetected roles in the interaction of phages and bacteria or even in higher organisms.

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ST023

Autophosphorylation of ArlH and its influence in the archaellum motor complex

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Motile archaea are propelled by the archaellum, a rotating filament powered by a membrane-embedded motor. This motor, which is unrelated to the bacterial flagellum, has a core formed by three protein types: ArlI, ArlJ, and ArlH. ArlI is the only ATPase of the archaellum, and it powers the assembly and the rotation of the filament. ArlJ is homologous to the platform protein of the type IV pili PilC, and as such it is hypothesised to relay the conformational changes of the ATPase ArlI across the membrane. No ArlH homologues are found in type IV pili; instead, the closest and best understood homologue of ArlH is KaiC, a kinase/phosphatase central for the cyanobacterial circadian rhythm regulation. Thus, we questioned whether ArlH shares biochemical properties with KaiC. In this work we show that ArlH can authophosphorylate. We show by single-molecule fluorescence measurements that the oligomeric state of ArlH is closely linked to its interaction with ArlI which, in turn, depends on the phosphorylation status of the former. Additionally we show that optimum phosphorylation activity of ArlH is essential for archaellation and motility in *Sulfolobus acidocaldarius*.

ST024

2-oxoglutarate activates the glutamine synthetase of the methanogenic archaeon *Methanothermococcus thermolithotrophicus* via an allosteric site

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The glutamine synthetase (GS) is a key enzyme for ammonia assimilation spread through all domains of life [1]. It catalyzes the ATP-dependent conversion of ammonia and glutamate to glutamine, an irreversible reaction that must be tightly controlled, especially in energy limited organisms like methanogenic archaea [2]. While bacterial GS have been studied extensively there is little known so far about archaeal GS, especially regarding their regulation mode that seems to differ strongly from bacteria.

Here, we natively purified the GS from *Methanothermococcus thermolithotrophicus*, a thermophilic methanogen. Biochemical characterization revealed a strict dependency of GS activity on 2-oxoglutarate (2OG) with a Ka of 170 µM. No activation was detected with analogues such as malate and succinate. To decipher the activation process at the molecular level, three crystal structures were obtained: an apo form at 1.65-Å and two 2OG bound forms with and without ATP at a resolution of 2.15 and 2.90-Å, respectively.

GS organized as a 0.6 MDa dodecamer, as predicted by native PAGE and gel filtration. The apo state is inactive with a hindered ATP binding site. 2-oxoglutarate binds in an allosteric pocket formed by the dimeric interface, located at 20-Å to the active site. Its binding leads to a conformational rearrangement of the substrate binding site allowing catalysis to proceed.

With these results, our group solved the whole ammonia assimilation pathway of this methanogen. While the glutamate synthase uses reduced F420 to produce glutamate from 2OG and glutamine, the GS shows a feedback activation by 2OG. Such a coupled system can precisely deliver nitrogen to the cell upon demand by using 2OG as a sensor. 2OG has also been reported to stimulate activity of GS in *Haloferax mediterranei* [3] and *Methanosarcina mazei* [4] and further studies will have to determine whether the activation mechanism found in *M. thermolithotrophicus* applies to other archaeal groups.

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ST025

Effects of ydrogen in anaerobic, extremely halophilic microbial cultures from the Zechstein formation

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Storage of green hydrogen is key in a climate-neutral economy. Salt caverns are artificial storage cavities that are created through solution mining in underground salt formations of thalassohaline origin. Long-term experience was gained during operation with natural gas. Contrary to natural gas, hydrogen (H2) is a favorable electron donor for microorganisms and might trigger microbial activity in the presence of a suitable electron acceptor. As sulfate salts are common components of underground salt deposits, indigenous halophilic prokaryotes may oxidize H2 using sulfate as electron acceptor, subsequently producing hydrogen sulfide (H2S). This interferes with operation due to the corrosive and toxic features of H2S. However, knowledge on the indigenous microbial community composition of salt caverns, their physiological characteristics and behavior to H2 is scarce. Therefore, future H2 underground gas storage requires careful examination of the cavern microbiome.

A field sampling campaign was designed to retrieve brine sample of five different caverns in the Zechstein Formation of Central Germany charged with natural gas. The limited amount of sample volume was split to investigate i) its hydrochemical composition ii) microbial diversity and iii) the microbial response to H2. Latter required incubation with labelled bicarbonate and water (13CO2, 2H2O), continuous measurement of H2S production and H2 oxidation through GC-IRMS. Finally, the single-cell isotopic composition was measured with nanoSIMS.

The salt cavern brines were colonized by a diverse community of putative halophilic prokaryotes. Amendment of H2 resulted in an increased uptake of 13CO2 and 2H2O by several community members, but sulfide production was not stimulated in the original microbial community. Nevertheless, addition of an extremely halophilic H2-oxidizing enrichment culture from a different origin to the brine resulted in sulfate reduction and H2-oxidation at a NaCl concentration of 27%. It is therefore necessary to monitor microbial community and activity in salt caverns intended for H2 storage.

ST026

Hmx1 and Hmx2 function as manganese importer in cyanobacteria

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1. Introduction
   Photosynthetic organisms rely on sufficient supply of manganese (Mn) to form the oxygen evolving complex (OEC), which is essential for oxygenic photosynthesis. The OEC drives the oxidation of H_2O and thus serves as electron donor for photosystem II. Nevertheless, Mn has detrimental effects if accumulating inside the cell, since Mn excess may cause mismetalation of metalloproteins. Hence, proper homeostasis is crucial to avoid an excess on the one hand, but also ensure sufficient Mn supply on the other hand [1]. Our group has identified the thylakoid Manganese exporter (Mnx, [2]), a member of the Unknown Protein Family 0016 (UPF0016), in the model cyanobacterium *Synechocystis* sp. PCC 6803. Besides Mnx, two additional members of the UPF0016 are encoded in the genomes of cyanobacteria. Due to their structure and size, they are called Hemi-manganese exchanger (Hmx) 1 and 2.

2. Objectives
   We test the hypothesis that Hmx1 and Hmx2 interact and function as another Mn transporter in cyanobacteria. We furthermore aim at unraveling the function of this transporter in the Mn homeostasis network in *Synechocystis*.

3. Material and methods
   Localization studies with *Synechocystis* strains expressing Hmx1:CFP or Hmx2:CFP fusion proteins were conducted as described in [2]. Using knock-out strains in *hmx1* and/or *hmx2* quantification of the intracellular and periplasmic Mn pools and drop-test assays under Mn depletion (0 and 4.5 µM $MnCl_2$), sufficient (9 µM), and excess

(45 and 90 µM $MnCl_2$) conditions [2] were performed.

4. Results
   Fluorescence microscopy indicated that both, Hmx1:CFP and Hmx2:CFP, reside in the plasma membrane. On medium supplemented with 0, 4.5, or 9 µM $MnCl_2$ the knock-out mutant lines showed retarded growth in comparison to the wild type. The growth phenotype was compensated by application of excess $MnCl_2$. Furthermore, intracellular Mn pools were reduced in the mutant lines.

5. Conclusion
   Hmx1 and Hmx2 assemble as heteromers in the plasma membrane. Here they likely function as importer for Mn under Mn deplete and sufficient conditions.

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ST027

Effect of biotransformation reactions as heterologous electron sinks on the cyanobacterial metabolism

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With their excellent selectivity and often very mild reaction conditions, oxidoreductases play an important role in the chemical and pharmaceutical industries. Widely-used enzymatic redox transformations such as C-H oxyfunctionalization or C=C double bond reduction require a stoichiometric supply of electrons, which is usually provided from petrol-based or agricultural cosubstrates such as isopropanol or glucose. The use of an equivalent amount of organic cosubstrates for a process is highly problematic for its atom economy, an important metric for sustainability. Whole-cell redox biocatalysis in cyanobacteria uses electrons from photosynthetic water-splitting and saves organic cosubstrates [1].

Above a certain reaction rate, heterologous biotransformation reactions are limited by the intracellular electron supply. We have shown that the deletion of flavodiiron proteins as competing electron outlets can significantly increase the reaction rate of whole-cell photobiotransformations such as ene-reductases or Baeyer-Villiger monooxygenaseses. The physiological consequences of a very strong heterologous electron sinks on the cyanobacterial metabolism will be discussed.

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ST028

Photosynthetic driven H_2 production in *Synechocystis* sp. PCC 6803 with the help of an oxygen tolerant hydrogenase

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Cyanobacteria are potential candidates to couple oxygenic photosynthesis and H_2 production with the help of hydrogenases. One of the major challenges in using native cyanobacterial hydrogenases is their high oxygen sensitivity. We addressed this challenge, by the introduction of an O_2-tolerant hydrogenase from *Ralstonia eutropha* into a phototrophic bacterium, namely the cyanobacterial model strain *Synechocystis* sp. PCC 6803 (Lupacchini et al. 2021). We characterized the strain *Syn_Re*SH^+ in detail and found that activity is limited by a low enzyme production and maturation. Therefore, we designed an advanced ReSH expression system in *Syne*6803, using the CyanoGate cloning systemc (Vasudevan et al. 2019), whereby we achieved a higher level of synthesized protein and elevated activity *in vivo*. Furthermore, we determined limitations and improved enzyme activity in *Syn_Re*SH^+ by optimizing physiological conditions, in particular the light availability, substrate (H_2) supply and a sufficient electrons sink (CO_2). To improve the H_2 production several strategies were followed and data will be presented.

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ST029

Continuous Stirred-Tank Bioreactor for the Winning of Zinc and Indium by Bioleaching

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The Erzgebirge region is, as its name suggests, very rich in ores. The reopening of a mine in the region is considered to extract tin primarily. However, zinc and indium could also be won from the sulfide fraction that contains sphalerite and arsenopyrite. Bioleaching is a well-established technique to dissolve sulfide minerals and it has the potential to treat also arsenic-containing material. Acidophilic bacteria that are able to oxidize iron generate ferric ions that are powerful leaching agents for the ore. A bioreactor cascade was set up to perform bioleaching reactions with the sulfidic flotation concentrate. The consortium of iron and sulfur oxidizing bacteria was composed of Leptospirillum ferriphilum, Acidithiobacillus caldus and Sulfobacillus thermosulfidooxidans. After several months of adaptation to high concentrations of metals, bacteria were fed with 60 g/L of sulfide concentrate in a continuous stirred-tank reactor system consisting of 3 vessels of 1.4 L working volume each. The flow rate was set to 0.3 L/day resulting in a hydraulic retention time of 14 days. After reaching steady-state,

recoveries of zinc and indium of 96 % and 92 %, respectively, were achieved. These results demonstrate the feasibility of the process to valorize zinc sulfide flotation residues providing an alternative route to pyrometallurgy for complex arsenic-containing ores and concentrates.

ST030

Productivity of *Nannochloropis salina* and *Synechocystis* sp. PCC 6803 in autotrophic batch cultures with CellDEG cultivators and balanced media with high nitrate concentration.

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Question: To obtain a high final biomass concentration of an autotrophic batch culture, limitations by depletion of essential nutrients have to be considered. This requires either controlled feeding of mineral nutrients or the use of a high salt medium with balanced concentrations of mineral elements. Although the use of high salt media is common for culturing *Spirulina platensis* or marine algae, it unknown, whether lab strains like *Nannochloropis salina* and *Synechocystis* 6803 can grow rapidly to high cell density, when the initial nitrate concentration is high enough to enable the formation of concentrated biomass.

Methods and Results: To study this problem, the CellDEG membrane technique of CO_2 feeding was combined with exponential increase in photon flux density (PFD) and the use of balanced and bicarbonate-buffered mineral media (HD 8110) containing 121 mM nitrate. Using shaken membrane cultivators (HD 10) and the CellDEG growth control unit (GCU), dissolved CO_2 was maintained at high level and a high mixing rate of the cultures was established. To prevent photoinhibition at low initial cell density, the PFD was increased with a doubling time of 8h from initially 100 to finally constant 1000 µmol photons m^{-2} s^{-1}. After the exponential phase, a rather long phase of almost constant productivity (more than 8 g CDW L^{-1} d^{-1}) was observed for both organisms (linear growth phase). As a consequence of increasing light limitation by self-shading during this phase, the specific growth rate decreased in an approximately reciprocal ratio to the increasing biomass concentration. Growth termination occurred at a biomass concentration close to values expected assuming complete nitrate assimilation.

Conclusions: Our experiments show that common euryhaline lab strains, such as *Synechocystis* 6803 and *Nannochloropis salina*, can be cultivated with high productivity to high cell densities in shaken batch cultures using a balanced medium with high nitrate concentration, membrane-mediated CO_2 supply and a controlled increase in light intensity.

ST031

Identification of non-canonical terpene biosynthetic routes in bacteria

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Terpene production is found in all realms of life. All the more astonishing is the fact that bacterial terpene biosynthesis is only poorly studied. Nevertheless, there is increasing evidence that bacteria evolved a unique terpene biosynthetic route where canonical terpene synthase (TPS) substrates are initially modified by methyltransferases (MT) as it has been shown for the isoprenyl pyrophosphates IPP, GPP and FPP [1,2,3]. Most recently, we showed that an FPP-MT is involved in biosynthesis of the non-canonical sesquiterpene sodorifen which was not only able to perform methylation but also cyclization of the FPP molecule [3].

The aim of this study was to identify further examples for such extraordinary, non-canonical terpene biosynthesis in bacteria.

For sodorifen biosynthesis, we identified a gene cluster including a pair of MT and TPS genes. Using antiSMASH we were able to find further potential candidate organisms which contain a gene cluster of similar structure like the sodorifen cluster. First, the respective MT & TPS genes were cloned followed by heterologous overexpression in *E. coli*, Ni-NTA purification and finally enzyme assays. GC/MS analysis of the resulting extracts revealed production of non-canonical terpenes which shared significant similarities to sodorifen but are undoubtedly different compounds. After testing the MT & TPS enzymes separately, we were able to show that the MT used FPP as a substrate and performed simultaneous methylation and cyclization whereas the TPS specifically used only this modified FPP molecule to synthesize the final non-canonical terpene product, just like it was shown for sodorifen biosynthesis.

Accordingly, these results prove that the unique reaction mechanism shown for sodorifen biosynthesis appears to be more widespread in the bacterial kingdom than previously thought. Moreover, it will be exciting and challenging to further explore the bacterial terpenome for such non-canonical terpene biosynthetic routes and to examine whether these mechanisms are specific for the bacterial kingdom.

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ST032

Temporal expression of biosynthetic gene clusters in the myxobacterium *Sorangium cellulosum* Soce836

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The ever-increasing spread of multidrug-resistant pathogenic bacteria demands the discovery of novel antibiotics. Myxobacteria are a valuable source of novel secondary metabolites, including antibiotics. While genome studies of myxobacteria indicate a large number of biosynthetic gene clusters (BGCs) with unknown secondary metabolites, many

of these metabolites are not produced under standard laboratory conditions. Improved understanding of the molecular regulation of secondary metabolite synthesis will facilitate the targeted induction and manipulation of metabolite production and hence could accelerate the discovery of novel antibiotics.

We have investigated the temporal expression of BGCs of the myxobacterium *Sorangium cellulosum* Soce836 and related it to its secondary metabolite production. In our study, we combined genome mining with time-course RNA-seq and LC/MS approaches over five growth times, covering all growth phases. Additionally, we tested growth conditions with and without an adsorbent resin.

As a result, we found that both, metabolite production and BGC gene expression varied dynamically over time and growth condition. The majority of core genes from individual nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) BGCs commonly followed similar temporal expression patterns, suggesting their co-regulation. Our results on cell counts showed a clear difference in cell growth between conditions with and without the adsorbent resin.

We show that core BGC genes in batch cultures of the myxobacterium *Sorangium cellulosum* are expressed most strongly during early growth phases. Furthermore, the adsorbent resin has a strong effect on the temporal gene expression and the production of secondary metabolites.

ST033

Evolving *Pseudomonas putida* as robust production platforms for the synthesis of bioactive natural products

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Pseudomonas species have become promising cell factories for the production of natural products due to their inherent robustness. However, efficient high-level microbial production is still challenging due to the chemical stress caused by high substrate or product concentrations. Although bacteria have naturally evolved different strategies to cope with toxic chemicals, biotechnological applications additionally benefit from the engineering of optimized *chassis* strains with specially adapted tolerance traits[1]. Accordingly, this work aimed to investigate resistance mechanisms for enhanced production of various natural products in *Pseudomonas putida* KT2440, with particular emphasis on outer membrane vesicle (OMV) formation[2]. Adapting CRISPR interference[3], we have developed a tool for efficient screening of genetic targets that are putatively affecting OMV formation. Using protein- and lipid-based quantification assays and dynamic light scattering (DLS), we confirmed that several newly developed *P. putida* strains showed a 5- to 10-fold increased OMV formation compared to the wild type. These new *chassis* strains were tested as production platforms for different classes of natural products including pyrrole-alkaloids and indolocarbazoles, which are valuable sources of exceptionally bioactive compounds. Accordingly, we installed biosynthetic gene clusters encoding such pathways from different bacteria for heterologous expression in *P. putida* KT2440. Triggering vesiculation in the newly engineered recombinant production strains resulted in an increased production of the respective compounds, for example 2- to 4-fold in case of the antibiotic and cytostatic prodigiosin or the cyclin-dependent kinase 4 inhibitor arcyriaflavin A. Our findings suggest that increased vesiculation in *P. putida* KT2440 can lead to higher production levels of recombinant natural compounds. Thus, the construction of robust *chassis* strains with increased OMV formation might be a powerful tool for significantly improving hitherto limited yields of natural products for biotechnological applications.

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ST034

Generation of novel pristinamycin derivatives by mutasynthesis approaches

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Infections caused by antibiotic resistant bacteria are one of the biggest threats to human lives in the decades to come. Apart from approaches to find new antibiotics, one strategy to combat drug-resistant bacteria is to modify known antibiotics and thereby improve their anti-infective properties. Drug modification can be achieved by the so-called mutasynthesis concept, whereby a biosynthetic mutant is fed with alternative biosynthetic building blocks (= mutasynthons), which results in the formation of novel antibiotic congeners. We report on a mutasynthesis approach towards generating novel congeners of the streptogramin antibiotic pristinamycin. Pristinamycin, produced by *Streptomyces pristinaespiralis*, is composed if the structurally distinct cyclohexadepsipeptide pristinamycin I (PI) and the macrolide pristinamycin II (PII) and used as an antibiotic of last resort against Gram-positive and some Gram-negative pathogens. However, increasing incidences of resistance against Pristinamycin are reported, endangering its usability as a therapeutic [**1**]. In our mutasynthesis approach we seek to alter the structural composition of PI. Specifically, we concentrate on the substitution of L-phenylglycine (L-Phg), which is the final amino acid that is incorporated into the peptide backbone during PI biosynthesis. It has been shown that Phg-like residues are important for the structure and bioactivity of various antibiotics [**2**]. By the incorporation of different Phg derivatives we aim to generate novel PI variants with better antibacterial and/or resistance-breaking properties. For mutasynthesis studies a double mutant was generated, in which the L-Phg-biosynthesis gene *pglA*, as well as the PII-specific gene *snaE1* [**3**] are deleted. HPLC-MS analysis of double mutant culture extracts showed that pristinamycin biosynthesis is abolished completely, while feeding with L-Phg restores PI biosynthesis solely. HPLC-MS/MS analysis also showed, that PI-like production was restored after feeding with multiple Phg derivatives as well, suggesting the formation of PI congeners. For congeners that showed bioactivity against test strains, larger amounts are still extracted and purified for structure confirmation by NMR and further examination of antimicrobial properties against, amongst others, pristinamycin resistant strains.

[**1**] Mast et. al (2014)

[**2**] Al Toma et. al (2015)

[**3**] Mast et. al (2011)

ST035

Double use of *Alternaria alternata* polyketide synthase A (PksA) for melanin and perylenequinone (PQ) biosynthesis

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The fungus *Alternaria alternata* is a wide-spread food contaminant and a weak plant pathogen. It is known to produce large numbers of different secondary metabolites, many of which are recognized as relevant toxins in food and feed, such as the polyketide alternariol[1]. Another important polyketide is the black pigment DHN melanin. Three genes involved in DHN-melanin biosynthesis are clustered in *A. alternata*[2], namely the polyketide synthase gene *pksA*, the 1,3,8-trihydroxynaphthalene (THN) reductase gene *brm2* and the transcription factor gene *cmrA*. Other genes required for DHN-melanin biosynthesis are scattered in the genome. *A. alternata* is also known as a main producer of mycotoxins of the perylenequinones (PQs) family, such as altertoxin I, II, III (ATX I-III) and some other derivatives. Among them, ATX II is reported as a very powerful mutagen and DNA strand-breaking agent in cultured mammalian cells, exhibiting at least 50-fold higher mutagenicity than AOH[3]. The PQs biosynthesis gene cluster is still unknown.
In this study, we constructed a large number of mutant strains using the CRISPR/Cas9 technology and show that the DHN-melanin and the PQs biosynthesis pathway share most of the biosynthetic pathway. Whereas melanin is formed in aerial hyphae and spores, PQs are produced in substrate hyphae. We found that AT4HN and T4HN (1,3,6,8-tetrahydroxynaphthalen) are likely synthesized by PksA. T4HN is probably the substrate for PQs biosynthesis in substrate hyphae, while the biosynthesis of melanin in arial hyphae and spores uses AT4HN and T4HN. We identified 1,8-DHN as the branch point between melanin and PQ biosynthesis. The transcription factor CmrA strictly controls the biosynthesis of PQs, but not the formation of melanin. The biosynthesis of PQs and melanin is an example how spatial regulation of certain steps of a biosynthetic pathway increases the complexity of secondary metabolites.

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ST036

[S,S]-EDDS: Insights into its biosynthesis and its role in mediating bacterial interactions

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Actinomycetes are filamentous soil bacteria that produce not only antibiotics, but also a variety of other secondary metabolites. For optimal growth microbes need to acquire essential metal ions from the environment, in particular iron and zinc. However, in many habitats, the availability of zinc and iron is limited due to low solubility. In order to respond to this nutrient deficiency, microbes synthesize chelating compounds to sequester metal ions from the environment. In addition to their function in provision of metal ions, ionophores are also known to mediate signalling among strains in the microbial community, playing an important role in the ecology of microorganisms. An example of a bacterial ionophore is the zincophore [*S,S*]-ethylene diamine-disuccinate (EDDS), which is produced by *Amycolatopsis japonicum*, under zinc-limited growth conditions. EDDS is a biodegradable EDTA isomer. Its biodegradability and excellent chelating properties make EDDS also attractive for many industrial applications.

In this project, we first aimed to analyze the ecological role of EDDS in a soil community. For this purpose, actinomycetes were isolated from soil samples and used for pairwise interactions with the EDDS producer. The influence of EDDS on e.g. growth on different plates was monitored. HPLC, HPLC/MS and metabolomic analysis were employed to investigate whether EDDS affects the production of secondary metabolites in the isolated strains.

Since EDDS is a suitable alternative to EDTA, our second goal is to establish an industrial bioprocess for the production of EDDS. This is only achievable if the individual steps of the biosynthesis have been elucidated. To date, it is known that the EDDS biosynthetic genes are regulated by the zinc uptake regulator Zur, and that L-aspartic acid and O-phospho-L-serine serve as precursors. In this study, we provided the first evidence that L-ornithine is also a precursor for EDDS biosynthesis by using NMR and HPLC-MS analyses combined with feeding experiments.

Altogether, this work will contribute on the one side to better understanding of the natural role of bacterial zincophores. On the other side, knowledge of biosynthesis will allow us to increase production rates further, bringing us one step closer to replacing the environmentally harmful EDTA with EDDS in many industrial applications.

ST037

Identification, biosynthesis and ecological role of secondary metabolites produced by Actinobacteria associated with fungus growing termites

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Introduction: *Macrotermitinae* cultivate a mutualistic food fungus for nourishment in so called "fungus gardens". Symbiotic bacteria presumably defend the nutrient-rich fungus garden by secretion of antimicrobials, which prevent growth of the insects" microbial competitors and

pathogens[1]. Bioassay-guided metabolomics analyses of gut-associated actinomycetes led to the characterization of novel natural products with promising biological activities.

Objectives: We combined genomic and metabolomic techniques to explore the biosynthetic potential of termite-associated Actinobacteria and to assess their role as defensive symbionts.

Methods: Bacteria from the termite gut and fungus garden were isolated and their bioactivities were assessed in co-cultivation studies against fungal competitors and entomopathogenic fungi. Bacterial strains with antifungal activity were chemically analyzed and the produced secondary metabolites structurally characterized. Genome mining in combination with heterologous expression and knock-out studies revealed their biosynthetic potential.

Results: Comparative genome studies of actinobacterial species in combination with co-culture studies uncovered their important contributions to sustain a healthy fungus garden for nourishment. In frame of these studies, the novel species *Actinomadura rubterolonii* produced the structurally intriguing PKS-derived maduralactomycins and actinospirols[2]. Genome mining, feeding and gene expression studies uncovered their putative biosynthetic pathway that encoded unique enzymes catalyzing oxidative rearrangement reactions.

Conclusion: Isolated actinobacterial species are important contributors sustaining a healthy termite colony. The unique environment harbors a reservoir of new microbial species that produce natural products with unique chemical scaffolds, which contribute as second layer of defense against invading species.

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ST038

Back to soil: Awakening the production of cryptic antibiotics in *Streptomyces*

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Introduction:

Most of the antibiotic biosynthetic gene clusters (BGCs) in *Streptomyces* are not expressed under laboratory conditions, however these clusters are maintained in the genomes of these strains, therefore indicating that they must play important roles in adaptation and survival within their ecological niches. These cryptic pathways represent an untapped resource in terms of new metabolites and novel chemistry that could be very useful in the clinic if we can awaken their expression and production under large scale production conditions.

Objectives:

Understanding the global regulation patterns that affect transcription of antibiotic BGCs in these strains under soil conditions versus laboratory conditions allows us to identify novel routes for up- or down- regulation in order to trigger expression of these BGCs and therefore production of these antibiotics under controlled laboratory and fermentation conditions.

Methodology

We are using RNA sequencing to analyse global transcriptional patterns of *S. venezuelae* and *S. rimosus* grown in sterile soil, non-sterile soil and standard laboratory solid medium. We also plan to use an unbiased metabolomics approach to identify compounds produced in soils.

Results:

We have identified a number of BGCs that are upregulated in both soil conditions compared to standard rich medium, some of which have unknown products supporting the hypothesis that understanding them in their natural habitat will help discovery of new natural products. Of particular interest are those clusters that are triggered in non-sterile soil alone as these may be a result from the interaction with the microbial community present in the soil.

Conclusion:

This knowledge derived from the RNAseq will enable us to generate genetically modified strains engineered to increase the yield of both well characterised and cryptic clusters which encode potentially novel compounds under industrial fermentation conditions. The transcriptional data may also help shed light on the complex regulation of the life cycle of these *Streptomyces* strains in an ecologically relevant environment. Furthermore, the non-sterile soil condition in particular, will allow planning specific interaction studies between *Streptomyces* and a natural soil community.

ST039

A synthetic carboxylation module to improve photosynthesis

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Key words: Photorespiration, carboxylation module, CO_2 fixation

Photosynthetic organisms play a crucial role in the global carbon cycle as they convert CO_2 into organic compounds that in turn are used as feedstock by heterotrophic organisms. However, the efficiency of photosynthesis via the Calvin-Benson cycle is diminished by the inability of Rubisco to reliably distinguish between O_2 and CO_2. Photorespiration that is required to recycle the toxic product of Rubico"s oxygenation side reaction involves the undesired re-release of carbon.

Our new-to-nature carboxylation module circumvents the loss of carbon and fixes additional CO_2, converting photorespiration into a carbon-positive pathway that

increases the carbon efficiency by up to 150 % while reducing its theoretical energy demand. It starts with glycolate, the dephosphorylated product of the oxygenation reaction and involves in total three enzymes that activate, carboxylate and reduce the substrate yielding glycerate that is channeled back into the Calvin-Benson cycle. The key enzyme, a non-natural glycolyl-CoA carboxylase (GCC), was engineered by combining rational design, high-throughput microfluidics and microplate screens. We improved its catalytic efficiency by three orders of magnitude to match the properties of natural CO_2-fixing enzymes. We also obtained a 1.96 Å cryo-EM structure for GCC that confirmed our active-site design. For tartronyl-CoA reductase, the enzyme that catalyzes the subsequent reaction in the carboxylation module, we obtained a 3.4 Å cryo-EM structure that provides novel insights into its architecture. We demonstrated that besides bypassing natural photorespiration, the module is also applicable as a general glycolate assimilation pathway during ethylene glycol degradation and as an extension for the artificial CO_2-fixing CETCH cycle. This showcases how expanding the solution space of natural metabolism provides new opportunities for biotechnology and agriculture.

ST040

Synthetic Co-Culture for the Production of Bioplastics from Light and CO2: Deciphering the Interplay of *Synechococcus elongatus* and *Pseudomonas putida*

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In the last century, axenic cultures have been the state of the art for microbiological studies and biomanufacturing due to simpler safety control. This view on the microbial world does not reflect the actual lifestyle of microorganisms nor does it consider the complex interaction and communication in inter-phylum consortia. In consequence, the next step in biotechnology is to employ well-defined synthetic co-cultures, where the metabolic labor is partitioned between the partners to make a specific bioproduction performance either possible or improve it.

In times of climate change, at best synthetic co-cultures are designed in a way to ensure sustainable production of biotechnological products. Along that line, we have set up a highly robust synthetic co-culture consisting of the cyanobacterium *Synechococcus elongatus cscB* PCC7942, which converts CO2 to sucrose and exports it into the culture supernatant and a derivative of *Pseudomonas putida* KT2440, which turns the sucrose, in the intracellular biopolymer polyhydroxyalkanoate (PHA, bioplastic) [1] & [2]. To this end, *P. putida* was modified to expand its substrate spectrum towards sucrose. This was achieved by the genomic introduction of the *cscRABY* gene cluster from *Pseudomonas protegens Pf5,* encoding the regulator *CscR,* the invertase *CscA,* the transporter *CscB* and the por*in CscY [3].* As PHA accumulates in conditions of carbon overflow and nitrogen limitation, a special focus was set on nitrogen availability (C/N-ratio). A Design-Build-Test-Learn (DBTL) driven approach with cycles of genetic and operational modifications was used for identifying a setup that allows us to selectively provide nitrogen for either strain to optimize PHA production and increase co-culture controllability (44 mg day-1 L-1 & 449 mg L-1). Furthermore, we investigated in more detail potential mutual interactions of the bacterial species by applying transcriptomics, as we observed enhanced cyanobacterial growth in the presence of the heterotrophic partner in comparison to axenic cultures.

Our results present a robust co-culture for the sustainable production of a biotechnological product from renewable feedstock on the example of PHA production from light and CO2. With the information gained on the process itself and on the interspecies interaction, we aim to refine the synthetic co-culture and expand its product portfolio.

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[2] DOI: 10.1128/AEM.07901-11

[3] DOI: 10.1111/1751-7915.13283

ST041

Lights on and action – Optogenetic on- and off-switches for light-induced control of microbial processes

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In the field of synthetic biology, both a stringent and straightforward orchestration as well as a simple and robust visualization of biological processes are essential. Light seems to be especially suited for these tasks, as it represents a non-invasive, precise, and exactly tunable external stimulus. Here, we report on the establishment of optochemical and optogenetic switches that can be used to control various microbiological processes at different cellular levels.

On the transcriptional level, novel variants of the light-responsive inducer molecule photocaged IPTG were successfully applied for controlling gene expression in several expression hosts, including *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis* [1] *and Rhodobacter capsulatus*. These photocaged inducer variants either differ in their water-solubility or exhibit bathochromatically shifted absorption maxima. Additionally, photocaged benzoate derivatives were evaluated for light-mediated gene expression in *E. coli* [2] and *P. putida*. Further, metabolic pathways, such as the biosynthesis of plant terpenes, were established in *R. capsulatus* [3] and optogenetic modulation of precursor accumulation could subsequently be demonstrated for the carotenoid biosynthesis. To implement light control at the post-translational and cellular level, fluorescent proteins were characterized for their suitability as genetically encoded photosensitizers [4]. It could be demonstrated that some fluorescent proteins generate high amounts of different reactive oxygen species thereby allowing to control enzyme activity and cell viability of various Gram-positive and Gram-negative bacteria as well as cancer cells.

Conclusively, versatile light-responsive tools for controlling biological functions on various cellular levels were established in *E. coli* and successfully utilized in alternative expression hosts such as *P. putida* or *B. subtilis.* These results thus clearly demonstrate a broad applicability of both the novel photocaged inducers and the genetically encoded photosensitizers as versatile optogenetic control systems for biotechnological and biomedical applications.

[1] Hogenkamp *et al.* 2020, ChemBioChem. 2021;22: 539–547.

[2] Hogenkamp *et al.* 2021, ChemBioChem. 2021, accepted.

[3] Hilgers *et al.* 2021, Microorganisms. 2021;9(1):168.

[4] Hilgers et al. 2019, Int J Mol Sci. 2019;20(18): 4608.

ST042

Unraveling the Physiological Response of *Escherichia coli* Under High ATP Demand

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A major task of the central metabolism of a cell is to provide energy in form of ATP. Perturbation experiments that manipulate the ATP pool can be of high value to determine the maximal capacity and to uncover possibly unknown regulatory mechanisms of the central metabolism.

In this study [1], we analyzed systematically the impact of different levels of uncoupled ATPase activity (and thus of ATP demand) in *Escherichia coli* under aerobic as well as anaerobic conditions, both with and without cell growth. As one key finding, in all conditions tested, we observed that the glucose uptake rate shows a biphasic response curve with respect to increasing ATPase activity, reaching a maximum value at a medium ATPase level, but dropping markedly when this level is exceeded.

Focusing on anaerobic growth, we used metabolomic and proteomic data in combination with a kinetic model of *E. coli*"s central metabolism for an in-depth analysis of the different strains and to reveal the underlying mechanism of this behavior. The model indicates that the dual dependency of the phosphofructokinase (PFK) on ATP as substrate and ADP as activator is likely the source of the biphasic response curve of the glycolytic flux. The PFK mechanism can buffer increased ATP demands by a higher glycolytic flux but, as shown herein, it collapses under high ATP demands with low ATP concentrations. Model analysis also led to hypotheses explaining the unexpected accumulation of intracellular glycolytic metabolites and it predicted two major rate-controlling steps of the glycolytic flux in *E. coli* under high ATP demand. We overexpressed the genes of the associated metabolic enzymes (*pfkA* and *pflB*) and a significant increase in glucose uptake could indeed be confirmed.

Our work provides new insights on the functioning of glycolysis under extreme (ATP-demanding) conditions and is of great value for rational engineering of highly productive cell factories.

[1] S. Boecker, G. Slaviero, T. Schramm, W. Szymanski, R. Steuer, H. Link, S. Klamt, Deciphering the Physiological Response, **2021**, *submitted*.

ST043

A Natural Language Processing tool for information extraction of microbial phenotypes from unstructured text

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The BacDive database (https://bacdive.dsmz.de/) is worldwide the largest database for structured, manually curated information about bacterial and archaeal strains and their taxonomy, morphology, physiology, origin, molecular data, as well as their cultivation conditions. In general, the large-scale integration of microbial biodiversity information has been hindered due to heterogeneity and fragmentation of data sources along with limited documentation, variable standards, low interoperability of data, and lack of specific computational approaches. Today Natural Language Processing (NLP) and Deep Learning (DL) based tools can efficiently extract information from text corpora. With the aim to mobilize information from microbiological literature and integrate them into BacDive, we have developed a combination of rule-based and DL-based models coupled with classical NLP approaches of dependency tree parsing and Part-Of-Speech (POS) tagging for entity recognition and relationship extraction based on the comprehensive manually curated information from BacDive. The results from a rule-based method of information extraction provide relevant input to develop unsupervised methods of Information Extraction (IE) using word embeddings and other deep learning methods.. We work towards a fully automated system of extracting information from unstructured text and making it robust by incorporating feedback loops from experts during the development.

ST044

Development of a modular T7 RNA polymerase based genetic platform for the efficient production of heterologous proteins in *Pseudomonas putida*

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The T7 RNA polymerase has proven itself to be one of the most powerful tools for heterologous gene expression in the golden-standard biotechnological host *Escherichia coli*. The exploitation of this tool in other prospective biotechnological hosts however is still very scarce. To this intent, a modular T7 RNA polymerase-based system for stable heterologous protein production in *Pseudomonas putida* was established and characterized. In this system, the T7 RNA polymerase gene was introduced into the genome of the host whilst the gene of interest was located downstream of the T7 promotor on a plasmid. The model protein eGFP was employed as an easy quantifiable reporter, facilitating *in vivo* tracking of the systems behaviour during microbial cultivations. The initial genetic set-up of the system suffered from slow growth and low protein production rates. After experimental confirmation that the limitations associated with our system were transcriptional problems rather than translation problems, the influence of the terminator sequence downstream of the eGFP gene on the transcription process was evaluated. Upon performing *in vitro* transcription assays, it became apparent that the T7-phage inherent TΦ terminator displayed low termination efficiency and caused read-trough transcription of the T7 RNA polymerase. By replacing the T7-phage inherent TΦ terminator downstream of the heterologous gene with the synthetic tZ terminator, growth and protein production rates improved drastically, enabling the T7 RNA polymerase system to reach a productivity level comparable to that of an intrinsic RNA polymerase based system. Additionally, a T7 RNA polymerase ribosome

binding site library was constructed to tune heterologous protein production by varying the amount of T7 RNA polymerase. Experiments with this library led to the identification of a T7 RNA polymerase saturation in the system. Therefore, the choice of ribosome binding site in front of the T7 RNA polymerase gene is a key factor in the optimization of the system, and it should be chosen in a way that the system operates at the verge of saturation, enabling maximum eGFP production rate and growth, without wasting cellular resources on surplus T7 RNA polymerase production.

ST045

Controlled interkingdom communication: crosstalk between bacteria *Bacillus subtilis* and the eukaryote *Saccharomyces cerevisiae* by utilizing bacterial quorum sensing peptides

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Quorum sensing is substantial for cell differentiation within a bacterial population and is mediated by small self-produced secreted peptides. In Gram-positive *Bacillus subtilis* W168, one of these quorum sensing peptides is CSF (competence and sporulation factor), encoded by the gene *phrC*. CSF indirectly regulates the activity of the transcriptional regulator ComA and thereby the expression of ComA-dependent genes responsible for e.g. secondary metabolites, natural competence development or sporulation.

In this study, we used CSF to establish a controlled interkingdom communication between yeast and *B. subtilis*. For this purpose, we have engineered *B. subtilis* to serve as a reporter strain, which responds to the CSF heterologously produced by the yeast *Saccharomyces cerevisiae*.

The reporter strain was constructed by fusing the ComA-dependent *srfAA* promoter with the *luxABCDE* cassette as a bioluminescence reporter for promoter activity. The generated fragment was then integrated into the genome of *B. subtilis*. Properties of different reporter strains were tested through a multi-mode microplate reader assay by measuring OD_{600} and bioluminescence in chemically defined MNGE media and complex LB media.

First results of promoter activity measurements in combination with CSF-producing yeast culture supernatant or synthetic CSF showed a specific response of the reporter strain to both sources of CSF. Then, systematic mutagenesis of the genes that regulate the activity of ComA in the reporter strain resulted in an increased sensitivity of the promoter and thereby higher activity to the heterologously produced or synthetic CSF.

Altogether, a quorum sensing-driven interkingdom crosstalk between bacteria and yeast was successfully generated. Currently, we are exploring other signal peptides in Phr family of signal peptides to establish more reliable interkingdom crosstalk. In future, we hope to fine-tune this communication in bacteria-yeast co-cultures and use it for processes like controlled protein expression and establishing biological sensor-actor systems.

ST046

Modulating bacterial data models – how databases can improve research

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In a time where high throughput techniques reign supreme, not only the amount of data but also the complexity has been increasing significantly. With multiple dimensions of data being generated, it is of upmost importance to catalogue them in an ordered manner and have proper ways of displaying them to the community. A way to address this is by creating specialized databases. More than a way to catalogue information for a certain organism or strain, databases allow users to have access to top quality data curation and their perfect integration with an interactive visualization. We have developed an extensive framework for the annotation of prokaryotic genomes that stores current information on genes, proteins, protein-protein interactions, expression data, metabolic pathways, regulatory elements, protein homology and annotated literature. Beyond putting all information together, we integrate it with each gene or protein's biological context, giving every scientist complete power over the organism's state of the art, providing with the necessary tools to postulate new hypotheses. As an example, we take a guided tour through our database dedicated to the model microorganism *Bacillus subtilis*, *Subti*Wiki (https://www.subtiwiki.uni-goettingen.de/)[1] and through *Syn*Wiki (http://synwiki.uni-goettingen.de/)[2], a database for the first synthetic microorganism with a minimal genome, JCVI-syn3A.

[1] - Pedreira, T, Elfmann, C, Stülke, J. The current state of *Subti*Wiki, the database for the model organism Bacillus subtilis. *Nucleic Acids Research*, 2021; gkab943. https://doi.org/10.1093/nar/gkab943

[2] - Pedreira, T, Elfmann, C, Singh, N, Stülke, J. *Syn*Wiki: Functional annotation of the first artificial organism Mycoplasma mycoides JCVI-syn3A. *Protein Science*, 2021; 1–9. https://doi.org/10.1002/pro.4179

ST047

Ecology impacts the decrease of Spirochaetes and *Prevotella* in the fecal gut microbiota of urban humans

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Question

Compared to the huge microbial diversity in most mammals, human gut microbiomes have lost diversity while becoming specialized for animal-based diets – especially compared to chimps, their genetically closest ancestors. The lowered microbial diversity within the gut of westernized populations has also been associated with different kinds of chronic inflammatory diseases in humans. To further deepen our

knowledge on phylogenetic and ecologic impacts on human health and fitness, we established the herein presented biobank of stool samples from diverse mammalian orders as well as its comprehensive microbiota analysis.

Methods

In total, 368 stool samples from 38 different animal species, including Homo sapiens, belonging to four diverse mammalian orders were collected at seven different locations. Microbiota composition was determined by 16S rRNA gene amplicon sequencing. Subsequently, comprehensive data analysis was performed to not only determine the overall impact of host phylogeny vs. diet, location, and ecology, but also to examine the general pattern of fecal bacterial diversity across captive mammals and humans.

Results

By using a controlled study design with captive mammals we could verify that host phylogeny is the most dominant driver of mammalian stool microbiota composition. However, the effect of ecology appears to be able to overcome host phylogeny and should therefore be studied in more detail in future studies. Most importantly, our study could observe a remarkable decrease of Spirochaetes and *Prevotella* in urban humans and platyrrhines, which is probably not only due to diet, but also to the social behavior of these communities.

Conclusions

Our study highlights the importance of phylogenetic relationship and ecology within the evolution of mammals stool microbiota composition. Particularly, the observed decrease of Spirochaetes and *Prevotella* in urban communities might be associated to lifestyle dependent rapid evolutionary changes, potentially involved in the establishment of dysbiotic microbiomes promoting the etiology of chronic diseases.

ST048

News from the cyanosphere – Metagenomic insights into low complexity microbiomes

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Introduction: High throughput sequencing and metagenome analyses paved the way for the current view on the tree of life. Microbiome studies provided fascinating insights into the microbial composition of various habitats ranging from the deep sea to the human gut. Non-axenic cyanobacteria, which were formerly regarded as the "grubby urchins" of microbial collections, are now considered as valuable resources. They represent connecting links between the environment and pure isolates.

Objectives: (1) Solving technical challenges in the generation of metagenome-assembled genomes [MAGs] and their reliable taxonomic classification. (2) Metagenome sequencing of non-axenic cyanobacteria in order to fill the gaps in the cyanobacterial tree of life and to reveal the composition of the cyanosphere. (3) Characterization of metagenomes from two enrichment cultures of predatory bacteria that are actively killing *Anabaena*.

Methods: DNA extraction, Illumina short-read/PacBio long-read sequencing, binning/assembly with novel pipelines and taxonomic assessment.

Results: (1) High quality DNA from fastidious cyanobacteria was extracted with a classical CTAB protocol in combination with a hydroxyapatite purification step. A novel text mining approach was developed for a rapid taxonomic assessment of the MAGs. (2) Illumina sequencing and binning of three non-axenic limnic cyanobacteria allowed us to establish high quality genomes of the phototrophs and provided comprehensive insights into the complex composition of the cyanosphere. *Stigonema ocellatum* DSM 106950 with a genome size of 10.3 MB is the first sequenced representative of the family *Stigonemataceae*. More than two dozen associated heterotrophic bacteria and one amoeba were also discovered in the *Stigonema* culture, which reflects the presence of a food chain that is stably maintained since five decades. (3) The predator project was conducted in cooperation with the group of Karl Forchhammer. Illumina sequencing revealed nearly complete MAGs of both predators representing two closely related species in a new family of *Bacteroidetes*. PacBio sequencing from one enrichment culture resulted in complete circular genomes of the *Anabaena*-killer and nine associated heterotrophic bacteria.

Conclusion: Our metagenome analyses provided first insights into the complexity of the cyanosphere and showed that non-axenic cyanobacteria as well as enrichment cultures are treasure troves of hidden microbial diversity.

ST049

Impact of Quantitiy and Quality of Dietary Fibre on the Gut Microbiome in Mouse Studies

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Introduction: Dietary fibre (DF) is an essential part of the diet both in humans and rodents. The intestinal microbiota (IM) utilizes DF as substrate and degrades the large polysaccharides to short chain fatty acids and other end products which serve as important energy sources for intestinal epithelial cells and offer a health benefit to the host. Rodent diets in experimental studies therefore include DF, but mostly insoluble DF like cellulose, which cannot be fermented by the IM. This can have huge effects on the gut physiology, e.g. lead to reduced cecum volume and colon length, potentially affecting physiology of the animals and the study outcome. We therefore evaluated the impact of different quantities and qualities of DF on the fecal IM composition in the mouse model. **Methods:** 120 male C57BL/6(J) mice (aged 4 weeks) were divided in 5 groups receiving one of 5 diets ad libitum: SC (standard chow = control group), AG (AIN-93G purified rodent diet), A30, A50, A70 (AIN-derived purified diets with increased DF content and a ratio of soluble:insoluble DF 30:70, 50:50, 70:30, respectively). The IM composition was determined from fecal samples at week 0 and 5, and colon and cecum contents at week 12. Variable region V4 of the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq platform. Sequence data were analysed using dada2, phyloseq and ALDEx2, and classified taxonomically using Silva v132. **Results:** Fecal IM composition shifted between week 1 and week 5 leading to significant differences between the dietary groups, which were also seen in cecum and colon samples at week 12. The AIN93G diet (AG) resulted in lower abundances of Muribaculaceae and *Bacteroides* in comparison to SC, while in contrast *Bifidobacterium* and

Faecalibaculum were higher. The three groups A30, A50 and A70 displayed an intermediate composition between AG and SC showing a gradual difference related to the DF composition. Most notably, the abundances of *Akkermansia* and Prevotellaceae group NK3B31 increased with the fraction of soluble fibre, while Lachnospiraceae group NK4A136 decreased. **Conclusion:** Both quantity and quality of DF in diets used in the mouse study modulate the composition of the fecal IM. A purified diet with increased amounts of soluble DF results in a microbiota composition that closer reflects the microbiota of mice on a standard chow diet, with a range of biological effects on host physiology.

ST050

Introducing the National Research Data Infrastructure for the Research of Microbiota (NFDI4Microbiota)

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Microbes have a strong influence on every aspect of human life including health, agriculture, biotechnology, biogeochemical cycles and climate. Due to this, various research is performed to study microbial species such as bacteria, archaea, unicellular eukaryotes and viruses. NFDI4Microbiota (https://nfdi4microbiota.de/) has started its activity in October 2021 together with several other NFDI (National Research Data Infrastructure) consortia. It consists of 10 well established partner institutions and more than 50 participating institutions and networks. Its goal is to serve the German microbiology research community through dedicated training and facilitating the generation, management, interpretation, sharing, and reuse of microbiological data. NFDI4Microbiota will achieve this by different community activities as well as by creating a cloud-based system that will make the storage, integration and analysis of microbial data, especially omics data (including (meta-)genomics, (meta-)transcriptomics, (meta-)proteomics and (meta-)metabolomics), consistent, reproducible, and accessible across all areas of life sciences. NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable and Re-usable) principles and Open Science with all its facets.

In this workshop we would like to present the planned working program as well as give the change for further discussion and exchange.

ST051

An increase in surface hydrophobicity formed by *N*-chlorinated residues mediates chaperone activity of RidA from *Escherichia coli*

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Under physiological conditions, *Escherichia coli* RidA is an enamine/imine deaminase, which promotes ammonia release from reactive enamine/imine intermediates of the PLP-dependent threonine dehydratase (IlvA). However, RidA, when modified by HOCl ($RidA_{HOCl}$), turns into a potent chaperone holdase that can effectively protect the cytosolic proteome during oxidative stress caused by neutrophil phagocytosis. In a previous study using mass spectrometry of the undigested protein, we determined that up to ten amino acid residues are modified in fully active $RidA_{HOCl}$. However, it remained unclear, which particular residues (from a total of eight lysines and five arginines) need to be chlorinated for RidA chaperone function. We performed a combination of direct LC-MS/MS analysis of N-chlorinated residues, a novel chemoproteomic approach to stably modify N-chlorinated lysines, and a mutagenesis study to probe the role of the individual arginine and lysine residues in the activation of RidA"s chaperone function. Chlorination of arginine R51 could be confirmed by direct LC-MS/MS evidence. Other *N*-chlorinated residues could not be detected, most likely due to the instability of the modification and its interference with trypsin cleavage (trypsin cleaves peptides on the C-terminal side of lysine and arginine). Therefore, we established a chemoproteomic approach using 5-(Dimethylamino) naphthalene-1-sulfinic acid as a probe to detect *N*-chlorinated lysines. Using this probe, we were able to identify *N*-chlorination of six additional lysine residues. In a mutagenesis study, we identified two arginines, R105 and R128, whose lack is concomitant with significantly reduced chaperone activity upon HOCl-treatment, but no synergistic effect was observed in an R105_R128 double mutant. Overall, our results indicate that the chaperone activity of RidA is not mediated by one or two modified amino acids acting as a switch, but N-chlorination leads to an overall loss of positive charges on the protein surface, leading to increased overall protein hydrophobicity. Molecular modelling of $RidA_{HOCl}$ and the rational design of a RidA variant that shows chaperone activity even in the absence of HOCl further supports our hypothesis. Our data provides us with a molecular mechanism of HOCl-mediated chaperone activation in RidA and other HOCl-activated chaperones.

ST052

Super-resolution microscopy to decipher the dynamic subcellular localization of pneumococcal proteins during an immunological challenge

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Introduction: *Streptococcus pneumoniae* is a gram-positive human pathogen that colonizes the upper respiratory tract and the causal agent of community-acquired pneumonia and also the human kidney disease Hemolytic Uremic Syndrome (HUS). On its surface, *S. pneumoniae* harbors several proteins protruding to the extracellular space with diverse functions (transport channels, iron receptors, immune evasion, etc). A group of these surface proteins is the so-called Choline-binding protein (CBP) group. CBPs have a choline-binding domain (CBD) which is responsible for the anchoring of the protein to the cell-wall (via phosphorylcholine moieties).

Objectives: In this work we focus on three CBPs, the hydrolase LytB and the immune evasion proteins PspA and PspC and their subcellular localization under different conditions at the single-cell super-resolution level.

Material&Methods: The subcellular localization of the three CBPs was deciphered by performing super-resolution microscopy, namely Structured Illumination Microscopy (SIM) and Stimulated Emission Depletion (STED) microscopy coupled with batch processing image analysis.

Results: LytB preferably localizes at the septal region, in contrast PspC and PspA rather avoid the mid-cell point and appear on the surrounding areas and poles of the cell. However, during an immunological challenge, PspA surprisingly presents a septal instead of a polar localization. The septal localization was not homogenous among all the single individuals, in fact a certain degree of heterogeneity was observed. Moreover, the 3D-spatial distribution on the cell-wall of the target proteins was characterized by developing modelling systems that determine the correlation between signal intensity, position, and neighborhood, corroborating that the subcellular localization of PspA, PspC and LytB is not random and strong intensity signals are observed in the septum (LytB) or surrounding areas (PspA and PspC). Finally, the composition, structure and localization of CBPs varies among clinical isolated *S. pneumoniae* strains from young patients with the kidney disease HUS. These results argue for a complex dynamic remodeling of the cell-wall architecture when *S. pneumoniae* is in contact with the human host.

Conclusion: Our study provides an understanding of the structural function of these pneumococcal virulence proteins, how they follow the dynamics of the cell-wall and open doors to the selection of new vaccination targets.

ST053

Deciphering the role of host-derived extracellular vesicles in the fight against *Aspergillus fumigatus*

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Invasive Aspergillosis is a life-threatening disease caused by the fungus *Aspergillus fumigatus*. Every day we inhale hundreds of fungal spores, which easily reach the lung alveoli due to their small size (2-3 µm). Depending on the immune status of the host they can be cleared by immune cells or grow out as hyphae, destroying the lung tissue and spreading to the vascular system. Despite epithelial cells and macrophages belong to the first line of defense against *Aspergillus* spores, the intervention by polymorphonuclear granulocytes (PMNs), or neutrophils, is decisive for full clearance of the pathogen. Neutrophils can neutralize the conidia via phagocytosis, degranulation and production of neutrophils extracellular traps (NETs). More recently a new mechanism to counteract pathogens was discovered: the production of antifungal extracellular vesicles (afEVs)1,2. These small vesicles can reach the fungus and limit its growth by delivering antifungal cargo. We are interested in eliciting the mechanism by which the EVs can damage the fungus and on how specific the EV response is to particular pathogens. To serve this aim we are now combining the work on primary neutrophils, isolated from donors, with work on a neutrophil cell line called PLB-985. Our studies suggest that these cells can be used as a model for aspergillosis since they behave in many ways like primary neutrophils when confronted with *A. fumigatus*. A better understanding of the function of these antifungal EVs will help in the treatment or diagnosis of aspergillosis.

1 Shopova *et al*. 2020, *mBio*

2 Brakhage *et al.* 2021, *mLife*

ST054

Role of nuclear targeted effectors of Brassicaceae smut fungus *Thecaphora thlaspeos* in modulation of host transcription

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Thecaphora thlaspeos rises as a novel infection system of *Brassicaceae*, specifically infecting *Arabis* species and the model plant *A. thaliana. T. thlaspeos* establishes a long-lasting biotrophic interaction with its host plants without causing any macroscopic symptoms. It has conserved and unique effectors repertoire like other biotrophic smut fungi which is recently identified from the genome analysis.

My project deals with the characterization of nuclear localized effectors of *T. thlaspeos*. Among the 40 effector candidates (Courville et al, 2019) I have identified 7 effectors with a predicted nuclear localization sequence (NLS) and verified them in heterologous expression systems. From this list, our prime candidate Tue1 has their signal present only in the nucleus, while two additional candidates, Tue10 and Tae2, accumulate in both nucleus and nucleolus. Three candidates have dual localization both in the nucleus and cytoplasm, while the 7th effector candidate is found in the nucleus and chloroplast. To focus, effectors with dual localization were excluded for further analysis. Next, the deletion of NLS in Tue1 leads to impaired nuclear localization and the signal was only accumulated in the cytoplasm, which indicate the specificity of NLS function in Tue1. Interestingly, Tue10 and Tae2 NLS mutants show exclusion of the signal only from the nucleolus. Furthermore, virulence activity of these 3 candidates were checked in a bacterial heterologous expression system and they have a significant effect on bacterial proliferation pointing towards interference with plant transcription during infection. Stable expression of GFP-tagged Tue1 in planta leads to

morphological phenotypes including dwarf rosette and induction of late flowering. These developmental phenotypes might suggest that Tue1 could interfere with plant regulatory mechanisms in order to promote pathogen virulence.

Transcriptome analysis of Tue1-overexpressing lines show alter gene expression with 93 up-regulated and 12 down-regulated genes. Notably, prediction of transcription factor binding sites in differentially regulated genes and DNA binding residues in Tue1 protein sequence lead to the hypothesis that Tue1 might acts as a transcription factor. Further analysis of DNA binding activity and protein-protein interaction for Tue1 are in progress via ChiPSeq and Yeast-Two Hybrid respectively. These data sets might assist in studying the interference of NLS effectors with plant immune responses.

ST055

RNA-Seq based transcriptomic profiling to understand off-target effects of antisense antibiotics

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Conventional antibiotics generally work against a broad spectrum of bacterial pathogens. This promotes the development of antibiotic resistance and damages our protective microbiota, which can have unwanted effects on our health. New antibiotics are therefore needed that can directly target individual pathogens, leaving beneficial bacteria unharmed.

Peptide nucleic acids are RNA-like molecules that bind to messenger RNA through complementary base pairing and thereby inhibit the production of proteins. When applied to essential genes, they can serve as sequence specific antibiotics killing species of interest. This approach has already been confirmed to be effective in preclinical studies, but there are many open questions, for instance about the rules for programming such antibiotics, mechanisms of resistance development, and possible toxicity to host cells and non-targeted members of the microbiome. Importantly, the short length of PNAs, in the range of 10-13 bases, could lead to off-target effects in untargeted regions of the mRNA or affect non-targeted species in communities. It is crucial to be aware of these off-target binding sites when designing ASO sequences.

To understand the effect of mismatches on target-binding activity, we took a scanning mutagenesis approach to a model PNA targeting an essential gene. We designed PNAs containing mismatches to the target sequence of an essential gene in *Salmonella* to see how the PNAs efficiency is affected. We then performed MIC assays and RNA-Seq to quantify growth inhibition and the downregulation of target genes, respectively. We found that PNAs with up to 3 mismatches are still able to both inhibit growth and significantly downregulate targeted genes if the mismatches occur at the end of the sequence, suggesting new rules for evaluating off-target effects of PNAs.

Our study highlights the importance of considering off-target potential when designing PNAs, and provides experimental methodology with which to evaluate them. We incorporated these results into MASON (**M**ake **A**nti**S**ense **O**ligos **N**ow). MASON is a web-based tool which facilitates the design of ASO sequences for any bacterial gene of interest. MASON calculates the melting temperature of PNA-RNA interaction and predicts possible off-targets within the targeted species and in other species of the microbiome. We hope it will become an evolving resource for the bacterial antisense community.

ST056

Presence of human pathogens of the *Borrelia burgdorferi* sensu lato complex shifts the sequence read abundances of tick microbiomes in two German locations

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The distribution of human Lyme borreliosis is assumed random in Germany, indicating that the human pathogenic species of the *Borrelia burgdorferi* sensu lato complex (Bb) are similarly distributed as part of the tick microbiome. The aim of this study was to differentiate if the presence of Bb occurs with a defined tick microbiome composition. Furthermore, the effect of location on tick microbiome composition was addressed for two German locations. Therefore, nucleic acid extracts from 82 *Borrelia*-positive and 118 *Borrelia*-negative *Ixodes ricinus* ticks sampled from human hosts in both districts were selected. Nucleic acid extracts were used for human pathogenic Bb species diagnostics based on qPCR and multilocus sequence typing and bacterial 16S rRNA gene amplicon sequencing followed by network analyses. As a result, the presence of Bb shifted the sequence read abundances of *Candidatus* Midichloria, *Rickettsia*, *Pseudomonas*, *Staphylococcus*, and *Candidatus* Neoehrlichia and their topological roles in the tick microbiome. Moreover, the location was less important in the tick microbiome composition but shifted significantly sequence read abundances of *Pseudomonas* and *Wolbachia* as well as the topological role of microbial members. Since the presence of human pathogenic Bb species with other tick-associated pathogens varies regionally, we suggest that a bacterial 16S rRNA gene-based microbiome survey should be implemented in the routine diagnostics for both tick and host if human pathogenic species of Bb were detected. This diagnostic extension will help to optimize therapeutic approaches against Bb infection and co-occurring pathogens.

ST057

Fungal Fight: Biotic Interactions based on the Biosynthesis of Secondary Metabolites associated with *Neonectria ditissima.*

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The wound parasitic ascomycete *Neonectria ditissima* is a causal agent of tree canker and fruit rot. Plant diseases, which lead to impaired growth and crop losses in fruit cultivation. Beside the main host *Malus*, a wide range of hardwood plants are affected. To date, there is no effective method to control the disease and little is known about virulence factors. In this study we are interested in secondary metabolites as determinants of the biotic interaction between the pathogen *N. ditissima* and fungal endophytes within the plant environment. The chemical communication between

host and pathogen may give hints for plant protection strategies. Our research is based on the antagonistic and mutualistic balance of the pathogen *N. ditissima* and the endophytic consortia in different host trees.

Bioactivities of crude extracts resulting from pathogen submerse cultivations under different conditions were tested in an apple infection assay and against the vegetative growth to other fungi. Various meroterpenoids, based on an ilicicoline-like structure, such as p-hydroxybenzoic acid, were detected. In addition, the corresponding gene cluster responsible for the ilicicoline biosynthesis was bioinformatically identified and validated via quantitative pcr.

A broad variety of endophytes were isolated from healthy fruit tree plants and the organisms were tested for antagonistic activity against the pathogen. Several secondary metabolites were identified from submerse cultures of various endophytic organisms with an inhibiting effect against *N. ditissima*. We were able to elucidate the structure from a still unknown compound belonging to the class of resorcinosides, which was produced by a undefined endophytic candidate. We will now further characterize the specific activity against ascomycetes, the cellular target and biosynthesis of the new natural product. This offers an future opportunity to develop a system treating the apple canker disease.

ST058

Unconventional suppression of plant defence responses by the signal peptide peptidase Spp1 in the *Ustilago maydis* - maize interaction

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Secreted effector proteins are central components for communication between biotrophic fungi and their host plants. In the biotrophic fungal plant pathogen *Ustilago maydis,* the plant-specific activation of the unfolded protein response (UPR) generates an optimised intracellular infrastructure, crucial for efficient processing and secretion of effectors. To gain insight into how individual UPR regulated factors contribute to fungal virulence, we used a combined RNAseq/ChIPseq approach to identify direct UPR targets. Screening of more than 40 deletion strains for altered ER stress resistance and virulence function identified the signal peptide peptidase Spp1 as an essential virulence factor. SPPs are ER-membrane localised aspartic proteases, cleaving type II oriented transmembrane domains, including remnant signal peptides that were previously processed by the signal peptidase complex. Deletion of *spp1* results in a massive induction of plant defence responses upon infection of its host plant maize, suggesting an important role of Spp1 in fungal plant communication. Spp1 is dispensable for vegetative growth, filament formation and ER stress resistance. Importantly, the essential virulence function requires the conserved catalytic activity but can neither be attributed to known physiological roles of SPPs, such as ER-associated degradation (ERAD) or hypoxia adaptation, nor to the altered secretion of effectors. This suggests that suppression of plant defence responses by Spp1 likely involves a previously unknown mechanism of fungal plant communication, which is currently further explored. To this end, we are utilising advanced proteomics approaches for the identification of potential Spp1 substrates and combine dual RNAseq with plant metabolomics for the detailed analysis of plant defence responses.

ST059

cross-kingdom RNAi in the interaction of smut fungi with model plants

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Smut fungi are an important group of biotrophic pathogens from the Basidiomycetes that undergo an intimate interaction with their host plant during infection. In particular, the corn smut fungus *Ustilago maydis* has been studied for years. It is genetically tractable and therefore it is an excellent model organism for fungal and infection biology. We recently established the Brassicaceae smut fungus *Thecaphora thlaspeos* as a novel tool to investigate smut fungal infection in model plants. It uses a distinct set of effector proteins compared to the grass smut fungi to modify its host plant. Notably, in contrast to *U. maydis*, *T. thlaspeos* contains the RNAi machinery, so that in addition to effector proteins cross-kingdom RNAi can determine the outcome of infection. We now aim at (1) compiling an inventory of fungal sRNAs from *T. thlaspeos* and the fungal or plant target genes, which would be the first inventory for smut fungi, (2) confirm the relevance of cross-kingdom RNAi for smut fungal infection, since so far only the effector proteins are functionally characterized, and (3) investigate the delivery of sRNAs from *T. thlaspeos* to its host plants via extracellularvesicles (EVs), which could be compared to effector protein translocation. So far, initial sRNA sequencing gives insights into potential plant pathways that the fungus targets during infection. Currently, we are confirming the role during infection. Understanding the role of cross-kingdom RNAi for fungal pathogens will open the avenue for novel plant protection strategies.

ST060

Identification of biosynthetic pathways of volatile organic compounds in the mushroom *Cyclocybe aegerita*

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The edible fungus *Cyclocybe aegerita* (syn. *Agrocybe aegerita*) of the division Basidiomycota is one of the most valuable cultivated mushrooms. It is commonly known as Samthaube, pioppino or black poplar mushroom. Because of its ability to form fruiting bodies under laboratory conditions [1], its enzymatic portfolio [2] and its versatile production of aroma compounds [3], this fungus serves as a basidiomycetous model system to study different aspects of mushroom biology. Due to the diverse set of volatile organic compounds (VOCs), which apparently are involved in intra- and interspecific communication, our group employs *C. aegerita* to elucidate the respective biosynthetic pathways.

The identification of enzymes responsible for VOC formation is achieved via multi-*omics* combining metabolomic, genomic and transcriptomic data. To reveal the biosynthesis of volatile oxylipins and terpenoids, the whole set of VOCs, also known as volatilome, was analyzed at different developmental stages of *C. aegerita* by means of gas chromatography-mass spectrometry (GC-MS). In parallel, gene expression levels at corresponding time points were examined using RNA sequencing. Furthermore, metabolomic data was obtained at

different stages of fungal development through biotransformation experiments using labeled pre-cursors.

By combining these metabolome and transcriptome data with genome mining, several genes with expression patterns matching the time dependent production of VOCs were recognized. With this information, biosynthetic pathways were deduced and gene clusters putatively responsible for the production of terpenoids or single genes coding for oxylipin modifying enzymes were identified.

Finally, the *omics*-deduced biosynthetic pathways were partly confirmed by biochemical characterization of various enzymes such as lipoxygenases, ene-reductases and sesquiterpene synthases. This proves that our approach for studying biosynthetic pathways for VOCs in higher fungi of the phyla Basidiomycota is encouraging.

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ST061

Multicellularity in fruiting body development of the mushroom *Coprinospsis cinerea*

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The dung fungus *Coprinopsis cinerea* is an excellent model mushroom for studying complex fungal developmental processes. It completes its life cycle very fast within two weeks and can be easily cultivated on an artificial media under laboratory conditions. Fruiting development begins on the dikaryon with monocentric growth of vegetative hyphae to form a primary hyphal knot by massive formation of short hyphal sidebranches. The primary hyphal knot transforms into a more compact light-induced aggregate, the multicellular secondary hyphal knot. From there, tissue differentiation starts to produce a primordium that continues over 5 days to finally develop into a mature mushroom. Fruiting bodies fully open their umbrellas in the last night phase of development to shed spores. However, at the following morning the cap autolyses to release the majority of the spores in liquid droplets that fall to the ground.

In this study, the complex fungal multicellularity in tissue formation and cell differentiation in the various stages of primordia is cytologically analyzed using histochemical techniques over the whole period of development. In *C. cinerea*, there are probably > 30 cell types that differentiate in sequence during the stages of development. The developmental process of fruiting body formation is a dynamic process in which different cells and tissues communicate with each other, together with various environmental signals, in order to coordinate the formation of the complete multicellular structure. Failure of communication within cell and tissue formation together with lack of environmental signals lead to malformation of the fruiting bodies, e.g. to short stipes or overlong stipes and underdeveloped cap structures. Communication between tissues and cells begins already in the initial phase of the formation of secondary hyphal knots, as soon as a light signal is received. Further differentiation in cell formation takes place in a coordinated manner, both in and between the cap and stipe regions. For instance, rapid elongation of the stipe must be timely coordinated with the opening of the cap for it to uplift for efficient spore dispersal. Communication between cap and stipe along with necessary nutrient transfer (glycogen) is mediated here by a special type of inter-connective tissue, called the lipsanoblema, a thin layer of stipe tissue connecting to the inner edges of the primary gills.

ST062

CLR-3 as a key factor in the cross-talk between cellulose and hemicellulose signaling in *Neurospora crassa*

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Fungi have developed elaborate systems to perceive the complex lignocellulosic substrates they grow on. Several interconnected signaling pathways are involved, which we could demonstrate to bear the potential for inhibitory crosstalk. Although this crosstalk is species-specific, the inhibitory effect between cellulose and mannan signaling was found to be conserved between the reference system *Neurospora crassa* and industrially relevant species, including *Trichoderma reesei* and *Thermothelomyces thermophilus* (formerly: *Myceliophthora thermophila*). Our current objective is to elucidate the underlying molecular mechanisms of this crosstalk between cellulose and hemicellulose perception. Published data revealed that the intracellular balance between cellulose- and mannan-derived oligomers is essential for the activation of cellulase expression mediated by the transcription factor CLR-1. Since CLR-1 itself is repressed by the transcriptional regulator CLR-3, we hypothesized that CLR-3 could be the receptor for either cellulose- or mannan-derived signaling molecules and binds to CLR-1 directly in the absence of cellulose. After heterologous expression and purification of CLR-3, isothermal titration calorimetry confirmed that CLR-3 can bind cellobiose (a cellulose-derived disaccharide) and mannobiose (the corresponding molecule from mannan), but with differing kinetics/affinity. In yeast two-hybrid assays, we could furthermore demonstrate that the directly interaction of CLR-1 and CLR-3 can be blocked by addition of cellobiose to the media. Moreover, deletion of *clr-3* in the background of a mannobiose-overaccumulating mutant (Δ*gh2-1*) with strongly impaired ability to grow on cellulose, restored growth on media with cellulose, even exceeding wild-type levels. Our data strongly support the hypothesis that CLR-3 is a key factor involved in the repressive crosstalk between cellulose and mannan perception. Accumulating mannan-derived oligomers might prevent cellulose-derived signaling molecules from binding to CLR-3, rendering it unable to dissociate from CLR-1 for its activation. These insights improve our understanding of the signaling pathways involved in the perception of complex lignocellulosic substrates and could lead to new strategies of rational engineering of filamentous fungi for uninhibited cellulase production.

ST063

Crop host signatures are constituted by co-association patterns of keystone bacteria in the rhizosphere microbiota

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The rhizosphere microbiota is a key functional trait of plant hosts in regard to nutrient acquisition and stability against abiotic and biotic stresses. The rhizosphere microbiota assembly is dynamic over plant growth stages and is impacted by soil parameters. Hence, it is difficult to identify those microbial species from thousands that are most important to establish beneficial symbiotic networks with its plant host. Modulating the rhizosphere microbiota assembly of crop plant hosts is nonetheless awaited to improve sustainability of agricultural plant production.

We resolved the metabolically active bacterial microbiota (SSU RNA) of the rhizosphere of four major crop species *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), *Secale cereale* (rye), *Brassica napus* L. (oilseed rape) grown widely in Europe, and investigated those growth stages at which plants rely most on root nutrient uptake from soil. We hypothesized that an individual crop host species comprises bacterial taxa as part of its core microbiota specific for each crop species, (b) that keystone bacteria occur among the core microbiota of each host and (c) that these keystone bacteria orchestrate differential associations as host-specific signatures.

A glasshouse experiment was conducted with summer cultivars grown in pots (n=8) with the same agricultural soil. The rhizosphere was sampled at booting and flowering. The active bacterial microbiota were examined using metabarcoding of the bacterial 16S rRNA. We identified keystone bacteria by combining LEfSe biomarker and network analyses.

Keystone bacteria were *Massilia* (barley, wheat) and unclassified *Chloroflexi* of group "KD4-96" (oilseed rape). Host plant-specific interconnections were uncovered for specific genera, e.g. *Tepidisphaeraceae* "WD2101 soil group" by the determination of differential associations between networks of each crop species core microbiota. Subnetworks of the keystone bacteria and their differential associations reflected the host species effect on the rhizosphere microbiota assembly.

These host-specific signatures were not evident from abundance differences of single bacterial taxa enriched or unique to a specific plant host. We concluded that co-occurrence patterns within the rhizosphere microbiota emerged in the observed crop host-specific microbiota. These co-occurrence patterns reflect the host species effect and not the specific identity of single bacterial taxa.

ST064

Microplastic-polymer specific modulation of the soil microbial community: Selection of a plastisphere microbiome

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Background: Microplastic (MP) particles < 5 mm have a high surface area and are ubiquitously detected in aquatic and terrestrial habitats. The huge surface area of MP represents an important, yet often overlooked, artificial habitat for microbes. Despite their importance, mechanisms of MP colonization and succession are largely unknown as well as potential effects on functional potentials of the plastisphere microbiomes. Thus, we addressed the hypothesis that MP polymer type is a major deterministic factor impacting MP community assembly, succession and biofilm formation on MP in a landfill habitat.

Methods: Five different MP types were incubated in mesh-bags buried in a dumping site for 5, 9 and 14 months. Recovered MP was analyzed using scanning electron microscopy and confocal laser scanning microscopy to evaluate surface changes and biofilm formation. Sequencing of 16S rRNA gene amplicons provided information about the bacterial and archaeal composition of the plastisphere microbiome and indicative prokaryotic groups depending on the polymer type. Marker genes for the N and CH4 cycle were quantified to elucidate changes in biogeochemical relevant gene potentials.

Results: The bacterial phyla *Chloroflexi* and *Gammaproteobacteria* explained differences among MP polymers and time points. Differences in community structure of polyethylene, polypropylene and polyamide MP to the soil community increased with time, while poly styrene and polyethylene terephthalate showed the highest distance after 5 months of incubation. *In silico* predictions of functional potentials and quantitative PCR of functional marker genes suggested that MP polymers exerted an effect on metabolic potentials related to greenhouse gas turnover, i.e. on methane and nitrous oxide metabolism.

Conclusion: Collectively the data indicates that (i) MP is quickly colonized by soil microbes leading to extensive multi-species biofilm formation, (ii) polymer type as deterministic factor rather than stochastic factor determines plastisphere community assembly as well as succession *in situ*, (iii) MP impacts greenhouse gas metabolism, and (iv) MP serves as an ideal model system to study fundamental questions in microbial ecology in terrestrial environments.

ST065

Microbe-substrate interactions following simulated microbial inoculation to thawed yedoma permafrost in anaerobic environments

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The relative roles of ancient versus modern microbial communities in yedoma permafrost carbon decomposition and subsequent greenhouse gas (GHG) production are poorly understood. We anaerobically incubated sediments collected from a 12-m yedoma profile in Interior Alaska to examine: (1) interaction between thawed substrate and microbial community composition (16S RNA) and function (metagenomics); (2) how mixing modern CH4-producing communities with microbial communities present in frozen permafrost affects community composition and function following thaw; and (3) subsequent effects on CO2 versus CH4 production. Inoculation with modern CH4-producing communities from surface sediment collected from an adjacent thermokarst lake (*Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales*) altered both microbial community development and organic matter utilization. For most depths, the inoculation increased CH4 (7.6 - 390x) and CO2 (1.0 - 2.7x) production and decreased CO2:CH4 ratios (36 – 99 % decrease) compared to controls. Combined data from our metagenomic functional pathway and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) analyses show this increase in

anaerobic GHG production following inoculation is the result of enhanced intermediate organic matter (OM) degradation (carbohydrate-active enzyme classes) breaking down more recalcitrant OM classes (carbohydrate-like compounds and lignin-like compounds) compared to the controls. Yedoma sediments with the highest initial substrate potentials (high relative abundance of aliphatic- and peptide-like compounds) that had not thawed since their formation experienced the strongest effects from inoculation, supporting previous suggestions that GHG production in thawed yedoma is microbially-limited. Interestingly, however, metagenomic data showed differing effects to the same inoculate at the two yedoma depths. Changes in microbial community composition (R2 = 0.90 and 0.51 for CO2 and CH4, respectively) and organic matter characterization (R2 = 0.68 and 0.33) provided better fits for estimating anaerobic GHG production potentials than initial microbial community composition (R2 = 0.32 and 0.41) and organic matter characterization (R2 = 0.49 and 0.29). This suggests predicting the evolution of microbial communities following thaw in conjunction with substrate potential will yield more accurate estimates of GHG production potentials compared to characterizing initial communities alone.

ST066

Microbial necromass in soil organic matter - implications for soil microbial ecology

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Microorganisms are known to be essential for soil organic matter (SOM) formation as catalysts, but their matter contribution was rarely analysed. They transform plant litter as a carbon and energy source for biomass formation. After cell death, necromass partially enters microbial feeding cycles, and partially is stabilised in SOM. A meta-analysis of data on microbial biomarkers, e.g. amino sugars (AS) and phospholipid fatty acids, under various land uses showed that the amount of microbial necromass in SOM based on AS concentrations ranged from 62% at grassland sites to 56% at arable land and 33% at forest sites (Liang et al, 2019). Presumably, these data would be much higher, if AS concentrations are steady states of formation and turnover, and shows a significantly higher contribution of necromass to SOM than previously considered. This has important implications for understanding SOM stabilisation processes and soil functions which will be reviewed here. Well-known SOM stabilisation mechanisms are organo-mineral interactions, in particular encapsulation and physical separation. The chemical structures of microbial necromass materials provide inherent features for additional stabilisation processes, such as aggregations of biomolecules, incrustation by metals and carbonates. In particular, proteins show a high meta-persistency in soils presumably due to post-mortem conformational changes and the spatial arrangement of microbial necromass material. These materials cover mineral particles, modifying their surface properties and water storage capacities. However,

Carbon retention in soils critically depends on substrate availability, energy flux, maximum growth and carbon use efficiency of the organisms within the microbiomes and environmental boundary conditions. In addition, soil microorganisms presumably optimize their energy fluxes and thus recycle biomass building blocks avoiding energy expenditure for biomass synthesis. This adds a resource function to SOM and may explain N cycling in soil microbial communities while wasting C. However, microorganisms then need the capability to overcome sequestration and stabilisation mechanisms.

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ST067

Analysis of microbial populations in plastic–soil systems after exposure to high poly(butylene succinate-co-adipate) load using high-resolution molecular technique

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Bio-based and biodegradable plastics are considered as plastics of the future owing to their ability to decompose under various environmental conditions. However, their effects on the soil microbiome are poorly characterised. In this study, we aimed to investigate the effects of an important bio-based and biodegradable plastic, polybutylene succinate-co-adipate (PBSA), on soil microbial diversity and community composition using high-resolution molecular technique (Illumina sequencing) targeting all three microbial domains: archaea, bacteria, and fungi. Adding high load of PBSA to soil (6% (w/w)) caused a significant decline in archaeal (13%) and fungal (45%) richness and substantial changes in both bacterial (Proteobacteria, Actinobacteria, and Acidobacteria) and fungal (Eurotiomycetes, Sordariomycetes, Leotiomycetes, and Dothideomycetes) community composition compared with no PBSA addition to soil. The combined effects of PBSA and $(NH_4)_2SO_4$ fertilisation on the soil microbiome were much greater than the effects of PBSA alone. We only detected opportunistic human pathogens in low abundance on PBSA and in the surrounding soil. However, some plant pathogenic fungi were detected and/or enriched on the PBSA films and in surrounding soil. Apart from plant pathogens, many potential microbial control agents and plant growthpromoting microorganisms were also detected/enriched owing to PBSA addition. Adding high load of PBSA together with $(NH_4)_2SO_4$ fertilisation can either eliminate some plant pathogens or enrich specific pathogens, especially *Fusarium solani*, which is economically important. We conclude that high load of bio-based and biodegradable PBSA plastic may negatively affect soil microbiome.

ST068

Soil properties influence the emission of the microbial volatile sodorifen of *Serratia plymuthica*

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Microbial volatile organic compounds (mVOCs) are products of primary or secondary metabolism. Currently more than 2000 mVOCs emitted by about 1000 microbial species are known. mVOCs are lipophilic, low molecular weight, and low vapor pressure compounds, hence they can easily diffuse over short and long distances in the air and in soil spaces. Therefore, mVOCs may function as infochemicals e.g. microbe-microbe, microbe-plant, microbe-nematode interactions. Under laboratory conditions, it is proven that mVOC profiles are affected by microbe interactions and

nutrient availability, among other factors. However, no clear information exists concerning the influence of soil properties on mVOC emissions. *Serratia plymuthica* 4Rx13 is a Gram negative proteobacteria which was isolated from the rhizosphere of *Brassica napus.* Its volatile profile is dominated by the unique compound sodorifen, a polymethylated, bicyclic sesquiterpene of C16H26. It is known that the emission quantities of sodorifen vary upon the presence of microbial interactors, are inverse depending on glucose supply, and increase towards the stationary growth phase. As soils are composed of various particles (e.g. loam, sand, clay), litter as well as various metals, in a first set of experiments we investigated the influence of various salts/metals commonly found in the soil on sodorifen emission of *Serratia plymuthica* 4Rx13.

Serratia plymuthica 4Rx13 was cultured in minimal medium (DMM) supplemented with succinate and various salts of different concentrations. The growth (48 hours) of the bacteria was monitored with a microplate reader or followed in Erlenmeyer flasks.

Serratia plymuthica 4Rx13 achieved 50% growth at 500 mM sodium chloride (NaCl), while at 200 mM NaCl the growth was slightly reduced (OD600 and CFU) but a sharp reduction in sodorifen emission was observed. At 50 mM magnesium chloride growth was not altered but sodorifen emission decreased significantly. On the contrary, calcium chloride application had no significant effect on sodorifen emission. Additional salts/metals will be studied in the future to understand how mVOCs emission and mVOC profiles (exemplified by sodorifen) are altered in bacteria growing in the soil and rhizosphere.

ST069

A closer look into cellular organization, physiological limits, and genomic features of cold-tolerant *Nitrotoga* strains

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Introduction and Objectives: Nitrite oxidizing bacteria (NOB) of the genus *Nitrotoga,* which was first enriched from permafrost soil, are widely distributed and important in cold nitrogen removal systems. Due to the scarcity of cultivated representatives, however, data like species-specific physiological features, ultrastructural and biochemical information, as well as inter-ecosystem genomic comparisons are limited. Here, we present these kind of data of *Nitrotoga* strains from various habitats

Methods: In total seven *Nitrotoga* strains were used for physiological experiments and ultrastructural and immunocytochemical analyses using transmission electron microscopy. Metagenomes of four strains were assembled and used for pangenomic comparison and phylogenetic analyses.

Results: Novel strains were enriched from permafrost soil (frozen since ~3 ka), a subsurface artificial tunnel system (ca. 500 m depth) and one from a marine aquaculture system, which is the first cultivated marine *Nitrotoga*. In physiological tests, the *Nitrotoga* cultures showed differing tolerance limits towards dissolved inorganic nitrogen concentrations and confirmed the generally psychrotolerant nature of the genus, with temperature optima between 17 and 22 °C. All non-marine strains were sensitive towards elevated salt concentrations. Phylogenetic analysis based on the concatenated alignment of 92 single copy core genes revealed at least three main groups within *Nitrotoga*. Except for the marine strain that clusters together with a metagenome-assembled genome from a submarine oil seep, the phylogenetic analysis did not infer any clear patterns based on shared habitat type. Electron micrographs of the different cultures showed the typical irregularly shaped periplasmic space and loose cell aggregates. Intriguingly, we observed cells sharing a common periplasm forming multicellular complexes. Immunogold labelling of monoclonal antibodies showed that the key enzyme complex of *Nitrotoga* is detached from the cytoplasmic membrane and soluble, as genomic information have predicted. Overall, these data largely broaden our knowledge about the phylogenetic spectrum, morphological and physiological features of this group of nitrite-oxidizers.

ST071

Endophytic actinobacteria as a biological tool to improve phytoremediation of heavy metals contaminated soils in the United Arab Emirates

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Phytoremediation is a promising alternative to rehabilitate polluted sites but due to poor biomass and slow growth, the metal-reclamation potential is often reduced. Conversely, the interactions of plant-microbe-metals have attracted much attention because of its biotechnological potential to remove heavy metals from polluted ecosystem. Due to wider physiological properties, endophytic and soil actinobacteria can act as bio-remediators. In an effort to gain the advantages provided by 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, in the phytoremediation of heavy metals from the environment, the ability of actinobacteria with ACC deaminase activity to promote the growth of Rhodes grass in soils contaminated with heavy metals was evaluated under greenhouse conditions. To achieve this, 28 different endophytic isolates obtained were selected and tested for their ability to produce ACC deaminase, plant growth regulators (PGRs) as well as to tolerate different concentrations of heavy metals. Only five isolates were able to produce ACC deaminase and PGRs. Under greenhouse conditions, three out of the five isolates were recovered from inside the roots at all samplings, up to eight weeks after inoculation. The application of a mixture of these three endophytic actinobacteria either as endophytes or as soil drench increased Rhodes grass growth in contaminated soils compared to the control treatments. Inoculations with the mixture of the three actinobacteria either as endophytes or as soil drench in the contaminated soils significantly enhanced heavy metals uptake by Rhodes grass from heavy metals contaminated soils compared to control treatments. Applications of the same three isolates either as endophytes or as soil drench also resulted in the reduction of the endogenous levels of ACC in both roots and shoots and was correlated with increased of endogenous PGRs *in planta*. Plant growth promotion and phytoremediation of heavy metals of Rhodes grass were most pronounced in the presence of endophytic actinobacteria compared to soil drench actinobacteria. Our results suggest that plant growth promoting endophytic actinobacteria containing ACC deaminase offer promise as bacterial inoculum for improvement of plant growth and phytoremediation of heavy metals particularly under unfavorable environmental conditions such as heavy metals contamination in soils.

ST072

Molecular multitasking – Aminoglycoside antibiotics protect bacteria from phage infection

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Bacterial populations face the constant threat of viral predation exerted by bacteriophages (or phages). In response, bacteria have developed multiple lines of defense that can collectively referred to as the bacterial "immune system". The currently known antiphage defense mechanisms are mainly mediated by protein complexes and act primarily at the unicellular level[1]. Nevertheless, there is a variety of phage defense systems providing protection on a community level. One of these multicellular defense mechanisms relies on the secretion of small molecules as chemical defense[2].

In this study, we are focusing our efforts on the antiviral activity of small molecules produced by *Streptomyces*, which is well-known for its complex secondary metabolism and its huge repertoire of bioactive molecules. In particular, we demonstrate that aminoglycosides, a major class of antibiotics produced by *Streptomyces*, are potent inhibitors of phage infection in diverse bacterial species. Using qPCR, RNA-sequencing and fluorescence microscopy, we could show that inhibition of phage infection most likely occurs early on, between phage DNA injection and replication. Interestingly, the antiviral effect of the pure aminoglycoside apramycin could be reproduced using supernatants from the natural producer *Streptomyces tenebrarius*, suggesting a potential role of these compounds in chemical defense against phage infection on a community level. Moreover, we showed that in vitro acetylation of apramycin decouples the antibacterial and antiviral properties of the compound by showing no more effect on bacterial growth, but still a complete inhibition of *Streptomyces* phage infection[3].

Altogether, these findings highlight the multifunctionality of this class of antibiotics, as they are not only used by their producers as a powerful weapon against bacterial competitors, but also protect them against phage predation at the community level.

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ST073

Interrogating the Thoeris novel anti-phage defense system in its native bacterial host.

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Bacteria and phages have been locked in a perpetual arms race, driving the evolution of a diverse arsenal of prokaryotic immune systems and viral countermeasures. Recent advances have expanded this arsenal to include a wide variety of previously unknown and functional diverse set of bacterial anti-phage defense systems. One of those systems, called Thoeris, is characterized by an NADase domain of its effector protein ThsA. Additionally, it encompasses one or more ThsB proteins containing Toll/interleukin-1 receptor (TIR) or TIR-like domains, which are also associated with eukaryotic innate immunity. It has recently been hypothesized to function as an abortive infection system, inducing NAD depletion leading to cell death. We investigate a Theoris system encoded in *Escherichia coli* purported to belong to a distinct subtype based on domain architecture. This *E. coli* strain carries six different defence systems in total, allowing us to investigate the role of Thoeris as part of the whole anti-phage arsenal. We identified Thoeris-mediated protection against different phages and found differences between Thoeris in its native context and when heterologously expressed in a different *E. coli* strain. By applying a cell-free transcription-translation (TXTL) system, we investigated the effector domain architecture and infection induced NADase activity. The observed NADase kinetics do not support abortive infection through NAD depletion, further promoting the hypothesis of a distinct subtype. Our goal is to investigate the role of Thoeris as part of the anti-viral repertoire in its native host, the interplay with other existing defense mechanisms, as well as modes of escape deployed by phages to circumvent the system. By investigating the system in its native host, we hope to broaden the understanding of antiviral immunity beyond any single defense system.

ST074

Harnessing synthetic sRNAs to unravel phage resistance mechanisms

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Vibrio cholerae is a major human pathogen causing the life-threatening diarrheal disease, cholera. Bacteriophages (or short phages) are viruses that infect bacteria and are tightly linked to various aspects of *V. cholerae*'s lifestyle. For example, the interplay of *V. cholerae* with phage CTXφ resulted in the development of toxigenic *V. cholerae* strains, whereas other, mostly lytic, phages quench cholera outbreaks in endemic settings. One of these lytic phages, vibriophage N4 (*Podoviridae* family), is able to infect and lyse various *V. cholerae* variants, however, the underlying molecular mechanisms are currently unknown.

In this project, we aim to identify and characterize host cell factors that allow N4 infection of *V. cholerae* as well as possible defense mechanisms that inhibit this process. To this end, we employed a recently developed library of synthetic sRNA variants that we harnessed to screen for phage-resistant *V. cholerae* variants. Indeed, using high-throughput sequencing, we discovered hundreds of sRNAs providing phage-resistance and follow up experiments showed that resistance can either block phage adsorption or interfere with downstream processes that occur following the injection of phage DNA.

Detailed characterization of the interactions of the most abundant sRNA variants with its respective target transcripts revealed a role of the *V. cholerae's* extracellular polysaccharides (EPS) in the phage adsorption process. Additionally, the resistance provided from other abundant sRNA variants implied the involvement of potential antiphage defense systems. Thus, our data indicate that synthetic sRNAs could be exploited to characterize phage-host interactions and identify phage resistance mechanisms.

ST075

Interaction between haloarchaeal virus HFTV1 and its *Haloferax* host

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Like all domains of life, archaea are susceptible to viral infections. Archaeal viruses are characterised by high diversity and complex morphologies. However, relatively little is known about their infection strategies, due to the lack of genetically accessible virus-host models. Archaeal viruses face different challenges during viral entry and egress, compared to their bacterial counterparts, as the archaeal cell envelopes and their appendages fundamentally differ from those of bacteria. For only a few archaeal viruses, the entry and egress mechanisms have been studied, so far. To investigate the main principles underlying infection strategies of euryarchaeal viruses, we use the recently isolated Haloferax tailed virus 1 (HFTV1) and its host *Haloferax gibbonsii* LR2-5 as a model system.

To study the interaction between *Hfx. gibbonsii* LR2-5 and HFTV1, we sequenced and analysed the host genome and determined optimal conditions for host growth, motility and virus production. We further investigated host cell characteristics like the surface layer, filamentous surface structures, and cell shape that might play a role in its virus susceptibility. Examinations with transmission electron microscopy showed that *Hfx.* LR2-5 exhibit pili and archaella, which might act as initial attachment sites for viruses (Tittes *et al.*, 2021; Tittes, Schwarzer and Quax, 2021).

HFTV1 is one of the first known virus infecting a *Haloferax* strain and was isolated together with its host from saline Lake Retba (Senegal) in 2011. HFTV1 has a double-stranded DNA genome and an icosahedral capsid with a long non-contractile tail (siphovirus morphotype) (Mizuno *et al.*, 2019). The characterization of the halophilic virus isolate HFTV1 showed a lytic life cycle hampering the host cell growth. Analysis of its reproduction showed that the release of progeny virions resulting from cell lysis occurs after ~6 hours. Remarkably, HFTV1 virions adsorb to the host cell surface much faster than other haloarchaeal viruses. We characterized the details of the adsorption process and examined the impact of viral infection on the host cell with a combination of light and electron microscopy. This experimental analysis of the viral life cycle enables a detailed characterization of molecular mechanisms underlying infection strategies of the relatively unexplored haloarchaeal viruses. This information is important to understand the diversity and evolution of virus infection strategies in general.

ST076

Prevalence, traits and fate of phage-plasmids

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Mobile genetic elements are pivotal drivers of bacterial evolution, with phages and plasmids as the two main classes. Despite their diversity, phages and plasmids are typically clearly distinguished. Temperate phages tend to integrate the chromosome whereas plasmids are extrachromosomal, autonomously replicating DNA elements. Phage-plasmids, however, share features of both groups, blurring these definitions.

We developed a method using comparative genomics and machine learning to identify phage-plasmids among phages and plasmids. This method revealed an unexpected number and diversity of phage-plasmids, raising the possibility that they are key drivers of bacterial adaptation by horizontal gene transfer. As phages and plasmids tend to carry different types of accessory genes, we wondered if phage-plasmids could carry both, thereby being a melting pot of phage and plasmid-related accessory genes for bacterial adaptation. This would be in agreement with our observations that phage-plasmids have variable frequencies of phage and plasmid genes. The careful study of the presence of virulence factors and antibiotic resistance genes, accompanied by experimental verifications, does suggest that phage-plasmids are important drivers of bacterial adaptation.

ST077

Viruses' potential roles in carbon and nitrogen cycling during benzene degradation under nitrate-reducing conditions

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The role of viruses in the anaerobic degradation of hydrocarbon has been long overlooked by microbiologists.

In this study, we monitored the succession of viruses and prokaryotes in a microcosm experiment during different phases of benzene mineralization under nitrate-reducing conditions in coarse sand containing mixed culture enriched from a benzene-contaminated aquifer. Nitrate-dependent benzene mineralization was monitored by the addition of ^{13}C-labelled benzene and subsequent analysis of generated $^{13}CO_2$. We collected solid (sand) and liquid samples at five-time points (three biological replicates per time point) for DNA extraction and shot-gun whole-genome sequencing. A total of 24 metagenomes (Illumina, 2x125 bp, 25 Mio reads) were analyzed.

Using MetaWrap we recovered almost 2000 prokaryotic metagenome-assembled genomes (MAGs). These MAGs were separated into 193 prokaryotic Operational Taxonomic Units (pOTUs) based on average nucleotide identity with a 0.95 cutoff (a proxy for species level). Taxonomic assignment by GTDB-tk revealed Gammaproteobacteria, Ignavibacteria, and an unclassified class of Zixibacteria as the most dominant phyla.

VirSorter, VirFinder, and VIBRANT were used to recover a total of 605 complete and 365 high-quality Uncultivated Viral Genomes (UViGs) as defined by CheckV. After dereplication (Stampede-ClusterGenomes), we defined a total of 222 viral Operational Taxonomic Units (vOTUs). Taxonomic analysis with vConTACT2 suggested that all vOTUs belong to novel viral groups. Analysis using WisH indicated that 72 pOTUs (37.35%) were infected with phages ($p < 0.05$).

Functional annotation of the 222 vOTUs identified 91 genes encoding 13 proteins relevant to benzene mineralization coupled to nitrate reduction. Regarding carbon cycling, genes related to anaerobic degradation of benzoyl-CoA, and CO_2 fixation using the Wood-Ljungdahl pathway were found

in our vOTUs. Similarly, genes related to nitrate reduction to nitrite, nitrite reduction to N_2, and nitrite reduction to ammonium indicate the viruses' potential contribution to the nitrogen cycle. Preliminary analysis using PHACTS suggested that 160 vOTUs (72.1%) have a lytic life cycle, indicating a potential role of viruses in carbon and nitrogen cycling.

Our data demonstrate the potential relevance of viruses in anaerobic benzene degradation and open new doors for the study of viruses in anaerobic ecosystems contaminated with hydrocarbons.

ST078

PspA Adopts an ESCRT-III-like Fold and Remodels Bacterial Membranes

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Introduction

The phage shock protein A (PspA) is a member of the PspA/IM30(Vipp1) protein family. The PspA/IM30(Vipp1) family is wide spread among the bacterial phyla and has also been identified in chloroplasts as well as in some archaea, where it is involved in maintaining the integrity of cellular membranes. A hallmark of this protein family is the formation of MDa-sized homooligomeric ring and rod structures. However, the molecular structure and function of this protein family is mostly unknown.

Objectives

In this study we aimed to resolve the structure of PspA rod structures at near atomic resolution and to elucidate the interaction of PspA and bacterial membranes in an in vitro system.

Methods

Therefore, we used a cryo-EM SPA approach to solve the structure of PspA and cryo-ET to visualize how PspA remodels membranes.

Results

Here, we present the 3.6 Å resolution cryo-EM structure of PspA assembled in helical rods, which reveals that PspA adopts a canonical ESCRT-III fold. Using cryo-EM and cryo-ET, we show that PspA interacts with small unilamellar bacterial membrane vesicles by fusion and fission events, resulting in µm-sized vesicles with distorted shapes and internalized membranes. These activities are mediated by PspA rods engulfing lipid bilayers and amorphous PspA assemblies acting as lipid transfer zones.

Conclusion

As similar structures and membrane remodeling activities are well-established for ESCRT-III proteins, we suggest that bacterial PspA belongs to the evolutionary ancestry of ESCRT-III proteins.

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ST079

Multiscale characterization of the nitrite oxidoreductase (NXR) of anaerobic ammonium-oxidizing bacteria

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Nitrite oxidoreductases (NXRs) are key enzymes in the global nitrogen cycle oxidizing nitrite to nitrate [1]. While it was first observed in nitrite-oxidizing bacteria (NOB), NXR has also been found in comammox microorganisms performing complete ammonium oxidation to nitrate, as well as in anaerobic ammonium-oxidizing (anammox) bacteria [2]. These bacteria, which were discovered in the early 1990s, have the unique metabolic ability to combine ammonium and nitrite (or nitrate) to form dinitrogen gas, a process that takes place in a special cellular compartment, the anammoxosome [3]. Anammox bacteria are now believed to be responsible for up to 30 to 70% of the annual nitrogen removal from the oceans. Despite its importance in the biogeochemical nitrogen cycle, essential issues on NXR functions remain unanswered, particularly due to the lack of structural information. To meet this challenge, we used a multiscale approach combining cryo-electron tomography, crystallography, single-particle cryo-EM together with reconstitution studies and enzyme kinetics to characterize NXR from the anammox bacterium *Kuenenia stutgartiensis* [4]. We show that, in contrast to what was shown for NXR in NOB, NXR of anammox bacteria forms tubule-like structures inside the anammoxosome held together by a novel subunit NXR-T. Together with describing the active site, we are able to shed light on a newly defined bidirectional reaction specificity of the enzyme.

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ST080

Structural insights into photosystem II assembly

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Membrane protein complexes play a central role in cyanobacterial bioenergetics. We have recently solved several structures of cyanobacterial membrane protein complexes by cryo-electron microscopy and complemented the work by spectroscopic analysis or molecular dynamics simulation (Schuller et al. 2019, Schuller et al. 2020). Photosystem II (PSII) is one of the best studied photosynthetic multi-subunit membrane protein complexes so far. It catalyzes a very unique reaction: the light-driven oxidation of water. Many details of PSII structure and function have been unraveled already. However, the molecular details of its biogenesis, particularly the role auf auxiliary proteins, which assist the assembly of the various cofactors and subunits, remained unclear. Using cryo-electron microscopy, we solved the first structure of a partially functional PSII assembly intermediate from the thermophilic cyanobacterium *Thermosynechococcus elongatus* at 2.94 Å resolution (Zabret et al. 2021). It contains three assembly factors (Psb27, Psb28, Psb34) and provides detailed insights into their molecular function. Binding of Psb28 induces large conformational changes at the PSII acceptor side, which distort the binding pocket of the mobile quinone (QB) and replace the bicarbonate ligand of non-heme iron with glutamate, a structural motif found in reaction centers of non-oxygenic photosynthetic bacteria. These results reveal novel mechanisms that protect PSII from damage during biogenesis until water splitting is activated. Our structure further demonstrates how the PSII active site is prepared for the incorporation of the Mn_4CaO_5 cluster, which performs the unique water splitting reaction.

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ST081

Growth-rate dependency of ribosome abundance and translation elongation rate in *Corynebacterium glutamicum* differs from *Escherichia coli*

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Introduction

Bacterial growth rate is directly linked to the translational capacity of the cell, which is determined by nutrient availability and ribosome abundance. Ribosomes are large multimeric RNA-protein complexes that constitute a significant fraction of the cellular RNA and protein content. Therefore, ribosome abundance must be tightly controlled to avoid loss of fitness due to resource misallocation. This complex regulation has been mostly studied in *Escherichia coli*, a fast-growing bacterium that exhibits a broad range of growth rates (up to 2.14 h^{-1}). *Corynebacterium glutamicum* is an important model organism in industrial biotechnology that although robust and easy to use presents a maximal growth rate (up to 0.6 h^{-1}) that lags far behind that for *E. coli*.

Objective

To identify potential growth rate constrains and enable better growth models for *C. glutamicum* we performed a systematic analysis of the growth rate dependency of ribosome abundance and translation elongation rate.

Methods

Ribosome abundance at different growth rates was determined via super-resolution microscopy using fluorescently tagged ribosomal proteins as well as total RNA-protein ratio. Translation elongation rate at different growth rates was measured via an inducible fluorescence-based assay.

Results

In *C. glutamicum*, the growth rate and ribosome abundance showed a marked non-linear relationship with little change in ribosome abundance below a growth rate of 0.4 h^{-1} and a steep increase at higher growth rates. The translation elongation rate varied from 9 amino acids s^{-1} at maximal growth rate to less than 2 aa s^{-1} during slow growth. Unlike in *E. coli*, in *C. glutamicum* at low growth rates, the majority of the ribosomes were found to be kept in an active state and the translation elongation rate decreased to very low levels.

Conclusion

A model-based approach showed that depletion of translation precursors during slow growth can explain the observed decrease in the translation elongation rate. Furthermore, maintenance of a pool of slowly translating ribosomes allowed for a quick recovery response, as shown by nutrient up-shift experiments, and may reflect a different evolutionary strategy from that of *E. coli* to cope with periods of nutrient deprivation.

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ST082

Deletion of SMC renders FtsK essential for completing DNA segregation in *Corynebacterium glutamicum*

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Structural maintenance of chromosomes (SMC) is an important protein involved in chromosome organization and essential in many organisms. However, it can be deleted in *Corynebacterium. glutamicum*. We reasoned that in *C. glutamicum* other proteins might be rendered essential for chromosome organization when SMC was deleted.

Therefore, we generated a high-density Tn-5 library based on wild-type and SMC-deleted *C. glutamicum* strains. The Tn-seq data revealed that the *ftsK* gene had an obvious change of transposon insertion numbers, suggesting that the FtsK protein became essential after SMC was deleted. Cells with depletion of Ftsk were elongated and have more DNA-free cells. Localization analysis showed that FtsK was localized at the septa and cell poles. While FtsK localized at two sides of the pole in the wild-type strain, it major localized one pole in the SMC-deleted strain. Single-molecule tracking revealed that FtsK had a greatly reduced mobility after SMC was deleted. In addition, tracking data also showed an increase in the dwell time of FtsK in an SMC deletion background, indicating that it is maybe active at the septum for a longer time compared to the wild type. We further show that deletion of the centromere-binding protein ParB, which is necessary to load SMC to parS sites, render SMC and FtsK molecules more mobile. Thus, we demonstrate here that FtsK is essential for chromosome organization after SMC deletion. The lack of SMC is compensated by a longer localization of FtsK at the cell septum, where the process of DNA segregation of a less structured chromosome takes longer. FtsK is apparently able to sense how long its activity is required before septation can be completed.

ST083

Archaeal SepF is essential for cell division in *Haloferax volcanii*

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In most bacteria, cell division depends on the tubulin homolog FtsZ and other proteins, such as SepF, that form a complex termed the divisome. Cell division also depends on FtsZ in many archaea, but other components of the divisome are unknown. Here, we demonstrate that a SepF homolog plays important roles in cell division in *Haloferax volcanii*, a halophilic archaeon that is known to have two FtsZ homologs with slightly different functions (FtsZ1 and FtsZ2). SepF co-localizes with both FtsZ1 and FtsZ2 at midcell. Attempts to generate a *sepF* deletion mutant were unsuccessful, suggesting an essential role. Indeed, SepF depletion leads to severe cell division defects and formation of large cells. Overexpression of FtsZ1-GFP or FtsZ2-GFP in SepF-depleted cells results in formation of filamentous cells with a high number of FtsZ1 rings, while the number of FtsZ2 rings is not affected. Pull-down assays support that SepF interacts with FtsZ2 but not with FtsZ1, although SepF appears delocalized in the absence of FtsZ1. Archaeal SepF homologs lack a glycine residue known to be important for polymerization and function in bacteria, and purified *H. volcanii* SepF forms dimers, suggesting that polymerization might not be important for the function of archaeal SepF.

ST084

Restoring functionality of a compromised *Bacillus subtilis* biofilm activator protein

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Undomesticated strains of the Gram-positive soil bacterium *Bacillus subtilis* can differentiate into complex, 3-dimensional structures, referred to as biofilms. In these biofilms, genetically identical but physiologically different cell types are encased in a self-produced matrix consisting of extracellular polysaccharides and the fibrillar TasA protein. Biofilm formation relies on a highly integrated network of signaling cascades, and involves a multitude of transcriptional regulators. Mutational analysis has previously identified a new component of this network, RemA, a crucial activator for biofilm formation (1). However, the central role of RemA in this cellular differentiation process has been highly underappreciated since its discovery by D.B. Kearns and co-workers in 2009 (1). Great strides have recently been made in our understanding of RemA function through its crystallographic analysis (2). This approach revealed a unique structure of a bacterial transcriptional activator, as RemA displays an octameric organization into a ring-like complex. Eight LytTR-related DNA binding motif are exposed on the surface of the ring. Although no structure of a RemA:DNA complex is available, the overall topology of the octamer suggest that the RemA activator protein wraps DNA around it (2).

The Arg-18/Trp variant of RemA is defective in biofilm formation (1), yet it can still bind to DNA (2). Amazingly, this single amino acid substitution causes the conversion of the authentic RemA octamer into a heptamer (2). To further our understanding of the structure/function relationship of RemA, we exploited the inability of this RemA variant to promote biofilm formation by selecting *B. subtilis* strains that had regained the ability to form biofilms on solid surfaces and at liquid/air interfaces. Intragenic suppressors were found in this screen that contained, in addition to the original Arg-18/Trp mutation, single amino acid substitutions at different positions of the RemA protein. Biochemical and structural analysis of some of them revealed the potential to form either heptameric or octameric assemblies. We will discuss these findings in light of the structural plasticity of the RemA activator protein and its biological consequences for gene regulation and the ensuing cellular differentiation of *B. subtilis*.

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ST085

Single molecule dynamics of bacterial GTPases suggests ribosome assembly lines at polar regions of non-compartmentalized cells

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Introduction

Eukaryotic cells transcribe ribosomal RNA and largely assemble ribosomes in a structure called the nucleolus, where chromosomal regions containing rRNA operons are clustered. In bacteria, many rRNA operons cluster close to the origin regions that are positioned on the outer borders of nucleoids, close to polar areas where translating 70S ribosomes are located. Because polar regions of the nucleoids contain the highest accumulation of RNA polymerase, it has been hypothesized that bacteria contain "nucleolus-like" structures. However, ribosome subunits freely diffuse through the entire cells, and could thus be assembled and matured throughout the non-compartmentalized cell.

Objectives

We wished to obtain deeper insight into the ribosome assembly process by learning where it is localized, because most of the rRNAs are synthesized close to translation regions. Maturation could be confined to translation zones, or pre-mature subunits might diffuse throughout the whole cytoplasm like matured free ribosomal subunits.

Materials & Methods

Protein dynamics, their localization and the duration of dwell events of essential GTPases involved in ribosome assembly were determined by utilizing single molecule tracking and epifluorescence microscopy.

Results

By tracking single molecules of GTPases Era and Obg in *Bacillus subtilis*, we show that this process takes place at sites of translation, and thus predominantly at the cell poles. Induction of the stringent response led to a change in the population of GTPases assumed to be active in maturation, but did not abolish nucleoid occlusion of assembly and translation. Dynamics of Era and Obg were similar to each other, supporting that both subunits are matured in a confined manner. Addition of an ectopic Era unable to hydrolyse GTP did not affect growth or localization of ribosomes, indicating that abolishment of switch activity is not dominant negative.

Conclusion

Our findings strongly support the idea of the conceptualization of nucleolus-like structures in bacteria, i.e. rRNA synthesis, ribosomal protein synthesis and subunits assembly occurring in close proximity at the cell poles, possibly involving phase separation effects.

ST086

The exo-β-N-acetylmuramidases NamZ1 and NamZ2 from the oral pathogen *Tannerella forsythia* are exo-lytic peptidoglycan processing enzymes with distinct substrate specificity

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ABSTRACT - The anaerobic, Gram-negative periodontal pathogen *Tannerella forsythia* is auxotrophic for *N*-acetylmuramic acid (MurNAc), which is an essential constituent of the peptidoglycan (PGN) of the cell wall of bacteria. Thus, to thrive in the oral habitat, *T. forsythia* strictly depends on the salvage of exogenous MurNAc or sources of MurNAc, such as polymeric or fragmentary PGN derived from cohabiting bacteria, to build up its cell wall. In our efforts to elucidate how *T. forsythia* satisfies its demand for MurNAc, we recognized that the organism possesses putative orthologs of the exo-β-*N*-acetylmuramidase BsNamZ from *B. subtilis.* TfNamZ1 and TfNamZ2 were characterized as exo-lytic β-*N*-acetylmuramidases, both cleaving terminal MurNAc entities from the non-reducing ends of the artificial substrate pNP-MurNAc and the naturally-occurring disaccharide substrate MurNAc-*N*-acetylglucosamine (GlcNAc), albeit the activity of TfNamZ1 was much lower compared to TfNamZ2 or BsNamZ. Unexpectedly, we revealed striking differences in the specificity and mode of action of these enzymes when peptide-free PGN glycans were used as substrates. TfNamZ1, but not TfNamZ2 or BsNamZ, released GlcNAc-MurNAc disaccharides from these glycans, which characterizes TfNamZ1 as a unique disaccharide-forming exo-lytic β-*N*-acetylmuramidase, whereas in contrast, TfNamZ2 and BsNamZ, constitute MurNAc monosaccharide-forming exo-β-*N*-acetylmuramidases.

IMPORTANCE - TfNamZ1 and TfNamZ2 from *T. forsythia* are two exo-*N*-acetylmuramidases belonging to the glycosidase family 171 (www.cazy.org). This work revealed that they have different substrate specificities and reaction products when cleaving peptide-free, PGN glycans: either they predominantly release disaccharides (GlcNAc-MurNAc) or monosaccharides (MurNAc) from the non-reducing ends, respectively. Thus, a functional characterization of glycosidases of family 171 requires the thorough analysis of the activities using polymeric PGN glycans. Our results provide a better understanding of how *T. forsythia* gains access to the essential growth factor MurNAc via the salvage of PGN fragments from cohabiting bacteria in the oral habitat. This knowledge may provide avenues to the development of anti-periodontal drugs. Apart from this special case, the utilization of PGN as a nutrient source via exo-lytic *N*-acetylmuramidases with different modes of action, appears to be a general feature of bacteria, particularly within members of the Bacteroidetes.

ST087

Discovery and biosynthesis of the mycobacterial redox cofactor mycofactocin

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Introduction

Mycofactocin (MFT) is peptide-derived natural product that was proposed by bioinformatics to represent a novel redox cofactor [1]. It has attracted attention due to the widespread presence of its biosynthetic gene cluster (BGC) in actinobacterial genomes, including *Mycobacterium tuberculosis* (Mtb). It was shown that MFT is involved in ethanol metabolism of mycobacteria [2] and the BGC was connected to both enhanced and reduced the survival of Mtb in the host organism [3]. *In-vitro* studies of biosynthetic enzymes have suggested a redox-active keto amide core unit of the molecule. However, the structure of the natural mycofactocin remained elusive [4].

Objectives

Given the intriguing physiological functions of MFT and its potential role as drug target, we aimed to identify the natural MFT molecule in the model organism *Mycolicibacterium smegmatis* and to elucidate its chemical structure. Furthermore, its biosynthetic pathway was to be established *in vivo.*

Methods

We used metabolomics based on high-resolution LC-MS/MS, functional genomics as well as biochemical methods to study the MFT biosynthetic pathway as well as MFT-dependent enzymes *in vivo* and *in vitro*.

Results

We identified MFT as a redox-active ketoamide that existed in the cell as oxidized and reduced forms supporting its role as a redox cofactor [5]. The natural product was decorated with up to nine *β*-1,4-linked glucose residues. We demonstrated that oligoglycosylation is linked to the *mftF* gene encoding a glycosyltransferase. In addition, we have identified methylation of the second glucose moiety as another maturation step that requires a factor encoded outside of the BGC. Furthermore, we showed that the level of MFT increases upon cultivation on ethanol and we observed cofactor activity using an MFT-dependent carveol dehydrogenase. Recently, further MFT-dependent oxidoreductases were discovered.

Conclusions

We elucidated the structure of natural MFT and established a model of its biosynthesis *in vivo*. These results will inspire future studies on the biochemical function and physiological roles of MFT. Lastly, they might inspire efforts to exploit MFT as a drug target or disease marker.

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ST088

Glutamate fermentation in Enterobacteria

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The 2-hydroxyglutarate and methylaspartate pathways are the two main routes of bacterial glutamate fermentation. Whereas the 2-hydroxyglutarate pathway was found only in bacteria inhabiting strictly anaerobic environments like gut or marine sediments, the methylaspartate pathway is more oxygen tolerant, being widespread in soil bacteria as well as in several pathogens [1]. In our former study [2] we showed that the genomes of most of the enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) strains possessed a cluster coding for the key enzymes of the methylaspartate pathway. Nevertheless, our attempts to detect the activities of corresponding enzymes in cell extracts of *E. coli* O157:H7 strain ATCC 700728 failed as this strain could not ferment glutamate [2]. However, this strain is classified to Biosafety level 2 and might be deficient in some pathogenicity-related features in comparison to other EHEC strains (Biosafety level 3). Here we decided to study another enterobacterium, *Raoultella planticola* JCM 20069, in which the activity of one of the enzymes of the methylaspartate cycle, methylaspartate ammonia lyase, was measured previously [3]. The genome sequencing of *R. planticola* JCM 20069 showed the presence of a gene cluster similar to the glutamate fermentation cluster found in the EHEC strains. This strain was able to ferment glutamate and glutamine, and proteomic and metabolomic data indicated the functioning of the methylaspartate pathway. Activities of the key enzymes of the pathway were detected during anaerobic growth on glutamate, and a novel citramalate lyase was identified and characterized in this strain. This novel citramalate lyase consists of two subunits that were previously annotated as domain of unknown function proteins, being phylogenetically not related to the known bacterial citrate and citramalate lyases. We conclude that the methylaspartate pathway is used by *R. planticola* and probably by many pathogenic enterobacteria for glutamate fermentation. The usage of glutamate and glutamine known to be present in intestinal tract [4] may promote their pathogenicity and fuel the invasion.

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ST089

Functional characterization of the putative tryptophan substrate-binding protein TrpA in *Streptococcus suis*

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Streptococcus suis, an important zoonotic pathogen, causes invasive disease in pigs and humans with symptoms including pneumonia, arthritis, meningitis, endocarditis, and acute sepsis.

The aim of this study was to functionally characterize the putative tryptophan substrate-binding protein TrpA of *S. suis*. The *trpA* gene had been previously identified as conditionally essential by intrathecal experimental infection of pigs with a transposon library of *S. suis* strain 10 where it was shown to play an important role in bacterial survival during invasive infections.

Bioinformatics analysis revealed that *trpA* is part of a putative tryptophan/tyrosine ABC transporter system in which it encodes the substrate-binding protein. Since *S. suis* is auxotrophic for tryptophan, TrpA could play a relevant role in bacterial metabolism during the infection process by mediating tryptophan import.

First, we analyzed the growth of *S. suis* strain 10 and its *trpA*-deficient mutant in chemically defined medium (CDM). After defining the maximum tryptophan concentration in CDM that did not result in growth of the mutant, we added a tryptophan-containing tripeptide as well as a tyrosine-containing tripeptide to assess growth restoration. These results revealed that growth of the *trpA* mutant is dependent on tryptophan concentration, i.e., the mutant lacks the capability of high affinity tryptophan uptake, and its growth was restored by adding a tryptophan-tripeptide to the media.

Analyzing the operon structure of this transporter demonstrated that *trpA* is co-transcribed with a gene encoding a putative permease and a putative ATPase gene. 5" rapid amplification of cDNA ends revealed an approximately 680 bp 5" untranslated region located upstream of the operon. Bioinformatics analysis identified a putative tryptophan T-box riboswitch in this region. T-box riboswitches are cis-regulatory elements monitoring the aminoacylation status of the tRNA to induce expression of regulated downstream genes. Finally, a reporter assay demonstrated an increase of *trpA* promoter activity under tryptophan-limited conditions.

In summary, our study suggests that TrpA is the substrate-binding protein of a tryptophan ABC transporter whose transcription might be regulated by a T-box riboswitch.

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ST090

The Old Yellow Enzyme (*ofrA*) contributes to *Staphylococcus aureus* stress response, virulence, and metabolism

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Old yellow enzymes (OYEs) reduces activated C=C bonds in α,β-unsaturated carbonyl compounds. Although OYEs are widely spread in the plantae, fungi, and bacteria kingdoms, the role of OYEs in bacterial stress response and the infection situations remained enigmatic. In the mode of pathogen, the gram-positive bacterium, *Staphylococcus aureus* (*S. aureus*), adapts to numerous stress conditions at the host-pathogen interface. In *S. aureus* genome, two paralogous genes (*ofrA* and *ofrB*) encode for two OYEs. *ofrA* is conserved among all publicly available staphylococcal genomes. We constructed a chromosomally encoded reporter system in a transcriptional fusion manner. Our reporter system indicated that *ofrA* is induced under reactive electrophilic, oxygen, and chlorine species (RES, ROS, RCS) stress conditions. Through RNAseq analysis, we concluded that *ofrA* mutation lowered the carotenoid pigment (staphyloxanthin) production. Functional analysis shows that *ofrA* is a stress-mediating factor against RES, ROS, and RCS. Furthermore, *ofrA* contributes to *S. aureus* virulence in whole human blood and in long-term RAW 264.7 macrophage cell line. In summary, *ofrA* plays an important role in *S. aureus* fitness in the infection conditions through contribution to stress response.

ST091

THCz - Synthetic small molecules that target the bacterial cell wall precursor lipid II

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Tackling emerging antibiotic resistance requires the identification of novel resistance breaking compound classes. A pneumococcal autolysis-based whole-cell screening for bacterial cell wall biosynthesis inhibitors identified a hit class of synthetic small molecules with a 1-amino substituted tetrahydrocarbazole (THCz) scaffold. THCz displayed potent activity against a broad spectrum of Gram-positive and selected Gram-negative pathogens and were not prone to resistance development *in vitro*. Mode of action studies comprising comprehensive whole-cell analysis in combination with *in vitro* assays revealed that THCz inhibit cell wall biosynthesis by simultaneously targeting undecaprenyl pyrophosphate-containing lipid intermediates of peptidoglycan, wall teichoic acid and capsule biosynthetic pathways. SAR studies suggest that THCz represent a promising scaffold for the development of novel bacterial cell wall inhibitors.

ST092

Actinobacterial Glutamine synthetase-like enzymes as novel drug targets

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The actinobacterial model organism *Streptomyces coelicolor* as well as the related human pathogenic actinobacterium *Mycobacterium tuberculosis* have to cope with limited nitrogen availability in their ecological niches. Our investigations showed that assimilation of polyamines and monoamines like ethanolamine can allow the survival of these bacteria under such conditions. We analyzed the function of three proteins annotated as glutamine synthetase-like enzymes (GS-like): GlnA2$_{Sc}$, GlnA3$_{Sc}$ and GlnA4$_{Sc}$ and demonstrated that in *S. coelicolor* GlnA2$_{Sc}$ and GlnA3$_{Sc}$ are involved in the catabolism of polyamines and that GlnA4$_{Sc}$ is involved in the catabolism of ethanolamine[1]. Transcriptional, phenotypical, structural and biochemical analysis of these enzymes demonstrated a dual function of GlnA2$_{Sc}$, GlnA3$_{Sc}$ and GlnA4$_{Sc}$ in ensuring both nutrients availability (*C*- and *N*-source) and resistance against high poly- or ethanolamine concentrations, which are toxic for bacteria[1]. Further analysis revealed the presence of these enzymes also in *M. tuberculosis*. Structural and biochemical studies demonstrated the glutamylation of the polyamine spermine by the mycobacterial GlnA3$_{Mt}$. Investigations of macrophage metabolism showed the induced polyamine production during the infection. We demonstrated the involvement of GlnA3$_{Mt}$ in the polyamine catabolism during the proliferation of *M. tuberculosis* in macrophages. Since GlnA3$_{Mt}$ ensures survival of Mycobacteria in their host, inhibition of GlnA3$_{Mt}$ might be an effective therapeutic

strategy that is safe and specific and thus kind of enzymes represent promising drug targets. Our current studies aim the development of novel anti-tubercular drugs based on the validation of GlnA3$_{Mt}$ as a target.

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ST094

Engineering Nitrogenases for CO_2 Fixation inside *Rhodobacter capsulatus*

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Nitrogenases are the only known family of enzymes that catalyze the reduction of molecular nitrogen (N_2) to ammonia (NH_3). The N_2 reduction drives the biological nitrogen fixation and the global nitrogen cycle. Besides the conversion of N_2, nitrogenases catalyze a whole range of other reductions, including the reduction of the small gaseous substrates carbon monoxide (CO)[1-3] and carbon dioxide (CO_2)[3-6] to hydrocarbons.

Nitrogenases are a family of complex metalloenzymes that are distinguished in three classes: the "conventional" molybdenum (Mo) nitrogenase, and the homologous "alternative" vanadium (V) and iron (Fe)-only nitrogenases. The isozymes differ in their metal content, structure and substrate-dependent activity, despite their homology. Both nitrogenase reactions, the well-known formation of NH_3 (fertiliser) and the newly discovered hydrocarbon formation reaction are environmentally relevant and mimicked by large scale industrial processes: the Haber-Bosch and the Fischer-Tropsch process, respectively. **Thus, understanding and engineering the fundamental mechanisms of the metal cluster in nitrogenase will also open new paths for the use of nitrogenase in bioremediation (CO_2 fixation) and renewable hydrocarbon and energy production.**

My lab focuses on engineering and the characterizing the CO_2 conversion by nitrogenases. One the one hand, we are evolving nitrogenases towards an increased CO_2 fixation rate. On the other hand, we are characterizing the evolved enzymes by catalytic, spectroscopic and structural means. **Our long-term goal is to engineer nitrogenases for the fixation of CO_2 and the production of hydrocarbons inside *R. capsulatus***.

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ST095

Towards a holistic monitoring system for protein production and secretion in *Bacillus subtilis*

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Bacillus subtilis is an industrially well-established host for recombinant protein production and secretion. Nevertheless, optimization campaigns are still performed using, for instance, signal peptide libraries [1]. In such campaigns, cheap and simple photometric or fluorometric enzyme activity assays are used for screening, but these methods are not generally applicable for each target protein and, additionally, secretion of recombinant proteins often creates stress, which can hinder further optimization. Thus, it is highly desirable to develop assays for simultaneous online monitoring of protein production, secretion and a putative stress response. To this end, we have combined the activity-independent split GFP assay [2] for protein quantification with a fluorescence-based secretion stress biosensor [3, 4].

For the split GFP assay, the eleventh β-sheet of GFP (GFP11 tag) is C-terminally fused to a target protein and can complement a truncated non-fluorescent GFP (GFP1-10) to form fluorescent holo-GFP. Based on our previously developed split GFP assay for secreted proteins [5] we devised a non-invasive *in vivo* assay for online monitoring the production of *Fusarium solani pisi* cutinase. Additionally, we have established the iSplit GFP assay for *in vivo* quantification of cytosolic target proteins [6].

Furthermore, an mCherry secretion stress biosensor based on the CssRS two-component system [7] was established, which responds to secreted but misfolded protein in *B. subtilis.* This biosensor was evaluated by monitoring secretion of *F. solani pisi* cutinase with different signal peptides and at varying translation rates.

Finally, we have combined all three biosensors using different fluorescent variants of split GFP to enable a holistic view on protein production and secretion in this industrially important bacterium.

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ST096

Recombinant production of bacteriocins using *Corynebacterium glutamicum* – obstacles and solutions

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Bacteriocins are antimicrobial peptides produced by bacteria to inhibit competitors in their natural environments. Due to the rapid increase in antibiotic resistant bacteria bacteriocins are discussed as alternatives to antibiotics to treat infections for therapeutic purposes. Currently, industrial production of bacteriocins is performed exclusively with natural producer organisms on complex substrates and products are commercialized as semi-purified preparations or crude fermentates used in food preservation. To allow clinical application and entry of novel bacteriocins into the market, efficacy of production and purity of the product need to be improved. One possibility is to shift production to recombinant biotechnological production host. Here, we report on our recent efforts to establish recombinant production of bacteriocins using the widely used industrial workhorse organisms *Corynebacterium glutamicum* as production host, associated problems and proposed solutions.

As a first demonstrator product we selected pediocin PA-1, a class IIa bacteriocin produced naturally by *Pediococcus spp.* and *Lactobacillus spp.* strains. *C. glutamicum* CR099 is resistant to high concentrations of pediocin PA-1 and does not show proteolytic degradation of the anticipated product. We successfully established recombinant of pediocin PA-1 using a *C. glutamicum* strain expressing a synthetic *pedACD* operon. Further experiments suggest that low levels of dissolved oxygen are favourable for production of pediocin. Subsequently, we successfully implemented production of a second class IIa bacteriocin and were able to determine pH and salt as critical parameters to increase product titers.

More interesting bacteriocins from a scientific and commercial perspective are class I bacteriocins e.g. nisin, which is the most intensively studied bacteriocin to date. Nisin is widely used as a food preservative with a global market volume projected to exceed 500 Mio. USD in 2025. Moreover, nisin has a high degree of posttranslational modification and it it is also active against *C. glutamicum* rendering production more challenging. To overcome these obstacles, we implemented a two-step process to produce inactive prenisin with subsequent activation using a soluble NisP protease.

In summary, we introduce *C. glutamicum* as a suitable host for production of antimicrobial peptides of different classes.

ST097
Microscale cultivation of *Trichoderma reesei* strains enables strain phenotyping and bioprocess development in batch and fed-batch mode with higher throughput

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As our society transforms from fossil resources to renewable alternatives, processing of biomass remains a key challenge. Plant biomass conversion requires hydrolytic enzymes such as cellobiohydrolases (CBHs) and β-glucosidases (BGLs); thus, cost-effective production of these enzymes is critical. Filamentous fungi are excellent enzyme producers due to their high production capacity and secretion efficiency. However, due to morphological challenges, high-throughput, parallelized cultivation of filamentous fungi using micro-bioreactors (MBRs) is not widely established. One important producer of cellulolytic enzymes is *Trichoderma reesei* RutC30. In contrast to other industrially relevant filamentous fungi, RutC30 is used with a microfilamentous morphology, leading to culture broth with higher viscosity.

In this study, a MBR workflow at mL-scale for time- and cost-efficient characterization of *T. reesei* strains is established. The applicability of MBRs in batch and fed-batch mode with online measurement of scattered light (SL; measure for biomass) and dissolved oxygen (DO) was examined. To this end, the strains RutC30 and RutC30D*gel3* were cultivated, the latter strain showing altered morphology with preserved hyperproducing properties. CBH and BGL activity were measured by photometric assays on a liquid handling robotic platform.

A protocol for reproducible MBR batch cultivation of RutC30 and RutC30D*gel3* was successfully developed. Round Well Plates shaken at 1000 rpm and 35 % oxygen were found to work best for both strains, showing microfilamentous morphology and only minor wall growth. Furthermore, the applicability of a microfluidic fed-batch cultivation with different feeding rates of 0.3-0.75 g/(l*h) lactose was shown. For RutC30, CBH and BGL activities in fed-batch mode were increased 2.8-fold and 4.4-fold respectively compared to a batch cultivation under the same conditions. For RutC30D*gel3* no significant increase in CBH or BGL activity was observed. A morphological change from microfilaments only to a mixture with pellets was found for both strains during fed-batch.

To conclude, MBRs are a promising tool for parallelized cultivation workflows of *T. reesei*. The presented cultivation workflow can be applied for strain library characterization, providing advantages in terms of increased phenotypic information and reduced experimental resources. Moreover, bioprocess optimization under industrially relevant fed-batch conditions is shown to be feasible.

ST098
ThermoCas9: a new tool for the genomic manipulation of the extreme thermophilic bacterium *Thermus thermophilus* HB27

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Hyperthermophiles have unique biochemical and physiological characteristics that make them very attractive for several applications as for example biocatalysis under harsh conditions. They have received much interest also as suitable hosts for metabolic engineering; however, their use is limited by the low number of developed tools for their genetic manipulation[1]. Among thermophilic bacteria, *T thermophilus* HB27 is a good candidate as a platform for metabolic engineering due to its high growth rates, high biomass yields, constitutive expression of a very efficient natural competence, and metabolic versatility. Here we describe the set-up of a highly efficient hyperthermoactive-

Cas9 based editing tool for the manipulation of the *T. thermophilus* HB27 genome[2]. The developed genetic system is active up to 65°C and this is the highest temperature reported to date for a Cas9-based editing tool. Based on our previous knowledge on the arsenic resistance system of *T. thermophilus* HB27[4], we developed a *whole-cell* biosensor for the monitoring of arsenic[5]. The bioreporter is a genome-modified strain, containing the gene encoding a thermotolerant yellow fluorescent protein integrated under the control of an arsenic-responsive promoter. The system can be easily adapted for editing the genomes of other thermophilic bacteria, thus representing a boost for metabolic engineering in hyperthermophilic microorganisms.

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ST099

CRISPR-Cas9 as a versatile molecular biological tool for genome editing in *Paenibacillus polymyxa*

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Paenibacillus polymyxa is a highly interesting non-model organism which has great potentials as an unconventional host for industrial biotechnology based on its versatile metabolism and production of various types of natural products, such as exopolysaccharides, 2,3-Butanediol, as well as different antimicrobial compounds like polymyxin and fusaricidin. For a long time, targeted strain development of *P. polymyxa* was hindered by the limited availability of genetic engineering tools. For this, we focused on the development of genetic tools to perform reliable genetic modifications in *P. polymyxa*. By harnessing the high versatility of CRISPR-Cas-based systems, we have managed to realize manifold genetic modifications in this bacterium. The first approaches targeted single-gene deletions by use of our Cas9-based tool, but thus far, no studies have conducted a systematic investigation of how the utilization of this tool can be extended towards deletion of larger genomic regions or multiplex genome editing. In our recent study, we demonstrate the utilization of the Cas9-based system for targeted deletions of various biosynthetic gene clusters, by the use of only one single targeting sgRNA. By that approach, we achieved efficiencies up to 100 % for the deletion of several large biosynthetic clusters. We were also able to identify an influence of the targeted position within the clusters, in which it becomes obvious that targeting a region in the middle or closer to the 3′ end is much more beneficial for large cluster deletion in *P. polymyxa*. Furthermore, we harnessed the system for multiplex editing of single genes and large genomic regions. Remarkably, multiplex deletion of two large clusters could also be realized with an efficiency of more than 80 %. Moreover, we also exploit the system to facilitate multiplex gene integration, what is rarely described in bacteria. Overall, our findings have surpassed the previous reported applications of the system and thus would greatly extend the state-of-the-art of genome editing in *P. polymyxa*. We are highly convinced that genetic toolbox development is not solely about designing a new tool, but also about advancing the knowledge and understanding of the tool itself. We believe that our study is of high interest and importance for advancing the strategies for strain engineering purposes. Finally, we anticipate a wider utilization of our Cas9-based system for future research in *P. polymyxa* as well as related species.

ST100

Generation of novel glycopeptide antibiotics by using the mutasynthesis approach

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Balhimycin is a vancomycin-type glycopeptide antibiotic (GPA) produced by *Amycolatopsis balhimycina*. It differs only in the glycosylation pattern of vancomycin, the GPA which is used as antibiotics of last resort against multi resistant pathogens. The common mode of action of GPAs is based on their binding to the terminal D-alanine-D-alanyl (D-Ala-D-Ala) moiety of the bacterial peptidoglycan (PG) precursors lipid II via five hydrogen bonds, resulting in the inhibition of the transpeptidation and transglycosylation reactions at the late stages of peptidoglycan formation. Reprogramming of the biosynthesis of the PG biosynthesis resulted in the formation of resistant lipid II molecules ending on D-Ala-D-lactate (Lac) and thereby to a decreased affinity of GPAs to their targets by 1000-fold. This effect is because a hydrogen bond cannot be formed between the carbonyl group of 4-hydroxy-L-phenylglycine (Hpg; position 4 aa in GPA) and the ester oxygen moiety of Lac.

Mutasynthesis is a suitable method to construct new GPA derivatives that also act against resistant pathogens. The prerequisite for this is the construction of a block mutant that is no longer able to produce the Hpg. In *A. balhimycina* this was achieved by the deletion of *hmaS* and *hmaO* genes. *A. balhimycina Δhpg* was cultivated under GPA production conditions supplemented with various Hpg derivatives, which were either commercially available, enzymatically or chemically synthesised by cooperation partners. The production of new balhimycin derivatives was verified by (High-resolution) LC-ESI-MS analysis.

Feeding of Δ*hpg* mutant with 4-hydroxy-2-fluoro-L-phenylglycine and 2-fluoro-L-phenylglycine resulted in the formation of new fluorinated balhimycin derivatives. Their biological function is under investigation.

With the established mutasynthesis approach further GPA derivatives, acting against resistant pathogens, can be produced.

ST101

Identification of aldehyde oxidizing enzymes in *P. taiwanensis* VLB120 and generation of a tolerance-optimized chassis strain

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Introduction

Pseudomonads are of growing interest as biotechnological workhorses due to their distinct robustness against various toxicants, such as aldehydes.1 Among these substances are promising bio-based platform chemicals like furfural and 5-hydroxymethylfurfural (HMF) causing electrophilic and oxidative stress. To cope with aldehyde stress, pseudomonads feature a large set of redox enzymes allowing quick removal of the toxic chemicals by conversion to the less noxious alcohol or preferentially acid derivatives.2 Being capable of rapidly oxidizing aldehydes renders the bacteria particularly attractive for whole cell biocatalytic production of terephthalate substituting bio-based plastic monomer furan dicarboxylic acid (FDCA) from HMF.3

Objectives

1. Identification of aldehyde oxidizing enzymes of *P. taiwanensis* VLB120
2. Targeted optimization of *Pseudomonas*" oxidative properties
3. Improvement of FDCA production from HMF

Materials and Methods

1. Gene deletions
2. Genome editing
3. Whole-cell biocatalysis

Results

We could identify two important members of *Pseudomonas"* built-in enzymatic toolbox for aldehyde oxidation by gene deletions, a molybdenum-dependent periplasmic oxidase and a cytoplasmic dehydrogenase. A double knockout of both enzymes led to dramatic reduction of HMF oxidation in *P. taiwanensis* VLB120. This discovery enabled us to specifically improve *Pseudomonas"* aldehyde oxidation machinery by overexpression of the respective enzymes, either plasmid-based or *via* genomic exchange of the native promoter regions for strong constitutive synthetic promoters.

Conclusion

The optimized strains are useful hosts for biotechnological synthesis of FDCA from HMF, since aldehyde toxicity as main drawback of whole-cell catalysis is attenuated due to boosted oxidation. Futhermore, the non-oxidizing double knockout is well suited for the elucidation of secondary aldehyde tolerance mechanisms different from fast conversion.

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ST102

Monovalent cations kill preferentially motile *B. subtilis* cells

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Bacillus subtilis has evolved several strategies for better survival under stress. Vegetative *B. subtilis* populations comprise a mixture of motile cells and sessile cells. Whether and how the different subpopulations vary with their response to stress is poorly studied. Here we show that monovalent cations efficiently kill motile cells under conditions of nutrient deprivation. Cell-type-specific lysis results from the upregulated expression of the *lytABC* operon in motile cells. The forced induction of the same operon in sessile cells also leads to cell lysis. Our data suggest that sessile cells are not necessarily better protected from stress. Instead, motile cells seem to be sensitized to stress. Moreover, our results raise the possibility that *B. subtilis* could selectively induce lysis of motile cells by means of electrical signals.

ST103

From extreme environments on Earth to space: *Buttiauxella* sp. MASE-IM-9 and *Salinisphaera shabanensis* as new model organisms in Astrobiology

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Background

Mars analogue environments are some of the most extreme locations on Earth. Their unique combination of multiples extremes (e.g. high salinity, anoxia, and low nutrient availability) make them a valuable source of new polyextremophilic microbes in general and for exploring the limits of life. These are seen as vital sources of information for Astrobiology, with implications for planetary protection and the search for life outside our planet.

Mars, especially the surface, is still considered very hostile to life. Nevertheless, there are probably geological niches where the occurrence of life is conceivable. Current knowledge on the capability of (facultative) anaerobic microbes as single strains or in communities to withstand Martian conditions is still very sparse. Therefore, space experiments are needed to substantiate the hypotheses of habitability on Mars which is one of the main goals of the project MEXEM (Mars EXposed Extremophiles Mixture). Selected model organisms will be exposed to space in a 3-month passive experiment and survivability will be evaluated after their arrival back on Earth.

Methods and Results

The survivability of two strains originating from extreme environments was investigated after exposure to Mars relevant stress factors: *Salinisphaera shabanensis*, isolated from a deep-sea brine pool within the Red Sea, and of *Buttiauxella* sp. MASE-IM-9, isolated from a German sulphidic spring. Both organisms showed survival after anoxic desiccation up to three months. Survival after desiccation could even reproduced if the cells were mixed, as an artificial community, before desiccation treatment.

The desiccation tolerance could be further extended (nearly doubled) by adding artificial Mars regolith (MGS-1S; 0.5 % wt/vol) and sucrose (0.1 M). The addition of these two components resulted in an elevation of the survival rate after desiccation up to three orders of magnitude in general and for longer time periods. The presence of these two components also influenced the survival after exposure to polychromatic UV (200 - 400 nm) up to 12 kJ/m^2 in liquid and in a desiccated form positively. To discuss the reasons for this positive change in survivability, different fluorescent-based microscopic techniques were applied.

The survival capabilities of the two strains after Mars relevant stress factors make them valuable new model microorganisms in Astrobiology in general and for space experiments in specific.

ST104

Laboratory evolution of *Thermoanaerobacter kivui* towards reduced growth temperatures

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The origin and evolution of life on Earth is still a controversial field of research today. Phylogenetic studies indicate that live on earth might have originated in a hydrothermal setting with the last universal common ancestor (LUCA) being an autotrophic thermophilic anaerobe using the Wood-Ljungdahl pathway for CO2 fixation [1]. Proceeding from a thermophilic LUCA growing optimally at temperatures over 45 °C, organisms that grow at moderate temperatures (mesophiles) possibly emerged out of a thermophilic lifestyle. This would require alterations regarding membrane structure or stability of nucleic acids and proteins.

Thermoanaerobacter kivui is the most thermophilic acetogenic bacterium characterized yet with an optimal growth temperature (T_{OPT}) of 66 °C [2]. Its physiology possibly resembles that of LUCA, making it a perfect model organism to study the evolutionary changes necessary to grow at moderate temperatures. Therefore, we carried out growth experiments at suboptimal growth temperatures. We found that *T. kivui* is able to grow at a temperature of 39 °C, more than 20 °C below its T_{OPT}. A strong variability during growth of individual colonies was observed regarding growth rates, final optical density and morphology. To possible obtain an individual better adapted to lower temperatures, we selected the largest colonies grown in agar at 50 °C. One promising isolate (Iso50) with a higher growth rate at suboptimal growth temperatures was genotypically and phenotypically characterized. Analysis of the fatty acids of wild type and Iso50 provided information about the changes in fatty acid content of *T. kivui* growing at 50 °C.

The phenotypic changes as well as single nucleotide polymorphisms in the adapted strain gave first ideas of the required alterations needed for growth of *T. kivui* at suboptimal temperatures. Moreover, we are currently testing different strategies of adaptive laboratory evolution (ALE) towards the adaption to lower growth temperatures.

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ST105

Exploring viral infections of uncultivated archaeal cells using correlative microscopy

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Studying viral infections of microbes and associated morphological changes in environmental samples remains a challenge for environmental virology and microbiology[1]. First steps towards filling this gap of knowledge has been performed via virus-targeted direct-geneFISH (virusFISH), a technique that is able to label small genomic fragments like those of viruses linking information from metagenomes to fluorescence microscopy of environmental samples[2]. However, higher magnification of the sample is needed in order to describe morphological changes of the host cell during infections.

Here we characterize viral infections of deep subsurface biofilms of *Candidatus Altiarchaeum hamiconexum*[3] via a combination of fluorescence microscopy (FM) and scanning electron microscopy (SEM). After performing virusFISH[2], we correlated the results to SEM images via glass slides with a coordinate system engraved on the surface. The shape and the coordinates of the biofilms were documented and sample preparation for SEM was performed by dehydration using acetone after osmium fixation, followed by critical point drying and coating with platin/palladium. Overlays of the micrographs were produced using GIMP.

We determined that the correlation of fluorescence microscopy and scanning electron microscopy was successfully established. Our recorded images of cells with viral signals undergoing cell lysis further underpinning the evidence of active lytic viruses in the deep biosphere. Interestingly, some intact cells with strong viral signal appeared to have a strong morphological change with increased cell volume.

These results provide evidence that this correlative virusFISH SEM technique can be used to study uncultivated prokaryotes regarding their morphological changes during viral infection *in situ*.

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ST106

Monitoring of the effects of a simulated hydrogen leakage on the groundwater geochemistry and microbiome in a shallow aquifer

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The future hydrogen economy requires a hydrogen gas grid as well as large deep underground stores, which, however, can be accompanied by leakage incidents, potentially affecting groundwater resources. The consequences of an unintended spread of gas, e. g., through leaky pipes or subterranean gas migration, on subsurface ecosystems are poorly understood. The aim of this study was to monitor whether hydrogen affects the geochemistry of and the microbiome in groundwater by injecting hydrogen into a shallow aquifer on the TestUM test field near Wittstock/Dosse (Brandenburg, Germany), thus, simulating a hydrogen leakage incident. Hydrogen-exposed and non-exposed wells were regularly monitored by analysing groundwater for geochemical and microbiological parameters. When sampling 76 – 77 days post injection, the hydrogen concentrations were again below the detection limit in the investigated hydrogen-exposed wells. Hydrogeochemical data indicated that microbial hydrogen consumption took place. Although the beta diversity of microbial communities altered and the growth of some prokaryotes was stimulated, a strong enrichment of specific putative hydrogenotrophs was not observed. Microbial hydrogen consumption by various taxonomically different prokaryotes seemingly resulted in minor changes in the groundwater microbiome but these were possibly masked by its diversity and heterogeneity.

ST107

Monitoring sulfonamide resistance in a river from pristine source to wastewater treatment plant impacted sites

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The WHO stated the spread of multidrug-resistant bacteria with antibiotic resistance genes (ARGs) as a threat to global public health. Even though many pharmaceuticals, including antibiotics, were proven to cause antibiotic resistance, currently there are no regulations regarding the discharge of antibiotics from wastewater treatment plants (WWTP) into aquatic ecosystems, making surface waters a reservoir for antibiotics and ARGs [1]. To better understand factors that influence the fate of ARGs in the environment and to foster surveillance of antibiotic resistance spreading in such habitats, several indicator genes have been proposed. These genes include *sul1* and *sul2*, which encode sulfonamide resistance, and the integrase gene *intI1.* Both, *intI1* and *sul1,* are components of the clinical class 1 integron, a mobile genetic element accumulating and carrying various ARGs [2]. Here we used quantitative real-time PCR to monitor the abundance of these indicator genes as well as the 16S rRNA gene in a river from pristine source to wastewater treatment plant (WWTP) impacted water. The gene cassettes in class 1 integrons were investigated by PCR and sequencing with Oxford Nanopore Technology to determine their alteration along the river stream. The dynamics of the microbial communities were studied via Illumina-based sequencing of 16S rRNA gene amplicons. In addition, the concentration of the broad-range antimicrobial sulfamethoxazole (SMX) was quantified using solid phase extraction (SPE) and high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) and conventional water parameters were monitored with standard analytical methods. WWTP effluent was the principal source for the three target genes and SMX. Downstream the WWTP water quality improved constantly, including lower levels of SMX and decreasing abundances of *sul1* and *sul2*. However, the relative abundance of *intI1* increased, contradicting previous studies, where direct correlation between *sul1* and *intI1* was observed. Overall, the combined data indicate an increase in integrase activity in bacteria downstream of the WWTP. Our findings emphasize the knowledge gap regarding the parameters that determine the fate of those indicator genes in aquatic ecosystems.

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ST108

Stieleriacines, small molecules produced by the planctomycete *Stieleria maiorica*, potentially act as biosurfactants that influence the bacterial community composition in marine biofilms

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Stieleriacines were identified as one of the first secondary metabolites in a member of the bacterial phylum *Planctomycetes* and were named after its producer *Stieleria maiorica* (Kallscheuer et al., 2020). Stieleriacines are composed of a derivative of the aromatic amino acid tyrosine and a C_{12} fatty acid. Ligation of the fatty acid to the amino group of the tyrosine derivative places the compounds in the class of long-chain *N*-acylated amino acids. *N*-acylated tyrosine derivatives structurally related to stieleriacines have been previously identified from heterologously expressed environmental DNA and in some marine bacteria (Brady and Clardy, 2000; MacIntyre et al., 2019), however, their exact function remains enigmatic. In cultivation experiments with *S. maiorica*, we could show that a supplementation of stieleriacines leads to shortening of its lag phase. In biofilm formation assays with two natural competitors of *S. maiorica* belonging to the "Roseobacter group", *Phaeobacter inhibens* and *Sulfitobacter dubius*, the presence of stieleriacines led to different effects: they stimulated the biofilm formation capacity of *P. inhibens* but reduced the biofilm formation of *S. dubius*. The observed effects point towards complex allelopathic interactions between planctomycetes and other marine bacteria, which justifies an investigation of the regulatory function of stieleriacines in greater detail. Extensive optimization of cultivation conditions now allows co-cultures of *S. maiorica* with *P. inhibens* and *S. dubius*, both in liquid medium and on agar plates, which in turn is the basis to study this complex interaction in real time. An *in silico* analysis of the putative stieleriacine biosynthetic gene

cluster in *S. maiorica* suggests a functional link to genes relevant for exopolysaccharide biosynthesis, reinforcing the relevance of these secondary metabolites during biofilm formation. Molecular biological experiments based on the identified biosynthetic cluster target the functional analysis of individual genes by an expression in heterologous hosts as well as the construction of mutant strains of *S. maiorica* that are incapable of stieleriacine biosynthesis.

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ST109

Microbial cell abundances in surface water and lower atmosphere along a 10.000 km transect in the Atlantic Ocean

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Louis Pasteur already described airborne microbes in the 19th century but the impact of bioaerosols – such as fungi, bacteria, viruses, and pollen – on global biogeochemical cycles and climate remains largely unknown. Marine bioaerosol interaction may be of particular importance as oceans cover more than 70 % of Earth's surface. Furthermore, airborne microorganisms have been shown to act as cloud condensation and ice nuclei, and can be metabolically active in clouds. Their presence and activity can thus directly influence atmospheric and oceanic chemistry, hydrological cycles and the Earth's radiation budget.

Over the last 17 months we used the sailing research vessel *Eugen Seibold* to investigate microbial abundance, spatiotemporal distribution, community structure, metabolic capabilities and function of the air and water microbiome at the ocean-atmosphere interface. On a transect from the polar circle to the equator (67 to 3 °N) we collected a total of 118 discrete samples to characterize the air and surface water microbiome. Together with the continuous measurements of aerosol physicochemical properties and water biogeochemical parameters, this provides insights into the biotic and abiotic processes occurring in the boundary layer between ocean and atmosphere.

First insights into microbial cell abundances and distribution along this transect will be presented and integrated with atmospheric particle counts, size distribution and surface water measurements. These results show the spatiotemporal distribution of airborne microorganisms in the context of surface ocean water conditions. Especially in a time of climate change, ocean warming and acidification, it is necessary to understand which environmental conditions affect oceanic airborne microbial communities, and how airborne microbes in turn affect the weather, climate, and global biogeochemical cycles.

ST110

Oligo- and copiotrophy – are (coastal) SAR11 really oligotrophs?

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Oligo- and copiotrophy is an analog to r and K strategies in microbial ecology. Oligotrophic organisms dominate substrate poor environments. They are characterized by slow growth rates and little responses to a changing environment. Bacteria of the SAR11 clade account for about a third of the surface ocean microbial community and are an often referenced oligotroph. They generally depict slow growth rates and their small, streamlined genome suggests little capability to react to environmental cues. *Vice versa*, microbes of the phylum *Bacteroidetes* are suggested copiotrophs with fast growth rates and great responses to environmental cues.

Using 16S CARD-FISH and high throughput image analysis, we studied microbial activity patterns during a phytoplankton spring bloom off Helgoland, German Bight, in 2018 and 2020. We assessed cell volumes and ribosome densities as well as the frequency of dividing cells *in situ*. We, further, conducted grazing experiments that allowed us to calculate taxon-specific growth rates based on the frequency of diving cells.

We found that cells with faster growth rates are on average bigger and contain more ribosomes, which we expected. *Bacteroidetes* cells reacted to increased organic matter during the phytoplankton bloom and reached maximum growth rates of 2.1 d-1. Interestingly, SAR11 cells were also highly responsive to the phytoplankton bloom, had high growth rates throughout the sampling period, with a maximum growth rate of 1.7 d-1. The here studied SAR11 cells were much bigger than those previously reported from open ocean studies. Additionally, metagenomic data supports our findings of high activity in SAR11 cells.

All in all, we introduce an easy to implement way of studying activity patterns *in situ*. We further question, whether (coastal) SAR11 cells should be considered oligotrophic.

ST111

Timeseries analysis of Arctic pelagic bacterial and eukaryotic communities

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Introduction

Microbial communities as central elements of marine ecosystems show distinct spatiotemporal variability and

harbor diverse interactions, shaped by environmental parameters. However, these dynamics in polar waters remain poorly understood, but are essential to characterize for assessing the fate of the future Arctic Ocean.

Objectives

In this study we analyze amplicon sequences of prokaryotes and eukaryotes autonomously collected over four years in the West Spitsbergen and East Greenland Currents within the FRAM long-term observatory and combine this with the MOSAiC project. MOSAiC started with the icebreaker Polarstern, which drifted with the sea ice over the central Arctic over one year. The aim of the this study is to perform an explanatory spatiotemporal analysis of eukaryotic and prokaryotic dynamics. Additional cooccurrence analysis and time series forecasts allow us to describe community changing over time considering climate conditions.

Methods

Beside the various descriptive analyses we analyze the time series components of each organism and their community. Ordering the abundance data by time shift allow to characterize the appearance of each species over the year. In addition we evaluate the dynamic of prokaryotes and eukaryotes using Fourier transformation and regression analysis. This enables us to describe the individual time series components, such as seasonality and trend. Cooccurrence of species is described using a network approach based on similar species dynamics.

Results

Our results point to a fine tuned biological clock which is best described by temperature change. However, temperature explains only 60% of the variance. We identify daylight duration and eukaryotic/prokaryotic interaction as additional explanatory variables.

Conclusion

The integrated analyses of annual time series data will improve our overall understanding of the dynamics of the Arctic pelagic microbial community over the annual cycle. The narrow temperature range that enables species dynamics of primary producers and their consumers shows how sensitive the ecological system is to climate change. Comparison of species communities between the different locations revealed the uniqueness of the ecological niches.

Ref:
https://www.biorxiv.org/content/10.1101/2021.04.08.436999v2.full/

ST112
Nucleotide salvage as an energetic trade-off in slow-growing nitrifying prokaryotes
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Purines and pyrimidines play a crucial role in the nucleic acid and nucleotide metabolism of all living cells. De novo biosynthesis of nucleotides is very costly, and some microorganisms have adapted to salvage exogenous nucleobases from their environment to offset this metabolic burden. Nucleotide salvage pathways could play an important role in aquatic and engineered systems, where dissolved nucleobases are released daily by virus-mediated lysis of microbial cells. This could explain why the biomass of nitrifying prokaryotes is high in nature, while growth rates are extremely low under laboratory conditions. Understanding the process of assimilation of exogenous nucleobases by nitrifiers could be an important point for understanding global nutrient cycling. Our genomic analyses show that most nitrifying prokaryotes have the genetic potential to utilize exogenous nucleobases and express genes encoding the salvage pathway in freshwater and marine ecosystems. The presence of nucleotide salvage genes means that they are able to scavenge exogenous nucleobases for replication and growth. Using species of the genus *Nitrosomonas* as model nitrifiers, we have investigated whether exogenous supply of nucleobases affects growth and the kinetics of ammonia oxidation, which is involved in energy generation. In this presentation, I will highlight the genomic and transcriptomic landscape of nucleotide salvage in various nitrifying prokaryotes and discuss the effects of exogenous nucleobases uptake on the ecology of ammonia-oxidizing prokaryotes.

ST113
Degradation of the marine polysaccharide laminarin by a particle-associated *Maribacter forsetii*
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Flavobacteriia constitute a significant component of bacterioplankton in the marine environment. The free-living members play a dominant role in the mineralization of polysaccharides of lysed algae. They often encode carbohydrate-active enzymes (CAZymes) in polysaccharide utilization loci (PUL), operon like genetic regions encoding the proteins for hydrolysis and transport of polysaccharides (Grondin *et al.*, 2017). The fierce competition among free living bacteria has resulted in a genomic streamlining often with a focus on few polysaccharides. In contrast, particle-associated bacteria (PA bacteria) are challenged with a variety of substrates. Some can sense nutrients and move towards them, others live attached to particulate organic matter (POM) (Heins *et al.*, 2021). Their genomes are larger than the ones of free-living bacteria (Kappelmann *et al.*, 2018).The polysaccharide utilization of PA bacteria has so far received little attention.

Maribacter has been observed in particle-associated fractions in marine systems. To close the gap in physiological studies, we applied proteomics to investigate polysaccharide utilization. Laminarin is the most abundant polysaccharide storage compound of microalgae. Here we report the proteome of the PA bacterium *Maribacter forsetii* grown with laminarin as a carbon and energy source.

We grew *Maribacter forsetii* in liquid medium on several different mono- and polysaccharides, including laminarin. The analysis of the proteome was performed by a liquid chromatography coupled to an orbitrap mass spectrometry system. Protein identification and quantification was done using MaxQuant and a strain-specific protein sequence database (Tyanova *et al.*, 2016).

Growth on laminarin coincided with the induction of a glycosyl hydrolase family 16 and SusC/D outer membrane oligosaccharide transporter and several candidate CAZymes. Hydrolysis is expected to release glucose. *Maribacter forsetii* uses the glycolysis pathway to supplement their citrate cycle.

The induced CAZymes of *Maribacter forsetii* were not located in a PUL on the chromosome.

Free living and particle-associated bacteria are challenged with different environmental circumstances. Our study showed that *Maribacter forsetii* has not streamlined the genes for laminarin degradation in a PUL.

ST114
eCetch – a CO2-fixation enzyme cascade powered by electricity
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Introduction

Carbon dioxide (CO2) is an important carbon feedstock for a future green economy. To use its full potential, new strategies for an efficient CO2 conversion into multicarbon compounds are required and need to be developed.

The herein presented approach combines a CO2 fixating-synthetic enzyme cascade with an electrochemical recycling system. The integration of catalysts (Ferredoxin-NADP+-Reductase(FNR)) and NADPH-dependent enzymes of the CETCH cycle into redox matrices opens up a wide range of new possibilities and advantages like the merge of energy production (in form of NADPH) and product formation in the same place.

Objectives

This study aimed the electricity-driven regio- and stereoselective incorporation of CO2 into crotonyl-CoA by an NADPH-dependent enzymatic reductive carboxylation. Co-Immobilization of a ferredoxin NADP+ reductase and crotonyl-CoA carboxylase/reductase (CCR) within a 2,2´-viologen modified hydrogel enabled iterative NADPH recycling and stereoselective formation of (2S)-ethylmalonyl-CoA.

Methods

We use a recently reported 2,2´-viologen modified polyvinyl alcohol (V2+-PVA) as low-potential redox polymer for the immobilization of FNR directing electrons towards the reduction of NADP+.

Additional we co-immobilized CCR on a glassy carbon electrode in the viologen modified hydrogel (V2+-PVA/FNR/CCR) for electrochemical generation of Ethylmalonyl-CoA. Product formation was measured by HPLC-MS.

Results

The amount of ethylmalonyl-CoA generated after one hour of electrolysis was used to determine the faradaic efficiency for the CO2 fixation as 92 ± 6%. A product rate formation of 1.6 ± 0.4µmol cm-2 h-1 and a TOF of 1.2 ± 0.3s-1 were calculated starting from 1 mM crotonyl-CoA. Using a previously established enzyme assay, the stereochemistry of the product was confirmed as (2S)-ethylmalonyl-CoA.

Finally, we scaled-up the system into a two compartment cell using a 1 cm2 Toray paper electrode with 0.8 mM of crotonyl-CoA (1.6 µmole). The reaction was completed after 10h with a product yield of 78%.

Conclusion

This biohybrid system fosters the role of bioelectrochemical CO2 fixation and represents an important step towards the synthetic applications of NADPH-dependent enzymes. Further extension of the system through additional enzymes might allow the realization of more complex reaction cascades and pave the way towards the development of advanced electrochemically-driven synthetic C1 chemistry platforms.

ST115
Characterization of advanced exoelectrogenic biofilms using microfluidic reactors and an autonomous robotic imaging platform
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Although biofilms are the most common growth form of microorganisms in nature, modern microbiology and biotechnology still studies and applies mainly microorganisms in their planktonic state. This can lead to biased results, for example when it comes to drawing conclusions about the behavior of bacteria in their natural habitat. However, due to their complex and temporally dynamic composition, neither the cultivation nor the analysis of biofilms is easy to accomplish. Therefore, we developed a machine assisted microfluidic cultivation platform which allows to study biofilms reproducibly in a highly parallel way. Our modular system offers versatility and precision in terms of the best possible simulation of different environments while allowing for a high-throughput of experiments. The system is equipped with a robotic optical coherence tomography-based imaging systems which allows to study biofilms without the necessity of using fluorescently labeled cells. We developed this platform foremost to study biofilms consisting of exoelectrogenic organisms thriving on anode surfaces within a bioelectrochemical system and focused in particular on biofilms of the exoelectrogenic bacterium *Shewanella oneidensis* and of *S. oneidensis* together with *Geobacter sulfurreducens*. Using this system, it was possible to correlate current density to biofilm volume and compactness over the whole time course of biofilm growth. Genetically engineered strains were developed in which the biofilm growth phenotype was tailored and the effect of these variations on current and biofilm production was studied. Moreover, the integration of solenoid valves and/or micropumps enabled the addition of conductive materials in alternating cycles to increase biofilm conductivity and further enhance biofilm growth and/or current production. In summary, electroactive biofilm growth under anoxic conditions using an anode as electron sink can be studied using the developed microfluidic cultivation platform. The manifold possibilities of this platform will be shown by selected examples.

ST116
Hydrogen-mediated extracellular electron transfer enables high performance microbial electrosynthesis in *Clostridium ljungdahlii*
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Microbial electrosynthesis has recently emerged as an alternative way of recycling carbon dioxide into valuable organic chemicals using electricity. Due to the current ecological transition, being able of substituting chemical production from fossil fuels by more sustainable processes is required. Nevertheless, microbial electrosynthesis is currently limited by the poor control and understanding over the biological aspects of the process. In order to increase the performance and applicability of microbial electrosynthesis, a deeper knowledge about the mechanisms of electron transfer is required. In acetogens, and more specifically in *Clostridium ljungdahlii*, biofilm-dependent direct electron transfer was initially proposed (1) while recent studies tend to assume hydrogen-mediated electron transfer as the main electron transfer mechanism involved (2). Using online and offline data collection, coupled with highly precised *in situ* measurements, it was possible to empirically prove for the first time that hydrogen mediates extracelullar electron transfer in *C. ljungdahlii*. Besides, the performance and the robustness of the process was dramatically increased by decoupling growth from biofilm formation, and actively promoting planktonic growth. Hence, the improvement of the performance of *C. ljungdahlii* and the deeper understanding about its electrophysiology, is an important step towards the goal of achieving scalable and feasible electro-fermentations that would allow carbon dioxide recycling and reutilization.

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ST117

Microbiome dynamics during *n*-caprylate production from ethanol and acetat.

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Ethanol and short-chain carboxylic acids (e.g., acetate) can be converted into medium-chain carboxylic acids (MCCAs) *via* chain elongation. With every elongation cycle, two ethanol-derived carbon atoms are added to the initial carboxylic acid. MCCAs are of high economic interest due to their versatile product function. Chain elongation has been demonstrated to work reliably and long-term with microbiomes up to a carbon chain length of eight atoms (*n*-caprylate) (Kucek et al. 2016). However, the pertinent populations in these microbiomes have not been linked to their function of *n*-caprylate formation. Here, we used process control and metagenomic approaches to study *n*-caprylate production with two open culture bioreactors. Both bioreactors were operated independently with identical operating conditions throughout an operating period of two years. One of the bioreactors was operated as an upflow anaerobic sludge blanket reactor with an active volume of 5 L, the other one was operated as an anaerobic filter and was filled with packing material (Kaldnes K1 25 Liter, KoiCompetence Witten, Germany), resulting in an active volume of 4.2 L. The bioreactors were fed with a basal medium with differing ethanol-to-acetate ratios and produced mainly *n*-caprylate and *n*-caproate. At a ratio of ethanol and acetate of 6:1, the *n*-caprylate to *n*-caproate productivity ratio was 2.5:1. The metagenome was analyzed in-house with a MinIon flow cell (Oxford Nanopore Technologies, Inc., Oxford, UK). The acquired metagenomic data revealed a dominance of *Methanobacterium congolense* (~30% abundance) and *Clostridium kluyveri* (~7% abundance). Members of the genus *Caproicipoducens* and *Oscillibacter* were also abundant (~1-2% abundance). The metagenomic results highlight the importance of hydrogen uptake by methanogens and the presence of chain elongating microbes in our bioreactor system. Current analysis on microbial community dynamics and *n*-caprylic acid production will determine the critical microbes involved. Knowledge of the link between presence and function will enable a more efficient bioreactor control.

Kucek, L. A., Spirito, C. M. and Angenent, L. T. (2016). *"High n-caprylate productivities and specificities from dilute ethanol and acetate: chain elongation with microbiomes to upgrade products from syngas fermentation."* Energy & Environmental Science **9**(11): 3482-3494.

ST118

Community dynamics during mixotrophic anaerobic fermentation with H_2, CO_2, CO, and ethylene supplementation

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Anaerobic fermentation produces carboxylates from organic feedstock and relies mostly on heterotrophic bacteria. Thus, these systems have their efficiencies limited by substrate quality and carbon and electrons routed into greenhouse gases (CO_2 and CH_4). Supplying H_2, CO_2, CO, and ethylene (syngas) can favor autotrophic and mixotrophic bacteria in microbial communities growing on organic substrates with the potential to steer carbon and electron flows and decrease dependence on the feedstock.

Here, we aimed to understand the effects, potentials, and limitations of supplementing H_2, CO_2, CO, and ethylene to anaerobic microbial communities feeding on organic substrates.

Data originated from experiments with anaerobic reactors operated with continuous gas recirculation and batch cultures in serum bottles[1-2]. Acetate/lactate or corn silage were used as organic substrates. Microbial communities were analyzed by 16S rRNA gene amplicon sequencing and Spearman correlations were obtained with $p<0.01$.

Methanogens (*Methanobacterium* and *Methanobrevibacter*) were present in all systems when H_2/CO_2 was co-fed and misrouted electrons from H_2 into CH_4. However, 1.5 kPa ethylene (which was not consumed), >9.0 kPa CO, or a mixture of both gases were effective at inhibiting methanogenesis. Without methanogens, *Clostridium* (related to *C. luticellarii*), *Oscillibacter*, and *Eubacterium* benefited most from H_2/CO_2 and $H_2/CO_2/CO$ by growing via homoacetogenesis and causing net carbon fixation. They were also linked to lactate/acetate consumption and production of butyrate, valerate, and caproate. CO inhibited strongly *Caproiciproducens* (a genus not linked to autotrophy) but did not inhibit Actinobacteria and lactic acid bacteria. *Acidipropionibacterium* appeared as an opportunistic propionate fermenter when clostridia were inhibited by high CO pressures (48 kPa) or by O_2 contamination.

Various clostridia were responsible for carbon fixation and for increasing the pool of carboxylates by consuming $H_2/CO_2/CO$, but could only thrive when methanogens were inhibited by ethylene and CO. Mixotrophy of anaerobic microbial communities can be employed with the help of such gases in order to improve sustainability and yields of anaerobic fermentation.

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2. Baleeiro, F. C. F.; Ardila, M. S.; Kleinsteuber, S.; Sträuber, H., Frontiers in Bioengineering and Biotechnology 2021, 9.

ST119

Synthetic co-culture of *A. woodii* and *C. drakei* for the production of caproate from $H_2 + CO_2$

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Introduction: The model acetogen *Acetobacterium woodii* is known for its high CO_2 assimilation capabilities and recombinant strains can produce bio-commodities such as acetone, isobutanol and lactate. Lactate is a suitable feedstock for a variety of bacteria. Chain elongating *Clostridia* such as *Clostridium drakei* can consume lactate and produce medium-chain organic acids via reverse β-oxidation. Co-cultivation of respective strains is an approach to facilitate conversion of $H_2 + CO_2$ to medium-chain organic acids via lactate.

Objectives: This work aims to construct an engineered fluorescent lactate-producing *A. woodii* strain and cultivate it in a synthetic co-culture with *C. drakei* to produce caproate from H_2 and CO_2. Furthermore, the fluorescence-activating and absorption-shifting tag protein (FAST) helps to characterize recombinant gene expression behaviour and to differentiate the involved strains.

Material & methods: The first step in construction of *A. woodii* Δ*lctBCD* Δ*pyrE* [p83_P_{bgaL}_NFP] was deletion of genes encoding the native lactate dehydrogenase complex. Secondly, plasmid-based expression of a codon-optimized fusion gene, encoding an N-terminally FAST-tagged lactate dehydrogenase (NFP) from *Leuconostoc mesenteroides,* facilitated lactate production and fluorescence emission. To ensure compatibility of *C. drakei* with the engineered *A. woodii* strain*, in silico* analysis for bacteriocins, using the Bagel4 software, as well as cross-streak assays were performed. Autotrophic batch co-cultivations were performed while tracking growth parameters, fluorescence and metabolic products.

Results: Co-cultivation of *A. woodii* Δ*lctBCD* Δ*pyrE* [p83_P_{bgaL}_NFP] and *C. drakei* resulted in 4 mM caproate and 18.5 mM butyrate from $H_2 + CO_2$ as substrate. Lactate served as an intermediate since it could not be detected above traces. Control experiments using *A. woodii* Δ*lctBCD* Δ*pyrE* [p83] in combination with *C. drakei* as well as monocultures confirmed the approach. The fluorescence of *A. woodii* Δ*lctBCD* Δ*pyrE* [p83_P_{bgaL}_NFP], mediated by the FAST-LdhD fusion protein, showed similar strength compared to the respective monoculture, which produced 20 mM lactate from $CO_2 + H_2$.

Conclusion: The synthetic co-culture produced caproate under autotrophic conditions. FAST mediated fluorescence proved the production of the recombinant lactate dehydrogenase and confirmed lactate production, which was subsequently consumed by *C. drakei*.

ST120

Metabolic engineering of *Vibrio natriegens* for the anaerobic succinate production

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The biotechnological succinate production bears serious potential to replace existing petrochemical approaches in the future. To meet economic viability, bioprocesses must operate at high titer, yield, and productivity, whilst making use of inexpensive substrates and cultivation media. *Vibrio natriegens* is known for its high biomass specific substrate uptake rate, which is a promising trait to achieve high productivities.

Here, we report a first approach aiming at the anaerobic succinate production with *V. natriegens*, which consists of essential metabolic engineering and an optimization of the production environment. The final producer strain *V. natriegens* Δ*dldh* Δ*lldh* Δ*pfl* Δ*ald* Δ*dns*::pyc_{Cg} (Succ1) retained a high growth rate of 1.41 h^{-1} under aerobic conditions (90% of the wild type strain) and yielded 1.46 mol of succinate per mol of glucose under anaerobic conditions (85% of the theoretical maximum). *V. natriegens* Succ1 revealed a particularly high biomass-specific substrate uptake rate of 1.38 g_{Glc} g_{CDW}^{-1} h^{-1} and succinate production rate of 1.33 g_{Succ} g_{CDW}^{-1} h^{-1}. Based on the quantified metabolites, we determined the intracellular carbon flux through applying carbon and redox balancing, and show that under the tested conditions the reductive TCA as well as the oxidative TCA and/or glyoxylate pathway contributed to succinate formation. Eventually, we exploited the high biomass-specific values of our engineered strain in a zero-growth bioprocess using minimal medium devoid of complex additives and expensive supplements, and obtained a final titer of 60.4 g_{Succ} L^{-1} within 7 h of fermentation with a maximum productivity of 20.8 g_{Succ} L^{-1} h^{-1}.

The key performance indicators (titer, yield, productivity) of this simple engineering approach to produce succinate in *V. natriegens* compete already with those of costly tailored microbial production systems and represent a good starting point for future process engineering.

ST121

Influence of biomass on hydrogen production with *Parageobacillus thermoglucosidasius* DSM 6285

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The replacement of fossil fuels into renewable energies is an undeniable step, and hydrogen has emerged as a versatile energy carrier with a high energetic yield.[3,8] Production of hydrogen can occur with the help of hydrogenogenic carboxydotrophs, able to perform the water-gas shift (WGS) reaction, where CO reacts with water to produce hydrogen and CO_2.[1,4] CO-driven H_2 production has been reported in some microorganisms, adapted to use CO as an energy and/or carbon source.[5]

It has been demonstrated that *Parageobacillus thermoglucosidasius* can produce hydrogen, performing WGS.[2,6] A lag phase between O_2 depletion and the

beginning of H_2 production has been described, and different parameters were optimized in batch experiments with *P. thermoglucosidasius* DSM 6285.[7] One of the parameters previously evaluated for the fermentation of *P. thermoglucosidasius* was the agitation rate, which is critical for the transfer of CO to the microorganism.

Based on the results from previous batch experiments, the aim of this study was to increase hydrogen yield and reduce the lag phase before hydrogen production with *P. thermoglucosidasius* DSM 6285. To achieve this purpose, a two-phase fermentation containing an aerobic and anaerobic step was performed, and different initial biomass concentrations and flow rates were evaluated. Batch fermentations were performed in 2.5 L stirred tank reactors for 7 days at 55 C, pH 7.0 and mLB media. The highest H_2 production rate was 0.6 mmol min^{-1} and corresponded to the OD600 of 1.21, using a continuous flow rate of air and CO, followed by a gas exchange to a mixture of nitrogen and CO.

The results underlay the importance of biomass and CO availability for an increase in hydrogen production, and can be used as a reference data for the evaluation of a continuous fermentation in the way of improvement of the biohydrogen platform.

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ST122

The glyoxysomal protease LON2 is involved in fruiting-body development, ascosporogenesis and stress resistance in *Sordaria macrospora*

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Microbodies, including peroxisomes, glyoxysomes and Woronin bodies, are ubiquitous dynamic organelles that play important roles in fungal development. The ATP-dependent chaperone and protease family Lon that maintain protein quality control within the organelle significantly regulate the functionality of microbodies. The filamentous ascomycete *Sordaria macrospora* is a model organism for studying fruiting-body development. The genome of *S. macrospora* encodes one Lon protease with the C-terminal peroxisomal targeting signal (PTS1) Serine-Arginine-Leucine (SRL) for import into microbodies. Here, we investigated the function of the protease SmLON2 in sexual development and during growth under stress conditions. Localization studies revealed a predominant localization of SmLON2 in glyoxysomes. This localization depends on PTS1, since a variant without the C-terminal SRL motif was localized in the cytoplasm. A ΔSmlon2 mutant displayed a massive production of aerial hyphae, and produced a reduced number of fruiting bodies and ascospores. In addition, the growth of the ΔSmlon2 mutant was completely blocked under mild oxidative stress conditions. Most of the defects could be complemented with both variants of SmLON2, with and without PTS1, suggesting a dual function of SmLON2 not only in microbody but also in cytosolic protein quality control.

ST123

Spore-type specific chemotropic growth to maize roots determines root infection of *Colletotrichum graminicola*

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Colletotrichum graminicola is a foliar maize pathogen that produces two morphological different asexual spores, which are oval and falcate shaped. The hemibiotrophic fungus causes the disease anthracnose characterized by lesions on leaves, stalk rot, dead of seedlings, and top dieback. Besides penetration of leaves and the stalk, falcate shaped conidia can enter plants via roots (Sukno et al., 2008). Our work shows that oval conidia can penetrate maize roots as well. However, the root infection process in the field involves the root recognition prior to infection by conidia, which are present in the soil. In a current project, we are investigating the process of root recognition in detail.

A different adaptation of oval and falcate shaped conidia of *C. graminicola* was shown previously for maize leaf infection. Falcate conidia secrete mycosporine-glutamine as a germination inhibitor to inhibit premature germination and are able to form appressoria from a single spore to penetrate maize leaves. A high density of oval conidia is required for formation of conidial anastomosis tubes (CAT) prior to leaf penetration, making them less efficient in leaf infection compared to the falcate conidia (Nordzieke et al., 2019). Also for root infection, a spore-type specific adaptation exists. We have analyzed *Zea mays* root infection outgoing from spore-enriched soil. Contrary to leaf infection, oval conidia are more efficient in this root infection process, indicated by a stronger reduction of plant length and biomass by oval compared to falcate conidia. Our analysis further provides evidence that oval conidia of *C. graminicola* grow chemotropically towards maize root exudate (MRE), whereas falcate conidia do not respond to MRE treatment. Current results of HPLC/MS analyzing extracted MRE indicate that a root-secreted diterpenoid might be the main attracting signal for oval conidia. The identification of the exact molecule is part of our current investigation. In summary, our results indicate that oval conidia are more efficient in root infection of *C. graminicola* of its host maize.

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ST124

RNA editing during fungal sexual development leads to new protein isoforms

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RNA editing is the selective insertion, deletion, or substitution of nucleotides and is conserved in all domains of life. RNA editing of protein-coding transcripts leads to sequence changes in the transcript as well as the protein that could alternatively be directly encoded in the DNA. In fungi, adenosine (A) to inosine (I) RNA editing was recently detected to occur in protein-coding transcripts during sexual reproduction. Our model system is *Sordaria macrospora*, a filamentous fungus that has been studied genetically since the 1950s. It reproduces sexually by generating complex pear-shaped fruiting bodies (perithecia) containing sexual spores (ascospores). RNA-seq as well as proteomic and proteogenomic analysis revealed a number of edited transcripts as well as affected proteins during fruiting body and ascospore formation. We named the respective genes *edited in fungal development* (*efd*) genes. Interestingly, in fungi, amino acid codons, but also stop codons tend to be affected by editing, the latter leading to a change of *TAG* or *TGA* codons to *TGG* tryptophan codons. As a consequence, proteins are extended at the C-terminus. We focused further functional analysis on transcripts that undergo stop-loss editing, because here we proposed to find a stronger effect on protein function then by a single amino acid variation. Deletion of several *efd* genes indeed revealed a function of these genes in late development during ascospore formation. Complementation studies with mutations of the native stop codon to a *TGG* (always long protein) or a *TAA* (always short protein) revealed possible functions for the editing sites. We show results on several *efd* mutants as well as on approaches to identify the fungal editing enzyme, which so far remains elusive.

ST125

Membrane proteins as virulence factors in the *Ustilago maydis* – maize pathosystem

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Smut fungi comprise a large group of biotrophic phytopathogens infecting important crops such as wheat and corn. The biotrophic parasite *Ustilago maydis* causes corn smut, a disease characterized by large tumors on all aerial parts of the maize plant. During infection, *U. maydis* secretes more than 300 effector proteins to suppress plant immune reactions and manipulate the metabolism of the host plant. Current research in the field of plant microbe interactions focusses strongly on these apoplastic and cytoplasmic protein effectors. However, membrane-associated virulence factors have been much less studied to date.

To fill this gap, we employed a computational approach to identify membrane proteins in *Ustilago maydis* that are linked to virulence as judged by elevated gene expression during infection of maize plants. To our surprise, this investigation revealed several novel proteins lacking domains of known function that are conserved among related smut fungi. Of this set, we further investigated seven membrane proteins that show elevated gene expression during the early stage of host colonization. We show that three of these, named Vmp1, Vmp2 and Vmp3 (for: **v**irulence-associated **m**embrane **p**rotein), are essential for the full virulence of *U. maydis* and deliver an initial characterization by combining genetic and biochemical methods (1). Our findings are a first step towards understanding how membrane proteins contribute to virulence in smut fungi and set the stage for an in-depth molecular characterization.

(1) Weiland P & **Altegoer F** (2021) Identification and Characterization of Two Transmembrane Proteins Required for Virulence of Ustilago maydis. *Front. Plant Sci.* 12, 669835. doi: 10.3389/fpls.2021.669835

ST126

Role of itaconate in the infection process of the human opportunistic pathogen *Candida albicans*

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The need to understand fungal infections leads us to investigate the role of the antimicrobial metabolite itaconate in human macrophage cell line where it is highly upregulated after infection with *Candida albicans*. In this context our preliminary results showed that *C. albicans* cells itself have a strong impact on the metabolic pathway and the immunemetabolism.

In eucaryotic cells IRG1 (immune response gene 1) is responsible for the conversion of *cis*-aconitate to itaconate. During microbial infections IRG1 is highly induced and the infected microorganisms are killed. In the case of macrophage-Candida engulfment we measured lower IRG1 levels with mass spectrometry analyses and lower itaconate concentration with mass spectrometry analyses. These results suggested that IRG1 maybe is somehow repressed after infection with this pathogen. After *Candida albicans* infection with the macrophage cell line RAW264.7 experimental studies with qPCR showed reduced level of pro-inflammatory cytokines like IL-1, IL-6 and TNF-alpha in contrast to the control with LPS stimulated cells. These results suggest that *Candida albicans* is able to evade the inflammatory pathways of the host with other virulence factors to develop from a commensal to a pathogen.

ST127

Identification of F-Box proteins involved in the regulation of sugar metabolism

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Filamentous fungi naturally thrive on decaying plant matter. Due to their ability to secrete a range of enzymes required for the deconstruction of lignocellulosic material, filamentous fungi are of particular interest for biotechnological processes based on the biotransformation of plant biomass, a renewable resource for the circular bioeconomy. A major drawback in fungal hydrolytic enzyme production is the carbon catabolite repression (CCR), the natural repression of genes in the presence of easily metabolizable carbon

sources such as glucose, a highly conserved and multi-faceted process.

F-Box proteins are known to be involved in many metabolic adjustments including CCR regulation. They destine proteins for the proteasomal degradation process, e.g. to make way for new cellular or metabolic states. F-box proteins are found in all eukaryotes and in fungi, they are, among other functions, involved in glucose sensing and induction of cellulolytic genes. Nevertheless, the specific function of the majority of the F-Box proteins present in filamentous fungi remains enigmatic.

To identify F-Box proteins involved in the switch between CCR and lignocellulose utilization, we employed the genetics reference organism *Neurospora crassa* to investigate 40 genes with putative F-Box protein function. *N. crassa* deletion strains were screened to identify aberrant phenotypes related to CCR. Enzymatic activity profiles on cellulose and glucose, a CCR-inducing sugar, revealed nine strains with a significant repression of cellulase activity, while ten showed a significant de-repression compared to the wild type strain. An additional screening of the F-Box gene deletion strains on glucose medium supplemented with allyl alcohol (becoming a toxic compound if metabolized by CCR-sensitive alcohol dehydrogenases) confirmed a CCR-deficient phenotype for some of these strains.

These findings led to the identification of candidate genes with a strong potential for regulatory importance in lignocellulose signaling pathways, which are currently investigated in more detail. A better understanding of the function of F-Box proteins will be essential to allow a targeted and rational modification, leading to improved enzyme-producing strains of high interest for the industry.

ST128

Comparative genomics of fungal hybrids in the anther smut complex *Microbotryum*

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Obligate pathogens like smut fungi often evolve intimate relationships to their host plants resulting in high host specificity. The anther smut complex of the genus *Microbotryum* on Caryophyllaceae is characterized by fungal pathogens specialized to different host species. Adaptation and specialization to these host species involve well adapted mating processes as well as repertoires of secreted proteins and effectors.

To identify genes essential for host invasion and coding for host specificity as well to understand chromosomal rearrangements in hybrids, we bred artificial hybrids between the host-specific smuts *M. lychnidis-dioicae* and *M. silenes-acaulis* or *M. coronariae*, and applied selection on corresponding host plants. Also, we analyzed the genomes of the respective parental strains.

Secretome comparison of selected *Microbotryum* species revealed that the majority of genes are orthologous, indicating similar biological function of similar biological features in these anther smuts. Interestingly, the count of orthologous genes solely shared between *M. lychnidis-dioicae* and the two host-specific species vary heavily which may have an effect on infection success.

Genomic analyses of interspecific fungal hybrids revealed that the majority of loci of assessed individuals were inherited from one parental species. Moreover, we detected signs of crossing over in the genomes of the analyzed hybrids. We identified species-specific candidate genes that occur in hybrid genomes posed under host plant-driven selection which may be crucial for infection and sporulation. In conclusion, first, our data indicate a strong influence of intra-genomic conflicts on the viability of hybrids, but, secondly, suggest that only a limited set of genes is required for successful host-specific interaction resulting in infection.

ST129

New insights into the architecture of the fission yeast kinetochore at a nanometer resolution

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The kinetochore is a multi-protein complex organizing and regulating the correct segregation of sister chromatids during cell division. It acts as a linker between the microtubules emanating from the spindle pole body (SPB) and the centromeric chromatin. A malfunctioning kinetochore can result in the incorrect distribution of chromosomes. The detailed investigation of its composition, structure and function promises new insights into aneuploidy-related diseases such as cancer, birth defects and cell death (Pfau and Amon 2012).

Recently, we have developed a multi-color single molecule localization microscopy (SMLM) imaging strategy that uses primed photoconversion and UV-photoactivation for quantitative, aberration-free imaging at the nanometer scale (Virant et al. 2017). We employed this method to image key structural proteins of the *Schizosaccharomyces pombe* kinetochore in relation to two reference proteins, one at the SPB and the other at the centromere. This arrangement allowed us to frame our single-protein work (Lando et al. 2012) in a multi-protein context. We then established a streamlined imaging and data post-processing pipeline that executes drift correction with fiducial markers (Balinovic et al. 2019), channel alignment, clustering, protein counting and Bayesian inference to determine protein distances within the complex. From this quantitative workflow, we constructed a map of the fission yeast kinetochore at a nanometer resolution and compared its insights to data from other organisms found in current literature.

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ST130

How a methanogen fixes Sulfate

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Hydrogenotrophic methanogens are strictly anaerobic archaea that thrive at the thermodynamic limit of Life and assimilate Sulfur by the direct incorporation of Sulfides (S^{2-}). It was accepted that methanogens cannot perform assimilatory sulfate (SO_4^{2-}) reduction due to obstacles such as toxic intermediates and energetic barriers. However, *Methanothermococcus thermolithotrophicus*, a marine thermophile, breaks this dogma: it can grow on SO_4^{2-} as sole Sulfur source [1]. Here, we investigated how these two ancient pathways, hydrogenotrophic methanogenesis and sulfate reduction, can coexist in this organism.

Using a complementary approach of physiological, biochemical and structural studies, we elucidated *M. thermolithotrophicus* complete SO_4^{2-} reduction machinery.

SO_4^{2-} is transported inside the cytoplasm where it is activated and phosphorylated by an ATP Sulfurylase and APS Kinase, which produce 3'-Phosphoadenosine-5'-phosphosulfate (PAPS). While these two enzymes are common to other organisms, the further steps are carried out by novel enzymes, which are, so far, unique to methanogens and ANME.

The PAPS will be converted into Sulfite (SO_3^{2-}) and 3'-Phosphoadenosine-5'-phosphate (PAP). Assimilatory (P)APS Reductases are phylogenetically and structurally distinct from the dissimilatory APS Reductases. Interestingly, the PAPS Reductase of *M. thermolithotrophicus* has a high structural homology with the dissimilatory version; however, the conserved residues recognizing the APS substrate are different and switch the specificity towards PAPS.

The toxic product PAP is efficiently and specifically hydrolyzed to AMP by a novel type of PAP Phosphatase, which, based on its structure, probably derived from an exonuclease.

SO_3^{2-} is a poison for methanogens. It must be quickly converted to HS^- by the coenzyme F_{420}-dependent Sulfite Reductase (Fsr, [2]), a single enzyme composed of a $F_{420}H_2$-oxidase and a novel class of Sulfite Reductase. Our crystal structures revealed an architecture similar to the dissimilatory Sulfite Reductases but an active site and enzymatic properties identical to assimilatory ones. Because of its primitive organization, Fsr would provide a plausible picture of a Sulfite Reductase prototype.

These results give the first insights into how a methanogen turns SO_4^{2-} into an elementary block of Life.

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ST131

Stress in the microbial community: unraveling carbon, nitrogen and sulfur metabolic pathways throughout disturbances in a bioreactor mimicking anoxic brackish coastal sediments

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Microbial communities are key drivers of carbon, sulfur and nitrogen cycling in coastal ecosystems, where they are experience to dynamic shifts in substrate availability and exposure to toxic compounds. However, how these shifts affect microbial interactions and function is poorly understood. Our research aims to better comprehend how such microbial community changes occur in a controlled laboratory reactor mimicking coastal sediment conditions. This will allow to shed light on the environmental distribution and resilience of different microbial communities under current and future disturbances.

We used metagenomics and metatranscriptomics to investigate microbial community structure and transcriptional responses to prolonged ammonium deprivation and sulfide and nitric oxide toxicity stresses. *Candidatus* Nitrobium versatile, identified in this study as a sulfide-oxidizing denitrifier, became a rare community member upon ammonium removal. The ANaerobic MEthanotroph (ANME) archaeon *Ca.* Methanoperedens showed remarkable resilience to both experimental conditions, dominating transcriptional activity of dissimilatory nitrate reduction to ammonium (DNRA). During the ammonium removal experiment, increased DNRA was unable to sustain anaerobic ammonium oxidation (anammox) activity. After ammonium was reintroduced, a novel anaerobic bacterial methanotroph species that we have named *Ca.* Methylomirabilis tolerans outcompeted *Ca.* Methylomirabilis lanthanidiphila while the anammox bacterium *Ca.* Kuenenia stuttgartiensis outcompeted *Ca.* Scalindua rubra. At the end of the sulfide and nitric oxide experiment, a Gammaproteobacterium affiliated to the family *Thiohalobacteraceae* was enriched and dominated transcriptional activity of sulfide:quinone oxidoreductase.

Our results indicate that some community members are more resilient to stresses than others, leading to dynamic microbial community shifts and novel functional states in coastal ecosystems. Anaerobic methane and sulfide oxidation could be ecosystem functions preserved across the investigated disturbances, while differing nitrogen cycling pathways might be favored in response to stresses. These insights will help to understand and predict coastal ecosystem responses to future disturbances.

ST132

Not all that glitters is gold: putative role of the unique genomic loci of carbon monoxide-oxidizing *Parageobacillus thermoglucosidasius* strains in modulating the WGS reaction

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Parageobacillus thermoglucosidasius is the first facultative anaerobe demonstrated to catalyse the biological water gas shift (WGS) reaction, which serves as a source of carbon and energy among various mesophilic and thermophilic taxa. The thermophile, *P. thermoglucosidasius* produces hydrogen (H_2) through carbon monoxide (CO) oxidation by the mediation of Ni-Fe-S CO dehydrogenase (Coo) and Ni-Fe hydrogenase (Phc). These enzymes are coded by genes (*cooCSF* and *phcA - L*) which are co-localised in a unique fifteen gene genomic region (1,2). Hence, the presence of this locus in all studied *P. thermoglucosidasius* genomes led to the prediction of the hydrogenogenic phenotype. However, "not all that glitters is gold", here, we show that under 50% CO and 50% air atmosphere, the majority of *P. thermoglucosidasius* strains are incapable of catalysing the WGS reaction. Of seven previously uncharacterised strains, only one, oxidises CO to yield H_2 despite the presence of the *coo - phc* genes. Comparison of seven available *P. thermoglucosidasius* genomes from three and four CO and non-CO oxidising strains, respectively, showed that the former harbour two unique loci. Annotation of the proteins encoded by the chromosomal hydrogenogenic strain unique locus (HSUL) 1 and the plasmid HSUL 2 revealed putative nickel uptake and catechol degradation functions for the respective loci. Combined, the reliance of the WGS reaction enzymes on nickel and the necessity of catechol detoxification, as consequences of CO induced siderophore-mediated iron acquisition (3), suggest a putative role of HSUL 1 and 2 in modulating the hydrogenogenic phenotype. Future studies will focus on investigating the *P. thermoglucosidasius* nickel and iron uptake systems to provide clearer insight into their roles in shaping the WGS reaction.

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ST133

Molecular basis of a novel electron bifurcation mechanism in the [FeFe]-hydrogenase HydABC

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Introduction: Flavin-based electron bifurcation is a novel mechanism to couple endergonic and exergonic redox-reactions widespread in anaerobes. The electron bifurcating [FeFe]-hydrogenase HydABC is a key respiratory enzyme that catalyses electron transfer from H_2 gas oxidation to both NAD^+ and ferredoxin (Fd), but the structure and molecular mechanism of this enzyme complex has remained enigmatic.

Objectives: We aimed to elucidate the molecular basis of electron bifurcation in the [FeFe]-hydrogenase HydABC.

Materials & methods: We have combined single-particle cryo-electron microscopy (cryo-EM), biochemical assays with mutagenesis experiments and molecular simulations to unravel the molecular basis of electron bifurcation.

Results: HydABC was produced homologously in *Thermoanaerobacter kivui* Δ*hydAB* using the pMU-based plasmid system. The enzyme was purified to apparent homogeneity and catalysed electron-bifurcation from H_2 to $NAD(P)^+$ and Fd with a specific activity of 20.6 U/mg. The reverse reaction could be observed with 8.9 U/mg. The cryo-EM model revealed a homodimer with one flavin and strings of Fe-S cluster leading from hydrogen to $NAD(P)^+$ and Fd. Site directed mutagenesis and molecular simulations identified clusters potentially involved in electron flow.

Conclusions: Combining the structural model with site-directed mutagenesis studies allows to explain how the single FMN cofactor switches between Fd and NAD^+ reduction and reveal how the electron-bifurcating [FeFe]-hydrogenase is able to perform bifurcation with just a single flavin cofactor.

ST134

Structure and function of a 1 MDa electron-bifurcating, dearomatizing enzyme complex from *Geobacter metallireducens*

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Benzoyl-CoA reductases (BCRs) are key enzymes in the anaerobic microbial degradation of aromatic compounds, catalysing the reduction of benzoyl-CoA to cyclohexa-1,5-diene-1-carboxyl-CoA. This reaction proceeds at a redox potential of $E°' = -622$ mV, far below any known physiological electron donors. Two classes of structurally unrelated BCRs have been identified: While class I BCRs couple the endergonic reduction of benzoyl-CoA to ATP hydrolysis in facultative anaerobes, ATP-independent class II BCRs catalyse this reaction in obligate anaerobes by a proposed flavin-based electron bifurcation.[1] Class II BCRs from *G. metallireducens* are membrane-associated and consist of eight subunits ($Bam[(BC)_2DEFGHI]_2$). The subunits share similarities with aldehyde:Fd oxidoreductases (BamB), electron-bifurcating heterodisulfide reductases (BamDE), and respiratory complex I (BamGHI). They harbour 4 tungstopterin, 2 selenocysteins, 6 FAD and >50 FeS clusters, making them one of the most complex metalloenzyme machineries known.[2]

Here, we applied cryo-electron microscopy to gain insights in the largely unknown structure and function of this complex, obtaining a solid model for the $Bam[(BC)_2DE]_2$ subcomplex at 2.6 Å. The $Bam(FGHI)_2$ components, though partially resolved, showed a high flexibility that hampered structure determination. The arrangement of electron input and output modules identified a NADH oxidoreductase as electron donor, a benzoyl-CoA reductase as low-potential electron acceptor, and, most possibly, a flavin-dependent oxidoreductase as high-potential electron output module.

Surprisingly, the modules similar to those involved in heterodisulfide reduction in electron-bifurcating enzymes from methanogens appear to have only structural or electron storage function. We propose that the high-potential electrons are transferred to menaquinone (MK) via electron-transferring flavoproteins (ETFs) and membrane-bound ETF:MK oxidoreductases. The latter do not only play a key role in the anaerobic degradation of aromatic compounds, but are abundant in many MK-containing microorganisms capable of β-oxidation, including syntrophs and pathogens such as *Mycobacterium tuberculosis*.

1Kung *et al.* 2009, *PNAS*

2Huwiler *et al.* 2019, *PNAS*

ST135

Tungsten containing Aldehyde Oxidoreductase from *Aromatoleum aromaticum* and Maturation of the Tungsten Cofactor

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Aldehyde Oxidoreductase from *Aromatoleum aromaticum* EbN1 is a tungsten containing enzyme and belongs to the subfamily of bacterial AORs. The enzyme catalyzes the oxidation of aldehydes to the corresponding carbonic acids and has been shown to be active on a broad variety of aliphatic and aromatic substrates, suggesting a physiological role in the elimination of toxic aldehyde intermediates [1].

AOR consists of three different subunits and contains a tungsten bis-molybdopterin cofactor in the active site. By transfer via five Fe_4S_4-clusters, electrons are transferred to a FAD cofactor. *In vitro*, AOR uses either NAD+ or benzyl viologen as electron acceptor, which suggests that NAD+ or ferredoxin act as the natural electron acceptor [1].

Using the close relative *Aromatoleum evansii* as host organism, we established a heterologous expression system for AOR that allows recombinant expression of highly active affinity-tagged AOR. The now accessible quantities of purified enzyme allow further biochemical and structure biological characterization.

Besides the enzyme itself and its potential biotechnological applications, we are also interested in the maturation process of the tungsten cofactor. As biosynthesis of the W-bis-MPT in *A. aromaticum* follows the well investigated pathway of molybdenum cofactor synthesis, the organism has to discriminate between molybdenum and tungsten. Therefore, we focused on the paralogues of molybdopterin molybdotransferase, moeA1 and moeA2. Our recombinant system allows expression of tagged AOR in genetically altered strains of *A. evansii* in order to investigate the role of these genes.

[1] Arndt et al. (2019) *Front. Microbiol.* 10:71

ST136

Hydrogen-dependent CO_2 reductase forms nanowire-like filaments and bundles inside cells to maximize enzymatic activity

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Introduction: Among the most fascinating and versatile organisms are obligate anaerobic microorganisms that can live on the metabolism of gases alone, even under conditions of extreme energy limitation. Typical examples of this organism are acetogenic bacteria that play a key role in the anaerobic food chain and in the global carbon cycle. Acetogens use acetate formation via the Wood-Ljungdahl pathway as a terminal electron-accepting, energy-saving CO_2 fixation process. The first step of the methyl-branch is performed by the filamentous hydrogen-dependent CO_2 reductase (HDCR), which is the only known biological catalyst that can directly reduce CO_2 to formate using molecular hydrogen as the sole electron donor. It thereby shows a higher activity than any other known biological or chemical catalyst and has therefore gained considerable interest in two areas of global importance: achieving renewable carbon neutral energy and combating climate change by removing CO_2 from the atmosphere.

Objective: We aim to determine the mechanistic basis of the high catalytic conversion rate of HDCR.

Materials & methods: We used cryo electron microscopy and mutational analyses in combination with biochemical data to understand the molecular basis of the unsurpassed HDCR activity. Additionally, to investigate what role the filament nature of HDCR plays *in-vivo* we imaged native *Thermoanaerobacter kivui* cells with cryo electron tomography.

Results: We could determine the structure of a short HDCR filament from the acetogenic bacterium *T. kivui*. The minimum repeating unit is a hexamer consisting of a formate dehydrogenase (FdhF) and two hydrogenases (HydA2) bound around a central core of one HycB3 and two HycB4. These small bacterial polyferredoxin-like proteins oligomerize via their C-terminal helices to form the backbone of the filament. Inside cells, HDCR filaments bundle to form large barrel-shaped superstructures attached to the plasma membrane.

Conclusion: By combining structure-directed mutagenesis with enzymatic analysis, we demonstrate that a network of [4Fe4S] clusters connects all catalytic centers along the filament. This central nanowire enables efficient electron transfer between the enzymatic reactions, explaining the unsurpassed catalytic activity of HDCR. The observed in-vivo supramolecular organization may further enhance HDCR activity and create specialized metabolic subcompartments, enabling metabolism at the thermodynamic limit of life.

ST137

Naphthalene Carboxylase - elucidating the first reaction mechanism of a polycyclic aromatic hydrocarbon carboxylating enzyme

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Polycyclic aromatic hydrocarbons (PAHs) are persistent and harmful pollutants with a low bioavailability in the environment. Degradation of PAHs is especially slow under anoxic conditions, which leads to the accumulation of PAHs in anoxic environments. Naphthalene is the simplest and smallest PAH and is therefore used as a model compound. Due to their stability, activation of PAHs is a very demanding enzymatic reaction. It was shown that the first reaction of naphthalene degradation is a carboxylation in position 2, catalysed by the enzyme naphthalene carboxylase.

Here we want to elucidate the reaction mechanism of the naphthalene carboxylase from the sulphate-reducing, naphthalene-degrading *Desulfobacterium* strain N47 isolated from a contaminated aquifer.

Carboxylase activity was determined in assays with naphthalene, ATP and bicarbonate using cell-free-extracts of cells grown with naphthalene as sole carbon source. Activity was measured by LC-MS analysis of the product 2-naphthoyl-CoA. The naphthalene carboxylation turned out to be oxygen-sensitive and ATP-dependent with a very low activity of approximately 0.27 nmol min-1 mg-1, which corresponds well to the very slow growth of this bacterium. When we performed the naphthalene carboxylase reaction in deuterated water, naphthoyl-CoA was produced that was labelled with one, two, or three deuterium atoms, indicating that the reaction is fully reversible. When the enzyme was incubated with the product 2-naphthoate and 13C-labelled bicarbonate, an isotope exchange occurred on the carboxyl group, which was ATP-independent with a specific activity of approximately 0.37 nmol min-1 mg-1.

We conclude that the naphthalene carboxylation follows a two-step mechanism with an activated intermediate. The first step is ATP-dependent and rate-limiting and produces an activated naphthalene intermediate. The second reaction step (carboxylation of the intermediate) is faster and ATP-independent, which might help to prevent loss of ATP through back-reaction of the first step.

ST138

Eeyarestatin 24 inhibits protein secretion and induces DNA damage in Gram-positive bacteria

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Eeyarestatin 24 (ES24) is a promising new antibiotic candidate with broad-spectrum activity. The compound shares structural similarity with nitrofurantoin (NFT), yet it appears to have a distinct and novel mechanism of action. Thus, ES24 was found to inhibit SecYEG-mediated protein transport and membrane insertion in Gram-negative bacteria (Steenhuis *et al.*, 2020). However, the activity of ES24 was much higher against Gram-positive bacteria, prompting the question if this is simply due to the Gram-negative outer membrane or whether there are additional targets in Gram-positive bacteria.

In this study, we investigated the mode of action of ES24 in comparison with NFT using Gram-positive *Bacillus subtilis* and compared our findings to Gram-negative *Escherichia coli*.

We used a combination of transcriptomic stress response profiling and cell biological assays to assess effects on DNA, RNA, and protein synthesis, membrane and cell wall integrity, and oxidative stress. Effects on protein secretion were studied using Western Blots and GFP fusions. *in vivo* efficacy of ES24 in comparison with NFT was evaluated in a zebrafish model of *Streptococcus pneumoniae* infection.

Using these assays, we could confirm inhibition of the Sec pathway in *B. subtilis*. While we could exclude off-target activity on the synthesis of DNA, RNA, protein, and cell wall components as well as direct and downstream membrane effects, we did observe evidence of DNA damage in this organism. Interestingly, DNA damage was not observed in *E. coli*. Moreover, ES24 did cause gradual dissipation of the membrane potential in *E. coli* but not in *B. subtilis*, suggesting that there are distinct differences in mode of action between Gram-positive and Gram-negative species. NFT, which is thought to generate reactive species that cause cellular damage similar to oxidative stress, was positive in almost all assays, illustrating the distinct mechanisms of the structurally related compounds. Strikingly, ES24 was superior to NFT in survival assays with *S. pneumoniae*-infected zebrafish embryos.

In conclusion, we show that ES24 inhibits the Sec-machinery in both Gram-positive and Gram-negative bacteria. Yet, in Gram-positives it additionally induces DNA damage while in Gram-negatives it additionally affects the membrane potential. While being structurally similar to NFT, it has an unrelated and unique mode of action and performs better in an infection model. These results underline the clinical promise of ES24.

ST139

***Chlamydia pneumoniae* Pmp21 fragments form Aβ-like oligomers, bind to the cellular prion protein and therefore provide a further link between a chlamydial infection and the development of the Alzheimer's disease.**

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Chlamydia are gram-negative, obligate intracellular pathogens that can infect humans as well as other mammals, birds and reptiles. Besides *C. trachomatis*, which leads to trachoma and subsequent blindness, *C. pneumoniae* (*Cpn*) is the second well-known human pathogen. Infection occurs from person to person *via* droplets through the olfactory route leading to pneumonia. In addition, *Cpn* is suspected of exerting an influence in chronic diseases one being Alzheimer's disease (AD). *Chlamydia* employ a unique biphasic replication cycle characterized by infectious elementary bodies (EB) and non-infectious and metabolically active reticular bodies (RB). Adhesins mediate binding of the EB to the host cell, while invasins lead to its uptake into the cell. Within a specialized membrane (inclusion), EBs differentiate into RBs. The RBs divide and subsequently redifferentiate into EBs. EB-release by exocytosis of the inclusion or lysis of the host cell completes the infection cycle. *Cpn* Polymorphic membrane protein (Pmp) 21 is an adhesin and invasin with the Epidermal Growth Factor Receptor (EGFR) as its human interaction

partner. We demonstrated that Pmp21_D (a C-terminal fragment of the Pmp21 passenger domain) generates oligomers und protofibrils comparable to structures generated by Aβ. Prominent candidates for the development of the AD are Aβ-oligomers and the prion protein (PrP). A possible role of a chlamydial infection in the development of AD has been discussed for over 20 years. Our study aimed to investigate a possible link. *Via* Thioflavin T fluorescence assays we demonstrated that Pmp21_D generates high molecular weight oligomeric structures with kinetics comparable to Aβ. Visualized by atomic force microscopy, Pmp21_D appeared as oligomers and protofibrils with a curvilinear structure. Adhesion studies with Pmp21_D revealed that it also binds to non-EGFR expressing CHO-K1 cells suggesting further binding partners. Interestingly, cells infected with *Cpn* showed a colocalization of EBs and the PrP in a short-term infection. Using pull-down experiments, it was possible to enrich PrP from cells binding Pmp21_D. This interaction was confirmed to be specific by mutation studies, as Pmp21_D oligomers only bound to PrP fragments that exhibited motifs already described as essential for binding with Aβ. In summary, our studies support the theory that *Cpn* may have an influence in the pathogenesis of AD and show for the first time a possible protein-receptor interaction.

ST140

Different modes of action of pneumococcal pneumolysin and *Staphylococcus aureus* alpha-hemolysin on platelets

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Background

Streptococcus pneumoniae and *Staphylococcus aureus* are causative agents of severe invasive diseases. Both pathogens express cytolytic toxins as major virulence factors: pneumolysin (Ply, *S. pneumoniae*) and alpha hemolysin (Hla, *S. aureus*). Ply is a cholesterol-dependent pore forming toxin (PFT) and recent results of our group demonstrated that the increase in activation markers is caused by impaired membrane integrity, instead of earlier reported activation, Ply perforates platelets, leading to loss of platelet function and viability. Pharmaceutical IgG (IVIG) completely inhibit Ply-induced platelet damage and rescue platelet function (Jahn et al., 2020). Hla similar to Ply, also forms pores in eukaryotic cell membranes and has been described to induce platelet activation and aggregation.

Objectives

This study aimed to decipher the mode of action of the PFT Hla on platelets, thereby comparing the effects with the pore forming pneumococcal Ply on platelet viability and function. In addition, we tested the intervention with pharmacological IgG with the aim to neutralize Hla and restore platelet function.

Methods

The impact of Hla and Ply on platelet activation and function was assessed by measuring CD62P expression, localization studies of CD62P, platelet aggregation, release of intracellular Ca^{2+} and platelet viability. The effect of Hla and Ply on thrombus formation in whole blood was measured in flow chamber experiments. All experiments were performed in the presence or absence of IVIG.

Results

Ply formed multiple pores in platelet membranes which were associated with staining of intracellular CD62P stores, Ca^{2+}-release and platelet lysis. In contrast, Hla pore formation led to real platelet activation as shown by extracellular CD62P staining, platelet aggregation and Ca^{2+}-release. Nevertheless, platelet activation is followed by loss of platelet viability and function in a time frame of minutes, leading to impaired thrombus formation and thrombus stability in whole blood. Hla and Ply induced platelet apoptosis in a caspase 3/7 dependent mechanism. IVIG neutralized Ply and rescued platelet function, whereas IVIG had no effect on Hla despite anti-Hla immunoglobulins are present in IVIG.

Conclusion

Pneumococcal Ply and the staphylococcal Hla highly impair platelet viability and platelet function, but via different mechanisms.

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ST141

Messengers outer space: extracellular vesicles of the fungus *Ustilago maydis* and their mRNA cargos

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Extracellular vesicles (EVs) are emerging as important mediators in plant-pathogen interactions. Plant-pathogenic microbes secrete effectors to manipulate the host. So far, only conventionally secreted effector proteins have been characterized in the maize smut fungus *Ustilago maydis* (Lanver et al., 2017). Since EVs can carry diverse cellular molecules, including RNA, examining the cargo of pathogen EVs could broaden the spectrum of effectors (Kwon et al., 2020). Since the *U. maydis* is a fungal model for membrane-associated mRNA transport (Niessing et al., 2018), we have characterized the repertoire of mRNAs associated with its EVs (Kwon et al., 2021). For this purpose, a robust EV isolation method was developed for filamentous cultures of *U. maydis*, designed to partially mimic infectious hyphae. Indeed, intact mRNAs were found protected inside EVs of *U. maydis*. EVs carry a different composition of mRNAs compared to the fungal cells, suggesting selective loading of cargos. Interestingly, the most highly enriched mRNAs in EVs encode metabolic enzymes that are upregulated during infection, inspiring the idea that EV-associated mRNAs may participate in reprogramming of host cell metabolism. We hypothesize that *U. maydis* secretes mRNAs encoding effectors via EVs, which can be delivered and translated in the maize cell during infection. This would transfer the cost of pathogen effector protein production to the host cell.

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ST142

Membrane stress induced by low pH decreases the susceptibility of *Pseudomonas aeruginosa* to antibiotics

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Introduction; Stress-induced-membrane modification is a common strategy for decreasing antibiotic susceptibility and developing the antibiotic resistance population in bacteria [1]. In low embedded pH, two major component systems, namely PmrAB and PhoPQ, are induced, which are responsible for changing the outer membrane composition of *P. aeruginosa* [2]. Besides, the bacterium benefits from membrane vesicles (MVs) production triggered by activation of the main bacterial envelope response system to accelerate the membrane remodeling process [3]. **Objective;** This study aims to disclose the effect of induced membrane stress in changing the bacterial susceptibility to antibiotics. **Methods;** To this end, the activation of envelope response factor (σE), membrane vesiculation, and alterations in membrane fatty acids production were monitored upon the bacterial growth in acidic media *via* qPCR and nanoparticle tracking analysis and gas chromatography, respectively. Moreover, the susceptibility of the bacteria to antibiotics was measured in acidic media, in wild type *P. aeruginosa* and the mutant for the components of PmrAB and PhoPQ systems and their regulatory elements. **Results;** Our results suggested, bacteria defend against membrane stress *via* membrane vesiculation and production of short-chain fatty acid. Also, it was revealed that membrane remodeling *via* the two-component systems is less efficient than changing the membrane lipid composition and membrane vesiculation. In this way, they become less susceptible to antibiotics. **Conclusion;** Chronic lung infection by *P. aeruginosa* provides an acidic environment for the bacteria, which decreases the effectiveness of the antibiotic treatment [4]. This study helps to have a mechanistic view of the effect of membrane remodeling to reduce the bacterial susceptibility to antibiotics in an acidic environment.

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ST143

The external pH regulates the activity of the *Yersinia enterocolitica* type III secretion system

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Introduction: *Yersinia enterocolitica* is a Gram-negative gastrointestinal human pathogen. One of its major virulence factors is the type III secretion system (T3SS), which is essential to evade predation of the bacterium by the immune system in the human host. The T3SS structure, also called injectisome, resembles a molecular syringe, which *Y. enterocolitica* uses to establish a direct connection between the bacterial and the host cytoplasm, allowing the translocation of effector proteins. While the structure of the injectisome is well-known and can be separated in static and mobile components, little is known about the molecular function and regulation of T3SS.

Objectives: We aimed to characterize the molecular regulation of T3SS activity and its physiological role during infection. To this aim, we combined live cell fluorescence microscopy, single particle tracking and functional assays. We discovered that the external pH, which strongly varies during *Y. enterocolitica*"s passage through the gastrointestinal tract, has a strong impact on the mobile cytosolic components of the T3SS and the function of the system.

Results: We show that at low external pH, encountered by the bacteria during the passage through the gastrointestinal tract, the cytosolic components of the T3SS are temporarily released, thereby preventing effector secretion. While the internal pH of the cell is only slightly affected, the drop in external pH is sensed in the periplasm by the membrane component SctD, which partially dissociates. This abrogates the binding interface of the dynamic cytosolic T3SS components, which consequently relocalize to the cytosol. Once neutral pH is restored, this effect is reversed, allowing for the reassociation of the cytosolic components. This leads to a fast activation of the T3SS at its neutral site of action in the lymphatic tissue.

Conclusion: Our findings indicate the presence of an adaptive regulatory interface between the membrane-bound and cytosolic T3SS components. This mechanism regulates T3SS activity in response to environmental conditions and provides a key layer of regulation in addition to the general host cell sensing. This allows to limit the otherwise unspecific secretion of the T3SS to favorable environmental conditions, thereby preventing the loss of valuable effectors or immune responses, and ultimately promoting successful infections. (Wimmi *et al.*, 2021) *Nat Commun* https://doi.org/10.1038/s41467-021-21863-4

ST144

scRadio-Seq: A novel method of selecting members from a microbial community with a specific metabolic function for single-cell genomics

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Current methods for identifying microorganisms that perform *in situ* degradation of an organic substrate are often hampered by insufficient sensitivity and habitat complexity. Here, we present an approach to identify single microbial cells metabolizing a specific organic compound with high sensitivity and without prior knowledge of the microbial

community. The method consists of the following steps: labeling individual cells of a community with a 14C substrate based on their metabolic activity; encapsulating cells in photoemulsion-hydrogels using a microfluidics system; microautoradiography to visually distinguish between encapsulated labelled and non-labelled cells; flow-cytometric sorting of encapsulated cells; and single-cell genomics of labelled and sorted cells. As a proof-of-concept, we were able to separate and sequence single cells of the benzene degrader *Pseudomonas veronii* from a mock microbial community. We anticipate that **S**ingle **C**ell capturing *via* microauto**Radio**graphy and genome **Seq**uencing (scRadio-Seq) will be highly beneficial for elucidating microbial identity, activity, and function in diverse habitats.

ST145

Persistence against benzalkonium chloride promotes rapid evolution of tolerance during periodic disinfection

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The global antimicrobial resistance crisis is a major concern for public health. This crisis is exacerbated by phenotypic heterogeneity in the form of tolerant persister subpopulations. Persisters can survive transient antibiotic treatment and facilitate resistance evolution. Disinfectants are an effective measure to prevent the transmission of bacterial pathogens in general, and antibiotic resistant bacteria in particular. To date, there are no reports that focus on persistence in the context of disinfectants and its consequences for the evolution of tolerance and resistance to disinfectants and antibiotics.

Here, we aimed to establish the concept of persistence in the context of disinfectants as exemplified by the widely used disinfectant benzalkonium chloride (BAC) in *E. coli*. We investigated the evolutionary consequences of persistence-mediated survival of periodic disinfection for the susceptibility to BAC and the genetic and mechanistic basis thereof. Furthermore, we aimed to link persistence to disinfectants to the evolution of antibiotic resistance.

We used genetic screens and survival assays to establish persistence to BAC and the mechanistic overlap with antibiotic persistence. We employed experimental evolution, combined with sequencing and extensive phenotypic analysis, to characterize the evolutionary response to periodic disinfection. Mathematical modelling was used to understand the population dynamics during evolution. We characterized the antibiotic susceptibility of the evolved mutants with respect to survival, resistance, and fitness.

We show that *E. coli* displays persistence against BAC. Periodic, persister-mediated failure of disinfection rapidly selects for BAC tolerance, which is associated with reduced cell surface charge and mutations in the *lpxM* locus, encoding an enzyme for lipid A biosynthesis. Moreover, the fitness cost incurred by BAC tolerance turns into a fitness benefit in the presence of antibiotics, suggesting a selective advantage of BAC-tolerant mutants in antibiotic environments [1].

Our findings highlight how cell-to-cell variability can affect disinfection processes and provide a link between persistence to disinfectants and resistance evolution to antimicrobials.

[1] Nordholt, N.; Kanaris, O.; Schmidt, S. B. I.; Schreiber, F. Persistence against benzalkonium chloride promotes rapid evolution of tolerance during periodic disinfection. Nature Communications, in press

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ST146

Rational engineering and Adaptive Laboratory Evolution of *Saccharomyces cerevisiae* to establish synthetic formatotrophy via the Reductive Glycine Pathway.

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Formate is a one-carbon acid that can be produced from the electrochemical reduction of CO2. *S. cerevisiae* is a biotechnologically relevant, simple eukaryote that naturally does not grow on formate as the sole carbon and energy source. Engineering formatotrophy in yeast could boost the emergence of a formate-based circular bio-economy. In this study, the Reductive Glycine Pathway (RGP) is employed to establish synthetic formatotrophy in baker"s yeast. This pathway can be subdivided into three modules: The formate assimilation and glycine synthesis module, the glycine to serine conversion module, and the serine to pyruvate deamination module, which connects formate assimilation to primary carbon metabolism.

A biosensor strain-based growth coupled selection strategy was used to implement the formatotrophy here. For this, we constructed a serine biosensor strain by disrupting the native serine and glycine biosynthesis routes to insulate serine, glycine, and C1 molecules from the central metabolic network. This strain cannot grow on glucose as the sole carbon source but requires the supply of glycine or serine to complement the engineered auxotrophies. Further, we implemented the glycine and serine synthesis modules of the RGP in the biosensor strain and tested for the synthesis of glycine and serine from formate and CO2 using growth as the readout. Since no growth was observed with the rationally constructed strain, we successfully applied Adaptive Laboratory Evolution **(**ALE), which yielded a strain capable of synthesizing glycine and serine from formate and CO2 and the growth is improved by ~35 folds. Carbon tracing experiments with 13C formate and 13CO2 confirmed the glycine and serine biosynthesis via the RGP from formate and CO2. Interestingly, labeling data also revealed partial labeling of alanine, which indicates pyruvate synthesis from labeled serine by native *Cha1p* activity in the evolved strains. Whole-genome sequencing of the evolved serine biosensor strains revealed mutations in *GDH1*, *PET9*, and *IDH1,* indicating demand for more reducing power to operate the RGP.

Although the 13C carbon tracing confirmed the activity of all three modules of the RGP, the overall flux might be limited by the supply of reducing power for complete formatotrophic growth. Hence, localizing the formate dehydrogenase to the mitochondria to improve the levels of mitochondrial reducing power could further improve the flux through the pathway to enable the complete formatotrophy.

ST147

Construction and characterization of the Sc2.0 tRNA neochromosome

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The Sc2.0 consortium aims to generate the first synthetic eukaryotic genome. All synthetic chromosomes have now been built and are currently being characterized. Merging multiple chromosomes in a single cell is in progress to ultimately construct the first synthetic eukaryotic cell. One major change to the Sc2.0 genome is the relocation of all tRNA genes to a new, *de novo* designed chromosome: the tRNA neochromosome. The 275 tRNA genes of *S. cerevisiae* are distributed throughout the whole genome. tRNA genes are highly transcribed and are preferred insertion sites for transposable elements. By this means, tRNA genes cause genomic instability. Removing the tRNA genes ensures that risk of transposon insertion into synthetic chromosomes is minimized during their construction. Here we present the design, construction and characterisation of the first *de novo* designed and constructed synthetic eukaryotic chromosome.

Methods: The tRNA neochromosome has been constructed and extensively characterized, including but not limited to growth phenotyping, global expression analysis and nuclear localization studies.

Results and conclusion Chromosomes can be designed *in silico* and build from scratch. The tRNA neochromosome is functional and allows in future to study tRNA biology from a new perspective. Interestingly the tRNA neochromosome seems to be unstable in haploid wild type cells but can provoke a whole genome duplication. In the genome-duplicated cells the neochromosome can be maintained stably in circular or linear form. In future, the tRNA neochromosome will supplement the final Sc2.0 strain with all necessary tRNA genes and will give new insights into tRNA biology.

ST148
Engineering synthetic formatotrophy via the Serine Threonine Cycle in E. coli
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To meet the demands of a growing world population while reducing CO2 emissions, it is necessary to establish a circular economy, where CO2, as the only scalable carbon source, is captured and converted into value-added products. This could be achieved by chemical reduction of CO2 to one-carbon (C1) compounds and microbial conversion of C1 compounds into products of interest. One promising C1 feedstock is formate as it can be produced from CO2 by various means and is easy to transport and store. The transition towards a C1-based bioeconomy requires engineering of bioindustrial microbes to grow on C1 feedstocks via natural or synthetic pathways.

The serine threonine cycle (STC) is a synthetic formate assimilation pathway that could support formatotrophic growth of *E. coli*. It is based on the naturally occurring serine cycle, following its general structure: formate + glycine → serine → PEP + CO2 → C4 → glycine + acetyl-CoA, but is modified to overlap with the metabolism of *E. coli*, requiring in theory the expression of only one foreign enzyme, formate tetrahydrofolate ligase. As in the serine cycle, formate is attached to the C1-carrier tetrahydrofolate before its carbon is donated to glycine to form serine. From serine onwards the two cycles take different directions. Instead of converting serine into hydroxypyruvate, in the STC serine is deaminated to pyruvate which is carboxylated to oxaloacetate. From oxaloacetate, the flux is directed to threonine, which is cleaved to recover glycine. As in the natural serine cycle, acetyl-CoA is the pathway product.

In this work, we show the stepwise engineering of STC in *E. coli* resulting in a strain capable of growing on formate as the sole carbon and energy source. After confirming the activity of the different pathway modules in individual selection strains, we combine all modules into a final STC strain. We apply adaptive laboratory evolution to enable formatotrophic growth via the STC and identify key mutations responsible for pathway activity. The evolved STC strain grows formatotrophically under ambient CO2 concentrations, a key advantage over other synthetic formatotrophs and autotrophs which require elevated CO2 concentrations for cellular growth.

This study presents for the first time the engineering of a synthetic formate assimilation cycle in *E. coli*. It also shows that it is possible to engineer complex cycles in the centre of metabolism and reveals important insights into metabolic flexibility.

ST149
Proteogenomics provides a powerful strategy to re-annotate and discover novel ORFs in the cyanobacterium *Synechocystis* sp. PCC6803
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Cyanobacteria are photoautotrophs that profoundly impact the biogeochemical cycles on Earth. Due to the fixation of atmospheric CO_2, they are of increasing interest for a sustainable economy. Knowledge on protein expression and regulation is a key for understanding of the cyanobacterial metabolism; however, proteome studies are still limited and cover only a fraction of the theoretical proteome. Here, we performed a proteogenomic analysis of the unicellular model cyanobacterium *Synechocystis* sp. PCC 6803 to characterize the expressed (phospho)proteome, re-annotate known and discover potential novel open reading frames (ORFs). By mapping extensive shotgun MS-proteomics data generated by the SCyCode consortium (www.scycode.net) onto a six-frame translation of the *Synechocystis* genome, we re-annotated 135 start sites and discovered 128 putatively novel ORFs, as well as 12 single amino acid variants. Through retrieval of external publicly available multi-omics datasets, we were so far able to verify 43 re-annotated or novel ORFs with high confidence. Our study resulted in the largest reported proteome and phosphoproteome dataset for *Synechocystis*, covering expression of approx. 80% of the theoretical proteome and several hundred phosphorylation events on Ser, Thr and Tyr residues under various growth conditions, such as nitrogen or carbon limitation. We are currently mining this dataset to gain a deeper insight into the frequency of protein phosphorylation in different biological processes, the characteristics of the dark (unidentified) proteome and genomic age of ORFs and related physiological processes. This dataset will serve as a resource for the cyanobacterial community with dedicated information on condition-dependent protein expression and phosphorylation.

ST150

Polyphosphate metabolism in the thermoacidophilic Crenarchaeon *Sulfolobus acidocaldarius*

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Inorganic Polyphosphate, a linear polymer of orthophosphate residues linked by phosphoanhydride bonds, occurs in all three domains of life and plays a diverse and prominent role in metabolism and cellular regulation (1). While the polyphosphate metabolism and its physiological significance have been well studied in bacteria (2) and eukaryotes including human (3), there are only few studies in archaea available so far. Until now, different types of polyphosphate kinases have been reported but despite intense investigation, the nature of the crenarchaeal polyphosphate kinase is still unknown (4). In Crenarchaeota including members of *Sulfolobales*, the presence of polyphosphate and degradation via exopolyphosphatase has been described and there is some evidence for a functional role in metal ion chelation, biofilm formation and motility (5). Here we used the crenarchaeal model organism *Sulfolobus acidocaldarius* to study the enzymes involved in polyphosphate metabolism. The recombinant exopolyphosphatase exhibited high specific activity with medium chain polyphosphates ($PolyP_{45}$, 857 U/mg protein) although highest activity was observed with long chain polyphosphates ($PolyP_{700}$). In addition, we identified a putative polyphosphate kinase by comparative bioinformatic analysis. The enzyme was expressed, purified and characterized using enzymatic assays as well as ^{31}P-NMR spectroscopy confirming the predicted polyphosphate kinase activity. The current insights in polyphosphate metabolism and function in *S. acidocaldarius* and novel phylogenetic implications will be discussed.

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ST151

Molecular basis for N_2-fixation in a thermophilic methanogen

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Hydrogenotrophic methanogens, belonging to Euryarchaeota, harbour yet uncharacterized nitrogenase (NifHDK) system. This N_2-fixation machinery is evolutionarily distant from characterized homologs[1], suggesting differences in structural and catalytic features. Even more interestingly, it can operate at high temperature (up to 92 °C), under high H_2 pressure (up to 2 bars), and extreme energy limitation (½ ATP/CH_4 formed). A tight regulation of the nitrogenase activity is ensured by $NifI_{1,2}$ belonging to the PII-family[2].

Here, we present physiological, transcriptomic, as well as structural studies of the nitrogenase system from the marine thermophilic methanogen *Methanothermococcus thermolithotrophicus*.

The fast and robust diazotrophic growth of this archaeon is strictly dependent on molybdenum and not inhibited by tungstate or vanadate in contrary to other closely related diazotrophs[3], implying a high specificity for metal insertion into the nitrogenase cofactor. This was supported by comparative transcriptomic, which showed that along the *nif* operon, *mod* operon encoding for molybdenum transporters is also significantly upregulated under diazotrophic condition. The stress response to the lack of available nitrogen in the early exponential phase is reflected in the upregulation of *glnK2*/*amtB2* genes (coding for NH_4^+ transporter and its regulator[4]) and suspected regulatory sRNAs.

The structure of the natively isolated nitrogenase reductase (NifH), solved at 1.95-Å, shows a very high conservation of its ligand binding sites. Overall conformational changes and [4Fe-4S]-cluster environment restructuration were observed upon nucleotide binding.

We also obtained the first crystal structure of $NifI_{1,2}$, which revealed an unsuspected oligomerization state. In contrast to the canonical homotrimeric organization of PII-family proteins, $NifI_{1,2}$ is organized as a dimer of $2xNifI_1$:$NifI_2$ that should be required to bind NifDK.

Our ultimate goal is to fully characterize and elucidate the molecular mechanisms and structures of the whole nitrogenase system. These future insights into the specific traits and adaptations of this unique system could be transferred to biotechnological application.

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ST152

New isolates of strictly hydrogen-dependent methyl-reducing *Methanosarcinales* from arthropod guts

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Metagenomic studies have uncovered an unexpected diversity of uncultured methanogens that are predicted to be obligately hydrogen-dependent methylotrophs. However, only a few representatives with this metabolism have been isolated in pure culture. One of them is *Methanimicrococcus blatticola* from the hindgut of the cockroach *Periplaneta americana* (Sprenger et al., 2000), a member of *Methanosarcinales* that possesses only a truncated version of the archaeal Wood–Ljungdahl pathway. We isolated four new species of obligately methylotrophic *Methanosarcinales*

from arthropod guts: two isolates from cockroaches are new species in the genus *Methanimicrococcus,* and two isolates from millipedes represent a new genus (93–94% 16S rRNA sequences similarity to *M. blatticola*). All isolates are irregular shaped cocci and produce methane by the reduction of methanol and methylated amines, with molecular hydrogen as an electron donor; details on their physiological properties will be presented. Comparative genome analysis of the isolates and several metagenome-assembled genomes (MAGs) of their closest uncultured relatives from termite guts with other members of *Methanosarcinales* provides new insights into the evolutionary transition from methyl-disproportionating to methyl-reducing methanogenesis.

ST153

Making Ribosomes in Archaea? Towards understanding common and specific principles of ribosome biogenesis across the different domains of life.

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Ribosomes are universally conserved molecular machines ensuring the decoding of the genetic information carried within mRNA intermediates into proteins. Remarkably, the ribosome molecular constituents, the ribosomal RNAs and ribosomal proteins, assemble into structural and functional entities conserved across the different domains of life. In addition, some general principles of ribosomal protein assembly seem to apply across the tree of life. In contrast, the ribosome biogenesis factor counterparts facilitating the ribosomal subunit assembly process apparently diverge between bacteria and eukaryotes. Interestingly, several eukaryotic-like ribosome biogenesis factor protein signatures are present and conserved in most archaeal genomes, suggesting that some aspects of ribosome biogenesis in archaea may resemble its eukaryotic counterpart. However, how ribosomes are made in archaea remain poorly characterized.

To better disentangle common and specific principles of ribosome biogenesis within and across the different domains of life, our work aims to systematically characterize the ribosome biogenesis pathway in archaea.

I will present some of our recent methodological developments and functional insights shedding light into eukaryotic-like, archaeal-specific and archaeal-divergent steps of ribosome biogenesis in archaea. Moreover, I will also discuss how our understanding of the ribosome assembly pathway across the tree of life can contribute to the emergence of unified conceptual frameworks on ribosome biogenesis and its evolutionary history.

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ST155

Infection with intracellular parasite *Amoeboaphelidium protococcarum* induces shifts in associated bacterial communities in microalgae cultures

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In recent years microalgae products have developed increasing market demand, but sustainable industrial production is still challenged by biological stability of large-scale production plants. Yet the relationships between algal hosts, associated microbiomes, and contaminants in photobioreactors remains widely understudied. The aim of this study was to investigate the temporal development of microbiomes of four freshwater microalgae species *Scenedesmus vacuolatus*, *Desmodesmus quadricauda*, *Chlorella sorokiniana*, and *Botryococcus braunii*, in presence and absence of the zoosporic parasite *Amoeboaphelidium protococcarum*. To compare the effects of sterile and nonsterile culture conditions, infection experiments were performed in sterile laboratory (sterile) and simulated industrial conditions (open). Algal growth (dry weight, optical density, and nutrient consumption) was observed for 21 days, and samples of the associated microbiome were collected for bacterial 16S rRNA gene Illumina MiSeq sequencing. Infection patterns of *A. protococcarum* were algae species-specific, irrespectively of culture conditions. Bacterial community analysis demonstrated distinct and stable bacterial communities for each algae species, which were mostly dominated by α- and γ-Proteobacteria. Upon aphelid parasitosis, bacterial diversity increased, and community compositions diverged algae-specific over time. Moreover, bacterial functional traits shifted to detoxification, degradation, and cellulolysis once algae were infected. This study provides a first insight into the close connection between algae, associated bacterial microbiomes and appearing contaminants in photobioreactor systems.

ST156

The evolution and consequences of RubisCOs interaction with- and dependence on its small subunit.

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1. **Introduction**

Most autotrophic microorganisms assimilate CO_2 using ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). RubisCOs are phylogenetically widespread and different forms of the enzyme exhibit vastly different catalytic parameters and complex stoichiometries. Aerobic autotrophs employ a form of RubisCO that assembles into a 16mer assembly of 8 large and 8 small subunits (SSU). Phylogenetic inferences show that this form evolved from

ancestors that did not yet interact with a SSU. Due to the enrichment of SSU-bearing RubisCOs in aerobic habitats, the SSU is considered an adaptation to oxygen and is thought to help suppress the oxygenation side reaction of RubisCO. However, no biochemical evidence of the SSUs function exists. This is because the SSU is essential for both the solubility and activity of SSU-bearing RubisCOs, which hinders studies surrounding its influence on catalysis.

2. **Objectives**

We aimed to decipher how the obligate interaction between RubisCO and its SSU evolved. This includes finding the functional implications of interacting with the SSU, answering why derived RubisCOs depend on the SSU even though it was ancestrally functional in isolation, and how the SSU modulated the evolutionary trajectories available to RubisCO.

3. **Materials & methods**

We identified novel RubisCO forms that are closely related to the evolutionary interval over which the SSU first evolved, use ancestral sequence reconstruction to resurrect ancestral RubisCOs that existed before and after the invention of the SSU and biochemically characterize these and intermediary ancestral RubisCOs.

4. **Results**

We show that modern day form I RubisCOs evolved from an octamer of large subunits that started to interact with- and depend on the SSU around 3 billion years ago. Immediately upon recruitment, the SSU improved RubisCOs catalysis and opened evolutionary paths that led to increased specificity. Thereafter, a single surface-exposed and functionally neutral substitution was able to cause RubisCOs dependence on the SSU for solubility. This substitution causes self-assembly of the octameric RubisCO complex into fibers via an interface that is shielded and sterically inaccessible in the SSU-bound form.

5. **Conclusion**

Our findings suggest that natural selection can fix protein-protein interactions for their beneficial effects and that such interactions can subsequently become essential via non-adaptive evolution. Additionally, we reveal the function of RubisCOs SSU.

ST157
Extrachromosomal location and horizontal transfer of ribosomal genes in the marine environment
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Ribosomal RNAs (*rrn*) are the gold standard for taxonomic identification and classification. Although it was shown that they can be horizontally transferred, previous analysis of bacterial genomes indicated that these transfers are rare. However, this conclusion was drawn from bacterial strains with high intragenomic diversity of the 16S rRNA genes (>2%). As recombination decreases with increasing sequence divergence in bacteria we investigated if horizontal transfer of *rrn* genes is also rare for strains that have highly similar 16S rRNA genes in the same genome. Therefore, we established high quality, closed genomes for 63 strains from the genus *Sulfitobacter*, which represent a range of species relationships (Average amino acid identity (AAI) 74 - 100%), from the marine roseobacter group. The strains contain one to four *rrn* operons but 41 of 187 operons analyzed are located on plasmids. The *rrn* genes varied in number, location and length within species and strains but the intragenomic 16S rRNA diversity was always below 1%. Comparative analysis of the 16S and 23S rRNA genes indicated a discordant evolution of the *rrn* genes. The phylogenetic networks of both genes show complex network structures indicating conflicting signals probably due to recombination. 16S rRNA variants are distributed over species borders and two bacterial strains from different species even contained completely identical 16S rRNAs. The usage of this marker would result in a wrong taxonomic classification. Our results indicate that horizontal transfer of *rrn* genes may be more frequent than expected between strains of closely related species und imply that selections of strains based on phylogenetic 16S rRNA gene classification may not be sufficient for speciation and microdiversity research.

ST158
From single cell growth to microbial ecosystems: Insights from mathematical modelling.
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Question: The evolution of oxygenic photosynthesis in the ancestors of modern-day cyanobacteria gave rise to perhaps the most important biological process within our biosphere. To this day, phototrophic microorganisms are primary engines of biogeochemical cycles and dominate primary production in marine ecosystems. The functioning of such ecosystems is crucial for the maintenance of our planet and facilitated by interactions between photo-, mixo-, and heterotrophic microbes.

While many properties of growth in axenic cultures are reasonably well understood, many properties of marine microbial ecosystems remain elusive. A number of recent landmark studies have challenged the way we think about microbial ecosystem. Rather than being mere assemblages of discrete species that interact haphazardly, many microbial communities seem to be more akin to co-evolved super-organisms.

Methods: Mathematical modelling can help us to understand how marine microbes interact and collaborate: what are the pre-requisites and energetic trade-offs for cooperation and division of labor? How do metabolic diversity and mutualistic relationships emerge? To tackle these challenging questions, we can build upon high quality quantitative models of microbial growth and resource allocation developed over the past decade. Our premise is that the perspective of cellular resource allocation offers a unique opportunity to understand the constraints and energetic trade-offs that govern the emergence of dependencies between photo- and heterotrophic microorganisms in marine environments.

Results: We will present a computational framework based on resource allocation models of microbial and cyanobacterial growth that helps to explain and understand the emergence of phototroph-heterotroph dependencies, as well as the constraints and energetic trade-offs that govern these dependencies.

ST159

A novel KaiA extends the KaiB3-KaiC3 system and gives hints about the evolution of the circadian clock in prokaryotes

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The Earth had rotated around its axis since its origin 4.6 billion years ago creating a stable daily rhythm of light and darkness. The resulting daily rhythm of intense UV radiation posed a challenge to the emerging life on Earth. However, this circumstance provided a strong selection pressure to anticipate those recurring cycles and thus a potential fitness advantage to the organisms. One of those adaptations is a circadian clock, which today is found in a wide range of organisms. In the cyanobacterium *Synechococcus elongatus* PCC 7942, which serves as a reference for circadian clocks in Cyanobacteria, the three proteins KaiA, KaiB, and KaiC constitute the central timing mechanism that drives circadian oscillations. However, large diversity within the protein sequences and the composition among Cyanobacteria has been detected.
In addition to the standard oscillator, named KaiAB1C1, *Synechocystis* sp. PCC 6803 harbors several, diverged clock homologs, whose functions are not yet fully understood. The nonstandard KaiB3-KaiC3 system was suggested to impact the metabolic switch in response to darkness. Here, I present Sll0485 as a potential new chimeric KaiA homolog that we named KaiA3. KaiA3 was initially annotated as a NarL-type response regulator due to its similarity to the response regulator receiver domains and thus overlooked for many years. However, its similarity to canonical NarL drastically decreases in the C-terminal domain, which resembles the circadian clock protein KaiA. KaiA3 as a novel, nonstandard KaiA homolog, extends the KaiB3-KaiC3 system in Cyanobacteria and other prokaryotes. This is in contrast to previous findings where KaiA was only found in Cyanobacteria. These newly identified orthologs share common features with standard KaiA proteins and form similar genetic clusters known for the KaiABC cluster. However, the differences and presumably modularity of KaiA orthologs challenge central paradigms and question previous assumptions about the order of events in the evolution of the circadian clock.

ST160

Insights into the microbial community structure and function of a unique terrestrial subsurface ecosystem located in a geologically active rift region

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With frequent seismic activity, consistently high CO_2 concentrations and fluxes, and a unique sedimentary composition, the Eger Rift in Western Bohemia, Czech Republic, represents a worldwide rare subsurface ecosystem and scientifically relevant location to study microbial behavior in the deep subsurface and bio-geo interactions under extreme conditions. Despite recent advances, the role of microbial communities and their metabolic processes in terrestrial subsurface settings remains poorly understood, leaving the question how these microorganisms thrive and survive in such unique environment unanswered.

To study the microbial composition and identify potential bio-geo interactions in this environment we collected sediment samples from a 240m-long core drilled through an active Mofette as part of the ICDP project "Drilling the Eger Rift". Applying an innovative DNA extraction approach we were able to recover DNA for genomic investigations from this low biomass environment. Microbial abundance was assessed using qPCR and microscopic cell counts, while 16S rRNA and metagenomic sequencing were utilized to explore microbial community composition and metabolic potential. In addition, anaerobic cultivations were used to enrich CO_2-utilizing methanogenic archaea.

Overall microbial abundance (10_2 to 10^6 16S gene copies) demonstrated microbial life to persist in the terrestrial subsurface below 200 meters. We discovered a distinct microbial community dominated by rhizome and soil associated Proteobacteria. In addition, we observed dominant Cyanobacteria and Chloroflexi signatures at specific depths. Amplicon sequencing and enrichments supported the presence of methanogenic archaea throughout the core. Reconstruction and annotation of several MAGs, from both core material and enrichments, provided first insights into microbial processes.

Our results highlight the presence of soil and water associated Proteobacteria and an unexpected emergence of Cyanobacteria, which could be attributed to the frequently changing ground water levels, and close proximity of the river Plesna. Furthermore, the identification of acidophilic and methanogenic taxa suggests CO_2 and H_2, the latter being released periodically through seismic activity, to be potentially important driving forces in this unique ecosystem. Altogether, this study advances the current understanding of microbial life in tectonically active subsurface systems and provides valuable data for future explorations.

ST161

Transcriptomic analysis of the ultramicrobacterium OP3 LiM in a methanogenic enrichment community provides insight into a predatory niche

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Introduction

Candidate division OP3, also known as *candidate phylum* Omnitrophica, is present in most anaerobic habitats, however, its exact niche within the environment remains unknown. The phylotype OP3 LiM was detected in a limonene-degrading methanogenic enrichment culture. Within this culture, OP3 LiM cells were found attached to *Methanosaeta* filaments and other bacteria, but also free-living. These free-living OP3 LiM cells appeared to be metabolically less active than attached living cells, based on the ribosome content as previously observed in in-situ hybridization images.

Objective

The transcriptome was analyzed to gain insights into the activities of attached and free-living OP3 LiM cells.

Materials & methods

The cells of the enrichment were separated by centrifugation, resulting in a fraction of normal-sized cells and aggregated cells (10,000 S-cells), a fraction of ultramicrobacteria (100 S-

Pellet) which after ultracentrifugation and resuspension formed aggregates (100 S-aggregates) and a fraction of single ultramicrobacteria (100 S-cells). RNA extracted from these fractions was sequenced by Illumina. The reads were trimmed using BBduk and mapped onto the OP3 LiM genome. The differential expression was calculated with Geneious Prime® 2021.0.1.

Results

A first analysis indicated a large difference in the transcriptomes of free-living and of attached OP3 LiM cells. Almost 1200 genes were 20x or higher expressed in the free-living OP3 LiM fraction, including those involved in pilus assembly and ribosomal silencing. In contrast, only four genes were highly expressed in attached OP3 LiM cells. Among these highly expressed genes were ribosomes and a homing endonuclease.

Conclusion

The transcriptome of OP3 LiM is indicative of different life styles. Free-living OP3 LiM cells were in a state of hibernation, keeping protein synthesis low using the ribosomal silencing factor, which prevents the ribosomal subunits from joining. Furthermore, they express pilin to attach themselves to a potential host. Attachment of OP3 LiM cells coincided with the induction of four genes, which indicate a metabolic active state. A detailed analysis is in progress.

ST162

Borgs use dynamic perfect nucleotide tandem repeats to create intrinsically disordered regions in proteins central to host association

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Introduction: Borgs are huge, linear extrachromosomal elements that were recently discovered in metagenomes from several anoxic ecosystems [1]. They associate with different phylogenetic groups of the *Methanoperedens* genus and carry genes that can augment the metabolism of their archaeal methane-oxidizing hosts.

Objectives: The objective of this work was to shed light on one outstanding Borg feature: perfect tandem repeats (TR) that are dispersed throughout Borg genomes.

Materials & methods: Six new metagenome-assembled Borg genomes were curated to completion to resolve local assembly errors and unmask TR regions. A database of TRs from ten Borg genomes was constructed. Many TRs were found in open reading frames (orf), resulting in novel repeat regions within proteins. These repeat proteins were bioinformatically analysed in terms of properties and functions.

Results: TR sequences of the repeat units are unique in virtually every instance. 55% of the nucleotide TRs are divisible by three and 85% of those are in orf, indicating a function on the protein level. These emerging repeat regions are often located in non-cytoplasmic proteins and are absent in non-Borg protein homologs. In most cases the TR regions are intrinsically disordered regions, which could be sites of post-translational modification and/or interaction domains.

Conclusion: This work demonstrates that nucleotide TRs are often masked in automated metagenome assemblies. The high frequency, abundance and within-population evolutionary dynamics of repetitive sequences indicate that they have an important biological function. We propose that TR regions serve functions related to regulation and protein complex formation and may be central to coexistence of Borgs and their hosts.

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ST163

How clear is our current view on microbial dark-matter? (Re-)assessing public MAG & SAG-datasets with "MDMcleaner"

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As of today, the majority of environmental microorganisms, designated as "microbial dark matter" (MDM), still remains uncultured. Insights into this uncultured majority are therefore predominantly limited to genome reconstructions via cultivation independent approaches such as single cell amplified genomes (SAGs) and metagenome assembled genomes (MAGs). Both, however, are sensitive to contaminations, potentially causing misattributed contigs to be included in the respective reconstructed genomes. Such contaminations may bias comparative genome analyses and cause false conclusions.

Consequently, strict contamination filtering needs to be applied. Current genome reporting standards, however, emphasize completeness over purity. Furthermore, current *de facto* standard genome assessment tools are most biased in the case of uncultured taxa and fragmented genomes, thereby discriminating against many SAGs and MAGs. The result is a potential gradual reference database corruption that may cause further error propagations and increasingly distort our view on microbial dark matter.

To combat this, we present a python implementation of a novel contig classification, screening and filtering workflow that tackles these issues: "MDMcleaner". This workflow reports potential contaminants not only in the subject genomes but also in the underlying reference datasets, allowing to simultaneously expand and refine genome databases.

Subjected to current "high quality" genome datasets, MDMcleaner revealed substantial fractions of preventable contaminations overlooked by current screening approaches. Affected genomes included selected "representative genomes" of curated public reference datasets. By sensitively detecting potential contaminants and eliminating error propagation from contaminated references, MDMcleaner can substantially enhance our view on "microbial dark matter" genomics

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ST164

Metabolic niche partitioning during cross-feeding is linked to diversity in leaf bacterial communities

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Introduction

Endophytic commensal microbes can establish in the intercellular space of leaves (i.e., the apoplast) where they strongly influence host health. Despite their importance, we know little about how they adapt to environment. While leaf pathogens have developed strategies to obtain nutrients from the plant, it is not yet clear how commensals, majorly deprived from these strategies, survive. In other systems, resource limitation promotes the uprise of cooperative or mutualistic interactions, stabilizing the microbial community. However, this has not been addressed in detail in leaves.

Objectives

Evaluate whether the nutrient environment influences metabolic exchange and assembly of the leaf bacterial communities in two *Flaveria* species.

Materials & methods

We enriched bacteria from leaves of *Flaveria robusta* and *F. trinervia in-vitro* in minimal media with sucrose as a carbon source and with or without amino acids. After 12 passages, we picked random isolates from each environment, tested their carbon preferences, and described their cross-feeding interactions using untargeted metabolomics. Next, we characterized the chemical landscape of the leaf apoplast of both species as well as the isolates' fitness *in-planta*.

Results

The richness of resources in the enrichment did not lead to increased diversity; however, in *F. robusta* enrichments we found that *Pseudomonas koreensis* strains cross-fed several compounds, including purines and polyamines from *Pantoea* sp. Although genetically very similar, the *P. koreensis* strains displayed clear niche partitioning which enabled them to co-exist during cross-feeding. Untargeted metabolomics analysis of the leaf apoplast of both species revealed contrasting nutrient environments, with greater concentrations of high-cost amino acids in *F. trinervia. Additionally, P. koreensis* strains were better able to survive in these apoplasts without a cross-feeding partner.

Conclusions

Our results suggest that cross-feeding may be widespread in leaves and can have an impact on the assembly of bacterial communities. Furthermore, the *in-planta* assays suggest niche-partitioned cross feeding might have help bacteria adapt to nutrient limitations in the host apoplast environment. Future work will focus on how these interactions may influence the establishment of pathogens and impact plant's health.

ST165
The Methane-Driven Interaction Network in Terrestrial Methane Hotspots
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The composition, activity and diversity of microbial communities is mainly affected by biological interactions. Concerning methanotrophy, aerobic methane oxidation is a community functioning, with emergent community traits arising from the interaction of the methane-oxidizers (methanotrophs) and non-methanotrophs. It is known that methanotrophs thrive better when heterotrophs are present, but little is known of the organization of these interaction networks in naturally-occurring complex communities. We hypothesized that the assembled bacterial community of the interaction network (interactome) in methane hotspots would converge, driven by high substrate availability that favors specific methanotrophs, and consequently influence the recruitment of non-methanotrophs. Such "hot spots" would also share more co-occurring than site-specific taxa.

To compare the site-specific methanotrophic interactomes, we applied stable isotope probing (SIP) using ^{13}C-CH_4 coupled to a co-occurrence network analysis to probe trophic interactions in widespread methane-emitting environments. Network analysis revealed predominantly unique co-occurring taxa from different environments, indicating distinctly co-evolved communities more strongly influenced by other parameters than high substrate availability. In the majority of all instances, the networks derived from the ^{13}C-CH_4 incubation exhibited a less connected and complex topology than the networks derived from the $^{unlabelled}C$-CH_4 incubations, likely caused by the exclusion of the inactive/non-replicating microbial population and spurious connections; DNA-based networks (without SIP) may thus overestimate the network complexity.

In contrast to our hypothesis, each environment contained distinct interactomes of methanotroph/methanotroph, as well as methanotroph/non-methanotroph. Such data indicate an influence of site-specific parameters over substrate availability, which leads to the assumption of different over all community functions.

ST166
The chemical and enzymatic fight of entomopathogenic *Photorhabdus luminescens* against phytopathogenic fungi
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Crop loss by phytopathogens is an everlasting threat in agriculture. Application of conventional pesticides harm the environment or humans. Therefore, new sustainable and biological alternatives are needed. The entomopathogenic bacterium *Photorhabdus luminescens* is already in use as symbiont of nematodes as biocontrol agent against insect pests. The bacteria are characterized by two phenotypic different cell variants: the symbiotic primary (1°) and non-symbiotic secondary (2°) cells. After insect infection a part of *P. luminescens* cells undergo a phenotypical switch from 1° to 2° cells, which remain in the soil specifically interacting with plant roots. There, 2° cells display increased antifungal activity against phytopathogenic fungi therefore suggesting a life in association to plant roots potentially protecting them from pathogens. Here, we showed that the antifungal activity of 2° cells is dependent on the exo-chitinase Chi2A and the chitin binding protein CBP, while both related genes are upregulated in presence of plant root exudates. The ability of Chi2A to digest chitin as well as the antifungal activity was successfully proven in bioassays using the phytopathogen *Fusarium graminearum*. Furthermore, we analysed the

secondary metabolites produced by *P. luminescens* on bioactivity against fungi. We could identify anthraquinones and an isopropyl stilbene showing a putative fungicidal effect since we observed antifungal activity of fractions containing these, repressing spore germination of *Fusarium graminearum* and *Magnaporthe oryzae*. As anthraquinones are produced by 1° cells during the infection of insect larvae the observed antifungal activity might also protect the insect cadaver from fungal concurrence. In summary, this work gives further insights in the molecular mechanisms of *P. luminescens* 2° cells as plant growth promoting organism promising new applicability in agriculture.

ST167

Bakta: Rapid & standardized annotation of bacterial genomes via alignment-free sequence identification

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Introduction

Command line software tools for the regional and functional annotation of bacterial genomes have gained popularity compared to centralized online services due to the worldwide increase of sequenced bacterial genomes. However, results of existing command line software tools depend on taxon specific databases or well annotated reference genomes.

Objectives

We addressed these issues via a new tool for the automated, taxon-independent, standardized but nonetheless fast annotation of bacterial genomes. Therefore, we aimed at a well-balanced tradeoff between runtime performance and comprehensive annotations. Furthermore, we addressed open gaps in contemporary software tools, *e.g.* lacking annotations of small proteins and assignments of stable public database identifiers.

Implementation

We implemented a new bioinformatics command line software tool called Bakta in Python 3 that runs on MacOS and Linux systems. It is freely available under a GPLv3 license at https://github.com/oschwengers/bakta and installable via BioConda packages and Docker container images. For further convenience, we have developed an accompanying web version providing interactive widgets and visualizations: https://bakta.computational.bio.

Results

Bakta conducts a comprehensive workflow annotating coding, tRNA, tmRNA, rRNA and ncRNA genes as well as CRISPR arrays, oriC, oriT and regulatory ncRNA features. The annotation of coding sequences is accelerated via an alignment-free sequence identification approach that also facilitates the precise assignment of stable identifiers to public databases like RefSeq and UniProt. In contrast to existing tools, Bakta is able to annotate small proteins of less than 30 amino acids. All features are rigorously filtered by annotations and overlaps. Final results are exported in human and machine readable standard file formats including INSDC-compliant EMBL and GenBank. We compared Bakta to other rapid contemporary command line tools in both targeted and taxonomically broad benchmarks on isolates and metagenomic-assembled genomes. We demonstrated that Bakta outperforms other tools in terms of functional annotations, assignments of functional categories and database cross-references whilst providing comparable wall clock runtimes.

Conclusion

We provide the community with a new software tool for the automated, comprehensive, taxon-independent but nonetheless fast annotation of bacterial genomes as a suitable alternative to Prokka and PGAP.

ST168

Membrane transport as crucial screw for metabolic engineering of microbial cell factories

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In our societies pursuit to substitute petroleum as carbon and energy source, industrial microbiology is one jigsaw piece. Microbial conversion of alternative carbon sources, such as plant derived sugars or municipal waste into fuels or chemicals of higher value is a promising approach. Such processes take generally place under conditions, which are far from the natural surroundings of microorganisms. So, the efficiency of many proposed processes is low, beacause the cell factories are stressed and concerned with survival instead of converting the substrate into our desired product. A key point to understand what stress means, is understanding the barrier, keeping the stress outside - the membrane.

On the one hand it should not be penetrable by unwanted substances, but on the other hand the substrates and products should be transported through the membrane with the highest possible rate and efficiency. This is a vast area for metabolic engineering and sysnthetic biology. The problem is, that our understanding of membranes and membrane transport proteins is far from complete, hindering rational engineering approached in many ways.

Even for many of our pet organisms, we do not even know, which proteins transport the most abundant substrates and products, let alone that we would understand how the mambranes work in detail.

Here, I want present some examples for membrane transport proteins, which we identified. Among them citric acid transporters and glycerol transporters from bacteria, yeasts and filamentous fungi. Some of the transporters can directly be used to improve bioprocesses - others cannot. There remains a lot to do and understand....

ST169

Engineering polyketide production routes in amoeba by exploiting native and synthetic hybrid enzymes

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Aromatic polyketides are natural polyphenolic compounds having a broad spectrum of pharmacological activities. Genome sequencing continuously expands the number of novel polyketide synthases (PKSs) in all kingdoms of life but often without a link to actual molecules, their biological activity or therapeutic potential. Heterologous expression of the producing enzymes in bacteria such as *Escherichia coli* or in the yeast *Saccharomyces cerevisiae* presents a widely known alternative to produce these molecules, but the lack of accessory secondary metabolic pathways in these model organisms often remains a limiting factor.

Social amoebae are intrinsic producers of such polyketides and harbor a large reservoir of PKS genes, two of them encoding unique types of natural hybrid enzymes of type I fatty acid synthases and type III PKSs. Here, we have exploited the social *Dictyostelium discoideum* as a chassis for the production of aromatic polyketides by expressing its native and cognate plant PKS genes. Extrachromosomal expression of natural hybrids led to the production of phlorocaprophenone and methyl-olivetol. Further genetic engineering using various plant polyketide synthases and a eukaryotic multi-gene expression tool led to the formation of naringenin, resveratrol, and olivetolic acid (OA). The latter acts as the central intermediate in the cannabinoid biosynthesis pathway. To further facilitate OA synthesis, we engineered a synthetic amoeba/plant inter-kingdom hybrid enzyme which produced OA from primary metabolites in only two enzymatic steps, providing a shortcut in the plant cannabinoid pathway. As the amoebae could also be successfully cultivated in high cell densities in bioreactors at the hundred liter scale, we are currently evaluating *D. discoideum* as a novel microbial host system for the production of polyketides.

Reimer C, Kufs JE, Rautschek J, Regestein L, Valiante V, Hillmann F. *Nat Biotechnol*, 2020, accepted

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ST170

UstiLisa – a Sars-CoV2 antigen test based on unconventional secretion and immobilization of *U. maydis* chitinase Cts1

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The Covid-19 pandemic has greatly impacted the global economy and health care systems. The need for quick and cheap responses to a pandemic threat like Covid-19 in the form of vaccines and antigen testing systems has become very clear during the last two years. The causative agent of Covid-19 is Sars-CoV2. It harbors a spike protein that interacts with the human angiotensin-converting enzyme (ACE2) via the so called receptor binding domain (RBD) and thereby facilitates virus to cell fusion. This RBD has become a target of great research interest, as a target for vaccines, therapeutical antibodies and for antigen testing systems. Right now antigen testing is generally done in one of two ways, either via flow chromatography using antibodies and gold or latex particles or via ELISA type assays. Both harbor increased cost due to the use of expensive materials like gold particles and protein adhesive polymers. In this study we present an alternative ELISA approach using cheap materials and quick detection. The chitinase Cts1 is unconventionally secreted in the smut fungus *Ustilago maydis* and binds chitin with a high affinity. By fusion of heterologous proteins to Cts1 its unconventional secretion pathway can be utilized for the export of biopharmaceuticals such as ScFvs and nanobodies. Here, we secreted several different anti-Sars-CoV2 RBD nanobodies fused to Cts1 and screened them for their binding affinity to the RBD *in vitro* and Sars-CoV2 *in vivo.* We present a straightforward study with the goal to generate an antigen test that is based on a chitin surface with immobilized nanobody-Cts1 fusions forming a RBD-trap and functionalized Sars-CoV2 nanobodies for detection. This study should be a clear proof of principle that cheaper materials can be incorporated into antigen testing systems to enhance testing capabilities worldwide, not only for Covid-19 but for viral epidemics and pandemics in general.

ST171

Generation of nano-magnetic hybrid materials by genetic engineering and functionalization of bacterial magnetosomes

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For orientation along geomagnetic field lines, magnetotactic bacteria like the alphaproteobacterium *Magnetospirillum gryphiswaldense* biomineralize magnetic nanoparticles (magnetosomes), which consist of a monocrystalline magnetite core that is surrounded by a proteinaceous phospholipid bilayer. Due to their strictly regulated biosynthesis, magnetosomes exhibit a number of unprecedented characteristics such as high crystallinity, strong magnetization, and uniform shape and size that can only hardly be achieved by chemical synthesis [1]. Moreover, both the crystal morphology and the composition of the enveloping membrane can be manipulated *in vivo* by genetic means. Because of these unique features, bacterial magnetosomes have the potential to yield biomaterials for use in numerous (bio)medical and biotechnological applications [2].

As the latter would benefit from particles that in addition to their magnetic properties display further functionalities on the surface, we developed a versatile "toolkit" for the generation of multifunctional magnetosomes with several entirely genetically encoded properties. Highly abundant magnetosome membrane proteins were used as anchors for the expression of foreign moieties as large hybrid proteins. In order to demonstrate the potential of this synthetic biology approach, a set of model particles was created that simultaneously displayed different catalytic activities, fluorescence and molecular connectors [3].

For their future use in the (bio)medical field, we investigated potential cytotoxic effects of magnetosomes on eukaryotic cells. The viability of different cancer cell lines as well as primary cells was not impaired when incubated with wildtype magnetosomes for up to 24 h, and particle accumulation in endolysosomal structures around the nuclei was observed. Furthermore, short-term incubation with magnetosomes was found to be sufficient to allow for magnetic separation of particle-loaded cells, with recovery rates of up to 60% [4].

Overall, our results demonstrate the versatile features of (engineered) bacterial magnetosomes, with enhanced potential for numerous biotechnological or biomedical applications, such as biosensors, magnetic drug targeting, or magnetic imaging techniques.

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ST172

Microalgae Microbiomes – A Natural Source for the Prevention and Treatment of Diseases in Aquaculture

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Aquaculture is one of the fastest growing food sectors in the world with land-based aquaculture gaining increasing interest. Diseases caused by bacteria like *Pseudomonas* sp., *Flavobacterium* sp.*, Aeromonas* sp. *or Edwardsiella* sp. are an obvious challenge to the aquaculture industry exacerbated by many pathogen"s biofilm building abilities. Antibiotics are still in use in many regions of the world, contributing to increased antimicrobial resistance. Our work builds on the hypothesis that a novel approach to disease treatment in aquaculture can be achieved by exploiting the healthy properties of microalgae and their associated microbiomes.

To advance this field, we performed both functional and sequence-based screening of microalgae and microbial consortia for anti-biofilm activities. Five promising candidates of microalgae which showed significant anti-biofilm traits were chosen for further investigation. Ongoing metagenomes-, metaproteomes- and metatranscription analyses gave first insights into the main enzymes involved in these antibiofilm effects.

ST173

Microbial risk assessment with focus on plant oil-derived fuels

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Objective

The legally required biological content of fuels is continuously increasing, and as consequence fuels get more susceptible to microbial contamination during long-term storage. Therefore, it is critical to develop tools that allow manufacturers to assess this vulnerability right after production which in turn indicates the need for action in the form of additives or consistent maintenance performance. We present here such a tool along with the resulting resistance difference of the vegetable oil-based fuels rapeseed oil methyl ester (RME) and hydrogenated vegetable oil (HVO) towards microbial contamination.

Materials & Methods

In the AIF-funded DGMK project 823, we are developing together with our project partners from the Department of Environmental Biology and Chemodynamics at the RWTH Aachen University and the OWI (Oil-Waerme-Institute) an approach for the assessment of microbial activity in heating oil storage systems. Our approach consists of the simulation of the oil tank situation in laboratory format, the use of a representative mixture of microorganisms and mobile BCP-CO_2 sensors (BlueSens). Hydrocarbons in fuels are degraded by microbes to CO_2 and H_2O. The infrared CO_2 sensor is attached airtight to the culture vessel and measures CO_2 formation. The CO_2 concentration indicates the extent of microbial activity. The extent of microbial activity in our test system indicates the vulnerability towards microbial contamination during long-term storage.

Results and Conclusion

Transesterification of rapeseed oil with methanol leads to rapeseed oil methyl esters (RME), which are simple carbon sources for microbes and store water in large quantities. Our test approach confirms a 2-3 times higher microbial activity for RME blends compared to fossil heating oils.

The hydrogenation of rapeseed oil leads to mixtures of alkanes and alkenes (HVO), which are difficult carbon sources for microbes and store low amounts of water comparable to fossil heating oils. Our test approach documents that pure HVO has a similarly low microbial risk as fossil heating oils and, if necessary, can be used to lower the risk in endangered batches by blending.

ST174

Powering an artificial enzymatic cascade for the synthesis of *N*-heterocycles with electrical energy

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Introduction: Saturated *N*-heterocycles are widespread in biologically active molecules. In particular, pyrrolidines and piperidines are increasingly attractive scaffolds found in agrochemicals, pharmaceuticals and natural alkaloid products. Only limited biological strategies exist for the preparation of saturated nitrogen heterocycles.

Objective: We aim to design an artificial enzymatic cascade for the synthesis of N-heterocycles and combine it with scalable platform that power the cascade in a continuous flow reactor with electricity.

Methods: Our artificial enzymatic cascade for the synthesis of *N*-heterocycles from diamines included an engineered O2-dependent putrescine oxidase [1], a designed NADH-dependent imine reductase and the O2-tolerant NAD+-reducing hydrogenase from *Ralstonia eutropha* [2]. Both required H2 for the hydrogenase and O2 for the oxidase were produced by electrolysis and transferred *via* a gas permeable membrane into the flow system. A gas-permeable tube enabled the separation of the electrolyte from the biocatalysts in the flow system, where H2 and O2 served as electron mediators for the biocatalysts.

Results: Through powering the cascade with electricity, substituted pyrrolidines and piperidines were obtained with up to 99% product formation in a one-pot reaction directly from the corresponding diamine substrates [3]. We extended the applicability of the system towards performing regioselective isotopic labeling providing useful insights into the enzyme mechanism of the hydrogenase.

Conclusion: This platform represents an important advance in the field for biocatalytic synthesis, and it can be expanded for powering various cofactor-dependent oxidoreductases.

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ST175

Lignin-based aromatic amino acid synthesis by aminomutases and ammonia lyases: Conversion studies, mutagenesis experiments, and evolutionary considerations

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Lignocellulose is an underused sustainable source of aromatic compounds. To valorize the low-value lignin monomers, we propose enzymatic conversion of cinnamic acid derivatives to generate value-added canonical and non-canonical aromatic amino acids. Among the latter, chiral β-amino acids are recognized as building blocks for natural compounds as well as for active pharmaceutical ingredients.

A library of enzyme variants of the *(R)*-selective phenylalanine aminomutase from the tree *Taxus chinensis* (TchPAM) was established by using Rosetta Enzyme Design and structure screening of the designed mutants. With multiple position mutations, this enzyme was successfully modified to accept β-tyrosine as substrate, as well as to generate *(R)*-β-tyrosine with excellent enantiopurity (*ee* >99%) as the unique product from *trans-p*-hydroxycinnamic acid. Kinetic parameters were determined for the synthesis of different phenylalanine and tyrosine enantiomers by four Y424 enzyme variants [1].

Based upon conservation analysis, computational simulation, and mutagenesis experiments, we report the proposed function of the highly conserved residues in the active site of TchPAM and their putative role for maintaining the enzyme"s strict enantioselectivity.

We performed an analysis of the phylogenetic relations between phenylalanine-/tyrosine aminomutases (PAM/TAM) and phenylalanine-/tyrosine-/histidine ammonia lyases (PAL/TAL/HAL) which demonstrated that these MIO-dependent enzymes most probably have a common evolutionary origin from ancient bacteria. Strong indications for a horizontal gene transfer between bacteria, fungi, and plants in symbiotic association after acquirement of the PAL gene from their ancestor leads us to propose an early function of these enzymes for acclimatization to adverse terrestrial environments under high UV light exposure [2].

In summary, we report a β-tyrosine synthesis process from a monolignol component, offering a new way for lignin valorization by biocatalyst modification.

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ST176

A multi-scale genomic approach to explore Acidovorax interaction with a model plant host, Lotus japonicus

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Acidovorax is a plant-associated genus of Bacteria, which has been known to promote plant growth or act as a pathogen. These effects are considered to be species or even strain specific, but so far no clear phylogenetic pattern has emerged. To better understand the interaction of different Acidovorax strains with a model plant, Lotus japonicus. We investigated Acidovorax genomic idiosyncrasies at pan-genome, community and isolate scale, as well as their connection to host health. At pan-genome level, genomes of 106 pathogenic, commensal and free-living strains were compared. At community level, we used in planta experiments to characterize the effects on Lotus growth. We studied a community of 52 Acidovorax strains which were previously isolated from the same host, and isolated genomic differences correlated with growth promotion and growth inhibition. At isolate level, we compared trancriptional profiles of the 2 isolates which were correlated with the most pronounced negative and positive effects on plant growth. These profiles were obtained by RNA sequencing after exposure to Lotus derived metabolites from uncolonised or arbuscolar mycorrhizae-colonised plants. Our pan-genome comparison retrieved protein families associated with commensals (371), pathogens (303), and differences in genome length, number of genes per kb and predicted secondary metabolism. At community level, 7 growth promoters were identified along with a set of 19 discriminating protein families, which were related to chemotaxis, uptake and metabolism of plant derived metabolites. At the isolate level, we found differences in the regulatory network, expression profiles, and a differential response to the arbuscolar mycorrhiza-altered metabolite landscape. Our results demonstrate the dominant role of genomic features in shaping the outcome of Acidovorax associations to its plant hosts and we isolated novel biomarkers to target in future investigations, thus promoting the understanding of plant-microbe interactions and the development of microbiome-augmented agricultural practices.

ST177

Planctomycetes In The Global Context: Comparison And Analysis Of Genomic Architectures Of Bacterial And Archaeal Clades

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The tree of life is filled with examples of the diversity created by nature. This diversity seems to be intensified when it comes to prokaryotic organisms, which defy the limits of what is possible. One of the rarities found in the bacterial domain are Planctomycetes. This bacterial phylum shows some particularly special physiological traits, such as extended cytoplasmic membranes, actual "prokaryotic organelles", such as the anammoxosome in anammox bacteria, unusual life cycles and cell division by budding. They also display a range of biochemical novelties in terms of both enzymes, such as hydrazine synthase, and molecules, such as the production of sterols or ladderanes as well as the encoded potential to produce secondary metabolites. Even their genomes seem to be special: they are 25% bigger on average than those of other planktonic bacteria, and some important biosynthetic gene clusters seem to be undetectable or misplaced, as well as those containing 16S rRNA gene sequences or cellular division genes. From these cell division genes, some essential, canonical ones seem to be missing altogether. These characteristics have raised the question of whether their genomic architecture is different to that of other bacterial species. Some authors have hypothesized that it is not, claiming that Planctomycetes lack operons or that their genomic rearrangement is faster than in other bacterial species. However, without a global analysis on genome architectures it is impossible to prove or refute those claims. Here, we compared the genomic architecture of Planctomycetes to that of both bacterial and archaeal clades. These analyses helped us determine whether planctomycetal genome architecture is similar to those of archaea and if it indeed differs significantly from other bacterial species. The results of our study contributed to shed light on the mystery that Planctomycetes still pose, as well as to highlight the diversity of prokaryotic genomic architectures.

ST178
EDGAR3.0 - Comparative genomics and phylogenomics on a scalable infrastructure

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The EDGAR platform, a web server providing databases of precomputed orthology data for thousands of microbial genomes, is one of the most established tools in the field of comparative genomics and phylogenomics. Based on precomputed gene alignments, EDGAR allows quick identification of the differential gene content, i.e. the pan genome, the core genome, or singleton genes. Furthermore, EDGAR features a wide range of analyses and visualization features required for phylogenomic inter- and intraspecies taxonomic analyses. EDGAR calculates core-genome-based phylogenetic trees as well as amino acid identity (AAI) and average nucleotide identity (ANI) matrices. Furthermore, it offers convenient visualization features such as Venn diagrams, synteny plots, and a comparative view of the genomic neighborhood of orthologous genes. Thus, the software enables a quick survey of evolutionary relationships and simplifies the process of obtaining new biological insights into the differential gene content of kindred genomes.
During the last few years, the average number of genomes analyzed in an EDGAR project increased by two orders of magnitude. To handle this massive increase, a completely new technical backend infrastructure for the EDGAR platform was designed and launched as EDGAR3.0. For the calculation of new EDGAR3.0 projects, we are now using a scalable Kubernetes cluster running in a cloud environment. A new storage infrastructure was developed using a file-based high-performance storage backend which ensures timely data handling and efficient access. The new data backend guarantees a memory efficient calculation of orthologs, and parallelization has led to drastically reduced processing times.
Based on the advanced technical infrastructure new analysis features could be implemented including POCP and FastANI genome similarity indices, UpSet intersecting set visualization, and circular genome plots. Also the public database section of EDGAR was largely updated and now offers access to 24,317 genomes in 749 free-to-use projects. In summary, EDGAR 3.0 provides a new, scalable infrastructure for comprehensive microbial comparative gene content analysis. The web server is accessible at http://edgar3.computational.bio.

ST179
Profiling the diversity of antiphage defense systems in nitrifying prokaryotes reveals host-specific viral defense strategies and infecting phage types

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Research in the last decades has provided important insights into the biology of nitrifying prokaryotes, including knowledge of environmental factors affecting their ecology, such as pH, nutrient availability, temperature, and salinity. However, an important but unanswered question is the extent to which environmental viruses influence the nitrification process and the ecology of key players. Nitrifying prokaryotes include ammonia-oxidizing Thaumarchaeota (AOA) and bacteria (AOB) that carry out the rate-limiting step of converting ammonia to nitrite. Current knowledge of potential viruses infecting nitrifiers comes mainly from bioinformatic studies fostered by single-cell genomics and metagenomics of the marine microbiome. To date, however, only a single phage has been isolated. The novel spindle-shaped virus is highly specific to the AOA host strain, replicates via a budding replication strategy that precludes host cell lysis, and is extremely rare in the oligotrophic ocean. Overall, this contributes to the lack of evidence for active lysis of AOA and AOB cells by phages despite the high biomass and diversity of nitrifying prokaryotes in aquatic and engineered systems. One possible reason for our current inability to isolate phages capable of lysing nitrifying prokaryotes is the possession of potent antiviral mechanisms that confer host resistance to phage predation. Accordingly, we performed extensive genome-wide profiling of antiphage systems in a variety of ecologically important nitrifying prokaryotes to elucidate innate and adaptive viral defense strategies and to identify the diversity of viruses that potentially infect these hosts. We found a wide range of antiphage mechanisms in nitrifying prokaryotes, although these are distributed differently in different taxa. Importantly, these pathways are also strongly expressed in freshwater and marine systems, suggesting that nitrifying prokaryotes are frequently targeted by viruses in nature. In this talk, I will review the mechanisms of host phage resistance and highlight the diversity of viruses that infect different nitrifying prokaryotes, including whether each virus can infect every host equally

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ST180

Self-transmissible plasmids are highly prevalent in members of the genera *Thauera* and *Aromatoleum* and form a novel IncP-1 subgroup

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Self-transmissible plasmids of the incompatibility group P (IncP) are considered important vehicles of genes for antibiotic resistance and various other metabolic functions. Many IncP plasmids were retrieved from environmental samples by exogenous plasmid capturing without knowing their native hosts. Previously, we found an IncP-1e plasmid in *Thauera aromatica* K172, a facultative denitrifier that can degrade various aromatic compounds. The related *Aromatoleum aromaticum* EbN1 also carries a replicon that was tentatively classified as IncP-1 plasmid. Therefore, we queried all publicly available single-isolate genomes of the family *Zoogloeacea*, to which *Thauera* and *Aromatoleum* belong, improved the already available genome assemblies of *Ar. anaerobius* LuFRes1, *Ar. buckelii* U120, *Ar. toluolicum* T, *Aromatoleum* sp. PAO1, *Th. phenylacetica* B4P, and *Thauera* sp. 2A1, and generated draft genome sequences of the three *T. aromatica* strains AR-1, LG356, and SP. Evolutionary histories were examined by phylogenetic tree building and comparing nucleotide signatures.

In total, we found 10 IncP-type plasmids in the 40 sequenced genomes of *Thauera* and *Aromatoleum*. Most of the plasmids form a novel IncP-1 subgroup to which we assigned the Greek letter q. While IncP-type plasmids are highly prevalent in *Thauera* and *Aromatoleum*, there was no indication for long-term co-evolution of plasmids and hosts based on nucleotide signatures. The plasmids in strains EbN1, PA01, and U120 carry the genes for anaerobic degradation of *o*-phthalate, whereas the plasmid in strain LuFRes1 harbors the genes for anaerobic resorcinol degradation. Both plasmids in strains K172 and B4P carry multiple antibiotic resistance genes. In contrast, no IncP-type plasmid was detected in 25 sequenced *Azoarcus* and *Zoogloea* strains, the other members of *Zoogloeacea*. Thus, it appears that members of *Thauera* and *Aromatoleum* are common hosts of IncP-type plasmids for reasons that are still unknown.

ST181

Functional Metagenomics as a tool to identify novel CODH enzymes

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Carbon monoxide dehydrogenases (CODHs) catalyze the reversible reaction between carbon monoxide and carbon dioxide. These enzymes can be involved in autotrophic carbon fixation and energy conservation by the reductive acetyl-CoA pathway (Wood-Ljungdahl pathway), which enables acetogenic bacteria, autotrophic sulfate-reducing bacteria, planctomycetes as well as methanogenic archaea to grow on inorganic carbon sources. Since the vast majority of microbes is currently not cultured, the exploration of a large biochemical potential remains limited using culture-dependent and sequence-based methods. Therefore, we have developed a function-based screening approach to seek CODHs from metagenomic fosmid libraries by detecting the oxidation of CO to CO2 and without the need to sequence the DNA beforehand. To test which CODHs from phylogenetically different microbial groups can be targeted using our newly developed screen, we applied it successfully to fosmid clones from genomic libraries constructed with material from *Moorella thermoacetica*, *Desulfovibrio vulgaris* and *Rhodospirillum rubrum*. Currently, the screen is applied to a metagenomic fosmid library constructed using anoxic marine sediments from the Eckernförde Bight (Baltic Sea, Germany) to discover new CODHs from anoxic environments.

ST182

Ecophysiological and metagenomic evidence for differences in the contribution of comammox *Nitrospira* clade A and clade B to nitrification in groundwater

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Complete ammonia oxidizing bacteria (CMX) of the genus *Nitrospira* are capable of performing both steps of nitrification in one single cell. Ecophysiological characteristics of CMX indicated specialization towards nutrient limited terrestrial environments by high affinity to ammonia and higher growth yields than canonical ammonia oxidizers. This may grant CMX a competitive advantage over terrestrial ammonia-oxidizing archaea (AOA) and bacteria (AOB) under oligotrophic conditions such as in groundwater. However, details are still lacking about the ecophysiological preferences of CMX clade A and B in the groundwater and the interplay of CMX with other nitrifying microbes as well as their contribution to nitrification. Therefore, we assessed the relevance of the three groups of ammonia oxidizers for nitrification activity in spatially heterogenous oxic and hypoxic shallow groundwater from carbonate-rich aquifers, including potentially distinct roles of the two CMX clades. Quantification and amplicon sequencing of the ammonia monooxygenase subunit A gene (*amoA*) showed that the abundances of CMX clade A and AOB were higher at sites with elevated ammonium and lower oxygen concentrations while we found an opposite trend for CMX clade B and AOA. In addition, the abundance of CMX clade A phylotypes and AOB *amoA* genes had a positive correlation to in situ nitrification activity (1.7 to 125 nmol N L^{-1} d^{-1}) measured by ^{15}N-based labeling assays. In groundwater incubations we observed growth of CMX and AOB with ammonium as electron donor but not of AOA suggesting that both groups are responsible for ammonia oxidation. Genome-resolved metagenomic analysis of both CMX clades indicated that clade B metagenome assembled genomes harbored the genetic potential for thiosulfate oxidation, formate uptake and genes involved in conversion of reactive oxygen species while clade A MAGs had a larger number of genes associated with nitrogen cycling. In conclusion, our findings suggest that both CMX and AOB contribute to overall nitrification activity in oligotrophic groundwater. We further propose ecophysiological differentiation of CMX clade A and B representatives in groundwater with a stronger involvement of clade A in nitrification and utilization of alternative electron donors by clade B.

ST183

Acquisition of a *scr*-gene cluster coding for sucrose metabolization enzymes enables isolates of *Vibrio parahaemolyticus* and *Vibrio vulnificus* to utilize sucrose

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Vibrio parahaemolyticus isolates are usually unable to utilize sucrose as primary carbon source, while a few exceptions were observed during routine investigations in the consultant laboratory for *Vibrio* spp. in food at the BfR. Based on the characterization of a representative sucrose utilization-positive isolate by whole-genome sequencing (WGS), a genomic island exhibiting sucrose utilization genes was determined. The island represents a 4.7 kb DNA-region exhibiting *scrA, scrK* and *scrB* encoding a sucrose uptake protein, a fructokinase, and a sucrose-6-phosphate hydrolase, respectively. The *scrAKB* region was amplified by PCR and inserted into the shuttle vector pVv3. Two recombinant plasmids, only differing in the orientation of the insert with respect to the pVv3-*lacZα*-fragment, were generated and introduced into *E. coli* resulting in transformants which are able to utilize sucrose. Furthermore, transformation of sucrose utilization-negative *V. parahaemolyticus* and *V. vulnificus* isolates also showed a change of their respective phenotypes.

To identify the dissemination of the *scr*-genes in *V. parahaemolyticus*, a multiplex PCR targeting *scrA, scrK,* and *scrB* was developed and used for molecular surveillance. A total of 45 *scr*-positive isolates were detected and phenotypically confirmed for its sucrose utilization ability. For *in-depth* characterization, 17 *scr*-positive isolates were subjected to WGS and bioinformatics analysis. The *scr*-containing island was identified in all isolates with a nucleotide identity of >95%. However, for some isolates variations in the *scr*-region were detected, represented by the occurrence of additional coding sequences (CDS). The identified CDS encoding a transcriptional regulator (*scrR*), a porin encoding gene and a gene product of unknown function. As sequence comparison showed a similar location of the *scr*-island on chromosome II of all analyzed isolates, we assume a directed, but a yet unidentified mechanism associated with the incorporation of the genes. Structural comparison of the genomes with sequences of the sucrose utilizing species *V. alginolyticus* revealed the same genomic island, which indicates a possible distribution of this genetic structure by horizontal gene transfer. The comparison of all genome sequences based on SNP differences reveals that the presence of sucrose utilizing genes is found in genetically diverse *V. parahaemolyticus* strains and is not restricted to a subset of closely related strains.

ST184

Contact-dependent predation processes of the predatory soil bacterium *Myxococcus xanthus* rely on protein secretion systems

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Myxococcus xanthus is an ubiquitous predatory bacterium, which feeds on a broad spectrum of microorganisms in soil. Bactericidal antibiotics, bacteriolytic proteins and membrane vesicles have been shown to be involved in myxobacterial predation, but the molecular basis of prey killing mechanisms and their efficacy against different prey remains largely unknown. Our recent findings show that only Gram-positive bacteria can be lysed by secreted bacteriolytic proteins. Gram-negative prey cells seem to be protected by their outer membrane, but can be killed and lysed by individual *M. xanthus* cells in a cell contact-dependent manner[1]. Contact-dependent prey killing occurs within minutes, which hints to a specific and targeted secretion of killing factors[2]. We hypothesized that dedicated protein secretion systems might mediate this process.

We deleted genes predicted to encode protein secretion system components in *M. xanthus* and screened for a putative role in predation. Two gene clusters encoding partial secretion systems were identified and their function was characterized in a range of co-culture assays. A main focus was put on the analysis of single cell predation in real time and the dynamic localization of secretion system components by fluorescence microscopy. Our observations suggest that both secretion systems have overlapping, but not identical, functions in *M. xanthus* contact-dependent prey killing. A double mutant, lacking components of both secretion systems, lost its ability to extract energy from Gram-positive and Gram-negative bacteria. These results emphasize the importance of contact-dependent prey killing by individual cells for the complex predation behavior of a *M. xanthus* population.

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ST185

Progression of the late-stage divisome is unaffected by the depletion of the cytoplasmic FtsZ pool

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The bacterial divisome is a highly dynamic and complex machinery that consists of a multitude of individual, closely interacting proteins coordinating the process of cell division to finally generate cell progeny. In this timely and spatially regulated process, FtsZ has been regarded the central pacemaker for regulating and building up the divisome machinery at the division site (1,2). In this study, we set out to further investigate the role of FtsZ and FtsZ ring dynamics in the course of divisome assembly and progression in rod-shaped bacteria. To this end, we made use of ADEP antibiotics as tools to disturb the divisome at the level of FtsZ ring assembly. Our previous studies showed that ADEPs preferably induce the degradation of FtsZ (3,4), resulting in a rapid depletion of the cytoplasmic FtsZ pool that is required for FtsZ ring dynamics. To study the role of the cytoplasmic FtsZ pool during division, *Bacillus subtilis* cells were treated with ADEP antibiotics while divisome formation and progression was simultaneously followed via time-lapse and super-resolution fluorescence microscopy (5). We observed that early FtsZ rings rapidly disintegrated upon ADEP treatment, which proves an essential role of the cytoplasmic FtsZ pool during initial assembly of the early divisome. However, unexpectedly, more progressed FtsZ rings constricted and finalized septum formation despite the depletion of the FtsZ pool, a turnaround that coincided with the arrival of late-stage divisome proteins, i.e. the septal peptidoglycan synthase PBP2b. Therefore, our data specifies the role of FtsZ in divisome assembly and progression in rod-shaped bacteria, revealing a two-step mechanism: while the FtsZ pool and FtsZ ring dynamics have an essential role during initiation and maturation of the divisome, both are considerably less critical during the late stages of divisome assembly and progression. Then, other triggers most probably take over to drive divisome progression, for example, peptidoglycan synthases that arrive at the divisome during the later stages of cell division.

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ST186

Tat transport of homodimers – two signal peptides for one protein

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The twin-arginine translocation (Tat) system transports folded proteins across the cytoplasmic membrane of prokaryotes, the thylakoid membrane in plant plastids, and the inner membrane in plant mitochondria. To be recognized by the translocon, Tat substrates are synthesized as precursors with N-terminal signal peptides that contain the eponymous "twin-arginine motif". The signal peptide usually is cleaved off after transport to release the mature protein. Many Tat substrates are homodimers, and a complete folding of these homodimers before transport would require the transport with two signal peptides, which mechanistically would be a challenge. We analysed this aspect with the periplasmic pyoverdine maturation protein PvdN, which is a dimer with two PLP cofactors at the subunit interface. The PLP cofactor is covalently bound to a lysine. We have found that substitution of this lysine by alanine results in a complete abortion of translocation, and PvdN is trapped in the membrane [1]. To examine the oligomeric state of PvdN in the membrane, we carried out cross-linking analyses. These experiments showed that PvdN is monomeric in the membrane-trapped state. In contrast, cross-linking confirmed the dimeric state of wildtype PvdN after transport into the periplasm. When produced without the signal peptide, PvdN efficiently formed dimers inside the cytoplasm, which was confirmed by MALS analyses after purification. However, when we produced PvdN with a mutated signal peptide that could not mediate transport anymore, the precursor protein was not dimeric, indicating a novel functional role of the signal peptide for prevention of cytoplasmic dimerization. This was further studied by co-translocation analyses. The data suggest that the covalently bound PLP cofactor masks a hydrophobic patch in PvdN subunits, which permits their Tat-dependent translocation. When PLP is not bound, i.e. in the Lys>Ala variant, this exposed hydrophobic patch aborts the membrane passage and results in membrane-trapped PvdN monomers.

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ST187

Characterization of a HilD induced Motility Defect in *Salmonella enterica* Serovar Typhimurium

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Background: *Salmonella enterica* serovar Typhimurium (*S.* Tm) is a Gram-negative enteropathogen that utilizes a number of virulence factors to establish a successful infection. The bacterial flagellum and the evolutionary related injectisome encoded on the *Salmonella* pathogenicity island 1 (SPI-1) are important virulence factors for the initial infection stages. A complex cross-regulation network controls expression of flagellar and injectisome genes and involves activation of the flagellar master regulatory operon *flhDC* by HilD, the main activator of SPI-1 gene expression [1].

Results: Contrary to the HilD-dependent activation of flagella gene expression, we describe and characterize here the unexpected finding that induction of *hilD* results in a dramatic loss of motility while flagellar assembly was not affected. A full deletion of SPI-1 was able to restore motility upon *hilD* induction. Single-cell analyses of *hilD* induced cells revealed a pronounced decrease of proton motive force (PMF) using the voltage sensitive dye $DiSC_3(5)$ and a coccoid morphology, which were also restored upon SPI-1 deletion. Additionally, induction of *hilD* enhanced the adhesion of *S.* Tm to ModeK epithelial cells and a transcriptome analysis revealed upregulation of several adhesin systems upon *hilD* induction. In support, overproduction of *pef* and to a lesser extent *saf* fimbriae resulted in decreased motility.

Conclusions: In summary, we conclude that the *hilD* induced motility defect is caused by multifaceted processes. First, HilD-dependent induction of injectisome expression and subsequent secretion of effector proteins might deplete the PMF available for flagellar rotation. Depletion of the PMF might further result in mislocalization of MreB and formation of coccoid cells. Coccoid cells are believed to be less efficient swimmers compared to rod-shaped cells. Second, HilD-dependent upregulation of adhesive structures might affect the near-surface swimming behavior. We suggest that the simultaneous upregulation of the SPI-1 injectisome and adhesins allow *S.* Tm to rapidly modulate flagella function inside the host and therefore enable efficient adhesion to host cells and delivery of effector proteins.

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ST188

Cell-cell communication structures in the cyanobacterium *Nostoc* sp. PCC 7120

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Nostoc PCC 7120 is a filamentous cyanobacterium with multicellular traits: the filaments consist of vegetative cells and heterocysts, allowing the simultaneous CO_2 fixation by photosynthesis and N_2 fixation by nitrogenase. This division of labor requires a cell-cell communication system which can be investigated using fluorescent marker molecules in FRAP (fluorescence recovery after photo bleaching) measurements (1). Recently, we revealed the *in situ* architecture of septal junctions (SJ) in *Nostoc* by imaging focused ion beam-thinned filaments with cryo-electron tomography (2). The SJ exhibited a five-fold symmetric cytoplasmic cap module, a membrane-embedded plug domain and a tube traversing the septum thru peptidoglycan nanopores. Furthermore, we

showed that under stress conditions the cap structure undergoes a conformational change leading to a closed state abolishing intercellular exchange. Interestingly, the cap can switch back to the open, communication-allowing state, which renders SJs gated cell-cell connections functionally analogous to metazoan gap junctions. The impact of the cap and plug module on SJ closure was further demonstrated by a *fraD* mutant, which lacked the cap and plug modules and was unable to abolish communication when exposed to stress conditions (1). We also showed that the FraD protein is a structural SJ component, which localizes to the plug domain.

Here, we identified via co-immunoprecipitation the new putative SJ protein SepN, which is essential for proper SJ assembly. A mutant in *sepN* exhibited a highly reduced rate of intercellular communication, but inability of septal junction gating. Cryo-electron tomography of *sepN*-mutant filaments revealed septal junctions lacking the plug module. The combination of missing plug but present cap allowed to deduce the importance of the plug module in ensuring the correct cap architecture and in sealing the diffusion area in the closed SJ state.

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ST189

Phenotypic plasticity, facultative multicellularity and an environmentally dependent life cycle

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Understanding the evolutionary transition to multicellularity is a key problem in evolutionary biology. While around 25 independent instances of the evolution of multicellular existence are known across the tree of life, the ecological conditions that drive such transformations are not well understood. The first known transition to multicellularity occurred approximately 2.5 billion years ago in cyanobacteria, and today"s cyanobacteria are characterized by an enormous morphological diversity, ranging from single-celled species over simple filamentous to highly differentiated filamentous ones. While the cyanobacterium *Cyanothece* sp. ATCC 51142 was isolated from the intertidal zone of the U.S. Gulf Coast as a unicellular species, we are first to additionally report a phenotypically mixed strategy where multicellular filaments and unicellular stages alternate. We experimentally demonstrate that the facultative multicellular life cycle depends on environmental conditions, such as salinity and population density, and use a theoretical model to explore the process of filament dissolution. While results predict that the observed response can be caused by an excreted compound in the medium, we cannot fully exclude changes in nutrient availability. The best-fit modeling results demonstrate a nonlinear effect of the compound, which is characteristic for density-dependent sensing systems. Further, filament fragmentation is predicted to occur by means of connection cleavage rather than by cell death of every alternate cell. The phenotypic switch between the single-celled and multicellular morphology constitutes an environmentally dependent life cycle, which likely represents an important step en route to permanent multicellularity.

ST190

Activation of the acid resistance network of *Escherichia coli* under consecutive increasing acid stress

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Adaptation to acid stress is an important factor in the transmission of intestinal microbes, such as the enterobacterium *Escherichia coli*. To counteract acid stress, *E. coli* mainly activates three inducible acid resistance (AR) systems: the glutamate decarboxylase, arginine decarboxylase, and lysine decarboxylase systems[1]. Each system relies on the activity of a proton-consuming reaction catalyzed by a specific amino acid decarboxylase and a corresponding antiporter. Activation of these three systems is tightly regulated by a sophisticated interplay of membrane-integrated and soluble regulators and at different external pH values and growth phases[2,3].

To answer the question why *E. coli* has three inducible AR systems, we visualized and quantified the activation and distribution of each of the AR systems by fluorescent translational fusions of the membrane-integrated antiporters GadC, AdiC and CadB[4], respectively, at single cell level. This three-color tagged strain was exposed to consecutively increasing acid stress and single cells were monitored by epi-fluorescence microscopy revealing a cellular individuality during activation of each AR systems. The Cad and Adi systems were heterogeneously activated in an *E. coli* population at pH 5.8 and pH 4.4, respectively. The degree of heterogeneity of the Adi system was influenced by the intracellular pH. The Gad system was heterogeneously expressed mainly in the stationary phase. However, its heterogeneous production was reduced at mild acid stress (pH 5.8). Activation of the Adi system correlated with the Gad system, but Adi and Cad were mutually exclusive systems in individual cells. We are now characterizing AdiY, a AraC-type transcription regulator, as a potential soluble pH sensor controlling induction of the Adi system.

Our studies highlight the advantages of *E. coli* in possessing three AR systems that enable division of labor in the population, which ensures survival over a wide range of low pH values.

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ST191

Unraveling microbial co-cultures at the single-cell level inside closed picoliter chambers

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Microbial communities play important roles in microbial life, human health and future biotechnology. It is clear that

microorganisms inside complex communities interact with each other in many unexplored ways. Microfluidic tools in combination with live-cell imaging enable researchers to cultivate and analyze natural and synthetic microbial consortia in a highly controlled way with single-cell resolution.

In the present project, we have developed an innovative single-cell analysis approach enabling to cultivate and analyze defined microbial co-cultures inside microstructured picoliter environments. Since the micro environment can be physically closed and isolated from the medium supply, metabolites and other molecules secreted by the community members stay inside the cultivation chamber enabling systematic investigation of cellular interactions (for example, cross-feeding).

The polydimethylsiloxane (PDMS) microfluidic cultivation device [1] incorporates hundreds of cultivation chambers, each 60µm x 100µm x 1µm (width, length, height) in size, in which cell growth is restricted to monolayers by the limited chamber height. Furthermore, monolayer cell growth allows single-cell analysis in which interactions can be resolved even between cells in direct proximity. Each cultivation chamber is connected to a medium containing cavity resulting in a total cultivation volume of approximately 600 pL. Furthermore, several microfluidic measures were implemented, such as an additional top layer perfused with water and water-filled side channels around the cultivation chambers, preventing evaporation of the low cultivation volumes.

In combination with automated fluorescence time-lapse microscopy, we successfully cultured and analyzed two commensalistic *Corynebacterium glutamicum* strains in our picoliter cultivation system for over 40 hours. Image data stacks were analysed by a semi-automated Deep Learning cell detection approach, thereby deriving cell numbers and growth rates.

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ST192

Implementing two synthetic pathways bypassing EMP glycolysis in *E. coli*

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The reactions of the central metabolism providing all essential building blocks and reducing-power are very conserved in all organisms. To identify and implement alternative routes bypassing the "classic" Embden-Meyerhof-Parnas (EMP) glycolysis in *E. coli*, we employed a combined approach including *in silico* design, rational engineering, and adaptive laboratory evolution. A genome-scale model identified two potential pathways within the metabolic network replacing canonical EMP glycolysis to convert phosphosugars into organic acids. The first of these glycolytic routes proceeds via methylglyoxal, whereas the second via serine biosynthesis and subsequent degradation. Therefore, we implemented both pathways in *E. coli* strains harboring deletions of key EMP enzymes. Surprisingly, while growing on glycerol, the pathway via methylglyoxal immediately operated in a triosephosphate isomerase deletion strain (Δ*tpi*). By contrast, in a phosphoglycerate kinase deletion strain (Δ*pgk*), the overexpression of methylglyoxal synthase was necessary for implementing a functional methylglyoxal pathway. Furthermore, we successfully engineered the "serine shunt" which converts 3-phosphoglycerate via serine biosynthesis and degradation to pyruvate, bypassing an enolase deletion (Δ*eno*). Finally, to explore which of these alternatives would emerge by natural selection, we performed an adaptive laboratory evolution study using an enolase deletion strain. The evolved mutants were shown to use the serine shunt.

ST193

Physiological Response of *Corynebacterium glutamicum* to Indole

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The aromatic heterocyclic compound indole is widely distributed in nature. Due to its floral odor, indole is used in dairy, flavor and fragrance products. In addition, it is an inter- and intracellular signaling molecule that affects cell division, sporulation, or virulence in some bacteria and can be synthesized from tryptophan by bacterial tryptophanases. *Corynebacterium glutamicum*, which is used for industrial production of amino acids including tryptophan, does not possess a tryptophanase. Here, we describe the characterization of the physiological response of *C. glutamicum* to indole. The regulatory and metabolic role of the aromatic compound were analyzed.

To characterize the physiological response, adaptive laboratory evolution (ALE) and RNAseq analysis were performed to identify genes involved in the indole response. The effects of identified mutations and regulators on the indole response were analyzed by characterization of deletion mutants, qRT-PCR, and electrophoretic mobility shift assays (EMSA). As shown by RNAseq analysis, indole, which inhibited growth at low concentrations, increased the expression of genes involved in the metabolism of iron, copper, and aromatic compounds. This may be due in part to iron reduction, as indole has been shown to reduce Fe^{3+} to Fe^{2+} in the culture medium. Three transcriptional regulator genes have been demonstrated to be responsible for the increased indole tolerance. These encode the regulator of iron homeostasis DtxR, the regulator of oxidative stress response RosR, and the previously uncharacterized Cg3388. Cg3388, renamed IhtR, was considered a potential repressor of the *iolT2-rhcM2D2* operon. Indole, hydroquinone, and 1,2,4-trihydroxybenzene may act as inducers of this operon in vivo because they interfere with DNA binding of IhtR at physiological concentrations *in vitro*. Taken together, indole has a major impact on the physiological response of *C. glutamicum*. Thus, indole inhibits growth even at low concentrations. In addition, indole leads to altered transcription levels of several genes involved in the

metabolism of iron, copper, and aromatic compounds, among others.

Keywords: Corynebacterium glutamicum; amino acids; indole; adaptive laboratory evolution; iron homeostasis; oxidative stress; aromatic compound catabolism

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ST194

Regulation of glycogen catabolism during the awakening from nitrogen starvation in cyanobacteria

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Regulation of glycogen metabolism is of vital importance in organisms of all three kingdoms of life. Although the pathways involved in glycogen synthesis and degradation are well known, many regulatory aspects around the metabolism of this polysaccharide remain undeciphered. We used the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 as a model to investigate how glycogen metabolism is regulated in dormant nitrogen-starved cells, which entirely rely on glycogen catabolism to restore growth. We found that the activity of the enzymes involved in glycogen synthesis and degradation is tightly controlled at different levels via post-translational modifications. Phosphorylation of phosphoglucomutase 1 (PGM1) on a peripheral residue regulates its activity and prevents premature degradation of the glycogen stores, which appears essential for survival of nitrogen starvation. Remarkably, this regulatory mechanism seems to be conserved from bacteria to humans. Moreover, phosphorylation of PGM1 influences its interaction with oxidative pentose phosphate cycle protein (OpcA) and glucose-6-phosphate dehydrogenase (G6PDH). Analysis of the steady-state levels of the metabolic products of glycogen degradation and with protein-protein interaction studies revealed that the activity of G6PDH and the formation of this complex are under additional redox control, likely to ensure metabolic channeling of glucose-6-phosphate to the required pathways for each developmental stage.

ST195

Using off-gas for insights: Monitoring the complete yeast volatilome online using SESI-Orbitrap MS

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Introduction & Objectives

The fermentation off-gas contains a myriad of microbially emitted volatile organic compounds. In contrast to the well-known liquid phase however, the volatilome is sparsely explored. Monitoring the entire volatilome in an online measurement helps to gain a deeper understanding of metabolic pathways and could help optimize fermentation processes. The monitoring can be done with a coupled Orbitrap MS/MS and secondary electrospray ionization (SESI) system in a second scale [1], which is faster than ever before. A textbook fermentation with yeast undergoing a C-source shift from glucose to ethanol will showcase the potential of this system.

Material & Methods

A 200 ml yeast culture was grown in Verduynmedium with 7.5 g/L glucose in a glass reactor at 30 °C and aerated with 2 vvm (0.4 lpm) compressed air. Off-gas (0.3 lpm) was fed into a SESI-Orbitrap MS unit for online gas-phase measurements in positive ionization mode. Liquid samples for classic HPLC and GC analysis were taken every 30 minutes.

Results & Conclusion

The metabolites glucose, ethanol, acetaldehyde, acetate, glycerol, and the biomass were measured using HPLC and GC techniques. The volatilome was measured over 16,000 times or every 2.6 seconds over the course of the 11.5 h fermentation. From the 2,600 measured features, close to 500 were biogenic of which 165 could be identified with a single sum formula. Clearly visible are the shifts at the end of the lag-phase (after 100 minutes, correlates start of acetate production) and during glucose depletion (after 500 minutes). Further, the system allows for continuous reaction monitoring with the measurement of metabolites, e.g. ethanol. This novel type of analytics opens the window for a new standard in off-gas analysis in small and large scale fermentations.

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ST196

Shutdown of secretory pathway by the bacterial alarmones (p)ppGpp

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The stringent response (SR) enables bacteria to respond to nutrient limitations (e.g., amino acids) amongst many other biotic and abiotic stress conditions through production of the guanosine nucleotide-based second messengers ppGpp and pppGpp (also: (p)ppGpp). We show here that (p)ppGpp inhibits the essential signal recognition particle (SRP)-dependent protein targeting pathway essential for membrane protein biogenesis and protein secretion. Using combined biochemical and structural analyses we discovered that (p)ppGpp interferes with the SRP machinery in a bimodal way: first, it binds to both SRP-GTPases, Ffh and FtsY, and inhibits the formation of the targeting complex, which is central for the coordinated binding of the translating ribosome to the SecYEG translocon. Second, (p)ppGpp locks SRP already on the ribosome in a conformation incompatible for productive interaction with the receptor. Thus, our study reveals a so far underappreciated layer of the complex molecular framework regulating the adaptation of bacteria to stress conditions.

ST197

Involvement of the MxtR/ErdR two-component system in the utilization of different carbon sources in *P. putida* KT2440

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Introduction: MxtR/ErdR is a two-component system important for acetate utilization in *Vibrio cholerae* (called here CrbS/CrbR) and some *Pseudomonas* species. Recently, our group reported its role in the consumption of acetate in *Pseudomonas putida* KT2440, and demonstrated the binding of the response regulator, ErdR, to the promoter region of some of the predicted target genes. However, our preliminary data also indicates a role for the utilization of other carbon sources.

Objective: Here, we aim to analyze the physiological role of this system in the consumption of different carbon sources and to identify and functionally characterize new target genes in the soil bacterium *Pseudomonas putida* KT2440.

Material and methods: We utilized previously generated *mxtR* and *erdR* mutants and tested the impact of these deletions on the ability of the bacterium to utilize propionate, butyrate, levulinic acid, among others, as carbon source. Additionally, we performed a bioinformatic analysis in order to identify new uncharacterized genes under the control of ErdR. Finally, the binding of the response regulator to the promoter region of these sequences was analyzed by EMSAs.

Results: Our results revealed that the MxtR/ErdR system is not only essential for the growth of *P. putida* KT2440 on acetate but also for additional substrates, such as propionate. Our analysis also identified new target genes of the system and shed some light about their function.

Conclusion: MxtR/ErdR is important for the utilization of various carbon sources, including acetate and propionate, in *P. putida* KT2440, and appears to be much more important than previously described. Further experiments are needed to fully understand the importance of the system for *P. putida* KT2440 metabolism.

ST198

Elucidating the role of protein load for the physiology of *Enterococcus faecalis*

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Understanding microbial growth has high biotechnological relevance and is one key aspect in understanding how to combat microbial pathogens. Although gene and protein expression as well as metabolic processes are studied in detail, still microbial growth cannot be understood in its entirety and complexity. In the last years, theoretical and experimental studies revealed that protein load - the total amount of cellular proteins - is limited and thus constrains microbial growth. When protein load is at its physical limit, e.g., expression of specific proteins for environmental adaptation can only be achieved by a reshuffling of the protein composition. The general aim of the project is to elucidate the role of protein load regulation in the cell for biotechnological purposes using *E. faecalis* as a model organism. To investigate this, *E. faecalis* was cultured in glucose-limited chemostats under conditions that perturb protein load. For this purpose, the plasmid-based inducible expression system pAGEnt was used to overproduce GAPDH and an inactive form of this enzyme in *E. faecalis* ATCC 19433. Changes in metabolic fluxes, intracellular proteome, secretome, and transcriptome, upon increased protein load were determined and will be integrated into genome-scale models to gain insight into resource allocation strategies in *E. faecalis*.

ST199

A novel metal-free CO synthase with distinct functions in prokaryotes and eukaryotes

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Introduction: CO biosynthesis under aerobic conditions is mediated by the bipartite HypX protein, which acts first as a formyl-THF:CoA formyltransferase and subsequently as a formyl-CoA decarbonylase. In aerobic H2-oxidizing bacteria, the released CO becomes incorporated as the CO ligand into the catalytic center of [NiFe]-hydrogenases, which catalyze the reversible cleavage of molecular hydrogen into two protons and two electrons [1,2]. However, HypX is also encoded in the genomes of a variety of unicellular and multicellular eukaryotes, such as pathogenic fungi and cnidarians [2].

Objectives: In this study we aimed to functionally analyze three eukaryotic HypX-like proteins and to compare their *in vitro* properties with those of HypX from the Knallgas bacterium *Cupriavidus necator* (*Ralstonia eutropha*). These included the HypX proteins from the sea shell *Lingula anatina* and the siphonophores *Nanomia bijuga* and *Physalia physalis* (Portuguese man o" war), which are likely to use HypX-produced CO for different purposes.

Methods: *In silico* analyses; cloning and expression of partial and complete *hypX* genes in *E. coli* Rosetta; affinity purification of full-length HypX as well as its two separate functional moieties. (Coupled) activity assays and reaction intermediate determinations adapted from [2].

Results: Compared to HypX from *R. eutropha*, the eukaryotic HypX variants show a 52-fold (*P. physalis*), 126-fold (*L. anatina*) and 348-fold (*N. bijuga*) higher CO production activity. Analyses of separate formyl group transferase and decarbonylase activities, as well as CO-dependent formyl-THF production activities, are the subject of ongoing studies.

Conclusion: The high activities of the siphonophore HypX versions are compatible with the abundance of CO gas (up to 90 %) in the pneumatophore for floating [3]. The versatile C1 group interconversion capacity of HypX should be of interest for the design of artificial carbon fixation pathways.

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ST200

Salinity exceeds dietary impact in shaping the gut bacterial community in an invertebrate model system

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The gut microbiome is one of the most important sites of host/microbe interactions, however, mechanisms governing its assembly in aquatic non-model organisms are poorly understood. In this study, we investigate the effect of dietary changes and environmental disturbances i.e. changes in salinity on the host-protected bacterial community of aquatic snail Ampullaceana balthica. A change of food source to the green algae Scenedesmus obliquus monoculture decreased the bacterial richness of the A. balthica gastrointestinal bacterial community and resulted in two different types of bacterial communities. Five of the S. obliquus fed snail gut microbiomes clustered to the salinity 3/S. obliquus treated samples whereas the seven were unrelated to any sample. Samples from the combined salinity 3/S. obliquus incubation had the smallest overlap in the bacterial community compared to reference samples suggesting a strong impact of salt on the bacterial community composition. Interestingly, the total energy pool of the host was significantly lower in all aquaria with exception of the salinity 3/S. obliquus incubation. Our study therefore showed a strong impact of food source on α-diversity and partially on β-diversity. Since the change in food source had a strong impact on the α-diversity and the biofilm bacterial community had an overlap with the gut microbiome, a deterministic bacterial community assembly is expected. In contrast to what we expected, a combined increase in salinity 3/S. obliquus seems to stabilize the gut bacterial community-host interactions. Due to this effect, mild salinification may have only little effect on the host viability of aquatic invertebrates.

ST201

The native microbiome is crucial for offspring generation and fitness of *Aurelia aurita*

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All multicellular organisms are associated with a diverse and specific community of microorganisms, ultimately forming a metaorganism. Several studies pointed out that the microbiome is of fundamental importance for the health and fitness of the multicellular host; particularly, immunological, metabolic, and behavioral benefits of the associated microbiota for the host were observed. A microbiome can influence the physiology of a host, and consequently, microbial community shifts can affect host health and fitness. However, studies on the microbiome contribution to host fitness are in their infancy, particularly for less well-established hosts such as the moon jellyfish and basal metazoan *Aurelia aurita*.

The present study aimed to evaluate the significance and functional role of the native microbiota for life cycle transitions and fitness of this cosmopolitan cnidarian jellyfish *A. aurita*.

We studied the impact of the native microbiome on asexual reproduction and other fitness traits (health, growth, and feeding) of *A. aurita* due to induced changes in its microbiome in a comprehensive host fitness experiment.

We observed a significant impact on all fitness traits analyzed, particularly in the absence of the native microbiome (protective microbial shield) and when challenged with marine potentially pathogenic bacterial isolates. Notable is the identified crucial importance of the native microbiome for the generation of offspring. In more detail, the generation of daughter polyps and the formation and release of ephyrae were highly affected in the absence of the native microbiota, ultimately resulting in a halt of strobilation and ephyra release. Moreover, changes in microbial community patterns were detected by 16S rRNA amplicon sequencing during the experiment. This demonstrated that six operational taxonomic units (OTUs) significantly correlated and explained up to 97 % of fitness data variability, strongly supporting the association of impaired fitness with the absence/presence of specific bacteria.

Thus, we conclude that the microbiome is essential for the maintenance of a healthy metaorganism.

ST202

Bacterial interactions and chitin degradation determine bacterial successions in the model cnidarian *Nematostella vectensis*

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Introduction: Multicellular organisms possess a microbiota that is largely specific for their species, but the factors controlling community membership and establishment are poorly understood. *Nematostella vectensis* undergoes a complex life cycle starting as a planula larva, which metamorphoses into a juvenile polyp, before reaching the stage of a sexually mature adult polyp. These three life stages all exhibit an age-specific microbiome[1].

Objectives: We wanted to investigate the host-microbe interactions of *N. vectensis* and its microbiome by (1) characterizing the recolonization process after transplantation of the host"s own developmental-specific microbiomes on gnotobiotic adult polyps, and by (2) identifying drivers of this recolonization process.

Materials & Methods: We used 16S rRNA sequencing and host RNA sequencing to track the bacterial recolonization process and the host"s transcriptomic response. We also reconstructed bacterial metabolic networks based on 64 sequenced bacterial genomes.

Results: In microbial transplantation experiments, we recolonized gnotobiotic adult polyps with the development-specific microbiota of the three life stages, respectively. Surprisingly, the recolonization dynamics with all three inocula mimicked the ontogenetic colonization pattern: during early recolonization the reconstructing

microbiome resembles a larval community, before it shifts to resembling a juvenile community. Eventually, during late colonization, the community resembles an adult microbiome. Metabolic pathway reconstructions of the colonizing bacteria hint towards chitin as driver of the recolonization dynamics as chitin degradation is more prevalent in early colonizers than in late colonizers. Fittingly, *N. vectensis* showed an increased expression of a chitin synthase during early colonization. **Conclusion:** Our results show that the dynamics of the microbial community composition during the development of *Nematostella vectensis* are highly dependent on a metabolic interplay between the host and its microbiome, underlining the importance of viewing the metaorganism as one unit.

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ST203

The Culturable Pacific Plastic Microbiome

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Each of the many millions of pieces of plastic in marine environments carries its own microbial community and, while there has been a large amount of research describing these plastic biofilm communities, nearly all this work has been conducted using amplicon sequencing on the 16S rRNA gene. We were interested in capturing whole bacterial organisms cultured from marine plastic to understand their unique physiology, metabolic and genomic features. During a cruise across the North Pacific Ocean on the R/V SONNE, including the Great Pacific Garbage Patch, plastic pieces were collected from the ocean surface and were plated on Marine Broth agar plates. In addition, portions of epi-plastic biofilms were scraped off and flash frozen for later DNA extraction and 16S rRNA sequencing of the whole community. Back in the lab, colonies were re-streaked to isolate individual strains, grown up to confluence in liquid media, and 69 were selected to have their entire 16S rRNA gene sequenced using Sanger sequencing. We were able to capture, among others, members of the *Pseudoalteromonas*, *Halomonas, Brachybacterium, Labrenzia, Qipengyuania, Kocuria* and *Vibrio* generas. Some of the bacteria we captured come from genera known to degrade hydrocarbons, such as *Pseudomonas* and *Marinobacter*, a possible response to the polycyclic aromatic hydrocarbons (PAHs) and other organic chemicals that may have accumulated on or in plastic in marine environments. Many of the colonies were highly pigmented, suggesting selection of microorganisms that can mitigate the damage caused by exposure to UV light at the ocean surface or those that can perform phototrophy. Interestingly, the majority of 16S sequences from isolated colonies matched with ≥99% sequence identity with OTUs from the community analysis, albeit representing rare members of their corresponding communities (<0.1% relative abundance). Thus, our results indicate that at least some of these rare members are viable, metabolically capable members of their communities. Subsequent and ongoing research includes whole genome sequence analysis of these cultivated plastic microbiome strains, with a goal of finding genomic elements and metabolic pathways involved with the plastic biofilm ecosystem.

ST204

Using OMICS-technologies to unravel the mystery of malodour formation in washing machines and on washed laundry

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Washing machines can be significantly contaminated with a great diversity of microorganisms, benefitting from the wet and nutrient-rich environmental conditions inside [1]. Locally, microbial densities can reach 337 000 CFU/cm2 [2]. Contaminations might pose a threat to susceptible persons and can cause aesthetic problems, such as staining on the machine as well as laundry malodour [2]. Interestingly, in particular the biochemical and microbiological basics of laundry malodour formation are still far from being understood.

In a recent pilot study [3], we used single-end RNA sequencing to generate *de novo* transcriptomes of the bacterial communities remaining on polyester and cotton fabrics washed in a domestic washing machine under mild conditions and subsequently incubated under wet conditions. We identified 17 genes that were differentially expressed between the two fabric types. Differential expression of genes affiliated with bacterial carbohydrate metabolism might indicate bacterial consumption of carbohydrates released from the cotton textile; a gene affiliated with *Moraxella osloensis*, a known malodour producing bacterium, was significantly more expressed on cotton tissue, suggesting a higher activity of this bacterium here.

In addition to the analysis of microbial gene expression on laundry, we aim to characterize the metabolic properties of common washing machine and laundry bacteria in more detail. In a preliminary experiment, we incubated four test bacteria [1,2] in rich and minimal medium with and without detergent solution from a domestic washing cycle. Metabolic profiling was performed using mass spectrometry and the Biocrates' AbsoluteIDQ p180 kit. Preliminary data analysis suggests that metabolites with potential relevance for malodour formation are not only produced but also consumed by the investigated bacteria.

In summary, we believe that modern OMICS-technologies, such as metatranscriptomics and metabolomics, are highly suited to better understand microbial formation of laundry malodour, which is a prerequisite to develop novel hygiene strategies to tackle this highly consumer-relevant phenomenon.

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ST205

How does draught impact soil (micro)biomes? A multi-proxy-comparison.

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16S rRNA gene has been primarily used as the microbe-proxy in microbiome studies. However, it only detects

prokaryotic fraction of microbiomes and the (micro)eukaryotic components are always underappreciated, especially regarding protists and metazoa, who play important roles in trophic interactions. Our previous study showed that eukaryotic rather than prokaryotic microbiomes changed over seasons in rewetted peatlands, underpinning the importance of under-characterized protists and metazoa in understanding the dynamics of soil food webs and ecosystem functionality. Later, a severe draught happened in the summer of 2018 in Europe, leading to an increased interest in the dynamics of soil biomes under the impact of draught. Here, we investigated both the prok- and eukaryotic community compositions in soils during the periods before, when and after the draught happened, using 16S and 18S rRNA gene amplicon sequencing as well as metatranscriptomic sequencing. We compared the community compositions using different molecular proxies, e.g. 16S/18S rRNA gene phenotypes (DNA), rRNA phenotypes (rRNA), mRNA taxonomy and mRNA function phenotypes (mRNA). Interestingly, no significant dynamics in prokaryotic community composition was found based on 16S DNA proxy, while an obvious significance was found based on rRNA and mRNA proxies. However, significant dynamics of eukaryotic communities was found based on all proxies. The variation explained by time points using different proxies is in the following order: mRNA > rRNA > DNA. These results indicate that RNA-based approaches are better proxies in showing microbial dynamics. What should be specifically aware is that 16S rRNA gene, the most widely used proxy, showed no significant dynamics in our study, strongly in contrast to the RNA proxies, which challenges studies using DNA-based approach to track the dynamics of prokaryotic communities.

ST206

Flooding Causes Dramatic Compositional Shifts and Depletion of Putative Beneficial Bacteria on the Spring Wheat Microbiota

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Extreme precipitation is expected to intensify with global warming, which may increase the frequency of flooding events, thus imposing heavy costs for plant production to terrestrial ecosystems. The plant-associated microbiota play a crucial role in plant growth and fitness, but we still have a limited understanding of the response of the crop-microbiota complex under extreme weather events, such as flooding. Here, we setup a pot experiment to explore the effect of flooding on the wheat plant-microbiota. Since plant phenology is known factor in the response of plants to hydrological stress, flooding was induced only once and at different plant growth stages, such as tillering, booting and flowering. After each flooding event, we measured in the control and flooded pots several plant and soil properties and characterized the bacterial microbiota associated with the rhizosphere and roots using a metabarcoding approach. We found that flooding caused a significant reduction in plant development and substantial shifts in bacterial microbiota composition at each plant growth stage when being hydrologically stressed. However, a pronounced disruption in community assembly occur at early plant growth stage. Hence, flooding caused (i) a significant increase of bacterial taxa with anaerobic respiratory capabilities, such as Firmicutes and Desulfobacterota, (ii) depletion of several putative plant-beneficial taxa, and (iii) an increase of the abundance of potential detrimental bacteria. These differences in microbiota composition between flooded and control treatments were correlated with changes in soil and plant parameters, with pH and total N as the soil, and, S, Na, Mn and Ca concentrations as the root properties that were most influencing microbial assemblage in the wheat microbiome under flooding stress. Our findings revealed that flooding leads to a substantial restructuring of the spring wheat microbiota, and highlighted the detrimental effect of this hydrological stress on plant fitness and performance.

ST207

Microbial metagenomics of chronic lung disease: Big Data mining to improve knowledge, diagnostics and patient care

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Question: Progressive respiratory diseases are characterized by chronic infections. Microbial communities have to date mainly been analyzed with 16S rRNA gene sequencing. Shotgun metagenomic sequencing overcomes the limitations of 16S rRNA gene sequencing as it provides quantitative information about the virome, the mycobiome and the bacteriome on species level. This project analyzes the airway metagenome in healthy subjects, acute respiratory tract infections and chronic lung diseases (asthma, bronchiectasis, bronchopulmonary dysplasia, and primary ciliary dyskinesia). The study aims to identify the relevant members and/or interactions in the community that distinguish between lung health and diseases.

Methods: DNA was isolated from sputum or throat swabs. Fragment libraries were sequenced on an Illumina NextSeq System. Quality filtering and alignment of the short reads to a reference database consisting of bacterial, fungal and viral genomes was performed by the pipeline https://github.com/MHH-RCUG/Wochenende, providing normalized read counts (bacterial-to-human-cell-ratio). Heat maps and heat trees were employed to visualize the quantitative composition of the microbiome. Data mining of the metagenome data sets will be performed by machine learning and both cluster and correlation analyses.

Results: Sputum samples of the bronchiectasis cohort (n=107) have been completely sequenced. Preliminary results showed a significantly reduced alpha diversity in patient samples, which were dominated by *Haemophilus influenzae*, *Moraxella catarrhalis* or *Pseudomonas aeruginosa*. Beta diversity analysis revealed a distinct clustering of patient samples based on the presence of either highly abundant *H. influenzae* or *P. aeruginosa*. A longitudinal collection of throat swabs from preterm-born infants (n=12) in their first week of life, first month of life as well as after 9 and 15 months of age has also been completely sequenced and is currently analyzed.

Conclusions: We identified distinct alpha- and beta-diversity patterns in bronchiectasis respiratory tract metagenomes. Some of the samples displayed a metagenome signature consisting of a few dominant airway pathogens and lacking commensal bacteria. In the next step, patients' metadata will be incorporated into the downstream analysis enabling the application of machine learning techniques and hence

extraction of disease-specific microbial features for clinical diagnostics and personalized medicine.

ST208

Aerobic bacteria produce nitric oxide through denitrification during microbial interactions

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Microbial denitrification is a fundamental metabolic pathway in which bacteria reduce nitrogen species for respiration. In the marine environment, denitrification is conducted by strict and facultative anaerobic microbes that occupy low oxygen niches. It is therefore puzzling why strict aerobic bacteria from the marine Roseobacter group carry denitrification genes?

We found that *Phaeobacter inhibens* bacteria, members of the Roseobacter group, are strict aerobes that express denitrification genes, produce and secrete nitric oxide (NO), which is the key intermediate of the denitrification process.

P. inhibens bacteria are commonly found associated with micro-algae. In a model system for co-cultivation of *P. inhibens* with the micro-alga *Emiliania huxleyi,* we found that nitrite, an algal exudate, triggers the expression of bacterial denitrification genes. We show that nitrite secretion occurs in a peak that corresponds to the algal growth phase. Importantly, nitrite is the precursor for production of NO in the bacterial denitrification pathway.

NO is a highly reactive, short lived, potent molecule, involved in intercellular signaling and cell death. Indeed, our results indicate that bacterial NO triggers a programmed cell death (PCD)-like process in the algal partner.

Taken together, we show expression of denitrification genes and production of denitrification intermediates in strict aerobic bacteria, under oxygenated conditions. Metabolic products of this denitrification process appear to be involved in algal-bacterial interactions. Our data reveals a novel route of microbial metabolic exchange based on inorganic nitrogen, and point to a possible wider role of NO as an inter-kingdom signaling molecule.

ST209

***Candidatus* Pumilisymbium abstrusum, a highly reduced and deeply branching Alphaproteobacterium in symbiosis with marine invertebrate gutless oligochaetes**

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Bacterial symbionts with highly reduced genomes are well known from terrestrial animals but few representatives in the marine realm are described. Here, we present *Candidatus* Pumilisymbium abstrusum, a deeply branching marine Alphaproteobacterium that is an endosymbiont of the gutless oligochaete *Olavius algarvensis* (Tubificidae, Annelida). Phylogenetic analyses place *Ca.* P. abstrusum and its closest relative, a symbiont of the arctic ice worm, as a novel order-level clade distantly related to Rickettsiales *sensu stricto*. *Ca.* P. abstrusum has the smallest genome reported from a marine animal-microbe symbiosis. The high quality metagenome-assembled genome has a size of only 641 817 bp with a GC content of 34.74% and comprises 3 rRNAs, 36 tRNAs and 658 protein coding genes. *Ca.* P. abstrusum has a streamlined metabolism that integrates with the host and other symbionts. While *Ca.* P. abstrusum uses available intermediates and waste products such as fructose and malate, it potentially supplies B-vitamins, co-factors and pyruvate. Core cell functions such as DNA and RNA metabolism, ribosomal protein biosynthesis and ATP synthesis via glycolysis and an ATP synthase are retained, whereas amino acid biosynthesis is almost absent. The reduced metabolic capabilities and a type III secretion system that can modulate host biology indicate an intracellular life-style. This combination of features is unprecendented in the bacterial world and suggests a novel type of integration into the host biology.

ST210

Reductive genome evolution in *Endomicrobia* – from intracellular mutualists of termite gut flagellates to energy parasites?

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Bacterial endosymbionts of eukaryotic hosts typically undergo progressive genome reduction. An excellent model for the underlying evolutionary processes is Endomicrobia, a family-level clade of mostly uncultured bacteria that have cospeciated with termite gut flagellates for the past 50 million years. Comparative analysis of 76 metagenome-assembled genomes from various termite species allowed us to document both the metabolic potential of Endomicrobia and multiple gene losses during the transition from a free-living to an endosymbiotic lifestyle. The latter were accompanied by the acquisition of new functions via lateral gene transfer, which enabled the endosymbionts to shift their energy metabolism from the fermentation of glucose to sugar phosphates. In one Endomicrobia lineage, the complete breakdown of glycolysis was compensated by the acquisition of an ATP/ADP antiporter from a termite-specific lineage of uncultured Alphaproteobacteria. Our findings shed new light on the biology of Endomicrobia and the evolution of obligately intracellular symbionts to energy parasites.

ST211

An unusual tripartite symbiosis in spirostomid ciliates (Heterotrichea, Ciliophora) reveals a genomically reduced, photosynthetic endosymbiont of the *Chromatiaceae* (*Gammaproteobacteria*)

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Oxygenic photosynthesizers (cyanobacteria and eukaryotic algae) have repeatedly become endosymbionts throughout evolution. In contrast, anoxygenic photosynthesizers such as purple bacteria are exceedingly rare as intracellular symbionts. The purple-green ciliate *Pseudoblepharisma tenue*, which was described in the early 20th century but never studied in detail, harbours green algal endosymbionts and hundreds of purple bacteria in its cytoplasm. This is a puzzling combination as these two groups of photosynthesizers typically occupy different niches in nature. Green algae are aerobic and oxygenic, whereas purple bacteria are primarily anaerobic and anoxygenic. To identify the symbiotic partners and to gain some insights into how this unusual tripartite symbiosis works, we studied the morphology, ultrastructure, lifestyle, and metagenome of

Pseudoblepharisma tenue. We found that the purple endosymbiont represents a genomically reduced member of the *Chromatiaceae* that lost known genes for sulfur dissimilation (a hallmark of its free-living relatives) and appears to be streamlined for carbon fixation. The purple bacterium, now described as "*Ca.* Thiodictyon intracellulare", retained transporters for glucose and nitrogen compounds, indicating metabolic exchange with the ciliate host. The absorbance spectrum of the purple endosymbiont is complimentary to that of the green algae, and the numerous "chromatophores" revealed by electron microscopy suggest photosynthetic activity under the symbiotic conditions. Hence, the ciliate cell is likely to combine (indirect) anoxygenic photosynthesis with phagotrophy, and forms a unique ecological niche in standing freshwater systems. Interestingly, its close relatives, the sediment-dwelling ciliates of the genera *Spirostomum* and *Pseudoblepharisma* display interesting endosymbioses as well, and might be the key to understand the evolution of *Pseudoblepharisma tenue*.

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eP001

Structure and function of the nitrogenase-like reductase CfbC/D involved in coenzyme F_{430} biosynthesis

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The metallo-enzyme nitrogenase and its functional and structural homologs are involved in essential biological processes, such as nitrogen fixation, photosynthesis and biological methane formation (methanogenesis). Nitrogenase catalyzes the biological nitrogen fixation, and thus, the reduction of dinitrogen to ammonia[1]. A structurally simpler, iron-sulfur cluster containing nitrogenase-like enzyme system called CfbC/D (Coenzyme F_{430} Biosynthesis) is involved in the biosynthesis of coenzyme F_{430}, the essential cofactor of methyl-coenzyme M reductase in methanogenesis. During the biosynthesis of coenzyme F_{430} the tetrapyrrole macrocycle of Ni^{2+}-sirohydrochlorin *a,c*-diamide (Ni-SHC-D) is reduced to Ni^{2+}-hexahydrosirohydrochlorin *a,c*-diamide (Ni-H_6-SHC-D) by CfbC/D by the addition of 6 electrons and 7 protons. In homology to nitrogenase, CfbC/D consists of a reductase component and a catalytic component. The reductase component is a homodimer of CfbC carrying an intersubunit [4Fe-4S] cluster. The catalytic component is composed of a homodimer of CfbD bridged by an intersubunit [4Fe-4S] cluster, in contrast to the heterotetrameric catalytic component of the nitrogenase[2].

CfbC and CfbD from the hyperthermophilic methanogen *Methanocaldococcus jannaschii* was produced in *Escherichia coli*. Enzyme activity assays after purification and reconstitution of the proteins demonstrated the conversion of the substrate Ni-SHC-D to the reduced product. In contrast to nitrogenase, the reductase unit CfbC showed ATP hydrolysis in the absence of the catalytic component CfbD. However, ATP hydrolysis was stimulated by adding CfbD. Using MST analysis, the K_d values for the interaction between CfbC and CfbD with and without nucleotides were determined. In the absence of nucleotides, a K_d value of 123 ± 3 nM was determined. The addition of nucleotides (non-hydrolysable ATP analog or ADP) led to a substantial increase of the K_d value.

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eP002

A cobalamin-dependent Radical SAM enzyme is responsible for a unique C_α-methylation of methyl-coenzyme M reductase

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The archaeal enzyme methyl-coenzyme M reductase (MCR) plays an important role in the global carbon cycle by its ability to catalyze the formation as well as the consumption of methane in methanogenic archaea and anaerobic methane-oxidizing archaea, respectively [1]. Crystal structures and mass spectrometry analysis of MCRs of methanogenic archaea revealed the presence of several unusual amino acid modifications within the alpha subunit of MCR including a 2-methylglutamine [2]. As the methylation of the unreactive sp^3-hybridized C2 atom of glutamine is chemically challenging, a predicted radical *S*-adenosylmethionine (SAM) methyltransferase (MT) may be responsible for the introduction of the methyl group. The gene encoding the suspected MT is often located in close vicinity to the *mcr* genes and also next to the gene encoding a Radical SAM enzyme responsible for the C5-methylation of an arginine residue, another modification found in MCRs [3]. Besides the Radical SAM domain, amino acid analysis revealed an N-terminal cobalamin-binding domain. Therefore, the enzyme most likely uses methylcobalamin as an additional cofactor.

In this project, we aimed to characterize the predicted glutamine C-methyltransferase (QCMT) *in vitro* starting with the production and purification of recombinant protein in *Escherichia coli*. Analysis of purified and reconstituted QCMT by EPR and UV/Vis spectroscopy revealed the presence of two cofactors, a single [4Fe-4S] cluster as well as methylcobalamin (MeCbl), which is bound to the protein in a base-off/His-off mode. Both cofactors are essential for QCMT activity in our *in vitro* activity assay. We could demonstrate that QCMT catalyzes the transfer of a methyl group to the glutamine residue of a peptide substrate *via* a radical-based mechanism. During the methylation reaction, two molecules of SAM are consumed and 5'-deoxyadenosin (DOA) and *S*-adenosyl-L-homocysteine (SAH) are formed as coproducts in a ratio of nearly 1:1. The methyl group itself originates from enzyme-bound methylcobalamin, which is re-methylated by SAM.

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eP003

Biosynthetic pathways of unusual respiratory quinones from *Sulfolobus acidocaldarius*

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Respiratory quinones are lipids functioning as electron and proton carriers in the electron transport chains of almost all forms of life. Respiratory quinones can be distinguished according to their redox potential into low- and high-potential quinones, used primarily for anaerobic and aerobic metabolism, respectively. It has been assumed that aerobic bacteria evolved high-potential quinones to avoid generation of reactive oxygen species through autoxidation of low-potential quinones by molecular oxygen. Like bacteria, many archaeal lineages have evolved aerobic metabolisms. Yet, within Archaea high-potential quinones have only been reported from the order Sulfolobales. The major high-potential quinones of Sulfolobales are sulfolobusquinone (SQ) and caldariellaquinone (CQ), which contain unusual methylthio and thiophene moieties. Although these compounds have been known for four decades, they have received little attention compared to bacterial quinones. Consequently, the biosynthetic pathways leading to CQ and SQ have not been described. Here, I show preliminary results from stable isotope probing experiments with the model organism *Sulfolobus acidocaldarius* DSM639 that elucidate some of the steps in CQ and SQ biosynthesis. Further, I suggest candidate genes involved in the CQ and SQ biosynthetic pathways. Phylogenetic analysis of these genes suggests that CQ and SQ biosynthesis evolved from a low-potential quinone biosynthesis pathway common to most archaea, but that there was also significant evolutionary innovation in the last common ancestor of Sulfolobales. These preliminary results provide starting points for the genetic and biochemical characterization of the CQ and SQ biosynthetic pathways and for unraveling the evolution of aerobiosis in archaea.

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eP004

Selenium utilization in *Methanococcus maripaludis*: A genomic analysis

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The archaeal model organism *Methanococcus maripaludis* employs hydrogenotrophic methanogenesis as primary energy metabolism. For the methanogenesis it employs two homologous sets of enzymes: one set contains selenocysteine (Sec), while the second set contains cysteine (Cys) instead. The usage of either of the isoform sets was shown to be governed by the selenium status of the cell. In a selenium depleted environment, or when the pathway for selenocysteine synthesis and incorporation is disrupted, the Cys-isoforms are used, as evidenced by their up-regulation and the concomitant down-regulation of the selenoproteins on the transcriptional level. It is not known, which selenium species (beside selenite) *M. maripaludis* utilizes, and how they get transported into the cell. To address these questions, an omics-based analysis of *M. maripaludis* cultures grown under selenium adequate and depleted conditions has been performed.

The obtained data confirm selenium-dependent regulation of the Sec- and Cys-isoforms. Interestingly, mRNAs for the Sec-isoforms are not depleted completely during selenium starvation, which leads to their $\log_2$-fold change of "only" -4. Conversely, differential abundances of mRNAs for the Cys-isoforms are dramatically reduced under selenium adequate conditions, which leads to their higher $\log_2$-fold change of -13. Additionally, transcripts of several putative transporters were increased under selenium depleted conditions, which might be interpreted as the organism"s effort to tap into alternative sources of selenium. Their encoding genes are being deleted, and the resulting mutants analyzed for their ability to transport various selenium species by using a selenium-regulated reporter system.

In this study, omics-generated observations were used for genetic and physiologic experiments in *Methanococcus maripaludis* JJ, to gain further insights into its selenium metabolism.

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eP005

Improving shuttle vector pWM321 for protein production in different *Methanosarcina* species

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Among various groups of microorganisms on earth, methanogenic archaea represent one of the most widely spread class and are thought to be the only microorganisms producing significant amounts of methane, which makes methanogens a key player in the global carbon cycle. But it also gives them the potential to contribute to a solution for future energy problems by producing methane as a renewable, carbon-neutral fuel and other higher value-added chemicals. To advance solution pathways, the BMBF funded MethanoPEP consortium (031B0851B) addresses gaps in basic research such as insufficient knowledge about DNA defence systems, plasmid replication and induced gene expression in methanogens.

For gene expression in *Methanosarcina* mostly stably replicating shuttle vectors are used. For this purpose, plasmids that occur naturally in methanogens like the plasmid pC2A from *Methanosarcina acetivorans* (Sowers & Gunsalus, 1998, doi: 10.1128/jb.170.10.4979-4982.1988) are fused to an *Escherichia coli* replicon, which allows rapid cloning in *E. coli* and subsequent transfer of the plasmids to *Methanosarcina* for gene expression. When Metcalf *et al.* (1997, doi: 10.1073/pnas.94.6.2626) generated the commonly used shuttle vector pWM321 the function of pC2A and its open reading frames and untranslated regions were not fully understood and in some *Methanosarcina* species pWM321 showed stability problems most likely due to its large size.

Here we show how pWM321 was optimized by reducing its size of about 30% by identifying and deleting redundant regions, including both ORFs and UTRs. The resulting plasmid which showed higher stability in *Methanosarcina mazei* might allow the introduction of longer ORFs and various inducible promotors and consequently might lead to higher protein yields. One region, which is essential for plasmid replication and stability in *M. mazei* is the ORF coding for the replication protein RepA. To further investigate the mechanism and origin of replication RepA was heterologously expressed and purified. In a subsequently performed nickase assay using supercoiled pWM321 or derivatives, RepA showed significant ssDNA nuclease activity. This finding strongly suggests that pWM321 might be replicating via a rolling circle mechanism.

Overall, the shuttle vector pWM321 was improved for protein production in different *Methanosarcina* strains and, to our knowledge, the reported results shed light on the replication mechanism of a methanogenic plasmid for the first time.

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eP006

Heme synthase AhbD from *Methanosarcina barkeri* contains two auxiliary iron-sulfur clusters

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The cyclic tetrapyrrole heme is an essential prosthetic group for almost all organisms on earth. The biosynthesis of heme starts with 5-aminolevulinic acid, which is stepwise converted into uroporphyrinogen III. In *Archaea*, the so-called siroheme-dependent pathway is used to transform uroporphyrinogen III into heme.[1]

The last step of the siroheme-dependent route is the conversion of iron-coproporphyrin III to heme, which is catalyzed by the heme synthase AhbD. This enzyme belongs to the Radical SAM superfamily and contains at least two [4Fe-4S] clusters.[2] AhbD catalyzes the oxidative decarboxylation of two propionate side chains of iron-coproporphyrin III to the corresponding vinyl groups of heme by a radical mechanism.

The N-terminal [4Fe-4S] cluster is required for the binding of *S*-adenosylmethionine (SAM) and radical formation. The C-terminal cluster was proposed to be involved in electron transfer. Determination of the iron and sulfide content of recombinant AhbD from *Methanosarcina barkeri* and amino acid sequence analysis indicate the presence of a third [4Fe-4S] cluster. To investigate this hypothesis and the function of the additional clusters, different single cluster variants of AhbD from *M. barkeri* were created. These variants were analyzed using an *in vitro* enzyme activity assay, a SAM cleavage assay and a substrate binding assay. Also, EPR measurements were conducted. The exchange of cysteine residues indeed suggested the presence of a third, central [4Fe-4S] cluster, which is absolutely required for heme synthase activity and appears to be involved in substrate binding. In contrast, it was observed, that the C-terminal cluster is not essential for heme synthase activity and substrate binding.

In addition to the iron-sulfur cluster characterization, we aimed to detect a potential substrate radical during the AhbD catalyzed reaction. Indeed, substrate radicals were detected by EPR spectroscopy using three different substrate analogs.

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eP007

Natural transformation and type IV pilus biogenesis in *Thermus thermophilus*: DNA binding and interaction of pilins with the inner membrane platform

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Introduction

Natural transformation of *Thermus thermophilus* HB27 is mediated by a macromolecular machinery that contains more than 16 different proteins, including minor pilins PilA1-3 and an inner membrane (IM) type IV pilus (T4P) assembly platform formed by PilM, PilN and PilO which interacts with the motor ATPase PilF. Despite the identification of key components of the DNA translocator functional details remain to be elucidated.

Objective

We addressed the distinct roles and the interactions of the pilins with components of the IM assembly platform of the DNA translocator.

Materials & Methods

Molecular cloning, affinity chromatography, electromobility shift assays, western blot, co-elution analysis.

Results

The minor pilins PilA1-3 were found to be essential for T4P biogenesis and functions. The pilins were detected in the IM and outer membrane (OM) but were absent in pilus fractions. PilA1 and PilA2 were found to bind dsDNA. PilA2 was found to bind to the IM assembly platform via PilM and PilO.

Conclusions

We propose that the minor pilins PilA1-3 form a DNA translocation pseudopilus connected via the IM assembly platform to the cytosolic motor ATPase PilF. The pseudopilus is suggested to extend from the IM through the periplasm into a secretin channel in the OM thereby binding the incoming DNA.

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eP008

Functional dissection of structural regions of the *Thermus thermophilus* competence protein PilW: Implication in secretin complex stability, natural transformation and pilus functions

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Introduction

Uptake of DNA from the environment into the bacterial cytoplasm is mediated by a macromolecular transport machinery that is similar in structure and function to type IV pili (T4P). The secretin PilQ forming dynamic outer membrane channels plays a dual role in pilus extrusion and DNA uptake. The localization of PilQ in the outer membrane was found to be strictly dependent on the unique PilW protein which is essential for natural transformation and piliation.

Objective

We aimed to elucidate the role of PilW and structural regions in PilW in DNA uptake, PilQ complex formation and T4P functions.

Materials & Methods

Site directed mutagenesis, transformation studies, western blot analyses, twitching motility, piliation analyses by electron microscopy.

Results

PilW and the secretin PilQ form high molecular mass complexes in the outer membrane. In a Δ*pilW* deletion mutant only PilQ monomers were detected. PilW comprises of an intrinsically disordered region. Deletion of this disordered region (*pilW*Δ163-216) did not affect piliation but significantly reduced transformation frequencies, abolished cell-cell adhesion and T4P-mediated twitching motility [1]. Distinct topological regions in PilW were found to be essential for functionality of the DNA transporter and T4P.

Conclusions

PilW is either important for PilQ complex assembly and stability. Deletion of a central disordered region of PilW impaired natural transformation as well as biofilm formation and abolished T4P mediated twitching motility. These findings suggests that the disordered region in PilW induces conformational changes in the PilQ complex affecting pilus dynamics and DNA uptake.

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eP009

***Metallosphaera* sp. J1, a novel species of thermoacidophilic archaea**

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Thermoacidophilic archaea, like *Acidianus* spp., *Sulfolobus* spp. and *Metallosphaera* spp. have high potential for application in bioleaching processes due to high dissolution rates and enhanced metal recovery of e.g. copper from chalcopyrite. To improve and further understand the role of the microorganisms in the bioleaching process, the novel archaeon *Metallosphaera* sp. J1 was chemotaxonomically and physiologically characterized. Strain J1 was originally isolated from a hot pool in a volcano area in Java and has been previously demonstrated to extract metals from sulfidic ores with high efficiency, exhibiting a high copper tolerance (Norris *et al.*, 2015). Cells of the strain were irregular, motile cocci of 1.0 to 1.2 µm diameter, growing aerobically at temperatures between 55 and 78°C with an optimum at 70°C. While growth could be observed between pH values of 1.5 and 5.0, the optimal pH was found to be at 3.0. Strain J1 was able to use complex organics (e.g. yeast, peptone) for heterotrophic growth, oxidized reduced sulfur compounds (S0, K2S4O6) for chemolithoautotrophic growth, or casamino acids (L- aspartanic acid, L-glutamic acid, L-alanine) in the presence of FeSO4 to grow mixotrophically. Additionally, the strain utilized a remarkable variety of sugars compared to other species of the genus. The organism contained the respiratory quinone caldariellaquinone. The DNA G+C content of 47.3 % is the highest among the known *Metallosphaera* species. Based on these results and the comparison with their closest relatives, it was concluded that strain J1 represents a novel species of the genus *Metallosphaera*.

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eP010

Establishing an *in vivo* reporter for analyzing SECIS-dependent UGA recoding in Archaea

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Introduction: The mechanism of recoding a UGA stop codon for co-translational selenocysteine incorporation is not known in Archaea. The selenocysteine insertion signal (SECIS) element, a secondary structure on selenoprotein mRNAs is directly located 3" of the UGA in Bacteria. In Archaea, such signals can only be found in the untranslated regions.

Objective: Addressing structure-function relations of factors involved in Sec synthesis and incorporation, like the SECIS element, is currently possible in *Methanococcus maripaludis,* an archaeal model organism, only through elaborate experimental approaches, like determining anaerobically the activity of formate dehydrogenase, or in vivo labeling using [^{75}Se] and subsequent analysis of the selenoproteome. Besides being tedious, these methods lack the possibility for quantitative probing of selenium insertion.

Results: Here we present a reporter system, which directly corresponds enzyme activity to selenium insertion. We engineered a bacterial reporter protein into a selenoprotein and show that its production *in vivo* depends on the availability of selenium and the presence and structural integrity of a SECIS element. We measure enzyme activity directly from lysed *M. maripaludis* cells. We address the structure-function relations for the SECIS in archaea by connecting structural changes to enzyme activity.

Conclusion: The reporter presented here, now allows for fast and convenient in vivo probing of structure-function relations during UGA recoding for selenoprotein synthesis in Archaea. The engineered reporter is also a proof-of-concept that bacterial enzymes can be converted into selenoproteins and produced in Archaea.

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eP011

Expanding the genetic toolbox for *Methanothermobacter thermautotrophicus* ΔH

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Methanothermobacter spp. are a group of thermophilic, chemolithotrophic methanogens with high relevance for basic science and biotechnology. They serve as model microbes for methanogenesis, which is a large contributor to greenhouse gas emissions. Furthermore, they are applied in biotechnology as biocatalysts for power-to-gas processes. In these processes, methane (*i.e.*, natural gas) is produced with

hydrogen that is generated *via* electrolysis of water with surplus electricity from renewable sources, and carbon dioxide from industrial off-gases. For these reasons, *Methanothermobacter* spp. was selected as the microbe of the year by the VAAM 2021. However, further investigation of *Methanothermobacter* spp. and the expansion of the power-to-gas process to produce value-added chemicals (power-to-x) has long been hindered by the lack of genetic tools. After the successful establishment of a genetic system for *Methanothermobacter thermautotrophicus* ΔH in our lab, we here present additional genetic tools in the form of novel selective markers and prospects on genome editing. We demonstrate that the formate dehydrogenase (*fdh*) operon from *Methanothermobacter thermautotrophicus* Z-245 enables *M. thermautotrophicus* ΔH to utilize formate as growth substrate and that the *fdh* operon can also be used as a selective marker. We also show that a mutated version of the *ileS* gene from *Methanothermobacter marburgensis* increases the resistance to mupirocin in *M. thermautotrophicus* ΔH and investigate its usability as a selective marker. Finally, we illustrate how a CRISPR/Cas9 system, which is based on a thermostable Cas9 from a *Geobacillus* strain, could be used for genome editing in *M. thermautotrophicus* ΔH. These findings are important additions to the genetic toolbox for *M. thermautotrophicus* ΔH, which can be used to answer basic research questions and create further industrial applications.

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eP012
Impact of viral infection on motility and adhesion in haloarchaea

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Over the last years, increasing attention has been paid to the virus-host interactions in archaea. Several studies have demonstrated that viruses bind to surface appendages of archaea such as type IV pili [1]. Adhesive type IV pili are involved in biofilm formation. The archaellum is a rotating type IV pilus that functions as the motility structure of archaea[2] and allows for swimming in liquid medium. The protein subunits that form these appendages are called pilins and archaellins, respectively. Most euryarchaeota contain multiple archaellin and pilin gene copies in their genome. It was shown that in several cases, surface appendages built from a single gene are completely functional [3,4]. We aim to study how viral infection impacts the physiology of the cells, with a special focus on the alterations in the presence of cell surface appendages. The euryarchaeon *Haloarcula hispanica* and several of its viruses serve as a model in this project. To this end, we aim to develop reliable assays to monitor changes in motility and biofilm behavior of *H. hispanica*. Previous work has successfully established biofilm assays for different crenarchaeotes and the halophile *Haloferax volcanii*. However, no such assay exists to study biofilm formation in *Haloarcula hispanica*. This work aims to design an approach for studying the effect of infection on the formation and stability of biofilms by *Haloarcula hispanica*.

[1] Tittes, C., Schwarzer, S. and Quax, T., 2021. Viral Hijack of Filamentous Surface Structures in Archaea and Bacteria. Viruses, 13(2), p.164

[2] Chaudhury, P., Quax, T. and Albers, S., 2017. Versatile cell surface structures of archaea. Molecular Microbiology, 107(3), pp.298-311.

[3] Syutkin, A., Pyatibratov, M., Galzitskaya, O., Rodríguez-Valera, F. and Fedorov, O., 2013. Haloarcula marismortui archaellin genes as ecoparalogs. Extremophiles, 18(2), pp.341-349.

[4] Esquivel, R., Xu, R. and Pohlschroder, M., 2013. Novel Archaeal Adhesion Pilins with a Conserved N Terminus. Journal of Bacteriology, 195(17), pp.3808-3818

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eP013
Intermolecular gene conversion in the polyploid haloarchaeon *Haloferax volcanii*

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Gene conversion is defined as the non-reciprocal transfer of genetic information between two homologous but not identical DNA sequences. It occurs in all three domains of life and can be part of different biological processes, like "antigenic variation" or "concerted evolution of gene families". We are focusing on "intermolecular gene conversion". This process results in an equalization of multiple genome copies and has hardly been studied in prokaryotes so far. Furthermore, a highly efficient correction of mutations via intermolecular gene conversion would be an escape from "Muller"s ratchet".

An experimental approach has been established to characterize unselected intermolecular gene conversion in *Haloferax volcanii*[1]. To this end, we made use of deleting genes encoding enzymes that are involved in carotenoid biosynthesis, resulting in white instead of the usually red cells. Protoplast fusions of red and white cells were used to produce heterozygous cells. Afterwards, unselected intermolecular gene conversion between different genomes could be easily analyzed by screening of phenotypes or/and by PCR.

We could show that unselected intermolecular gene conversion is extremely efficient in haloarchaea. Notably, its frequency is independent from the extent of genome differences, i.e. even a one nucleotide difference triggers gene conversion. In addition, gene conversion also operates between genes of different species. It was verified that gene conversion tracts in haloarchaea are much longer than in antigenic variation or concerted evolution in bacteria and can exceed 15 kbp. Furthermore, we could identify some proteins that are important for gene conversion in *H. volcanii.* Future work will concentrate on elucidating factors that determine the direction of gene conversion and on further characterization of the involved proteins and the molecular mechanism.

[1] Wasser et al. (2021) Frontiers Microbiol. 12:1477

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eP014
Translational coupling via termination-reinitiation in *archaea* and *bacteria*

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

In a previous study we have shown that TeRe operates at several native gene pairs of the archaeon *Haloferax volcanii* and the bacterium *Escherichia coli*, and we have characterized the dependence on intragenic Shine Dalgarno motifs in the upstream genes[1]. Translational efficiencies were quantified using reporter genes fused to native gene pairs, and enzyme assays and northern blotting were used to determine protein and transcript levels. In the present study, this experimental approach was applied to unravel the correlation between the intergenic distance and the efficiency of translational coupling. Several native gene pairs of *H. volcanii* and *E. coli* were chosen, and the intergenic distances were varied via site-specific mutagenesis. As expected, efficient coupling required very short distances, and relatively small distances already totally inhibited coupling.

Furthermore, we investigated whether local mRNA structures at gene overlaps in *E. coli* are required to inhibit novel initiation at the downstream genes and guarantee coupling. To this end, we generated several constructs with truncated or mutated gene pairs, with the aim of destabilizing local mRNA structures close to the overlaps. The effects of these genetic perturbations on the efficiencies of translation and coupling will be reported.

Taken together, translational coupling via TeRe operates at many gene pairs in archaea and bacteria, and various aspects of the molecular mechanism could be characterized using native gene pairs fused to reporter genes. Future experiments will aim to unravel additional sequence motifs and structural requirements that influence translational coupling via TeRe.

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

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eP015

Important roles of Zinc Finger µ-Proteins in *Haloferax volcanii*

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Zinc finger proteins are known to fulfil many different roles in eukaryotes, e.g. zinc finger proteins can be transcription factors, ribosomal proteins, regulators of membrane proteins, or molecular scaffolds. In archaea, they are severely understudied. In addition, most well-studied eukaryotic zinc finger proteins are large proteins containing several small zinc finger domains. In contrast, in archaea the majority of putative zinc finger proteins are very small and await experimental characterization.

Haloferax volcanii is a halophilic and mesophilic archaeon that can easily be cultivated, making it a frequently used model organism. Its genome encodes 282 small proteins (less than 70 amino acids), most of them having no annotated function yet. 43 of those proteins contain at least two C(P)XCG-like motifs, making them putative one-domain zinc finger proteins of unknown function.

For the analysis of these zinc finger µ-proteins more than 30 single gene *in frame* deletion mutants have been generated until now. They were compared to the wildtype concerning e.g. growth behaviour in different media, stress resistance, swarming, and biofilm formation. Most of the mutants showed a strong phenotype under at least one of the conditions tested, revealing that the zinc finger -proteins fulfil various important functions in haloarchaea1. For further analyses, currently co-affinity purification of his-tagged versions has been initiated to screen for interaction partners (proteins, DNA, RNA).

One of the proteins, HVO_2753, has been studied in detail2. An RNA-Seq analysis (coll. with C. Sharma, Würzburg) revealed that movement/chemotaxis genes are downregulated, in excellent agreement with its inability to swarm. Determination of the NMR solution structure (coll. with H. Schwalbe, Frankfurt) revealed that only one of two putative zinc finger domains binds zinc, in agreement with a biochemical zinc assay. Nevertheless, all four C(P)XCG motifs were shown to be essential for structure and function.

Taken together, we could show that haloarchaeal zinc finger µ-proteins are important for various biological functions, e.g. glycerol metabolism, Fe(III) homeostasis, biofilm formation and swarming. More in-depth studies selected proteins are underway.

1 Nagel et al. (2019) Genes 10:361

2 Zahn et al. (2020) FEBS J. 288:2042

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eP016

Mucithermus cthulhu sp. nov. represents a novel genus of marine, hyperthermophilic Archaea within the family _Desulfurococcaceae_ with tentacle-like protrusions

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The Archaea Centre Regensburg has a long tradition and experience in the isolation and characterization of hyperthermophilic microorganisms, especially from the domain of the Archaea. This includes *Pyrodictium occultum*, the first isolated organism with an optimal growth temperature above 100 ° C, but also the unique, intimate association of *Ignicoccus hospitalis* and *Nanoarchaeum equitans*.

Here, we describe a newly isolated strain *Mucithermus cthulhu* Mex13E-LK6-19. This strain was enriched in 2019 from a deep-sea sample taken in 2008 near a black smoker in the Gulf of Mexico at a depth of 2507 m. A pure culture was prepared by isolating a single cell employing an optical tweezer.

Both electron tomography and scanning electron microscopy revealed that the coccoid cells have unusual tentacle-like cell appendages composed of multiple vesicles with varying size and shape. In addition, the cells are surrounded by a mucoid

matrix and appear to form a branched cell-cell network connected via these branches. Optimal growth was observed at 95 °C, neutral pH and salt concentrations matching the conditions in the marine habitat. A phylogenetic classification using the 16S rRNA gene showed that the new isolate represents a novel genus within the *Desulfurococceae* (*Desulfurococcales*, Thermoprotei, Crenarchaeota). The genome of *Mucithermus cthulhu* Mex13E-LK6-19 is 1.91 Mbp in size and encodes 1926 genes. In addition, the genome contains two proviral regions, which appear to be active. Surprisingly, no canonical CRISPR-Cas-locus or other known defense systems against viruses could be identified which could be detected in >95% of completely sequenced genomes of hyperthermophilic archaea. Currently, we are studying the composition of the vesicles in more detail, to explore if they are associated with one of the two proviruses.

Mucithermus cthulhu has been dreaming waiting a decade to be isolated from its house MEX13E*. Taken together, it represents a novel genus of marine and hyperthermophilic Archaea with unusual tentacle-like protrusions. Moreover, the organism can potentially serve as an excellent and unique archaeal system to study virus-host interactions within a genomic background without any so far described prokaryotic defense systems against viruses.

* "Ph'nglui mglw'nafh Cthulhu R'lyeh wgah'nagl fhtagn": "In his house at R'lyeh, dead Cthulhu waits dreaming" (Lovecraft H. P., 1928)

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eP017

Genetic engineering of *Methanosarcina acetivorans* for the production of isoprene and isoprenoids

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Isoprene and isoprenoids are used in a wide range of industries, for example for production of cosmetics, foods, drugs and as fuels. Until now, most of the isoprene is produced from petroleum but bio-production of these chemicals is desirable. This perspective is exemplified by the National Research Strategy BioEconomy 2030, which financially supports research on using renewable raw materials and energy resources for industry.

We want to establish *Methanosarcina acetivorans* as a producer of isoprene and isoprenoids. In contrast to other organisms that have been used for this purpose, *M. acetivorans* requires less complex growth media and has the ability to grow on carbon monoxide (CO) which is part of syngas, a common industrial byproduct. Growth of *M. acetivorans* on CO leads to the production of high amounts of acetyl-CoA which is the central precursor required for formation of isoprene providing a good basis for successful isoprene production in this organism. Based on these in general favourable characteristics, we heterologously expressed the gene encoding the isoprene synthase of *Populus alba* in *M. acetivorans*. The recombinant strain produced low levels of isoprene (50 µM). To overcome potential limitations in the availability of important intermediate compounds and, thus, boost isoprene production in *M. acetivorans*, we overexpressed several genes coding for enzymes involved in the mevalonate pathway in this strain. Having validated the applicability of *M. acetivorans* for isoprene production, we also aim to overexpress several selected isoprenoid synthases in this organism to expand its potential use for industrial production of additional molecules derived from the mevalonate pathway.

In summary, our data demonstrate the applicability of genetically modified strains of *M. acetivorans* for production of isoprene and potentially also other related molecules.

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eP018

Archaeal biofilms: Exopolysaccharide composition, size and synthesis in *Sulfolobus acidocaldarius*

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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 exist predominantly in the biofilm mode, knowledge about archaeal biofilm formation, structure, EPS composition and synthesis is limited [1].

Sulfolobus acidocaldarius is a thermoacidophilic (76°C, pH 3), aerobic Crenarchaeon that was isolated from acid hot springs [2]. The organism is easy to cultivate under laboratory conditions and a genetic system is established. In this study, we investigate *S. acidocaldarius* biofilms with a special focus on exopolysaccharide (PS) composition, size and synthesis. PS constitute a major EPS component beside proteins and extracellular DNA, suggesting an important role in *Sulfolobus* biofilms. Changes in PS amounts and composition were observed in response to environmental stress before [3].

In order to perform detailed structural PS analyses, high amounts of biomass were required. Therefore, a new cultivation method was established, growing archaeal biofilms on membranes, floating on the surface of liquid medium. EPS components were isolated and quantified [4], and the monomeric composition of *S. acidocaldarius* MW001 PS was defined by acidic hydrolysis, chromatographic separation and mass spectrometry. Size exclusion chromatography was used to determine the nominal weight of PS species.

A gene cluster comprising 11 glycosyltransferases and 7 membrane proteins likely involved in PS synthesis was identified in *S. acidocaldarius* [5]. Several deletion mutants have been constructed and phenotypes of wildtype and mutant strains were compared using the established analytical methods. The current insight in the composition and size of the *S. acidocaldarius* PS, the function of selected proteins encoded by the PS gene cluster as well as a model for PS synthesis and export will be presented.

[1] van Wolferen et al. (2018). *Nature Rev Microbiol* 16(11), 699-713.

[2] Brock et al. (1972). *Arch Mikrobiol* 84, 54-68.

[3] Benninghoff & Kuschmierz et al. (2021). *Appl. Envionm Microbiol* 87:e02988-20.

[4] Jachlewski et al. (2015). *Front Bioeng Biotechnol* 3, 123.

[5] Orell er al. (2013). *ISME J* 7, 1886-1898.

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eP019

Characterization of unusual kinases in *Methanosarcina acetivorans* and their role in signal transduction

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Methanogenic archaea acquire energy by producing methane out of hydrogen, acetate and one-carbon compounds. *Methanosarcina acetivorans* belongs to only a few methanogens, which are capable to metabolize different substrates by the carboxydothrophic, acetoclastic and the methylotrophic pathway. Archaea often live in hostile habitats with rapidly changing environmental conditions such as nutrient depletion. The metabolic state of the cell, including its energy status and the availability of substrates, is thought to be measured by sensor kinases [1]. Signal transduction allows cells to change the properties of their current proteome in a way that ensures adaptation without relying on the synthesis of new proteins. Bacteria often uses two component systems, which consist of a sensor kinase and a response regulator. However, whereas signal transduction is well studied in eukaryotes and bacteria, the knowledge in archaea is still rather scarce [2]. In the genome of *M. acetivorans* several genes encoding for sensor kinases are present. Three of these genes encode for the methylsulfide methyltransferase-associated sensor (MsmS), the redox dependent methyltransferase-associated sensor (RdmS), and MA4377. Downstream of these sensor kinase genes, transcription regulators (MsrG/F/C) and corrinoid/methyltransferase fusion protein (MtsF/D/H) are encoded. Previous studies showed a cross-regulation of these three systems, which led to the assumption that this is a multi-component system rather than a typical two-component system. Protein-Protein interaction studies using the bacterial two hybrid system were performed suggesting an interaction of the sensor kinases with the three regulators. Band shift Assays has shown that the regulators interact with the promotor region of the *mts* genes. Furthermore, radioactive kinase assays were performed to show intramolecular signal transduction within the sensor kinases. We currently investigate by qPCR analysis how the sensor kinases affect the transcription of the methyltransferases of *M. acetivorans* knock-out strains grown on different substrates. Overall, with our work we aim to contribute to the understanding of archaeal signal transduction and methanogenic physiology with our work.

[1] Deppenmeier *et al.* (1996) Archives of Microbiology

[2] Esser *et al.* (2016) FEMS Microbiology Reviews

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eP020

Using the endogenous CRISPR-Cas Type I-D system for genetic engineering in the thermoacidophilic archaea *Sulfolobus acidocaldarius*

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CRISPR-Cas is a small RNA-based defense mechanism of prokaryotes, enabling them to protect themselves against viral infections through the acquisition of small spacers and their insertion into the genome between clustered short palindromic repeats via CRISPR-associated proteins (Cas). Most *Sulfolobales* posses CRISPR type I and III systems, with type I-A, I-D, III-B and III-D as the most common subtypes. Because of the thermoacidophilic environment of *Sulfolobales*, genetic manipulation with exogenous CRISPR-Cas systems, like CRISPR-Cas9, is not feasible, because these derive from mesophilic organisms. Therefore, the aim of our study is to use the endogenous CRISPR-Cas Type I-D system of the crenarchaeotal model organism *Sulfolobus acidocaldarius* as a platform to perform genetic engineering in this archaea. To accomplish this, an artificial mini CRISPR array, consisting of a designed spacer flanked by specific repeats, identical with the *S. acidocaldarius* CRIPSR repeats, is introduced to the cell via plasmid. Additionally, approximately 500 bp long up- and downstream flanking regions of the gene of interest are introduced to the plasmid for homologues recombination.

In our study we were able to efficiently generate knock-out mutants using our CRISPR-Cas system alongside with establishing an optimized protocol, shortening the time necessary to obtain mutants in comparison to previous developed tools for *S. acidocaldarius*.

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eP021

Investigations on high CO2 concentration adapted microbial communities and their physiology

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Despite recent advances in the field, insights on how microbial populations are shaped by the extreme conditions predominating in terrestrial subsurface environments remains limited. The Eger Rift in Western Bohemia, Czech Republic, is characterized by frequent seismic activity, consistently high CO_2 concentrations and fluxes and thus represents a worldwide unique natural laboratory to study the interactions of geological processes driven by seismic events and microbiological processes. Exploring the composition, physiology and metabolism of subsurface communities from this region will provide valuable insights on the long-term effect of such extreme, high CO_2 condition on the local microbial communities, and also contribute to the description of the deep biosphere.

To investigate microbial distribution and behavior, we took sediment samples from a 240m long drill core through an active Mofette. To obtain information on cell abundance and physiology cell counts were conducted. Furthermore, the ability of microbes to live under high CO_2 concentrations and potentially metabolize CO_2 was investigated via enrichments using minimal mineral media and H_2/CO_2 headspace. Active microorganisms in sediment and enrichment samples were targeted using Hybridization Chain Reaction Fluorescence in situ Hybridization (HCR-FISH).

Drilling efforts were carried out following contamination control guidelines, thus providing stringent quality control. We utilized a fluorescent tracer, which allowed the identification and exclusion of contaminated samples from downstream analysis. Total cell numbers throughout the core were generally low, ranging from 10^2-10^5 cells per gram of sample, with a small peak around 80 m below surface, and were found to increase in the deeper sediments. Indigenous Pseudomonadaceae at the deepest sample was observed through HCR-FISH. First round of culturing allowed the enrichment of methanogenic archaea.

Cell counting data suggest that this extreme environment is characterized by low biomass, but some microbes could still find their niche at specific depths. Emergence of methanogenic archaea in enrichments despite low abundance in sediments suggests that a dormant population forced by the extremely oligotrophic condition may quickly thrive once H2 becomes available. Considering H_2 is occasionally brought up through seismic event, the "invisible" minority of the in situ microbial community may seize the opportunity and dominate temporarily.

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eP023

HcgA is a radical SAM enzyme catalyzing formation of the pyridinol precursor in biosynthesis of the [Fe]-hydrogenase cofactor.

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In the hydrogenotrophic methanogenic pathway, [Fe]-hydrogenase (Hmd) catalyzes the hydride transfer from H_2 to methenyl-tetrahydromethanopterin (Methenyl-H_4MPT$^+$) to produce methylene-H_4MPT. Hmd contains a unique prosthetic group, the iron-guanylylpyridinol (FeGP) cofactor. The FeGP cofactor consists of a low spin Fe(II) coordinated with a pyridinol-N, an acyl group, two carbon monoxide, and the sulfur of a cysteine of the enzyme. The cofactor is biosynthesized by the reactions catalyzed by the enzymes HcgA-G. In the proposed biosynthesis sequence, 6-carboxymethyl-5-methyl-4-hydroxy-2-pyridinol (**1**) is converted to the 3-methylated-form (**2**), and then 4-guanylylated to form a guanylylpyridinol (**3**). Previous studies indicated HcgA is a new type of radical *S*-adenosylmethionine (SAM) enzyme; however, its role in the biosynthesis of the FeGP cofactor is unknown. Here, we report the *in vitro* biosynthesis of the FeGP cofactor using a Δ*hcgA* strain of *Methanococcus maripaludis* to analyze the function of HcgA in the biosynthesis of the FeGP cofactor. The cell extract of the Δ*hcgA* strain of *M. maripaludis* did not show [Fe]-hydrogenase activity. We did not observe formation of the FeGP cofactor in an *in vitro* biosynthesis assay containing the cell extract of the Δ*hcgA* strain and the co-substrates including SAM. Addition of each pyridinol precursor (**1**, **2** or **3**) complemented the biosynthesis of the FeGP cofactor *in vitro*, which indicates that HcgA catalyzes a biosynthetic reaction before biosynthesis of **1**. Heterologously produced HcgA complemented the biosynthesis in the absence of any of the pyridinol precursors. Mass spectrometric analyses indicated that **1** was the product of the HcgA catalyzed reaction from the compound in the cell extract from *M. maripaludis* and also *Escherichia coli*. These results indicate that HcgA is a novel radical SAM enzyme catalyzing formation of the pyridinol precursor **1**.

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eP024

Molecular identification of *Alternaria alternata* causing black spot of pecans (*Carya illinoinensis*) and the antifungal activity effects of leaves and husk crude extracts against the pathogen

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South Africa is the third-largest producer of pecans (*Carya illinoinensis*) in the global market. The development of pecan trees and nuts are highly vulnerable to the attack of *Alternaria alternata* that causes black spot disease also known as Alternaria black spot (ABS) disease. It is crucial to mitigate the challenges by understanding the risk these endophytic fungi possess to the industry. In this study, 30 *Alternaria* isolates causing ABS were identified based on molecular identification. The virulence of *A. alternata* isolates were also tested on detached nuts cultivars (cv.) of Wichita and Ukulinga, detached leaves cv. Wichita, and seedling blights. Furthermore, we investigated the antifungal activity of acetone and ethanolic leaves and husk extracts of both cultivars against *A. alternata*. Finally, we conducted HPLC analysis to screen for relevant phenolic acid composition and the total phenolic content present in leaves and husk for both cultivars. The result of the multi-locus DNA sequencing analysis of (*gapdh*, *rpb2*, *tef1*, *Alt a 1*) gene regions revealed that the 30 *Alternaria* isolates were *A. alternata* sensu stricto, forming part of the *Alternaria* species complex. The pathogenicity bioassay results differed significantly between cultivars of the detached nuts (wounded and unwounded) for all the tested isolates. Similarly, the wounded detached leaves were significantly different from the unwounded leaves, seedling bioassay confirmed that the isolates cause seedling blights. Contrary to ethanolic crude extracts assay, the acetone crude extracts with concentration levels at 60 - 90 mg/ml was shown to have more antifungal efficacy against all the tested *A. alternata* isolates, with inhibition zones ranging from 11-39 mm. Scanning electron microscopy (SEM) showed major morphological alteration/ damages on the conidia from assayed cultures, which resulted in inhibition. Twelve individual phenols were detected through HPLC analysis in the respective extracts. The total phenolic content was higher in the leaves extracts of cv. Wichita (102.19 mg GAE/g) and cv. Ukulinga (110.13 mg GAE/g) relative to the husks extracts of cv. Wichita (62.03 mg GAE/g) and cv. Ukulinga (85.07mg GAE/g). We confirmed that *A. alternata* causes black spot disease and seedling blights of pecans in South Africa. The study shed light on the importance to select the best extract with the goal of developing natural bioactive antifungal agents to control ABS disease of South African pecans.

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eP025

Using the filamentous ascomycete *Sordaria macrospora* to study the conserved histone chaperone ASF1

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ASF1 is a conserved eukaryotic histone chaperone and is involved in the assembly and disassembly of nucleosomes during transcription, replication and DNA-repair. Its importance is underscored by studies showing that all non-DNA-bound histones are bound to ASF1. *S. macrospora* is one of the very few multicellular organisms where *asf1* deletions are viable which makes it exceptionally useful for *in vivo* analysis of ASF1 functions. Prior studies of our group have shown that *asf1* deletions lead to sterility and vegetative growth defects but don"t affect nucleosome positioning.

We focused on mapping areas of ASF1"s highly conserved core and divergent C-terminal tail that are relevant for histone interaction and sexual development of *S. macrospora*. Furthermore, we studied the effect of ASF1 on histone modification and tested its relevance for genomic stability.

By using Co-IP and complementation analysis we were able to show that substitutions at the conserved core of ASF1 abolish histone binding and lead to strains resembling a

deletion mutant. A mutation in a putative HIRA-binding site didn"t inhibit histone binding, but produced strains with growth aberrations. Truncations of the C-terminal tail after position 210 had no effect on histone interaction or sexual development, while truncations at positions 185 and 152 severely disturbed histone binding and inhibited development. ∆asf1 strains proved to be sensitive to the DNA-damaging agent MMS, while complementation strains retained the wild type resistance despite the inability of ASF1 variants to interact with histones. Semiquantitative western blots showed that the deletion of *asf1* leads to a significant increase in overall H3K27Me3 and a significant decrease in H3K56Ac compared to the wild type.

In summary our data indicates that interaction of ASF1 with histones is an essential factor for sexual development in *S. macrospora*. The divergent C-terminal region is also essential for histone binding as truncations before position 210 abolish this function. Interaction with histones isn"t necessary for protection against DNA damage, but the exact mechanism remains elusive. A connection with histone modifications which undergo a significant change in ∆asf1 seems likely.

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eP026

Analysis of the fungal STRIPAK complex: Function of putative target proteins

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The STRIPAK (striatin-interacting phosphatases and kinases) complex is a highly conserved signaling complex that performs a similar function in humans and micro-organisms; it regulates proteins functions by phosphorylation and dephosphorylation which is essential for biological activities [1]. In humans, dysfunction of the STRIPAK subunits leads to several diseases such as cancer, while in fungi, it controls vegetative growth, hyphal fusion, and sexual development. However, it had not yet been understood, which proteins exactly are posttranslationally modified by the complex. STRIPAK interacts with other conserved signaling protein complexes such as the highly conserved septation initiation network (SIN) complex, which is homologous of the Hippo signaling pathway from animals. Recent proteome and phosphoproteome studies, using wild-type and mutant strains from *S. macrospora*, have identified putative STRIPAK target proteins such as CDC7, SmKIN3, DBF2, which are components of SIN [2,3].

To analyze the function of genes encoding subunits of the SIN signaling pathway, we have constructed deletion and complementation strains. These will be the basis to investigate phosphomimetic and -deficient variants of SIN subunits for identifying conserved (de)phosphorylation residues. The investigation presented here will contribute to the overall mechanistic understanding of how STRIPAK controls cellular development in euascomycetes [4].

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eP027

Biofouling and Biocorrosion of Aerospace Materials by the black mold *Aspergillus niger*

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Since human space exploration started around 1961, microbial life has been inevitably carried alongside astronauts (Checinska Sielaff et al., 2019) to the space stations Mir and ISS (International Space Station). A space station is a confined living environment with controlled environmental parameters. This is an ideal scenario for microorganisms to grow and spread. In 2008 researchers identified 39 different mold species in dust collected from HEPA Filters inside the ISS (Vesper et al., 2008). Among these species, *Aspergillus* ssp. and *Penicillium* ssp. were predominant. Growth of fungal colonies inside the ISS can create health predicaments for the astronauts, that are known to be immunocompromised over a long-term stay in space, as filamentous fungi can release a high number of mycotoxins and allergens. Additionally, the surface colonization of filamentous fungi can lead to structural integrity loss of the spacecraft built materials.

Using the black mold *Aspergillus niger,* we perform research on its biofouling capacities towards aerospace-relevant materials e.g., silica aerogels as insulation materials in standardized biodegradation assays. Here, we investigate not only the durability of the materials, but we aim to enhance our understanding towards the fungal physiology behind the process of biofouling. Additionally, we characterize the antifungal effects of antimicrobially active copper and copper alloy surfaces, which have been topographically modified via Ultrashort Pulsed Direct Laser Interference Patterning (USP-DLIP). The antifungal activity will be determined by performing specific contact-killing experiments. First results indicate the necessity of improving antifungal properties of the tested materials due to the high resilience of *A. niger* spores and their ability to thrive on aerospace-relevant materials, even under suboptimal growth conditions. The standardized experimental platforms used for the studies aim to enhance testing preventive systematic countermeasures against fungal contaminations in confined aerospace-built environments and apply the findings for improvement of future built materials in those settings.

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eP028

A novel binding platform consisting of three MademoiseLLE domains links the key RNA transporter to endosomes

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Transport of mRNAs coupled with local translation ensures the spatiotemporal regulation of gene expression. Active transport along the cytoskeleton is a common mechanism of mRNA localization in highly polarized cells such as filamentous fungal hyphae, plant endosperm, and neurons. Transport of mRNAs require the involvement of specific RNA binding proteins, molecular motors and adaptor proteins.

In our model organism *Ustilago maydis*, a phytopathogenic filamentous fungus, Rrm4 is the key RNA binding protein that mediates the long-distance transport of mRNAs. Loss of *rrm4* leads to disturbed hyphal growth and reduced pathogenicity. Rrm4 forms the messenger ribonucleoprotein (mRNP) complex together with the accessory Poly[A] binding protein (Pab1, PABPC in human), and, is transported along the microtubule cytoskeleton by hitchhiking on early endosomes. Both Rrm4 and Pab1 have RNA recognition motifs (RRM) in their N-terminal for interaction with RNA, whereas in the C terminal they have MademoiseLLE (MLLE) domain(s) for direct interaction with the essential endosomal adaptor protein Upa1. Deletion of C terminal MLLE domain affects the endosomal attachment which in turn results in disturbed mRNA transport and polar growth.

MLLE domains are conserved across eukaryotes, mediate protein-protein interactions and oligomerization. In humans, MLLE domains are found in PABPC and E3 ubiquitin ligase (Ubr5). MLLE of PABPC interacts with proteins containing PAM2 motif whereas MLLE of Ubr5, interacts with PAM2 motif as well as PAM2 like (PAM2L) motif. In *U. maydis* MLLE of Pab1 interacts with PAM2 motif of Upa1 whereas MLLE of Rrm4 interacts with PAM2L motif of Upa1 specifically.

By computational structure prediction we found that C-terminal of the Rrm4 has a sophisticated multi MLLE domain binding platform. Later we confirmed it with structure determination by SAXS and X-ray crystallography. We characterized the role of the individual MLLE domains by *in vitro* biochemical and biophysical assays. Further, using fluorescently labeled Rrm4 mutant variants in combination with Upa1 mutant variants we investigated the role of individual MLLE domains *in vivo* and characterized their effects on hyphal growth and endosome mediated RNA transport.

With the combined approach of structural biology and fungal genetics we show that the third MLLE domain of Rrm4 is essential for the endosomal attachment and polar growth whereas the first and second MLLE domains most likely play an accessory role.

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eP029
Antimycotic effects of 11 essential oil components and their combinations on 13 food spoilage yeasts and molds
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Food safety is important to reduce food spoilage microorganisms and foodborne pathogens. However, food safety is challenging, as customers" demand for natural preservatives is increasing. Essential oils (EOs) and their components (EOCs) are alternative antibacterial and antimycotic food additives. In this study, the minimal inhibitory concentrations (MIC) of 11 different EOCs against 13 food spoilage molds and yeasts were investigated via the microdilution method. Cinnamaldehyde (CA) revealed the lowest MIC for all tested strains and all EOCs (32.81–328.1 □g ml[1]). However, CA is organoleptic and was therefore combined with other EOCs via the checkerboard method. Overall, 27 out of 91 combinations showed a synergistic effect, and both respective EOC concentrations could be reduced by maintaining MIC. Thereby, the combination with citral or citronellal showed promising results. The concentration-dependent effect of CA was studied in further detail on *Saccharomyces cerevisiae*, with CA causing delayed growth-kinetics and reduced total cell numbers. In addition, flow cytometric measurements combined with live–dead staining indicate the fungicidal effect of CA, due to decreasing total cell numbers and increasing relative amount of propidium iodide-positive cells. In this study, we demonstrated that CA is a potent candidate for the use as a natural preservative against food-relevant mold and yeasts showing fungistatic and fungicidal effects. Therefore, CA and EOC combinations with respective lower EOC concentrations reduce organoleptic reservations, which ease their application in the food industry.

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eP030
Proximity-dependent labeling of cellular microenvironments in the ascomycete *Sordaria macrospora*
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Biotin Identification (BioID) is a powerful tool to map protein-protein interactions *in vivo*. This method relies on a promiscuous biotin ligase which covalently labels nearby proteins through the attachment of biotin to lysine side chains. When fused to a protein of interest, the proteinaceous neighborhood within a radius of the bait protein is biotinylated. Biotinylated proteins are then enriched through biotin affinity capture and identified by liquid chromatography followed by tandem mass spectrometry. Contrary to pulldowns, the BioID method does not require protein complexes to stay intact, and even weak or transient interaction partners can be identified.

Here we report the successful establishment of BioID in the filamentous ascomycete *S. macrospora* using the striatin-interacting phosphatase and kinase (STRIPAK) complex as a proof of principle. This multiprotein complex is highly conserved in animals and fungi. The STRIPAK complex is involved in the phosphoregulation of cellular and developmental processes such as cell growth, -fusion and apoptosis. Deletion of SmSTRIPAK subunits in *S. macrospora* causes severe defects in fruiting body formation, cell fusion and vegetative growth.

For establishment of BioID, the TurboID biotin ligase was fused to the STRIPAK complex interactor 1 (SmSCI1), which is a known component of the SmSTRIPAK complex. The SmSCI1-TurboID fusion protein was expressed in the SmSCI1 knockout strain Δsci1 under control of the native *sci1* promoter. An unfused TurboID ligase was used as control for unspecific biotinylation. In the subsequent BioID experiment, we identified the SmSTRIPAK subunits PRO11, SmMOB3, PRO22 and PP2Ac1 as interaction partners of SmSCI1. By identification of these proteins, which are known to be in the vicinity of SmSCI1, we were able to show

functionality of the BioID method, further expanding the molecular toolbox of *S. macrospora*.

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eP031

The isoprenyl chain length of coenzyme Q mediates nutritional resistance of fungi to a predatory amoeba

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Prey predatory interactions among microorganisms are main drivers in microbial evolution and can influence the diversity of entire microbial communities as well as evolutionary adaptations of single microbial species. *Protostelium aurantium* is a fungivorous amoeba that co-exist with a variety fungal species in nature and feeds on a wide range of fungal species including filamentous and yeast-like ascomycetes and basidiomycetes. Food source screenings identified the *Saccharomyces* clade including the fungal pathogen *Candida glabrata* as one of the very few taxonomic groups that showed resistance to the amoebae, despite rapid uptake by the phagocyte (1, 2).

One of the very few fundamental molecular differences of this clade in comparison to other fungi and bacteria is the different length of the side chain of the major mitochondrial electron carrier, ubiquinone or coenzyme Q. While most fungi and bacteria use coenzyme Q with chain lengths of 8-10 isoprenyl units (Q8 to Q10), *Saccharomyces sp*. generally use only Q6. The biological reason for this evolutionary switch to shorter isoprenyl chain lengths is currently unknown. Genome analyses of *P. aurantium* suggested the absence of a functional biosynthetic pathway for the coenzyme and indicated that this vital cofactor is supplied only via predatory feeding on its fungal prey. External feeding of Q8 or the introduction of a functional pathway for Q8 biosynthesis restored amoeba predation on *S. cerevisiae.* Hence, the inability of *P. aurantium* to feed on members of *Saccharomyces* clade indicates that a nutritional resistance (switch to Q6) can function as an efficient strategy to escape environmental predation and may be easily achieved by a fermentative microorganism which could potentially tolerate a less efficient version of coenzyme Q.

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eP032

Sugar uptake of the Brassicaceae smut fungus *Thecaphora thlaspeos* during biotrophic interactions

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Smut fungi are biotrophic plant pathogens, which grow as endophytes in their host to produce teliospores in the plant"s reproductive organs. During *in planta* growth, the fungi depend on taking up all required nutrients, including sugars, from their respective host plant. Sugars are necessary nutrients, providing energy for survival and growth, are used as building blocks for biomolecules, and function in intracellular signaling processes.

The smut fungus *Thecaphora thlaspeos* infects the natural host plants *Arabis hirsuta* and *Arabis alpina*, proliferating along the plant"s vasculature. Under lab conditions, colonization of the model plant *Arabidopsis thaliana* is possible, providing an ideal platform to study smut fungal plant-pathogen interactions in the model host plant.

In my PhD, I will investigate how plant pathogenic fungi ensure sugar uptake for their own survival during plant infection and whether manipulation of plant sugar transport can change the outcome of infection. Therefore, I performed a functional analysis on the *T. thlaspeos* sugar transporterome, giving me first hints about the impact of these transporters during virulence. *T. thlaspeos* contains 21 putative sugar transporters, of which five are upregulated during infection. Among these five, the putative Hexose-transporter-1 contains a conserved region to the yeast"s glucose sensors Rgt2p and Snf3p, and is therefore thought to function as a transceptor during pathogenicity. To analyse Hxt1p and the remaining four upregulated candidates further, complementation analysis in the relative smut *U. maydis* is attempted.

Subsequently, prime candidate transporters of *T. thlaspeos* will be knocked out in the fungus and tested in an infection assay. *T. thlaspeos* can infect its host plants via roots. To make use of this mechanism, I am establishing a root infection setup, in which the plants are grown hydroponically. With this tool, I will be able to investigate the influence of smut infection and sugar uptake on plant carbon partitioning, for example by using *A. thaliana* sugar transport mutants.

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eP033

Mg^{2+} transport and its role for virulence in the smut fungus *Ustilago maydis*

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Magnesium is the most abundant bivalent cation in the cell. It is involved in versatile cellular processes, for example, it functions as enzymatic co-factor and influences macromolecular structures. Thus, the role of magnesium in plant infection is difficult to study. While there are several examples of pathogens were Mg^{2+} availability impacts disease severity, Mg^{2+} transport is not described in smut fungi so far. Smut fungi are plant pathogens with high agricultural and economical importance, and *U. maydis* is the model smut fungus to study plant-pathogen interactions. Previously, it was shown, that Mg^{2+} is reduced in *U. maydis* infected maize tissue although these are sink tissues with increased phloem flow. The aim of this study was to create an inventory of Mg^{2+} transporters of the CorA family in *U. maydis* to set the foundation to elucidate a potential contribution to virulence. We showed that UMAG_00361 is the plasma membrane Mg^{2+} transporter homologue of the well characterized *Saccharomyces cerevisiae* ALR1p. In *U. maydis alr1* deletion results in a filamentation defect which blocks infection on the plant surface. We hypothesize that Mg^{2+} transporters contribute to *U. maydis* virulence by taking up Mg^{2+} at the points of cell-cell passage to weaken the plant cell wall and to alter the Mg^{2+} concentration at the infection site to redirect sugar partitioning. Thus we suggest that fungal specific Alr1 inhibitors could be promising drugs for pest control.

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eP034

Action of Extracellular Proteases of *Aspergillus flavus*, *Aspergillus ochraceus* and *Aspergillus terreus* Micromycetes on PlasmaHemostasis Proteins

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Micromycetes of the *Aspergillus* genus are known as producers of extracellular proteases with different activities, including activities against proteins of the hemostatic system. This enables the potential application of these enzymes in diagnosis and disease treatment. Therefore, further investigations of the *Aspergillus* proteases is required. Here, we studied the effect of micromycetes *A. flavus* 1, *A. ochraceus* L-1 and *A. terreus* 2 proteases on proteins of the hemostatic system.

Three strains of micromycetes, *A. flavus* 1, *A. ochraceus* L-1 and *A. terreus* 2, with previously indicated activities toward protein components of hemostasis, were used.

For activity determination, an integrative approach was applied. The first stage included cultivation on different nutrition media: Skim milk agar (SMA), Blood agar (BA) and Plasma agar (PA), named after their main components, respectively. The cultivation was performed at 24, 28 and 37℃, and after 5 days after inoculation, hydrolysis of substrates around the colonies was visualized. The second stage included the determination of the proteolytic activity of enzyme preparations. These were obtained after two-stage submerged fermentation of the micromycetes by salting out, dialysis, lyophilization and isoelectrofocusing. Activities were determined with suspensions of Human and Bovine Serum Albumin and Horse hemoglobin and measured spectrophotometrically. Fibrinolytic activity was revealed using fibrin zymography.

The optimal growth on different media was observed at 28℃ for *A. flavus* 1 and *A. ochraceus* L-1 and 37℃ for *A. terreus* 2. Growth and clearly visible hydrolysis zones were registered for SMA, proving the proteases secretion. Among the determined, all the strains showed direct fibrinolytic, albuminolytic and hemoglobinolytic activities. The highest activity against hemoglobin was registered for *A. terreus* 2, reaching 1139.4 UTyr/mg. The highest activities against human and bovine serum albumin were 482.4 UTyr/mg and 604.7 UTyr/mg for *A. flavus* 1 and *A. ochraceus* L-1, respectively.

Thus, proteases of studied micromycetes exhibit a complex effect on the proteins of the hemostasis system, performing the proteolysis of a significant number of its components. Considering the results, these proteases may be applied for diagnostic purposes rather than in therapy and disease treatment.

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eP035

Sustainable *in vivo* biosynthesis of 7-aminocephalosporanic acid in the cephalosporin C producing fungus *Acremonium chrysogenum*

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Cephalosporins are a class of β-lactam antibiotics with a broad spectrum against both, Gram-positive and Gram-negative bacteria. Most of the highly effective semi-synthetic cephalosporins are produced by modifying the side chains of the core molecule 7-aminocephalosporanic acid (7-ACA), which is derived from cephalosporin C (CephC) by deacylation. In nature, CephC is exclusively produced by the filamentous fungus *Acremonium chrysogenum*. In industrial applications, CephC is used to generated 7-ACA in two-or three step enzymatic conversion processes *in vitro*. Due to the high cost of the complex enzymatic process, researchers have identified CephC acylases from bacteria for a one-step deacylation of CephC. However, the industrial applications are still restricted to *in vitro* applications. Therefore, the objective of this project is to introduce the bacterial CephC acylase into the CephC producer *A. chrysogenum* to achieve an efficient *in vivo* one-step conversion of CephC to 7-ACA.

An acylase from *Pseudomonas* was discovered to be highly active to glutaryl-7-ACA, but low activity to CephC. In previous studies, this acylase has been optimized for the improvement of its substrate specificity towards CephC via site-directed mutagenesis. To express the acylases in *A. chrysogenum*, codon optimized CephC acylase genes were integrated into a fungal gene expression vector. The genes were modified to generate recombinant proteins with an N-terminal His6-tag and a C-terminal HA-tag. DNA-mediated transformations were done using conventional transformation protocols. Western blot analysis of the transformants showed that the CephC acylase genes were successfully expressed in *A. chrysogenum*, as well as the autocatalytic cleavage was processed well, which is crucial for activating the enzyme. Supportive analyses were done using protein HPLC-MS for the confirmation of the full-length enzyme expression. Most importantly, via HPLC analysis, we were able to detect significant amounts of the acylase product 7-ACA from culture supernatants. Moreover, the optimizations of enzyme reaction were conducted in terms of pH, temperature and incubation time of *A. chrysogenum* culture supernatants.

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eP036

Characterization of the potential phosphatidylserine transporter SmOSH6 in the filamentous ascomycete *Sordaria macrospora*

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Eukaryotic cells are organized into separate membrane-bound compartments to segregate diverse metabolic processes, which are mediated by different sets of proteins. Each of these compartments have specialized functions and a unique protein and lipid composition. The glycerophospholipid phosphatidylserine (PS) is present in lesser amounts in these membranes. However, the low abundance is outweighed by its physiological importance, due to its negative charge. Externally exposed PS has an important role in apoptosis and blood clotting. Although, in healthy cells it is normally not exposed to the outside and involved in several intracellular signaling pathways. PS is distributed unevenly within the cell, which is achieved by a combination of vesicle-mediated transport and non-vesicular transport for example in form of lipid transfer proteins (LTP). In yeast, two LTPs with a high affinity to PS were discovered: OSH6 and OSH7. Recently, we found a potential homologue of OSH6 and OSH7, SmOSH6, in the filamentous ascomycete *Sordaria macrospora*. Here, we aim to analyze the function of SmOSH6.

First localization studies showed a net-like localization of SmOSH6 in the hyphal tip, co-localizing with marker proteins for the ER, early and late endosomes, and a degradation in older hyphae. To monitor the pattern of PS and to perform co-localization between the putative phosphatidylserine transporter and PS in *S. macrospora*, the C2 domain of the glycoprotein bovine lactadherin (LactC2) will be established as a PS lipid marker. The potential function of SmOSH6 as a PS transporter will be analyzed using a fat blot assay to determine its specificity to PS and by identification of possible interaction partners using LCMS/BioID.

To better understand the role of SmOSH6 during the development of *S. macrospora*, the knockout strain ΔSmosh6 was generated. It showed no distinct phenotype compared to the wild type under normal conditions, most likely due to redundancy with other lipid transfer proteins. However, even small changes in PS abundance resulted in impaired fruiting-body development under temperature stress.

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eP037

Histone acetylation affects the production of soluble metabolites secreted by *Candida albicans* to escape macrophages

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Introduction: Tissue-resident macrophages represent key players for host defense in specific organs during early stages of fungal infections since they work as immune sentinels to detect infections at the front-line. *C. albicans* has evolved several strategies to evade host immune response and escaping from macrophages is the basis for establishing systemic infection. Chromatin modifying enzymes have been shown to epigenetically modulate pathogenicity of the opportunistic pathogen *C. albicans*. The most abundant epigenetic modification in *C. albicans* is the H3K56ac which results from the activity of two opposite enzymes, the histone acetyltransferase RTT109 and the histone deacetylase Hst3p. Despite the well-known host immune evasion mechanisms, *C. albicans* secretes soluble factors which could have an immunomodulatory role influencing the macrophages' recruitment to the infection site.

Objectives: The aim of this work was to assess if the metabolites produced by *C. albicans* to escape the host immune response can be affected by H3K56ac levels.

Methods*: C. albicans* conditioned medium (CM) or *C. albicans* conditioned medium +10 mM NAM (CM NAM) used as Hst3p inhibitor, were collected from overnight cultures and used for the treatment of J774A.1 macrophages, that were then stained with TRITC-Palloidin at different time points to follow changes in actin cytoskeleton by confocal microscopy. Moreover, to verify the effect of such metabolites on the phagocytic activity, the macrophages pre-stimulated with CM or CM NAM were infected with live *C. albicans*. Finally a Mass Spectrometry analysis was performed to identify metabolites potentially modulated by the treatment.

Results: We found that the exposure of J774A.1 to CM NAM leads to changes in actin cytoskeleton structure resulting in activated cells with linear filopodia (already after 4 h of exposure), although in cells treated with CM the actin filaments are more concentrated around the nucleus. This marked difference was also observed in the infection experiments as the macrophages pre-stimulated with CM NAM displayed an improved phagocytic activity. The subsequent MS analysis allowed us to identify some candidate metabolites responsible for mitigating the macrophages' response.

Conclusion: The present study highlights the relevance of the epigenetic modifications in the regulation of *C. albicans* virulence leading the way to the study of new strategies used by the fungus to escape the host-immune response.

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eP038

Investigation of the posttranscriptional regulatory role of the RBP Khd4 during the infectious phase of *U. maydis*

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Several pathogenic fungi require a morphological transition from yeast to hyphal form for successful infection. This switch facilitates specialized infection structures, proliferation in the tissue and acquisition of nutrients. In the hyphal growth form, membrane trafficking is critical not only for the correct translocation of proteins and constant supply of membrane remodeling proteins at the polar tip. This transition of morphology and the further virulence require precise gene regulation. RNA-binding proteins (RBPs) are important for the posttranscriptional control of mRNA, thereby adjusting protein levels within the cell. Interaction with RBPs regulates translation, subcellular localization and the lifetime of the target mRNA. One group of RBPs are the multi-KH domain containing proteins, which are widely conserved and tend to bind to AU-rich RNA targets.

In the maize smut fungus *U. maydis* the RBP Khd4 is important for morphology and pathogenicity. Homologues are known in mammalian fungal pathogens such as *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. Deletion of *khd4* in *U. maydis* is linked to delayed hyphal growth and a complete loss of pathogenicity. Previous studies revealed that Khd4 binds hundreds of RNA via an AUACCC motif. Various target mRNAs are important for membrane trafficking, thus, Khd4 has a regulatory function for the polar growth of the hyphae. However, the exact role of Khd4 during the infection is still unknown.

The aim of this study is to determine the regulatory function of Khd4 during infection, using high throughput experiments, such as *in planta* HyperTRIBE. This will be a powerful tool to study posttranscriptional regulation during the virulent phase *U. maydis* more in detail and will reveal further functions of RBPs regarding pathogenicity in the future.

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eP039

Effect of bacterial volatiles on the mycelium growth of mushrooms

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Bacteria play an important role in the life cycle of fungi showing remarkable effects on *inter alia* morphological features, mycelial growth and fruiting body induction.

Nevertheless, little is known about the underlying mechanisms of effects and how species-specific these are.

To tap these topics, several bacterial strains, isolated from commercial spawn, mycelial colonized straw and fruiting bodies of *Pleurotus ostreatus* were used to analyze the bacterial effetcs on mycelial growth of four *Pleurotus species*, namely *P. ostreatus*, *P. eryngii*, *P. sapidus* and *P. citrinopileatus* as well as on mycelial growth of *Cyclocybe aegerita*, *Lentinula edodes* and *Kuehneromyces mutabilis*.

Interestingly, in co-cultivation on agar plates significant growth promoting effects of bacteria were only observed on fungi from the genus *Pleurotus*. In early cultivation stages, the *Paenibacillus* strain M48F remarkably increased mycelium growth of *P. ostreatus*, *P. eryngii* and *P. sapidus*, to about 47%, 32% and 27%, respectively. To elucidate the growth effects of bacterial volatile organic compounds (VOCs) on the fungi, *P. ostreatus* and *P. eryngii* were cultivated along with the bacteria on divided petri dishes. VOCs from strain M48F showed, with about 50% and 20% more growth of the *P. ostreatus* and *P. eryngii* mycelium, respectively, the strongest growth enhancing effects of the tested bacteria. Hence, VOCs of strain M48F were analyzed solely as well as in combination with *P. ostreatus*, *P. eryngii*, *P. sapidus* and *L. edodes* using divided petri dishes combined with a SPME-GC-MS approach. The volatilome of strain M48F were dominated by 2,5-diisopropylpyrazine throughout all sampling days. In strain M48F samples as well as in co-cultivation with *L. edodes*, the amount of 2,5-diisopropylpyrazine remained quite constant whereas with the *Pleurotus* species the amount decreased remarkably, especially with *P. ostreatus.* Interestingly, cultivation of *P. ostreatus* in the presence of 2,5-diisopropylpyrazine resulted in significantly enhanced mycelium growth though the growth promoting effect was not as pronounced as during co-cultivation with strain M48F.

The results of the present study revealed that mycelium growth promoting effects of bacteria are species-specific and that bacterial VOCs, such as 2,5-diisopropylpyrazine, can enhance fungal growth.

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eP040

A mycophagous amoeba uses fungolytic vesicles to kill fungal pathogens

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Predatory interactions among microbes are considered to be a major evolutionary driving force for biodiversity, but may also have contributed to the acquisition and maintenance of microbial virulence determinants. The Amoebozoa are a major group of ubiquitous, eukaryotic micro-predators that commonly feed on microbial prey by phagocytosis and subsequent intracellular killing. Here, we have used the amoeba *Protostelium aurantium* to study the impact of micropredation on fungi and elucidate recognition and killing mechanisms.

Food source screenings identified a wide food spectrum of ascomycete and basidiomycete yeasts, but also included filamentous fungi. *P. aurantium* efficiently ingested and killed various pathogenic yeast, but was also able to kill the filamentous fungal pathogen *Aspergillus fumigatus* via an active invasion to the fungal filaments (1). Fluorescence microscopy using GFP-labelled cells of *Candida parapsilosis* revealed that the yeast prey was rapidly internalized and exposed to reactive oxygen species, before the cells were lysed within minutes inside the phagolysosomes of *P. aurantium*.

To identify the lytic factors, the amoebae were grown on large scales using the yeast pathogen *C. parapsilosis* as a single food source. The analysis of the cellular extracts from cultures of *P. aurantium* showed that the fungolytic activity was largely retained in intracellular vesicles of the amoebae and induced immediate killing and lysis of *C. parapsilosis in vitro.* Proteomic analyses identified 4 cell wall-associated proteins of *C. parapsilosis* and at least 56 different vesicular proteins from the amoeba (2). Novel proteins were dominant among the amoeba proteins. Yet, many of them could be categorized as hydrolytic enzymes targeting the fungal cell wall, indicating that fungal cell wall structures are under predatory selection pressure in the natural environments and may have acted as an early promoter to withstand phagocytic killing. Identification of the lytic factors is currently ongoing and will elucidate the highly efficient killing strategies employed by this fungus-specific predator. This may also provide a new perspective on antifungal therapy or biocontrol.

1. Radosa et al., 2019; *Environ Microbiol,* 21,1809-1820.
2. Radosa et al., 2021; *Cell Microbiol,* 23, e13389.

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eP041

Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*

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In the filamentous fungus *Magnaporthe oryzae* the cellular response mechanism to different external changes in the environment, e.g. osmolarity, is detected by the high osmolarity glycerol (HOG) signaling pathway. The pathway consists of a phosphorelay system and a downstream MAPK cascade. In response to high osmolarity, *M. oryzae* produces the compatible solute arabitol to compensate for the extracellular osmotic imbalance. *M. oryzae* "loss-of-function" (lof)- mutants of the HOG pathway are unable to cope with osmotic stress.

Long-term cultivation of these *M. oryzae* lof- mutants at high osmolarity for about 4-8 weeks resulted in "suppressor"-mutants, which arose as individuals being restored in osmoregulation. That"s valid for not only one specific lof-mutant of the HOG pathway, but rather for every single lof-mutant in which the different genes of the pathway have been individually inactivated. Interestingly, the major compatible solute being produced in these "suppressor"-mutants when exposed to osmotic stress is now glycerol instead of arabitol. In addition, it can be observed that most of all these "suppressor"- mutants can memorize the capability even after growing without stress.

In order to understand the molecular basis of the adaptation phenomenon, first results of modern quantitative (phospho)-proteomic techniques in combination with RNA sequencing results led to a set of candidate genes which may play an

essential part in the rewiring of the osmoregulation pathway. Genomic DNA sequencing did not show any apparent alterations. Initial reversed molecular genetics will be the next step to get closer to understanding the rapid adaptation process. We are convinced that this phenomenon is essential for understanding rapid evolutionary processes in signal transduction in eukaryotes and can be applied to other organisms apart from *M. oryzae* as well.

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eP042

Alternative splicing as an element of signal transduction in multi-step phosphorelay systems in fungi

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One of the best-characterized stress response signaling cascades in *Saccharomyces cerevisiae* is the high osmolarity glycerol (HOG) signaling pathway. Extracellular changes are received by one sensor histidine kinase and transmitted *via* a phosphotransfer protein ScYpd1p to the downstream response regulator.In contrast to yeast, in the genomes of higher fungi are many sensor histidine kinases coded, whereas only one single copy gene for the Ypd1 phosphotransfer protein exists. According to the current understanding, it is unknown how many different sensor proteins can receive various signals and how these different signals can be transmitted independently to distinct targets by only one phosphotransfer protein.

Alternative splicing (AS) could be one answer to explain signaling variability. AS is a fundamental process, especially in higher eukaryotes, for generating multiple transcripts from a single gene, which increases protein diversity. Recent studies indicate that AS is more widespread in fungi than previously assumed. Although there is evidence that AS occurs more frequently in pathogenic fungi than in nonpathogenic fungi, the production of multiple protein isoforms from a single gene in response to environmental stress, as previously described in plants, has not been extensively studied.

Our project focuses on the molecular mechanisms of AS by using the filamentous phytopathogenic fungus *Magnaporthe oryzae*. In our preliminary work, we observed an increased structural variation of mRNA from *MoYPD1* and amplified putative *MoYPD1* isoforms at cDNA level. We generated mutant strains overexpressing these isoforms and identified two peptide fragments unique to a novel *MoYPD1* transcript by using HPLC-MS/MS Peptide analysis. Furthermore, we study the relationship between AS and signal transduction by using different bioinformatics tools to predict additional MoYpd1p isoforms.

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eP043

Data from more than 5000 metagenomes suggest an increase in Eukaryotic reference genomes, and long-read sequencing data are required to recover high-quality fungal metagenome-assembled genomes.

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As most (micro)Eukaryotic genomes are yet to be sequenced, the mechanisms underlying their contribution to different ecosystem processes remain untapped. Although approaches to recover Prokaryotic genomes have become common in microbiology, few studies have tackled the recovery of Eukaryotic genomes from metagenomes.

This study aimed to assess the reconstruction of Eukaryotic genomes from public-available metagenomes found at the TerrestrialMetagenomeDB (https://webapp.ufz.de/tmdb/). We selected a total of 5149 metagenomic libraries containing a minimum of eight million paired-end reads and biome information in their metadata. We performed quality control of raw reads and assembly using metaWrap, while we recovered (micro)Eukaryotic metagenome-assembled bins using the EukRep tool.

A total of 948 Eukaryotic bins were recovered from 493 of the 5149 metagenomes. On average, each eukaryotic bin contained 2695 contigs with an average 12603 bp. Eukaryotic bins were classified into 19 classes (e.g., Saccharomycetes, Sordariomycetes, and Mamiellophyceae) and 23 orders (e.g., Mamiellales, Saccharomycetales, and Hypocreales). However, we only obtained matches to 13 different genera (spread across 126 bins) and ten species (spread across 49 bins) due to the limited number of eukaryotic reference genomes in public databases. Almost 68% of the eukaryotic bins were recovered from host-associated, water and forest samples. Only 361 bins contained completeness and contamination measures as most of the Eukaryotic diversity is not present in reference databases. The remaining 361 bins showed on average 42.64% (±28.09) completeness and 4.91% (±7.24) contamination. The bins with the highest quality belonged to the fungal phylum Ascomycota with average completeness and contamination of 94% and 0.55%, respectively.

Our results highlight the two significant constraints to reconstructing high-quality Eukaryotic genomes from metagenomes. These are: (i) the lack of reference genomes and marker genes in reference databases; and, (ii) the assembly of Eukaryotic chromosomes from short reads. The genomic structure of eukaryotes contains introns and higher rates of gene and genome duplication events, making it challenging to assemble eukaryotic genome using *de novo* assembly. We postulate that using long-read sequencers to produce metagenomes may circumvent these constraints as they facilitate the assembly of a longer fraction of the chromosome.

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eP044

Mycoparasites infecting mushrooms in nature

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Fungicolous mycoparasites causing distinct diseases in mushroom farms are well investigated due to economic interests. In contrast, mycoparasites in nature tend to be much ignored. The majority of infestations on macrofungi comes from the Ascomycota and, secondly, from the Basidiomycota. Certain mycopathogenic species exhibit board host ranges, while others show adaptation and are restricted to an order, family or fungal genus. Mycoparasites can be biotrophs which obtain nutrition from living host cells or necrotrophs that kill the host cells consumed by them. The most destructive necrotrophic mycoparasites belong to

certain conidiogenous ascomycete genera which spread rapidly via masses of asexual spores and infect and completely colonize their fungal hosts in a few hours to days. In this study, we observed such mycopathogens in nature. Humid weather with repeated rainfalls at comfortable lower temperatures is of advantage for both, mushroom formation and mushroom infestations. Mycopathogens of e.g. the ascomycetous genera *Cladobotryum* and *Mycogone* were variably detected on polypores and agarics. Deformations of primordia and carpophores by cobweb and wet bubble disease were observed on different mushroom species. Mycopathogens tended to grow from the ground over the stipe up to the pores or the lamellae with the hymenia and spores. We harvested infected mushrooms and analyzed the infections on the mushrooms. Strains of the *Hypomyces oderatus* were isolated from *Agaricus macrosporus* and identified by conidiophore morphology, sclerotia formation, production of aurofusarin pigment yellow to red in color dependent on the pH, and molecular markers [1]. They infested carpophores of different *Agaricus* species while *Pleurotus* mushroom caps were resistant. *Agaricus* and *Pleurotus* mycelium was also resistant to the strains unlike mycelium of the dung fungus *Coprinopsis cinerea*. Such mycopathogens from nature are potential sources for infestations in commercial mushroom cultures.

[1] Lakkireddy et al. (2020) Mycoparasite *Hypomyces odoratus* infests *Agaricus xanthodermus* fruiting bodies in nature. AMB Expr. 10:141

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eP045

A secreted protein from Maize smut pathogen suppresses reactive oxygen species response in the host plant

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Plant pathogens deploy secreted molecules to supress plant host immunity and to change the physiology of the plant host to favour the pathogen"s proliferation. Plants in turn have developed the sophisticated immunity responses, including production of reactive oxygen species (ROS), which allow to prevent the pathogens expansion in the host (1). The ROS production is usually triggered by the detection of fungal chitin or conserved part of bacterial flagella (flg22) by cell surface receptors (2). There are nearly 550 effector genes predicted in the *U. maydis* genome. These genes" products are predicted to have different biochemical activities and their expression is tightly regulated to allow the successful infection and disease development (3).

Here we focus on elucidation of molecular mechanisms behind *U. maydis* ability to suppress plant ROS-burst response by using secreted proteins.

We have identified the ROS burst inhibiting effector 3 (Rosie3) which suppresses ROS-burst response when overexpressed in *N. benthamiana* leaves upon flg22 perception.

In transgenic *Arabidopsis thaliana* plants an induction of Rosie3 expression leads stunted growth phenotype indicating that Rosie3 is either recognised in *A. thaliana* or it is targeting a plant metabolic pathway related to ROS-production and conserved in Arabidopsis, Maize and Tobacco. Using the *U. maydis rosie3* knock-out and overexpression lines we confirmed the secretion of this effector. Using a Co-IP (co-immunoprecipitation) followed by LC-MS/MS analysis of the Rosie3 we could predict the putative Rosie3 interactors in Tobacco. Virus induced gene silencing to knock down of these interactors in *N. benthamiana* showed an altered ROS generation upon flg22 perception. This hints these interactors as possible targets of Rosie3 and their role in ROS production process.

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eP046

Dark stipe mutants in fruiting body development of *Coprinopsis cinerea*

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Fruiting body development in the life cycle of the basidiomycete *Coprinopsis cinerea* normally occurs on the dikaryon and follows a conserved scheme defined by seven day and night phases, with well predictable distinct stages over the time. Fruiting starts with primary hyphal knot (PK) formation in the dark, followed by light-induced aggregation into compact round secondary hyphal knots (SKs) in which stipe and cap tissues differentiate. Primordia development (stages P1 to P5) takes five days to culminate on day 6 of development in karyogamy (K) and meiosis (M) within the basidia in the hymenium on the gills and the subsequent basidiospore production which parallels fruiting body maturation (stipe elongation and cap expansion). Fruiting bodies are fully opened in the last night phase to shed their spores while at the morning on day 7 the cap autolyses to release the majority of the spores in liquid droplets that fall to the ground. Fruiting body development is a complex cellular process and the fungal complexity increases from the P1 to P5 stages. This development is strictly regulated by environmental conditions including nutrients, light, temperature, humidity and aeration. Failure in light signaling or in aeration leads to formation of so-called "dark stipes", under proliferation of stipe tissues and blocks in cap development. UV or REMI mutants in four different genes (*dst1*, *dst2*, *dst3* or *dst4*) are available from the self-compatible homokaryon AmutBmut, with abnormal "dark stipe" phenotypes expressed under standard fruiting conditions (12 h light/12 h dark, 25 °C, high humidity, aeration). *dst1* and *dst2* mutants have defects at earliest stages of primordial development and are also blind with respect of light-induced oidiation. These mutants have known defects in the white collar 1 (WC1) photoreceptor and a FAD/FMN-containing dehydrogenase, respectively. Later stages in primordium development are sensitive to high CO2 concentration. In wildtype cultures, "dark stipe" phenotypes are formed from P3 and P4 stages under non-aerated conditions with normal light-dark changes. Scavenger experiments of CO2 with KOH however recovered the normal phenotypes in fruiting body development. The *dst3* and *dst4* mutants are defective in regulation at these later

oxygen-dependent stages of primordial development while they are not blind with respect of light-induced oidiation. Functions in metabolic pathways producing CO2 and CO2 signaling pathways will be discussed.

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eP047

Amylase activities in cultures of white-rot basidiomycetes

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Amylases are enzymes able to catalyze the hydrolysis of starch into sugar by attacking the glycosidic bonds in amylose and amylopectin, the two main components of starch. Three classes of amylases are distinguished in this process. The metalloenzyme α-amylase cleaves randomly 1-4 glycosidic bonds in amylose and amylopectin. Its function strictly depends on the presence of the cofactor calcium. β-Amylase as exoamylase breaks the second α,1-4 glycosidic bond from the terminal ends of starch molecules whereas γ-amylase primarily breaks the α,1-6 glycosidic linkages of amylopectin. In this examination, the ability of wood-decay fungi from the Agaricomycetes (Basidiomycota) to produce amylases was analyzed and the dependency of the amylases to the cofactor calcium determined. Five basidiomycetes, *Pleurotus ostreatus*, *Ganoderma resinaceum*, *Schizophyllum commune*, *Trametes hirsuta* and *Trametes versicolor* were selected for this study to investigate (1) if selected basidiomycetes are able to produce amylases for the degradation of starch as a nutrient source, (2) the influence of the cofactor calcium for the production and functioning of the fungal amylases, and (3) to test whether an elevated level of calcium leads to a higher amylase activity and probably also a higher fungal growth rate. Genes for Ca-depending α-amylases were found in the genome of all five fungi. The turnover of starch to maltose and glucose was determined in liquid fungal cultures under different concentrations of $CaCl_2$ supplied by the nutrient medium. All fungi showed an increased amylase activity upon addition of $CaCl_2$, albeit at individual $CaCl_2$ concentrations of best enzymatic activity. Tests with $MgCl_2$ and $CaCO_3$ were made in parallel to distinguish potential effects mediated by Ca^{2+} and Cl^{2-}. However, both addition of $MgCl_2$ and $CaCO_3$ did not lead to a higher amylase activity in liquid cultures as compared to fungal cultures without added supplements. Growth rates and mycelium patterns of all fungi were analyzed in $CaCl_2$ supplied agar cultures. The growth response of the fungi differed by species and according to the concentration of $CaCl_2$. i.e. the optimal concentration varied between the fungi. The mycelium morphology was affected for *P. ostreatus* with less aerial mycelium for under elevated $CaCl_2$ concentration while *T. versicolor* showed a contrary effect with increased production of fluffy aerial mycelium.

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eP048

The multi KH domain RNA binding protein Khd4 orchestrates membrane trafficking to promote polar growth of infectious hyphae

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Many fungal pathogens require the formation of highly polarised hyphae for successful infection. Such complex morphological development requires robust control of gene expression. Besides regulation of mRNA expression at the transcriptional level, the precise spatiotemporal control of protein expression is intensively achieved through post-transcriptional regulation of RNAs by RNA binding proteins (RBPs). Here we demonstrate that in the phytopathogen *Ustilago maydis*, the multi-KH RNA binding protein, Khd4, regulates the expression of a distinct set of mRNAs to orchestrate the membrane trafficking process during polar growth of infectious hyphae.

Using RNA-editing based hyper TRIBE technique we set to identify *in-vivo* target mRNAs bound by Khd4. Our analyses show that Khd4 binds to target mRNAs enriched for proteins associated with membrane trafficking, such as GTP binding proteins. Analysing the mRNA abundance revealed that ~40% of the target mRNAs show altered expression with the majority being upregulated in the absence of Khd4. Mainly, the upregulated target mRNAs show high enrichment of the Khd4 binding site at the 3´UTR. Subsequent analysis of the target mRNAs such as small GTPases *arl1*, endosomal cargo adapter *hok1*, vacuolar ATPase assembly factor *vma21* in the wildtype hyphae revealed that the mRNA expression is strongly downregulated when Khd4 interacts with the target mRNAs through the 3´UTR binding site. Furthermore, the analysis of the hyphal transcriptome also revealed that the deletion of *khd4* causes severe misregulation of genes involved in vesicle transport. These findings confirm the importance of RBP Khd4 in intense post-transcriptional regulation during the membrane trafficking process. Interestingly, the loss of *khd4* also induces adverse effects on vacuolar biogenesis. The aberrant vacuoles were minuscule, scattered throughout the hyphae and missorted to the cell cortex upon deletion of *khd4*. Thus, an unregulated post-transcriptional regulation of the endomembrane system leads to defective vacuole biogenesis.

Our study illustrates that the membrane trafficking mediated vacuolar biogenesis is regulated at the posttranscriptional level and it is important for the polar growth during fungal infection.

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eP049

Immunoproteome analyses identify *Aspergillus fumigatus* protein antigens specifically recognized by infected cystic fibrosis patients

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The opportunistic human pathogenic fungus ***Aspergillus fumigatus*** is able to cause a variety of diseases ranging from invasive to locally-restricted forms of infection and allergic disorders. It frequently colonizes the airways of patients with the genetic disorder **cystic fibrosis (CF)**, causing hypersensitivity responses/allergies to this fungus. One of the most serious manifestations of fungal allergy in CF patients is **allergic bronchopulmonary aspergillosis (ABPA)**. Diagnosis of ABPA is difficult, but it is especially challenging in CF patients who often suffer from various respiratory infections simultaneously. This raises the need for new diagnostic and therapeutic approaches for these

patients. Although the patients" elevated IgE and IgG titers to *A. fumigatus* are indicative for an *A. fumigatus* infection, the serum IgG/IgE antibody response to crude *A. fumigatus* antigens is in many cases inconsistent. However, the usage of a defined set of *A. fumigatus* protein antigens could improve the diagnostic accuracy in CF patients. To identify *A. fumigatus* protein antigens recognized by IgG antibodies from CF patients and thus find **putative biomarkers**, a **serological proteome analysis (SERPA)** was applied. With this method **44 immunogenic proteins** of *A. fumigatus* were identified and **nine** of those were **exclusively recognized by sera of CF patients.** Subsequently, an **enzyme-linked immunosorbent assay (ELISA)** was established to investigate the potential of the most promising immunogenic proteins to serve as putative biomarkers for the diagnosis of ABPA in CF patients. Additionally, the respective genes of these immunogenic proteins were deleted in *A. fumigatus* in order to elucidate their function, in particular their possible role in infection. The phenotypic characterization of the mutants is presented.

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eP050

Identification and functional characterization of *Ustilago maydis* effectors targeting auxin signalling

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Biotrophic plant pathogens employ secreted molecules, called effectors to suppress their host immune system and to redirect the metabolism and development in their own favour. The biotrophic fungus *Ustilago maydis* causes smut disease in maize (*Zea mays*) and teosinte *(Zea mays ssp. parviglumis)*. Its genome encodes approximately 550 predicted secretory proteins that likely function as *effectors,* of which round one fifth are localized to gene clusters. During plant colonization *U. maydis* also secretes certain plant growth substances including auxins. Auxin levels are increased in the infected tissues of maize and auxin signalling and auxin responsive genes are transcriptionally up-regulated. Our understanding how *U. maydis* employs auxin signalling is just recently emerging.

We analysed the ability of *U. maydis* putative effectors to induce growth hormone auxin signalling in plants and identified a set of five, genetically clustered effectors. By using different molecular and cell biological approaches we identified central corepressors of the Topless family as targets of these effectors. By performing various knockout strain analysis, we demonstrate that the auxin signalling inducing sub-cluster effectors play a role in virulence. Topless Interacting protein effectors (Tips) interacts solely with the N-terminal TPD domain and compete with Aux/IAA transcriptional repressors for their binding. Our findings reveal that Topless proteins, key-regulators of growth-defense antagonisms, are a major target of the *U. maydis* effectome.

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eP051

Structural studies on the key mRNA transporter on endosomes in *Ustilago maydis*

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Transport of mRNAs coupled with local translation ensures the spatiotemporal regulation of gene expression. Active transport along the cytoskeleton is a common mechanism of mRNA localization in highly polarized cells such as filamentous fungal hyphae, plant endosperm, *Drosophila* embryo, *Xenopus* oocyte, and neurons. Transport of mRNA requires the involvement of specific RNA binding proteins, molecular motors and adaptors that connect these proteins.

In our model organism *Ustilago maydis*, a phytopathogenic filamentous fungus, Rrm4 is the key RNA binding protein that mediates the long-distance transport of mRNAs. Loss of *rrm4* leads to disturbed hyphal growth and reduced pathogenicity. Together with the accessory Poly[A] binding protein Pab1 (PABPC in human), and an essential adaptor protein Upa1, Rrm4 forms the messenger ribonucleoprotein (mRNP) complex and, is transported along the microtubule cytoskeleton by hitchhiking on Rab5a-positive early endosomes. Both Rrm4 and Pab1 have RNA recognition motifs (RRM) in their N-terminal for interaction with RNA and MademoiseLLE (MLLE) domain(s) in their C terminal for protein-protein interaction (PPI). Deletion of C terminal affects the endosomal attachment which in turn results in disturbed mRNA transport and polar growth.

MLLE domains are conserved across eukaryotes, participate in PPI and oligomerization. In humans, MLLE domains are found in PABPC and E3 ubiquitin ligase (Ubr5). MLLE of PABPC interacts with proteins containing PAM2 motif whereas MLLE of Ubr5, interacts with PAM2 motif as well as PAM2 like (PAM2L) motif. In *U. maydis* MLLE of Pab1 interacts with the PAM2 motif of the endosomal adaptor protein Upa1 whereas MLLE of Rrm4 interacts with two PAM2L motifs of Upa1 specifically.

From our earlier structural studies we found that C-terminal of the Rrm4 is made of a sophisticated multi MLLE domain binding platform. Using *in vitro* and *in vivo* studies we found that the third MLLE domain is essential for the endosomal attachment and polar growth whereas first and second MLLE domains of Rrm4 most likely play an accessory role. We are currently investigating the MLLE-PAM2L/PAM2 in detail, in addition we are studying other uncharacterized inter and intra molecular interactions in the Rrm4 associated mRNP complex using a combined approach of structural biology, biochemistry, biophysics, bioinformatics and fungal genetics which should enable us to understand their molecular mechanism and evolutionary relationship.

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eP052

The fungal root endophyte *Serendipita vermifera* displays inter-kingdom synergistic beneficial effects with the microbiota in *Arabidopsis thaliana* and barley

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Plant root-associated bacteria can confer protection against pathogen infection. By contrast, the beneficial effects of root endophytic fungi and their synergistic interactions with bacteria remain poorly defined. We previously demonstrated that both local and systemic colonisation by the Sebacinales endophyte *Serendipita vermifera* (syn. *Sebacina vermifera*, hereafter Sv) afford protection against infection of the soil-borne plant pathogen *Bipolaris sorokiniana* (syn. *Cochliobolus sativus*, hereafter *Bs*) in *Hordeum vulgare* (barley). Here we explore how *Sv* and *Bs* colonisation capacities in two plant species, barley and Arabidopsis, are modulated by the presence of individual members of the core bacterial microbiota or SynComs isolated from the barley rhizosphere or Arabidopsis roots. The finding that *Bs* also

infects and causes disease symptoms in Arabidopsis roots motivated us to develop a set of physiological measurements to characterize disease severity and plant growth in Arabidopsis under different microbe treatment regimes. These measurements include ion leakage (quantified via electric conductivity) and photosynthetic activity (measured using pulse amplitude modulation fluorometry) as readouts for host cell death progression and biotic stress during the host-microbe interaction. Analyses of inter-kingdom activities in barley and Arabidopsis revealed that *Sv* can functionally replace root-associated bacteria by mitigating pathogen infection and disease symptoms in both hosts. Additionally, we show that cooperation between bacteria and beneficial fungi leads to inter-kingdom synergistic beneficial effects. We found that inter-kingdom protective benefits are largely independent of the host while synergism leading to early growth promotion is driven by host species and microbiota composition. Finally, RNA-seq experiments with selected bacterial strains alone or combined with *Sv* and/or *Bs* provide insights to how microbes synergistically protect plants. We conclude that plants have evolved to preferentially accommodate communities that support their health and that root-associated prokaryotic and eukaryotic microbes can act synergistically with the plant host in limiting fungal disease.

Mahdi, L.K., Miyauchi, S., Uhlmann, C. *et al.* The fungal root endophyte *Serendipita vermifera* displays inter-kingdom synergistic beneficial effects with the microbiota in *Arabidopsis thaliana* and barley. *ISME J* (2021). https://doi.org/10.1038/s41396-021-01138-y

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eP053
Cell death regulation and function in plant-fungal symbiosis
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Intracellular colonization of plant roots by the beneficial fungal endophyte ***Serendipita indica*** follows a biphasic strategy. After an early biotrophic phase the interaction switches to a **host cell death** phase restricted to the root epidermis and cortex layer. This host cell death is required for fungal accommodation and the establishment of a long-lasting beneficial interaction in barley and Arabidopsis. However, how this cell death is activated and regulated is unknown. Here we show that two fungal enzymes, the ecto-5-nucleotidase ***Si*E5NT** and the nuclease ***Si*NucA** act synergistically in the apoplast at the onset of cell death to produce deoxyadenosine (**dAdo**), a potent cell death inducer in animal systems. Uptake of extracellular dAdo, but not of the structurally related adenosine (Ado), activates a previously undescribed cell death mechanism in Arabidopsis and several other plants. Mutation of the root-expressed *A. thaliana* Equilibrative Nucleoside Transporter **ENT3** confers resistance to extracellular dAdo-induced cell death and leads to decreased fungal colonization at the onset of the cell death phase. Taken together, by using transcriptomics, metabolomics and suited readouts for fungal- and chemical-elicited plant cell death we show that an apoplastic metabolite produced by the combined activity of two secreted fungal enzymes is sufficient to trigger cell death in different host species.

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eP054
Towards a self-healing hydrogen fuel cell_1: Suitable enzymes
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Polymer electrolyte fuel cells (PEM-FCs) convert chemical energy from hydrogen oxidation to electrical energy. Due to their light weight and compactness as well as their low operating temperature (60-85°C), PEM-FCs are most suited for transportation applications and portable electronics.
Frequent start-up cycles – often given in applications in passenger cars – lead to pinholes in the proton-conducting polymer membranes (mostly Nafion TM). Since there is no possibility to repair this type of defect when the fuel cell is already assembled (besides the disassembly of the fuel cell, which is highly inefficient economically), the lifetime and performance of the fuel cell stack is continuously reduced.
The project "BioHealing" (funded by the German State of Baden-Württemberg) pursues the objective of creating a biohybrid system that uses nature"s mechanism of self-healing to selectively plug pinholes in the membrane of fuel cells. This is enabled by a condensation reaction catalyzed by hydrolytic enzymes which are functionally immobilized on the polymer membrane.
In this study, different enzymes are tested for their ability to catalyze the synthesis of suitable polymers to plug the pinholes as well as to withstand the harsh reaction conditions in a PEM-FC at elevated temperatures and acidic pH values.

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eP055
Towards a self-healing hydrogen fuel cell_2: Immobilization techniques
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Polymer electrolyte fuel cells (PEM-FCs) convert chemical energy from hydrogen oxidation to electrical energy. Due to their light weight and compactness as well as their low operating temperature (60-85°C), PEM-FCs are most suited for transportation applications and portable electronics.

Frequent start-up cycles – often given in applications in passenger cars – lead to pinholes in the proton-conducting polymer membranes (mostly NafionTM). Since there is no possibility to repair this type of defect when the fuel cell is already assembled (besides the disassembly of the fuel cell, which is highly inefficient economically), the lifetime and performance of the fuel cell stack is continuously reduced.

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In this study, different immobilization techniques are tested to functionally attach suitable enzymes to the NafionTM polymer membrane using high density peptide arrays.

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eP057

Laterite bioleaching for the recovery of nickel and cobalt in Brazil

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Metals like Ni and Co are of commercial importance, both are e.g. used in batteries, with the projected demand for Ni in electrical vehicle battery manufacturing outpacing the Li demand by 2030. Laterite ores, mainly found in tropical countries, contain approximately 70% of the worlds Ni and Co, as well as other critical raw materials. However, only about 40% of the current production is derived from these ores. Industrially used methods for the recovery of Ni and Co generally include high energy and/or reagent costs, and associated expensive equipment cost. Considering economic efficiency and environmental impact, an integrated low-energy and environmentally benign biohydrometallurgical process for the recovery of these metals from oxidized laterites in Brazil is developed. The bioleaching of limonite will be developed based on the published Ferredox concept using microorganisms to reduce the insoluble metal compound to a water-soluble form at low pH. Suitable acidophilic bacteria able to reduce ferric iron include chemolithotrophic *Acidithiobacillus* spp., mixotrophic *Sulfobacillus* spp., and obligate heterotrophic *Acidiphilium* spp.. Many of these organisms are able to use S as electron donor and couple the oxidation of S to the reduction of ferric iron, thereby generating sulfuric acid and the acidic milieu that is needed to keep iron soluble. Bioreactor experiments in laboratory scale are used to optimize parameters including pH, temperature and aeration for the recovery of Ni and Co. In addition, a bacterial consortium most suitable for the process will be investigated. Eventually, the process will be upscaled and reach pilot stage, leading to an increase in the recovery of metals for existing mines, transforming unexploited ores and limonite stockpiles into valuable resources, whereby new reserves of raw materials are unlocked.

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eP058

High- throughput screening and real-time CO2 production monitoring devices for isolation ethanol production microorganisms

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Bioethanol"s importance as a renewable energy source led to developing of new devices to involve potential screening strains, monitoring ethanol production, and process optimization. As carbon dioxide is a metabolite that is produced together with ethanol production in fermentation. Therefore, measuring carbon dioxide (CO2) could achieve ethanol concentration. Here a novel and rapid high-throughput screening (HTS) strategy is introduced to identify high ethanol producer strains for industrial purposes. The ethanol- HTS (E-HTS) method was established in a microplate format to isolate fast and precise bioethanol-producing microorganisms. It is based on using a reagent that contains bromothymol blue as pH indicator dye and a 3D-printed mold to cast special silicone lid that conduct and isolate two wells in plate. The color was converted from blue to yellow by transforming produced CO2 in fermentation well toward reagent well. Moreover, we develop a mini fermentor system with monitoring the bioethanol production process is applied by a CO2 flow-meter (CFM) device. The CFM device is a valuable tool for real-time detecting ethanol production concentration and surveying the impact of a different component during fermentation on a laboratory scale. Furthermore, the data provided by CFM helps determine the stopping time during ethanol production. It contains four chambers to simultaneously apply different fermentation processes. This device could develop to monitor the ethanol production process on an industrial scale via CO2 measurement, which avoids any risk during fermentation and batch-to-batch variation.

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eP059

Medium-throughput screening of a production library for sustainable Perspex biosynthesis

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3,000 kilotons of Perspex, or methyl methacrylate (MMA), are made every year. Of this huge market, 96% is manufactured from 6.7 million tonnes of acetone annually. An alternative is production of MMA using consolidated bioprocessing from a lignocellulosic carbon source. Bioprocessing can occur under mild, aqueous conditions, relies on cheap and sustainable carbon sources. Heterologous gene expression from microorganisms has been successfully implemented to produce low concentrations of MMA precursors (MMPs), which can be chemically converted into MMA indistinguishable from its petrochemical-based counterpart.

Carbon flux analysis has demonstrated that product inhibition and enzymatic promiscuity prevent significant MMP formation from a biocatalyst during the final two steps in MMP biosynthesis, catalysed by an oxidase (OX) and a transferase (TFS) respectively. *In vitro* oxygen electrode assay demonstrated that OX is inhibited by its product at µM concentrations several hundred fold lower than the K_M of TFS. While little to no connection has been found between the structure of TFS and its specificity towards its "preferred" substrate *vs.* those from the MMP pathway.

Using bioinformatics and Golden Gate assembly, we created a combinatorial library of alternative OX and TFS enzymes to catalyse the final stages in MMP formation. In this work, we interrogate the enzyme library using a medium-throughput fluorescence screen. We are able to quantitatively identify hits from this library that demonstrate improved MMP production, and validate these hits in flask fermentation. The future development of a successful biocatalyst for MMP production will allow the methyl methacrylate industry to begin a shift towards sustainable and increasingly environmentally conscious production methods.

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eP061

Engineering *Corynebacterium glutamicum* for the production of itaconic acid from acetate

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The shift from a petroleum-based to a climate-neutral and sustainable way of life is one of the major challenges for the future. Industrial biotechnology represents an important pillar for this transition and aims to convert biogenic, preferably non-food resources, into chemicals and fuels. Acetate is one promising substrate for this purpose, as it can be derived from biomass and/or synthesis gases by various methods (Reviewed in [1]). For the biotechnological conversion of acetate, *C. glutamicum* is ideally suited as production host, as it tolerates high concentrations and is able to effectively uptake and metabolize acetate [2]. In this study, we engineered *C. glutamicum* for the production of itaconate from acetate. Itaconic acid is an unsaturated dicarboxylic acid of industrial and medical interest that can replace petroleum-derived polymer production. For this purpose, *C. glutamicum* ATCC13032 was transformed with a pEKEx2 plasmid encoding a fusion construct of maltose-binding protein (*malE*) and cis-aconitate decarboxylase (*cad*) derived from *E. coli* and *A. terreus*, respectively [3]. All strains were screened in small-scale 1-mL CGXII cultures containing 20 g acetate L^{-1} using the BioLector® instrument, for 72 h. By reducing the amount of nitrogen in the production medium to a C:N ratio of 20:1, a yield of 7 ± 2 mmol mol^{-1} was obtained. Reduction of isocitrate dehydrogenase activity increased the yield up to 52 ± 4 mmol mol^{-1} with a final titer of 1.86 ± 0.14 g L^{-1}. Deletion of the regulator of acetate metabolism RamB in the wild type background increased the yield to 33 ± 2 mmol mol^{-1} with a final titer of 1.43 ± 0.11 g L^{-1}. Additional deletion of glutamate dehydrogenase in the latter strain doubled the yield to 63 ± 8 mmol mol^{-1} with a final titer of 2.52 ± 0.55 g L^{-1}. With this we achieved 12.5 % higher yields and 300 % higher titers compared to similar cultivations performed with engineered *E. coli* [4].

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eP062

Immobilization protects enzymes from inactivation in plasma-driven biocatalysis

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Peroxygenases, which carry out one-electron oxidation reactions and stereoselective oxyfunctionalizations, are a promising enzyme class for biocatalysis. Their industrial application thus far is limited since they require H_2O_2 as the driving substrate while an excess of H_2O_2 also leads to their inactivation. We previously reported on a novel non-invasive approach for in situ H_2O_2 management by production using non-thermal atmospheric pressure plasma [1]. Still, in plasma-driven biocatalysis enzyme stability was identified as one of the main limitations. Besides hydrogen peroxide, plasma generates various reactive oxygen and nitrogen species (ROS/RNS) leading to enzyme inactivation [1,2]. Exploration of several protection strategies revealed immobilization as the most promising one [3]. Thus, we set out to test whether immobilization is suitable to generally protect proteins from plasma-mediated inactivation. Plasma stability of a panel of six different enzymes (including the promising biocatalyst unspecific peroxygenase from *Agrocybe aegerita Aae*UPO) immobilized on six different carrier materials was tested using suitable photometric activity assays. We found immobilization to protect all tested enzymes, with the combination of covalent immobilization on a hydrophobic carrier surface presenting the highest level of plasma protection. Promising residual enzyme activities were observed after prolonged treatment times of one hour, while free enzymes were completely inactivated after 10 min.

We conclude that immobilization can be considered a universal strategy for enzyme protection under plasma-operating conditions.

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eP063

Application of the fluorescent reporter proteins greenFAST and redFAST for multicolor approaches at single cell level in the anaerobic solventogen *Clostridium saccharoperbutylacetonicum*

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Introduction: Solventogenic clostridia such as *C. saccharoperbutylacetonicum* naturally produce industrially relevant alcohols since they perform an acetone-butanol-ethanol fermentation. Moreover, *C. saccharoperbutylacetonicum* is genetically accessible, and several molecular tools are available. However, the usage of fluorescent reporter proteins is still limited in most clostridia since commonly used reporters such as GFP and its derivatives need oxygen for chromophore maturation. A promising alternative is the fluorescence-activating and absorption-shifting tag (FAST), which was established for several anaerobes and caused bright fluorescence. We established the two green- and red-fluorescent reporters greenFAST and redFAST as multicolor-fluorescent reporters for different applications in *C. saccharoperbutylacetonicum.*

Objectives: In this study, greenFAST and redFAST were applied in synthetic *C. saccharoperbutylacetonicum* co-cultures to track dynamics of both metabolically engineered strains during growth. Moreover, we established a tightly regulated inducible two-plasmid system based on the P_{tcdB}-*tcdR* promoter system and used greenFAST and redFAST to track coexistence and interaction of both plasmids under anaerobic conditions.

Materials and Methods: The metabolically engineered *C. saccharoperbutylacetonicum* strains *Cspa*-greenFAST and *Cspa*-redFAST were constructed and used to inoculate a

synthetic co-culture. In addition, the strain *Cspa* [P_{thlA}_redFAST-P_{bgaL}_tcdR][P_{tcdB}_greenFAST] was constructed harboring two plasmids able to produce both reporter proteins. Fluorescence of cells was determined at single cell level using flow cytometry.

Results: The synthetic *Cspa*-greenFAST-*Cspa*-redFAST co-culture showed bright green- and red-fluorescence and a constant strain-to-strain ratio during growth. *Cspa* [P_{thlA}_redFAST-P_{bgaL}_tcdR][P_{tcdB}_greenFAST] constitutively produced redFAST controlled by P_{thlA}, which resulted in red fluorescence. Only when TcdR gene expression controlled by P_{bgaL} was lactose-induced, cells showed bright green fluorescence as well.

Conclusion: The establishment of greenFAST and redFAST extended the genetic toolbox of *C. saccharoperbutylacetonicum* and opens the door for further multicolor approaches to investigate cell dynamics, gene expression, or protein localization.

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eP064

Identification of L-histidine-producing *Corynebacterium glutamicum* strains via biosensor-based high-throughput screenings and comparative genome analysis

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Keywords: Biosensor, FACS, high-throughput screening, genome sequencing, comparative genome analysis, strain engineering, *Corynebacterium glutamicum*

Classical strain development strongly relied on "random" approaches, typically based on the random generation of genetic diversity and subsequent screening for the desired production phenotype. However, with growing knowledge of the cellular metabolism and its regulation, less laborious "rational" metabolic engineering approaches were more favored in recent decades. Nevertheless, with the availability of biosensor-based FACS-screening strategies, random approaches are coming back into fashion.

In this study, we set out to use biosensors in combination with FACS to identify novel mutations contributing to product formation in the genome of an existing *Corynebacterium glutamicum* L-histidine producer. This strain was already rationally engineered, making identification of novel and beneficial genomic mutations challenging, as they might not be directly linked to L-histidine biosynthesis.

With the aim to generate a large set of 100 improved variants for comparative genome analyses and to avoid the isolation of genetically identical sister clones, we performed each step more than 100 times: Chemical mutagenesis, biosensor-based FACS-screening and strain characterization (cultivation, product analysis). In this manner we performed > 200 FACS-screenings, isolated > 50,000 variants with increased fluorescence, and characterized > 4,500 variants with regard to biomass formation and L-histidine production. Based on a comparative and combinatorial genome analysis of 100 variants accumulating 10-80 % more L-histidine, we selected more than 30 mutations for further analyses. Currently, we perform reverse engineering to assess the contribution of individual mutations (and selected combinations) to the production phenotype.

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eP065

Hexanoate production with metabolically engineered *Acetobacterium woodii* strains using hydrogen and C1-carbon sources

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Introduction: Hexanoate is a platform chemical used for the production of e.g. food additives. Currently, hexanoate is mainly produced petrochemically. However, some anaerobic acetogenic bacteria such as *Clostridium carboxidivorans* or *C. drakei* are naturally capable of producing hexanoate from C1-carbon sources via reverse β-oxidation. The enzymes of the respective pathway are encoded in the *hcs* (hexanoyl-CoA synthesis)-operon including Crt (crotonase), Hbd (3-hydroxybutyryl-dehydrogenase), Thl (thiolase), Bcd (butyryl-CoA dehydrogenase) as well as EtfB and EtfA (electron-transferring flavoproteins B and A). Thus, the corresponding genes of the *hcs*-operon in combination with the greenFAST (fluorescence-activating and absorption-shifting tag) encoding gene (*fegG*) were recombinantly expressed in *Acetobacterium woodii* and resulting recombinant strains used to produce hexanoate autotrophically.

Objectives: The aim of this study is recombinant hexanoate production using metabolically engineered *A. woodii* strains, by performing batch experiments with different C1 carbon sources and H_2 as energy source. Moreover, *fegG* expression and hexanoate production strains are used to elucidate population dynamics and heterogeneity of cell cultures.

Material and methods: The *fegG gene* was cloned downstream of the *hcs*-operon under the control of either the lactose-inducible promoter P*bgaL* or the constitutive promoter $P_{pta\text{-}ack}$ resulting in plasmids pMTL83251_P_{bgaL}_hcs_greenFAST and pMTL83251_$P_{pta\text{-}ack}$_hcs_greenFAST. *A. woodii* was electroporated using the respective plasmids and the resulting recombinant strains were characterized in growth experiments using CO_2, formate, or methanol in combination with H2. During growth, the greenFAST mediated fluorescence was determined via spectral photometry and flow cytometry.

Results and conclusion: Growth experiments were performed using the recombinant strains *A. woodii* [pMTL83251_P_{bgaL}_hcs_greenFAST] and *A. woodii* [pMTL83251_$P_{pta\text{-}ack}$_hcs_greenFAST], and hexanoate was produced from the substrates fructose as well as CO_2, formate, or methanol in combination with H_2. Green fluorescence indicated the expression of the *hcs*-operon and the subpopulations were quantified. *A. woodii* is an ideal model acetogen for production of higher value biocommodities or fuels from a variety of C1 substrates.

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eP066

The Role of Microbial Mats in the Removal of Hexavalent Chromium and Associated Shifts in Their Bacterial Community Composition

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Question: Microbial mats are rarely reported for chromium-polluted ecosystems, hence information on their bacterial diversity and role in chromium removal are very scarce. This study was undertaken to investigate the ability of chromium-contaminated microbial mats from excavation quarry sumps to remove Cr(VI) and to reveal the composition of their

bacterial communities. The main question of this research is are microbial mats able to remove Cr(VI)?, is this done aerobically or/and anaerobically? And what is the underlying mechanism of Cr(VI) removal by microbial mats?

Methods: We investigated the role of nine microbial mats, collected from three quarry sumps of chromium mining sites, in the removal of hexavalent chromium (Cr(VI)). Bacterial diversity in these mats and community shifts after incubation with Cr(VI) have been investigated using MiSeq sequencing.

Results: In nature, a chromium content of 1,911±100 mg kg-1 was measured in the microbial mats, constituting the third highest source of environmentally available chromium. The mats were able to remove 1 mg l-1 of Cr(VI) in 7 days under aerobic and anaerobic conditions. MiSeq sequencing of the original mats yielded 46-99% of the sequences affiliated to Proteobacteria, Firmicutes and Actinobacteria. When the mats were incubated with Cr(VI), the bacterial community shifted in the favor of Alphaproteobacteria and Verrucomicrobiae. We conclude that microbial mats in the quarry sumps harbor diverse microorganisms with the ability to remove toxic Cr(VI), hence these mats can be potentially used to remove chromium from polluted waters.

Conclusions: We conclude that the microbial mats in Nakhl and Samail quarry sumps were able to decrease Cr(VI) concentration under aerobic conditions and, thus, playing a vital role in chromium removal. These mats harbored diverse microbial communities, with the dominance of the bacterial classes Proteobacteria, Firmicutes, Actinobacteria and Verrucomicrobiae and these communities shifted in the favor of Alphaproteobacteria and Verrucomicrobiae when incubated in the presence of Cr(VI).. Further research should attempt isolating aerobic Cr(VI)-reducing bacteria from these mats.

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eP067

Expanded Toolbox for Convenient High-Throughput Identification and Characterization of Antimicrobial Peptides

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Food-spoiling organisms and opportunistic pathogens such as *Listeria monocytogenes* represent a major threat in food industry. One approach to prevent contamination of food with these organisms are antimicrobial peptides (AMPs) secreted by many lactic acid bacteria (LAB) to inhibit competitors in their natural environment. Many AMPs inhibit their target organism by formation of pores in the cytoplasmic membrane while the producer is protected by expression of immunity proteins. AMPs or AMP producer strains are thus widely used as food preservatives. Moreover, AMPs represent a potential alternative to antibiotics that may help to overcome the global problems with emerging antibiotic resistances.

Identification of novel AMPs and their producers using classical approaches is a tedious procedure. We thus have developed an integrated pipeline for identification and characterization of antimicrobials. A previously described assay for detection of membrane damage in *L. monocytogenes* using the ratiometric pH-sensitive fluorescent protein pHluorin (Crauwels *et al.*, 2018) was transferred to the BSL1 organism *L. innocua*. Sensitivity of the assay was increased by using pHluorin2, a variant with enhanced fluorescence intensity, and optimized assay conditions. Moreover, an protocol for high-throughput screening of supernatants of potential AMP producers was developed. Also, we expanded the assay by integrating growth measurements to allow detection of AMPs that inhibit growth of *L. inncua* without pore formation. The final assay was used to screen supernatants of a collection of 400 LAB strains and allowed identification of about 30 potential new AMP producer strains.

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eP068

Low-biomass concept for industrial biotechnology with engineered *Vibrio natriegens*

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In recent years, *Vibrio natriegens* gained attention in biotechnology due to its very fast growth and associated high specific substrate uptake rate (q_S) [1]. To reach a high volumetric productivity (Q_P) most industrial production processes must operate at a high final biomass concentration, wasting significant amounts of carbon for biocatalyst generation. Therefore, the choice of future production processes will be low-biomass processes with non-growing but metabolically highly active catalysts.

To achieve this, we deleted the *aceE* gene encoding the E1 subunit of the pyruvate dehydrogenase complex (PDHC) in the prophage-free strain *V. natriegens* Δ*vnp12* [2]. This PDHC-deficient strain is acetate auxotroph with a decoupled catabolite repression, allowing the simultaneous consumption of glucose and acetate. Moreover, the biomass formation can be adjusted by the initial amount of acetate, splitting the whole process into a growth and production phase in which pyruvate is secreted into the medium. Already during growth on 7.5 g glucose L^{-1} and 1 g acetate L^{-1} in shaking flasks, up to 4.0 ± 0.3 g pyruvate L^{-1} were excreted. Therefore, a fed batch fermentation divided into a growth and production phase was performed. To supply the cell´s energy demand in the non-growth production phase a constant acetate feed of 8 mM h^{-1} was applied. With a total time of 11 h and a final biomass concentration of only 3.8 g_X L^{-1}, 28.6 g pyruvate L^{-1} were produced with a yield of 0.4 g pyruvate g^{-1} glucose and a Q_P of 2.6 g pyruvate L^{-1} h^{-1}, which are already in the range of other reported processes (Reviewed in [3]). Furthermore, the non-growing cells showed a high q_S value of 2.7 g glucose g_X^{-1} h^{-1}, which is 45 times more than the reported maintenance glucose requirement [4].This combination of productivity with volumetric production per biomass and time shows the potential for cost-efficient productions with *V. natriegens*.

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eP069

Fatty alcohol production in *Corynebacterium glutamicum*

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Fatty acid (FA)-derived products such fatty alcohols (FAL) find growing application in cosmetic products, lubricants or biofuels. As the biosynthetic pathways of FA and FAL synthesis are similar until they diverge at the acyl-CoA node, a *Corynebacterium glutamicum* ATCC 13032-derived strain with deregulated fatty acid synthesis was constructed in this study for the microbial production of FAL from glucose. In that pursuit two fatty acyl-CoA reductases (Maqu_2220 and Maqu_2507) of *Marinobacter hydrocarbonoclasticus* VT8 were used.

All strains were cultivated under nitrogen-limiting conditions with 20 g glucose L-1 as carbon source and 1.45 g urea L-1 as sole nitrogen source. FA and FAL samples were analyzed on a GC-FID (Agilent 8890 GC System) equipped with a DB-FATWAX UI (30 m x 250 µm x 0.25 µm) and quantified using authentic FA and FAL standards.

Plasmid-based expression of the two reductases led to a reduction in fatty acid synthesis by over 50 % in the strains harboring pEKEx2_*maqu_2220* or pEKEx2_*maqu_2507* compared to the control strain *C. glutamicum* Δ*fasR* pEKEx2. Simultaneously, FAL production was observed solely in both reductase-harboring strains, reaching titers of 482 ± 27 mg FAL L-1 and 356 ± 67 mg FAL L-1 for the *maqu_2220* and the *maqu_2507*-expressing strain, respectively. The observed product distribution, which was in agreement with previously reported fatty acid distribution of *C. glutamicum*, appeared to be independent of the expressed reductase. This observation indicated that both reductases do not have a bias towards one of the three available acyl-CoA substrates making both suitable candidates for FAL production in *C. glutamicum*. Subsequently, the best-performing reductase Maqu_2220 was genomically integrated into the landing pad CgLP11 (Lange et al., 2018), resulting in the plasmid-free strain *C. glutamicum* Δ*fasR* CgLP11::(P*tac-maqu_2220*-T*rrnB*). However, this strain solely produced 31 ± 2 mg FAL L-1 of cell culture, indicating that a high expression level of *maqu_2220* is essential for efficient FAL production. To our knowledge this is the first report of FAL production in *C. glutamicum* and will serve as a basis for further metabolic engineering in that context.

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eP070

Modular specialized enzyme for biomass degradation from *Thermoclostridium stercorarium*– combination of modules creates thermostable enzyme that synergistically acts on arabinoxylan

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1. Introduction. *Ts*_Axh43A, secreted from *Thermoclostridium stercorarium,* exhibits rare Axh-md2,3 activity: it is able to cleave O2-, or O3-bound arabinoses from xyloses of the backbone in arabinoxylan (AX), that are either mono-substituted, or double-substituted. The multimodular enzyme contains four domains named GH43_10 (c1), GH43_C2 (link), GH43_16 (c2) and CBM6 (cbm).
2. Objectives. The aim of this study was to determine if similar catalytic domains in *Ts*_Axh43A have the same or different specificities when produced individually. In addition, the function of the non-catalytic domains were examined.
3. Materials & Methods. Variants from *Ts*_Axh43A were produced in *E. coli*, purified by His6-affinity chromatography and incubated with different substrates (*p*NP-α-l-arabinofuranoside, AX, arabinoxylooligosaccharides/AXOS). The hydrolysis products were analyzed via High Performance Anion Exchange Chromatography and Pulsed Amperometric Detection (HPAEC-PAD) to determine activity and specificity. Differential scanning fluorimetry (DSF) was performed to determine melting temperatures and stability.
4. Results. The full-length *Ts*_Axh43A exhibits *p*NPAse, as well as Axh-md2,3 activity at its Topt = 60°C on all tested AXOS. The analysis after incubation with AX and subsequent de-polymerization using a GH11 xylanase implies Axh-md2,3 activity on the polysaccharide. The combination of the first two domains (c1link) only cleaved arabinose from double-substituted xylose residues, whereas c2cbm only de-arabinosylated mono-substituted AXOS. Degradation products generated from AX also confirmed these Axh-d and Axh-m specificities acting on the polysaccharide. DSF revealed instability in terms of reduced melting temperatures for c1, c2, c2link and one module of c1linkc2, likely the c2 domain. Moreover, the individual catalytic domains c1 and c2 showed no activity against polysaccharides and only minor activity on AXOS at lower temperatures.
5. Conclusion. The 135 kDa *Ts*_Axh43A comprises two modules, which exhibit individual specificities against AX and its derived oligosaccharides. Both modules are thermostable and adequately active when fused to their original c-terminal domain (c1link, c2cbm). Evolutionarily, the combination of two specificities in one enzyme is reasonable and applicable in rational design of enzymes for saccharification of agricultural wastes.

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eP071

Characterization of the cellulose synthesis genes of two *Komagataeibacter hansenii* strains

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Bacterial cellulose (BC) overcomes some biotechnological limitations of plant cellulose, as the microcrystalline cellulose is free from non-cellulosic polymers like lignin and hemicellulose.

We sought to resolve the contribution of each of three cellulose synthase (CS) genes that are encoded by *K. hansenii* ATCC 23769 and *K. hansenii* ATCC 53582. Although both strains have an analogous organization of the regions of CS operons, the second strain produces much higher amounts of cellulose.

Markerless in-frame deletions of combinations of the CSs *bcsAB1*, *bcsAB2* and *bcsAB3* as well as other contributing genes in both strains were constructed using a *codBA* counter selection system developed in our lab. The strains were characterized by studying their phenotypes, by quantification of the cellulose, by electron microscopy, by assessing the transcription of CS genes as well as by measuring the protein expression by proteomic analysis. Transcription was determined for *K. hansenii* ATCC 23769 and *K. hansenii*ATCC 53582 by absolute quantification of gene expression using qRT-PCR. The proteome was characterized by measuring relative abundances of the CSs

and its associated proteins using label-free quantitative proteomics.

The resulting data revealed that BcsAB1 is the most abundant and most important CS for synthesis of BC, because BcsAB1 was identified to be responsible for synthesis of microcrystalline fibers forming the main portion of BC. Expression of *bcsAB2* is clearly reduced as compared to *bcsAB1* in both wild type strains. BcsAB2 seems to be responsible for the production of a non-fibrous EPS (nfEPS) in *K. hansenii* ATCC 23769, whereas its product could not be visualized in *K. hansenii* ATCC 53582. Interestingly, the expression of *bcsAB3*, the CS encoded in the least complex gene cluster, is strongly reduced and the protein was not identified in the proteome.

In summary, BcsAB1 contributes most to biofilm formation in two cellulose-producing *K. hansenii* strains by synthesis of microcrystalline cellulose fibers. BcsAB2 forms a smaller amount of cellulose that seems to modify the network, possibly for prevention of drought stress or for alteration of the mechanical properties. The function of BcsAB3 remains unclear under the cultivation conditions of this study, despite transcription, no corresponding protein could be detected.

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eP072
Identification of novel glycosyltransferases for biotechnology
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Introduction: Glycosylation is a ubiquitous trait to introduce posttranslational modification in nature of proteins and other molecules. It can be found in all domains of life and is mainly performed by glycosyltransferases (GTases). These enzymes catalyze the transfer of sugar moieties from activated donor (aglycone) to specific acceptor molecules. The products of glycosylation are named glycosides and the glycosidic linkages can be oxygen-, nitrogen-, sulfur- or carbon-based. Thereby glycosylation can affect their acceptor molecules and increase bioavailability, water solubility and induce new bioactivity. Therefore, GTS are valuable tools with a broad application in biotechnology.

Objectives: Currently only very few active GTs are known that are involved in modifying phenolic compounds and increasing their solubility. This study aims to enrich the diversity of phenol-active GTases and establish a large collection (GTAse Tool box) for biotechnology.

Materials and Methods: In order to find new glycosyltransferases, we choose two different approaches. A sequence-base screening and a function-base screening. For the sequence base screening we created a Hidden Markov Model and used for the search the UniProt-Database and the non-redundant Database of the NCBI. The function-base screening based on the method, which was developed in our working group by Rabausch *et al.*, and was used for fosmid libraries from environmental samples [1]. As screening method a biotransformation approach and using thin layer chromatography for detection were used.

Results and Outlook: As a first step we designed a Hidden Markov model specific for GTases. Using this we identified and partially characterized 5 new glycosyltransferases by the sequence-based screening and in addition 3 promising candidates by the function-based screening. The proteins were synthesized and or amplified from metagenomes and genomes using PCR. These amplified genes were cloned and transformed into different expression systems. Subsequently the expressed proteins were functionally tested and their activity was verified by TLC analyses. Continuing characterization and analysis of the glycosyltransferases are in process.

[1] Rabausch, U., Juergensen, J., Ilmberger, N., Bohnke, S., Fischer, S., Schubach, B., Schulte, M., Streit, W.R., 2013. Functional screening of metagenome and genome libraries for detection of novel flavonoid-modifying enzymes. Appl. Environ. Microbiol. 79, 4551–4563.

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eP073
Metabolic engineering of *Corynebacterium glutamicum* for the production of anthranilate
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Anthranilate and its most important derivative aniline are important basic chemicals used for the synthesis of various compounds with many applications in the chemical, pharmaceutical and food industries. Today, aniline production is not sustainable as it is mainly chemically synthesized from petroleum-derived benzene. However, aniline can also be obtained by decarboxylation of microbially produced anthranilate, which represents a more sustainable option.

In this study, a *Corynebacterium glutamicum* strain was engineered for the microbial production of anthranilate from a carbon source mixture of glucose and xylose. In order to enable the accumulation of anthranilate, a feedback-resistant variant of DAHP synthase from *E. coli* was functionally introduced into *C. glutamicum*. Additionally, xylose utilization was achieved by employing the isomerase pathway, which increased the anthranilate titer manifold. Any formation of the by-products glycerol and quinate was prevented by the deletion of *nagD* and *qsuD* to further improve product formation. Genomic introduction of a feedback-resistant anthranilate synthase (ANS-S38R) resulted in an increased formation of anthranilate. Finally, the carbon flux through the shikimate pathway was improved by a start codon exchange of *aroK* (GTG to ATG) encoding shikimate kinase. The final strain synthesized up to 3.1 g/L (22.4 mM) anthranilate in defined CGXII medium from a mixture of glucose and xylose.

We believe that the constructed *C. glutamicum* strain also represents a robust host for the synthesis of other shikimate pathway intermediates or any aromatic compound derived thereof.

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eP074
Characterization of dihydropyrimidinases for the biocatalytic synthesis of chiral beta amino acid.
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Chiral beta amino acids are important components of pharmaceutical drugs, peptide mimetics and fine chemicals (Rudat, Brucher, & Syldatk, 2012). The so-called *hydantoinase process* is already industrially used for the production of chiral alpha amino acids (Engel, U., Rudat J., & Syldatk C., 2014). Applying a cascade of up to three enzymes (a hydantoinase, a carbamoylase and a hydantoinase racemase) a racemic 5'-monosubstituted hydantoin is converted to a chiral alpha amino acid. The aim of this project is to produce chiral beta amino acids using *a*

modified hydantoinase process starting from 6'-substituted dihydropyrimidines.

It was already demonstrated in previous work that hydantoinases/dihydropyrimidinases can hydrolyze racemic 6'-substituted dihydropyrimidines to the corresponding *N*-carbamoylated beta-amino acids (Engel, Syldatk *et al.* 2012). Based on this result, several enzymes are examined and compared for their ability to stereoselectively hydrolyze hydantoin and dihydropyrimidine derivatives. Furthermore, optimal reaction conditions (cofactor, temperature- and pH-optima) are investigated.

The dihydropyrimidinase showing optimal performance is to be combined with a suitable carbamoylase to establish an enzyme cascade (modified hydantoinase process) and produce chiral beta amino acids.

Rudat, J., Brucher, B.R. & Syldatk, C., *AMB Express*, **2**(1), (2012) p.11.

Engel, U., Rudat J., & Syldatk C., *Industrial biocatalysis. Singapore: Pan Stanford Publishing Pte. Ltd* (2014).

Engel, U., Syldatk, C. & Rudat, J., *Appl. Microbiol. Biotechnol.,* **94**(5), (2012), 1221-1231.

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eP075

Enzymatic synthesis of non-canonical amino acids by carbamoylases

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Non-canonical amino acids are important building blocks for bioactive peptides and pharmaceuticals [1]. By using dihydropyrimidine derivatives as substrate instead of the hydantoin derivatives, the hydantoinase process was modified to establish a universal biocatalytic synthetic pathway for chiral beta-amino acids [2]. The aim of this project was the investigation of carbamoylases responsible for the second process step, producing beta-amino acids from N-carbamoyl amino acids.

For this reason, several carbamoylases were evaluated. The enzymes were recombinantly expressed and an optimized MBP-tag purification procedure was established. The purified enzymes were studied regarding stability, temperature- and pH optima, and the suitable cofactors were determined using N-carbamoyl beta-alanine as a model substrate.

The main focus were the individual substrate spectra, particularly regarding the conversion of a wide variety of N-carbamoyl amino acids, especially N-carbamoyl beta-homo amino acids. Therefore substrate specificity and stereoselectivity were investigated for alpha- and beta-N-carbamoyl amino acids.

The carbamoylase showing optimal performance is to be combined with a suitable hydantoinase to establish an enzyme cascade (modified hydantoinase process) and produce chiral beta-amino acids.

[1] Rudat, J., Brucher, B.R. & Syldatk, C., *AMB Express*, **2**(1), (2012) p.11.

[2] Engel U, Syldatk C, Rudat J. 2012. Stereoselective hydrolysis of aryl-substituted dihydropyrimidines by hydantoinases. *Applied microbiology and biotechnology* **94**(5):1221–1231.

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eP076

Investigating the effect of linker mutagenesis in a fusion protein comprised of an azoreductase and a formate dehydrogenase

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1. Introduction

The combination of different enzymes, interconnected through a peptide linker into a fusion protein, enables the possibility of coupling various enzymatic reactions. The proximity of the enzymes, as well as the mutual substrate or cofactor supply can lead to significantly refined cascades, although the effect is dependent on multiple factors and hard to generalize. Azoreductases catalyse the reduction of azo bonds (N=N) and use NAD(P)H as a co-substrate for the bond cleavage. Since NAD(P)H is quite costly for practical application, implementing a regeneration system through usage of the formate dehydrogenase could be beneficial for the overall catalysis.

2. Objectives

The objectives of this project include the further investigation of using the azoreductase from *Rhodococcus opacus* 1CP together with the formate dehydrogenase from *Candida boidinii* as a fusion protein to possibly untap improvements of the overall reaction through NADH recycling. The linkers between the two enzymes were exchanged with varying properties and length to observe the effect on the combined enzymatic reactions against selected azo dyes.

3. Materials and Methods

The utilized linker constructs were acquired through site-directed mutagenesis of the originating fusion protein. The investigation on the effect of the linker mutagenesis was carried out using spectrophotometer analysis of different parameters, as well as the evaluation of the enzyme activity against Brilliant Black for every used linker variant.

4. Results

The obtained results indicate differences in the solubility and the activity of the different fusion protein constructs. Regarding the degradation of the diazo dye Brilliant Black, some of the observed linker constructs displayed decolorization up to almost 100%, while other variants only showed marginal activity, implying only partial decolorization.

5. Conclusion

Overall, the investigation of the effect of linker mutagenesis on the observed fusion protein showed promising findings and indicated linker variants to be more suitable than others in the observed set of constructs. The evaluation also demonstrated multiple candidates indicating to outperform the originating fusion protein. Additional testing with methods like liquid chromatography-mass spectrometry (LC-MS) may provide a deeper understanding of the overall reaction and assist towards analysing the impact of linkers in the observed fusion protein.

eP077
Adjusting the surface properties of bacterial magnetosomes by the display of artificial peptides
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Magnetic nanoparticles are of increasing interest for a number of (bio)medical applications, for instance as nano-sized drug carriers or as agents for magnetic imaging techniques. The latter usually require nanoparticles with a uniform size and shape as well as stable magnetic moments. However, controlling the particle size distribution, and the synthesis of particles with a uniform crystallinity is still challenging [1]. An attractive alternative might be provided by bacterial magnetosomes, biogenic nanoparticles synthesized by magnetotactic bacteria. In the model organism *Magnetospirillum gryphiswaldense* they consist of a monocrystalline magnetite core enveloped by a biological membrane that consists of phospholipids and a set of magnetosome specific proteins. Due to a strictly genetically regulated biomineralization process, magnetosomes exhibit characteristics that can only hardly be achieved by chemical synthesis [2].

So far, magnetosomes showed only low cytotoxicity when incubated with mammalian cells and thus, were considered to be biocompatible [3]. However, for future *in vivo* applications further toxicity factors have to be evaluated as the particles are of bacterial origin. Thereby, the antigenicity and endotoxicity might play an important role as the magnetite cores are surrounded by a membrane of bacterial lipids and proteins, and are supposed to contain certain amounts of endotoxins acquired unspecifically during particle isolation.

Bacterial magnetosomes are accessible to genetic engineering techniques, enabling a selective and controllable modification of the particle surface. Utilizing highly abundant magnetosome membrane proteins as anchor molecules we expressed peptide arrays consisting of the "shielding" tripeptides GSA and PAS on the magnetosome membrane. In addition, we explored the expression of coupling groups on the particle surface to immobilize serum proteins, thereby generating a protein corona. Both approaches aim to modify the particle surface and to camouflage toxic compounds, which is evaluated in cytotoxicity tests on mammalian cells as well as endotoxicity assays.

Thus, genetic engineering might not only be used to engineer the magnetosome surface properties, but also to improve the biocompatibility for future *in vivo* applications.

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[2] Uebe R., Schüler D. *Nat. Rev. Microbiol.*, 2016, *14*, 621

[3] Rosenfeldt S., et al. *Acta Biomater*. 2021, *120*, 293

eP078
Recombinant nisin production with *Corynebacterium glutamicum* using a two-step process
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The lantibiotic nisin is a bacteriocin naturally produced by *Lactococcus lactis* species. It is synthesized as an inactive prepeptide (prenisin) and posttranslationally modified by dehydration and cyclization, resulting in five characteristic (methyl-)lanthionine rings. Export and processing by proteolytic cleavage of the leader peptide results in release of active nisin[1]. As the receptor of nisin is the universal peptidoglycan precursor lipid II, nisin has a broad target spectrum including food-spoilage bacteria and human pathogens such as *Listeria monocytogenes*[2] or methicillin-resistant *Staphylococcus aureus* (MRSA)[3]. Consequently, nisin is used as food preservative and discussed as alternative to classical antibiotics. Industrial nisin production is currently performed in natural producer strains fermented on complex milk or whey substrates resulting in difficult and expensive downstream processing and low product purity[4].

Production with recombinant strains used in biotechnology and pharmaceutical industry, which are able to grow in defined media, may overcome these drawbacks. We established heterologous nisin production using the biotechnological workhorse organism *Corynebacterium glutamicum* in a two-step process. We successfully demonstrate synthesis and export of fully modified prenisin following expression of a synthetic *nisZBTC* operon. Activation of prenisin to mature nisin was achieved by a purified, soluble nisin protease NisP produced in *Escherichia coli*. Active nisin was detected using a highly sensitive *L. lactis* sensor strain expressing the fluorescent protein mCherry in a strictly nisin-dependent manner. Following standard batch cultivation and activation of prenisin by addition of soluble NisP, at least 1.25 mg/l of active nisin was detected in supernatants of the recombinant *C. glutamicum* producer strain.

The presented two-step process for production of active nisin using *C. glutamicum* may serve as a blueprint for further production processes of other bacteriocins harboring complex posttranslational modifications. Moreover, our results provide a basis for further studies to optimize product yields, transfer production to sustainable substrates and purification to obtain pharmaceutical grade nisin.

1 Lubelski, et al. 2008. *Cell. Mol. Life Sci. 65*, 455–476.

2 Benkerroum, et al. 1988. *Dairy Sci. 71*, 3237–3245.

3 Brumfitt, et al. 2002. *J. Antimicrob. Chemother. 50*, 731–734.

4 Abbasiliasi, et al. 2017. *RSC Adv. 7*, 29395–29420.

eP079
Submerged and solid-state fermentation to obtain keratinolytic enzymes of fungal origin
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The demand for agricultural products leads to the accumulation of organic waste, including those rich in keratin, a hard-to-decompose fibrillar protein. Its content is high in the epidermis and its derivatives such as feathers, which are a major by-product of the poultry industry. The development of biotechnological approaches to the utilization of such waste is necessary for a sustainable economy and the obtaining of products with high added value. Filamentous fungi are one of the most promising producers of extracellular proteases with keratinolytic activity, due to the

developed capacity for secretion and the ability to grow not only under submerged fermentation (SF) conditions, but also under solid-state fermentation (SSF) conditions on animal waste too.

The ability of the new *Aspergillus clavatus* strain to synthesize extracellular keratinases under SF and SSF was studied. Cultivation was carried out in a liquid modified Czapek medium with milled chicken feathers (SF) and on whole chicken feathers with the addition of salts solution (SSF). Proteolytic activity was measured in the culture liquid (or eluate) and with complex preparations of extracellular proteins obtained after salting out using ammonium sulfate. Enzymatic activity was examined with casein solution and alpha-keratin suspension. The formed products amount was measured spectrophotometrically at 275 nm and 280 nm, respectively. One unit (U) of activity was defined as an increase of A275 (or A280) by 0.01 under the assay conditions (37 °C, pH 8.2, 600 rpm). The activity of complex preparations was expressed in specific units per amount of protein measured spectrophotometrically at 280 nm.

The maximum accumulation of *A. clavatus* proteolytic enzymes was reached on the 4th day of cultivation under SF and SSF conditions. The keratinolytic activity (SF – 55.6 U; SSF – 50.9 U) slightly decreased with the growth of the producer on the feathers in comparison with the cultivation in a liquid medium, while the caseinolytic activity (SF – 47.5 U; SSF – 64.6 U) increased. However, the specific activity against keratin of the complex preparation obtained by SSF raised 2.5 times compared with extracellular proteins synthesized using SF, and the activity against casein increased 4 times.

The data obtained indicate not only the potential of the new producer as a source of keratinolytic enzymes for biodegradation, but also the perspective of using SSF to produce industrially significant proteases.

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eP080
Catalyzed Disulfide Bond Formation in *Bacillus subtilis*
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Biotherapeutics and enzymes are a huge worldwide market, and disulphide-bonded proteins are a significant portion of that. While bacteria such as *Bacillus subtilis* are very commonly used workhorses for the secretion of proteins in large industrial quantities, their expression and secretion capacity for complex disulphide-bonded proteins, including antibodies, is limited. Consequently, one important step for constructing a *Bacillus subtilis* strain capable of producing these proteins concerns the modulation of the cells" thiol-disulfide oxidoreductases (TDORs), repsonsible for proper protein oxidation and folding. Also, the integration of recombinant TDORs from yeast and mammalians into the *B. subtilis* cell machinery are tested, as exemplified by the CyDisCo technology in *E. coli.*

In addition, strains from the genome-reduced mini*Bacillus* strainline have shown to represent a production platform of choice for difficult-to-express target proteins in terms of yield and quality. Besides serving industrial requirements as being depleted from prophages, extracellular proteases and genes for sporulation, these minimized strains furthermore simplify transcriptomics, proteomics and data modelling. Applying modern genome engineering, different promoter systems, secretion signal peptides and gene copy numbers are tested for the different TDORs and proteins of interest.

Here, we show first results of the expression of Gaussia luciferase, a model protein with five disulfide bonds used for benchmarking the performance of disulfide bond formation. Thereby, genome-reduced strains do achieve significantly higher titers of active enzyme than wild-type *B. subtilis*.

In conclusion, the overexpression of TDORs shows to be a challenging act. A balanced membrane redox-chemistry is crucial for cell growth and protein secretion, while excessive overexpression of oxidizing TDORs can cause a distorted redox equilibrium leading to cell death.

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eP081
Sequential fermentation of C1-compounds to value-added products
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Introduction

With a growing population, declining resources and global warming, alternative and sustainable methods to produce industrially relevant chemicals are urgently needed. Fermentation of climate-damaging C1 compounds like CO_2 by anaerobic acetogenic bacteria is one such method. However, autotrophically growing acetogens like *Acetobacterium woodii* are known to be energetically limited, rendering the synthesis of higher-value products difficult. This problem could possibly be overcome by applying a two-step fermentation process with acetate production from CO_2 and H_2 by *A. woodii* in a first step and subsequent acetate conversion to higher-value products by an aerobic organism like *Corynebacterium glutamicum* in a second step.

Objective

Objective of this study was to combine an anaerobic fermentation of acetogenic bacteria with an aerobic cultivation of *C. glutamicum* to produce at long last higher-value products from CO_2, methanol or formate in a two-step process.

Methods

Media optimization for *A. woodii* was done to enable efficient formation of acetate from either CO_2 (with H_2) or from methanol or formate. *C. glutamicum* was metabolically engineered to produce 3-hydroxypropionic acid (3-HP) by introduction of a codon-optimized malonyl-CoA reductase from *Chloroflexus aurantiacus* and by exchanging the native promoter of the citrate synthase gene and thus reducing the carbon flux into the tricarboxylic acid cycle. Formation of glutamate and of 3-HP was analyzed by HPLC.

Results

Optimization of *A. woodii* medium resulted in formation of up to 290 mM acetate from CO_2 and H_2, of 102 mM from 200 mM methanol and of 60 mM from 200 mM formate. In the second fermentation step, *C. glutamicum* was used to produce up to 10 mM glutamate from CO_2 (and H_2)-derived acetogenic acetate by induction with Penicillin G. When the spent medium included the *A. woodii* biomass and was autoclaved before inoculation, glutamate concentrations increased to 13 mM. A metabolically engineered *C. glutamicum* strain produced up to 4.8 mM 3-HP from commercial acetate as sole carbon source and about 2 mM 3-HP from biological CO_2-derived acetate.

Conclusion

We here show proof of principle of a sequential fermentation process enabling the synthesis of higher-value products from CO_2 (and H_2), methanol or formate using *A. woodii* and *C. glutamicum* strains. This approach constitutes a promising climate-friendly alternative to established sugar-based fermentations.

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eP082
Genomic modification of non-competent *Bacillus* sp. by using a novel vector
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Natural competence describes the ability of bacteria to take up foreign DNA and its use as nutrient or to compete with other microorganisms (e. g. antibiotic resistance). Additionally, natural competent microorganisms are of big advantage for the use as model organisms under laboratory conditions in terms of simple intention of genome modifications. *B. subtilis* W168 is such a model strain, which belongs to the genus *Bacillus*. Other strongly related *Bacillus* species e. g. *B. subtilis* subsp. *spizizenii* ATCC 6633, *B. thuringiensis* 407, *B. cereus* ATCC 14579 or *B. licheniformis* ATCC 10716 are not naturally competent. Properly DNA implications in such strains can be done so far by conjugation only.

In the last few decades, methods for genetic engineering of non-transformable *Bacillus* sp. strains have improved and became of interest for biotechnological applications such as protein production and biosensor molecule detection.

In this work, we show how the novel conjugation plasmid helps to introduce genetic rearrangements in non-competent *Bacillus* strains. The plasmid contains a tunable origin of replication and thereby the plasmid copy number (PCN) can be controlled. Furthermore, by plating the cells on selective media, the genomic integration of the plasmid can be forced successfully and result in a e. g. markerless deletion of the gene of interest.

Altogether, we established an efficient protocol for genomic modifications in naturally non-competent *Bacillus* sp. that can be further applied to a broad range of biotechnologically relevant strains (e. g. *B. pumilus*) as well as to strains of special interest like *B. anthracis.*

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eP083
BM3-catalyzed testosterone hydroxylation by whole cells is boosted by co-expression of hydrophobic outer membrane pores
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Biocatalytic steroid hydroxylation has been targeted by applying various cytochrome P450 monooxygenases in different biocatalyst formats. However, the implementation of steroid hydroxylation processes at an industrial scale still suffers from low conversion rates. In this study, we selected variants of the self-sufficient cytochrome P450 monooxygenase BM3 from *Bacillus megaterium* for the hydroxylation of testosterone either at the 2β- or 15β-position (Kille *et al.*, 2011). Recombinant *Escherichia coli* cells were used as biocatalysts to provide a protective environment for recombinant enzymes and to ensure continuous cofactor recycling. However, only low initial whole-cell testosterone conversion rates were observed for resting cells. Results obtained with different biocatalyst formats (permeabilized cells, cell-free extracts, whole cells) indicated a limitation in substrate uptake, most likely due to the hydrophilic character of the outer membrane of *E. coli*. Thus, we co-expressed nine genes encoding hydrophobic outer membrane proteins potentially facilitating steroid uptake. Indeed, the application of five candidates led to increased initial testosterone hydroxylation rates. The highest activity of 34 U g_{CDW}^{-1} was obtained for a strain containing the hydrophobic outer membrane protein AlkL and the BM3 variant KSA14m. Overall, we show that the straightforward application of hydrophobic outer membrane pores can boost whole-cell steroid conversion rates and thus be game-changing with regard to industrial steroid production efficiency.

References:

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eP084
Examination of substrate specificity of a novel CYP 153A from *Gordonia rubripertincta* CWB2
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Cytochrome P450 monooxygenases are a huge enzyme family, known for oxyfunctionalization of non-activated C-H bonds. Especially the enzymes of the subfamily CYP 153A are known to perform terminal hydroxylation of linear substrates and are therefore also designated as alkane hydroxylases [1]. Moreover, these enzymes are capable not only of hydroxylation but also of epoxidation of double bonds or hydroxylation of heteroatoms [2].

The current study primarily aimed to examine the novel CYP 153A from *Gordonia rubripertincta* CWB2 with regard to its ability to hydroxylate alkanes and epoxidate alkenes of different chain lengths. We therefore performed *in vivo* whole cell biocatalysis upon heterologous gene expression in *Escherichia coli*. Additionally, we studied the influence of glucose addition on substrate conversion.

In summary, our study revealed that this CYP 153A enzyme is able to perform both reactions, hydroxylation and epoxidation, of distinct substrates. Using different electron transport systems has an effect on the amount of product produced. Furthermore, co-produced CYP with the electron transport partners or using a fusion protein of these components also affects the turnover rate. Supplementation of the system with glucose has a positive effect on the substrate turnover.

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eP085

High-yield production of prebiotic inulin-type fructooligosaccharides using crude inulosucrase from *L. gasseri* DSM 20604

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Inulin-type fructooligosaccharides (IFOS) stimulate the growth and activity of beneficial gut microbes. The global market for IFOS is constantly growing due to their health-promoting effects and functional properties. Hence there is a continuing demand for new, efficient biotechnological approaches for IFOS production. The aim of this work was the high-yield production of IFOS from sucrose using a crude inulosucrase from *Lactobacillus gasseri* DSM 20604. To this end, the optimal process conditions were determined and employed in bioconversion reactions. Heterologous enzyme production of the inulosucrase InuGB-V3 was performed in *Escherichia coli* BL21. Cleared cell lysate (crude inulosucrase) was then applied in IFOS synthesis reactions. Substrate conversion and product synthesis was analyzed by isocratic aminophase HPLC.

Supplementation with 1 mM CaCl2, a pH of 3.5 - 5.5, and an incubation temperature of 40 °C were found to be optimal production parameters at which crude inulosucrase showed high conversion rates, low sucrose hydrolysis, and excellent stability over 4 days. Bioconversion of 800 g L-1 sucrose resulted in very high product titers of 400 g L-1 IFOS within 20 hours of incubation. In summary, more than 8 kg IFOS can be obtained when cell extract from 1 L *E. coli* culture (expressing InuGB-V3) is used for bioconversion. The predominant product was 1,1-kestotetraose (degree of polymerization (DP) 4) with 120,3 ± 10,8 g L-1 followed by 1,1,1-kestopentaose (DP5), which amounted to 95,0 ± 3,6 g L-1. Products with a DP > 6 were present in only small amounts. Increasing the reaction volume from 1 mL to 10 mL and 10 L confirmed the consistent performance of the enzyme at a larger scale.

Thus, the crude inulosucrase exhibited excellent properties that make it suitable for biotechnological IFOS production. The product titer obtained is the highest reported to date for a bacterial inulosucrase, demonstrating that the enzyme is competitive with existing systems derived from fungi. The use of crude enzyme bypasses expensive enzyme purification steps, reducing production costs and improving the economics of the process.

eP086

Energy on demand - Characterization of a novel Electron Transport System for P450 Monooxygenases

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Many enzymes need electrons as energy source for catalysis. Some enzymes cannot directly utilize electrons from natural donors like NADH. Instead, the electrons are harvested and transferred to the enzymes by electron transport chains, consisting of one or more proteins. One enzyme family which relies on electron transport systems (ETS) are cytochrome P450 monooxygenases (P450s). One of the most common ETS is the bacterial system, consisting of a flavin containing reductase (FR) and a ferredoxin (FD) or flavodoxin. This study focuses on the characterization of an ETS for a P450 of the subfamily CYP153A, consisting of a FR and a FD from *Acinetobacter* sp. OC4 which was already used in a whole cell format (Fujii et al. 2006).

Aiming to produce, purify and test the system towards different conditions, the proteins were synthesized, cloned into an expression vector and produced in *E. coli*. After purification *via* affinity chromatography, the proteins were tested for activity towards cytochrome c reduction. Furthermore, experiments for buffer and pH optima, as well as temperature and storage stability were executed. To further check if the ETS was suitable for combination with not only the P450 from *Acinetobacter* but also with other proteins of the subfamily CYP153, the system was co-produced with different P450s in *E. coli* and used for whole cell catalysis to test for activity.

Results showed that the system could be produced in a sufficient amount, 30 mg/l for FD and 28 mg/l for FR, with a pET-expression system in *E. coli* and also purified via nickel-affinity chromatography. Purity was checked via SDS-PAGE. Based on a simple activity test for the FR, the pH optimum was determined to be at pH 3 using Britton Robinson buffer. Due to interactions between different proteins, standard buffers for pH between 6-8 were also tested, with HEPES at pH 6 working best. A thermal shift assay was done for both proteins to check for temperature stability within different buffers. The ETS was applicable for all tested P450s within a whole cell system but unstable when used in purified form in presence of alcohols. Thus, a new ETS for P450s could be established.

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eP087

The microorganisms as the possible solution of degradation high-lipid food waste

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Introduction

While in some countries there is persevering problematics of hunger and malnutrition, other countries are the exact opposite. The excess of food turns into food waste, which makes up to one third of produced food. More than half of the overall food waste origins from the households; retail and food service make together about 17%.

Objectives

The eukaryotic microorganism, yeast *Yarrowia lipolytica*, is well known for production of lipases. Its potential usage on olive mill lipid-rich wastewater was already examined [1], but the composition of oils commonly used in European cuisine and in restaurants, especially central Europe, is various, containing rapeseed oil, sunflower oil, palm oil, olive oil or

lard. In this work we measured microbial respiration of this yeast growing on different oil substrates.

Materials & methods

The yeast *Yarrowia lipolytica* W1 was pre-grown in malt extract broth (MEB) medium for 24h. Washed cells were then added to the media with substrate. The respiratory activity was measured by WTW OxiTop® Control device following OECD protocol Test No. 301: Ready Biodegradability. The supernatant obtained by centrifugation after cultivation was analysed on HPLC.

Results

Yarrowia lipolytica proved capable of growing on provided substrates. Respiration rate of the yeast growing on rapeseed oil was significantly ($p<0.05$) higher than on oleic acid. Addition of glucose was negatively affecting the respiration rate leading to decrease of rate. The analysed products observed were acetic acid, formic acid and ethanol, and their amount depended on the substrate used.

Conclusion

This poster presents preliminary results leading to further research and establishing new possible way of pre-treatement high-lipid kitchen waste. With the use of *Yarrowia lipolytica*, we could decrease the oil content in food waste for next processing steps.

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eP088

Strain optimization of solventogenic Clostridia for simultaneous sugar uptake from hemicellulosic substrates like milling by-products.

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Complex biomass with high hemicellulose and cellulose content is still a challenge for biotechnological solvent fermentation. The complex sugar composition and structure of hemicellulose allows only inefficient biological conversion, especially in the presence of glucose.

Rye and wheat bran are hemicellulose substrates which are high abundant and cheap, but in solvent fermentations the remaining starch from the milling process is inhibiting the utilization of the other sugars. This effect is due to carbon catabolite repression. To circumvent the problem, deoxyglucose resistant mutations in *C. acetobutylicum* were identified from a continuous fermentation on a mixture of xylose and glucose. The resulting mutants were characterized by their fermentation profiles on monosaccharides and were ordered into groups. Several mutants, classified as group 1 mutants, were able to utilize glucose together with arabinose, galactose and xylose and convert them completely into butanol. By complete sequencing, a high number of mutations were observed in the glucose specific PTS system. To verify the role of those specific mutations for the phenotype, they were reintroduced into the wild type strain using a markerless genome editing strategy based on 5-fluorocytosine counter-selection. The strains were also characterized regarding the utilization of the different complex milling by-products. To this end the sugar composition of the initial substrate and the leftovers were analyzed after acidic hydrolysis. Homologous mutations will also be introduced in *C. saccharobutylicum* to test the portability of the phenotype for solventogenic Clostridia.

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eP089

Lactose transport in the filamentous fungus *Neurospora crassa*

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Introduction

Lactose is a by-product of the dairy industry, which is utilized for the induction of cellulase enzyme expression in some fungi (e.g. *Trichoderma reesei*), but which could also serve as a substrate for fungal biotransformations into products for the bio-economy. The yeast *Saccharomyces cerevisiae*, widely used in industrial fermentation processes, cannot metabolize lactose natively. Several metabolic engineering approaches have been used to construct lactose-metabolizing *S. cerevisiae* strains, including genes from other fungi able to assimilate lactose, such as *Kluyveromyces lactis*. However, more knowledge is needed to optimize the system for high productivity. The filamentous fungus *Neurospora crassa* is also able to grow on lactose as sole carbon source, and lactose uptake and metabolism have been demonstrated. However, although the *N. crassa* cellodextrin transporter CDT-1 was demonstrated to enable lactose uptake in yeast, the identification and in-depth characterization of specific lactose transporters in this fungus remains an enigma.

Objectives

Based on available data, it appears that *N. crassa* harbors several transporters with lactose uptake capability, including both specific and unspecific transporters. The aim of this study is to identify and characterize all transporter proteins involved in lactose metabolism in this reference system.

Materials & methods

Gene expression data of *N. crassa* genes of the major facilitator superfamily (MFS) were analyzed and *N. crassa* gene deletion strains obtained from the Fungal Genetics Stock Center. Growth experiments on media with different lactose concentrations and lactose uptake assays were performed.

Results

Several single mutants of *N. crassa* were found to exhibit strong growth defects in lactose-containing medium compared to WT, and their lactose uptake capacity is being analysed. Furthermore, after testing different induction conditions, we found several conditions for high lactose uptake, which do not match the induction conditions for *cdt-1*. These results suggest that CDT-1 is not the only protein responsible for lactose transport in *N. crassa*, and we now focus on genes which are up-regulated under these conditions. The results of the lactose uptake assays will be discussed.

Conclusion

The elucidation of all genes involved in lactose transport in *N. crassa* will provide insights to optimize the reuse of dairy industry by-products in order to create economic value and reduce their environmental impact.

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eP090

From unexplored strains to robust *chassis* platforms – evolving novel stress tolerant *Pseudomonas* species

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During the last decade, several *Pseudomonas s*trains were identified that share a notably small genome and limited metabolic flexibility[1,2,3,4]. However, they exhibit remarkable robustness in challenging environmental conditions, like wide temperature and salinity ranges. In addition, their harsh natural habitats (like oil- or metal-contaminated sites or the deep sea) indicate tolerance to physical and chemical stressors. These species are currently classified as the *P. pertucinogena* lineage and form a unique phylogenetic branch within the genus *Pseudomonas*, although this classification is controversially discussed[5,6,7]. Because of the innate robustness, these strains are considered as promising candidates for biotechnological applications. However, the microbial and molecular methodology can often not readily be transferred from other Pseudomonads to these unique species. Hence, we selected four particularly interesting strains to establish cultivation conditions and manipulation methods to assess their application potential as robust microbial cell factories. Specifically, we developed suitable cultivation media for *P. aestusnigri*[1], *P. bauzanensis*[2], *P. litoralis*[3], and *P. oceani*[4] using a microbioreactor system resulting in higher growth rates compared to complex media. Furthermore, we verified the applicability of DNA transfer protocols using electroporation and conjugational horizontal gene transfer. We could demonstrate the stable replication of a remarkable diversity of vector backbones harbouring different origins of replication. Additionally, various promoter systems were used for the successful expression of heterologous target genes, e.g., fluorescent reporters or enzymes. The analysis of the response to different stressors by HPLC revealed that all selected strains produce the high-value osmolyte ectoine. For *P. bauzanensis* and *P. litoralis* we observed the production of hydroxyectoine in response to high salt concentrations. The production of both osmolytes is not common in *Pseudomonas sp.* Our findings thus corroborate the presumed inherent robustness towards some stress factors and demonstrate the strains' potential as promising platform organisms.

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eP091

Enhancement of soluble heterologous protein expression in *V. natriegens* by co-expression of chaperones

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For recombinant enzyme production, *Escherichia coli* is one of the most widely used bacteria. However, many proteins are difficult to express. A common drawback is the intracellular accumulation of misfolded protein, forming inclusion bodies. Several strategies may be followed to overcome this issue, one being the co-expression of molecular chaperones. A commercial chaperone plasmid [1] serves the overexpression of genes coding for GroEL/ES, Trigger factor (TF), DnaK/J/GrpE and combinations thereof. This strategy is well established in *E. coli* with success. Recently, *Vibrio natriegens* has drawn biotechnological attention as an alternative expression host to *E. coli*. The marine bacterium has attained the status of the fastest growing microorganism. Moreover, *V. natriegens* is genetically easily accessible and many techniques can be transferred from *E. coli* to *V. natriegens*. Particularly, many genetic elements are functional is both organisms. The Takara Bio chaperone plasmid set uses the pACYC ori and the *araB* promoter, which were found to be functional in *V. natriegens* [2].

The goal was to evaluate co-expression of chaperones with the example of a difficult to express aminoacylase from *Mycolicibacterium smegmatis*. In *E. coli*, expression yielded only insoluble inclusion bodies when induced with IPTG. Preliminary experiments showed that, when switching to autoinduction with lactose, soluble protein could be obtained. Furthermore, the co-expression with pGro7 further enhanced the expression. Motivated by this, we investigated all five chaperone plasmids in *E. coli* BL21 (DE3) and *V. natriegens* Vmax for co-expression. The strains were cultivated in shake flasks and aminoacylase activity in the cell free extracts were measured with a ninhydrin-based assay.

In *E. coli* BL21 (DE3), only the co-expression of GroEL/ES from pGro7 resulted in an increased aminoacylase activity in the cell free extract of approx. 50 %, while DnaK/J/GrpE had a detrimental effect. In *V. natriegens*, the effect was even more pronounced, abolishing aminoacylase activity with DnaK/J/GrpE and enhancing activity 3-fold with GroEL/ES. In conclusion, the chaperone plasmid set works in *V. natriegens* and enhanced soluble expression of an aminoacylase.

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eP092

Semi-synthetic multistep enzyme cascade for *N*-hydroxytriazene synthesis

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N-hydroxytriazenes are small molecules which have been widely used in spectrophotometry and complexometry as chelating agents since the 70"s. Recent studies of these chemicals showed antimicrobial and anti-inflammatory activities among others, increasing their clinical and industrial

relevance.[1] So far, the synthesis is solely chemical and is limited to aromatic compounds, due to the instability of the aliphatic substrates. Moreover, the synthesis requires expensive and toxic chemicals. The exact mechanisms of action and full potential of these chemicals remain unknown, so an optimized synthesis method and easier access to these chemicals might benefit further research in these fields.

Therefore, the objective was the enzymatic conversion of L-ornithine (Orn) to *N*-hydroxyputrescine (hPut) through a decarboxylation and subsequent *N*-hydroxylation step catalyzed by the decarboxylase GorB and the NADPH and FAD dependent *N*-hydroxylating monooxygenase GorA, both originating from *Gordonia rubripertincta* CWB2. To regenerate NADPH a formate dehydrogenase (FDH) from *Candida boidinii* was chosen. Due to the inability of the wild type FDH to accept NADP+, the FDH mutant (D195Q/Y196H) was used for regeneration. In the final step, the aim was to synthesize the *N*-hydroxytriazene through chemical coupling of hPut with diazonium salts and verify the product through LC-MS/MS.

After several optimization steps, in a sequential approach, conversion rates of 89% for the decarboxylation and 99% for the *N*-hydroxylation step after 18 h were achieved. However, hPut degradation over time was also observed. Chemical coupling hPut with diazonium salts resulted in the desired *N*-hydroxytriazene as confirmed by LC-MS/MS, giving consistent product ions. Furthermore, the Fe3+-chelating ability of the *N*-hydroxytriazene was confirmed by CAS-assay.

In conclusion, the synthesis of *N*-hydroxytriazenes through a semi-synthetic cascade was successful, giving proof-of-concept. It represents a more sustainable approach which does not involve toxic chemicals and harsh conditions. However, there are still some challenges to overcome such as the instability of hPut and the lack of a purification method for the final product so far.

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eP093

Lactate and ethanol as co-electron donors in a chain-elongating microbiome

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Microbes can utilize both ethanol and lactate as electron donors for chain elongation *via* reverse beta-oxidation into even-chain medium-chain carboxylic acids (MCCAs) such as *n*-butyric acid (C4), *n*-caproic acid (C6), and *n*-caprylic acid (C8). Understanding the relationship between ethanol-based and lactate-based chain-elongating bacteria when ethanol and lactate serve as co-substrates in microbiomes is beneficial for controlling the bioprocess of MCCAs production. In addition, some bacteria ferment lactate into acetic acid (C2) and propionic acid (C3), which compete with lactate-based chain elongation. This pathway should, thus, be prevented. Propionic acid is used as an electron acceptor during chain elongation to result in odd-chain products (*e.g.*, *n*-valeric acid [C5] and *n*-heptanoic acid [C7]). This study aimed to investigate the effect of substrate ratio and operating temperature on MCCA product composition. For this, we studied: (1) the interactions between ethanol-based and lactate-based chain-elongating populations; and (2) the microbial competition between the conversions of lactate into *n*-caproic acid or lactate into propionic acid. We operated two 5-L bioreactors for several years to shape the microbiome. Our results showed that with the increasing ratio of ethanol to lactate from 1:1 to 3:1, the ratio of even to odd products increased (at 30°C). In addition, the 3:1 ratio of ethanol to lactate resulted in the highest specificity for *n*-caprylic acid. Then, we investigated four different temperatures ranging from 25°C to 42°C. When we increased the temperature, we found that the ratio of even to odd products increased, which is advantageous, and showed that propionic acid production became inhibited. *n*-Caproic acid reached its highest specificity at 42°C with *n*-caprylic acid production becoming virtually zero. The *n*-caprylic acid production recovered again after we reduced the temperature back to 30°C. Therefore, we can control the production of either *n*-caproic acid or *n*-caprylic acid for ethanol-based chain-elongating by setting the temperature. We will also present the bacterial community dynamics throughout the operating period of several years to better understand the mechanism of MCCA bioproduction *via* chain elongation in microbiomes.

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eP094

Fluorescence *in situ* hybridization for the monitoring of syngas fermenting clostridium co-cultures

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Clostridium co-cultures are currently under investigation for biotechnological production of chemicals and fuels. Examples are the combination of cellulolytic or solventogenic species or of acetogenic clostridia that can grow autotrophically with carbon monoxide (CO) and carbon dioxide (CO2). Acetogenic clostridia allow the conversion of synthesis gas into short-chain intermediates and, in combination with chain-elongating clostridia, to produce high-value medium-chain fatty acids and alcohols.

For monitoring of a synthetic co-culture of *C. carboxidivorans* and *C. kluyveri* to produce medium-chain alcohols from CO in syngas Fluorescence *in situ* Hybridization (FISH) was used, to achieve an individual labeling of *C. kluyveri* and *C. carboxidivorans* cells. The application of FISH for cell enumeration in co-cultures provides high sensitivity and does not require a genetic modification of the strains, which is typical for other labelling techniques.

To this end, two species-specific oligonucleotide FISH probes, ClosKluy for *C. kluyveri* and ClosCarb for *C. carboxidivorans,* were designed using the ARB software package and a calculated consensus tree of 23S rRNA gene sequences from *Firmicutes* and *Clostridiales* from the large subunit SILVA database. Derived oligonucleotide probe sequences were checked by an *in silico* probe match analysis against all three domains. The optimal hybridization condition of 30% (v/v) formamide was determined in a stepwise formamide series. The new 23S rRNA gene probes ClosCarb and especially ClosKluy revealed a high specificity in online probe match as well as in cross-hybridization experiments with different reference strains, excluding a cross-reactivity with non-targeted species. They allowed specific quantification of *C. kluyveri* and *C. carboxidivorans* in an autotrophic co-culture in a stirred-tank bioreactor by

epifluorescence microscopy and by cell counting using flow cytometry. Furthermore, both new probes could also be applied to environmental samples.

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eP095

Automated atline activity measurement of antimicrobial peptides for bioprocess development

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In recent years antimicrobial peptides (AMP) have gained more interest due to their ability to effectively combat pathogens in food matrices. Although AMP have been safely used for a long time, only few are commercially available. To overcome this bottleneck, faster identification and characterization of AMP and their production processes are needed.

In the past, methods for the measurement of antimicrobial activity were often based on laborious growth inhibition assays, which are error-prone in terms of reliable quantitative data. The fluorescent protein pHluorin allows measurement of antimicrobial activity induced by pore formation (Crauwels et al., 2018; doi.org/10.3389/fmicb.2018.03038). Based on the pH-dependent change of fluorescence intensity, pore formation caused by antimicrobial peptides can be measured for various AMP producing strains. This approach using simple fluorescence measurement forms an essential basis for automation of antimicrobial activity measurement to increase reliability, robustness, and quantitative value of the data, whilst also offering the potential for substantial decrease of hands-on labor and increase of sample throughput.

To deepen insight into cultivation processes of natural AMP producers, such as *Lactococcus lactis* or *Lactobacillus sakei* being producers of Nisin and Sakacin, bioprocess characterization is conducted using miniaturization and parallelization in microplate cultivation to increase experimental throughput, thus also raising the demand for higher analytical throughput. This is tackled by using a laboratory liquid handling robotic system to automate the AMP activity measurement that enables handling of large sample sizes as well as continuous and autonomous sampling to broaden the scope covered by sampling timepoints.

The fluorescence assay was established and fully automated on a liquid handling platform with a built-in microtiter plate reader. After placing the necessary reagents and labware on the robotic platform, the measurement can run autonomously, including dilution, incubation, fluorescence measurement, and a fully automated data analysis workflow. Automated sampling from a connected microplate cultivation device can be included in the workflow.

A method for automated online measurement of antimicrobial activity was successfully demonstrated and forms the foundation for future workflow expansions towards automated sampling, sample processing and further analytical workflows, such as flow cytometry.

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eP096

Marine fungi as a source for mycoremediation of manure and sewage sludge

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The application of manure and sewage sludge on farmland is one key element in building up soil organic matter, and is thus contributing to carbon sequestration (Flessa et al., 2018). These organic fertilizers, however, are in many cases contaminated with anthropogenic pollutants that can cause serious harm to ecosystems (Roskosch & Heidecke, 2018). For this study, marine filamentous fungi selected from a vast academic collection (Flensburg strain collection of marine fungi) and various white-rot fungi are first screened for their ability to tolerate and degrade organic pollutants (e.g. pharmaceuticals, PCBs, PAHs) in a simple growth medium. Marine fungi may have advantages in terms of growth in osmotically challenging manure and sewage sludge matrices. Screening is performed in a miniaturized format to allow for the examination of as many strains as possible. In a second step, favorable fungal candidates are employed to degrade organic contaminants in manure and sewage sludge. Degradation rate and products are assessed with HPLC-DAD and UPLC-MS and kinetics are determined. This study provides an example of an applied bioremediation technique as a sustainable, eco-friendly, and inexpensive way to deal with pollutants and thereby promoting increased use of organic fertilizers in agriculture to scale up carbon sequestration.

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eP097

Targeting three birds with one stone: RcsF-mimic peptide as a potential antimicrobial agent.

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Introduction: The rising problem of antibiotic resistance urges the scientific community to find innovative solutions for the development of new antimicrobials. The Gram-negative bacterial cell envelope is a complex macromolecular structure harboring key proteins for bacterial survival and adaptation. Using a single agent to interfere with several cell envelope proteins might represent a promising strategy to affect bacterial growth. Interestingly, a 14 kDa outer membrane lipoprotein, called RcsF is conserved in *Esherichia coli* and many Enterobacteria. It is the stress sensor of the Rcs two-component system, a complex signalling cascade that responds to envelope stresses. RcsF interacts with 2 essential envelope proteins BamA (involved in the insertion of beta-barrel outer membrane proteins) and IgaA (the negative regulator of the Rcs system). Also, it interacts with the porin OmpA, which is important for

membrane integrity and homeostasis. Accordingly, we hypothesized that an RcsF-mimic peptide will likely perturb one or more of these interactions thus affecting *E. coli* cell envelope homeostasis and bacterial viability.

Objective: Designing and developing an RcsF-mimic peptide of small molecular mass affecting *E.coli* growth/ viability.

Methods: We designed a 36 amino acids peptide (RcsFΔ19-92) harboring the key residues involved in RcsF- IgaA interaction (based on in silico docking) and BamA- RcsF interaction (based on the available crystal structure). We predicted RcsFΔ19-92 binding to its potential targets. We overexpressed *rcsF*Δ19-92 in wild-type *E.coli* K12 MG1655 and assessed the effect on *E.coli* Rcs system activation and growth. We attempted to decipher the probable target by which this new peptide affects *E.coli* growth.

Results: Overexpression of tagged *rcsF*Δ19-92 caused significant activation of the Rcs system without competing with chromosomal *rscF* for binding with IgaA. However, *rcsF*Δ19-92 could not complement *rcsF* null mutant but caused a significant delay in *E. coli* growth independently of Rcs system activation. The effect of *rcsF*Δ19-92 overexpression on bacterial growth could be a direct consequence of envelope damage, membrane destabilization or toxic complex formation.

Conclusion: RcsFΔ19-92 represents a potential antimicrobial peptide, targeting the cell envelope of *E. coli* and probably other Enterobacteria. We anticipate it could be imported by viable intact *E.coli* causing a dose-dependent bacteriostatic effect.

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eP098

A manually curated compendium of expression profiles for *Corynebacterium glutamicum*

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Introduction: *Corynebacterium glutamicum* is a Gram-positive soil bacterium used for production of amino acids in the multimillion tons scale and has become an intensively studied model organism in industrial biotechnology. Over the decades, rational strain construction by metabolic engineering has led to a continuous improvement of established producer strains and simultaneous expansion of the substrate and product spectrum. The success of these approaches is based on detailed knowledge of the metabolism as well as transcriptional regulation by using, for example, comprehensive studies of the transcriptome.

Objectives: Presentation of a manually curated compendium of expression profiles for *C. glutamicum*.

Methods: Over the past 20 years, researchers of the Institute of Bio- and Geosciences 1: Biotechnology (IBG-1, until 2010 named Institute of Biotechnology 1) at Forschungszentrum Jülich (Germany) have performed and collected the results of 927 microarray experiments in an in-house database[1]. Here, we manually re-evaluated all experiments according to the Minimum Information About Microarray Experiments (MIAME) standard[2], categorized them according to specific criteria (e.g., media composition, strain background, plasmid-based overexpression) and uploaded them as a superseries to *Gene Expression Omnibus* (*GEO*).

Conclusion: In recent years, digitalization, big data generation and analysis have become important issues in scientific research. For researchers the demand for data exchange to also address research questions going far beyond the individual studies is constantly increasing. Making our expression profiles **F**indable, **A**ccessible, **I**nteroperable and **R**e-usable (FAIR[3]) allows other researchers to use our data for future studies that will further elucidate the complex transcriptional regulatory network of *C. glutamicum*. Furthermore, the resulting knowledge gained for *C. glutamicum* will also benefit the understanding of other phylogenetically linked Actinobacteria.

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eP100

Online profiling of population dynamics in a model filamentous co-culture: A valuable approach for optimizing the biotechnological production of bioactive compounds

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Co-cultures of microorganisms are an emerging strategy to stimulate the production of new natural compounds thereby overcome the deceleration in the discovery of bioactive substances. However, the influence of differing population dynamics on the natural product formation in co-cultures is still largely unknown due to analytical challenges. Hereby, we propose that monitoring of population dynamics can be a valuable tool to optimize and understand natural product formation in a synthetic co-culture of filamentous microorganisms. For this purpose, a dual fluorescent tagged co-culture of the cellulolytic fungus, *T. reesei* RUT-C30 – mCherry and the pigment-producing bacterium *S. coelicolor* A3(2) – mNeonGreen was established. In the proposed system a small amount of glucose is used to boost initial growth, while cellulose is employed as main carbon source to establish a relation of dependency. Thereby, *S. coelicolor* is dependent on cellulolytic enzymes produced by *T. reesei* to feed on cellulose. The population dynamics were monitored in a 48-well microbioreactor by online fluorescence measurements. Several *T. reesei* : *S.coelicolor* spore inoculation ratios were tested, covering orders of magnitude from **10^0:10^{-4} to 10^{-4}:10^0**. Both fluorescence tags effectively described the individual biomass contributions. In the 10^{-4}:10^0 and 10^{-3}:10^0 co-cultures, there was a clear dominance of *S. coelicolor* with online signals resembling those of axenic *S. coelicolor* cultures. As *S. coelicolor* quickly outcompeted *T. reesei* under these conditions, no cellulases were formed and no cellulose could be consumed, which resulted in absence of pigmentation. In contrast, for the co-cultures with ratios of 10^0:10^{-4} and 10^0:10^{-3}, fluorescence signals indicated that both partners sustained growth throughout culture and strong pigment formation was visible. In these cultures, extracellular protein concentration was clearly higher than those of *S. coelicolor*-dominated co-cultures, indicating efficient cellulase production. Hence, the delayed growth of *S. coelicolor* in these conditions enabled *T. reesei* to grow and produce sufficient cellulase enzymes

which enabled *S. coelicolor* access to cellulose. These findings highlight the importance of analyzing population dynamics during the optimization of co-culture processes and further demonstrate the benefits of mimicking natural environments to stimulate natural product formation.

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eP101

Production of tailored glycolipid biosurfactants in *Pseudomonas putida*

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Biosurfactants comprise a large number of amphipathic biomolecules useful for industrial and medical applications. The production in natural hosts is often challenging due to complex regulatory networks and difficult cultivation. As a solution, recombinant expression strains can be used that feature excellent laboratory access and optimized production conditions. Here, *Pseudomonas putida* KT2440 stands out because of its robustness and a particularly versatile metabolism.

We have applied *P. putida* KT2440 as a safe platform for the recombinant biosynthesis of biosurfactants such as rhamnolipids and yet unexplored sugar-amino acid lipids. The aim is to produce a large variety of biosurfactants with tailored chemical compositions for different applications.

Rhamnolipids were produced using different expression cassettes with a differing arrangement of biosynthetic genes obtained from different organisms. We succeeded to produce pure 3-(3-hydroxyalkanoyloxy-)alkanoic acid (100%), mono-rhamnolipid (100%) and mixtures of mono- and di-rhamnolipids (14-94% mRL) with potentially different properties. Further, a *P. putida* strain producing sugar-amino acid conjugated lipids was constructed using genes from *Alkanivorax borkumensis* that were predicted by *in silico* analysis to encode parts of the respective biosynthetic pathway.

While the validation of the full biosynthetic pathway is still ongoing, *P. putida* already proved suitable for unveiling the involved proteins. Our results show the potential of *P. putida* to produce different biosurfactants thus increasing the diversity of these compounds for a variety of biotechnological applications.

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eP102

Enzymatic Hydroxylation to Produce Medium-Chain Glycols

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The production of simple molecules as bulk chemicals for industrial applications requires processes that are cost- and resource- efficient at the same time. In this context, petrol-based synthesis strategies are classically the methods of choice. In view of a circular (bio)economy and atom efficiency, a reconsideration is required and feedstocks have to be altered. This can result in completely new synthesis routes towards established bulk chemicals.

In view of this rethinking of processes, the production of glycols with different chain lengths becomes a challenge that can be tackled by selective hydroxylation of non-activated carbon atoms of simple aliphatic precursors. Our approach to produce such compounds in a biotechnological setting uses oxygenases for the terminal hydroxylation of alcohols.

Towards this aim, cytochrome P450 monooxygenases from versatile origins were tested for their functional expression in *Escherichia coli* expression strains. A screening for the selective hydroxylation of simple alcohols identified suitable catalysts. The tolerance of the host cells towards alcohols and glycols was investigated in detail. To enable an effective turnover of terminal alcohols into glycols, reaction engineering addressed challenges that have to be met in a whole-cell format. We will present our results with view towards the feasibility of incorporating such reactions in bulk chemical production.

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eP103

Z-Parts: A Golden Gate Modular Cloning Toolbox for heterologous gene expression and genetic engineering in *Zymomonas mobilis*

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The facultative anaerobic Alphaproteobacterium *Zymomonas mobilis* has many properties that are advantageous for the production of biofuels. It is characterized by exceptionally high glucose uptake and ethanol production rates. Moreover, it produces very little biomass but has a close to maximal yield of ethanol exceeding that of most other production organisms. Though *Z. mobilis* holds great promise, its use in bioproduction is hampered by its limited native substrate range, its weak resistance towards inhibitory substances and by a lack of efficient tools for genetic engineering. Genetic engineering would allow to expand the product range and to broaden its substrate spectrum. Golden Gate cloning allows for rapid, efficient and directed combination of DNA modules. A Golden Gate cloning-based modular toolbox made for *Z. mobilis* is suited to bring together all elements needed for genetic engineering. The creation of fine-tuned transcription units is possible by combining different promoters, regulators, ribosomal binding sites and terminators. Libraries of such elements can easily be built and due to the modular character, the toolbox enables an efficient testing of different elements and combinations. The approach is also suitable for applications of genome editing that demand for a combination of parts, like upstream and downstream homology arms, crDNA transcription units, inserts and/or antibiotic resistances. We are offering a collection of native and synthetic constitutive promotes of varying strength, as well as inducible promoters, an array of terminators and synthetic rbs, reporter genes and target genes selected for metabolic engineering. Additionally, over a golden braid-based system an assembly of operons consisting of up to four genes is possible. Further, we provide a set of shuttle vectors for *E. coli* and *Z. mobilis* as parts of our modular toolbox. These vectors contain different antibiotic resistances and origins of replication, some from broad range plasmids and some from native *Z. mobilis* plasmids. We quantified the levels of mCherry expressed by different combinations of elements of our library and can achieve a broad range of expression levels. Also, we were able to achieve different levels of lactate production by *Z. mobilis* expressing lactate

dehydrogenase from different constructs. The modular cloning system for *Z. mobilis* will be further expanded and holds great promise as a basis for the future of metabolic engineering of *Z. mobilis*.

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eP104

Microbiome-based anaerobic fermentation of glycerol and carbon dioxide into succinic acid

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Glycerol was once considered a desirable chemical but is now regarded as a waste stream with a disposal cost. The reason for this shift is the rise in biodiesel production during the last decade because glycerol is produced as a side product of the transesterification process at a rate of 10% (w/w). One way of making use of the surplus of glycerol is to biologically convert it together with carbon dioxide *via* anaerobic fermentation into value-added chemicals such as succinic acid. This chemical is a four-carbon dicarboxylic acid and is recognized as a Top-10 platform chemical for high value-added derivatives by the European Commission and the United States Department of Energy. Our objective is to develop a microbiome-based biotechnological production process to anaerobically ferment glycerol and CO2 to succinic acid. Our microbiome was shaped in a 5.5-L continuously fed bioreactor, with automatic control of the operating parameters. Furthermore, an in-line electrochemical product-extraction system (i.e., electrodialysis) was connected to the bioprocess. We have operated this integrated bioprocess for more than two years, shaping the microbiome towards an increased selectivity of succinic acid. The average volumetric succinic acid production rate during the latest operating period was 25 mM C·L·d-1 (0.185 g·L·d-1), which is still too low for an economically viable process, but was relatively stable throughout a longer operating period.

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eP105

Enriching the diversity of polyethylene terephthalate (PET) degrading enzymes from metagenomes

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Polyethylene terephthalate (PET) is a durable, chemically and thermally stable polyester. Due to these desirable properties, PET is used for a variety of applications in industry. However, PET plastics do not degrade to a large degree under natural conditions (1). Its accumulation in the environment has become one of society´s major concerns. The use of microbial enzymes offers a novel environmentally friendly option for the recycling of PET materials. Certain members of the Actinobacteria and Proteobacteria are known to degrade PET. Here, we describe the first functional PET-active enzymes from the Bacteroidetes phylum. This project mainly focuses on the isolation and characterization of the novel enzymes that have the ability to degrade PET.

To identify possible new PET-degrading enzymes, environmental samples were collected from an area in Wietze (Lower Saxony, Germany), where soils are heavily contaminated by crude oil. With these samples, enrichment cultures were set up, of which the metagenomic DNA was subsequently isolated and sequenced. Additionally, global metagenome datasets have been accessed. Both sequence data sets were subjected to a Hidden Markov Model-based search specific for PETases. From a large number of hits, the DNA of 80 promising candidates has been synthesized.

Among them, three promiscuous and cold-active esterases derived from *Aequorivita* sp. (PET27), *Kaistella jeonii* (PET30) and *Amycolatopsis* sp. (PET40) showed depolymerizing activity on polycaprolactone, bis(2-hydroxyethyl) terephthalate and amorphous PET foil. The crystal structure of PET30 without its C-terminal Por-domain (PET30ΔPorC) was solved at 2.1 Å and displays high structural similarity to the *Is*PETase. PET30 shows a Phe-Met-Tyr substrate binding motif and PET27 Phe-Met-Trp, which seem to be unique features, as *Is*PETase, LCC and PET2 all contain Tyr-Met-Trp binding residues. Microscopic analyses showed that *K. jeonii* cells are able to bind on and colonize PET surfaces after a few days of incubation. Homologues of PET27 and PET30 were detected in metagenomes, predominantly aquatic habitats, encompassing a wide range of different global climate zones and suggesting a hitherto unknown influence of this bacterial phylum in man-made polymer degradation.

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eP106

Protein dynamics and structure guided engineering of an active site loop of an ene/yne-reductase from *Cyclocybe aegerita*

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The applications of ene-reductases (ER) in the chemical landscape are very diverse. Especially ERs of the flavin mononucleotide (FMN) depending Old Yellow Enzymes (OYE) were extensively studied in recent years.[1] An overlooked enzyme family is the medium-chain dehydrogenase/reductase superfamily. Recently, we described the first ene/yne-reductase (CaeEnR1) from a filamentous fungus of the phylum Basidiomycota as a member of the MDR-superfamily displaying novel biocatalytic activities like highly efficient and regioselective reductions of α,β,γ,δ-unsaturated aldehydes as well as the reduction of activated alkynes to their saturated alkanes.[2] Importantly, only the ER from *Nicotiana tabacum* of this superfamily was also extensively characterized for its biocatalytic application. Taken together, both MDR-related ERs show a broad substrate scope, displaying their high potential as a biocatalyst. In comparison with OYEs, both biocatalysts show significantly poorer conversion rates of cinnamaldehyde-like substrates (3 – 29%).[2, 3] Nevertheless, cinnamaldehyde-like compounds are of high value in various industrial processes or as fragrances.

Hence, we investigated the molecular reason for this circumstance. First, we successfully crystallized CaeEnR1 and were able to solve the crystal structure with a resolution of 1.4 Å. Most importantly, with this structure we identified a

flexible loop which is not resolved in already published crystal structures. Secondly, we performed HDX-MS measurements underlining the flexibility and thorough access of this loop to the surrounding solvent indicating the importance in substrate recognition. Via semi-rational engineering of this active site loop we were able to increase the conversion rates of various relevant cinnamaldehyde-like substrates such as 2-methoxycinnamaldehyde, 4-methoxycinnamaldehyde or 4-hydroxy-3-methoxycinnamaldehyde by the factor of 2 – 15 compared to the wild-type enzyme.

These results confirmed our hypothesis that this loop is indeed involved in substrate recognition. This provides valuable information for future projects and opens up new opportunities in protein engineering of MDR-related ene-reductases.

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eP107

Engineering a fluorinated C2 metabolism in *Escherichia coli.*

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Fluorine-containing organic molecules (fluorochemicals) are valuable compounds, widely used in many application fields. Yet, no bio-based process has been developed to produce fluorochemicals. There is a critical need for such bioprocesses using safer and sustainable substrates, supported by predictions of rising demands. This study aims to pave the road toward a bio-based economy for fluorochemicals by engineering and characterizing a synthetic C2 fluorine-based metabolism in the well-characterized biotechnological workhorse *Escherichia coli*. To this end, synthetic biofluorination pathways were designed based on the fluorinase enzyme of *Streptomyces cattleya.* The unique activity of this enzyme catalyzes the formation of a covalent bond between the F^- ion and *S*-adenosyl-L-methionine, generating 5'-fluoro-5'-deoxyadenosine (FDA). In the following reactions, FDA is further metabolized and cleaved, generating dihydroxyacetone phosphate (DHAP, an essential metabolite) and fluoroacetaldehyde (a C2-F moiety). Central metabolism in *E. coli* was re-wired to accommodate biofluorination, providing not only fluorinated C2 compounds but also DHAP for biosynthesis. Gene deletions were introduced to isolate DHAP from the endogenous metabolism (in a selection strain) and the biofluorination module was introduced as the sole route for DHAP biosynthesis. This operation facilitates the assessment of the synthetic pathway performance, since its activity can be directly gauged from growth rates and biomass yields. Consumption of the pathway´s substrates was confirmed by HPLC measurement, proving further that synthetic biofluorination is active in *E. coli.* Parallel to the engineering of C2 fluorochemicals biosynthesis, top-down biofluorination was implemented by external feeding of fluoroacetate. Quantitative physiology was explored in the presence of fluoroacetate and a clear correlation between the fluorochemical concentration and growth impairment could be observed. Furthermore, the occurrence of evolutionary events as well as rational metabolic re-wiring generated fluoroacetate-tolerant strains. These results are the first step into exploring a novel fluoro-metabolism towards consolidated microbial chassis for bio-based production of fluorochemicals.

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eP108

Metabolic Engineering of *Pseudomonas taiwanensis* for the production of 4-coumarate and derived aromatics

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Introduction

A bio-based production of aromatics is needed for the shift to a sustainable economy. We applied *Pseudomonas taiwanensis* as microbial host for whole-cell biosynthesis of a range of aromatics from renewable substrates[1-4]. In this work, we focused on the production of 4-coumarate (4CA) and derived aromatics.

Aims

- Establishing efficient 4CA production
- Avoiding the formation and accumulation of precursors and byproducts
- Diversifying the product spectrum by extending the biosynthetic pathway

Methods

- Metabolic engineering
- Cultivations
- Analytics (HPLC)

Results

Pseudomonas taiwanensis was engineered to increase metabolic flux to tyrosine, via phenylalanine, to enable production of aromatics from renewable substrates[1,2]. Multiple ammonia-lyases were evaluated for specific deamination of tyrosine. All tested tyrosine ammonia-lyases (TALs)[5] were highly specific for tyrosine, but 4CA production was limited by the enzymes" activity, indicated by residual tyrosine. When a phenylalanine ammonia-lyase (PAL) with PAL/TAL activity was used, no tyrosine was detected. Instead *t*CA accumulated as a major byproduct (2.7 mM) even surpassing the titer of 4CA (1.0 mM).

To make use of the higher activity of the PAL, but limit *t*CA accumulation, a point mutation was introduced in the prephenate dehydratase. This reduced the flux to phenylalanine, limiting byproduct formation on the level of substrate availability. Thereby, 2.7 mM 4CA were produced from glucose with a yield of ~20 % (Cmol/Cmol). Only ~0.1 mM *t*CA was produced by this strain.

In the following, the 4CA producer was used as a basis for the biosynthesis of 4CA-derived aromatics (e.g., 4-hydroxybenzoate) through the expression of additional heterologous enzymes.

Conclusions

The previously generated tyrosine producer is a useful platform for the production of several aromatics through a plug-and-play expression of product-specific modules. However, best 4CA production was achieved with an unspecific PAL/TAL enzyme whose efficient application required a reduced phenylalanine biosynthesis to limit *t*CA byproduct formation. This was achieved by targeting the prephenate dehydratase activity.

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eP109

Bioleaching of waste materials with a newly isolated cyanide-producing *Pseudomonas* strain

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Introduction: Large amounts of electronic waste are produced annually, of which only a moderate fraction is being recycled. Gold (Au) and other precious metals are solubilized chemically by cyanide leaching, which leads to toxic waste water. Some bacteria produce cyanide as secondary metabolite, which is used for precious metal bioleaching from urban wastes at 10-20-fold lower concentrations compared to chemical leaching. Apart from the non-pathogenic *Pseudomonas protegens* CHA0, the best-known cyanide-producing bacteria are *Chromobacterium violaceum* and *P. aeruginosa*, which are biosafety level-2 organisms.

Objectives: The aim of this study was to identify a biosafety level-1 strain with similar or even higher Au-leaching rates compared to the level-2 representatives and to optimize bioleaching of precious metals.

Methods: Samples collected from forest soils in Darmstadt were serially diluted and spread on cyanide-containing solid media. Cyanide production was assayed by the methemoglobin method. Bioleaching experiments conducted with several ground urban wastes and ore samples were assayed by ICP measurements for precious metal recovery. Media optimization via the Design of Experiments (DoE) strategy was done using the software DesignExpert 8.0 and executed with a pipetting robot.

Results: The newly isolated *P. donghuensis* G25 strain produced cyanide to a concentration of 4 mg / ml in solution. Bioleaching capabilities were observed for five of eight urban wastes/ore samples such as crucible particles with an Au-leaching efficiency of nearly 50 %. A waste incinerator bottom ash (2.5 % w/v) with a high precious metal content resulted in an initial Au-leaching efficiency of 8 % after 48 h, whereas an alkaline washing step enhanced the recovery to 16 %. DoE media optimization for the *P. donghuensis* strain with the pre-treated ash (1 % w/v) increased the Au-leaching efficiency to ≈70 %, compared to the standard growth medium that resulted in 40 % Au-leaching efficiency. The main effects were achieved by the optimization of the amino acid set and pH adjustment. The results were confirmed in 100 ml shaking flask experiments.

Conclusions: The Au-bioleaching capability of the newly isolated *Pseudomonas* strain was demonstrated with different materials including a pre-treated bottom ash. Optimization via DoE led to a medium with a doubled Au recovery.

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eP110

An activity-based protein profiling (ABPP) approach for biocatalyst screening in the white rot fungus *Phanerochaete chrysosporium*

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Activity-based protein profiling (ABPP) has emerged as a widely used proteomic method for basic biology research [1] in which covalently binding chemical compounds known as activity-based probes (ABPs) are used to label active enzymes under native conditions [2]. In comparison to sequence homology-based biocatalyst screening approaches, e.g. by analyzing genomic data to find novel enzymes, this approach exploits the established enzyme target selectivity of ABPs to elucidate novel biocatalysts. In the present work, we aimed to showcase the potential of the ABPP biocatalyst screening technology in a complex and biotechnologically relevant experimental setting. For this, we used the ABPs FP-alkyne for targeting of serine hydrolases and JJB111 for targeting of glycoside hydrolases (GH) to identify biocatalysts in the lignocellulose degrading white rot fungus *Phanerochaete chrysosporium*. We cultured *P. chrysosporium* in the presence of beech wood as the sole carbon and energy source and analyzed all secreted proteins (soluble and substrate-bound) via an ABPP approach. Using this approach, we were able to identify a set of carbohydrate esterases (CE) and glycoside hydrolases from different families that are most likely involved in the degradation of hemicellulose, according to their sequence homology. Afterwards, we expressed and purified a set of target enzymes to confirm activity and found the CE1 family protein 126075 to function as an acetyl-xylan esterase, while the GH5 2915237 was able to degrade both xylan and lichenan. In addition, we were able to label the putative glutaminase 3002168 via our GH-specific probe JJB111, and analyzed whether this protein takes part in lignocellulose degradation. In total, our experiments demonstrated that ABPP allows for a straight-forward and technically simple targeted identification of active biocatalysts, including enzymes with previously unannotated gene sequences.

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eP111

Development of tools for genetic engineering of *Clostridium cellulovorans*

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Lignocellulosic plant biomass is the most abundant renewable carbon source on earth. It offers great potential as raw material for biotechnological production of chemicals and biofuels and does not compete with food production. But cellulose fermentation with the anaerobic cellulolytic organism *C. cellulovorans* results mainly in butyrate, acetate, H2 and CO2. To achieve solvent formation, a stable co-culture of *C. cellulovorans* with the solvent producing *Clostridium acetobutylicum* will be established. Additionally, the formed CO2 will be reassimilated by adding the homoacetogen *Clostridium carboxidivorans* to the co-culture. All organisms will be engineered to make them interdependent on each other. Therefore, a system for genome editing of *C. cellulovorans* is needed. To this end a protocol for efficient transconjugation from *E. coli* to *C. cellulovorans* via triparental conjugation with *E. coli* CA434 as helper strain was established. As selection marker for *C. cellulovorans* thiamphenicol resistance by the the *catP* gene from the plasmid pMTL83151 was used und *E. coli* was removed after conjugation by using cellobiose as carbon source together with the presence of 50 µg mL-1 D-cycloserine. To circumvent restriction in *C. cellulovorans in vivo* methylation by the methyltransferases Clocel_4007&4008 was used. Successful conjugation of the thiamphenicol resistance was observed and validated by PCR and re-transformation in *E. coli*. This provides the basis for establishing a marker-less deletion system using *codBA* counter-selection with 5-fluorocytosine, which will be used for metabolic engineering of *C. cellulovorans.*

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eP112
Exploring the biochemical potential of new subtilisins from halotolerant *Bacillacea*
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Proteases, especially alkaline proteases, are of great importance primarily as detergent additives, but are also useful in many other industries, such as leather and feather processing, waste water treatment, and food manufacturing [1]. Several *Bacillus* derived alkaline proteases were developed to meet industry requirements [2]. The needs were high or low specificity depending on the application, good stability towards pH, salt, temperature, organic solvent, metal ions and surfactants. The search for these enzymes is facilitated by data mining of the increasing number of deposited genome sequences. Selecting microorganisms with extreme habitats will provide access to their robust proteases that can meet these diverse extreme conditions. In particular, microorganisms from halotolerant habitats provide proteases that are in demand for various applications due to their ability to function under these harsh conditions.

Considering the current need for more efficient proteases, the present study aims to find novel candidates in the genome databases (NCBI identical protein groups). The cloning, production and purification of several new proteases was performed to investigate their biochemical potential for these extreme conditions. First promising results will be presented.

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eP113
Regulatable expression systems for the acetic acid bacterium *Gluconobacter oxydans*
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Question: For the acetic acid bacterium (AAB) *Gluconobacter oxydans*, and AAB in general, hitherto no system was available that allowed highly regulatable plasmid-based target gene expression in basic and applied AAB research[1]. The strictly aerobic Gram-negative *G. oxydans* serves as a cell factory for oxidative biotransformation and as a model organism for elucidating the metabolism of AAB. This study aimed to test and establish regulatable expression systems in *G. oxydans* that allow highly tunable target gene expression in an effector-dependent manner.

Methods: Expression plasmids were constructed based on the pBBR1MCS plasmid family to test the well-characterized heterologous AraC-P_{araBAD}, TetR-P_{tet}, and LacI-P_{lacUV5} systems in *G. oxydans* 621H. As expression reporters, the β-D-glucuronidase UidA and the fluorescence protein mNeonGreen were used.

Results: With a pBBR1MCS-5-based plasmid, the L-arabinose-inducible AraC-P_{araBAD} system showed very low basal expression of the reporters and up to 480-fold induction with L-arabinose and well tunability from 0.1 to 1% (w/v) inducer[2]. In *G. oxydans*, the P_{araBAD} promoter was not active without AraC, and thus did not require repression by AraC. The Tn*10*-based TetR-P_{tet} system from *Escherichia coli* also showed extremely low non-induced basal expression allowing anhydrotetracycline-dependent tunable induction up to more than 3500-fold[3]. In contrast to P_{araBAD}, P_{tet} was highly active in *G. oxydans* in the absence of TetR, indicating that P_{tet} was highly repressed by TetR when not induced. The LacI-P_{lacUV5} system was tunable by IPTG only up to 40-fold induction due to relatively high leakiness of P_{lacUV5} when not induced[3]. This basal expression appeared to be independent of potential read-through from the *lacI* promoter upstream and thus suggested insufficient repression of P_{lacUV5} by LacI.

Conclusions: After 35 years of constitutive target gene expression in *G. oxydans*, now the first highly regulatable expression systems are in hand. Besides transcriptional activation, also a pure de-repression-based system was performing very well in *G. oxydans*, which could potentially also be achieved in other AAB.

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eP114
Screening for algae cell wall degrading enzymes with biotechnological traits
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Background: The sustainable use of marine resources requires not only a reliable source of organic material but also an efficient way of processing high amounts of macro- and microalgae. Cell walls of algae consist of a complex network by structure and protection giving polymers like Cellulose, Fucoidane, Laminarine and Alginate. To hydrolyze the molecular connections, the bacterial community associated with the macroalgae *Fucus vesiculosus* is characterized. Furthermore, sequence and function-based searches reveal the composition of responsible enzymes for the hydrolyzation of algae cell walls carbohydrates. Additionally, based on the chemical structure of the cell wall polymers we conclude an application of those enzymes on structural similar synthetic polymers.

Materials and Methods: Enrichment cultures in microcosms with *F. vesiculosus* samples from the Kiel Bight were designed to characterize the microbiome that is responsible for the degradation of the brown algae by conducting metagenomic-, proteomic and transcriptomic analyses. While the degradation products will be analyzed by HPLC, HPLC-MS, TLC and several sugar assays measuring released sugars like glucose and the L-Fucose of the carbohydrate Fucoidan, the responsible glycoside hydrolases were screened by sequence-based searches from the corresponding metagenome. Furthermore, glycoside hydrolases were screened in a *Nannochloropsis salina* microbiome culture metagenome. Potential algae cell wall degrading genes are cloned and tested on *F. vesiculosus* substrates as well as Tributyrin, Polycaprolacton, Bis(2-Hydroxyethyl) terephthalate.

Results and outreach: Microbial degrading processes took place within 10-15 days in the microcosm set ups, observable by an increased sugar release and visual structural changes of the macroalgae. The rapid release of Glucose and L-Fucose in the first 10 days and the following decrease leads to the assumption of an active hydrolyzing microbiome on the start of the enrichment cultures, which the bacteria, which graze on the released monomers, suppress. Genes for putative L-Fucosidases and Cell wall associated hydrolases were cloned and expressed to be tested on the targeted substrates.

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eP115

Lignin degradation by filamentous fungi – searching for new aromatic compounds

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Lignin is one of the most abundant biopolymers. In addition to cellulose and hemicellulose, it can be found in vascular plant cell wall and is therefore a particularly common waste product in the paper and pulp industry. Approximately 50 million tons of lignin are burned annually instead of being used as carbon source.

Due to its aromatic structure, lignin is a raw material with a lot of potential. There are already various industrial or chemical approaches how aromatic precursors are formed from the lignin, which then can be used for the industrial production of for example drugs, plastics or other fine chemical products.

If these new precursors could be synthesized through a biotechnological process, using industrial waste from the pulp and paper industry, a much better CO_2 balance will be achieved.

The degradation of lignin by fungi, mainly basidiomycetes, which break down the lignin with the help of enzymes such as laccases and peroxidases, is a possible environmentally friendly approach. Therefore, we are looking for suitable filamentous fungi that decompose the ligninolytic waste products into usable aromatic building blocks, which then can be used for further industrial application. Several fungi are fermented separately with lignin sulfonate or kraft lignin and the new products are then analyzed by HPLC and GC-MS.

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eP116

Current status of the BIOFILMS ISS experiment: testing functionalized antimicrobial surfaces in space

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Microbial biofilms are universally present in our environment. However, since they can damage materials and pose a health risk, they are unwanted in certain settings. Especially during space travel, where crew health is top priority and failure of equipment is detrimental, methods for preventing biofilm formation are vital. Antimicrobial active metals, such as copper, have already shown their potential on Earth [1]. Furthermore, additional surface functionalization of copper surfaces through direct laser interference patterning using ultra short pulses (USP-DLIP) can increase the antimicrobial efficiency and biofilm inhibition for certain types of bacteria [2].

The spaceflight project "BIOFILMS" investigates the antimicrobial effect of these functionalized copper surfaces in microgravity by using a unique experimental setup on board the ISS. For this, three bacterial species *Staphylococcus capitis* subsp. *capitis*, *Cupriavidus metallidurans* and *Acinetobacter radioresistens* were selected. Copper and brass were selected as antimicrobial metals and steel is used as an inert reference. All three metals are tested with and without surface functionalization. For the experiment, specific hardware was developed that allows bacterial biofilm formation in liquid medium in immediate contact to the different surfaces. The first results from the Science Verification Test and Experiment Sequence test showed that the hardware is biocompatible with the selected bacterial species and is suitable for the scientific requirements and proposed experimental sequence. The first launch for BIOFILMS was in August 2021 with SpX 23 with the first set of samples. Aboard the ISS, the hardware was integrated into the KUBIK incubator inside the Columbus laboratory. In this incubator, the experiment was incubating at 20 °C for 14 days in microgravity (Space), 0.4 x g (Mars) and 1 x g (Earth)

as control. The second and third set of samples will be launched in 2022. Preliminary experiments on Earth indicated that the functionalized copper surfaces have altered antimicrobial effects on the selected bacteria.

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eP117

Production of mandelic acids by using recombinant *Escherichia coli* cells expressing hydroxymandelate synthase (HMS) from *Amycolatopsis mediterranei*

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Introduction

An important class of industrial valuable compounds is the aromatic alpha-hydroxy carboxylic acids like mandelic acid (MA) (1). MA and its derivatives are used in a broad field of applications. It can be used as an important precursor and building block for the synthesis of ionic liquids (2) or the preparation of semisynthetic cephalosporin antibiotics (3, 4).

The enzyme hydroxymandelate synthase (HMS) is a non-heme iron dioxygenase that converts *p*-hydroxyphenylpyruvate and other 3-aryl pyruvates by decarboxylation to the corresponding mandelates (5, 6). This study aimed to construct and establish an *in vivo* cascade in *E. coli* for the synthesis of MAs by using HMS.

Methods

We cloned and heterologous overexpressed the gene *hms* encoding the hydroxymandelate synthase from *Amycolatopsis mediterranei* in *E. coli* BL21(DE3).

Results

We successfully used the recombinant cells for the production of *para*-hydroxymandelate in a whole cell biotransformation approach with L-tyrosine. Furthermore, by utilizing different halogenated phenylalanine derivatives, the corresponding MAs were obtained with high conversion (21–87 %) and high *ee* (38–97%). This whole cell catalysis process is an alternative and attractive way to get access to a variety of mandelic acids (7).

Conclusion

We demonstrated that *E. coli* is a suitable microorganism to produce MAs by using HMS in a whole cell biotransformation approach.

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eP118

Bioelectrochemical System for flexible Biogas Production

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Biogas plants in which biomass is converted by microorganisms to biogas are already widely applied. However, the biogas production is rather sensitive to an accumulation of organic acids that inhibits methanogenesis. To optimize this process, one approach is to integrate microbial electrolysis cells (MEC) into existing biogas plants. In the envisioned MEC-biogas hybrid reactor, exoelectrogenic organisms will oxidize organic acids in a respiratory process to carbon dioxide. The terminal electron acceptor of the microorganisms is the MEC-anode while hydrogen is produced on the cathode side. By adjusting the potential of the anode, it will be possible to direct electron flow from organic acids in the biogas reactor either towards cathodic hydrogen or biogas production.

A 10 L MEC-reactor was developed for scalable process development. The reactor was constructed according to the blueprint of a biological contactor with the rotating discs as anodes of the MEC system. A model biofilm was pre-grown on the rotating discs. The biofilm consisted of the exoelectrogenic organisms *Shewanella oneidensis* and *Geobacter sulfurreducens*. After reaching a stable current production, linear sweep voltammetry was used to determine the limits of potential-controlled electrode transfer. Here, a potential below -0.24 V vs. normal hydrogen electrode (NHE) led to a complete inhibition of anodic electron transfer. Alternating potentials of -0.2 V and 0.2 V vs. NHE were applied over different time periods to determine how exoelectrogenic organisms can cope with rapid changes in the applied anode potentials. The conducted experiments revealed that these rapid potential variations did not interfere with long-term activity of the anode biofilms as it was possible to regain maximum current densities shortly after readjusting the potential towards maximum electron transfer kinetics. The experimental data is currently evaluated to identify kinetic parameters for the development of a mathematical MEC model that can be integrated into existing models for anaerobic digestion.

eP119

In-situ resource utilization (ISRU): Microbial biomining of Lunar regolith EAC-1A

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Planned return missions to the Moon will certainly mark the future of space exploration, as it is also a steppingstone to Mars (International Space Exploration Coordination Group, 2018). However, the reduced access to supplies, and the increased costs of mobilizing the materials required for the continuous study and exploration are deeply related to the distance to Earth, making In-Situ Resource Utilization (ISRU) the only viable option towards sustainable exploration in the long term (Sanders, 2018). The current study tests microbial biomining of a lunar regolith simulant (EAC-1A) as an ISRU approach to extract minerals and metals accessible on the regolith. To fullfill this goal, two space-relevant fungal strains, *Penicillium simplicissimum* (DSM 1097) and *Aspergillus niger* N402, and an iron-resistance cyanobacterium (LEGE 06123) will be grown together with the lunar regolith simulant and their biomining capabilities will be evaluated. The first step involves biocompatibility assays of each microbial strain with representative EAC-1A Lunar regolith simulant. This is followed by the establishment of bioreactor optimal culture conditions for the biomining process, which is based on the exposure of microbial liquid cultures to the EAC-1A regolith simulant and promote its bioleaching. Biomining potential will be analysed by X-ray fluorescence and X-ray diffraction to provide insight into the final elemental and mineral composition of the leached regolith simulant. Morever, Inductively Coupled Plasma Mass Spectrometry and High-Performance Liquid Chromatography will be used to identify the successfully extracted compounds, such as nickel or iron, on the leachate and the organic acids involved in the process. Results will be applied in the design of a miniaturized biomining setup to be tested aboard a mission on Low Earth Orbit, thus providing valuable insight on the potential of microbial biomining for the sustainable generation of essential resources on a future lunar-colony.

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eP120

Investigating the processing potential of novel Ethiopian agricultural residue Enset/Ensete ventricosum for biobutanol production

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Lignocellulosic biomass is considered a promising raw material for biobutanol production [1]. Enset plant (Ensete ventricosum (Welw.) Cheesman) (false banana) is a herbaceous monocarpic plant that belongs to the Musaceae family [2]. The Enset plant is a potential food source for around 20% of the Ethiopian population. A massive amount of residual byproducts is discarded from traditional food processing in Ethiopia [3]. The aim of this study was to analyze the composition of the Enset plant residue and to examine the processing potential for biobutanol production. The biomass samples were collected from Wolkite, Ethiopia, and examined for their physical and chemical characteristics. The pulverized samples were pretreated with 2% H_2SO_4 (v/v) or NaOH (w/v) and subjected to enzymatic hydrolysis. The enzymatic hydrolysates were then fermented anaerobically by C. saccharoperbutylacetonicum DSM 14923. In samples pretreated with alkali, 49g/l glucose was found in the enset fiber after 36 hours of treatment with cellulase, while 48.2 g/l glucose was found in Midirib after 48 hours. In the case of leaf sheath peel and Mixed enset waste, however, 47 g/l and 46.5 g/l glucose, respectively, were found after 72 hours. For Enset fiber and leaf sheath peel hydrolyzate, a slight difference in glucose concentration between acidic and alkaline pretreated samples was found, which was less than 10 g/l. However, for Midirib and Mixed enset waste hydrolyzate, a 19 g/l and 15 g/l higher glucose concentration, respectively, was obtained from the alkaline-pretreated hydrolyzate. In addition, 10 g/l biobutanol was produced from Mixed enset waste hydrolyzate that has been pretreated with an alkaline solution, which was similar to that obtained when using commercially available glucose. These results suggest that wastes from Enset plant are suitable raw materials for butanol production.

Keywords: Enset plant, Biobutanol, *C. saccharoperbutylacetonicum DSM 14923,* ABE fermentation

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eP121

Engineering microbial communities for the conversion of lignocellulose into medium-chain carboxylates

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Production of medium-chain carboxylates (MCCs) by anaerobic fermentation of lignocellulose with microbial consortia is environmentally friendly, because lignocellulose is an abundant and renewable resource. The two bottlenecks in the production of MCCs from lignocellulose are efficient cellulose hydrolysis and internal production of lactate that is needed as electron donor for microbial chain elongation. Lignocellulose is highly resistant to enzymatic hydrolysis, and so far, no anaerobic lactate producing strain has been discovered, that is able to hydrolyze cellulose efficiently.

A major goal of the ERA CoBioTech project Cell4Chem is to establish and study anaerobic mixed cultures by engineering synthetic consortia consisting of recombinant strains, wildtype strains and enrichment cultures. The strains and cultures have different metabolic functions including cellulose and hemicellulose hydrolysis, lactate fermentation and microbial chain elongation, and are intended to overcome the bottlenecks and produce MCCs from lignocellulosic biomass in a concerted manner.

Recombinant strains of *Ruminiclostridium cellulolyticum* and *Lactococcus lactis* provided by cooperation partners are employed to increase cellulose degradation and lactate production. The synthetic consortia are cultivated anaerobically with defined substrates (cellulose, hemicellulose) and complex biomass (straw) in microtiter plates. The most efficient consortia will be scaled up stepwise from 100 mL batch cultures to continuous fermentation in 1-L-reactors. Growth of microbial biomass (optical density, protein), pH development, substrate consumption and product formation in liquid and gas-phases are measured (HPLC and GC). Community composition and dynamics are assessed based on 16S rRNA gene amplicons, while the functional potential and actual metabolic activity of the consortia is analyzed by metagenomics and -proteomics.

MCC production from lignocellulose is expected to be enhanced by combining recombinant and wildtype strains that complement each other in their metabolic properties to overcome the bottlenecks. Key players and metabolic interactions will be identified and the ecological understanding of such consortia will be extended.

The construction of synthetic consortia using strains with known metabolic traits is a promising approach to improve the anaerobic fermentation of lignocellulose to produce valuable platform chemicals.

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eP122
Employing a terephthalic acid biosensor for screening of potential polyethylene terephthalate (PET) degrading enzymes

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The ongoing pollution and accumulation of plastic waste in marine and other environments poses a problem of increasing importance to our society. As synthetic polymers are degraded slowly and degradation is mainly limited to the mechanical disruption into smaller plastic particles, efficient means to break down the polymer chains on a molecular level are needed. A promising approach to achieve degradation of plastics is the use of enzymes, which are capable of cleaving the polymer chains, yielding molecules which are more readily degradable or could be used as source materials for further reactions or processes.

One of the most abundant plastic pollutants is polyethylene terephthalate (PET) which is a polyester made up of terephthalic acid (TPA) and ethylene glycol. Because of its poor biodegradability in natural environments, the identification of PET-degrading enzymes – so called PETases – is one major field of interest.

In this work, the focus lies on the screening of enzyme candidates and their host organisms regarding potential PET-degradation activity. Furthermore, it is of interest, whether the original hosts of these enzymes actually contribute to PET degradation in their natural environment or if PET breakdown is merely a promiscuous side activity of the isolated enzymes.

In order to assess PET-breakdown by enzyme candidates *in vitro*, established techniques like (U)HPLC analyses of reaction supernatants are employed. However, the identification in environments such as biofilms of potential PET-degrading communities and organisms poses challenges such as lower detection limits and a less homogeneous distribution of breakdown products and therefore calls for alternative evaluation methods. Here, a bacterial reporter strain shall be employed to assess PET-degradation, not only of isolated enzyme candidates, but also *in situ* of single-species biofilms and microbial communities inhabiting PET substrates.

For this purpose, a fluorescent biosensor reacting on terephthalic acid was established based on *Comamonas thiooxidans* and its responsiveness to different TPA levels in solution was verified down to 50 µM. Additionally, the responsiveness to TPA released from enzyme-mediated degradation of PET was tested. Further efforts are directed towards the transfer of the system to autologous hosts and the application in more nature-like environments.

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eP123
Searching for new plastic-degrading enzymes, bacteria and microbial consortia

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Plastics are broadly used in our daily life, but they are also a main contaminant of our environment accumulating in both the ocean and the land. In the last years, only few enzymes and microorganisms have been identified with the ability to degrade some synthetic polymers. However, more active species and enzymes need to be discovered and characterized in order to enlighten protein adaptation towards the degradation of human-made polymers.

Within this work, new strategies are applied to screen for enzymes, bacteria and communities that can degrade various polyurethanes (PUR), as well as polyethylene (PE) and polypropylene (PP) for which efficient catalysts remain yet undiscovered.

For the discovery of polyurethanases, a series of Hidden Markov Models were optimized from the very few available protein sequences to screen public databases [1]. Additionally, several PET-hydrolyzing esterases identified in our group were assayed on anionic aliphatic polyester-polyurethane dispersions. Subsequently, various enrichment cultures were set up with sediments from the river Elbe (Hamburg, D) on PE, PP and other substrates resembling oligomers of those.

A total of 17 genes coding for putative PU amidases (PUa) and esterases (PUe) were synthesized and are being characterized biochemically. The most promising candidate was able to hydrolyze at least 7 differently substituted carbamates at 40 and 60 °C. The promiscuous PETases were able to efficiently degrade Impranil® DLN and DLN-SD within a broad pH range. Furthermore, microbial consortia within the enrichment cultures were observed to grow on all assayed hydrocarbons as the only available C-source. Few single microorganisms could be isolated. Sequencing results identified microorganisms affiliated to the phylum Bacteroidetes and the class γ-Proteobacteria as the main enriched bacteria. Still, individual genes or gene clusters involved in polymer oxidation and degradation need to be identified.

The results of this research will allow us to comprehend the way nature could be dealing with the consequences of

human-caused environmental pollution, as well as to develop new strategies for the recycling of synthetic polymers.

[1] Danso et al. (2018) Appl. Environ. Microbiol. DOI: https://doi.org/10.1128/AEM.02773-17

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eP124
Rejoining two separated wastes: co-fermentation of syngas and pyrolysis aqueous condensate.
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The need to minimize the anthropogenic impacts on the environment has promoted the surge of a new philosophy of economics, the circular economy, aiming to the valorisation of those resources that would otherwise be considered as waste in a linear economy. Biotechnology plays a crucial role: the ability of microorganisms to transform monomers from waste streams into complex, industrially relevant molecules represents a part of the techno-economical change required for our economy.

The large amounts of lignocellulosic waste produced worldwide represents a great economic opportunity. Technologists of every sort have been trying to develop processes to optimize the use of lignocellulosic biomass: one alternative is pyrolysis. Pyrolysis products are charcoal, bio-oil, pyrolysis aqueous condensate (PAC) and syngas. While charcoal, bio-oil and syngas maintain a high calorific value and can be combusted in CHP plants or used as fuels, PAC has a high water content and high toxicity, preventing its further use [1]. The bioremediation of PAC has been attempted before with single and mixed strain fermentations [1,2,3] but no attempt was done before to ferment syngas and PAC together by a mixed community.

The basic idea of our work is to exploit the resilience and syntropy of interactions within an anaerobic microbial community to minimize gaseous emissions and bioremediate PAC while producing relevant platform chemicals.

This study tested the co-fermentation of syngas and PAC, identifying kinetic inhibitions and the effects of increasing PAC concentrations on the microbial behaviour. The experiments were run in 250 mL serum bottles, incubated at 55°C and stirred at 200 rpm. The bottles were inoculated with 10% inoculum from an anaerobic digester and were pressurized with 21 kPa CO, 6 kPa H2, 26 kPa CO2. Gases uptake and production and metabolic profiles were taken into account to determine the inhibition by PAC on the inoculum.

The results show that co-fermenting syngas and PAC is possible up to high raw PAC concentrations. These data are promising for the development of an integrated process to transform lignocellulosic waste into heat, biofuels and biochemicals whilst minimizing emissions and noxious by-products.

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[3] Hübner et al. (2015). Bioresource Technology. doi.org/10.1016/j.biortech.2015.02.037

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eP125
Engineering hyper-solvent-tolerant *Pseudomonas taiwanensis* chassis strains
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Introduction

Organic solvents within the logPO/W range of 1.5 to 4 are highly toxic for microorganisms [1], but some bacteria such as *Pseudomonads* show a high natural tolerance primarily mediated by solvent efflux pumps. The *Pseudomonas taiwanensis* GRC strains [2] feature improved bioprocess performance and provide a platform for investigation of tolerance mechanisms against organic solvents such as styrene. Further increase of already high host intrinsic solvent tolerance is a key requirement to achieve microbial production of solvents in the future.

Objectives

- Implementation of a solvent-proof cultivation system based on the Growth Profiler platform
- Generation of strains with improved styrene tolerance
- Identification of biochemical solvent-tolerance mechanisms
- Laboratory evolution and reverse engineering of solvent hyper-tolerant strains

Material and methods

- High throughput online growth profiling
- Adaptive laboratory evolution (ALE)
- Whole genome sequencing
- Genetic engineering

Results

We implemented a system for small scale two-liquid-phase cultivation with organic solvents featuring online growth monitoring. The system was designed for the Growth Profiler (EnzyScreen) platform using commercially available glass vials and 3D-printed racks in 24 well format, enabling online growth monitoring of up to 240 cultures in parallel.

This system was utilized for characterization of the *P. taiwanensis* GRC strains revealing that *P. taiwanensis* GRC2 is able to grow in presence of a second phase of styrene in mineral medium without prior adaptation using glucose as a carbon source, whereas *P. taiwanensis* GRC3 is not. An ALE approach aiming at increasing the styrene tolerance of *P. taiwanensis* GRC3 yielded multiple strains capable of growing in presence of a second phase of styrene without adaptation and outperforming *P. taiwanensis* GRC2 in terms of growth. Genome sequencing revealed promising mutations likely to contribute to increased solvent tolerance which were subsequently tested in a reverse engineering approach.

Conclusion

The developed cultivation system is a powerful tool for high throughput strain characterization and ALE experiments involving solvents. Obtained strains from styrene tolerance

ALE show improved performance and revealed promising mutations for reverse engineering approaches.

References

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eP126

Valorization of an aqueous pyrolytic condensate for L-malic acid production with *Aspergillus oryzae* DSM 1863

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Introduction

Currently, malic acid is mainly produced via fossil-based production routes. An important step towards making a microbial production of the acid more economically attractive is the utilization of inexpensive side and waste streams as substrates.

The pyrolytic aqueous condensate (PAC) is formed as an unexploited side stream during the fast pyrolysis of wheat straw, but might still serve as potential C-sources for microbial fermentations as it contains acetol and acetate as its main components. The latter has recently been identified as suitable substrate for microbial L-malate production with *Aspergillus oryzae* [1], which is known to be a natural malic acid producer and characterized by a high tolerance towards pyrolysis products [2].

Objectives

The aim of the study was to evaluate the potential of a detoxified PAC to serve as substrate for growth and L-malic acid production with *A. oryzae*. The influence of the initial medium pH on the acid production should be assessed and a scale-up of the process to a bioreactor should be performed.

Materials & methods

The suitability of the condensate as substrate was first examined in 100 mL shake flask cultures containing 100 % PAC. In a follow-up experiment, the initial medium pH was adjusted to 5.5 using CH_3COOH or H2SO4 to improve product titer. The process was then scaled up into 500 mL bioreactors using H2SO4 for pH adjustment. The acetate consumption and product formation were analyzed via HPLC.

Results

*A. o*ryzae was able to grow in 100 % PAC medium with a maximum CDW of 5.21 ± 0.46 g/L. In a first main culture a malate titer of 3.37 ± 0.61 g/L was observed after 192 h, which was further increased to 9.77 ± 0.55 g/L and 8.29 ± 0.26 g/L by pH adjustment with CH_3COOH and H2SO4, respectively. In the bioreactor fermentations 7.31 ± 0.29 g/L malic acid were detected after 144 h of cultivation, which was 2.7 times higher than in the acetate controls.

Conclusion

This work shows that the PAC is a suitable substrate for L-malic acid production with *A. oryzae*. The acid formation was improved considerably by decreasing the medium pH and the process was successfully scaled up into a 0.5-L bioreactor.

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eP127

Anaerobic digestor sludge from biogas plants as a valuable phytosterol source for biotechnological applications

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Biogas production is a promising technology for energy generation from renewable resources. The main feedstocks in most biogas plants are plant-based commodities from agriculture or organic waste streams. Although a significant amount of the feedstock"s organic fraction is transformed into methane and carbon dioxide by the methanogenic community, many organics cannot be fully degraded and remain in the final digestate. Today, residues from biogas plants are mostly incinerated for heat and energy production or used as fertilizer. For a more sustainable use of digestor sludge, it would be desirable to establish procedures for the downstream utilization of valuable organic compounds remaining in the sludge to be used in biotechnological applications.

Plant sterols are in high demand for the production of pharmaceutically active steroid drugs, as food additives and for the cosmetics industry. Plant-based feedstocks likely introduce relevant amounts of these phytosterols into the biogas process. However, little is known about the fate of steroids under the anoxic conditions of methanogenesis. In this study, we analyzed the fate of sterols under methanogenic conditions by quantifying the contents of phytosterols and other steroids throughout the biogas process. Analyses of samples from different stages of a biogas plant in Germany by organic extraction and GC-FID analyses revealed that relevant amounts of phytosterols and cholesterol (around 0.8 g and 0.1 g (kg dry weight)-1) were introduced into the biogas process. These concentrations remained stable in the active sludge and in the final digestor sludge, suggesting that sterols are not degraded by the methanogenic community. This was confirmed by methanogenic batch cultures containing active biogas sludge, which were spiked with different steroids and incubated for up to four weeks. In a proof-of-principle experiment, dried, phytosterol containing digestor sludge was fed to cell suspensions of *Mycobacterium neoaurum*, an established, industrial producer of precursors of androgenic and estrogenic steroid drugs from sterols. In this setting, *M. neoaurum* produced androstadienedione in the millimolar range.

Our results show that sterols are introduced in relevant quantities into biogas plants but are not degraded under the prevailing methanogenic conditions. Thus, final digestor sludge is an untapped source for these valuable compounds, which could be of interest for the pharmaceutical industry.

eP128
Overexpression of the Calvin-Benson-Bassham cycle regulator CP12 increases hydrogen production in the cyanobacterium *Synechocystis* sp. PCC 6803
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More than 16% of all proteins in prokaryotes might actually be smaller than 100 amino acids and many of them serve important regulatory functions. Those small regulatory proteins (SRPs) play important roles in the control of diverse metabolic pathways by simultaneous binding of several interaction partners and the integration of different environmental and metabolic signals to control their activity. Hence, SRPs might pose an interesting opportunity to metabolically engineer bacteria towards enhanced flux into desired routes (Brandenburg and Klähn, 2020).

In this project, we made use of the so-called "light switch" to obtain control over the Calvin-Benson-Bassham (CBB) cycle of phototrophic cyanobacteria. For this, the small protein CP12 (8.2 kDa; 74 aa) was overexpressed in the model strain *Synechocystis* sp. PCC 6803 by putting it"s gene under control of the strong nickel-inducible promoter of the *nrsB* gene. Under dark conditions, the protein forms a supramolecular complex with the enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK) and thereby inhibits their activity. Both enzymes are important regulatory points of the CBB. Our hypothesis is that the shut-down of the CBB, one of the major sinks for reduction equivalents to fix CO_2, would increase the availability of electrons for the production of molecular hydrogen via hydrogenases.

Indeed, we could detect a significant increase of the production of molecular hydrogen (H_2) in our CP12 overexpression strain CP12::oex in comparison to the WT control. These results were supported by metabolite profiling targeting key intermediates of the CBB cycle such as ribulose-5-phosphate, ribulose-1,5-bisphosphate, glyceraldehyde 3-phosphate and 1,3-bishphospoglycerate. Moreover, the impact on the cellular redox status, i.e. the redox couples NAD(P)H/NAD(P)+ was determined by hydrophilic interaction chromatography (HILIC).

Besides the production of H_2, this approach might prove useful for the production of other value added compounds in cyanobacteria which rely on the availability of reduction equivalents. Given the plethora of uncharacterized small proteins in cyanobacteria, this work might be a hint on a whole unexplored universe of SRPs with potential biotechnological applications.

Brandenburg F, Klähn S (2020) Small but smart: On the diverse role of small proteins in the regulation of cyanobacterial metabolism. Life **10**: 1–15

eP129
The Impact of the Outbreak of Tomatoes disease Tuta Absoluta in Nigeria
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The outbreak of tomatoes disease called Tuta absoluta, locally called 'tomato ebola' in Nigeria. And this resulted to adverse scarcity of tomatoes supply in the various markets of the affected states in the year 2016. The pest is known all over the world, as it originated from South America far back 1912 and having ravaged tomato farms in so many countries of the world till date including Europe, Middle East, Asia and Africa. It entered Nigeria through Niger Republic, via some flying insects called moth that bores into the fruits and stem of the tomato plant, and invaded into farms in Nigeria in Kastina, Kano, Kaduna, Jigawa, Nasarawa, Lagos, Oyo and Ogun States and other tomato producing states . The Infestation by the tuta has ravage and wide out tomato farms within 48 hours. Though various pesticides cannot control the disease as it easily develops resistance. This paper therefore studies the Tuta Absoluta, locally called 'tomato ebola', a leaf miner/moth that bores hole into the fruits and stem causing enormous food loss, the Nigeria experiences of the adverse. scarcity of tomatoes and the Federal Government effort. Farms were visited and market to ascertain the effect on supply, prices and consumption, oral interviewed were conducted. Further research too study the effect and analyzed the losses made by farmers and needs for experts and farmers will need to receive government support to continue sustainable production of fruits for local consumption.
Keywords, Tomatoes , Tuta Absoluta, farmers , Nigeria

eP130
Root-Soil contact areas are critical for rhizosphere characteristics
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Introduction

The root system is providing a favourable habitat for soil microorganisms (Fierer 2017). Moreover, it is crucial for anchoring the plant in the soil as well as taking up nutrients (Ryan et. al. 2016). For these functions, the root-soil interface is an important aspect (Stewart et al. 1999), but open questions remain regarding the microbial life at these interfaces and its relevance for nutrient uptake and plant performance.

Objective

We investigated the root-soil interface using maize genotypes with differing root growth strategies in order to analyse the effect of root-soil contact areas on the microbiota and on plant performance.

Material & Methods

In a pot experiment, wildtype maize plants were compared to the mutant *rth3*, which is deficient in root hair elongation and should thus exhibit less root-soil contact. We visually determined root-soil contact for the two genotypes using pore endoscopy and destructive sampling. Further, we analysed variation in plant performance and the microbial colonization as a function of root-soil contact. Root surface areas with and without soil contact were compared regarding the microbial colonization using fluorescent microscopy.

Results

Significant evidence is present for a lower root-soil and root-root contact within pores from the mutant plants compared to the wildtype plants, indicating the importance of root hairs for the establishment of root-soil contact. Bacteria were only detectable on root surface regions with direct soil contact, no

matter the phenotype. To study how root-soil contact influences the composition of the rhizosphere and root microbiota in these contact areas compared to the bulk soil, a microbial community analysis by amplicon sequencing is ongoing.

Conclusion

Thus, our findings underline the importance of root hairs for the establishment of soil contact as well as the necessity of soil contact for root colonization by microorganisms.

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eP131

Interactions between microbial activity and enhanced benthic weathering of carbonate and olivine in the Baltic Sea

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This project is part of the DAM (Deutsche Allianz Meeresforschung) mission CDR mare – RETAKE, where the overall goal is to study the potential, benefits and environmental risks of oceanic alkalinity enhancement as a means of removing CO2 from the atmosphere.

Our subproject focuses on analysing the microbial community structure in alkalinity enhancement scenarios in the Baltic Sea. Marine sediments are treated with olivine and carbonate minerals in core incubations and mesocosm experiments. We study the interactions and feedbacks between benthic weathering of these minerals and microbial activity. The microbial community composition is analyzed using 16S RNA/DNA tags and the microorganisms belonging to major microbial groups are quantified via CARD-FISH and qPCR. For selected samples, transcriptomics is applied to capture potential shifts in gene expression caused by alkalinity enhancement through benthic weathering.

We expect that changes in the microbial community structure relate to artificially enhanced alkalinity which can be related to, and help explain, the potential changes in benthic geochemistry and alkalinity fluxes from the sediment. Microbial activity and chemical alterations are discussed in this poster in the context of mitigating global climate change.

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eP132

The membrane cardiolipin (CL) phospholipid in *Pseudomonas fluorescens* UM270 plays an important role in promoting plant growth under salt stress

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The membrane cardiolipin (CL) phospholipid plays a fundamental role in the adaptation of bacteria to multiple environmental soil conditions, including saline stress. However, its role is unknown in plant growth-promoting bacteria (PGPB), like *Pseudomonas*. Here, we constructed deletion mutants in two CL synthetase genes, *clsA* and *clsB*, in the PGPB *Pseudomonas fluorescens* strain UM270, and evaluated their role on plant growth promotion under salt stress. *P. fluorescens* UM270 Δ*clsA* and Δ*clsB* mutants showed a significant reduction in CL synthesis, compared to the UM270 wild-type strain (58% Δ*clsA* and 53% Δ*clsB*), and were not affected in their growth rate, except when growing at 100 and 200 mM NaCl. Concomitant with the deletion of *clsA* and *clsB* genes, some physiological changes were observed in the UM270 Δ*clsA* and Δ*clsB* mutants, such as a reduction in the indole acetic acid and biofilm production. On the contrary, an increase in the siderophores biosynthesis was observed. Such changes are under evaluation by using transcriptomics analysis. Additionally, inoculation of the UM270 wild-type strain in tomato plants (*Lycopersicon esculentum* cv ¨Saladette¨) grown under salt stress conditions (100 and 200 mM NaCl), exhibited an increase in root and shoot length, as well as chlorophyll content and dry weight. On the contrary, when each of the mutants (Δ*clsA* and Δ*clsB*) was inoculated in tomato plants, it was observed a reduction in root length when growing at 200mM NaCl, but the shoot length, chlorophyll content and total plant dry weight parameters were significantly reduced either growing under normal or saline conditions (100 and 200 mM NaCl), compared to UM270 wild type-inoculated plants. In conclusion, these results suggest that CL synthesis in *P. fluorescens* UM270 have important roles during promotion of tomato plant growth in normal, but at a greater extent, under salt-stress conditions.

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eP133

Frequency of colonization and bacterial activity in the leaves of *Arabidopsis thaliana* are influenced by plant defense metabolites

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Introduction

Microbial colonization of the apoplast - the interior - of leaves is critical for plant health, but the factors how hosts shape these microbial communities remain largely unknown. A well-studied class of defense metabolites in *Arabidopsis thaliana* (*At*) are glucosinolates (GLs) whose breakdown products, e.g. isothiocyanates (ITCs), can have antimicrobial properties and inhibit leaf pathogens. However, their composition varies across *At* genotypes. *At* NG2, a local population from Jena, and the widely used *At* Col-0 harbor distinct microbial leaf communities and produce mainly allyl-GL and 4MSOB-GL, respectively, making them interesting targets to understand genotype-dependent leaf colonization.

Objectives

We hypothesize that different GLs profiles in *At* have different effects on microbial leaf colonization and thus influence the assembly and activity of microbial communities in the apoplast.

Materials & methods

Mutant line *myb28/29* in Col-0 background and our generated *myb28* in NG2 background do not produce aliphatic GLs. We inoculated all plants with very low levels of individual bacterial strains and evaluated them for disease and growth phenotypes, colonization frequency and bacterial loads. In addition, we tested toxicity of the main ITCs. In ongoing experiments, we are evaluating how aliphatic GLs influence leaf microbiome assembly.

Results

We observed that commensals like *Plantibacter* sp. 2H11-2 colonized less frequently on Col-0 compared to NG2 plants, and we linked colonization frequency to aliphatic GLs in Col-0 since most strains tended to colonize *myb28/29* plants more often. Most strains were slightly more virulent on *myb28/29* compared to Col-0 WT, but independently of bacterial load. We connected reduced colonization to toxicity of 4MSOB-ITC for *Plantibacter* sp. 2H11-2. In contrast, in NG2 aliphatic GLs had no effects on colonization frequency and disease phenotypes. Similar to Col-0, they had no effects on bacterial loads but appear to suppress other activities like growth promotion, e.g. *Plantibacter* sp. 2H11-2 increased leaf fresh weights significantly more in NG2 *myb28* than NG2 WT.

Conclusion

Our results support the hypothesis that *At* defense metabolites play distinct roles in bacterial leaf colonization. While aliphatic GLs in both genotypes might affect bacterial activity, they have different effects on colonization frequency and help to explain differences in plant-microbe interaction phenotypes between Col-0 and NG2.

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eP134
Microbial mineralization of tire-wear particles and its leachates
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Recent studies have shown that tire-wear particles (TWPs) resulting from abrasion of tires are the major source of microplastics in the environment (Sommer et al., 2018). These particles are composed of natural and synthetic rubber followed by carbon black, sulphur, zinc and several additives like plasticizers or mineral oils. However, the fate of TWPs in the environment remains largely unknown.

Here we investigate if TWPs and its leachate (compounds leached from TWPs) can be degraded by microorganisms. To this end, microcosms were set-up with TWPs obtained from a tire test bench. Microcosms were filled from a Rainwater Retention Basin and the river Wupper located in proximity to the Autobahn and amended with leached TWPs and leachates as sole carbon source. The microcosms were regularly sampled to monitor tire-wear mineralization to CO2 throughout the incubation period. The CO2 evolution was measured with Reverse Stable Isotope Labelling (Dong et al., 2017) through assessing the carbon stable isotope ratio with an Isotope Ratio Infrared Spectrometer.

During the incubation period of 230 days, significant amounts of CO2 evolved in microcosms with leached tire-wear in comparison to abiotic (without bacteria) and biotic controls (without tire-wear). A similar degradation activity was observed in microcosms incubated with the leachate. These results indicate that microorganisms can mineralize tire-wear particles and leachate.

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eP135
Early bacterial plant colonizers influence plant phenotypes and final microbial community outcome in a plant genotype-dependent way
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Introduction

Variation is omnipresent in nature and plays an important role in evolution. Plant-associated microorganisms can strongly alter plant phenotypes and in nature are highly variable between individual plants. Therefore, they have the potential to underlie some of this variation.

Objective

We tested the hypothesis that phenotypic variation in plant populations is caused in part by stochastic colonization processes, including the heterogeneous nature of soil, a main inoculum for plants.

Material & Methods

We identified highly plant-adapted bacterial strains by enriching them from a leaf-derived inoculum on two wild *A. thaliana* genotypes from Jena and the reference Col-0. Bacteria that established on the plants from a single to a few cells were chosen as efficient early colonizers, namely *Pseudomonas sp*., *Xanthomonas sp*., *Curtobacterium sp*., *Plantibacter sp*., and *Rhodococcus sp.* We then tested how increasing their abundance in the soil inoculum influences heterogeneity of the microbial community of mature leaves and plant phenotypes (rosette size and *Pseudomonas syringae DC3000* infection) of one wild *A. thaliana* genotype (NG) and Col-0.

Results

Surprisingly, increasing the abundance of individual strains in the soil increased the variation of microbial communities of mature leaves relative to plants without an early bacterial application. *Pseudomonas sp*. soil treatments resulted generally in control-like leaf microbiomes. The microbiomes with *Curtobacterium sp.* or *Plantibacter sp.* treatments on the other hand were the most variable, whereby they sometimes were similar to the control but often different. Col-0 plants inoculated with *Pseudomonas sp.* and *Plantibacter sp*. show more consistent and less *Pst DC3000* infection. In NG plants this occurred only in plants inoculated with *Pseudomonas sp.*, which also had bigger and less variable rosette diameters. In Col-0 bacterial treatment tended to increase the variation of rosette sizes compared to the uninoculated control independent from the identity of the enriched bacterium.

Conclusions

We observe that early colonization of highly efficient leaf colonizers can influence the plant phenotypes and final microbial communities depending on the plant genotype. However, observed effects are not always inoculum specific and can vary within the same treatment, suggesting that other processes besides microbial interaction and competition also play an important role.

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eP136
From water into sediment - tracing freshwater cyanobacteria via DNA analyses
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Since the beginning of the Anthropocene, lacustrine biodiversity is influenced by climate change and human activities. These factors advance the spread of harmful cyanobacteria in lakes around the world, which affects water quality and impairs the aquatic food chain. In recent times, sedimentary ancient DNA (sedaDNA)-based studies have been used to probe centuries of climate and environmental changes and determine how they have affected cyanobacterial assemblages in temperate lakes. To extend the resolution of sediment record interpretation, we used high-throughput sequencing, amplicon sequence variant (ASV) analysis, and quantitative PCR to compare pelagic cyanobacterial community dynamics to those of sediment traps (both collected monthly) and sediment communities from a well-studied climate archive and hard water lake with annual mixing in northeastern Germany. The cyanobacterial community composition (CCC), species richness and evenness did not change significantly between pelagic and surface sediments ($p > 0.05$), indicating that the overall sediment-deposited CCC reflected the water column community. However, the total cyanobacterial abundance (qPCR) decreased from the metalimnion down the water column. The aggregate (*Aphanizomenon*) and colony-forming taxa (*Snowella*) showed pronounced sedimentation. Contrastingly, *Planktothrix* was only very poorly represented in sediment traps (meta- and hypolimnion) and sediments, despite its highest relative abundance at the thermocline (10 m water depth) during periods of lake stratification. Our study identified a skewed sediment deposition of single filament taxa, such as *Planktothrix,* and a relative overrepresentation of aggregate and colony-forming taxa in the sediment because of alteration and/or deterioration of DNA during transport. This is relevant for future sedaDNA paleolimnological investigations because *Aphanizomenon* and *Planktothrix* are potential bloom forming and toxin producing taxa and their near-accurate reconstruction from sediments is crucial, especially in lakes lacking long-term monitoring data. These results lay the foundation for pre-Holocene and Holocene cyanobacteria sediment reconstruction devoid of human influences, to decipher the role of natural climate changes on their dynamic and structure. Altogether, our findings highlight the potential of high-resolution amplicon sequencing in investigating the dynamics of past and present cyanobacterial communities in freshwater ecosystems.

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eP137

Strep-CAMP – a computational tool for comparison of unknown *Streptomyces* isolates based on image analysis of colonies

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In the need for novel drugs, the search for new natural product producers in nature has been revived. Soil bacteria of the genus *Streptomyces* still have a high potential to produce novel bioactive compounds[1]. Nevertheless, due to the high diversity of microorganisms in soil, fast and low-cost strategies are needed to identify interesting strains for natural product discovery. Herein, we present the tool Strep-CAMP that allows the comparison of bacteria by content-based image analysis due to shape, size and color of bacterial isolates. Furthermore, the tool serves as database to save and evaluate characteristics, such as the production of bioactive compounds by the organism.

To test the tool, we isolated 100 *Streptomyces* strains from divers soil samples. The growth of all strains was characterized, as were the siderophore and antibiotic production employing a chrome azurol S based assay[2] and cocultivation with gram-positive and gram-negative test strains. The robustness of the tool was tested by scrutinizing the calculations, and assessing the dispersion and biological variation in our dataset. The 16s rDNA was extracted from the sequenced genomes using RNAmmer 1.2[3]. Distances of extracted 16s rDNA were calculated using TYGS[4].

Calculated values for size, color, and shape showed minimal deviation for geometric shapes: 0% for size, 1 – 2% for color, and 11% for shape. Within the dataset, the dispersion of all chosen parameters was low allowing the identification of statistical outliers. On average the variation was low for biological replicates for the parameters color (8.7%) and shape (7.4%), but higher for colony size (24.33%). The biological variation over time (9 months) was higher with similar distribution for color (13%), shape (10%), and size (42%). In conjunction with the 16s rDNA-based classification, content-based image analysis and the information about the production of bioactive compounds can guide the selection of divers strains for further in depth characterization.

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eP139

Thermophilic composting of human feces: fate of human pathogens, antibiotic resistance and virulence genes

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In times of tremendous global risks caused by climate change, which is also endangering food security, practicing a form of sustainable, climate-smart and productive agriculture is of primordial importance. Compost could be one form of sustainable fertilizer, increasing humus, water holding, and nutrients contents of soils. This in turn makes the consideration of different organic resources, now treated as wastes, a worthwhile undertaking. However, the safety of the produced compost with regard to human pathogens, pharmaceutical contents (e.g., antibiotics) and related resistance genes have to be considered. In this context, we have been investigating the effect of up to 154-days thermophilic composting on the hygienisation of a mixture of human faeces, saw dust and toilet paper from dry toilets together with straw and green cuttings.

Compost samples were analyzed with regard to human pathogens, as well as antibiotic resistance and virulence genes, using shotgun metagenomic sequencing. Most pathogens show a decrease in relative abundance from start to end of composting. Strong decreases were observed for the genera *Enterococcus* and *Staphylococcus*, several *Pseudomonas* species, as well as *Alcaligenes faecalis*, *Listeria monocytogenes*, and *Stenotrophomonas maltophilia*. Declines are observed for genes involved in type III, IV and VI secretion systems, as well as the functional groups of quorum sensing, bacterial motility, flagellar assembly, chemotaxis, toxins, invasion of epithelial cells, and biofilm genes of *P. aeruginosa*, *V. cholerae*, and *E. coli*.

Although some individual genes exhibit a different tendency, the total number of resistance genes towards different classes of antibiotics declines in relative abundance. Trimethoprim and sulfamethoxazole resistance genes exhibit strongest decreases of over 90 %. To complement these findings, qPCR analyses were conducted for selected resistance genes that revealed decreases in copy number over composting.

Our data shows that severe human pathogens decrease in relative abundance and also a reduction of genes involved in bacterial pathogenicity. Lower relative abundance of type IV secretion system and antibiotic resistance genes indicate that the potential to spread resistance among the bacterial community is reduced through thermophilic composting. Thermophilic composting of fecal material could therefore serve as a pretreatment to help reduce the risks of fecal-oral contamination and spread of antibiotic resistances.

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eP140

Iron amendment to hydrothermal plumes induces shifts in microbial communities and provide first insights into the microbial organic iron-ligand production

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Iron is an essential micronutrient often limiting the growth of marine microorganisms in wide areas of the world"s oceans. In high concentrations, iron, by contrast, is potentially toxic and usually leads to irreversible cell encrustation followed by cell death. To counteract both, microorganisms have evolved the strategy of producing organic iron-binding molecules, so called iron-ligands, enabling them to improve the bioavailability and uptake of iron as well as to mitigate its potentially toxic effects. Hydrothermal vents are among the major sources of iron in the oceans. These dynamic habitats host a variety of metabolically highly specialized and versatile microbes that not only have to cope with partially high iron concentrations but may also be able to mediate the availability of inorganic hydrothermal iron by actively producing iron-ligands.

However, hardly any information exists to-date describing the impact of increasing iron concentrations on hydrothermal plume microbial communities and their potential to form iron-ligands. We therefore set up microcosm experiments with hydrothermal plume material in artificial seawater along an iron gradient ranging from 0 to 10 mM. We found that the microbial community at low iron concentrations (0.1 to 100 µM) differs significantly from that found in the original non-treated plume sample, allowing a certain group of Epsilonproteobacteria to become dominant (up to 93% of the overall community). The microbial community detected at 10 mM is by contrast more similar to that found in the original plume sample and consists mainly of one gammaproteobacterial group (up to 97% of the overall community). We further analyzed these results in the context of ligand concentrations and structural diversity and found indications for microbially mediated iron-ligand formation. This is the first holistic experimental approach linking studies of hydrothermal vent microbial community composition with the geochemistry involved in organic iron-ligand formation.

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eP142

Functional characterization of the Radical SAM enzyme NirJ involved in heme d_1 biosynthesis

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During denitrification, nitrate is stepwise reduced and finally released as dinitrogen. Nitrite reductases catalyze the second step of denitrification, the one-electron reduction of nitrite to nitrogen monoxide. There are two structurally unrelated dissimilatory nitrite reductases, distinguishable by their use of different cofactors [1]. NirS, a cytochrome cd_1 nitrite reductase, harbors the tetrapyrrole cofactors heme *c* and heme d_1. Heme d_1 is a dioxoisobacteriochlorin with a central iron ion. Compared to other tetrapyrroles, heme d_1 contains two unusual keto groups at rings A and B, as well as an acrylate side chain at ring D [2]. All steps of heme d_1 biosynthesis were elucidated in recent years, except the chemically challenging introduction of both keto groups. The Radical SAM enzyme NirJ might be a possible candidate for this particular reaction. The *nirJ* gene is located together with other genes required for heme d_1 biosynthesis in the *nir*-operon. NirJ catalyzes the removal of the two propionate side chains at tetrapyrrole rings A and B [3].

However, it is unknown whether NirJ is also responsible for the subsequent introduction of the two carbonyl functions. Also, the mechanism of the propionate removal and the actual NirJ reaction product remain unresolved, because the tetrapyrrole seems to form lactones at rings A and B under aerobic extraction conditions. Therefore, esterification experiments prior to aerobic extraction were performed and evaluated via HPLC to elucidate the true nature of the extracted tetrapyrrole products.

Based on amino acid sequence alignments of homologous NirJ enzymes, conserved cysteine residues indicate the presence of a second iron-sulfur cluster besides the Radical SAM iron-sulfur cluster [3]. For further investigation, Mössbauer spectroscopy was used. NirJ and its substrate were co-produced with ^{57}Fe supplementation of the growth medium and co-purified. The obtained Mössbauer spectra led to the identification of a second $[4Fe\text{-}4S]^{2+}$ cluster in NirJ.

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eP143

A multi-level approach to study cyanobacterial summer blooms in the Baltic Sea

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Introduction and Question. In the last decades the occurrence of toxic cyanobacterial blooms in the Baltic Sea has been increasingly observed and attributed to global warming and eutrophication1. In addition to physical parameters, low concentrations of dissolved inorganic nitrogen (DIN) and a surplus of dissolved inorganic phosphorus (DIP) are considered favorable factors to enhance the bloom formation of diazotrophic cyanobacteria2. Under inorganic nutrient depletion, the ability of specific cyanobacterial species such as *Nodularia* spp. to take up DIP or organic phosphorus3 and to control their buoyancy4 may represent an advantage over other aquatic microorganisms. A multi-level study has been initiated to particularly understand the role of DIP as trigger for the occurrence of cyanobacterial summer blooms in the Baltic Sea.

Methods. The study includes three approaches: (1) controlled conditions were applied in laboratory experiments with *Nodularia spumigena* CCY9414 to characterize acclimation mechanisms towards long-term DIP starvation via RNA sequencing data analysis at defined experimental time points; (2) semi-controlled conditions were applied in mesocosm experiments conducted at the Tvärminne Zoological Station (Finland), in which organic (Dissolved Organic Carbon) or inorganic nutrient (DIN) sources were supplied in a 15-day experiment. This experiment aims to identify the members of the phytoplanktonic community that benefit from DIP naturally available before establishment of summer blooms; (3) uncontrolled, field conditions were investigated using an Automated Fixation multi-Sampler Systems (AFISsys) for *in-situ* samplings at the IOW-MARNET research stations (Southern Baltic Sea). This approach allows cyanobacterial field sample processing for "meta-omics" studies to assess their role in the Baltic Sea microbial communities.

Results and Conclusion. Preliminary results from all three approaches of the study will be presented. Particularly, polyphosphate accumulation and transcriptomic profiles of long-term DIP-starved *N. spumigena* CCY9414 cultures will be illustrated. Furthermore, nutrients consumption and chlorophyll-a data from the different treatments of the mesocosm experiment, along with microbial community composition from samples of the first AFISsys deployment in the Baltic Sea will be shown.

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eP144

Changing the atmosphere every 35-minutes: Viral and microbial interactions in a cold-water CO2-geyser with periodic eruptions

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Intro: CO2-driven, cold-water geysers are microbial hotspots that can be completely fueled by carbon fixation, yet the influence of viruses and microbial symbionts on these communities is little understood.

Objective: Here, we decipher the microbial and viral dynamics of the cold-water geyser Wallenborn (Germany, Eifel).

Methods: Subsurface fluids discharged via CO2-eruptions were sampled over ten consecutive days and analyzed by applying genome-resolved metagenomics and viral predictions.

Results: We identified 17,105 viral genomes (>3 kb) across the 27 metagenomes showing strong fluctuations of the viral community over time. After dereplication, 243 viral genomes could be linked to 135 microbial hosts via CRISPR-spacer to protospacer matches. Genomes of Campylobacterales, Nitrosomonadales and *Bacteroidetes* hosted a significant portion of the viral matches. The microbial community was rich in the potentially symbiotic Gracilibacteria, a phylum known for their alternate coded genomes. Using co-correlation networks of 125 high-quality metagenome-derived genomes, we identified potential interactions of microbial species in the 27 metagenomes and linked putative hosts to the Gracilibacteria.

Conclusion: Overall, these analyses demonstrate a complex interplay of microorganisms and viruses that sustains the biota in an extreme environment high CO2 concentration, undergoing CO2-discharge and rapid pressure changes in approximately 35-minute intervals.

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eP145

Identification of a novel, putative metal-free hydrogen-converting enzyme drawn from a deep-sea hydrothermal vent metagenome

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Molecular hydrogen can be an important energy source for microorganisms thriving in various habitat types, especially in those exhibiting elevated hydrogen concentrations such as deep-sea hydrothermal vent systems. The enzymatic interconversion of molecular hydrogen to protons and electrons is catalyzed by hydrogenases. So far, all known hydrogenases have been described as metalloenzymes, harboring nickel and/or iron atoms at their respective hydrogen-binding sites and requiring a complex maturation apparatus.

By means of an activity-based screen, we recovered a fosmid clone from a hydrothermal vent derived metagenomic library exhibiting hydrogen uptake as well as hydrogen evolution activity. The respective metagenomic insert, however, does not show any homologies to known hydrogenase genes in common public databases. Partial purification of protein extracts revealed that the putative novel hydrogen converting enzyme is located in the membrane of the host in which it is recombinantly expressed (a mutant of *Shewanella oneidensis* MR-1). Stability tests of the respective protein extracts suggest a high oxygen tolerance, wide pH-range and high temporal stability of the metagenomic enzyme. In order to identify which of the five open reading frames encoded on the metagenomic insert confer the hydrogen uptake and evolution activities, PCR- and restriction-based subclonings of the insert were performed. Subsequent activity-measurements of the resulting subclones indicate that a single open reading frame suffices to obtain the activity of the original fosmid clone. The

respective amino acid sequence does not exhibit any motifs which are typical for the coordination of metal atoms and may indicate that this gene encodes a metal-free hydrogenase enzyme. In iron and/or nickel limited habitats a metal-free hydrogen-converting enzyme, which does not require extensive maturation processes, could serve as a valuable tool for the utilization of hydrogen by diverse microorganisms.

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eP146

Microaerobic degradation of xylene: an enrichment approach coupled with genome-resolved metagenomics

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Introduction

Among aromatic hydrocarbons xylenes are considered one of the most common environmental contaminants. Since many microorganisms can use xylenes as source of carbon and energy under aerobic conditions, biodegradation of the contaminated environments is usually an obvious solution. However, in subsurface environments the availability of oxygen is always restricted.

Objectives

We investigated microbial communities of enrichment cultures degrading xylenes under either aerobic or microaerobic conditions to reveal differences between degrading bacterial populations.

Materials & methods

Triplicate aerobic and microaerobic enrichment cultures were set up with xylene isomer mixture and inoculated with groundwater sample of the Siklos xylene-contaminated aquifer (Hungary). Composition of the enriched bacterial communities was revealed by Illumina 16S rDNA amplicon sequencing, strain isolation and whole-genome sequencing of selected isolates. In the case of a microaerobic enrichment, genome-resolved metagenomics was applied to uncover the metabolic capabilities of the uncultivated community members.

Results

The Illumina 16S rRNA gene amplicon sequencing based analysis of the enriched bacterial communities revealed that members of the genus *Sphingobium* were only abundant in the aerobic enrichments, while presence of the genera *Rhodoferax* and *Azovibrio* was characteristic of the microaerobic enrichments. Members of the genus *Pseudomonas* were the most dominant in both type of enrichments, but significantly higher dominancy of these bacteria could be observed in the aerobic cultures. However, identification of bacterial isolates revealed that different lineages of the genus *Pseudomonas* were dominant in the microaerobic enrichments than in the aerobic cultures. From metagenomics data of a microaerobic culture we were able to assemble 12 high-quality bacterial genomes, including a *Rhodoferax*-related genome as well. This genome encoded a toluene-monooxygenase gene cluster and a complete *meta*-cleavage pathway for the degradation of monoaromatic compounds.

Conclusions

Our results revealed that distinctly different xylene-degrading bacterial communities evolved under aerobic *versus* microaerobic conditions. Under clear aerobic conditions members of the genera *Pseudomonas* and *Sphingobium*, while under microaerobic conditions yet undescribed members of the genera *Pseudomonas* and *Rhodoferax* were identified as key xylene-degraders.

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eP147

Characterization of the novel styrene-degrading actinobacterium *Gordonia rubripertincta* CWB7

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The ubiquitously distributed phylum *Actinobacteria* belongs to one of the largest and most diverse groups of microorganisms in nature. They show a great metabolic potential, as they are versatile producers of bioactive natural products. Due to this, actinobacteria show a high capability for biotechnological, medical or agriculture applications in general. The bacterial genus *Gordonia* is a member of the actinomycete family. Herein, several species were isolated due to their degradation capability for slowly biodegradable natural polymers, environmental pollutants, or xenobiotics. Moreover, some species exhibit the ability to synthesize or transform industrial-relevant compounds. Since a diverse range of enzymatic reactions are catalysed, which are known to be rare in other organisms, some *Gordonia* species become attractive candidates for biotechnological applications.

Recently, the actinobacterium *Gordonia rubripertincta* CWB2 was isolated from soil by enrichment with styrene as carbon source. Further studies showed its ability to metabolize the plastic precursor styrene and related compounds. In contrast to other styrene degraders, strain CWB2 uses a "hybrid" degradation pathway, which leads to the central metabolite phenylacetic acid, a compound with a high industrial demand. Strain CWB2 also showed its ability to produce ibuprofen from 4-isobutyl-α-methylstyrene in a co-metabolic process. Resultant, related *Gordonia* species like *G. rubripertincta* strain CWB7 became of interest to unlock the species potential regarding biotechnological use. Since an anthropogenic release is caused due to the use of styrene in industry, enzymes involved in styrene-degradation pathways seem to be promising catalysts in biotechnological applications for bioremediation. In contrast to strain CWB2, strain CWB7 shows an altered growth behaviour, allowing easier operations for future applications. In this study, strain CWB7s genome was analysed and compared to strain CWB2. Furthermore, this novel strain was initially characterized with regard to ongoing studies on its styrene-degradation pathway. Overall, this study presents the characterization of the genome as well as its morphological and metabolical properties of the novel styrene-degrading actinobacterium *G. rubripertincta* CWB7. It also aims at the phylogenetic classification of this actinomycete. First whole-cell biotransformations of styrene and related compounds are promising and pointing towards platform chemicals.

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eP150
How did anoxic conditions affect nitrogen fixing Cyanobacteria on early Earth?
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Introduction:

The early Earth"s atmosphere was without free oxygen until the Great Oxygenation Event, thought to have been driven by cyanobacterial oxygenic photosynthesis, ~2.4 Ga. This project investigates the effect of an anoxic atmosphere on cyanobacterial nitrogen fixation rates compared to cultures grown under present day atmospheric conditions.

Objectives:

The effects of an anoxic Archean atmosphere on the growth of the nitrogen fixing Cyanobacterium *Nostoc* sp. PCC7524 was compared to control cultures grown under present day atmospheric levels (PAL) of O2 and CO2. Additionally, we assessed whether the early Archean atmosphere affected the gas diffusion barrier of the heterocyte, consisting of heterocyte glycolipids, as well as nitrogen fixation rates.

Materials and methods:

For this comparison we made use of a variety of techniques including Chlorophyll *a* determination, quantitative real-time PCR, Bradford assay, Glycogen determination, Western blotting, PAM fluorometer for oxygen levels and fluorescence microscopy.

Results:

While no significant changes were observed for growth rates under N-depleted conditions in the experimental and control atmospheres, upregulation of the C- and N2-fixation associated genes, were observed under Archean conditions relative to PAL. This correlated with increased levels of the C-fixing Rubisco protein and O2 production. The glycogen and protein content of the Archean endpoint culture material showed raised levels of these long-term storage compounds compared to those grown under PAL conditions. No significant changes in the heterocyte glycolipid content or composition were observed, although the ratio of heterocytes to vegetative cells was higher in cultures grown under PAL compared to those in the Archean.

Conclusion:

This data suggests that diazotrophic Cyanobacteria were able to fix nitrogen and carbon more efficiently under the anoxic conditions of theArchean, thereby releasing more biologically available carbon and nitrogen into the immediate environment than under PAL conditions. The factthat no significant changes in the heterocyte glycolipid content occurred suggests they are suitable biomarkers for cyanobacterial N2-fixation ingeological records.

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eP151
uBin – a manual refining tool for metagenomic bins designed for educational purposes
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Introduction

Resolving bacterial and archaeal genomes from metagenomes has revolutionized our understanding of Earth"s microbiomes. While automated binning software allows high-throughput generation of metagenome-assembled genomes, the resulting metagenomic bins are frequently fraught with contaminating sequences. Hence, manual genome curation is necessary, which is frequently challenging.

Objectives

We aimed to develop a user-friendly, open-source bin refinement tool that facilitates genome curation through a selection of interactive and interconnected plots revealing discrepancies in GC content, coverage and scaffold taxonomy, guided by completeness and contamination metrics. Consideration was given to make it user-friendly and usable for both non-bioinformaticians and students in classrooms.

Materials & Methods

Here, we present uBin, a GUI-based, standalone bin refiner that runs on all major operating platforms. We tested uBin"s bin curation performance on both simulated as well as real world datasets. Additionally, the viability of uBin as a standalone binner was explored.

Results

When applied to the public CAMI dataset, refinement of bins was able to improve 78.9 % of bins by decreasing their contamination. We also applied the bin refiner as a standalone binner to public metagenomes from the International Space Station and demonstrate the recovery of near-complete genomes, whose replication indices indicate active proliferation of microbes in Earth"s lower orbit.

Conclusions

uBin is an easy to install software for bin refinement and binning of simple metagenomes. Additionally, uBin facilitates communication of metagenomic results to other scientists and in classrooms. The software is open source and available under https://github.com/ProbstLab/uBin.

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eP152
The heterotrophic potential of microbial communities regarding chitin degradation in Arctic deep-sea hydrothermal sediments
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Deep-sea hydrothermal vents are among the most extreme habitats on Earth and represent interesting targets for marine bioprospecting and biodiscovery. Microbial communities at deep-sea hydrothermal vents are often dominated by chemolithoautotrophic microorganisms that use simple chemical compounds as energy sources. However, the full extent of their heterotrophic capabilities is still being

explored. Metagenomic studies already found that previously uncultured heterotrophic microorganisms control the carbon flow in marine and hydrothermal deep-sea sediments through organic matter decomposition. To find new microbial solutions that are urgently needed in the bioprocessing industry, where the degradation of complex organic materials is often a major challenge, novel *in situ* incubators have been developed and tested. Using these we investigated if chitin material causes changes in the microbial community structure in hot marine hydrothermal sediments. Since chitin is one of the most abundant biopolymers in aquatic environments and has a direct impact on carbon and nitrogen cycles it is crucial to understand its impact on the microbial community composition. Here we report on sequence data of *in situ* enrichments on chitin with samples from the Jan Mayen vent field that gives an indication of which groups of microorganisms are promising candidates involved in chitin degradation. We could observe high abundances of Bacteroidetes, Proteobacteria, Thermotogae, Firmicutes and Acidobacteria throughout the whole sequencing data. Furthermore, we identified potential chitin degraders of the candidate phylum KSB1. These microorganisms are of particular interest, because they could neither be cultivated before, nor their role in carbon metabolism was described. Our metagenomic and phylogenetic analyses indicate a relationship of our candidate KSB1 MAGs with MAGs assembled from the Guyamas Basin hydrothermal sediments. Both AAI and ANI analyses clearly point out completely new lineages of KSB1 from Arctic deep-sea sediments. These results indicate that chitin-degrading microorganisms were enriched *in situ* and play a critical role in the sediments carbon cycling. It is suggested that the mentioned microorganisms are important players in organic matter utilization/breakdown in deep-sea hydrothermal vents and the identified novel lineages are likely adapted to dealing with the industrial substrates in the incubation chambers, and thus provide novel sources for enzyme mining.

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eP153

Microaerobic enrichment and description of benzene degrading bacteria

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Introduction

Monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes (BTEX) are the most common contaminants of groundwater. The concentration of dissolved oxygen in the soil decreases rapidly because the BTEX-degrading bacteria use molecular oxygen as a co-substrate for mono- and dioxygenases enzymes require for the hydroxylation and the cleavage of the aromatic ring. The I.2.C-type catechol 2,3-dioxygenase (*C23O*) genes adapted to low oxygen concentrations resulting a large diversity of these genes in such environments. Several members of the family Comamonadaceae are known to play an important role in the biodegradation of petroleum hydrocarbons and showed a possession of diverse *C23O* genes.

Objectives

The objectives of this study was to investigatethose bacteria and their functional genes that can play a critical role in BTEX, specially benzene biodegradation in oxygen-limited subsurface environment.

Materials & methods

Triplicate microaerobic enrichments were established using benzene as the sole carbon and energy source and BTEX-contaminated groundwater of the Siklós site (Hungary) as inoculum. The composition of the bacterial community was investigated by T-RFLP and Illumina 16S rRNA gene amplicon sequencing. Bacterial strains were also isolated from the enrichments. BTEX degradation of the enrichments and selected strains were carried out using GC-MS. The functional genes of some selected strains involved in BTEX degradation were determined. In addition, other experiments necessary to describe a new bacterial species were performed.

Results

Results of the 16S rRNA gene amplicon sequencing have showed that the microaerobic benzene-degrading enrichment was dominated by the members of the genus *Rhodoferax* followed by *Pseudomonas* and *Acidovorax*. A member represents a novel species of the genus *Ideonella*, belonging to the family Comamonadaceae was isolated successfully. The name *Ideonella benzenivorans* sp. nov. was proposed for the strain designated as B7T. A *C23O* gene was found in a gene cluster, encoding for a complete a *meta*-cleavage pathway. This strain is able to utilize benzene, toluene and ethylbenzene as sole source of carbon and energy both under aerobic and microaerobic conditions.

Conclusions

In conclusion, exploration of bacteria and their functional genes involved in BTEX-degradation in hypoxic environment has a current importance, because very few known cultured bacteria are available yet for the purpose.

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eP154

Isolation of Diazotrophic Bacteria which are significant for Sustainable Agriculture

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Microbial biostimulants containing plant growth promoting rhizobacteria are perspective products for sustainable agriculture. Nitrogen fixing bacteria help to reduce amounts of mineral nitrogen fertilizer in agriculture and the negative impact on the environment and humans caused by the misuse of chemical fertilizers. It is important to select appropriate microorganisms for microbial biostimulants production. Microorganisms have to pose a beneficial effect on plants, should be easy applicable on an industrial scale, easily stored and remain stable after application. In this research nitrogen fixing bacteria were isolated from the spring wheat rhizosphere by using different nitrogen free media. Phylogenetic analysis of 16 rRNA sequences showed that isolates belong to *Bacillus* spp., *Paenibacillus* spp., and *Azotobacter* spp. The effect on development and growth of spring wheat of three isolated strains were investigated. It was found that Bacillus sp. MVY-028 has the best effect on spring wheat growth promotion and significantly increased the amount of ammonium in the soil and nitrogen accumulation in grain, furthermore, higher root biomass was determined. *Bacillus* sp. MVY-028 strain was selected for cultivation in 5 L bioreactor and the most suitable medium

and fermentation conditions for biomass production were detected.

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eP155

Site-specific microbial biological soil crust colonization of a potash salt heap gradient

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Globally, potash mining is a huge industry, which continues to grow in demand due to the need for agricultural potassium fertilizer. However, this mining creates large piles of highly saline residual waste, mainly sodium chloride, that are difficult to revegetate and can have detrimental ecological impacts on surrounding areas. Recent studies have found biological soil crusts (biocrusts) on and around these heaps, suggesting some type of resilience to the extremely saline environment. In this study, we propose that (i) the initial establishment of biocrusts with filamentous phototrophs such as cyanobacteria primes the environment for heterotrophic colonization because it reduces potentially toxic features such as high salinity, and (ii) microbial community composition will converge as biocrusts develop regardless of site location. To achieve this, we sampled transects of emerging to established multi-species biocrust communities on and around heaps from two separate sites (Oedesse "OD," Wietze "WT") in Lower Saxony, Germany. We employed chemical analyses and metabarcoding to determine soil properties and prokaryotic community composition, respectively. As predicted, all established biocrusts had the highest chlorophyll and DOC content, alpha diversity, and lowest pH and electrical conductivity (EC), as compared to emerging biocrusts and bare heap material. Community composition was greatly affected by site and developmental stage of biocrusts. On bare heaps, site-specific halotolerant and extremophilic taxa such as *Truepera* (8.8%, WT), *Rubrobacter* (6.4%, WT) and *Halomonas* (12.3%, OD). In emerging biocrusts, we found cyanobacteria (23-24%) made up the largest proportion, with family *Nodosilineaceae* comprising 11-12.3% relative read abundance in both sites. Established biocrusts were very diverse, with common phyla Chloroflexi (17-23%), Proteobacteria (15-26%), Planctomycetes (12-14%) Actinobacteria (7-19%) and Bacteroidetes (7-9%), with ammonia oxidizing family *Pirellulaceae* found in the OD (5.3%) and the WT (2.5%). However, on ASV level, community composition diverged and established biocrusts formed outgroups (using Pearson distance and Ward clustering), while emerging biocrusts and bare heap communities clustered together. These findings show that biocrusts can potentially help change hypersaline heaps into more habitable environments by promoting microbial diversity; however, the specific microbial colonization can be dependent on site location.

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Bio-UGS - Biological conversion of green hydrogen and CO2 to methane in porous underground storages (UGS)

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As the integration of wind and solar energy increases, so does the need for large-volume and efficient storage to balance fluctuations in power generation and demand. Many industrial processes produce CO2 as a by-product which"s impact on the climate must be reduced. Existing natural gas storage facilities offer the potential for a biological methane generation (bio-methanation).

The objective is to investigate the feasibility of biological methane formation in porous UGS. Elevating the microbial conversion of CO2 and H2 into CH4 by hydrogenotrophic methanogens, the existing UGS as well as network infrastructure will stay in operation without any restrictions or additional risks.To quantify the bio-methanation potential, the influence of H2 and CO2/H2- mixtures on microbial processes in UGS were examined by comprehensive literature survey, laboratory investigations and simulation works.

Simulation experiments with representative samples of formation water and rock material of German reservoirs were carried out to study a potential Underground Bio-Methanization process (UBM). Therefore, anaerobic cultivation tests and molecular biological analyses were used. Turnover rates of metabolic processes were determined by gas and ion chromatography. All batches were incubated under H2 and CO2 atmospheres as well as under normal and high-pressure conditions.

Samples from 11 UGS were examined. Microorganisms were detected in almost all of them. Active methanogenic archaea were enriched from three reservoirs and their ability for methanogenesis was investigated and confirmed under various conditions (pressure, temperature, salinity). However, microorganisms of competing metabolic pathways have also been detected (i. e. sulfate reducers and acetogens). It was shown under near in-situ conditions that methanogenesis with autochthon microorganisms is possible in UGS, and favourable as well as unfavourable conditions were identified, such as risks due to competing microorganism groups.

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The rhizosphere microbiome´s role in carbon sequestration of oilseed rape (*Brassica napus* L.) – the CropRhizoSOM project

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The soil microbiome plays a key role in the decomposition, stabilization and formation of Soil Organic Carbon (SOC), discussed with concepts such as the microbial carbon pump in which microbial necromass is the main constituent of freshly formed, stable SOC. Arable agricultural soils store about 10% of the estimate 3500-4800 Pg of Carbon (C) in soils. Globally, tillage erosion stresses the capability of agricultural soils to function, but it has also been shown that eroded soils can rapidly sequester atmospheric C. The objective of our project CropRhizoSOM is to quantify the distribution and dynamics of C in the oilseed rape rhizosphere and bulk soil over several growth stages and link this to the structure and functions of the microbiome.

A pot experiment with an eroded Nudaric Luvisol from Dedelow, NE Brandenburg will be used. Tillage erosion will be simulated by subsoil admixture of 0% (control), 12% (moderate erosion) and 24% (severe erosion) of Bt subsoil horizon to Ap topsoil. 14C radioactive isotope labeling of oilseed rape will be applied to trace plant C fluxes into the microbial biomass, soil respiration and C distribution in soil. Abundance of bacteria, archaea and fungi will be determined by qPCR. Soil enzyme assays, amplicon sequencing and metagenomics will reveal taxonomic composition and functional traits of the rhizosphere and bulk soil microbiome.

Our results suggest that the rhizosphere is a hotspot of freshly assimilated C with approximately three time higher 14C signature in microbial biomass compared to bulk soil during flowering. Lower microbial carbon (Cmic), DNA and gene marker (16S rRNA gene, ITS1) abundance in the simulated high erosion treatment indicate a reduced microbial activity, a main factor for the preservation of existing SOC.

The project CropRhizoSOM will resolve the ecological importance of the plant associated soil microbiome in SOC formation and decomposition in agricultural soils. Further significance of the research is the improvement of modelling of C fluxes in soils, remediation strategies in eroded soils and development of integrated C farming approaches to mitigate climate change.

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Response of Aerobic Methane Oxidation and Associated Methanotrophs to a Step-wise Increase in Ammonium Stress

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High methane emitting environments like wetlands and landfills are facing the effects of elevated nitrogen input (e.g., N runoff from agriculture, waste deposit). Aerobic methanotrophs belong to a unique group of microorganisms capable of using methane as a sole carbon and energy source, chiefly at oxic/anoxic interfaces, and thus play a key role in mitigating methane emissions from various environments. Ammonium-induced effects on aerobic methane oxidation are still contentious considering that both, stimulatory and inhibitory effects were reported. Although ammonium is known to act as a competitive inhibitor for the methane monooxygenase, supply of ammonium can also relief nitrogen limitations, and thus enhance methane oxidation.

Here, we hypothesized that the effect of ammonium on methane oxidation is dose-dependent and leads to a microbial community shift towards an ammonium tolerant methanotrophic community, with continuous exposure to increasing ammonium concentrations. To address our hypothesis, laboratory-scale incubations using a landfill cover soil and freshwater river sediment were performed. Exposing these environmental samples to a step wise increase in ammonium to induce stress (0.5 – 5.0 g NH_4Cl/l, in 0.25 – 0.5 g NH_4Cl/l increments), we detected stimulation of methanotrophic activity at low ammonium levels (< 2.5 g NH_4Cl/l for the landfill cover soil, < 1.75 g NH_4Cl/l in the river sediment), while high ammonium levels (> 2.5 g NH_4Cl/l for the landfill cover soil, > 1.75 g NH_4Cl/l in the river sediment) inhibited the methane uptake rates, in line with our hypothesis. Unexpectedly, methane oxidation was also observed in incubations with up to 5.0 g NH_4Cl/l, albeit with significantly lower methane uptake rates after a prolonged lag period (2 days for both landfill cover soil and river sediment). The *pmoA*-targeted amplicon sequence analysis revealed that *Methylocystis* and specific gammaproteobacterial methanotrophs were favored by NH_4Cl supplemented at higher concentrations. Additionally, a co-occurrence network analysis will be performed to elaborate the interplay between the methanotrophs and non-methanotrophs, in response to ammonium stress.

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eP161

Intracellular and extracellular DNA: An improved method for separation and extraction from diverse terrestrial habitats

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Environmental DNA comprises a mixture of DNA coming from active or dormant cells (intracellular DNA, iDNA) and free DNA that could come from the lysis of dead cells, autolysis and/or active secretion systems of living cells (extracellular DNA, eDNA). iDNA represents the living community at the current time, while part of the extracellular DNA may represent past communities so it has been considered to bias the composition of the living microbial community. Therefore, discrimination of eDNA and iDNA can be a valuable attribute in studying microbial diversity, ecology and functionality. Alawi et al. (2014) developed a novel method to discriminate between these two DNA pools. However, no further systematic studies were done on different terrestrial samples to solve specific problems, i.e., low biomass content, cell attachment to the soil, DNA adsorption to minerals, and cell lysis due to osmotic stress. For this reason, we analysed a broad number of samples (i.e., low and high biomass, low and high organic carbon content, high salinity) to adapt and optimize this methodology to specific sample types. To improve the method, we evaluated the effect of different buffers (sodium phosphate/NaP and EDTA), molarities (120 mM and 300 mM) and a mixture of both to evaluate the DNA recovery. Furthermore, the use of detergents and vortexing to improve the cell detachment was evaluated. Additionally, we used a mock community with halophilic microbes and different salt concentrations to improve the cell lysis due to osmotic stress. To evaluate the efficiency DNA quantification methods such as PCR, qPCR and DNA sequencing were applied. Regarding DNA adsorption, an increasing concentration of NaP and the NaP/EDTA mix positively affected the eDNA recovery. While the use of detergents increased the recovery of iDNA. The combination of NaP/EDTA mix and detergents showed the best results. The halophilic mock community showed that an increase in salt concentration improved the iDNA recovery by avoiding the lysis of the halophilic cells. These results indicate that specific adaptations should be applied to samples from different environments depending on the purpose and design of the study. Finally, we propose adaptations for different types of terrestrial samples according to their composition.

Alawi, M.; Schneider, B.; Kallmeyer, J. A procedure for separate recovery of extra- and intracellular DNA from a single marine sediment sample. J. Microbiol. Methods 2014, 104, 36–42

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eP162

Finding novel biocatalysts in environmental samples using functional meta-proteomics

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Biocatalysts are important for a wide range of industries, such as food processing, pharmaceuticals and biofuels. To increase cost-effectiveness and production capacities for these growing sectors, we need novel enzymes with high conversion rates and pH- and temperature stabilities suitable for the application.

Traditionally, new biocatalysts are identified by the creation of metagenomic expression libraries or by searching for similar structural motifs in a sequence database. The former can be time consuming with low success, the latter is a "what you search for, is what you get" approach that makes it difficult to find truly novel enzymes. To overcome these issues, we recently developed a functional metaproteomic method, in which we combine cultivation-independent -omics methodologies with the immediacy of activity screening. It has the potential to discover all enzymes with a given activity expressed in an environmental community and does not require prior assumptions about the biocatalyst"s structure.

In our approach, proteins are isolated from environmental samples collected in promising habitats. We then utilize two-dimensional polyacrylamide gel electrophoresis to separate the metaproteome. After refolding, activity is directly assessed by in-gel zymography. Active spots can be excised, tryptically digested and analyzed by mass spectrometry. In parallel, we isolate DNA from the very same sample to obtain its metagenome, which is used as reference to reassemble the proteins from the mass spectrometry data. The identified protein can then be obtained recombinantly from a synthetic gene.

We have now applied this method to discover novel amylases, cellulases and hemicellulases, as these enzymes are key to a sustainable utilization of renewable plant-based resources. We extracted protein and DNA from several different habitats, including compost, decaying wood, forest soil and hot springs and were able to identify activity in all of them with our established, robust zymography methods.

Taken together, these results lay the groundwork for a quickly adaptable method. We can now screen multiple enzyme classes in environments that naturally select for desired enzyme properties, making them accessible for industrial applications.

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eP163

Deciphering the electromicrobiology of *Desulfopila corrodens*

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Electroactive microorganisms are increasingly recognized as important contributors to microbially driven processes in ecological, medical and biotechnological settings. For example, *Desulfopila corrodens* strain IS4 has the ability to oxidize elemental iron and couple this to sulfate reduction or a methanogenic partner[1]. Such anaerobic corrosions can cause serious economic problems, e.g. in oil production, but at the same time serve as important model systems to understand extracellular electron transfer. In this study, we want to understand how *D. corrodens* IS4 performs extracellular electron transfer when coupled internally to sulfate reduction or externally to a methanogenic partner. We grew *D. corrodens* IS4 on Fe(0) granules as pure culture under sulfate reducing or as co-culture with a methanogen under methanogenic conditions. Under sulfate reducing conditions approximately twice as much Fe(0) was oxidized compared to methanogenic conditions. Interestingly, hydrogen production from Fe(0), which also occurs abiotically, was increased twofold in the presence of strain IS4 with subsequent rapid consumption. Under methanogenic conditions, the presence of IS4 increased methane production three-fold as compared to experiments when the methanogen was grown alone on abiotically forming hydrogen from Fe(0) oxidation. Currently, we follow the transcriptional response of strain IS4 under sulfate reducing and methanogenic conditions in pure and co-culture, respectively, to understand the molecular mechanisms behind Fe(0) oxidation and stimulation of methane production. This is accompanied by CARD-FISH and SEM analyses to gain insights into the growth dynamics of IS4 (and the methanogenic partner) and the spatial organization of this surface-driven process. In summary, this will help us to understand extracellular electron transfer mechanisms and provide a model system for other iron transformation mechanisms with proposed intermediate hydrogen production such as the recently discovered FeS oxidation to pyrite coupled to methanogenesis[2].

References:

[1]Dinh, Hang T., et al. "Iron corrosion by novel anaerobic microorganisms." Nature 427.6977 (2004): 829-832.

[2]Thiel, Joana, et al. "Pyrite formation from FeS and H2S is mediated through microbial redox activity." Proceedings of the National Academy of Sciences 116.14 (2019): 6897-6902.

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eP164

Spatiotemporal dynamics of AOA-driven nitrification and CO_2 fixation in a perialpine lake

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Large populations of planktonic *Thaumarchaeota* inhabit deep oligotrophic lakes. They are thought to be the key ammonia oxidizers in these freshwater reservoirs and as such responsible for the rate-limiting step in nitrification. In addition, their autotrophic metabolism is hypothesized to influence the carbon budget of these habitats. However, the impact ammonia-oxidizing archaea (AOA) have on nitrogen and carbon cycling in oligotrophic lakes is yet to be quantified. Using FISH and qPCR analyses in combination with ^{15}N-ammonium and ^{13}C-dissolved inorganic carbon (DIC) incubations, we followed spatiotemporal activities of these planktonic AOA in Lake Constance, Germany, which is an important drinking water reservoir for >5 million people. Our previous work has shown that AOA are the numerically predominant ammonia oxidizers in this lake[1]. Stable isotope probing revealed that AOA also incorporated significantly more ^{15}N-labeled ammonium than most other microorganisms at near-natural conditions and oxidized ammonia at an average rate of 0.22 ± 0.11 fmol cell^{-1} d^{-1}. Overall nitrification rates were 6.1 ± 0.7 nmol l^{-1} d^{-1} at the

center of the hypolimnion (85 m depth) and increased by one order of magnitude just below the thermocline (20 m depth). Currently, we combine ^{13}C-DIC incubations with selective inhibition of ammonia oxidation to assess the fraction of AOA-driven dark CO_2 fixation at different depths. Those experiments will be accompanied by CARD-FISH analyses to quantify AOA. Our final goal is to assess the role AOA play in the overall primary productivity of deep oligotrophic lakes.

[1]Herber et al. 2020. A single Thaumarchaeon drives nitrification in deep oligotrophic Lake Constance. Environmental Microbiology 22:212-228.

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eP165
The Degradation of Antibiotics and Reduction of ARGs in Mesophilic and Thermophilic Anaerobic Digestion System

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Antibiotic resistance is one of the ten most important threats to global health according to WHO. Animal agriculture is a crucial sector using antibiotics. Animal manure is a common substrate for large-scale anerobic digestion (AD) systems. Depending on the medical treatment of the animals the manure is often contaminated with antibiotics and antibiotic resistant genes (ARGs).

It was pointed out that AD systems can be potential reservoirs for multi-resistant bacteria considering the continuous load of antibiotics and various resistant bacteria from livestock manure. However, the survival of resistant bacteria from livestock could be reduced in a well-managed AD process. We hypothesize that the management of operational parameters and the application of various post-treatments can enhance the degradation of antibiotics and effectively reduce the abundance of ARGs.

In our research, we investigate the effect of temperature and hydraulic retention time (HRT) on the fate of the three selected antibiotics (sulfadiazine, chlortetracycline and enrofloxacin) and three selected ARGs (*sul2, qnrD* and *tetM*). A mesophilic system, representing the German biogas plants, was compared to a thermophilic system, more frequently used in other countries (e.g. Denmark and Sweden). In each system, five conditions were investigated in triplicates. In the thermophilic system, the methane production was better and the pH was higher and more stable. A process failure was observed at short HRT under mesophilic condition due to the accumulation of volatile fatty acids while the thermophilic system was effective even at short HRT. In addition, a better elimination of ARGs are observed at a higher temperature. The relative abundance of target ARGs showed slight increase at the end of 3rd HRT in mesophilic, whereas in thermophilic system it has never shown an increase. It is also expected that the target antibiotics are degraded faster in thermophilic system than in mesophilic. The temperature, antibiotic addition and HRT had major influences on the structure of the microbial communities.

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eP166
Impact of aspect and climate on soil bacterial community composition along the Chilean Coastal Cordillera

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Soil bacteria play a fundamental role in the pedogenesis and functioning of soil ecosystems. It is well known that they contribute to mineral weathering, organic matter decomposition, nutrient cycling, plant nutrition, and stabilization of soil aggregates. Even so, there is a lack of knowledge about the impact of aspect and climate on bacterial community and their consequences in pedogenetic processes. With this in mind, our main objective is to explore changes in bacterial community diversity and structure along the climatic gradient and different slope aspects and to understand their role in pedogenetic processes. Therefore, soil bacterial communities were analyzed from four sites with similar bedrock, substrates, and two different aspects along the unique climatic gradient of the Chilean Coastal Cordillera. Using the 16S rRNA high-throughput sequencing method, the structure, diversity, and degree of specialization of bacterial communities from soil samples from arid, semiarid, mediterranean, and humid climates were analyzed. The sequencing data obtained were correlated with physicochemical properties. Results revealed seven dominant phyla, which showed an increase of *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*, and *Planctomycetes* from arid to humid climates, while *Actinobacteria* and *Gemmatimonadetes* showed a decrease along the climatic gradient. The highest alpha diversity among the bacterial communities was found in the mediterranean climate. The structure of bacterial community and diversity varied with climate and aspects and besides these changes correlated with soil physicochemical properties such as pH, bulk density, C/N ratio, plant-available phosphorous, clay, and total soil organic content. Higher bacterial specialization was found in the arid and humid climates and on the south-facing slope, likely promoted by more stable soil environments. Identification of specialists was associated with ecosystem functional traits that changed from pioneers accumulating organic matter in the arid climate to recyclers or organic decomposers in the humid climate. These findings suggest that aspects and climate influence the bacterial composition, specialization, and functional capabilities, most of which are involved in pedogenetic processes and soil ecosystem development, providing a new perspective on bacterial compositions and functions.

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eP167
Methanogenic community dynamics in Philippine rice field soil

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Background

Rice field soils are a major anthropogenic source of methane. Straw is commonly used as a fertilizer in commercial rice cultivation, but in particular, the late community responses to the addition of complex carbon are not yet well studied. Here, we aimed to decipher the structural and functional responses of the methanogenic community to rice straw addition during an extended incubation period (120 days) of Philippine paddy soil in anoxic flooded microcosms. The research combined process measurements, quantitative real-time PCR and RT-PCR of

particular bio-markers (16S rRNA, *mcrA*), and meta-omics (environmental genomics and transcriptomics).

Results

Unexpectedly, qPCR and RT-qPCR, but also metatranscriptomics, revealed two major bacterial and methanogenic activity phases defined as early (days 7 to 21) and late (days 28 to < 60 days) community responses, separated by a significant transient decline in microbial gene and transcript abundances. The two methanogenic activity phases corresponded to the greatest transcript abundances of the *Methanosarcinaceae* but differed in the methanogenic pathways expressed. While three genetically distinct *Methanosarcina* populations contributed to acetoclastic methanogenesis during the early activity phase, the late activity phase was defined by methylotrophic methanogenesis performed by only a single *Methanosarcina* population. Mapping of environmental transcripts onto metagenome-assembled genomes (MAGs) revealed a population closely related to *Methanosarcina* sp. MSH10X1 to be the key player in both acetoclastic and methylotrophic methanogenesis. Members of the *Methanocellaceae* were the key players in hydrogenotrophic methanogenesis, while the acetoclastic activity of *Methanotrichaceae* members was detectable only during the very late community response.

Conclusions

In conclusion, the detection of methylotrophy, in addition to the occurrence of acetoclastic and hydrogenotrophic methanogenesis, is a crucial new finding showing that the methanogenic community responses in flooded rice field soils are more complex than previously thought.

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Landslide chronosequences as emerging model systems for soil microbial community succession and greenhouse gas flux research

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Landslides represent the main erosion force in mountain regions around the globe, actively shaping mountain morphology, facilitating sediment and organic carbon export, bedrock weathering, pedogenesis and ecological succession. Landslide soil chronosequences have long been used as model systems for studies of vegetation succession in initial ecosystems, yet the development of belowground microbial communities remained largely underexplored. Consequently, the role of landslide soils in greenhouse gas fluxes remains unknown as microbial activity is the main driver of soil CO_2, CH_4 and N_2O fluxes. In this study, we investigate the succession of microbial communities including bacteria, archaea, fungi and algae in landslide chronosequences (ranging from 3 years to 250 years after landsliding) in the Southern Alps of New Zealand. Microbial communities are investigated on the basis of ribosomal and functional genes using specific primers. Greenhouse gas fluxes will be measured by using soil-gas flux chambers and gas chromatography. Our preliminary results indicate the rising N, TOC and total 16S rRNA gene contents in soils along the chronosequence, corroborating previous studies. Based on 16S rRNA gene taxonomy, the potential for N cycle processes at all sampled landslides was dominated by nitrification with most abundant groups comprising ammonia oxidizing archaea (AOA) of the families Nitrosotaleaceae, Nitrosopumilaceae, Nitrososphaeria. Younger soils were dominated by the orders Thermoplasmata Marine Group II and Nitrosopumilales. Older soils were also dominated by the orders Nitrososphaerales Group 1.1c and Nitrosotaleales. Also, sequences of Bathyarchaeota and Methanomassiliicoccales were detected in older soils in higher abundances. Putatively anaerobic groups became increasingly abundant in older soils. The bacterial community was dominated by the orders Burkholderiales, Rhizobiales, Vicinamibacterales, Acidobacteriae Subgroup II, Gemmatales, Salinisphaerales.

In the view of increasing anthropogenic influences and climatic changes caused by global warming, soil is becoming a critical resource in the future world. Landslides offer an ideal model system for studying soil development processes in different climates and bedrock types.

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eP169

Phthaloyl-CoA decarboxylase, a key enzyme in the anaerobic degradation of plasticizers

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The environmentally relevant xenobiotic phthalic acid esters (PAEs) are annually produced at the million ton scale and are mainly used as platicizers that are non-covalently incorporated into high-molecular weight polymers such as polyvinyl chloride[1]. After migration of PAEs into the environment, microbial hydrolysis via esterases into respective alcohols and phthalates occurs under aerobic and anaerobic conditions. Denitrifying and sulfate-reducing bacteria activate PA to the possibly most instable CoA ester present in nature – phthaloyl-CoA (half-life ~7 min), followed by decarboxylation to the central intermediate benzoyl-CoA catalyzed by the UbiD-like phthaloyl-CoA decarboxylase (PCD)[2]. Among UbiD-enzymes it is unique in using a CoA-ester for the difficult dexcarboxylation reaction at an aromatic ring.

Here, we present the cryo-electron microscopy structure phthaloyl-CoA decarboxylase at 1.95 Å, that contains a prenylated FMN (prFMN), Fe2+ and K+ as cofactors. Based on structural and kinetic data by the use of inhibitors we postulate an unprecedented 1,3-cycloaddition mechanism via covalent catalysis at the prFMN cofactor. The unique active site architecture guarantees that decarboxylation precedes rearomatization of a covalently bound carboxy-diene intermediate.

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eP170

Functional diversification of *Staphylococcus aureus* on the skin of atopic dermatitis patients and healthy individuals

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Staphylococcus aureus is a common commensal colonizer of the human skin and nose, but has been often associated with skin diseases. One of the most prevalent is atopic dermatitis (AD), which is characterized by chronic and recurrent inflammatory infections. AD has been associated with dysbiosis of the skin microbiome typically characterized by a reduction of microbial diversity, accompanied by an increase in *S. aureus* abundance. Recent studies have reported that AD skin harbors specific clonal complexes (CCs) of *S. aureus* rarely detected in healthy skin. However, functional implications of CCs and consequences for AD development and progression are still unclear. It is also not clear whether these CCs are specifically pathogenic AD-causing strains or newly emerging variants from commensal strains.

Here, a set of 48 *S. aureus* strains from skin and nose of AD patients and healthy individuals were physiologically characterized and sequenced. First tests using the API® ID 32 STAPH and 50 CH series already indicated a strongly personalized pattern of *S. aureus* strains, as well as a differentiation in response to the disease status of the subjects. Whole genome phylogeny confirmed this observation, however when isolates from the same subject were analyzed, a high degree of similarity of *S. aureus* indicated a transmission between nose and other skin sites. This was further proven by Average Nucleotide Identity (ANI) and core genome Single Nucleotide Polymorphism (SNP) analyses. Functional differences between strains from healthy and AD-affected individuals differed mostly in the presence of virulence factors (VF). Out of 199 VFs identified, the staphylococcal enterotoxin SEB, enterotoxins SETs, and clumping factor A (which can enhance cutaneous binding and exacerbate AD infections) preferentially occurred in AD strains. In contrast, all strains possessed 8-15 antimicrobial resistance genes with no clear differentiation between strains from AD patients or healthy individuals. Further study needs to be performed to investigate consequences of genotypic variations for the phenotype of *S. aureus* strains under different conditions present at the skin of healthy individuals and AD patients.

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eP171

The type VI secretion system is involved in phenotypic heterogeneity and interbacterial interaction of *Photorhabdus luminescens*

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Secretion systems are protein complexes widespread among bacteria. Recently, the discovery of the type VI secretion system (T6SS) and its potential in application for plant protection strategies has received more attention. T6SSs are membrane-bound, contact-dependent nano weapons with a broad range of functions regarding bacteria-bacteria or bacteria-host interactions and competition. It is found in approximately 25% of all Gram-negative bacteria. Through bioinformatic analysis, we identified several T6SS related genes in the genome of *Photorhabdus luminescens*. *P. luminescens* is a Gram-negative entomopathogenic enterobacterium characterized by phenotypic heterogeneity and it is a model organism for bacteria-host interactions. It is characterized by two phenotypically distinct cell forms: i) the symbiotic and pathogenic cell form (1° cells) interacting with entomopathogenic nematodes for infecting insect larvae, and ii) the free-living form in the soil (2° cells), which colonizes plant roots. To gain first insights whether the T6SS related genes of *P. luminescens* play a role during its symbiotic or its free-living soil stage, diverse analyses were carried out. In order to identify T6SS related genes in *P. luminescens* we used NCBI blasts and further HMMER, to identify protein domain similarities to known T6SS components. Hereby, two gene clusters, each encoding all core components of the T6SS and four auxiliary clusters encoding several additional T6SS core components scattered on the genome of *P. luminescens* were identified. The deletion mutants of a baseplate component to knockout both T6SSs main clusters (respectively TssA1b and TssA2) were generated. The inactivation of either one of the T6SSs led to an increase of interbacterial competition of 1° cells and shifts in secondary metabolite production analysed by HPLC. Moreover, different motility behaviour of 1° and 2° cells was observed if one of the T6SS was no longer functional. Considering these results, we conclude that *P. luminescens* harbours two active T6SSs with distinct functions in phenotypic heterogeneity and interbacterial interactions. Whether both T6SSs are necessary for fitness, the interaction with the different hosts or plant protection is currently under study.

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eP172

Picolitre droplet cultivation of the unculturable subsurface microbes Candidate Phyla Radiation from groundwater

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Introduction

Cultivation of microbial dark matter residing in complex environments such as the terrestrial subsurface remains challenging. Candidate phyla radiation (CPR), comprising more than 15% of the bacterial domain[1], are abundant in groundwater but mostly lack isolated representatives. CPR are depicted as a monophyletic branch with massive lineage diversity according to the bacterial tree of life[2]. Their ultra-small size and reduced genomes render them with limited biosynthetic capacities thereby indicating a symbiotic or partnership-dependent lifestyle.

Objective

Cultivation and enrichment of the microbial dark matter CPR harvested from groundwater using droplet microfluidics.

Methods

Microbes from groundwater were harvested via filtration and encapsulated in monodispersed pL droplets. Compartmentalization in droplets reduces competition between fast- and slow-growers leaving room for the CPR and their putative hosts to flourish. Cultivation conditions were evaluated with various nutrient supplements and oxygen availability. CPR were quantified at different time

points using qPCR. Amplicon sequencing was performed to characterize the microbial population enriched in droplets.

Results

The findings indicated that CPR were successfully cultivated in pL droplets after 30 days incubation. Highest CPR enrichment was achieved with the addition of cell-derived nekromatter as supplement without pre-incubation of R2A in anoxic condition, resulting in an 8-fold increase in absolute abundance when compared to Day 0. Among CPR classes, *Candidatus* Parcubacteria and ABY 1 were particularly enriched. Amplicon sequencing revealed that several phyla, identified as putative hosts of CPR in a metagenomic network analysis, were enriched simultaneously in this condition.

Conclusion

Droplet cultivation was demonstrated to maintain and enrich native CPR from subsurface groundwater. Nekromatter-supplemented condition without R2A pre-incubation was found to be the most efficient in enriching CPR population. Genomic characterization of microbial community in droplets enabled deeper insights into CPR phyla enriched and the potential correlation with the enrichment of putative hosts. Further work focuses on the maintenance of enriched co-culture and investigating the effect of different nekromatter on cultivating distinct CPR classes.

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eP173

Isolation and Characterization of an *Acidiphilium acidophilum* Strain Capable to Grow Lithotrophically with Arsenite at pH 2

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Arsenic (As) is relatively abundant in the earth"s crust, occurring, for example, in the form of metal arsenides or arsenosulfides. In aqueous solutions As is normally found as arsenite (As (III)) or arsenate (As (V)) of which the former is more toxic and mobile. Arsenite oxidizing bacteria have been found in many different environments. Nonetheless, so far no chemolithotrophic acidophilic arsenite oxidizing bacterium has been described. Acidophilic iron and sulfur oxidizers are used in biomining operations to leach metals, and in such operations, arsenic may also be released. Thus, finding new ways to oxidize and immobilize arsenic could be very useful for biomining. Thus, the objective of this work was to isolate and characterize new As (III) oxidizing acidophiles. For that objective, samples from Reiche Zeche mine (Freiberg, Germany) were cultured in enrichments with As (III) and medium suitable for such bacteria. Arsenite oxidation was detected by ion chromatography, and cell growth was observed with a Neubauer chamber under a phase-contrast microscope. Liquid cultures positive for arsenite oxidation were spread on agarose plates, and material from single colonies were cultured again in liquid medium. Of several isolates obtained, strain CRJ1 was investigated in more detail. 16S RNA gene sequencing identified this isolate as an *Acidophilium acidophilum*. The optimum As (III) concentration for growth was 0,5 g/L (6,6 mM), while the pH optimum was 2 and the optimal temperature was 30°C. This new isolate is the first acidophile bacteria that is able to oxidize As (III) chemolithotrophically and at lower temperatures than other acidophile microorganisms that have been described before.

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eP174

An optimized culture based approach for the detection of antibiotic-resistant bacteria in the aquatic environment

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The emergence of antibiotic-resistant bacteria (ARB) in the environment has become one of the most important global health challenges. ARB have been found in surface waters and wastewater (WW) treatment plants but monitoring of ARB is lacking in all currently existing guidelines. However, standardized methods for the detection of ARB exist for clinical investigations of human materials. These methods cannot be used directly for environmental samples because of the magnitude and diversity of bacterial background flora which interferes with the detection of human-relevant ARB. Aim of this study was to propose methodological approaches for future normative standard operation procedures with international relevance, for the culture based detection of clinically-relevant ARB in aquatic environmental samples. CHROMagar and other selective agar plates were used for detection and differentiation of gram-negative bacteria resistant against 3rd generation cephalosporins and against carbapenems, gram-positive vancomycin-resistant *enterococci* and methicillin-resistant *Staphylococcus aureus* in WW and surface water. The main adaptation from standardized cultivation methods included an increase in incubation temperature, which inhibits the environmental background flora on agar plates while desired target species survive. All grown colonies were first grouped according to morphological characteristics and subsequently putative target colonies were further characterized by morphological and physiological (oxidase and catalase activity) differentiation. Final species confirmation was performed using matrix-assisted laser desorption/ionization time of flight mass spectrometry. Odds ratios and quality performance values have been calculated for a data set of 3867 samples. [LF1] Depending on the target species and sample type, sensitivity of up to 100% was achieved, and specificity ranged from 91.1% to 99.7%, while the positive predictive value, negative predicted value and accuracy rate were >90%. A simple, time and cost-optimized testing scheme was developed to effectively detect target isolates in (waste)water samples. The method provides an excellent consistent approach for harmonized and standardized investigations of ARB dissemination within the environment.

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eP175

Microbial diversity in different Opalinus Clay facies at the Mont Terri rock laboratory

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Clay is an important material in many deep geological repository (DGR) designs for high level nuclear waste. As part of a multibarrier system, it is the primary candidate for

buffer material but is also considered as host rock due to its favourable properties. Clay formations have a high sorption capacity for radionuclides, a high swelling pressure that enables sealing of fractures, and a low water activity that suppresses microbial growth. However, excavation activities and perturbation of the host rock create favourable conditions for microbial activity by providing increased space, water and substrates. Thus, effects resulting from microbial activity might be expected in the proximity of a geological repository.

In order to understand the mineral-associated microbial communities and their potential effects on the performance of a DGR in Opalinus clay host rocks, we studied the culture-dependent and -independent (amplicon based sequencing of 16S rRNA) microbial community composition of two different Opalinus clay facies (sandy and shaly) at the Mont Terri underground research laboratory (URL) in Switzerland. Drilling and sampling were performed under anaerobic conditions. Results of the microbial community analysis were related to mineralogical and geochemical parameters of the Opalinus clay.

Unlike the shaly facies, the mineralogy and the microbial community of the sandy facies were highly heterogenic. Differences between the communities of both facies resulted from a higher abundance of Bacteroidota and a lower abundance of Actinobacteria in the sandy facies as compared to the shaly facies. In general, Actinobacteria, Bacteroidota, Firmicutes and Proteobacteria were the dominant phyla in Opalinus clay, thus resembling the microbial communities of other clay formations.

Our data give first insights into the mineral-associated microbial community of Opalinus Clay and the physicochemical key drivers controlling the community structure. A metagenome study will improve our understanding of the community functions and their effects on the performance of Opalinus clay as host rock for a DGR.

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eP176
Influence of operating parameters on the nitrifying community and their activity in biofilters of marine aquaculture plants (RAS)

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Introduction

A key process in the treatment of water in recirculating aquaculture systems (RAS) is nitrification to avoid toxic effects of ammonium and nitrite by biofiltration.

Objectives

The focus of this study is to investigate the influence of microalgae diets and stress due to increasing salinity on the activity and composition of nitrifying biofilms in biofilters of a marine RAS in northern Germany and enrichment cultures in order to contribute to animal welfare and to optimize nitrification processes in these systems.

Materials & Methods

Analysis of the nitrifying community is done by amplicon sequencing, determination of nitrifying activity as well as Fluorescence in situ hybridization (FISH) and electron microscopy (TEM). Furthermore, the influence of changing salinities on the bacterial community of enrichment cultures is investigated.

Results

Bacteriodetes, *Nitrospirae* and *Proteobacteria* were identified as the main phyla in the biofilters and *Nitrosomonas* and *Nitrospira* were the common AOB and NOB with characteristic species. *Nitrobacter* could only be found in low numbers during the start-up phase of one biofilter. The relative abundance of nitrifiers decreased after stocking with fish while the diversity of the whole bacterial community increased. Nevertheless, the activity of both AOB and NOB rose during the same period. After switching from conventional to microalgae feed the activity of NOB still increased, while AOB activity stagnated and the bacterial diversity decreased slightly. Amplicon data from nitrifying enrichment cultures could show a shift in the bacterial community with elevated salinity.

Conclusion

Operating parameters have a direct impact on the diversity and activity of the microbial community and its ecological niche formation. To limit disturbances in the aquaculture system, a detailed investigation of the driving forces will contribute to identification of the key organisms and their impact on the efficiency of nitrification.

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eP177
Effects of plastic surfaces on colonization and interactions within a biofilm community

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Plastic production and associated with it the pollution of plastic waste within the oceans is increasing rapidly (1). Considering the total amount of plastics produced in total annually polyethylene and polyethylene terephthalate constitutes almost 50% (38% PE and 11% PET) (2). While we now have a fairly good understanding of the colonization of those plastic surfaces, our knowledge of the signal exchange during colonization and its effects on the microbial community is rather sparse (3).

In order to shed light on this, we pursue different strategies. First, marine biofilms and marine model organisms (*Vibrio* spp.) are cultivated in the laboratory on different types of plastics like PE and PET and in addition on plastics having modified surface properties starting with previously performed plasma treatment. The transcriptomes of these biofilms are currently analyzed to identify dominant and differentially regulated signaling pathways in the microbial communities concerning surface colonization. Furthermore, the question arises to what extent degradation products and additives of the plastic can influence bacterial quorum sensing (QS) and other regulatory pathways. Therefore, we establish different reporter fusions of the QS systems of our model organism Vibrio gazogenes. These reporter strains

will be used directly in PE and other plastic biofilms to monitor expression and signal perception in vitro.

Microscopic analyzation has shown that especially the colonization of *Vibrio gazogenes* on previously plasma modified plastics like PE and PET is significantly increased compared to untreated plastics. Biofilm formation of *V. gazogenes* on treated surfaces establishes faster and in a higher number of cells. In addition to that, microorganisms from Baltic seawater incubated with untreated as well as modified plastics show similar growth behavior with regard to faster attachment of plastic surfaces after previous modification. Leading to the question to what extend considering the results of the transcriptomic data plastics surfaces can be modified enhancing surface attachment and degradation of PE and PET.

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eP178

Hydrogen recovery from brewery industrial wastewater via adapted and bio-augmented cultures in bioelectrochemical system

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The bioelectrochemical conversion of industrial wastewater into energy carriers such as hydrogen is still challenging due to substrate complexity and our less knowledge of exoelectrogens. In this study, we target the brewery industry as one of the water-intensive industries discharging organic-based wastewater. Using extracted biomass from real effluent samples, parallel isolation of exoelectrogens via ferric-citrate as electron acceptor and anodic enrichment in bioelectrochemical system (BES), at a poised potential of 0 V vs SHE, were investigated. Five consecutive batch cycles, each of 7-10 days, were employed to the BES until maximum current density (recorded when hydrogen is produced) and coulombic efficiency (CE) of 450 mA/m2 and 39% were achieved, respectively. Afterwards, continuous operation was conducted for 22 days, with increased flow rate, resulting in stable and similar current density. Initial, planktonic, and anodic microbial samples (after both the batch and continuous experiments) were collected and analyzed via next generation sequencing to identify the enriched microorganisms. Genus *Azospira* substantially developed with time in all samples, while genera *Christensenellaceae R-7 group* and *Sulfurospirillum* had high abundances in the anodic and planktonic samples, respectively. Moreover, metagenomic analysis was performed for the adapted anodic and planktonic communities, after the continuous operation, to get further insights into the functional annotation of genes. In a second step, a bio-augmentation using the model exoelectrogen *Geobacter sulfurreducens* (OD of 0.5) was examined through batch-mode experiments for 25 days. The maximum obtained current density gradually increased until reaching 2100 mA/m2 (CE of 80%), which was 4.7-fold higher than the control BES inoculated only with the adapted culture.

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eP179

The role of microbial pigmentation as resistance factor against a selection of state-of-the-art and novel decontamination methods

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Since 2019 the global SARS-CoV-2 pandemic is still severely affecting complex and multiple aspects of our human life around the globe leading in a high demand for efficient and feasible decontamination strategies to be applied in the most frequently visited places like public transportation. To prevent the transmission of potential infectious diseases, there is an essential need for research on microbial resistance patterns towards known decontamination strategies as well as for the invention of novel techniques for decontamination. Microbial pigments can be of different structure and color, but all of them can increase the virulence of microorganisms. Research on microbial pigments aimed to elucidate the resistance mechanisms of microorganisms, providing the basis of knowledge for further application of biosynthetic pigments for biotechnology purposes.

This study aims to understand the influence of pigmentation of different groups of organisms on resistance to different stress tests as well as to individually characterize and identify the studied microbial pigments.

We tested five different groups of organisms for their resistance potential towards liquid and nebulized H_2O_2, heat, UV-C, radiation and desiccation. The experiments included cells of *Escherichia coli* and *Staphylococcus capitis*, endospores of *Bacillus subtilis*, bacteriophage MS2 and *Aspergillus niger* spores. Specific organisms that displayed pigmentation were used for further investigations towards biomolecular stress tests and corresponding resistance behavior. Pigments of interest were characterized with UV-spectroscopy and MS spectroscopy.

It could be shown that most of the pigmented organisms were significantly more resistant to all measures tested. Regarding the identification of pigments, the carotenoid staphyloxanthin and its precursors were successfully detected in the ISS isolate *S. capitis* DSM 111179. Melanin was observed in *A. niger* and melanin-like substances in *B. subtilis* spores.

Based on this work we can state that it is a promising technique to include pigmented microorganisms in the evaluation of efficacy tests for decontamination measures. Their increased resistance against environmental factors due to their protective pigmentation validates them to be fitting model organisms for broad range decontamination studies.

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eP180

Occurrence of antibiotic-resistant bacteria in the aquatic environment - Association to the occurrence of antibiotic-active substances

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The WHO specified antibiotic-resistant bacteria (ARB) as a serious threat to modern medicine due to increased resistance and stagnation in development of new antibiotics. To evaluate the influence of wastewater (WW) different sites [ER1] with and without clinical WW were sampled as well as samples of different surface waters with a varying degree of

wastewater influence. The sampling point with the largest share of clinical WW contained WW of a maximum care hospital (>1,000 hospital beds). [ER2] In the clinical-urban study area all hospitals were connected to the southern influent of the investigated WW treatment plant (WWTP) while the northern influent has no health care facilities in its catchment area. Possible correlations between the presence of ARB and antibiotic-active substances (AAS) were statistically analyzed. Significant relationships were shown between the occurrence of ARB and AAS in WW, particularly between clinically used antibiotics and *P. aeruginosa* resistant against 3rd generation cephalosporins (3GCR) as well as ESBL-producing *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp[ER3] . In contrast, no specific clustering was observed for the sampling sites without clinical WW. Relationship scatter plots show a significant influence of clinical WW, which decreased within the sewage system until the water reached the WWTP effluent. Clinical WW contained significantly more multidrug-resistant bacteria than clinically unaffected WW. For all investigated resistance parameters, reduction rates of 3 to 4 Log10 levels could be found within the investigated WWTP. No statistically significant correlation between the concentration of ARB and the proportion of WW in the surface water could be calculated. A significant positive association of ARB and AAS in surface water could be calculated only for the antibiotic erythromycin in association with ESBL-producing *E. coli* and *Acinetobacter* spp. 3GCR. The pathway of a resistant bacterium from the environment to humans is generally possible wherever there is direct contact with a contaminated environmental compartment. It could be shown that treated WW can be a point source of ARB into the environment and thus pose a risk for dissemination of resistance and a risk for human health.

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eP181

Improving phytoremediation of oil polluted soil in the United Arab Emirates using ACC deaminase producing actinobacteria under arid conditions

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Many 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase-producing and oil-degrading rhizosphere actinomycetes were isolated from oil-contaminated soils in the United Arab Emirates. These actinomycetes isolates were further selected based on their ability to solubilize phosphorus, to produce siderophores, and to produce plant growth regulators (PGRs) including auxins and polyamines. The ability of the most-promising isolates to promote the growth of "Bermuda grass" in soil polluted with Light Arabian Crude Oil (LACO) was evaluated under greenhouse conditions. The application of the strongest actinomycete isolate which exhibited the maximum production of ACC deaminase and PGRs promoted Bermuda grass roots and shoots in oil-polluted soils compared to control plants grown in oil-polluted soils but without the application of actinomycete isolate. The application of the actinomycete isolate has also significantly increased photosynthetic pigment contents and promoted Bermuda grass growth characteristics compared with control plants. The application of the actinomycete isolate also significantly reduced the levels of ACC in the roots and shoots compared with control plants grown in oil-polluted soils without the application of actinomycete isolate. The application of actinomycete isolate with the hyper-accumulating plants significantly, reduced the levels of the total recoverable hydrocarbons (TRH) and polycyclic aromatic hydrocarbons (PAHs) and increased the levels of the total recoverable hydrocarbons (TRH) and polycyclic aromatic hydrocarbons (PAHs) in Bermuda grass roots compared with control Bermuda grass treatment without the addition of the actinomycete isolate. In conclusion, I report the production of ACC deaminase by different actinomycetes isolates and their ability to enhance the growth of the hyper-accumulating plants in the presence of LACO through the reduction in the *in planta* levels of endogenous ethylene levels. This study is the first report to demonstrate the potential of oil-degrading rhizosphere actinomycetes to produce ACC deaminase and to improve the phytoremediation capability of the hyper-accumulating plants grown in oil contaminated soil through the production of ACC deaminase and PGRs. The application of these beneficial actinomycetes may help in the phytoremediation of oil-polluted environments in the UAE.

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eP182

Exploration into the terrestrial plastisphere - taxonomy and polymer-colonizing potential of plastic-associated microbial communities in soil

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The concept of the "plastisphere" as a distinct microbial community arose from research on aquatic plastic debris, while the effect of plastics on microbial communities in soils remains poorly understood. Following the assumption that microbial communities in terrestrial habitats are substrate-dependent and influenced by their surrounding habitat, we hypothesize that plastic debris forms a selective habitat in soil in which the community structure is distinct from that of soil without a substantial plastic content. In our first study, we examined the inhabiting microbial communities of two plastic debris ecosystems with regard to their diversity and composition relative to plastic-free soils from the same area using 16S rRNA amplicon sequencing. By using standard plating techniques on media with polyethylene (PE) as only carbon source, a number of plastic-associated bacterial strains were isolated from the sampled material. Furthermore, we studied the plastic-colonizing potential of bacterial consortia originating from both study sites as a measure of surface adhesion to UV-weathered PE using high-magnification field emission scanning electron microscopy (FESEM). The 16S rRNA amplicon sequencing revealed that a high plastic content of the soils was associated with a reduced alpha diversity and a significantly different structure of the microbial communities. The presence of plastic debris in soils did not specifically enrich bacteria known to degrade plastic, as suggested by earlier studies, but rather shifted the microbial community towards highly abundant autotrophic bacteria potentially tolerant to hydrophobic environments and known to be important for biocrust formation. However, two of the newly isolated strains have been found to hydrolyze carbon-carbon bonds in n-Hexadecane (C16), an alkane whose basic chemical structure is identical to PE. In our recent transcriptome study of PE-colonizing bacteria, we aim to elucidate such enzymatic processes further and monitor the plastic-associated gene expression during biofilm formation on plastic debris in soil. In essence, this study suggests the concept of a "terrestrial plastisphere" as a diverse consortium of microorganisms including autotrophs and other pioneering species able to master this hydrophobic habitat. By using transcriptome and metagenome techniques in our

follow-up study, potential plastic-degrading taxa and their utilized enzymes and biochemical mechanisms can be identified.

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eP183

The deep biosphere in lake sediments: One core per habitat – is it enough?

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In the past few decades, the deep biosphere has received a lot of attention as the importance of microorganisms in elemental cycling and remodeling of this specific habitat was widely recognized. Research in the lacustrine environment is the newest addition to this field and of particular interest in the face of climate and environmental change. Lake systems, being smaller in size than their marine counterparts, react more quickly to 1) changing environmental conditions triggered by climate change, 2) different inputs from volcanic or seismic events and 3) productivity changes in the water column and the connected changes in water chemistry. On the one hand, these changes leave imprints in the sediment record but on the other hand the microbial community is still active and evolving after burial. Sampling of the deep biosphere is very demanding in respect to equipment, manpower and the number of cores that can be taken at a time. Therefore, studies often rely on technical replicates only. In this study, we want to describe the microbial community compositions and their habitat along a 3.4 m varved sediment sequence. In doing so, we compare between technical and biological replicates by sampling in replicate from two cores of the same habitat extracted from perialpine Lake Schliersee (Germany). Alongside the analysis of the microbial community by sequencing of the V4 region of the 16S rRNA gene, general microbial activity was assessed *in situ* using ATP measurements and in the laboratory via incubation. Pore water was extracted and preserved on site for IC- and photometric analysis of its chemical components. Furthermore, the sediment was dated and TIC, TOC and TIN were measured to describe the habitat.

In the overall analysis, we will compare the microbial communities among the technical replicates of each core and between cores. We will use the environmental parameters to evaluate any possible differences in habitat and discuss the feasibility of biological replicate sampling of deep lacustrine sediments. Furthermore, we will discuss the community composition in terms of their origin and indicative value of the environment each sampling depth represents.

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eP185

Identification of diclofenac, ibuprofen and carbamazepine degrading bacteria from a groundwater biofilm using shotgun metagenomic sequencing and analysis

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Diclofenac (Dic), ibuprofen (Ibu), and carbamazepine (Cbz) are some of the most commonly detected pharmaceutically active compounds (PhACs) in freshwater ecosystems globally. They can be detected in surface and groundwater, as well as finished drinking water. It has been demonstrated that even at environmentally relevant concentrations Dic, Ibu, and Cbz may have ecotoxicological effects on multiple organisms including rainbow trout, Zebrafish, mussels, and crustacean.

The objective of the present study was to selectively enrich, identify and isolate potentially Dic, Ibu and Cbz degrading bacteria that may be used in the development of sustainable biotechnological approaches applicable in the elimination of these emerging pollutants from aquatic ecosystems.

Selective mineral enrichment cultures containing Dic, Ibu, or Cbz as carbon source were set up, inoculated with biofilm samples and incubated for three months. After each month sub-cultivations into fresh selective media occurred. From the already enriched cultures bacterial community DNA extractions and bacterial isolations took place. Population dynamics that occurred due to the selective enrichments was assessed using shotgun metagenomic sequencing. In the case of three months old enrichment cultures deep metagenomic sequencing and reconstruction of bacterial genomes was performed (genome-resolved metagenomics). Bacterial isolates were identified at species level and tested regarding their pharmaceutical biodegradation capacities.

Metagenomic analyses indicated that members of the genera *Ferrovibrio*, *Hydrocarboniphaga*, *Zavarzinia* and *Sphingopyxis*, representatives of the genera *Starkeya* and *Methylibium,* and bacteria affiliated with the genera *Pseudonocardia*, *Sphingopyxis* and *Rhodococcus* were most probably degrading Dic, Ibu and Cbz, respectively. In parallel to metagenomics, a bacterial strain collection containing 31 isolates from these enrichment cultures was established. Two previously unknown bacterial species were isolated and affiliated to the genera *Nocardioides* and *Ancylobacter*. The genome of the novel *Nocardioides* species was also reconstructed through metagenome binning (100% completeness). Isolates *Stenotrophomonas* sp. DIC_5 and *Rhizobium* sp. IBU_18 were capable of complete Dic and Ibu degradation (1.5 mg/l), respectively. The highest Cbz biodegradation was found in the case of *Brevundimonas* sp. CBZ_3 and *Nocardioides* sp. nov. CBZ_1 isolates (~20% conc. reduction).

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eP186

Abundance, activity and community composition of methane-cycling microorganisms in the rhizosphere of *Fagus sylvatica* and *Pinus silvestris*

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Methanotrophic communities in upland forest soils are quite well characterized in general, but little is known about spatial heterogeneities within forest soils. Moreover, the role of methanogenic archaea in these soils is often neglected. We hypothesize that tree roots cause alterations in the abundance, activity, and composition of methanotrophic and methanogenic communities. To test our hypothesis, we collected bulk soil and rhizosphere samples from *Fagus*

sylvatica and *Pinus silvestris* in a forest near Hartheim, Baden-Württemberg, Germany. DNA and RNA were extracted simultaneously to evaluate the total and the active communities. Quantitative PCR and amplicon sequencing of the functional marker gene *pmoA* were used to examine differences in methanotrophic abundance and community composition. In addition, the abundance of methanogenic archaea was analyzed by qPCR based on the marker gene *mcrA*. Results indicate that the abundance of total and active methanotrophic bacteria was higher compared to methanogenic bacteria. Nevertheless, methanogenic bacteria were detected at all sites, and their abundance was even higher in the active fraction than in the total community. Surprisingly, the abundance of methanotrophic and methanogenic bacteria did not differ significantly between bulk soil and rhizosphere, in contrast to the community composition. Our results also suggest that *Fagus* and *Pinus* harbored distinct methanotrophic communities in the rhizosphere. In addition, the active communities differed from the total communities in dependence on the compartment. Taken together, our results suggest that methane-cycling communities in forest soils show spatial variability in terms of community structure, activity and abundance, which can be related to the presence of tree roots and is further modulated by tree species.

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eP187
Establishment of a bacterial necromass surrogate to assess nutrient-recycling within the subsurface microbial loop

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The biodegradation of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) in aquifers or oil reservoirs, can be limited by nutrient availability. We hypothesize that bacterial necromass fermentation, as part of the microbial loop, is relevant to overcome these growth limitations and, hence, facilitates degradation. To study nutrient-cycling through the microbial loop we first need a surrogate of necromass simulating natural cell death. Important criteria for a suitable necromass surrogate are its bioavailability for necromass fermenters.

We determined necromass degradation rates for the environmental Spirochaete *Rectinema cohabitans* using reverse isotope labelling (RIL) to monitor CO_2 evolution and qPCR for cell counts. *E. coli was* used as model organism for necromass surrogate. The employed necromass was prepared in different ways: mechanic pressure causing shear stress (French press) as a proxy for lysed necromass or pasteurization representing membrane-intact necromass.

Pasteurized *E. coli* necromass was not accessible for degradation (-9.8 µM CO_2 day^{-1}), while cells mechanically disrupted with French press resulted in 3.5-fold higher CO_2 production (43.5 µM CO_2 day^{-1}) in comparison to the control were *E. coli* was added untreated (12.2 µM CO_2 day^{-1}). At this point, it is unclear if *R. cohabitans* is unable to open intact, dead cells or if enzyme denaturation due to pasteurization hampered degradation.

The results indicate the importance of the necromass preparation method for its effects on bioavailability. Finding a necromass surrogate simulating natural cell death is important to investigate nutrient-cycling.

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eP188
Subsurface planctomycetes as sources for novel biotechnological applications

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The CRC AquaDiva revealed that bacteria from the phylum *Planctomycetes* play key roles in subsurface habitats. In the groundwater, they convert ammonium and nitrite in the anammox reaction into dinitrogen gas and contribute significantly to both, carbon fixation and nitrogen release. Furthermore, aerobic planctomycetes seem to be involved in carbon cycling. Planctomycetes are unusual bacteria that divide without FtsZ via budding and enlarge their periplasm for the uptake and digestion of large macromolecules. However, thus far only heterotrophic planctomycetes from habitats richer in organic compounds compared with the subsurface have been studied in detail. Thus, we are wondering if subsurface planctomycetes comprise traits different from their surface counterparts. Using enrichment and deep-cultivation techniques, we obtained the first planctomycetes from subsurface habitats as axenic cultures. We currently analyze their potential for biotechnological applications such as the production of bioactive small molecules.

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eP190
Water and salt dependent, living microbial communities in Late Pleistocene subsurface sediments of the hyperarid Atacama Desert, Chile.

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The extreme aridity of the Atacama Desert is generating challenging environmental conditions for microbial communities in soil such as low water availability, high salt concentrations, and strong temperature variations. This results in the formation of unique surface habitats for specialized organisms which can resist these harsh circumstances. However, despite the growing interest in the microbial communities of the Atacama Desert, no study so far explored the deeper subsurface habitat of these unique soils. Here we report on living microbial communities colonizing the subsurface of these extremely old sediments.

For this purpose, a novel DNA extraction method was applied which enables the separate recovery of intracellular and extracellular DNA from the same sediment sample and thus, allows the distinction between living and lysed cells. Community data from 16S rRNA gene sequencing was then linked to the geochemical soil parameters.

Shifts in microbial community composition correlated mostly to changes in salt content and water availability resulting in three characteristic zones of the profile: In the upper part are the surface zone (2-10 cm depth) and the shallow subsurface zone (20-80 cm depth) within fine grained playa

sediments. These zones are characterized by low microbial diversity including halophilic amplicon sequence variants (ASVs) belonging to the phylum Firmicutes. The deeper subsurface zone (170-420 cm depth), where the underlying alluvial fan sediments appear and salinity diminishes, is characterized by more diverse microbial communities.

The finding of this relatively moderate subsurface habitat within one of the most hostile environments on Earth could hint at a more diverse and active subsurface community in the Atacama Desert, especially in basins where water accumulates during rare rain events. Furthermore, the buffering of the extreme conditions from the surface could underline the significance of subsurface environments for the search of extraterrestrial life on other planets. This study is presenting the potential of iDNA analysis as a method to explore living communities in low biomass habitats which make other approaches like RNA analysis extremely challenging. The current development of this method on terrestrial samples will help to gain an understanding of active processes in extreme environments and is the subject of ongoing research.

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eP191

Interactomic studies of proteins involved in energy metabolism of *Clostridium ljungdahlii*

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The Rnf complex of the acetogenic bacterium *Clostridium ljungdahlii* plays an important role in energy conversion. Previous studies described an increased ATP gain with nitrate supplementation. Therefore, we are interested to elucidate an interplay between the Rnf complex and nitrogen metabolism. *In-vivo* interactomic studies could provide deeper insights into the energy metabolism of *C. ljungdahlii*. The approach is based on affinity purification of protein complexes coupled with mass spectrometry. Here, the RnfC subunit was used as a bait protein to capture Rnf interaction partners as prey proteins. For this purpose, we generated a recombinant clostridial strain harboring a pMTL-vector with the C-terminal strep-tagged *rnfC* sequence. After strain cultivation and expression of the strep-tagged bait protein, we performed an *in-vivo* cross-linking step followed by a mechanical cell lysis. The cell free extract was used for protein purification to capture bait-prey complexes. Finally, the proteomics of the eluted protein composition obtained possible interaction partners. Interestingly, proteins involved in nitrogen and alcohol metabolism show evidence to interact with the Rnf complex.

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eP192

Microplastic biofilms in marine environments around the world – a niche for phototrophic microorganisms

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Diverse microbial communities inhabit microplastics in the marine environment, but little is known about plastic-specific colonizers and their functional niches. With a cross-hemisphere comparison of plastic-colonizing communities we set out to discover common taxonomical signatures on plastics from the Baltic, Sargasso, and Mediterranean Seas, defining the plastic biofilm. Based on high-throughput 16S rRNA gene sequencing, we were able to identify geographically ubiquitous plastic-specific bacteria, which were not detected in the communities on natural particles and corresponding water samples. Most of these bacteria were members of the Alphaproteobacteria, esp. *Rhodobacteraceae,* with closest relatives often described in the context of oil spills or in association with eukaryotes. Overall, they represented low-abundant and so far uncultured microorganisms, indicating that microplastics are a reservoir for rare and understudied bacteria and traits. In order to better understand the traits of plastic biofilms, we explored the metagenomic and proteomic profiles of polyethylene- and polystyrene-associated communities from the Baltic Sea. The data suggest that the degradation of plastic polymers in the Baltic Sea is insignificant. Rather, photosynthesis-related proteins of both eukaryotic and prokaryotic origin were more abundant on plastic as compared to the reference material wood. Microplastics as a relevant niche for phototrophic microorganisms and the associated ecological implications warrant future investigations.

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eP193

Enrichment and isolation of syntrophic hydrogen-producing microbes from the human gut

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The fermentation of carbohydrates is one of the major functions of the gut microbiome, which results in the production of short-chain carboxylic acids and gasses such as hydrogen (H_2) and carbon dioxid (CO_2) (Oh *et al.*, 2003). Accumulation of H_2 disrupts gut function due to thermodynamic limitations of further carbohydrate fermentation and is even harmful to humans health. However, H_2 is an important energy source for gut microbes such as sulfate-reducing bacteria, acetogens, and methanogens (Carbonero *et al.*, 2012), which prevent the excessive accumulation of H_2 in the gut for well-functioning microbiomes. The thermodynamic limitation of H_2 accumulation predicts syntrophy in which carbohydrate degradation can only occur when a microbial partner consumes H_2 simultaneously. Therefore, it is important to investigate how these syntrophic microbes transfer or exchange molecular H_2 in the gut and how they benefit from this transfer. The outcomes of this study are essential to develop an isolation approach without the requirement of the syntrophic partner. Our ultimate goal is to better understand individual human gut microbes (Smith *et al.*, 2021). We utilize a bioelectrochemical system (BES) to actively remove H_2 by oxidation at the anode and to provide an environment that is favored by H_2-producing, carbohydrate-degrading bacteria. Furthermore, our BES provides a close interaction site of this microbe with the anode. For a proof-of-concept, an H_2-producing microbe that has been isolated from the gut, *Christensenalla minuta*, was used. It is known that *C. minuta* can support the growth of other gut microbes (Ruaud *et al.*, 2020). The enrichment and subsequent isolation of H_2-producing microbes in the BES will help further technical BES development as an isolation tool in the field of microbiology.

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eP194

Phage MS2 resistance to physical stressors of space travel

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Introduction

Pathogenic microorganisms can travel with astronauts to space, posing a threat due to weakening of the human immune system to space conditions e.g., microgravity. Therefore, it is expected that viral disease outbreaks on space missions would have devastating effects on astronauts. Since viruses possess high diversity of genomes and shapes, it is crucial to study their stability in space environment. This could lead to strategies to prevent viral transmission onboard spacecraft.

Objectives

Understanding the effect of stressors related to space travel in inactivation of a model virus, phage MS2.

Materials & methods

MS2 was treated in number of stress factors viruses would experience in space travel. Virus inactivation activity was determined for stress response to space vacuum, radiation, desiccation, heat either in buffer or lunar dust simulant EAC-1. The infectivity was determined by plaque assay.

MS2 was treated at 40, 50, 60, 70, and 80°C for 10 and 30 min. The 10-min treatment in LDS was also done.

A sample of MS2 stock solution was transferred to a tube and air-dried. For vacuum-desiccation, vacuum centrifugation was used for drying the same volume of MS2 solution. The dried MS2 was diluted in SM buffer and titer was determined.

MS2 virions were treated with X-rays to different doses (i.e., 100, 250, 500, 1000, and 2000 Gy).

The transmissibility of MS2 particles was tested by dispersing the virus in air while the plates containing the bacterial lawns were placed at distances 0.5, 1, and 1.5 m.

Results

Heat treatments show that MS2 are rapidly inactivated at the temperature higher than 60°C. The lunar dust simulant protected MS2.

The air-desiccation has higher virucidal activity (1.1 log reduction) than vacuum-desiccation (0.6 log reduction).

MS2 infectivity was still detectable even after radiation to 2000 Gy X-rays. LD90 is 2188.27 Gy according to the logarithmic model.

Phage MS2 is transmissible while suspended in air. MS2 might be used as a model for viruses that can spread by aerosols in space missions. This has to be confirmed by comparison to other viruses.

Conclusion

Space conditions are hard to sustain for viruses like MS2. The physical conditions explored all inactivated MS2. LDS protects MS2 from heat inactivation. MS2 can also be transmitted via aerosols. In future, different model viruses will be treated to determine the influence of genome type and shape on the stability in aerospace environments.

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eP195

Whole genome analysis of the first XDR *Mycobacterium tuberculosis* isolate from Algeria.

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Background: The anti-tuberculosis sensitivity testing remains essential for a complete diagnosis and provides better medical management of the patient. However, conventional methods remain complex and time consuming, while automated methods don"t identify all genetic loci associated with TB drug resistance. Whole genome sequencing (WGS) addresses this problem and allows reliable prediction of drug susceptibility phenotype within a short time with exhaustive results for better prescribing.

Material and Methods: In this study, we performed a WGS of the first Extremely Dug Resistant isolate of *M. tuberculosis* (XDR-TB), described in our works on tuberculosis. *M. tuberculosis* isolate was identified by direct examination, according to Ziehl- Neelsen (Z-N) staining and bacterial culture on Lowenstein Jensen (L-J) medium. Identification was confirmed by whole genome sequencing. Genomic DNA was extracted from 1 ml of MGIT culture, using a lysis buffer and protease K. Total DNA was using Promega Mxwell 16 cell DNA purification KIT (AS 1020). A library preparation was performed by using the Nextera xT DNA Library prep kit. All gDNA samples were fragmented, with the nextera XT transposome, and adapters sequences added to the both ends of each DNA fragment generated, then two index primers were added for all samples. Tagmented DNA was amplified with PCR. DNA was sequenced on illumina next seq500 system. Interpretation of the fasQ files generated was done by PHyresse analysis.

Results: The most frequent polymorphism detected in the *kat*G gene was Ser315Thr. In *rpo*B, the Ser450Leu amino acid change. Mutation in *emb*B gene with amino acid change of Met-Val at position 306 was also reported. Mutations were

also reported in *gid*, *rrs* and *rps*l. In *pnc*A gene, conferring pyrazinamide resistance, Ala146Pro was identified. For Fluoroquinolons, Asp94Asn mutation in *gyr*A gene identified. Many other mutations were detected.

Conclusion: Resistance to many drugs, never used in Algeria, were detected by WGS.

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eP196

The effect of allicin on the proteome of SARS-CoV-2 infected Calu-3 cells

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Introduction: Allicin (diallyl thiosulfinate) is the major thiol-reactive organosulfur compound produced by garlic plants (*Allium sativum*) upon tissue damage. Allicin exerts its strong antimicrobial activity against bacteria and fungi via *S*-thioallylation of protein thiols and low molecular weight thiols. Objectives: In this work, we were interested in the effect of allicin on SARS-CoV-2 infected Vero E6 and Calu-3 cells [1]. Materials & Methods: Cell viability, qRT-PCR, plaque titration and glutathione (GSH) assays were applied to study the toxicity of allicin in the Vero E6 and Calu-3 cell lines and the antiviral effect of allicin after infection with SARS-CoV-2. We further used LFQ proteomics to investigate the proteome changes in infected Calu-3 cells and the effect of allicin on the host-virus proteome. Results: Toxicity tests revealed that Calu-3 cells showed greater allicin tolerance, due to >4-fold higher GSH levels compared to the very sensitive Vero E6 cells. Exposure of infected Vero E6 and Calu-3 cells to biocompatible allicin doses led to a ~60-70% decrease of viral RNA and infectious viral particles. In the proteome, SARS-CoV-2 infection of Calu-3 cells caused a strong induction of the antiviral interferon-stimulated gene (ISG) signature, including several antiviral effectors (e.g. cGAS, Mx1, IFIT, IFIH, IFI16, IFI44, 2'5'-OAS and ISG15), pathways of vesicular transport, tight junctions and ubiquitin modification, as well as reprogramming of host metabolism, transcription and translation. Allicin treatment of infected Calu-3 cells reduced the expression of IFN signaling pathways and ISG effectors and reverted several host pathways to levels of uninfected cells. Allicin further reduced the abundance of the structural viral proteins N, M, S and ORF3 in the host-virus proteome. Conclusion: Altogether, our data demonstrate the antiviral and immunomodulatory activity of biocompatible doses of allicin in SARS-CoV-2-infected cell cultures. Future drug research should be directed to exploit the thiol-reactivity of allicin derivatives with increased stability and lower human cell toxicity as antiviral lead compounds.

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eP198

Deep RNA seq analyses in multispecies microbial communities involved in wound and lung infections

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Until not long-ago microbiology and biofilm research has mainly focused on single species microorganisms as the main drivers of infections. However today with an improved understanding on the human microbiome it has become clear that in lungs and wounds complex microbial consortia are drivers of infections. These consortia harbor different microbial species originating from all lineages of the tree of life. To improve our understanding on the infection process with relation to the complex interaction of the different microorganisms, we are establishing artificial communities that serve as model consortia and help us to address scientific questions related to infection, host defense and drug target delivery.

For this, we are working with fluorescence labelled strains of the pathogens *Staphylococcus aureus*, *Staphylococcus epidermidis, Pseudomonas aeruginosa* and the emerging pathogen *Stenotrophomonas maltophilia*. In addition, we have integrated *Candida albicans* into these multispecies consortia and have successfully established mixed species biofilms under flow and static conditions. Employing LSM imagining, a distinct distribution and arrangement of each species in these biofilms was observed.

Dual species and triple species RNA seq analysis of these multispecies biofilms and the application of promotor fusion constructs has shown specific and different expression patterns for each species as compared to the control single species biofilms, suggesting that the different species acknowledge and respond to the presence of others in these complex communities. By applying our multispecies model consortia, we will be able to address research questions regarding complex inter species interactions, interaction with the host and drug target delivery.

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eP199

Purification and characterization of the host defense protein S100A8/A9 (Calprotectin)

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S100A8/A9-heterodimer, called Calprotectin, plays a crucial role in the innate immune response to bacterial infection. Calprotectin has two binding sites for divalent metal ions like zinc, manganese, and also nickel at the interface of the dimer. One site (S1) is comprised of two histidines of S100A8 and four histidines of S100A9. Manganese, zinc, and nickel are bound here in an octahedral geometry. The other site (S2) consists of two histidines of S100A8 and one histidine and one aspartate of S100A9. At the site of bacterial infection, Calprotectin is released by neutrophils or activated macrophages. By binding manganese and zinc with high affinity, Calprotectin reduces the availability of these transition metal ions for bacteria. In consequence of these ions being essential for bacterial growth, Calprotectin exerts bacteriostatic activity (Damo et al., 2013). The sequestration of zinc and manganese also inhibits superoxide dismutases and catalases which function as bacterial virulence factors.

Here we present a new purification protocol which takes advantage of the ability of Calprotectin to bind nickel ions. This allows the use of a nickel loaded affinity column for the purification of untagged protein, simplifying this process and therefore aiding in the research on calprotectin and its role in fighting off bacterial infections.

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eP200
Differential prophage activity within *Clostridioides difficile* isolates
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Introduction & objective

Clostridioides difficile is a worldwide severe cause for nosocomial, antibiotic-associated infections. Studies aim to understand *C. difficile* pathogenicity, especially virulence heterogeneity within this species. It has been observed that *C. difficile* prophages affected toxin production. Further, lysogenic phages might alter *C. difficile* phenotype by harboring virulence-associated genes encoding e.g. antibiotic resistances. In addition, spontaneous prophage release has been detected. However, *C. difficile* phages were mainly isolated after induction by mitomycin C, which significantly increased phage yield in several studies. So far, the dynamic prophage activity within a *C. difficile* strain is almost unknown. In this study, differences in prophage presence and activity between spontaneous and induced release from *C. difficile* isolates were analyzed.

Materials & methods

Phages from four *C. difficile* strains were isolated from culture supernatants after cultivation with and without mitomycin C as inducing agent. Phage DNA was isolated, sequenced and mapped on the host genome. Further, phage particles were analyzed using electron microscopy.

Results

Mapping phage DNA on the host genomes confirmed active prophages in all *C. difficile* strains. Further, isolate-specific differences in phage activity between induced and non-induced conditions were observed. Two isolates belonging to clades 1 and 5 harbor an extrachromosomal phage that showed similar activity under both conditions. The Clade 2 strain possesses a highly active prophage with twofold higher activity after induction. The cryptic clade C-III isolate harbors four prophages with remarkable activities. Two of them were slightly active only when spontaneously released. The third phage was highly active with twofold higher activity under inducing conditions. The fourth, extrachromosomal phage showed strong activity only when spontaneously released (>30x compared to induction). Interestingly, this prophage encodes an antibiotic-associated gene, a penicillinase repressor.

Myoviridae and *Siphoviridae* phage particles were detected, and particle abundance and diversity matched with DNA mapping results.

Conclusion

The results provided first evidence for dynamic activity of prophages within *C. difficile* isolates. Consequently, the effect of prophages on host phenotype might be more dynamic as currently assumed and contributing to *C. difficile* virulence.

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eP201
Adhesion, Motility and Biofilm Formation: uncharacterised gene in the ancient story
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One of the leading gram-negative bacteria associated with nosocomial infections is *Pseudomonas aeruginosa.* The bacterium is frequently implicated in hospital-acquired urinary tract and bloodstream infections [1]. *P. aeruginosa* can adapt to various oxygen gradients due to its versatile metabolism, encompassing denitrification and oxidative phosphorylation. It is able to survive in this environment, thrive and establish a biofilm. Motility, adhesion and biofilm formation ability are essential for the course of infection. In a biofilm, state bacteria can withstand antibacterial agents by many folds compared to their sessile/planktonic state [2].

The function of approximately 40% of *P. aeruginosa* genes is not known to date. This makes the development of effective therapeutic methods against this bacterium difficult. By extension, this also makes understanding the mechanisms of infection, adaptation, and survival challenging.

In my project, I am devoted to the elucidation of hypothetical genes upregulated during different infection conditions. For the characterisation of the genes, I analyse e.g. the motility, adhesion and biofilm formation ability of the mutants under different conditions.

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eP202
***Bordetella bronchiseptica* infection of porcine respiratory epithelial cells is not affected by its dermonecrotic toxin**

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Bordetella bronchiseptica causes respiratory disease in many different animal species, as well as in (immunocompromised) humans. In swine, it contributes to the porcine respiratory disease complex (PRDC) which leads to high economic losses in the pig industry. The dermonecrotic toxin (DNT) is one of the toxins produced by *B. bronchiseptica* and has been associated with the turbinate atrophy in swine atrophic rhinitis. Many studies have been conducted with regard to its effect on osteoblastic differentiation, but only little is known about interactions with porcine respiratory epithelial cells and its role in the pathogenesis of PRDC.

Thus, the aim of our study was to analyze the effects of DNT on porcine respiratory epithelial cells in more detail. For this, we used two different *in vitro* models - the immortalized new-born pig tracheal epithelial cell line (NPTr) and porcine precision-cut lung slices (PCLS). We investigated the effects of DNT on colonization and cell damaging capacities of *B. bronchiseptica* by using a DNT-positive wild type strain and a DNT-deficient mutant strain. During infection, we determined the extent of induced cell damage using a lactate dehydrogenase release assay and the number of cell-associated bacteria by plating cell lysates. Additionally, ciliary activity was monitored by light microscopy (only PCLS) and immunofluorescence staining was performed. None of these assays revealed a clear phenotype of the mutant. Thus, we conclude that DNT does not contribute to the colonizing and cell damaging capacities of *B. bronchiseptica* in infection of porcine respiratory epithelial cells, in contrast to its previously described effects on osteoblasts.

Finally, this knowledge can contribute to further studies on the pathogenicity of *B. bronchiseptica* and it may well be translated to the closely related human pathogen *B. pertussis*.

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eP203
Intracellular *Staphylococcus aureus* induced cell death and cytokine release in human macrophages

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Professional phagocytes play a key role in the host defense against bacterial pathogens by recognizing, engulfing and eradicating invading bacteria. *S. aureus* has evolved strategies to avoid the host immune response, either by hiding inside of the phagocytic cells or escaping from within cells by the induction of a so far unknown type of cell death. We show that two major regulators of *S. aureus* namely AgrABCD and SaePQRS act in concert to facilitate escape of the bacteria after initial uptake by human macrophages. Sae-regulated two-component pore-forming toxins LeukocidinAB (LukAB) and/or Panton-Valentin Leukocidin (PVL) are required for the escape of *S. aureus* from within human macrophages after phagocytosis. Non-toxic strains were able to persist in macrophage without causing cell-death.

LukAB when added exogenously potently trigger the activation of the NLRP3 inflammasome, promote IL-1ß secretion and eventually kill primary human monocytes. Conversely, the role of LukAB, when expressed intracellularly by *S. aureus* and its effects on the cell-death pathways and NLRP3 inflammasome, are not well understood.

We could show that the pore-forming LukAB induced from within macrophages is enough to induce cell-death independent of pyroptosis and apoptosis. The intracellular activation of the NLRP3 inflammasome was also LukAB independent. However, necroptosis seems to be critical since p-MLKL was observed. Thus, intracellular toxin dependent cell-death and cytokine release are due to different mechanisms.

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eP204
CsrA coordinates compatible solute synthesis in *Acinetobacter baumannii* and facilitates growth in human urine

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Introduction

The opportunistic human pathogen *Acinetobacter baumannii* is one of the most concerning nosocomial pathogens and due to its high antimicrobial resistance rates new antibiotics targets are urgently needed [1]. CsrA is a global posttranscriptional regulator widespread in bacteria and known to be involved in different physiological processes, including pathogenicity. Recent studies revealed that deletion of *csrA* in the two *A. baumannii* strains AB09-003 and 17961 resulted in an increase in hyperosmotic stress resistance [2]. To overcome hyperosmotic stress *A. baumannii* accumulates compatible solutes [3]. However, the role of CsrA in compatible solute synthesis remained unknown.

Objectives

This study aimed to investigate the role of CsrA in compatible solute synthesis.

Materials & Methods

A Δ*csrA* mutant of *A. baumannii* ATCC 19606 was generated, growth studies have been performed and the intracellular compatible solute pool was analyzed.

Results

Deletion of *csrA* in *A. baumannii* ATCC 19606 resulted in a mutant that is impaired in carbon utilization and growth in human urine, but growth was not enhanced upon osmotic stress as reported earlier. We tried to unravel these contradictions and thereby observed striking differences in the ability of the different *A. baumannii* strains to cope with hyperosmotic stress. Strains AB09-003 and 17961 were strongly impaired in hyperosmotic stress resistance in comparison to strain ATCC 19606. These differences were abolished by deletion of *csrA* and are in line with the ability to synthesize compatible solutes. In the salt-sensitive strains AB09-003 and 17961 compatible solute synthesis was repressed by CsrA.

Conclusions

CsrA mediates inhibition of compatible solute synthesis in salt sensitive *A. baumannii* strains.

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eP205
Identification of Interaction Partners of *Legionella pneumophila* Mip

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Legionnaire"s disease is a severe pneumonia transmitted by *Legionella pneumophila* containing aerosols. The macrophage infectivity potentiator (Mip) protein on the outer membrane is one of the key virulence factors of this intracellular pathogen. Biochemical and molecular studies have shown that Mip is a basic 24 kDa surface protein (pI 9.8) that possesses an N-terminal signal sequence, which is cleaved off when the protein is transported through the cytoplasmic membrane. The protein consists of two domains that are separated by 6.5 nm long α-helix. While the N-terminus facilitates homodimerization, the C-terminal domain is closely related to the human FK506-binding protein (FKBP12), and like FKBP12, Mip exhibits peptidyl-prolyl-*cis/trans*-isomerase (PPIase) activity. Although the contribution of Mip to pathogenesis ranges from stress tolerance to initiation of cellular infection and dissemination in the body, until now no natural substrates have been identified. In this work, we have conducted diverse interactomics techniques that led to the determination of novel bacterial interaction partners affine to *L. pneumophila* Mip. Furthermore, we have validated these binders by undertaking various biochemical assays. Moreover, we predicted the interacting protein structures by using the deep learning based program AlphaFold v2.0. The results derived from biochemical assays and docking simulations consistently revealed specific protein regions, which mediate the respective Mip interactions.

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eP206
Revisiting *Klebsiella in* critically ill patients: New Insights on DNA Methylation and Phylogenetic Analysis

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Best practices in the treatment of sepsis patients include immediate administration of broad-spectrum antibiotics against Gram-positive bacteria. Consequently, treated critically ill patients have reduced gut microbiome diversity resulting in prolonged recovery times and increased lethality. Several antibiotic-tolerant, persistent bacteria have been identified in feces. Persisting bacteria are subpopulations of genetically identical cells that resist antibiotic treatment and are thus tolerant. This regulation of persistence is of primary interest, as persister will be the "starter bacteria" for the new patient gut microbiome.

In bacteria, most studied epigenetic regulatory mechanisms involve DNA methylation. DNA methylation regulates persistence, gene expression, and biofilm formation. Also, DNA methylation protects the integrity of prokaryotic genomes and plays a role in chromosome replication, nucleotide segregation, DNA repair, and transcription. The DNA methylation is not detectable with standard PCR-based sequencing, but with methylation-sensitive sequencing like PacBio and Oxford Nanopore Technology (ONT). Analytical methods based on mass spectrometry were tested to overcome these limitations. They can quickly detect modified DNA from whole-genome DNA and are relatively cheap. The presented method is based on Ultra Performance Liquid Chromatography High-Resolution Mass Spectrometry (UHPLC-HRMS) analysis of genomic DNA (gDNA) and reveals the effect of treatments with different antibiotics and bile acids on bacterial epigenetics. When treated with meropenem and ursodeoxycholic acid (UDCA), the methylation grade in DNA was increased.

Additionally, we characterized *Klebsiella* isolates from the same intensive care unit For exclusion of a hospital outbreak, the phylogenetic analysis with reference genomes was done. It was based on small and SNPs (kSNP) and significant differences as average nucleotide identity (ANI). Clustering was done with reference-independent, alignment-free methods taking small-scale and large-scale differences within the genomes into account. With affinity propagation clustering (APC), bacterial clusters could be assigned and enabled us to generate hypotheses about epidemiologic relationships within the *Klebsiella* genus.

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eP207
"Novel points of attack" – Exploiting capsule biosynthesis in *Streptococcus pneumoniae* for antibacterial treatment

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Synthesis of a polysaccharide capsule is crucial for pneumococci to resist the immune system during infection. Whereas capsules have been successful targets for vaccines, biosynthetic reactions and mechanisms regulating capsule expression have not been investigated as potential therapeutic targets so far.

Most *S. pneumoniae* serotypes produce capsular polysaccharides (CP) via the Wzx/Wzy-dependent pathway. In this case, CP building blocks are assembled on C_{55}-P at the inner side of the cytoplasmic membrane, translocated and polymerized in a non-processive manner on the exterior of the cell. Enzymes of the LytR-CpsA-Psr (LCP) family are presumed to catalyze transfer and covalent linkage of CP to peptidoglycan under release of the lipid carrier. However, biochemical evidence and molecular details for these reactions are mostly lacking.

Streptococcal capsular biosynthesis reactions are functionally reconstituted *in vitro* using purified recombinant enzymes and substrates. Furthermore, post-translational regulatory mechanisms, particularly phosphorylation, which allow the orchestration of CP and PGN reactions are investigated.

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eP208

Multiplication of the intracellular pathogen *Rhodococcus equi* depends on host ESCRT complexes

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Professional phagocytic cells such as macrophages ingest and kill microorganisms within microbicidal phagolysosomes. The Gram-positive bacterium *Rhodococcus equi* evades killing in phagolysosomes, replicates in host macrophages, and causes pneumonia in immunocompromised humans and especially in young foals. Intra-macrophage replication of *R. equi* is promoted by an extrachromosomal virulence plasmid which encodes virulence proteins including *Virulence associated protein A* (VapA). During infection, VapA permeabilizes the phagosomes for protons, leading to pH-neutral, spacious *Rhodococcus containing vacuoles* (RCVs) with intraluminal vesicles. Avirulent, plasmid-cured *R. equi* can be brought to multiplication by adding recombinant VapA to the growth medium. To understand the biogenesis of VapA induced vacuoles, we analyzed them by mass spectrometry. One enriched group of host proteins belonged to the *Endosome Sorting Complex Required for Transport* (ESCRT). As the ESCRT complex is involved in lysosome membrane repair we hypothesized that they might maintain VapA-permeabilized membranes. Intraluminal vesicles, which are formed ESCRT-dependent during repair might function in nutrient supply for intravacuolar *R. equi*. To test these hypotheses, we analyzed by fluorescence microscopy bacterial multiplication in macrophages treated with siRNAs against ESCRT I or ESCRT III or in macrophages overexpressing dominant-negative ESCRT I or ESCRT III proteins. This analysis revealed that *R. equi* depends on ESCRT III for intracellular multiplication, but surprisingly not on ESCRT I. An *Enzyme-Linked Immunosorbent Assay* (ELISA) for a type I immune response protein validated that the RCV stays intact during *R. equi* infection. Therefore, we wondered whether ESCRT repairs VapA-induced lesions, which would lead to lysosome macroautophagy. In macroautophagy-deficient macrophages treated with siRNA against ESCRT I and ESCRT III *R. equi* multiplied to the same extent as in wildtype macrophages, indicating that repair is not the major effect of ESCRT. These findings suggest that *R. equi* requires ESCRT III, but not ESCRT I, for intraluminal vesicle formation directly at the phagolysosome membrane, which is likely required for intracellular replication.

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eP210

Unravelling the microbial fitness and the acquired co-resistances towards environmental extremes of vancomycin resistant enterococci

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The COVID-19 pandemic displayed how vital international cooperation and preparedness are in a crisis with over 4 million deaths worldwide. Now is the opportunity to prevent future microbial pandemics and respond with awareness, knowledge and preparedness. Antimicrobial resistances (AMR) spread globally via international travel and supply chains and there is an essential need for detecting future pandemic threats like AMR. The WHO listed vancomycin-resistant enterococci (VRE) with high priority on the global priority list of antibiotic resistant bacteria. As a cause of endemic hospital outbreaks worldwide, VRE emerged as nosocomial pathogens which are life threatening for immunosuppressed patients. Weakened immune functions are also a health issue for astronauts exposed to various stressors on long-term space missions. Therefore, VRE can become a threat whether on Earth or in space. To prevent VRE infections and guarantee the health of the crew members it is important to understand the influence of microgravity on the development of antibiotic resistances. Hence, antibiotic resistances of clinical isolates of sensible, low-level and high-level vancomycin resistant enterococci were investigated after simulated microgravity. Therefore, the minimal inhibitory concentration (MIC) was determined before and after simulated microgravity by using clinorotation. Additionally, stress tests for characterization under extreme environmental conditions were performed. As a result, the majority of the VRE isolates showed no change in their resistance behavior after simulated microgravity. Furthermore, as one of the extreme environmental conditions, the isolates showed an increased tolerance to desiccation after simulated microgravity. The fact that the VRE resistance behavior was similar under simulated microgravity to the control (normal terrestrial 1 x g) stresses importance under both conditions and allows for similar containment and therapy strategies both, on Earth and in space. To estimate the risk of VRE and their role in an upcoming pandemic of drug resistant infections it is essential to investigate VRE fitness and narrow down methods against the arising challenge of antibiotic resistant bacteria. Moreover, analysis of multi-stress factors of VRE and research of co-resistance towards environmental extremes and space conditions will improve the understanding how to fight drug resistant bacteria and, therefore, minimize healthcare-associated infections worldwide.

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eP213

Tracking the expression of eight different flavodoxins of *Clostridioides difficile* under iron limitation and oxidative stress

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Clostridioides difficile is a vast problem in human health care, as it causes serious and recurrent inflammation of the intestinal epithelium often with a lethal outcome. It is considered to be an anaerobic bacterium, but previous studies have shown a high and strain-dependent oxygen tolerance of the pathogen. To understand this tolerance, we aim for identification and characterization of candidates in the genome of *C. difficile*, which are involved in the oxidative stress response.

We focused our investigation on flavodoxins – small proteins featuring a flavin mononucleotide cofactor. An involvement of flavodoxins in the oxidative stress response and their vital role as electron donors in essential metabolic processes has

been reported previously. The genome of *C. difficile* encodes for eight highly diverse flavodoxins.

Bioinformatical analyses of all flavodoxins in *C. difficile* 630 were carried out to collect information on the evolution and functional characteristics of these proteins. Therefore, sequences of flavodoxins have been aligned against well-known flavodoxins of other bacterial species.

Furthermore, transcription of all eight flavodoxin genes of *C. difficile* 630 was investigated by Slot Blot analyses at different conditions, including iron limitation and oxidative stress induced by H2O2, O2 and paraquat and O2 simultaneously.

Especially the flavodoxin *fldX* showed a high induction under various of the tested conditions. To reliably quantify the strength of flavodoxin induction, RT-qPCR was performed subsequently. Furthermore, a targeted mass spectrometry approach was used to determine the abundance and induction rate of flavodoxins in *C. difficile* 630 on protein level.

Our research sheds light on the function of the various flavodoxins in the pathogen *C. difficile* and could thus be a starting point for the development of novel treatment strategies.

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eP214
Identification and characterization of the choline oxidation pathway of *Acinetobacter baumannii*
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Introduction
In the last decades the number of *Acinetobacter baumannii* infections of intensive care patients increased significantly, due to multidrug resistance and diverse persistence mechanisms. An extraordinary trait of *A. baumannii* is its high desiccation resistance which is based on perfect adaptation to dry or hyperosmotic conditions. One strategy to cope with osmostress is the uptake of compatible solutes such as betaine or its precursor choline from the environment *via* specific transporters belonging to the betaine-choline-carnitine transporter (BCCT) family. Interestingly, these solutes are also accumulated under isoosmotic conditions, but nothing is known about the role of solutes in the absence of salt stress.

Objectives
The aim of the study was to elucidate the role of betaine and choline accumulation and the oxidation of choline to betaine under isoosmotic conditions and the importance of these osmostress adaption strategies in adaptation of *A. baumannii* to the human host.

Materials & Methods
Heterologous expression and purification of the choline dehydrogenase BetA, biochemical analysis and mutant studies.

Results
A. baumannii has six different BCCTs of which three are osmo-independent. One of these transporters is important for the uptake of carnitine, which is used by this pathogen as sole carbon source. The second transporter is responsible for betaine uptake and the last one transports choline under low salt conditions into the cell. We found that choline is not just oxidized to the solute betaine to stabilize the cellular turgor under hyperosmotic conditions but is also important for the adaption to the human host. During the oxidation of choline to betaine four electrons are liberated which are funneled into the respiratory chain leading to ATP synthesis. Furthermore, we characterized the choline dehydrogenase and could show that the enzyme uses FAD as cofactor and is stimulated by glutamate but inhibited by high salt conditions, which is unique for enzymes important for osmostress protection.

Conclusion
We identified and characterized the choline oxidation pathway in *A. baumannii.* We could show that the choline oxidation serves as a metabolic adaptation strategy of this pathogen to the human host leading to energy conservation. Choline in form of phosphatidylcholine is a abundant carbon and energy source in the human, which suggests, that choline oxidation is important for virulence and persistence of *A. baumannii* in the host.

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eP215
Characterization of the endosomal proteome during hantavirus infection
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Understanding infectious diseases in animals and humans is of utmost importance to be prepared for an emerging and potentially zoonotic virus. Hantaviruses are a good example of the tight relationship between the human population and the animal reservoir where the frequency of human infections and disease is not only driven by ecological and virological factors but influenced by human behavior.

In our multidisciplinary consortium we integrate virus-cell interaction and infection efficiency with a proteomic analysis to identify cellular receptors of hantavirus and pinpoint activated immune signaling cascades. Our specific interest is the isolation and proteome analysis of early and late endosomes, to identify specific cellular receptors involved in cell entry.

Our analysis comprised several time points after infection. Synchronized virus infection at the plasma membrane was achieved by using low-temperature incubation and raising the temperature subsequently. Further, we used Bafilomycin A to inhibit endosomal maturation and prevent virus uncoating. Cells were harvested and directly processed for proteomic characterization. Since endosomes represent only a minor fraction of the total cellular content, it was of significant importance to ensure separation of cellular organelles to obtain a pure endosomal fraction for mass spectrometric analysis. This was achieved by density gradient centrifugation. Subsequently, the fraction was processed by S-trap digestion and the eluates were measured at a Q Exactive mass spectrometer. Last, the profiles of the endosomal compartments of infected and uninfected cells will be compared, potentially allowing the identification of virus-receptor complexes. The knowledge gained from this experiment may help to unmask the still unknown binding receptor of the puumala virus.

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eP217

Antimicrobial Effects of Novel Gyrase Inhibitors Against Gram-Negative and Gram-Positive Bacteria

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Antibiotic resistance is a growing threat to human health. One strategy to overcome antibiotic resistance is to develop new potent antibiotics with broad-spectrum activity. The aim of this study was to characterize the antibacterial effects of a series of 113 novel compounds based on the norfloxacin lead structure. To this end, we tested their activity against Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa,* as well as Gram-positive bacteria, including *Staphylococcus aureus,* using microdilution minimal inhibitory concentration (MIC) assays. The most promising compounds were then further tested against ciprofloxacin and multiresistant strains. Fluorescence microscopy-based phenotypic analysis was used to validate the mechanism of action of the best compounds using *E. coli* as model. Finally, we assessed possible off-target activity on the outer and inner membrane using permeability studies. MIC assays showed that 91 out of 113 compounds possessed activity against at least one of the tested bacterial strains. Importantly, six candidates showed equal or higher potency than ciprofloxacin and norfloxacin. We then proceeded to investigate the mechanism of action of the 19 most promising candidates, 18 of which were confirmed to inhibit the function of the gyrase enzyme. Of these, none showed any off-target activity on the *E. coli* cell envelope, suggesting that they are specific gyrase inhibitors. In conclusion, our work validated promising broad-spectrum antibacterial activity of the novel compounds and confirmed their mode of action as gyrase inhibitors. More research is recommended to further study the antibacterial effects of these derivates against a broader range of bacterial pathogens.

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eP218

Development of antivirulence agents targeting the central regulator of *Salmonella* invasion-related pathogenicity

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The increasing spread of antibiotic resistant bacteria is a global public health concern. Development of novel antibiotics is challenging, and resistance mechanisms are usually identified among clinical isolates shortly after the introduction of a new molecule to clinical use. An alternative approach is the development of compounds that inhibit virulence mechanisms without affecting cell viability. This class of non-traditional antibiotics are thought to reduce the risk of emergence of resistance while being species-specific, avoiding adverse effects on gut microbiota. We sought out to identify inhibitors of Type III secretion system (T3SS) of *Salmonella enterica* serovar Typhimurium. An HTS-friendly, luminescence-based assay was developed to monitor the secretion of the effector protein SipA through T3SS. We were able to identify a small-molecule C26 which blocks the secretion of SipA and other effector proteins leading to a reduction of host cells invasion. Aiming to understand the mode of action, genetic evidence and *in vitro* interaction assays suggest that C26 targets HilD, the central regulator of *Salmonella* pathogenicity, causing a downregulation of downstream pathways responsible for the activation of virulence genes encoded in *Salmonella* pathogenicity islands. A structure-activity relationship analysis helped the identification of key structural elements important for C26-HilD interaction, leading the way for further rational design of optimized compounds. Antivirulence agents targeting HilD hold great promise as intervention for outbreak control, and for prevention of severe disease outcomes in acute invasive non-typhoidal *Salmonella* infections.

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eP219

Evaluation of Cytotoxicity effect and cell death mechanism of *Salmonella Typhimurium* Protein Fractions on breast Cancer Cell

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Evaluation of Cytotoxicity effect and cell death mechanism of *Salmonella Typhimurium* Protein Fractions on breast Cancer Cell

Background and target: Salmonella typhimurium is a gram-negative rod-forming bacterium belonging to the family Enterobacteriaceae and Is one of the most common foodborne pathogens, that With several pathogenic factors, including toxins, Secretory proteins, and secretory system T3SS(That Causes fluid secretion And inflammation), Etc It can a suitable candidate for this stud. The purpose of this study, Evaluation of the cytotoxicity effect of bacterial protein fractions on growth and proliferation of breast cancer cells, which is the most resistant type of skin cancer.

materials and methods: In this experimental study, the breast cancer cell line MCF7 was used. Different bacterial fractions were prepared by the ammonium sulfate method. Interaction of cancer cells with different concentrations of Salmonella typhimurium fractions was studied. Cell proliferation was assessed by MTT assay at 24 and 48 hours.

findings: MTT test results with fractions (Bacteria lysate in culture medium, 80% deposition of lysate proteins, 30% deposition of lysate proteins, 80% deposition on culture media, and 30% deposition on culture media) The highest effect was observed at concentrations of 9.25,30, 8, 72, 10.75 µg / ml, respectively.

Discussion and conclusion: Results show that bacterial fractions of Salmonella typhimurium have high toxicity and lethal effect on breast cancer cells. These compounds can be suggested and used as an alternative or complementary to cancer therapy.

key words: Cytoplasmic extract, Media, *Salmonella typhimurium*, Breast cancer

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eP220

Unraveling the molecular mechanisms determining type III secretion of transmembrane substrates

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Type III secretion (T3S) systems are needle-like molecular machines that allow the injection of bacterial proteins directly into host cell cytosol or membranes in order to promote bacterial infection. Among the injected proteins are those containing transmembrane domains (TMD). Strongly hydrophobic TMD-effectors are prone to be mistargeted to the bacterial inner membrane unless they bind to a T3S chaperone (T3SC). This binding was found as a critical step for discrimination between: the classical Sec-dependent co-translational targeting and membrane insertion; and T3S, which is widely believed to be a post-translational process. *Salmonella´*s pathogenicity island 2 encodes SscB and SseF which form a chaperone-effector complex. SscB was previously shown to be essential for the T3S of the TMD-effector SseF by avoiding its erroneous insertion into bacterial membranes. SscB binds to the chaperone binding domain (CBD) and first TMD of SseF. However, the processes underlying the targeting to T3S of TMD-effectors still remain elusive. Here, we aim to understand the features of SscB and the mode of interaction with SseF that ultimately prevent mistargeting.

Analysis of the stability of SscB and SseF in *Salmonella* revealed co-stabilization since both proteins were more stable when interacting. Also, adding a SseF peptide (part of the CBD) stabilized purified SscB as observed by Nano-DSF. Furthermore, SscB has tetratricopeptide repeats and a circular dichroism spectrum typical of α-helical proteins. These features resemble those of class II T3SC which bind translocators, also TMD-proteins. However, according to the current chaperone classification, SscB should be classified as class I, since it binds a bona-fide T3S effector. Another feature reminiscent of the class II T3SC is the interaction of SscB with TMD and its ability to form dimers in solution, as observed by SEC-MALS. Moreover, the interacting partners of class II T3SC bear a "P/VXLXXP" consensus amino acid sequence in the CBD. This sequence is also present on SseF. Overall, these observations suggest that SscB-SseF interaction may occur during translation due to the observed co-stabilization and a need for rapid protection of TMD to avoid mistargeting to the Sec-dependent pathway. Also, it indicates that T3SC may have evolved to accommodate the structural characteristics (i.e. TMD) of the respective interacting partners.

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eP221
Antimicrobial properties of Mentha piperita loaded in chitosan nanogels against *Acinetobacter baumannii*
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Background: Today, one of the problems in the treatment of bacterial infections is drug resistance and biofilm formation. Therefore, due to the ineffectiveness of existing treatments, the purpose of this study was evaluation of anti-biofilm properties of Mentha piperita essential oils loaded in chitosan nanogels (MPEO- CsNPs)against *Acinetobacter baumannii* in the catheter surface and its mutagenic potential.

Material and methods: In this experimental-laboratory study, after preparation of MPEO- CsNPs and differential calorimetry scanning, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of MPEO- CsNPs against *A.baumannii ATCC19606* isolate were determined using the micro-dilution broth method. Biofilm production was investigated by light absorption method. RNA was extracted, evaluation of *Bap* gene expression done by Real-Time PCR.. Then assessment of mutagenic potential was performed using Ames test.

Results: MIC and MBC of MPEO-CsNPs were 3.12 and 12.48 µg / mL, respectively. Antibiofilm inhibition of *A.baumannii* ATCC19606 occurred at 2.69µg/mL as the minimum dose of MPEO-CNs. This value for CNs without essential oil was obtained at the highest dose tested, namely 400 µg/mL. The lowest dose of unloaded-nanogel was observed at about 7.38 % biofilm inhibition, while MPEO-CNs in the same dose was able to inhibit 2.69% of biofilm formation. The result of Evaluation of *Bap* gene expression showed there are significant differences at concentrations of 50 µg/mL (P=<0.0001).

Conclusion: Due to the effect of MPEO-CsNPs on *A. baumannii*, this substance can be introduced as a candidate for the treatment of infections caused by *A. baumannii*.

Keywords: Mentha piperita, essential oils, chitosan nanogel, biofilm formation, *acinetobacter baumannii*, catheter surface

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eP222
Effects of the novel epilancin A37 on bacterial and artificial membranes
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Epilancins are a group of lanthibiotic peptides produced by *Staphylococcus epidermidis,* consisting of two already described members (K7 and 15X) and the novel epilancin A37. Assessment of antibiotic activity demonstrated excellent activity of A37 against a variety of gram-positive bacteria. *Corynebacterium glutamicum* showed the highest susceptibility and was therefore chosen as model organism for mode of action studies. Fluorescence microscopy was used to visualize cellular effects of A37 treatment and the fluorescently labelled derivative A37FL *in vivo*. A37FL was shown to localize in the cytoplasm, along with formation of intense fluorescence foci associated to the cytoplasmic membrane. Colocalization studies with a membrane dye revealed these foci to be intracellular lipid vesicles heavily loaded with the compound. Super resolution microscopy allowed detailed visualization of the vesicles protruding from the cell membrane into the cytoplasm, thereby causing massive membrane deformations. The effects of A37 on membranes were further investigated with *in vitro* experiments on Giant Unilamellar Vesicles (GUVs). The compound was found to bind to and penetrate GUV membranes, thereby disrupting membrane integrity, allowing for fluid exchange with the surrounding medium and causing GUV instability and disintegration. It can be concluded that the novel epilancin A37 causes substantial lipid membrane disruption both *in vitro* and *in vivo*, resulting in cell death of *C. glutamicum.*

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eP223
Identification, Isolation, and characterization of a novel epilancin produced by *Staphylococcus epidermidis* A37
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Drug and multidrug resistances are major factors contributing to growing numbers of bacterial infections that are increasingly difficult to treat with classic antibiotics, severely limiting the available treatment options. Development and regulatory approval of novel antibiotics are research-, cost-, and time-intensive. Antimicrobial peptides (AMPs) have emerged as promising alternatives to conventional antibiotics in recent years as they often show high antimicrobial activity against numerous pathogens and are potentially able to circumvent established mechanisms of resistance.

We identified *Staphylococcus epidermidis* A37, which was isolated from a nasal swab, as the natural producer of an antimicrobial compound, which was named A37 accordingly. By genetic analysis, we identified A37 as a novel AMP and member of the epilancin class of type A lantibiotics, which are elongated, flexible, and cationic peptides. Although it has been shown that lantibiotics frequently act by disintegration of the bacterial membrane, the way epilancins interact with these membranes has not been studied in detail and remains largely unknown. The previously described epilancins, K7 and 15X, and A37 are structurally highly similar but their amino acid sequences differ in several positions.

We established a multi-step production and purification process to obtain pure epilancin A37. The compound was isolated from *S. epidermidis* A37 culture supernatant via hydrophobic interaction chromatography and the desired purity was achieved by anion exchange and reversed phase chromatography. The identity of A37 was confirmed by mass spectrometry after purification. Activity studies revealed potent antimicrobial activity of A37 against a wide range of bacteria. Of these, *Corynebacterium glutamicum* was chosen as a model organism for further studies due to its high susceptibility toward A37. We performed *in vivo* and *in vitro* experiments to investigate A37"s antimicrobial potential and its mode of action. Based on our findings, we postulate a multifaceted mode of action for A37 and related peptides. This model adds to the fundamental understanding and may provide a basis for further investigations of the antimicrobial activity of AMPs.

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eP224

High-throughput screening of *Pseudomonas aeruginosa* genes of unknown function revealed novel drug targets

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Antimicrobial resistance is recognized as a major threat to human health. *P. aeruginosa* is one of the most problematic nosocomial pathogens whose multi-drug resistant clones have become prevalent worldwide. To overcome multi-drug resistance, new antibiotic drug targets and drugs need to be discovered. Genes of unknown function (GUFs) which encompass 40% of the *P. aeruginosa* genome represent a promising pool of genes for the discovery of novel drug targets.

We performed high-throughput *in silico* and experimental characterization of *P. aeruginosa* GUFs to identify novel drug targets. The putative biochemical functions were assigned to 1267 GUFs by comparison of their homology models with the structures of proteins with known functions. These results revealed that 556 GUFs encode putative hydrolases which were selected for experimental validation of predicted functions because these enzymes are common drug targets and virulence and antibiotic-resistance factors. We generated an expression plasmid library for 435 putative hydrolases using the newly constructed vector, pGUF. This broad-host-range vector was used for protein production in *Escherichia coli* and *P. aeruginosa*. High-throughput expression of putative hydrolases under several different conditions revealed expression success of 74%. A screening of enzyme activities from nine hydrolase families (phospholipases A and C, lipase, esterase, phosphatase, thioesterase, phosphodiesterase, protease, glucosidase) using lysates of cells expressing GUFs revealed 82 novel hydrolases whose functions were previously unknown. Analysis of *P. aeruginosa* transposon mutants linked several of those hydrolases with the virulence in a *Galleria mellonella*, biofilm formation, and resistance against ampicillin.

The here presented paradigm for determining the biochemical functions of a large set of proteins by untargeted high-throughput screening may be valuable for the identification of entirely new antibiotic targets and a better understanding of host-pathogen interactions.

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eP225

Structural and mechanistic insights into membrane phospholipid remodeling by phospholipase A of *Pseudomonas aeruginosa*

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Cells steadily adapt their membrane glycerophospholipid (GPL) composition to changing environmental and developmental conditions. While the regulation of membrane homeostasis *via* GPL synthesis in bacteria has been studied in detail, the mechanisms underlying the controlled degradation of endogenous GPLs remain unknown. Thus far, the function of intracellular phospholipases A (PLAs) in GPL remodeling in bacteria is not clearly established.

Here, we identified the first cytoplasmic membrane-bound phospholipase A1 (PlaF) from *P. aeruginosa* involved in GPL remodeling *via* the Lands cycle. PlaF is an important virulence factor, as a *P. aeruginosa* Δ*plaF* mutant showed strongly attenuated virulence in *Galleria mellonella* and macrophages. We present a 2.0-Å-resolution crystal structure of PlaF, the first structure that reveals homodimerization of a single-pass transmembrane (TM) full-length protein. PlaF dimerization, mediated solely through the intermolecular interactions of TM and juxtamembrane

regions, inhibits its activity. A dimerization site and the catalytic sites are linked by an intricate ligand-mediated interaction network which likely explains the product (fatty acid) feedback inhibition observed with purified PlaF. We used molecular dynamics simulations and configurational free energy computations to suggest a model of PlaF activation through a coupled monomerization and tilting of the monomer in the membrane, which brings the active site cavity into contact with the GPL substrates.

Thus, these data show the importance of the GPL remodeling pathway for virulence and pave the way for the development of a novel therapeutic class of antibiotics targeting PlaF-mediated membrane GPL remodeling.

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eP226

Novel oxidoreductases from *Pseudomonas aeruginosa*

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Reactions catalyzed by bacterial oxidoreductases (OXR) are involved in numerous essential biological processes. Therefore, these enzymes attracted considerable attention as drug targets indicated by the approval of several OXR inhibitors as antibiotics and the clinical development of new OXR inhibitors for the treatment of bacterial infections. We focus on the identification and characterization of novel OXR which might represent novel virulence factors and putative drug targets of *P. aeruginosa*, a critical human pathogen according to World Health Organization.

Previously, structure-based prediction of biochemical functions of uncharacterized proteins from *P. aeruginosa* revealed 240 putative OXR. High-throughput cloning of putative *P. aeruginosa* OXR genes into the pGUF plasmid, designed for recombinant expression in *Escherichia coli* and *P. aeruginosa*, resulted in a library consisting of 149 expression plasmids. Overexpression of 79 putative *P. aeruginosa oxr* genes in *E. coli* BL21 (DE3) and *E. coli* C43 (DE3) was confirmed by SDS-PAGE, dot blot, and Western blot analyses.

Spectrophotometric high-throughput screening of dehydrogenase activity using various substrates including alcohols, aldehydes, carbohydrates, organic acids, and amino acids, revealed the putative functions of eleven studied OXR. Among them, PA3306, PA2936, and PA2804 catalyzed the NADP+-dependent oxidation of carbohydrate substrates. Furthermore, PA4533 and PA1218 oxidized aldehydes, and PA1560 and PA4182 were active with organic acid and alcohol, respectively.

Characterization of the biochemical function and the role of newly identified OXR for the metabolism and virulence of *P. aeruginosa* should reveal their potential as drug targets.

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eP227

A novel intracellular phospholipase PlaB is a virulence factor of *Pseudomonas aeruginosa* which affects biofilm assembly

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P. aeruginosa is a severe threat to immunocompromised patients due to its numerous virulence factors and multiresistance against antibiotics. This bacterium produces and secretes various toxins with hydrolytic activities including phospholipases. However, the function of intracellular phospholipases for bacterial virulence has still not been established.

Here, we demonstrate that the hypothetical gene *pa2927* of *P. aeruginosa* encodes a novel phospholipase B named PlaB. This enzyme, isolated from detergent-solubilized membranes of *E. coli* after heterologous expression, rapidly degraded various GPLs including endogenous GPLs isolated from *P. aeruginosa* cells. Cellular localization studies suggest that PaPlaB is peripherally bound to the inner and outer membrane of *E. coli*, yet the active form was predominantly associated with the cytoplasmic membrane. *In vitro* activity of purified and detergent-stabilized PaPlaB increases at lower protein concentrations. The size distribution profile of PaPlaB oligomers revealed that decreasing protein concentration triggers oligomer dissociation. These results indicate that homooligomerisation regulates PaPlaB activity by a yet unknown mechanism, which might be required for preventing bacteria from self-disrupting their membrane. Results obtained with a *P. aeruginosa* Δ*plaB* deletion mutant revealed that PlaB is important for establishing an infection in *Galleria mellonella* larvae and for biofilm formation. A global proteomics analysis suggests that PlaB is rather indirectly affecting *P. aeruginosa* through its effect on essential metabolic pathways.

This novel intracellular phospholipase B with a putative virulence role contributes to our understanding of membrane GPL degrading enzymes for bacterial virulence.

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eP228

Mathematical modelling and metabolic engineering of the cyanobacterium *Synechocystis sp.* PCC 6803 for improved production of the sesquiterpenoid squalene

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Cyanobacteria are a promising host for the production of the C30 terpenoid squalene, a central intermediate for many sterol compounds. Squalene itself is widely used in cosmetic products and as an adjuvant in some vaccines, but its production is currently unsustainable. The production of squalene in a *Synechocystis* Δ*shc* knock-out strain was demonstrated previously and gene targets whose overexpression increase production were identified by Flux balance analysis.

The goal of this work was the systematic evaluation of the target genes by quantifying each gene's impact on squalene production. To achieve this goal, a mathematical model of ordinary differential equations (ODE) of the terpenoid biosynthesis pathway was constructed from literature data and used to predict the quantitative impact of the overexpressions on squalene production. Each target gene predicted by FBA was overexpressed in *Synechocystis* Δ*shc,* and its impact on squalene titres measured.

The overexpression of target genes successfully increased squalene titres, with several target genes providing increases to production and one strain reaching higher specific production rates of squalene than ever reported before for cyanobacteria. The increases in production achieved for the overexpressions closely matched the predictions from the ODE model, showcasing the ability of *in silico* work to predict experimental results. The *in vivo* observations made in this work can also improve the ODE model and enable the mathematical optimisation of squalene production.

In conclusion, a systematic overexpression of genes led to strong increases in squalene production, which matched the predictions made by an ODE model. A combination of the experimental and *in silico* findings will be used to design an optimised production strain, which may establish a sustainable production of squalene at industrial levels.

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eP229
Modular genetic engineering of cyanobacteria: expression of distinct heterologous genes from individual synthetic shuttle vectors
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Cyanobacteria have raised great interest in biotechnology due to their potential for a sustainable, photosynthesis-driven production of fuels and value-added chemicals. This demand has led to a concomitant development of molecular tools for genetic engineering of those organisms. In this regard, however, even model strains like *Synechocystis* sp. PCC 6803 (*Synechocystis*) still lag behind compared to their heterotrophic counterparts. For instance, extrachromosomal shuttle vectors that allow gene transfer independent of recombination into host DNA are still scarce. Here, we introduce the pSOMA shuttle vector series for comprehensive genetic engineering of *Synechocystis,* which is based on the small endogenous plasmids pCA2.4 and pCB2.4 as well as various combinations of different selection markers. We made use of genes encoding either green fluorescent protein (GFP) or a Baeyer-Villiger monooxygenase (BVMO) to demonstrate functional gene expression from pSOMA plasmids *in vivo*. Moreover, we have proven compatibility between the two sub-series as well as with another replicative plasmid, the RSF1010-based broad-host-range plasmid pSEVA351. Therefore, the pSOMA series allows combined genetic engineering by introducing distinct genes via individual plasmids into the same strain. We also show that pSOMA plasmids allow gene transfer into the filamentous model strain *Anabaena* sp. PCC 7120.

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eP230
Novel anti-phage resistance mechanisms channelled through transcriptional regulation
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Marine picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* are the most abundant photosynthetic organisms in the oceans and substantially contribute to marine primary production. The coexistence with highly abundant cyanophages, viruses that infect cyanobacteria, is impacting the abundance, diversity and evolution of the cyanobacterial hosts. This is likely possible due to effective mechanisms of resistance. Broad host-range T4-like cyanophages have identical transcriptional programs in multiple sensitive host strains and recruit their host RNA polymerase. However, the regulation of the transcriptional program remains largely unknown. Generalist cyanophages are able to attach to and enter resistant cyanobacterial cells but cannot complete the infection cycle whereas resistant cyanobacteria commonly show reduced transcription of phage genes. The vast majority of these marine cyanobacteria lacks known resistance mechanisms suggesting that a currently unknown intracellular defence system is at play. The objective of this study is to elucidate how the phage transcriptional program of marine T4-like cyanophages is regulated in sensitive cyanobacteria and to unveil the mechanisms of defence at the transcriptional level in resistant cyanobacteria. As model organisms we will use the T4-like cyanophage *Syn9* with the sensitive *Synechococcus* strain WH8109 and the resistant strain CC9311, all of which can be genetically modified. I will present first results on phage and host proteins that might be involved in the regulation of phage promoter activity.

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eP231
Deciphering primary events in the evolution of phototrophic endosymbionts in a *Synechocystis-Dicytostelium* model
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Eukaryotes harbour organelles such as mitochondria and chloroplasts that originate from endosymbiosis of a *proteobacterium* and a *cyanobacterium*, respectively. Although these events have been the foundation of eukaryotic life forms, there are several open questions regarding these initial processes of organelle formation.

The objective of the project is to identify conditions and molecular interactions that have favoured the establishment of a mutualistic partnership between a photoautotrophic prokaryote and a heterotrophic phagocytic amoeba. The cyanobacterium *Synechocystis* sp. PCC 6803 and the phagocyte *Dictyostelium discoideum* are both model organisms, each one offering the full array of molecular tools, which are necessary to tackle fundamental questions in the coevolution of host and endosymbiont. Moreover, they offer the possibility to study a bipartite interaction at the scale of entire populations down to the single cell level.

We initially found that *Synechocystis* was ingested by *D. discoiduem*, but not utilized as a preferred food source by the amoeba when compared to γ-proteobacteria. Following ingestion, the cyanobacteria were processed in phagolysosome of *Dictyostelium*. Processing resulted in two different major scenarios: The bacteria were either rapidly expelled from the phagocyte via exocytosis or resided in phagolysosomes. Cellular retention of the bacteria resulted in rapid loss of motility and finally lysis of the amoeba host cell. Analyses of cellular extracts of the cyanobacteria revealed no amoebicidal metabolic products suggesting an interactive biochemical process between the two partners. We next varied abiotic conditions, such as light and CO2. Loss of motility and cellular lysis were drastically accelerated in phagocytically active amoebae in the light. We hypothesize this is due to the photosynthetic production of

reactive oxygen species (ROS) of the cyanobacterium, making it plausible that detoxification of ROS in early eukaryotes were among the most significant challenges to overcome.

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eP233

Phycocyanobilin biosynthesis in an early diverging streptophyta

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1. Introduction

The multicellular eukaryote *Klebsormidium flaccidum* (lately reclassified as *Klebsormidium nitens*) is a member of the lineage from which land plants evolved, streptophyta. Besides being able to harvest light, land plants and streptophyta are also able to sense its quality and intensity through phytochromes. However, while land plants' phytochromes incorporate phytochromobilin (PΦB), some streptophyta's phytochromes, including *K. flaccidum*, were shown to use phycocyanobilin (PCB). The presence of PCB rather than PΦB implicated the encoding of the phycocyanobilin:ferredoxin oxidoreductase (PcyA) rather than the phytochromobilin synthase (HY2). Both enzymes belong to the family of ferrodoxin-dependent bilin reductases (FDBRs). Surprisingly, transcriptomic and genomic analysis have shown lack of any identifiable PcyA and the presence of only HY2-related transcripts in *K. flaccidum*.

2. Objectives

The aims are to provide the direct proof of the conversion of BV (biliverdin IXα) to PCB catalyzed by KflaHY2 (HY2 of *K. flaccidum*) and to identify the crucial residues for the activity.

3. Materials & methods

Site-directed mutants of KflaHY2 were generated in the pET28a_KflaHY2 vector using the QuikChange® Lightning kit (Agilent). KflaHY2 and its site-directed mutants were overexpressed in *E. coli* and purified by affinity chromatography. The activity of the proteins was tested in activity assays and the reaction products were identified via HPLC (Agilent 1100).

4. Results

Activity assay and subsequent HPLC revealed KflaHY2 was able to reduce BV to PCB. To gain further insights on the unusual behavior of KflaHY2, an amino acid sequence alignment and a structure alignment using different FDBRs were constructed. These revealed three residues putatively important for the activity: Asn105, Asp122 and Asp242. To investigate the influence of ionization on catalysis, Asp122 and Asp242 were replaced by Asn whereas Asn105 was replaced by an Asp. KflaHY2_D122N was able to bind BV but not to use it as substrate. KflaHY2_D242N retained the ability to bind BV and produce the radical BVH• but did not reveal any conversion. Surprisingly, KflaHY2_N105D produced PCB as the intermediate and PEB as the final product. The double mutant KflaHY2_N105D_D122N retained the wt activity.

5. Conclusion

KflaHY2 is a functional FDBR, catalyzing the reduction of BV to PCB. The residues Asp122 and Asp242 are essential. This conclusion fitted with the hypothesis made based on the alignments.

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eP234

Novel phycobiliprotein assembly in cryptophytes: The light-harvesting protein PE545 in *Guillardia theta*

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In cyanobacteria, red algae and cryptophytes, enhanced utilization of photon energy for oxygenic photosynthesis is facilitated by light-harvesting complexes consisting of phycobiliproteins (PBP). These PBPs include open-chain tetrapyrrole chromophores, called phycobilins, which bind to the apo-proteins. Formation of holo-proteins is ensured by covalent linkage via thioether bonds to conserved cysteine residues inside the polypeptide backbone. Proper stereochemical attachment of pigments is provided by so-called PBP lyases. In cyanobacteria, the majority of PBPs accumulate to macromolecular antenna complexes, known as phycobilisomes. In contrast, cryptomonad light-harvesting relies on a highly degenerated, single type of PBP, which is localized in the thylakoid lumen [1]. The cryptophytic light-harvesting apparatus employs the hetero-tetrameric (αβ)(α'β) phycoerythrin PE545 consisting of CpeA (α) and CpeB (β), however, the preceding formation to a fully mature PBP remains mostly elusive [2].

Here we present approaches for the functional characterization of the *Guillardia theta* PE545 post-translational modification machinery whereby *E. coli* and *Synechocystis* sp. PCC 6803 serve as bacterial hosts for heterologous expression of the PE545 subunits and its related lyases. Prior to pigment attachment to the α-subunit, a PBP lyase (CpeX), specific for the attachment of the chromophore DHBV to CpeA, was produced in *E. coli*. The biochemical activity of the heterologously synthesized CpeX was analyzed spectroscopically by chromophore-binding assays to evaluate its phycobilin-associating activity. Transfer of DHBV to CpeA requires further characterization. In an alternative approach, assembly of the complementary β-subunit CpeB is realized via synthetic operons in *Synechocystis* sp. PCC 6803. Consequently, PBP lyase mediated pigment transfer to recombinantly produced PE545 will be investigated by spectroscopic-, HPLC- and MS-analyses.

Being the first completely sequenced cryptomonad, *G. theta* provides a promising opportunity to identify novel PBP lyases and characterize their biochemical activity during maturation. This knowledge will offer an in-depth understanding of how a dramatically degenerated light-harvesting complex is still able to efficiently capture and transfer solar energy to the photosystems' reaction center.

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eP235

Characterisation of a cyanobacterial bacteriocin heterologously produced with *E. coli*

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Introduction

Microorganisms resistant to common antibiotics are an emerging health thread. Bacteriocins are considered as a potential alternative to classic antibiotics. Bacteriocins form a heterogeneous group and occur in several microorganisms, also in cyanobacteria. An example are the cyanobactins, small cyclic peptides with diverse bioactivities. (Sivonen *et al*., 2010). Moreover, bacteriocin-encoding gene clusters were identified in cyanobacteria (Wang *et al*., 2011).

Objectives

A putative bacteriocin gene-cluster was identified in the genome of the terrestrial cyanobacterium *Chroococcidiopsis cubana*. This organism attracted attention in preceding antimicrobial screenings, as its biomass and culture supernatant possessed growth inhibitory activity against *Micrococcus luteus*. The corresponding gene product was characterised and tested for antimicrobial activity.

Materials & Methods

The cluster was amplified by PCR and heterologously expressed in *E. coli* LEMO21 (DE3) (NEB, Ipswich, USA). The protein was purified by affinity chromatography (HisTrap HP 5 ml, Cytiva, Freiburg, Germany) and tested for activity by inhibition tests in well-plates (Nunc, Thermo Scientific, Waltham, USA).

Results

The purified compound showed potent bacteriolytic activity against the Gram-positive bacteria *M. luteus, Mycobacterium phlei* and *Staphylococcus auricularis* and slight activity against Gram-negative *Pseudomonas fluorescens*. The most significant impact was measured against *S. auricularis*; 6.25 µg ml- 1 was determined as the minimum inhibitory concentration. The bacteriocin had no cytotoxic impact on mouse neuroblastoma N2a cells. Temperature stability tests confirmed activity till 60 °C.

Conclusion

A cyanobacterial bacteriocin could be produced with *E. coli* and showed potent activity against several bacteria. The data suggests a potential use of the bacteriocin for treatment of specific pathogenic bacteria. The temperature stability could also facilitate the application as a food preservative.

Sivonen, K., Leikoski, N., Fewer, D. P., & Jokela, J., 2010. Cyanobactins-ribosomal cyclic peptides produced by cyanobacteria. Appl. Microbiol. Biotechnol. 86, 1213–1225.

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eP236

Analysis and modification of the cellulose synthesis in the cyanobacterium *Synechococcus elongatus* PCC 7942 for biotechnological application

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The increasing world population and the increasing climate change, demand for renewable energy sources, and sustainable production of bulk chemicals and high-value compounds. Currently, biotechnological productions rely on heterotrophic microorganisms, which require external carbon sources for their growth. Cyanobacteria are an attractive alternative to those traditionally used heterotrophic organisms. Among other advantages over heterotrophs, cyanobacteria are capable of performing oxygenic photosynthesis thereby using the energy of the sun to fix carbon dioxide into carbohydrates and release elemental oxygen to the atmosphere. Cellulose is the most abundant biopolymer on Earth and one of the most important bulk chemicals. Cellulose is primarily produced by plants, however, it is also a component of the cell wall of certain cyanobacteria. Further, it has been suggested that plants acquired the capability to synthesize cellulose through the endosymbiosis of a cyanobacterium (Nobles et al., 2001). The model organism *Synechococcus elongatus* PCC 7942 harbors three genes encoding copies of the bacterial cellulose synthase, namely *bscA1*, *bcsA2*, and *xcsA*. Here, we present initial results about the role and importance of cellulose synthesis in cyanobacteria. We performed different knock-out combinations of the three isoforms and analyzed the effect on the physiology of the cells. In addition, the composition of wild-type and mutant cell walls was analyzed by linkage analysis via gas chromatography and mass spectrometry.

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eP237

The conserved endoribonuclease RNase III affects formation of photosynthetic complexes in *Rhodobacter sphaeroides*

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Bacteria are frequently adjusting their transcriptome to adapt to changes in their environment. This does not only take place on level of transcription, but also involves ribonucleases (RNases) that control RNA processing and turn-over. RNase III (*rnc*) is a highly conserved endoribonuclease, which is present in all known eukaryotes (Dicer and Drosha are RNase III enzymes) and prokaryotes, and was shown to be an important regulator of gene expression in many organisms. The enzyme can contain one or two nucleolytic active RNase III domains (RIIID) that harbour a 9 amino acids RNase III signature motif. Bacterial RNase III enzyme typically contain a single RIIID, often followed by a C-terminal dsRNA binding domain.

Rhodobacter sphaeroides is a facultative phototrophic alpha proteobacterium that can perform aerobic respiration in presence of oxygen, as well as anoxygenic photosynthesis, anaerobic respiration or fermentation in absence of oxygen. To better understand the role of RNase III in adjusting the *R. sphaeroides* transcriptome to changes in the environment, we constructed a mutant strain lacking RNase III activity by exchanging two highly conserved amino acids in the signature motif. An obvious phenotype of this mutant was its

lighter red color indicating that formation of photosynthetic complexes differs to the wild type. Indeed, we could confirm lower amounts of bacteriochlorophyll and carotenoids and lower levels of photosynthetic complexes in the mutant. Quantification of several mRNAs encoding structural proteins of photosynthetic complexes or enzymes required for pigment synthesis, revealed that their levels are influenced by RNase III. Moreover, an elevated steady state level of the sRNAs PcrX and PcrZ, which act as post transcriptional repressor of photosynthesis genes, was observed in the mutant, which could explain the down regulation of some quantified mRNAs. Our transcriptome analyses by RNAseq based experiments suggest a widespread regulatory role of RNase III in *R. sphaeroides*, not only on level of photosynthesis gene expression, but globally. Together, these results demonstrate, how much a single RNase can influence important physiological processes.

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eP238

Understanding cellular rhythms under constant environmental conditions

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Cyanobacteria are well suited as model organisms for investigating fundamental processes such as photosynthesis and circadian rhythms. As phototrophic organisms, they are dependent on sunlight. A timing system that allows predicting light changes before sunrise or sunset and adjusting the expression of specific genes, e.g. for photosynthesis, is of great advantage. This protein system, the so-called Kai protein system, forms the central oscillator for time measurement. Various Kai proteins have been described within the cyanobacterial phylum, which together regulates cell processes (Wiegard et al. 2013).

The self-sustained oscillation of KaiC phosphorylation has been described in vitro and in vivo several times before. Novel methods for online OD measurement now provide a non-invasive way to map cellular growth behavior under constant reactor conditions.

Here we show the behavior of cyanobacteria under continuous light conditions by comparing growth data from wild-type and circadian clock mutants. We test whether cyanobacteria show oscillations under these conditions, advancing our understanding of the internal clock.

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eP239

Engineering the cyanobacterium *Synechocystis* into a synthetic organelle

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1. Introduction

Plastids in plants and algae evolved through the endosymbiotic incorporation of an ancient photosynthetic cyanobacterium. The reduction of the cyanobacterium to an organelle involved processes like endosymbiotic gene transfer, development of a protein targeting and import system, metabolic integration through the insertion of transporters, retrograde and anterograde signaling, and the synchronization of host and endosymbiont cell cycles [1]. It is suggested that the development of the plastid involved an additional partner, a Chlamydia-like intracellular pathogen. In this Ménage à trois hypothesis, the cyanobacterium and the bacterial pathogen coexisted within a parasitophorous vacuole and a hexose-phosphate and an adenine nucleotide transporter were acquired by the nascent plastid and cyanobacterial glycogen storage was lost early during this tripartite symbiosis [2].

2. Objectives

We test the hypothesis that losing the glycogen storage capacity while being externally supplied with energy is a basic step towards metabolic cooperativity between cyanobacterial endosymbiont and host.

3. Material and methods

We use the model cyanobacterium *Synechocystis* sp. PCC6803 (*Synechocystis*) in our experiments. A knock-out mutant in carbon storage metabolism was generated by insertional inactivation. This strain was tested for glycogen accumulation and survival under diurnal rhythm.

4. Results

We were able to generate a fully segregated mutant in ADP-glucose pyrophosphorylase (*glgC*). The ∆*glgC* mutant was impaired in glycogen accumulation and needed constant light for survival. Mutant strains with additional deletions in the carbon partitioning pathway and expression constructs for different transport proteins (e.g., ATP transporter, hexose-phosphate carrier) were generated. The metabolic and physiological effects on the engineered strains are currently under work and will be discussed with respect to its relevance for metabolic cooperativity.

[1] S.B. Gould *et al.* (2008). *Annu. Rev. Plant Biol.* 59

[2] F. Facchinelli *et al.* (2013). *TiPS* 12

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eP240

Elucidating the role of sodium bioenergetics during resuscitation from nitrogen chlorosis in the Cyanobacterium *Synechocystis* sp. PCC 6803

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Nitrogen is an essential macronutrient and a growth limiting factor of bacteria. To survive periods of combined nitrogen depletion, the non-diazotrophic cyanobacterium *Synechocystis* sp. PCC 6803 changes into a dormant like state, the nitrogen chlorosis. The recovery from chlorosis, termed resuscitation, starts when a source of combined nitrogen is available again.

It was discovered that the initial rise in the ATP level in the early resuscitation relies on sodium bioenergetics, without the contribution of photosynthesis. In the ongoing resuscitation, the increase in the ATP level is supported by

respiration from glycogen degradation, potentially involving an alternative respiratory chain (ARC).

The aim was to further investigate the role of the sodium bioenergetics during the resuscitation by the identification of the components that are part of the proposed ARC.

The energy content and the glycogen amount of various mutants throughout chlorosis and resuscitation were determined. Recovery drop assays, the measurement of the photosystem 2 activity with a PAM Fluorometer and the staining of cells with a fluorescent dye (DiBAC4(3)), able to enter depolarized cells, were used to test the growth and viability of resuscitating cells.

Cells of the mutant *Synechocystis* sp. PCC 6803 ∆*nhaS2* showed a conditionally impaired vegetative growth and a low survival rate during prolonged chlorosis. They also had strong problems in the recovery from chlorosis when added NaNO3, marked by a great decrease in the OD750 and poor growth in the recovery drop assay. The ATP-level and -increase during the resuscitation was lower than in the wild-type. Furthermore, the outcome of resuscitation from chlorosis without Nhas2 strongly depends on the nitrogen source.

The sodium/proton-antiporter NhaS2 seems to be very important not only in the vegetative state of growth, but in particular in the chlorosis and resuscitation. Furthermore, it seems to play a key role in sodium bioenergetics of chlorotic cells.

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eP241

Identification of HmxR as a novel Fe-S cluster protein in the cyanobacterium *Synechocystis* sp. PCC 6803

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1. Introduction
   As catalyst in the oxygen evolving complex, manganese (Mn) plays a central role in oxygenic photosynthetic organisms. In the model cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis*) the proteins Hmx1 and Hmx2 form a Mn transporter at the plasma membrane and are likely involved in Mn supply to the oxygen evolving complex. In many cyanobacterial genomes, one additional gene is organized in the same operon as *hmx1* and *hmx2*. We named the encoded protein Hmx Related (HmxR). HmxR belongs to the Unknown Protein Family 0153 (UPF0153) with a highly conserved cysteine residue motive, which is predicted to serve as a metal-binding site.

2. Objectives
   The aim of this project is to identify the biological function of HmxR in Mn homeostasis in cyanobacteria. To this end, we biochemically characterized the metal-binding site of HmxR and studied a *Synechocystis* mutant in *hmxR*.

3. Material & methods
   Biophysical properties of the protein were tested by expression and purification of recombinant GST::HmxR. UV/vis spectra and ICP-MS analysis of the protein reveal information about the cluster type and bound metal ions. To determine, which cysteines are involved in metal-binding, site-directed mutants were generated. The physiological function of HmxR was investigated by using knock-out mutants.

4. Results
   The UV/vis spectrum of HmxR was comparable to known [4Fe-4S] cluster proteins. ICP-MS analysis identified Fe as bound metal. Though the ∆*hmxR* mutant did not show a Mn sensitive phenotype, its survival during prolonged incubation was strongly reduced. Under high light conditions, the double mutant ∆*hmx1*/∆*hmxR* showed a lethal phenotype.

5. Conclusion
   We have identified HmxR as a founding member of the UPF0153, a novel family of Fe-S cluster proteins. Its biological function is unclear but the lethal phenotypes indicate involvement in managing stress conditions.

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eP242

Identification of a novel PII interactor that regulates Carbon flux under nitrogen deprivation in *Synechocystis* sp. PCC 6803

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Non-diazotrophic cyanobacteria undergo an important transition in the lifestyle stimulated during nitrogen limitations controlled by the nitrogen regulatory cascade including the ubiquitous signal processor PII (K. Forchhammer, K. A. Selim., 2020). In the case of nitrogen deficiency, the newly fixed CO2 is immediately channeled into glycogen synthesis (Klotz et al., 2016). Knowledge on how to regulate this redirection of the carbon flow or the formation of precursors is limited. We discovered a novel PII interactor with a major impact in this carbon flow regulation, the product of *sll0944*, which we named PirC, in the non-diazotrophic model cyanobacteria Synechocystis sp. PCC 6803. With co-immunoprecipitation assays using PirC as a bait and BLI measurements, we confirmed the PII interaction and we identified the 2,3-bisphosphoglycerate independent phosphoglycerate mutase (PGAM) as the second target of PirC. PGAM directs the carbon flow into lower glycolysis by converting 3-PGA to 2-PGA. In enzyme assays, we showed PGAM is inhibited by PirC which is controlled by the interplay of PII and the nitrogen limitation mediator 2-OG. Furthermore, a nitrogen-depleted PirC-deficient mutant (DpirC) showed much lower glycogen levels and a significant increase in polyhydroxybutyrate content than the wild-type. Additionally, LC-MS metabolome analysis revealed 2-fold lower 3-PGA levels and 14-fold higher pyruvate levels, in D*pirC* compared to the wild-type after 48 hours of nitrogen depletion. With our results, we suggest the model that PirC works as a switch for the direction of newly fixed carbon either into building block or glycogen synthesis. In vegetative growing cells, PirC is bound by PII and the fixed carbon is directed into lower glycolysis by the conversion of 3-PGA to 2-PGA. In nitrogen-deprived cells, the rapid accumulation of 2-OG triggers the release of PirC the associated inhibition of

PGAM prevents the 3-PGA interconversion. This leads to an accumulation of 3-PGA and therefore in the formation of the carbon storage polymer glycogen.

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eP243
The essential role of sodium bioenergetics and ATP homeostasis in the developmental transitions of the cyanobacterium *Synechocystis* sp. PCC 6803
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Dormant bacteria are widely present in natural environments. The ability of restoring growth after a period of dormancy is a survival strategy of high importance for the maintenance of bacterial biodiversity, the spread of pathogens and the development of antibiotic resistances. In order to reach a dormant state, bacterial cells must enter cell cycle arrest and minimize their metabolic activity. In this quiescent state, energy homeostasis is critical for cell survival. So far, the regulation of the energy homeostasis during dormancy had been poorly understood. *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) is a non-diazotrophic cyanobacterium that enters metabolic dormancy as a response to nitrogen starvation. In this study, nitrogen starved *Synechocystis* cells served as a model to investigate how bioenergetic processes are regulated during the entry into and awakening from dormancy.

Determining viability, growth, amino acid levels, and changes in ATP content upon nitrogen starvation and resuscitation under different conditions allowed us to unravel a critical role for sodium bioenergetics in dormant and resuscitating cells.

When cells are nitrogen starved, ATP levels are actively tuned down to facilitate metabolic quiescence. During the period of nitrogen-starvation, cells engage sodium bioenergetics to maintain the ATP content at the minimum level necessary to keep cell viability. When nitrogen becomes available, energy requirements rise, and cells immediately increase ATP levels, employing sodium bioenergetics and glycogen catabolism. These processes allow them to restore the photosynthetic machinery and resume photoautotrophic growth. In the absence of sodium, cells fail to restore photosynthetic activity, show increased respiration rates and metabolic dysregulation. This work revealed an essential role of sodium in bacterial survival during periods of nutrient deprivation.

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eP244
Self-sustaining cyanobacterial biofilms for technical applications
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Biofilms are microorganisms attached to each other on a surface embedded in 3-D extracellular polymeric matrix. Compared to their planktonic counterparts biofilm growing organisms are more robust and can reach higher cell densities. Cyanobacteria recently gained high attention in the field of biotechnology since they can use sunlight as energy source while reducing atmospheric CO_2 and N_2 which makes them truly self-sustaining biocatalysts. In this project we investigated various cyanobacterial strains focussing on their ability to form biofilms in a capillary biofilm reactor with the aim of establishing novel photo-biotech work horses. The influence of nitrogen fixation as well as the effect of combining the cyanobacterial strains with heterotrophic organisms like reported in (1) on biomass formation, biofilm stability and surface coverage have been examined. The key player emerging from this study was *Tolypothrix* PCC 7712 which grew up to 62.6 ± 6.34 and 57.5 ± 1.08 $g_{BDW}L^{-1}$ biomass under nitrate-enriched and nitrate-omitted conditions, respectively, with less detachment and excellent surface coverage compared to other species tested. These findings highlight the potential suitability of other cyanobacteria compared to model organisms like *Synechocystis* sp. PCC 6803.

[1] Bioresource Technology (2019), 282:171-178. Doi: 10.1016/j.biortech.2019.02.093

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eP245
Maximizing photosynthesis-driven Baeyer-Villiger oxidation efficiency
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Photosynthesis-driven whole-cell biocatalysis has great potential to contribute to a sustainable bio-economy since phototrophic cells use light as only energy source. Cyanobacteria have been used to connect different reactions to the photosynthetic light reaction [1],[2]. The use of oxygenases is of particular interest, since the photosynthetic apparatus yields required O2 and electrons *in vivo*, overcoming problems of conventional heterotrophic oxygenase biocatalysis [3],[4]

It has yet to be shown that cyanobacteria, as the model organism *Synechocystis* sp. PCC6803, can combine supply of high heterologous enzyme levels with allocation of sufficient reduction equivalents to enable efficient light-driven redox biocatalysis.

We engineered *Synechocystis* sp. PCC6803 with a Baeyer-Villiger-Monooxygenase (BVMO) gene from *Acidovorax* sp. CHX100 to turn it into an efficient oxyfunctionalization biocatalyst, deriving electrons and O2 from photosynthetic water oxidation. Several expression systems were systematically tested and yielded activities of up to 60.9 ± 1.0 U gCDW-1. Detailed analysis of reaction parameters, side reactions, and biocatalyst durability revealed i) high *in vivo* BVMO activity in the range of 6 ± 2 U mgBVMO-1 and ii) impairment of biocatalyst performance by product toxicity and by-product inhibition. Scale-up of the reaction to 2-liter fed-batch photo-bioreactors resulted in a stabilization of the bioconversion over several hours with a maximal specific activity of 30.0 ± 0.3 U g CDW-1 and the formation of 2.6 ± 0.1 g of ε-caprolactone. Process simulations based on determined kinetic data revealed that photosynthesis-driven cyclohexanone oxidation on a 2-L-scale was kinetically controlled and not subject to a limitation by photosynthesis.

[1] Appel, J., Hueren, V., Boehm, M., & Gutekunst, K. (**2020**). doi:10.1038/s41560-020-0609-6

[2] Lassen, L. M., Nielsen, A. Z., Ziersen, B., Gnanasekaran, T., Moller, B. L., & Jensen, P. E. (**2014**) 1-12. doi:10.1021/sb400136f

[3] Hoschek, A., Bühler, B., & Schmid, A. (**2017**) doi:10.1002/anie.201706886

[4] Jodlbauer, J., Rohr, T., Spadiut, O., Mihovilovic, M. D., & Rudroff, F. (**2021**) doi:10.1016/j.tibtech.2020.12.009

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eP246

Tackling *Syn-Re*SH limitations: expression system, physiological condition and H_2ase maturation system

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Cyanobacteria are potential candidates to couple oxygenic photosynthesis and H2 production with the help of hydrogenases. One of the major challenges in using native cyanobacterial hydrogenases is their high oxygen sensitivity. We addressed this challenge, by the introduction of an O_2-tolerant hydrogenase into a phototrophic bacterium, namely the cyanobacterial model strain *Synechocystis* sp. PCC 6803 (Lupacchini *et al*., 2021). The in detail characterization of *Syn_Re*SH$^+$ revealed some constrains: at first a low *Re*SH expression level in *Syn*6803 compared to its native system, which results in low cell specific activity. Therefore, we designed an advanced *Re*SH expression system in *Syne*6803, using the CyanoGate cloning strategy (Ravendran *et al*., 2019), whereby we achieved a higher level of synthesized protein and elevated activity in vivo. The high activity, meaning the H_2-driven NAD^+ reduction, could be furthermore improved by optimizing physiological conditions, in particular the light availability, substrate (H_2) supply and a sufficient electrons sink (CO_2). Additionally, we observed that the highest level of protein synthesis doesn"t correspond to the highest activity *in vitro* and *in vivo*. This evidence led to the hypothesis that the *Re*SH maturation might be impaired. Our results demonstrate that the maturation apparatus of *Synechocystis* is sufficient to maturate *Re*SH *in vivo.* Functional *Re*SH synthesis in *Synechocystis* was accomplished by co-expressing only the structural *hox* genes and *hoxW*, encoding a specific endopeptidase ensuring proper C-terminal processing of HoxH. However, heterologous expression of *Re*SH has only been reported in combination with the co-expression of all or a certain subset of the respective maturation genes. For *R. eutropha* under oxic conditions, HypX is known to be essential for the biosynthesis of CO and its insertion as a ligand into the Ni–Fe active site of *Re*SH. Therefore, we are testing if the expression of *hyp* genes and especially *hyp*X can improve *Re*SH maturation in *Syne*6803, in particular under oxygenic conditions.

Lupacchini, J. Appel, R. Stauder, P. Bolay, S. Klähn, E. Lettau, L. Adrian , L. Lauterbach, B. Bühler, A. Schmid, J. Toepel, *Metab. Eng.*, Volume 68, 2021, Pages 199-209.

Ravendran, G. A. R. Gale, A. A. Schiavon, A. Puzorjov, J. Malin, M. D. Gillespie, K. Vavitsas, V. Zulkower, B. Wang, C. J. Howe, D. J. Lea-Smith, A. J. McCormick, *Plant Physiol.*, Volume 180, Issue 1, May 2019, Pages 39–55.

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eP247

Antibiotic-induced amino acid release: A bacterial emergency response to membrane-targeting antibiotics

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Introduction:

Antimicrobial resistance has culminated in infections no longer treatable with any common antibiotic. Despite considerable efforts, the alarming emergence of resistant superbugs is still not matched by the comparatively slow development of innovative drugs. This problem motivates the need to find alternative strategies such as targeting bacterial stress responses to weaken bacterial defenses. In response to antibiotic stress, *Bacillus subtilis* synthesizes large amounts of the amino acids glutamate and aspartate and releases these molecules through mechanosensitive channels[1]. This release provides osmotic stabilization of the cell membrane and grants protection from antibiotics. While the roles of amino acid release during hypoosmotic shock are well-characterized, the role of this mechanism in antibiotic adaptation remains enigmatic.

Objectives:

Our aim is to further characterize this antibiotic-induced amino acid release and explore its function as defense mechanism against antimicrobial compounds.

Materials and methods:

We analyzed amino acids profiles of *B. subtilis* after treatment with a broad panel of antibiotics to explore which mechanistic classes trigger this stress response. To investigate whether antibiotic-induced amino acid release is conserved, we performed amino acid analysis on *Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Candida albicans*. Finally, we constructed *B. subtilis* strains expressing fluorescent protein fusions to each individual mechanosensitive channel as well as to selected amino acid synthases and performed phenotypic analyses under antibiotic stress.

Results:

We observed that a broad range of cell membrane-targeting antibiotics induced amino acid release, while cell wall-targeting antibiotics did not. We could further confirm that antibiotic-induced amino acid release is conserved among Gram-positive and Gram-negative bacteria as well as in yeast. Microscopic analysis revealed distinct changes in the localization patterns of both mechanosensitive channels and amino acid synthesis enzymes after antibiotic treatment.

Conclusion:

Our study indicates that amino acid release through mechanosensitive channels is a widely conserved and highly efficient stress response strategy against a range of antibiotics that will be interesting to further characterize.

[1]Wenzel *et al*., PNAS, 2014

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eP248

Synthesis of the unusual lipid bis(monoacylglycero)phosphate in environmental bacteria

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Synthesis of the unusual lipid bis(monoacylglycero)phosphate in environmental bacteria

Florian Heinrichs, Simon Czolkoss, Franz Narberhaus, Meriyem Aktas

Lyso-phospholipids (L-PLs) are mono-acylated members of the phospholipid family, which can be found in all domains of life. They are intermediates in membrane lipid synthesis and degradation and potent secondary messengers in eukaryotes. In plants, L-PL amounts increase in wounded tissues, possibly playing a role in systemic defence responses against herbivores or pathogens[1].

Bacteria usually display L-PL amounts of less than 1 %, originating from membrane turnover or exogenous sources and being controlled by the LplT/Aas (re-acylation) system or several phospholipases (PlaA or VolA, degradation)[2,3]. The plant pathogen *Agrobacterium tumefaciens* lacks the re-acylation system and homologues of these phospholipases, suggesting yet undiscovered mechanisms to deal with L-PL exposure.

Here, we found that feeding of *A. tumefaciens* cultures with lyso-phosphatidylglycerol (L-PG) led to the formation of two isoforms of the unusual lipid bis(monoacylglycero)phosphate (BMP), as identified by thin-layer chromatography and quadrupole time-of-flight mass spectrometry MS/MS[4]. Subcellular localization studies revealed BMP to be located in the inner membrane of *A. tumefaciens*, probably synthesized by a membrane-associated acyltransferase. We tested further microbes for L-PG-dependent BMP production and found that the plant symbiont *Sinorhizobium meliloti* is also able to use L-PG from the culture medium for BMP formation. The γ-proteobacteria *Escherichia coli*, *Pseudomonas fluorescens* and *Pseudomonas syringae* produced BMP only in crude extract preparations but not after culture feeding, indicating limited substrate access as a bottleneck in BMP synthesis. Attempts to identify genes involved in BMP production by screening a knock-in mutant library of selected candidates in *A. tumefaciens* failed, necessitating biochemical approaches in identifying the enzymes responsible for L-PG uptake and BMP synthesis.

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eP249

Phospholipid *N*-methyltransferases produce various methylated phosphatidylethanolamine derivatives in thermophilic bacteria

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One of the most common pathways for the biosynthesis of the phospholipid phosphatidylcholine (PC) in bacteria is the successive 3-fold *N*-methylation of phosphatidylethanolamine (PE) catalyzed by phospholipid *N*-methyltransferases (Pmts). Pmts with different activities have been described in a number of mesophilic bacteria, like *Agrobacterium tumefaciens* [1] or *Xanthomonas campestris* [2].

The objective of this study was to investigate the PC biosynthesis in a set of thermophilic bacteria [3]. We identified and characterized the substrate and product spectra of four Pmts, three out of which were purified in an active form. The Pmts from the actinomycetes *Melghirimyces thermohalophilus*, *Thermostaphylospora chromogena*, and *Thermobifida fusca* produce monomethyl-PE (MMPE) and dimethyl-PE (DMPE). *T. fusca* encodes two Pmt candidates, one of which is inactivated by mutation and the other is responsible for the accumulation of large amounts of MMPE. The Pmt enzyme from the proteobacterium *Rubellimicrobium thermophilum* catalyzes all three methylation reactions to synthesize PC. Analysis of the lipid spectrum of *R. thermophilum* revealed that PE, previously reported to be absent in *R. thermophilum*, is in fact produced and serves as a precursor for the methylation pathway. In an alternative route, the strain is able to produce PC by the PC synthase pathway when choline is available. In agreement with previous reports [4], the activity of all purified thermophilic Pmt enzymes was stimulated by anionic lipids, suggesting membrane recruitment of these cytoplasmic proteins via electrostatic interactions. Our study provides novel insights into the functional characteristics of phospholipid *N*-methyltransferases in a previously unexplored set of thermophilic environmental bacteria.

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eP250

Pink-pigmented *Arthrobacter* species use bacterioruberin to modulate membrane fluidity

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Carotenoids are associated with several crucial biological functions and are discussed to be part of the cold adaptation mechanism of some bacteria. To ensure growth at low-temperature, bacteria mainly modulate membrane lipid fluidity by modifying their fatty acid composition. However, some pink-pigmented *Arthrobacter* species have an increased content of the rare C_{50} carotenoid bacterioruberin at low-temperature growth, whose function in these bacteria is not yet clear and which is found mainly in halophilic archaea. *Arthrobacter agilis* strain DSM 20550^T and *Arthrobacter bussei* strain DSM 109896^T had an increased bacterioruberin content if growth temperature is reduced from 30 °C down to 10 °C. *In vivo* anisotropy measurements with TMA-DPH could substantially increase membrane fluidity and a broadening phase transition with increased bacterioruberin content in the membrane at low-temperature growth. Suppression of bacterioruberin synthesis at 10 °C using sodium chloride confirmed the function of

bacterioruberin in modulating membrane fluidity. Increased bacterioruberin content also correlated with increased cell resistance to freeze-thaw stress. These results strengthen that bacterioruberin is involved in regulating membrane fluidity and the upregulated synthesis of this carotenoid represents an additional adaptive response to low growth temperatures with a beneficial impact on bacterial cell resistance to freeze-thaw stress. The potentially beneficial effect of this rare C_{50} carotenoid on *Arthrobacter* species and other bacterioruberin-producing bacteria may partly explain the successful colonization of low-temperature environments.

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eP251

Listeria monocytogenes integrates exogenous fatty acids in its membrane

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Listeria monocytogenes (*L. monocytogenes*) is a food-borne pathogen that can grow at very low temperatures. Maintaining cytoplasmic membrane fluidity by changing the lipid membrane composition is indispensable during survival and growth at low temperatures. In *L. monocytogenes*, the dominant adaptation effect is the shortening of the fatty acid chain length. To date, no incorporation of exogenous fatty acids has been reported for *L. monocytogenes*. However, it is not known whether *L. monocytogenes* can use exogenous fatty acids to support membrane adaptation to low temperatures. We hypothesized that the intake of external fatty acids affects membrane adaptation by shifting the melting point of the membrane depending on the available fatty acids. Fatty acids from food could thus have a favorable or unfavorable effect on the membrane of *L. monocytogenes* concerning the particular melting temperature of the exogenous fatty acid. Therefore, we tested the effect of exogenous fatty acids on *L. monocytogenes*. We demonstrated that exogenous fatty acids were covalently bound to the membrane lipids and had positive or negative impacts due to their melting temperature. Thus, there is a previously unknown mechanism for influencing the psychrotolerance of *L. monocytogenes* by providing specific external fatty acids. This was demonstrated by supplementing the growth medium with polysorbate 60, polysorbate 80, and food lipid extracts, which resulted in a corresponding change in the membrane fatty acid profile. The integrated exogenous fatty acids had a significant effect on the fitness of *L. monocytogenes* strains, as demonstrated by analyses of membrane fluidity, growth rates, and resistance to freeze-thaw stress. The results indicate that the fatty acid content of the growth medium or food matrix affects membrane fluidity and thus the proliferation and persistence of *L. monocytogenes* in food under low-temperature conditions.

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eP252

A promiscuous phospholipid biosynthesis enzyme in the plant pathogen _Pseudomonas syringae_

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A promiscuous phospholipid biosynthesis enzyme in the plant pathogen _Pseudomonas syringae_

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The ability to modify membrane lipid composition in response to environmental changes is crucial for bacterial survival. Recent studies revealed a remarkable diversity in bacterial membrane lipid composition and biosynthetic pathways (1). The major membrane phospholipids in bacteria are phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL). Unlike most phospholipids, CL is a lipid dimer comprised of two PG moieties with four fatty acyl chains giving the lipid a unique structure. In all organisms, CL is produced from PG but the mechanism how PG is converted to CL differs among bacteria. Multiple CL synthases with different substrate specificities can operate in the same organism (2, 3).

Here, we identified a phospholipase D-type enzyme (PSPTO_0095) responsible for CL synthesis in the plant pathogen *Pseudomonas syringae* (4). *In vivo* and in *vitro* studies revealed remarkable multiple talents of PSPTO_0095. (i) This enzyme acts like a typical bacterial CL synthase using either two PG molecules or a combination of PE and PG for CL production. (ii) PSPTO_0095 converts also PE and glycerol to PG and (iii) can hydrolyze CDP-DAG to phosphatidic acid. Finally, (iv) PSPTO_0095 accepts ethanolamine and methylated derivatives to produce the corresponding phospholipids PE and methylated PE derivatives, respectively.

Our study adds an example of a promiscuous enzyme, able to synthesize a suite of lipid products depending on available substrates, which may allow *P. syringae* to rapidly adapt its membrane composition to environmental changes.

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eP253

Interplay of phage shock proteins and membrane microdomains: A new area to explore

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Introduction: Soil bacteria are at constant exposure to a multitude of stress factors, such as antimicrobial compounds, temperature, pH, and osmotic shifts. The first line of defense against such stressors is their cell membrane, which possesses different types of membrane domains, such as "regions of increased fluidity" (RIFs). RIFs are associated with lateral cell wall and lipid synthesis. Based on previous proteomic profiling studies, we hypothesized that RIFs may also be involved in sensing environmental stress through an association with phage shock proteins. The soil bacterium

Bacillus subtilis possesses two phage shock protein homologues, LiaH and PspA.

Objectives:

Here, we aim to rule out whether phage shock proteins are indeed associated with membrane domains and how this relationship plays together with their respective triggers and functions.

Methods:

We used *B. subtilis* strains expressing fluorescent protein fusions to PspA and LiaH and a selection of membrane dyes that report on fluidity. These strains were then subjected to various stress conditions like temperature shifts, antibiotic, osmotic, pH, and oxidative stress. Similar stress experiments were carried out with single and double deletion strains.

Results

Phenotypic characterization of deletion strains revealed that strains lacking one or both phage shock protein homologues appear to be more sensitive to conditions that increase the rigidity of the cell membrane. Similarly, clear localization changes were observed for a number of these triggers for both LiaH and PspA. However, the localization of the two proteins differed markedly from each other, both under unstressed and stressed conditions, suggesting distinct functions. Furthermore, we studied the localization of different proteins that are known to associate with membrane domains in the deletion strain backgrounds. Surprisingly, we could observe aberrant localization of several of these proteins in the mutant strains, even in unstressed conditions, suggesting that phage shock proteins may fulfill a role related to membrane domains, even during normal growth.

Conclusion

From these results, we conclude that phage shock proteins are functionally connected to membrane fluidity. Possibly, they are required to stabilize phase boundaries between membrane domains of different fluidity. The breadth of stress factors that seem to play a role in the phage shock protein response underlines our still very limited understanding of this stress response.

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eP254
LapB orchestrates protein-protein interactions at the interface of LPS and PL biosynthesis
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Bacteria are surrounded by at least one cell membrane, which protects the cell from harmful compounds such as antibiotics and detergents. While the inner membrane of Gram-negative bacteria is symmetrical, the outer membrane is characterized by its asymmetry due to the presence of lipopolysaccharides (LPS) on the cell surface. Since many Gram-negative bacteria are not viable without LPS, the essential LPS synthesis pathway represents a promising target for future antibiotics. Several essential membrane proteins such as YejM, LapB, and FtsH are involved in the maintenance of the optimal cellular level of LpxC via regulated proteolysis. LpxC is the cytosolic key enzyme that catalyzes the first irreversible step of LPS synthesis. It is unclear which signals control the demand-based degradation of LpxC and thus maintain the optimal ratio of LPS to phospholipids. Previous experiments suggested intricate crosstalk of both metabolic pathways (Thomanek et al., 2019). Our results now show that direct protein interactions with LapB link the two pathways. These were validated using four independent protein-protein interaction methods: the bacterial two-hybrid system, far western dot blots, pulldown assays, and microscale thermophoresis. Overproduction of LapB is known to destabilize LpxC and we found that it also destabilizes LpxD, but not FabZ and LpxA. Thus, LPS synthesis is not inhibited directly at the branch point but rather through the degradation of the subsequent enzymes. In summary, LapB is not only an adapter protein for the degradation of LpxC, but rather a central regulator of phospholipid and LPS synthesis, that acts through protein-protein interactions.

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eP255
Complexome profiling of membrane proteins from *Pseudomonas aeruginosa* PAO1 with focus on c-di-GMP modulating proteins
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Although membrane proteins are important for various cellular processes they are often underrepresented in research. *Pseudomonas aeruginosa* PAO1 is an opportunistic human pathogen and a model organism for the investigation of biofilm formation and dispersion. In our work, *Pseudomonas* membranes are analyzed by mass spectrometry and complexome profiling. With this method we aim to identify large membrane complexes and interacting regulatory modules. The data quality is assayed on already described membrane complexes involved in metabolism, cell division, secretion or motility.

Data analysis focuses on the regulatory network of c-di-GMP. The molecule c-di-GMP is a bacterial second messenger that controls various processes such as motility or biofilm formation in bacteria [1]. Synthesis and degradation of c-di-GMP is mediated by two types of enzymes, the diguanylate cyclases (DGC) containing a GGDEF domain and the phosphodiesterases (PDE) containing either an EAL domain or HD-GYP domain [1,2]. *P. aeruginosa* encodes 18 GGDEF, 5 EAL, 16 GGDEF / EAL, and 3 HD-GYP domain-containing proteins [3]. In total, 22 of these proteins are predicted to be membrane bound. In first experiments 15 out of 22 membrane bound c-di-GMP modulating proteins in complexes were detected from planktonically grown cells.

One example is the phosphodiesterase NbdA with a large N-terminal membrane domain, predicted to be a sensory domain, and a cytoplasmic GGDEF and EAL domain. Using confocal laser scanning microscopy, a polar localization of NbdA was observed, pointing towards a local function in the polar region of the *P. aeruginosa* cell. In detail colocalization studies and interaction analyses are in progress.

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eP257

Characterisation of the vancomycin resistance protein VanW

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Antibiotic resistance is a long-known and ongoing threat in the treatment of bacterial infections. Vancomycin, an antibiotic already discovered in the 1950s, was long thought to be a secure antibiotic with respect to the development of resistance due to its unique involvement of disrupting peptidoglycan (PG) synthesis. Vancomycin is binding to the PG precursor lipid II, thus preventing PG synthesis and cell growth. Vancomycin resistance is mainly divided into two classes, defined by the gene clusters VanA and VanB. The latter alters lipid II synthesis to contain a pentapeptide with a C-terminal Ala-Lac as opposed to the natural di-d-Ala C-terminus. VanW is located within the VanB resistance cluster, but close homologs can be found isolated or in related Vancomycin resistances. The function and structure of VanW are still unknown. When *Clostridioides difficile* is treated with an antimicrobial peptide specifically the VanW gene is upregulated. Our goal is the identification of substrates and functional characterization of VanW. To do this, we choose to take a heterologous approach by expressing the protein in *E. coli.* As VanW contains one anchoring transmembrane helix (TMD), we exchange the TMD by a deca-histidin tag. The purified protein was used for intrinsic tryptophane fluorescence measurements using compounds of the peptidoglycan layer: N-acetylmuramic acid (MurNAc), N-acetyl-D-glucosamine (GlcNAc) and the pentapeptide which is part of lipid II (L-Ala-D-Glu-L-Lys-D-Ala-D-Ala). Using increasing concentrations of compound, we were able to determine K_D-values for all compounds with the result, that MurNAc and pentapeptide have a four times higher affinity than GlcNAc towards VanW.

We succeeded in expressing and purifying the protein VanW and initiated a detailed biophysical characterization of the protein and its possible ligands.

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eP258

The nisin resistance operon in *S. agalactiae.*

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Lantibiotics are small antimicrobial peptides with high potency against human pathogenic Gram-positive bacteria. However, pharmaceutical usage of the lantibiotics is hampered by the presence of gene clusters in human pathogenic strains, which, when expressed, confer resistance. One example is the nisin resistance system found in *Streptococcus agalactiae.* By upregulating the genes encoded within the nisin resistance operon, the host is able to build up resistance via multiple mechanisms i.e., by using proteolytic degradation by the nisin resistance protein *Sa*NSR or by expressing the BceAB-type transporter *Sa*NsrFP.

Here we report two different approaches to overcome this resistance system: In the first approach the last cysteine residue of nisin was replaced by a proline and the variant was termed nisin$_{C28P}$.This mutant cannot be efficiently cleaved by the NSR protein while still showing nanomolar antibacterial activity against Gram-positive bacteria (Zaschke-Kriesche *et al.*, 2019). In the second approach, natural compounds were screened on their ability to inhibit the proteins *Sa*NSR and *Sa*NsrFP from *S.agalactiae*. The most potent small-molecule inhibitor of *Sa*NSR, a halogenated phenyl-urea derivative, NPG9 was taken as a model to design and screen for even more potent inhibitors. Preliminary results show potent compounds which inhibit both the nisin resistance protein as well as the ABC transporter *Sa*NsrFP.

By understanding this resistance system of *S.agalactiae* and finding methods to bypass it, we have the potential to tackle antibiotic resistance also in other bacteria.

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eP259

Investigation of the pyoverdine transport in *Pseudomonas putida* KT2440

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Pseudomonas putida KT2440 is a Gram-negative, rod shaped bacterium that colonizes the roots of plants and is part of freshwater communities. To cope with the harsh conditions present in its native environment, especially the scarcity of iron in its soluble form, pseudomonads produce and secrete siderophores to bind and import external iron (1). In recent studies, the ABC system PvdRT-OpmQ and the RND system MdtABC-OpmB have been described as the main contributors to the circuit of iron acquisition (2, 3). However, a double deletion of both systems still secretes 40% of the siderophore present in *P. putida* KT2440, pyoverdine. In order to elucidate all pathways of pyoverdine secretion, the aim of this work was to test and further characterize other tripartite efflux systems of *P. putida* KT2440. As a second step, initial attempts were made to biochemically characterize these transport systems.

Therefore, deletions of several additional tripartite efflux systems were introduced in the genetic background of the deletion mutant Δ*pvdRT-opmQΔmdtA*. Subsequent phenotypic analyses involved single cell analysis of pyoverdine accumulation, colony morphology assays and growth under iron depletion. As a second step, the inner membrane component PvdT and the adaptor protein PvdR were purified using immobilized metal ion affinity chromatography. First analyses of the purified proteins involved ligand binding studies and ligand-dependent ATPase activity measurements.

Our results showed that a deletion of additional tripartite systems in the Δ*pvdRT-opmQΔmdtA* background had no significant effect on pyoverdine secretion. We identified another RND system, TtgABC, as a main contributor for detoxification of 2'2-bipyridyl, a commonly used iron-chelator (4). Moreover, preliminary biochemical analyses of purified PvdT indicated binding of pyoverdine and antibiotics to the transporter. Furthermore, the PvdRT complex was shown to possess a ligand-dependent ATPase activity.

We provide first biochemical evidence for ligand interactions and activities of the tripartite efflux pump PvdRT-OpmQ of *P. putida* KT2440. Analyses of the substrate specificity of the complex are under way. The mechanism of pyoverdine secretion in the double mutant remains enigmatic.

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eP260
Morphological characterisation of shape-shifting environmental isolates
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Cocci, rods, and ovoid cells: Every microbiologist has seen bacteria that possess such cell shapes. But bacteria can comprise an amazing variability of shapes, which range from precisely geometrical to completely asymmetric. Yet, little is known about exceptionally shaped bacteria. For this reason, we focus on yet undescribed and unusually shaped bacteria as well as their morphogenesis.

In the second half of the last century, Heinz Schlesner (Kiel University) isolated over 500 strains based on their unusual morphology from various habitats and deposited them in his personal bacterial strain collection. From this collection we investigated 17 phylogenetically closely related strains, which are members of the Alphaproteobacteria and possess uncommon shapes. As most of these strains had not been studied in detail before, we examined their morphology by employing multiple light and electron microscopy techniques as well as their phylogenetic relationships. Furthermore, some isolates were found to change their shape depending on the environmental conditions. Therefore, we investigated the influence of different media components and compositions on their cell shape and growth. Finally, we studied the transition between their two shapes upon altering the growth medium with light microscopy.

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eP261
An SMC-like plasmid defence system in *Corynebacterium glutamicum*
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Chromosome organisation is universally essential in all kingdoms of life. Correct chromosome compaction affects many vital processes, such as replication, cell cycle progression and DNA repair. Structural maintenance of chromosome (SMC) complexes are key organisers of chromosome architecture. SMC complexes are molecular motors that condense DNA by loop-extrusion. Two homologous complexes have evolved: SMC are mainly found in bacteria and archaea whereas MukBEF complexes occur in γ- and δ-proteobacteria. Recently, a third family has been discovered MukBEF-like SMC proteins (MksBEF(G)). Surprisingly, in some organisms, it exists as an additional copy.

Condensins like SMC, MukB and MksB are highly conserved proteins and belong to the WalkerA-WalkerB type ATPases. Kleisin (ScpA/ MukF/ MksF) and kleisin-associated (ScpB/ MukE/ MksE) subunits are regulators for the ATPase activity of SMC/ MukB and MksB.

In *Corynebacterium glutamicum*, we have identified two condensin paralogs of different function: The SMC/ScpAB complex is involved in overall chromosome organisation. However, the additional MksBEFG complex is likely a novel system involved in plasmid defence or plasmid maintenance. The Mks complex is spatially organised by the polar scaffold DivIVA. So far it is unclear how this plasmid defence activity is exerted. Here, we investigate the molecular arrangement of the MksBEFG complex and its ATPase activity *in vitro*. We provide evidence that MksG is the catalytic subunit that acts on the plasmid DNA.

Böhm, K., Giacomelli, G., Schmidt, A. *et al.* Chromosome organization by a conserved condensin-ParB system in the actinobacterium *Corynebacterium glutamicum*. *Nat Commun* **11,** 1485 (2020). https://doi.org/10.1038/s41467-020-15238-4

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eP262
Towards characterising the putative ABC transporter EslABC in the human pathogen *Listeria monocytogenes*
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Transporters mediate many crucial processes in the cell. One conserved group of transporters are ATP-binding cassette (ABC) transporters, which usually facilitate ATP hydrolysis to translocate their cognate substrate across the membrane. Interestingly, recent studies have revealed alternative mechanisms not directly linked to import or export, resulting in a more dynamic use of the term. One candidate for this is the putative ABC transporter EslABC, which was recently identified in the human pathogen *Listeria monocytogenes*. Preliminary experiments demonstrate the involvement of the transporter in creating intrinsic lysozyme resistance and ensuring correct cell elongation. As part of the innate immune system, lysozyme serves as a natural antibiotic, thus, identifying proteins involved in its resistance will aid efforts to counteract pathogenic bacteria. Our findings also reveal that the transporter plays a crucial role in peptidoglycan biosynthesis and cell wall integrity. Even though, the mode of action of EslABC, as well as its exact connection to peptidoglycan biosynthesis and remodeling remains elusive, EslABC sheds light on cognate transporter functions and their versatility in cellular processes.

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eP263
Phylogenetic Distribution of Medial elongation in the Bacteria Domain
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Conventionally elongasome and divisome complexes are known to mediate cell growth in rod-shaped bacteria by inserting peptidoglycan into the cell wall. The peptidoglycan sacculus with alternating sugars of N-acetyl glucosamine and N-acetyl muramic acid, and the spatiotemporal regulation of the enzymes that are involved in growing the sacculus determine the bacterial morphology. The elongasome and divisome complexes consist of inner membrane-spanning elements, scaffolding cytoskeletal proteins, and peptidoglycan hydrolases and synthases along with other periplasmic enzymes. The elongasome facilitates the cell to elongate by the insertion of peptidoglycan throughout the length of the rod-shaped bacteria, whilst the divisome is responsible for the addition of novel peptidoglycan at the mid cell, resulting in constriction during cell division.

However, a third growth mode has been observed in the model organisms *Escherichia coli* and *Caulobacter crescentus*. This growth mode, where peptidoglycan is inserted the at mid cell, takes place between elongasome-mediated elongation and division and seems to commence with the recruitment of MreB to the FtsZ ring. Even though two decades have been passed since the discovery of this PBP3 independent peptidoglycan synthesis (PIPS) activity, studies regarding this growth mode are sparse. It remains unknown which species use this growth mode and which relative importance it has in cell elongation. Furthermore, the exact mechanism and the proteins involved in the medial elongation and their conservation throughout species remain unresolved.

Hence the goal of this study is to determine the explicit phylogenetic distribution of medial elongation in bacteria using the hydroxycoumarin-carbonyl-amino-D-alanine (HADA) fluorescent labeling in phylogenetically well-distributed bacterial strains. Further, the study aims to identify the effects of environmental conditions on medial elongation and to deepen the knowledge about proteins and mechanisms involved in this growth mode. Lucid understanding of these mechanics would hence unleash the potential for the development of novel antibiotics that can target peptidoglycan involved in the medial elongation of pathogenic bacteria.

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eP264

RmdB-mediated global and local c-di-GMP-signalling in *Streptomyces* developmental control

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The second messenger c-di-GMP is produced by GGDEF-domain containing diguanylate cyclases (DGCs) and hydrolyzed by EAL or HD-GYP-type phosphodiesterases (PDEs). In antibiotic-producing *Streptomyces*, c-di-GMP regulates the developmental transition from unicellular spores to multicellular hyphae by controlling the function of the master developmental regulator BldD and the sigma factor σ^{WhiG} [1,2]. However, our understanding of how the DGCs and PDEs affect cell differentiation in these bacteria is limited.

In this study, we focus on the functional and physiological characterization of the composite GGDEF-EAL domain protein RmdB. We show that RmdB is a membrane-bound PDE that is needed for the coordinated developmental transition since sporulation is severely delayed upon deletion of *rmdB*. The PDE activity of RmdB contributes to the control of the global c-di-GMP pool, which is increased in the *rmdB* mutant. In addition, our data obtained using co-elution experiments and *in vivo* bacterial two-hybrid assays demonstrate that RmdB directly interacts with the c-di-GMP effector σ^{WhiG}. During vegetative growth, σ^{WhiG} is blocked by its anti-sigma factor RsiG. C-di-GMP dimer mediates the formation and stabilization of the σ^{WhiG}-RsiG complex, thereby inhibiting sporulation. Thus, based on our data, we propose that using both, a global and local mode of action, RmdB stimulates sporulation initiation. The PDE lowers global c-di-GMP levels which likely inactivates BldD as a repressor of sporulation genes and degrades the c-di-GMP that stabilizes the σ^{WhiG}-RsiG complex leading to sigma factor release and transcription of developmental genes.

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eP265

Elucidating Auranofin's (AF) mechanism of action in bacterial cells.

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Bacterial resistance against antibiotics is one of the main challenges for our public health. Infections with resistant bacteria currently claim at least 700 000 lives per year, projected to increase to 10 million by 2050. Conventional *de novo* drug discovery is a lengthy and expensive process. A promising approach consists of screening libraries of approved compounds to repurpose these molecules to create new viable therapies. One candidate drug is Auranofin (AF). AF is a gold-based compound utilized to treat rheumatoid arthritis, and it is known to inhibit human thioredoxin reductase (TrxR) by binding to thiol and selenol groups in the active site of the enzyme. Studies using AF demonstrated its antibiotic potential against gram-positive bacteria, including multi-drug resistant pathogens. However, it lacks significant activity against Gram-negative species for reasons that remain unclear. Using *E. coli* as a gram-negative and *B. subtilis* as a gram-positive model organism, we aim to identify AF's target(s) in bacteria. Due to the well-established inhibition of human thioredoxin reductase, it stands to reason that AF has similar effects on the bacterial homologs of that protein. We tested this hypothesis with enzymatic assays *in vitro*. In *E. coli,* TrxR shows a Ki around 63 nM, while the level required to exhibit cytotoxicity against this bacterium is around 188 µM. We performed proteomic experiments studying *E. coli's* response to AF using sub-lethal concentrations to determine potential targets. We were able to identify 20 proteins that were upregulated. Those proteins are mainly involved in the oxidative stress response. However, *E. coli* mutants lacking these proteins did not show to be more sensitive to AF's treatment, which led us to conclude that AF might not have one specific target. We used genetically encoded redox probes to monitor AF's influence on the bacteria's redox state using WT and mutants in the Trx- and Grx-system. Neither system influenced the probe's oxidation by AF, suggesting that they are not essential for repairing AF-inflicted oxidative damage. So far, our data suggest that AF globally perturbs the bacterial thiol proteome. AF's mode of action seems to inactivate thiol-containing enzymes, among them those involved in the oxidative stress response, with subsequent induction of systemic oxidative stress and detrimental effects on bacterial metabolism.

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eP266

MurA escape mutations uncouple peptidoglycan biosynthesis from PrkA signaling in *Listeria monocytogenes*

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Gram-positive bacteria are protected by a thick mesh of peptidoglycan (PG) completely engulfing their cells. This PG network is the main component of the bacterial cell wall, it provides rigidity and acts as foundation for the attachment of other surface molecules. Biosynthesis of PG consumes a high amount of cellular resources and therefore requires careful adjustments to environmental conditions.

An important switch in the control of PG biosynthesis of *Listeria monocytogenes*, a Gram-positive pathogen with a high infection fatality rate, is the serine/threonine protein kinase PrkA. A key substrate of this kinase is the small cytosolic protein ReoM. We have shown previously that ReoM phosphorylation regulates PG formation through control of MurA stability (Wamp et al. 2020). MurA catalyzes the first step in PG biosynthesis and the current model suggests that phosphorylated ReoM prevents MurA degradation by the ClpCP protease. In contrast, conditions leading to ReoM dephosphorylation stimulate MurA degradation. How ReoM controls degradation of MurA and potential other substrates is not understood. Also, the individual contribution of the ~20 other known PrkA targets to PG biosynthesis regulation is unknown.

We here present *murA* mutants which escape proteolytic degradation. The release of MurA from ClpCP-dependent proteolysis was able to constitutively activate PG biosynthesis and further enhances the intrinsic cephalosporin resistance of *L. monocytogenes*. This activation required the RodA3/PBP B3 transglycosylase/transpeptidase pair as additional effectors of the PrkA signaling route. One *murA* escape mutation not only fully rescued an otherwise non-viable *prkA* mutant during growth in batch culture and inside macrophages but also overcompensated cephalosporin hypersensitivity (Wamp et al. 2021). Our data collectively indicate that the main purpose of PrkA-mediated signaling in *L. monocytogenes* is control of MurA stability during extra- and intracellular growth. These findings have important implications for the understanding of PG biosynthesis regulation and β-lactam resistance of *L. monocytogenes* and related Gram-positive bacteria.

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eP267

A bacterial dynamin-like protein confers a novel phage resistance strategy on the population level in *Bacillus subtilis*

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Many bacteria encode dynamin-like proteins (DLPs), however, the molecular role of DLPs remained enigmatic. We have shown before that DynA, a DLP from *Bacillus subtilis*, is involved in membrane remodeling processes. DynA was shown to catalyze full membrane fusion in vitro and it plays a role in membrane surveillance against antibiotics in vivo. We show here that DynA also provides a novel resistance mechanism against phage infection. Cells lacking DynA are efficiently lysed after phage infection and virus replication. DynA does not prevent phage infection and replication in individual cells, but significantly delays host cell lysis, thereby slowing down the release of phage progeny from the host cells. This protective effect requires functional GTPase activity of DynA. During the infection process, DynA forms large, almost immobile clusters on the cell membrane that likely support membrane integrity. Single molecule tracking revealed a shift of freely diffusive molecules within the cytosol towards extended, confined motion at the cell membrane following phage induction. Thus, the bacterial dynamins are the first anti-phage system reported to delay host cell lysis and the last line of defense of a multilayered antiviral defense. DynA is therefore providing protective effects on the population, but not on single cell level.

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eP268

Single molecule dynamics at a bacterial replication fork after nutritional downshift or chemically induced block in replication.

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Introduction

All cells need to adjust DNA replication, which is achieved by a well-orchestrated multi protein complex, in response to changes in physiological and environmental conditions. For replication forks it is extremely challenging to deal with conditions where amino acids are rapidly depleted from cells, called the SOS response, with inhibition of one of the centrally involved proteins, or with DNA modifications that arrest the progression of forks.

Objectives

By investigating the single molecule dynamics of replicative helicase DnaC, DNA primase DnaG, and of lagging strand polymerase DnaE in the model bacterium *Bacillus subtilis* we analysed single molecule dynamics of proteins in response to stress conditions or blocking of polymerase E.

Materials & methods

We were using Single Molecule Microscopy and tracking of the central proteins of *B. subtilis* replication forks, at 20 ms stream acquisition speed.

Results

DnaG appears to be recruited to the forks by a diffusion and capture mechanism, becomes more statically associated after arrest of polymerase, but binds less frequently to fork blocked by DNA damage or nutritional downshift. Thus, binding of the alarmone (p)ppGpp due to stringent response prevents DnaG from binding to forks rather than blocking bound primase. Dissimilar behaviour of DnaG and DnaE suggests that both proteins are recruited independently to the forks, rather than jointly. Turnover of all three proteins was increased during replication block after nutritional downshift, different from the situation due to DNA damage or polymerase inhibition, showing high plasticity of forks in response to different stress conditions. Forks persisted during all stress conditions, apparently ensuring rapid return to replication extension.

Conclusion

We show that replication forks do not dissipate but adapt during the stringent response, as well as after other blocks to replication. Single molecule dynamics of functional fluorescent protein fusions generated for DnaC, DnaG and DnaE show individual changes of bound versus unbound molecules at forks after inhibition of serine tRNA synthetase, in response to DNA damage-induced blocks of replication forks, or by specifically blocking PolC. By observing distinct changes at the single molecule level to the three scenarios described above, we reveal that replication forks have a high plasticity to deal with different stress situations in a bacterial cell, but tend to avoid replication restart.

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eP269

Single molecule dynamics of the DNA receptor ComEA and DNA uptake in competent *Bacillus subtilis* cells

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1. Introduction

Many bacteria can take up double stranded DNA from the environment, sometimes under certain conditions, in a process called natural competence. This can result in the uptake of novel genetic information leading to horizontal gene transfer. The model organism *Bacillus subtilis* uses a cell envelope spanning competence machinery to take up exogenous DNA at the onset of the stationary growth phase. This multiprotein complex facilitates the steps necessary for import of DNA into the cytoplasm where it can integrate into the genome through homologous recombination. DNA is transported across the cell wall by a pseudopilus that is formed by polymerization of the major pilin ComGC together with minor pilins. After transport, DNA is bound by the membrane receptor ComEA, a membrane protein with a DNA binding domain at its C-terminus located in the periplasm, before it is transported through the membrane by the ComEC channel.

2. Objectives

We wish to visualize the path DNA will take into competent *B. subtilis* cells.

3. Materials & Methods

In previous work pili have been labelled *in vivo* by introducing a cysteine substitution in the major pilin and subsequent labelling with maleimide dyes. We applied this staining technique to the pseudopilus of *B. subtilis*. In order to follow the movement and dynamics of fluorescently labelled DNA and the DNA receptor mVenus-ComEA, we used single molecule tracking (SMT).

4. Results

By *in vivo* labelling of the pseudopilus we observed that cells had one or more labelled filaments exposed to the surface, of a size of roughly 200 to 300 nm, suggesting that cells can take up DNA at many places on their surface. By tracking fluorescently labelled DNA we found that taken up DNA diffuses through the entire periplasm, with a mobility similar that of a large protein complex, and also comparable to that of mVenus-ComEA. In contrast, polarly localized ComEC appears to find its site of localization through a diffusion/capture mechanism.

5. Conclusion

Our data suggest that most of taken up DNA molecules are directly bound to ComEA. DNA/ComEA complexes can diffuse through the entire periplasm, indicating that this subcellular space can act as reservoir for taken up DNA, before its entry into the cytosol, occurring through the polar ComEC protein. According to that, DNA uptake in *B. subtilis* seems to be spatially, but not temporally coupled.

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eP270

Tn*FLXopen*: marker-less transposons for functional fluorescent fusion proteins and protein interaction prediction

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Fluorescence microscopy of cells expressing proteins translationally linked to a fluorophore can be a powerful tool to investigate protein localization dynamics *in vivo*. One major obstacle to reliably analyze biologically relevant localization is the construction of a fusion protein that is both fluorescent and functional. Here, we develop a strategy to construct fluorescent fusions at theoretically any location in the protein by using Tn*FLXopen* random transposon mutagenesis to randomly insert a gene encoding a fluorescent protein. Moreover, insertions within a target gene are enriched by an inducible gene-trap strategy and selection by fluorescence activated cell sorting. Using this approach, we isolate a fusion to the flagellar stator protein that is both functional for supporting motility and localizes as fluorescent puncta. We further modify Tn*FLXopen* to insert the coding sequence for the C-terminal half of mVenus for use in bimolecular fluorescence complementation (BiFC) and the *in vivo* detection of protein-protein interaction candidates. As proof-of-concept, the DivIVA polar scaffolding protein was fused to the N-terminus of mVenus, the C-terminus of mVenus was delivered by transposition, and a combination of FACS sorting and whole genome sequencing identified

the known self-interaction of DivIVA as well as other possible candidate interactors.

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eP271
Visualization of the secretion process in *Bacillus* species
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1. Introduction

Many bacteria secrete proteins in order to degrade polymeric substances into monomers that can be taken up as nutrients. The secretion of enzymes by bacteria is also highly significant in biotechnological application. *Bacillus* strains are frequently used for protein production due to their fast replication and natural secretory capabilities. The starch-degrading enzyme α-amylase is one of the most prevalent secreted proteins produced by various *Bacillus* species. While we have a good understanding of the transport processes of proteins through the cell membrane, it is still unclear how proteins move through the multilayered Gram positive cell wall.

2. Objectives

In this project we investigated the protein translocation through the bacterial cell wall in the Gram positive model organism *Bacillus subtilis*. We aimed to answer whether proteins can pass the cell wall everywhere or via specialized zones of secretion.

3. Materials & methods

To visualize the secretion pathway in Bacillus species we used an AmyE-mCherry fusion. Structured illumination microscopy (SIM) was used for super resolution imaging. Furthermore, Bacillus cells secreting α-amylase in the presence of the starch BODIPY-FL stain were examined via fluorescence microscopy.

4. Results

Studying the AmyE-mCherry fusion using SIM, we observed multiple distinct, fluorescent spots distributed over the cell envelope, at the transition to stationary phase growth, indicating that the secretion pathway does not take place at one point only. Furthermore, this observation suggest also that secretion does take place at distinct places that can be resolved by light microscopy. Foci showed extremely low mobility, suggesting that the cell wall passage takes an extensive amount of time. By visualizing the secreted amylase outside of the cells, we found amylase activity-induced fluorescence localizing predominantly at only one of the cell poles.

5. Conclusion

Amylase as a model secreted protein appears to be secreted at multiple sites of the cell, in agreement with several sites in the membrane containing the Sec translocon. Low mobility of AmyE foci indicates a slow passage through the wall, possibly employing a passive mechanism via inside out cell wall growth. Zones of high amylase activity at the cell poles are puzzling, indicating differences between cell wall synthesis between sites of lateral wall extension and of cell division.

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eP272
The pilotin protein controls assembly, but also substrate specificity of the Type III Secretion System
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Introduction: The type III secretion system (T3SS), also commonly referred to as injectisome, is a syringe-shaped molecular nanomachine. Essential for infection in important pathogens, the T3SS exports specific proteins directly from the bacterial cytosol into the host cytoplasm. Of the approximately 25 proteins that make up the T3SS, the pilotin has a specific genetic localization independent of the other components and is thought to be involved in the distribution of the T3SS during the early stage of T3SS assembly.

Question and methods: Besides, for the central role of pilotin in the T3SS assembly, many questions remain unanswered. Indeed, it is still unclear why pilotins are generally encoded independently of the other structural components of the T3SS and whether they are directly involved in the T3SS function. Therefore, we sought to characterize the overall role of the pilotin in the T3SS in *Yersinia enterocolitica*. To address this objective, we combined functional secretion assays, fluorescence microscopy, as well as interaction studies and experiments of dynamics.

Results: Our results show that the pilotin SctG (also called YscW in *Yersinia*) plays a distinct role in T3SS assembly and function. Surprisingly, SctG interacts only temporarily with the secretin and moves dynamically in the bacterial membrane where it forms transient patches. While the correct localization of the secretin and a cytosolic component is dependent on SctG, a subset of bacteria remain capable of constructing polar needles. Despite the specific location of its gene, SctG exhibits similar regulation to other components of the T3SS. Furthermore, in the absence of SctG, the T3SS exhibits a strongly altered secretion pattern. Finally, we identified specific interactors of SctG that may contribute to its role in assembly and function.

Conclusion: Our findings provide new insight into the role of pilotin SctG in the T3SS. In addition to its known function in localization and oligomerization of the T3SS secretin, the pilotin also influences early substrates secretion. Finally, the unexpected localization of the pilotin and its transient interaction with secretin and additional proteins outside the T3SS suggest previously unknown additional functions of pilotin proteins.

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eP273
The role of glutathione in the periplasm of *Escherichia coli*
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The tripeptide glutathione (GSH) is the most abundant low molecular weight thiol in many bacteria and eukaryotes. In bacteria GSH plays a key role in a variety of redox-processes, such as protecting against oxidative stress and maintaining the reduced state of the cytoplasm. Although recent studies identified GSH transporters in the inner membrane of Gram-negative bacteria, very little is known about GSH or its oxidized dimer GSSG, in the periplasm, a compartment more oxidized than the cytoplasm. The major thiol oxidase system in the periplasm of *E. coli* is the DsbA-DsbB pair, which can introduce disulfide bonds critical for

correct folding of many extracytoplasmic proteins. If and how GSH is involved in periplasmic disulfide formation or isomerization remains unclear.

Here we investigate the role of GSH in the formation of disulfide bonds and maintenance of the redox environment in the periplasm of *E. coli.* Therefore, we expressed a genetically encoded roGFP-2 redox-probe in the cytoplasm and periplasm of *E. coli*, allowing us to visualize changes in the redox potential.

Monitoring the oxidation state of roGFP-2 in the cytoplasm of a mutant lacking *gshA* (encoding for the first enzyme in GSH synthesis) revealed that absence of GSH caused a more oxidized milieu in the cytoplasm. External addition of GSH or GSSG quickly restored the reducing environment to WT level, suggesting GSH and GSSG uptake and transport. Using periplasmic roGFP-2 we verified the highly oxidative character of the periplasm. Interestingly, even in the periplasm of mutants lacking *dsbA* or *dsbB* roGFP-2 was oxidized indicating a secondary mechanism for the introduction of disulfide bonds besides the DsbA-DsbB system. However, the capacity to reoxidize roGFP-2 in the periplasm after reduction with DTT was diminished in cells lacking *dsbA* or *dsbB* and restored by addition of GSSG, but not by GSH. In a *gshA, dsbA* double deletion stain, the reoxidation of roGFP-2 is fully inhibited by the addition of GSH. Surprisingly, in contrast to that, GSH and other monothiols speed up the reoxidation of periplasmic roGFP-2 in WT, suggesting an activating effect of monothiols on DsbA. Future assays with purified DsbA will investigate this activation further.

Overall, our data suggest that GSH is not directly involved in the maintenance of the oxidative milieu in the periplasm, but rather plays a role in regulation of the oxidation state and adaption to changes in redox potential of the environment.

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eP274
Coordination of capsule assembly and cell wall biosynthesis in *Staphylococcus aureus*

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The Gram-positive cell wall consists of peptidoglycan functionalized with anionic glycopolymers, such as wall teichoic acid and capsular polysaccharide (CP). How the different cell wall polymers are assembled in a coordinated fashion is not fully understood. Here, we reconstitute *Staphylococcus aureus* CP biosynthesis and elucidate its interplay with the cell wall biosynthetic machinery.

We show that the CapAB tyrosine kinase complex controls multiple enzymatic checkpoints through reversible phosphorylation to modulate the consumption of essential precursors, which are also used in peptidoglycan biosynthesis. In addition, the CapA1 activator protein interacts with and cleaves lipid-linked CP precursors, releasing the essential lipid carrier undecaprenyl-phosphate. Hence, CapA1B1 is a key regulator of CP biosynthesis with a dual function in signaling and processing of capsular polysaccharide precursor.The Ser/Thr kinase PknB, which can sense cellular lipid II levels, negatively controls CP synthesis. Our work sheds light on the integration of CP biosynthesis into the multi-component Gram-positive cell wall[1].

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eP275
New insights into chlamydial cell division amidase function, architecture and inhibition

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Chlamydia trachomatis is the most widespread sexually transmitted bacterial pathogen and the leading cause of preventable blindness worldwide. Due to its obligate intracellular lifestyle, *C. trachomatis* does not have to withstand environmental challenges like free-living bacteria, and therefore lacks a cell wall. Instead, the genome-reduced organism synthesizes a transient peptidoglycan ring at the forming septum (Liechti *et al*., 2016), which is degraded after cell division. Constriction and degradation of the ring and recycling of peptidoglycan requires constant remodeling and includes multiple enzymatic steps. Here, we show that AmiA, the only cell division amidase retained in *Chlamydia*, is part of the remodeling process in *C. trachomatis*. Surrogate host experiments in *Escherichia coli* revealed that heterologously overproduced AmiA of *C. trachomatis* (AmiA_Ct) lyzes *E. coli* cells. *In vitro* experiments with the purified enzyme confirmed a hydrolytic activity on peptidoglycan. In contrast to AmiA from *C. pneumoniae (*Klöckner *et al.,* 2014*)*, AmiA_Ct showed monofunctional amidase activity on the peptidoglycan precursor Lipid II. Pulldown experiments showed that AmiA harbours a potential, yet unidentified peptidoglycan binding module besides its catalytic center. Using site-directed mutagenesis, we analyzed the zinc-coordinating active site of AmiA_Ct. Moreover, we identified potent inhibitory compounds of AmiA_Ct, including the antibiotic clioquinol. In future experiments, these compounds will be used as tools to study functioning of AmiA_Ct in cell culture infection models. Our work contributes to the understanding of chlamydial amidases and the role they play in the tightly orchestrated peptidoglycan biosynthesis and cell division machinery.

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eP276
Characterisation of LCP proteins of *Streptococcus pneumoniae* – Influence on teichoic acid biosynthesis, cell morphology and physiology

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Introduction: Teichoic acids (TAs), important components of the cell wall of the pathobiont *S. pneumoniae* are either bound to the peptidoglycan (PGN) as wall teichoic acids (WTA) or anchored in the cell membrane as lipoteichoic acids (LTA). The linkage to either PGN or a glycolipid is the only difference between pneumococcal WTA and LTA, as both share the same biosynthetic pathway and repeating units. While the teichoic acid ligase TacL has been identified as the enzyme responsible for anchoring of the LTA, the LytR-Cps2A-Psr (LCP) protein family is assumed to be involved in the anchoring of WTA and capsule to PGN.

Objectives: Aim of this study is to elucidate the role of the individual LCP proteins in the anchoring of WTA and capsule to PGN and to characterise the altered bacterial cell physiology of specific *LCP*/*tacL* deletion mutants.

Methods: Isogenic single mutants of the *lcp* genes were generated in *S. pneumoniae* D39 as well as double mutants of *cps2A*/*psr* and *cps2A*/*lytR*.

For phenotypic characterisation, pneumococcal growth in complex and chemically defined medium and autolysis behaviour were analyzed. Further, the susceptibility of the individual mutants to various cell wall antibiotics was tested. As LCP proteins are suspected to be involved in WTA and capsular polysaccharide (CPS) linkage, the TA and capsular content was investigated by flow cytometry and electron microscopy.

Our previous data suggested that the loss of TacL activity induces alterations in the membrane integrity (Heß et al., 2017), the abundance of various lipoproteins was compared between the mutant D39Δ*tacL,* its isogenic wild-type and the complemented mutant. Flow cytometry and Western blotting were used to quantify the amount of various lipoproteins.

Results: Taken together, our results suggest that LCP proteins have a direct influence on capsule anchorage and are able to compensate partially or completely for the loss of one LCP protein by another. In addition, the loss of individual LCP proteins leads to an altered cell morphology and physiology. Further, we showed that the loss of TacL leads to significant changes in the abundance of surface-exposed and membrane-anchored lipoproteins.

Conclusion: This study highlights the importance of LCP proteins in anchoring WTA and CPS to the PGN. This is an important step towards understanding the mode of action of individual LCP proteins and their influence on the WTA content as well as their influence on bacterial physiology.

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eP277
Mechanism of Action of the Cell Wall Targeting Antibiotic Hypeptin

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Hypeptin (HYP) shares structural features with teixobactin (TEIX). It is a cyclodepsipeptide antibiotic produced by previously uncultured Lysobacter sp. K5869, isolated from an environmental sample by the iChip technology. Via systematic in vivo and in vitro analyses, we showed that HYP exhibits potent activity against a broad spectrum of gram-positive bacteria and binds to multiple undecaprenyl pyrophosphate-containing biosynthesis intermediates, blocking bacterial cell wall biosynthesis without affecting membrane integrity. In addition, HYP triggers secondary effects, such as increased autolysis likely contributing to limited resistance development, as observed with TEIX [1].

[1] Wirtz DA, et al., Biosynthesis and Mechanism of Action of the Cell Wall Targeting Antibiotic Hypeptin. Angew Chem Int Ed Engl. 2021; 60(24):13579–13586.

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eP278
Hypeptin – a newly discovered lipid II binding antibiotic

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Infections with gram-positive pathogens have become a major global health burden worldwide. As resistance against available antibiotics is spreading rapidly, new antibacterial substances with novel mechanisms of action are needed. The newly detected cyclodepsipeptide antibiotic hypeptin (HYP), that was discovered with the help of the iChip technology for bacterial cultivation, showed great activity against a broad bacterial spectrum. Elucidation of the mechanism of action by assessment of bioreporter strains revealed that HYP inhibits cell wall biosynthesis. With further in vitro studies we could show that HYP interferes with important staphylococcal biosynthesis steps by binding to C55PP-containing lipid intermediates within peptidoglycan, wall teichoic acid, and capsule biosynthesis. Hypeptin forms a stoichiometric 2:1 complex with biosynthetic precursors. Alongside cell wall biosynthesis inhibition, complex formation of HYP with WTA precursors leads to deregulation of autolysis triggering rapid lysis. Therefore, HYP shows impressive bactericidal activity.

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eP279
Learning from predatory bacteria: from OMICS to molecular mechanisms

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1.Introduction:

As one way to tackle the antibiotic resistance crisis natural killers of bacteria like predatory bacteria have gained attention in the scientific community recently. Gram-negative predatory bacterium *B. bacteriovorus* has not only a big potential as a putative "living antibiotic" by invading a wide range of Gram-negative (antibiotic resistant) pathogens in/on different organisms, but as well as a biotechnological tool or as a source of potential new drugs due to its predatory and lytic capacity. *B. bacteriovorus* is a model organism for predatory bacteria that invade and replicate in the periplasm of other Gram-negative prey bacteria. Once nutrients become scarce *B. bacteriovorus* septates to produce progeny which escape the depleted host cell remnants in the exit phase.

2. Objectives:

To date the molecular mechanisms underlying this last, crucial phase remain enigmatic apart from a novel predatory lysozyme that I characterized in collaboration with Prof. R. E. Sockett (University of Nottingham) and Dr. A. L. Lovering (University of Birmingham). The prey exit mechanism involves degradation of other cellular structures with new predatory mechanisms to discover.

3. Materials & Methods:

To uncover important factors in the exit process, state-of-the-art "omics" technologies (transcriptomics & proteomics) are

used. In order to test for a delayed-exit phenotype knock-outs of candidate genes will be made. Further, genes of interest will be localized with fluorescent tags and epifluorescence wide-field microscopy.

4. Results:

Using semiquantitative-reverse transcriptase PCR upregulated genes in the prey exit process were identified. Current investigations aim at localizing the candidate proteins & investigate their function. It is planned that initial results from proteomics will complement the findings from transcriptomics.

5. Conclusion:

Using "omics" technologies we aim to learn from predatory bacteria and what molecular mechanisms they are using to escape the prey bacterial barriers. This knowledge is essential to use predatory bacteria or their novel enzymes in potential applications as therapeutic agents/drugs or biotechnological tools.

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eP281

The LytR-CpsA-Psr (LCP) protein-catalyzed capsule attachment in *S. aureus*

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The cell wall of Gram-positive bacteria is constructed from a thick peptidoglycan sacculus surrounding the cytoplasmic membrane, which is decorated with covalently attached teichoic acids, polysaccharides, and proteins. Playing a major role in bacterial physiology, the cell wall maintains cell shape and integrity during growth and cell division, and mediates interactions with the environment.

LytR-CpsA-Psr (LCP) enzymes catalyze the attachment of anionic glycopolymers such as wall teichoic acids (WTA) and capsular polysaccharides (CP) to peptidoglycan in Gram-positive bacteria. LCPs transfer lipid-linked teichoic acid and capsular precursors assembled on the membrane-bound lipid carrier undecaprenyl phosphate to the C6-hydroxyl group of *N*-acetyl muramic acid of the peptidoglycan network. WTAs are covalently attached via a phosphodiester linkage, whereas the chemical nature of the CP linkage in *S. aureus* is elusive [1, 2].

The genome of *S. aureus* encodes three LCP proteins LcpA (SA1195), LcpB (SA0908) and LcpC (SA2103). Knockout mutants display growth defects, deformed cell morphology, reduced polymer synthesis and attachment, and secretion of anionic polymers into the medium [1, 3]. *In vivo* analyses revealed that the triple LCP mutant entirely lacks attached CP material, a defect that is best complemented by expression of *lcpC* in *trans* [4].

We provide biochemical evidence that the attachment of glycopolymers is achieved by LCPs using the native peptidoglycan precursor lipid II as acceptor substrate. To investigate potential donor and acceptor substrates and visualize the transfer of glycopolymers onto peptidoglycan, we synthesized radiolabeled lipid-linked capsular and wall teichoic acid precursors. Our work sheds light on the integration of capsule and wall teichoic acid biosynthesis into the multi-component Gram-positive cell wall.

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eP282

Rethinking bacterial sugar uptake: Planctomycetes employ a molecular fishing rod-like mechanism for polysaccharide uptake

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Efficient carbon uptake is one of the fundamental steps for bacterial biomass and energy formation, which is reflected in an increased competitiveness against other species. Many bacteria employ a variety of specialized transport enzymes with binding affinities for several mono-/di- or oligosaccharides. In doing so, they can cover a broad spectrum of short-chain molecules as potential carbon sources. However, high uptake rates of such easily degradable carbon sources appears to be inefficient in environments rich in high molecular weight polysaccharides. Taking up these large molecules provides much carbon per single uptake event and removes the molecules before other organisms have the chance to take up the smaller subunits they consist of. Such an uptake mechanism could thus provide a fitness advantage, but requires a specialized protein machinery. Algae-rich environments are well-known for their high abundance of complex polysaccharides and bacterial strains of the phylum *Planctomycetes* are often found in close association with them. Indeed, a previous study showed that the planctomycetal strain *Planctopirus limnophila* is able to bind the polysaccharide dextran using fibres (Boedeker *et al.*, 2017). When fed with fluorescently labeled dextran, a respective signal could be detected after one hour in the periplasm of the model planctomycete *P. limnophila* (Boedeker *et al.*, 2017), implying an uptake of dextran via these fibres. For polymeric sugar uptake, these fibres need to retract, portraying a "fishing rod" for sugars. We show that dextran-primed cells take up fluorescently labeled sugar molecules faster than cells grown on smaller sugars. This leads to the conclusion that cells can adjust their fibre equipment in response to environmental clues. Bioinformatic analyses point towards the occurrence of many different major pilin subunits. This gives rise to the hypothesis that previously observed fibres are in fact type IV pili specialized in sugar uptake. Furthermore, it sparks the assumption that planctomycetal cells might be able to change the composition of pili according to the sugars present in the environment - similar to changing the bait of a fishing rod. For now, it remains unclear how bacteria are actually able to employ this novel mechanism and how different sugars can be bound and taken up by these "molecular sugar rods".

Reference:

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eP283

Mechanisms of β-lactam induced persistence in chlamydiae

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Chlamydiaceae are the leading cause of sexual transmitted bacterial diseases worldwide. Due their obligate intracellular lifestyle the pathogens do not need to resist osmotic challenges and thus have lost their cell wall in the course of evolution. Nevertheless, the genome-reduced bacteria build a transient peptidoglycan-ring (PGN) at the septum during cell division (Liechti *et al*., 2014). Unlike in other bacteria, treatment with β-lactams does not kill *Chlamydia*, but arrests cell division and leads to the reversible formation of non-dividing cells that persist within the host cell. *Chlamydia* harbor homologs of penicillin-binding proteins (PBPs) representing the primary target structures of β-lactams in most bacteria. Besides the two putative transpeptidases PBP2 and PBP3 and the carboxypeptidase PBP6 (Otten *et al*., 2015, Klöckner *et al*., 2016), our group identified LysM protein Cpn0902 of *C. pneumoniae* as a carboxypeptidase and cell division amidase AmiA as a novel target for penicillin (Klöckner *et al*., 2014).

Our aim was to gain insight in the underlying mechanisms of β-lactam induced persistence and why the otherwise bactericidal β-lactams trigger cell division arrest in *Chlamydia.*

In cell culture experiments, we found not only β-lactams but also β-lactamase inhibitor clavulanic acid (CLA) induce persistence. The determined MIC was <1 µg/ml and 32 µg/mL in *C. trachomatis* and *C. pneumoniae*, respectively, indicating differences in target proteins among the two species. Next, we analysed the affinity to various β-lactams for chlamydial PGN processing enzymes using fluorescently labelled bocillin. These experiments revealed differences in affinity of PBP6_Cp and PBP3_Ct for several of the tested compounds in comparison to the respective *Escherichia coli* homologs and identified PBP3_Ct as a target of CLA. Furthermore, we addressed inhibition of the enzymatic function using PGN precursor lipid II as a substrate. Here, we found LysM protein Cpn0902 to be inhibited by penicillin. We also established an adapted transformation system in *C. trachomatis* for future experiments on the localization of AmiA and other enzymes in active and persistent chlamydial infection.

Our research on β-lactam target proteins and other proteins of the chlamydial PGN-ring machinery will elucidate which enzymes and cellular mechanisms are targeted by β-lactams leading to arrested cell division and persistence in *Chlamydia*.

eP285

Kinetic characterization and comparative antibiotic susceptibility profiles of PBP4 variants from methicillin resistant *Staphylococcus aureus*

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The gram-positive bacterium *Staphylococcus aureus* poses one of the biggest challenges to modern medicine as it is able to spontaneous develop or acquire resistance to a wide range of antibiotics. As an opportunistic pathogen *S. aureus* is able to cause a variety of infections ranging from relatively mild soft tissue and skin infections to severe bone and bloodstream infections. In order to achieve high-level resistance towards ß-lactam antibiotics *S. aureus* acquired an additional penicillin binding protein encoded by *mecA* on the mobile element SCCmec. However, even in the absence of *mecA*, *S. aureus* is capable of achieving resistance towards ß-lactams. In these *mecA*-negative methicillin resistant *Staphylococcus aureus* (MRSA), Penicillin binding protein 4 (PBP4) has been found to contribute significantly to resistance. We therefore characterized PBP4 of a *mecA*-negative MRSA ($PBP4_{E183A,F241R}$) with regard to IC50 values for common ß-lactam antibiotics. In comparison to $PBP4_{WT}$, $PBP4_{E183A,F241R}$ was found to be more resistant towards penicillins and towards the majority of cephalosporins tested, whereas cefoxitin as well as carbapenems remained effective inhibitors of $PBP4_{E183A,F241R}$. Biochemically $PBP4_{E183A,F241R}$ was less active in comparison to $PBP4_{WT}$. However, kinetic analysis of the single mutations hints towards hyperactivity in $PBP4_{E183A}$ which may be counter-acted by a less active $PBP4_{F241R}$. In summary, we were able to further elucidate the molecular basis of ß-lactam resistance in *mecA*-negative MRSA and to expand the current knowledge of PBP4 as a resistance mediator in *mecA*-negative MRSA.

eP286

Linking endosomal mRNA transport and mitochondria

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More than 90% of mitochondrial proteins are nuclear-encoded. Hence, targeted delivery and import of mitochondrial proteins are essential for cell survival. This is especially important in polar growing cells like neurons and filamentous fungi where the energy demand at the growth site is very high. Maintenance of local protein expression is therefore vital for mitochondrial activity. A prominent example is the endosome-coupled mRNA transport in the basidiomycete *Ustilago maydis.* The key factor of this process is the RNA-binding protein Rrm4. Rrm4 binds to almost all mRNAs of nuclear-encoded subunits of the ATP synthase at or in proximity to the stop codon. Also, loss of Rrm4 strongly reduce the protein level of the respiratory chain components in the infectious hyphae. Besides fungal hyphae, the endosomal linked transport processes for mitochondrial proteins are also known in neurons where late endosomes act as translation platform to ensure the functionality of mitochondria in axons. However, the importance of endosome-coupled mRNA transport for mitochondrial activity is still unclear. Here, we address the differences on the physiological level of mitochondria in absence and presence of Rrm4. For this purpose, we used different mitochondrial inhibitors, non-fermentable C-sources, and specific mitochondrial visualization techniques to investigate the mitochondrial import efficiency of F1FO-ATP synthase subunit.

eP287

Giant unilamellar vesicles as a bacterial mimetic system for studying bacterial inactivation and bacteriophage infection.

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Lipopolysaccharide (LPS) is the major component of the outer membrane of Gram-negative bacteria. The large amphiphilic LPS has major impact on the outer membrane characteristics. Understanding its role is important for electric-field based inactivation of bacteria, exploration of new antibiotics and bacteriophage treatments. Due to the high complexity of the Gram-negative outer membrane, giant unilamellar vesicles (GUVs) are promising synthetic membrane systems to investigate membrane properties, while controlling parameters such as membrane composition and surrounding media [1].

The aim of this project is the construction of asymmetric, LPS containing GUVs (LPS-GUVs) and their application as *in vitro* membrane models of Gram-negative bacteria in bacteriophage interaction studies. *Salmonella* LPS containing GUVs were prepared using an inverted emulsion technique that allows individual formation of the two membrane leaflets and optimal incorporation of smooth LPS molecules. LPS distribution in GUVs was investigated with fluorescence recovery after photobleaching (FRAP), electroporation and micropipette aspiration. LPS concentrations incorporated into the GUV outer leaflet were below those found in Gram-negative bacteria. The consequences on bacteriophage interactions with the LPS receptor were analysed with *Salmonella* bacteriophage P22 and fluorescence microscopy [2]. In conclusion, the GUV system provides insights on the effect of LPS on the properties of phospholipid lipid membranes and is a promising platform to investigate bacteriophage-host interactions and the bacteriophage infection process.

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eP288

Activity of acyldepsipeptides on human mitochondrial ClpP

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In recent years, the importance of the proteolytic core (ClpP) of the caseinolytic protease as target structure of different antimicrobial compounds has increased substantially. ClpP is conserved in prokaryotes and eukaryotes, where it is located in chloroplasts and mitochondria but not present in the eukaryotic cytoplasm. The mitochondrial homolog of the protease has important functions in the homeostasis of the eukaryotic cell, such as protein quality control and the regulation of different stress responses, including the mitochondrial unfolded protein response (UPRmt). Aside from being a target structure for antibiotics in prokaryotes, ClpP is discussed as a target for the treatment of different types of cancer. In cancer research, studies showed that ClpP is upregulated and essential for proliferation and metastasis in different cancer cell types. In this project, we focus on the effects that antibacterial acyldepsipeptides (ADEPs) exert on mitochondrial ClpP. ADEPs are able to dysregulate mitochondrial ClpP in vitro and to enter the eukaryotic cell, but their impact on the physiology of eukaryotic cells has not been studied on the molecular level. ADEPs were previously shown to be bactericidal to a broad range of Gram-positive bacteria by dysregulating bacterial ClpP. The potency of ADEPs against bacteria is based on a dual mechanism. While all natural functions of the Clp protease are prevented, ADEPs stimulate the proteolytic capacity of the ClpP core for unspecific degradation of essential proteins. To investigate the effects of different ADEPs further on bacteria and human cells, we are in the process of determining the structure-activity-relationship by comparing the activities of a series of ADEP derivatives against different bacterial ClpPs and human mitochondrial ClpP. Further, we are interested in the natural functions of hClpP, its physiological role in the eukaryotic cell and the impact of ADEP on these functions. While different in vitro studies showed the degradation of unphysiological model substrates (e.g., casein) by the functional hClpXP complex, studies of the natural functions and physiological substrates are still in their infancy. Therefore, we are also interested in identifying natural substrates to better understand the overall role of the mitochondrial proteolytic complex ClpXP.

eP289

The periplasmic chaperone Skp is a potent mediator in folding of lipase A of *Pseudomonas aeruginosa*

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The Gram-negative bacterium *Pseudomonas aeruginosa* is a wide-spread opportunistic human pathogen. A broad range of secreted virulence factors, i.e. extracellular enzymes, such as exotoxins, lipases and elastases, ensure the pathogenicity of *P. aeruginosa* and facilitate the infection pathways. Here, we investigate folding and the chaperone-mediated activation of the primary lipase LipA associated with biofilms of *P. aeruginosa*. Our data demonstrate that the pre-activated LipA is prone to aggregation and assembly of amyloid-type structures, and the misfolding pathways are promoted by the helix 5, known as the "lid" domain. A single mutation within the "lid" domain, as well as LipA interactions with the foldase LipH greatly stabilize the lipase. Next, we demonstrate that the general periplasmic chaperones of *P. aeruginosa* affect the folding/aggregation balance of LipA, and the ubiquitous chaperone Skp efficiently prevents LipA misfolding. Using small-angle X-ray scattering and biophysical analysis, we describe the conformational dynamics of the trimeric Skp chaperone and reveal that two copies of Skp are required to bind single LipA. As Skp does not inhibit LipH-mediated activation of LipA, we suggest that Skp is a potent factor that ensures correct folding of the lipase in the dynamic environment of the bacterial periplasm.

eP290

Unsaturated fatty acids augment protein transport via the bacterial SecA:SecYEG translocon

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The translocon SecYEG and the associated ATPase SecA form the primary protein secretion system in the cytoplasmic membrane of bacteria. The secretion is essentially dependent on the surrounding lipids, but the mechanistic understanding of their role in SecA:SecYEG activity is sparse. Here, we reveal that the unsaturated fatty acids (UFAs) of the membrane phospholipids, including tetraoleoyl-cardiolipin, stimulate SecA:SecYEG-mediated protein translocation up to ten-fold. Biophysical analysis and molecular dynamics simulations show that UFAs increase the area per lipid and cause loose packing of lipid head groups, where the N-terminal amphipathic helix of SecA docks. While UFAs do not affect the translocon folding, they promote SecA binding to the membrane, and the effect is enhanced up to five-fold at elevated ionic strength. Tight SecA:lipid interactions convert into the augmented translocation. Our results identify the fatty acid structure as a notable factor in SecA:SecYEG activity, which may be crucial for protein secretion in bacteria, which actively change their membrane composition in response to their habitat.

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eP291

Microfluidic high precision k_S estimation for *Corynebacterium glutamicum* growing on glucose (as sole carbon source)

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The substrate affinity, commonly referred as k_S, is a crucial parameter for characterizing growth of cells and is desired for modelling of cellular growth processes. Conventional process engineering techniques are operated in batch or chemostat mode to quantify k_S by measuring either uptake or consumption rates. However, these bulk measurements are only capable of extrapolating the average behavior of cells with respect to k_S. Novel (microfluidic) methods are necessary to determine heterogeneity within k_S on single-cell level. One-dimensional growth channels allow the investigation of growth and division characteristics (Lindemann et al., 2019). In this contribution, we apply the substrate limiting microfluidic single-cell cultivation (sl-MSCC) technology, that enables growth studies under constant and defined perfusion condition even at $\mu g \cdot L^{-1}$ range. In combination with phase-contrast microscopy, cell division events within the lineage of cells can be followed with high spatio-temporal resolution allowing to investigate growth kinetics including cell-to-cell heterogeneity within growth. As a model system we used *Corynebacterium glutamicum* ATCC 13032 growing on glucose as sole carbon source. In this context, systematic growth studies within a range from 150 $g \cdot L^{-1}$ down to 0 $g \cdot L^{-1}$ were performed to display the growth rate of single cells as a function of glucose concentration. These growth data were used to estimate k_S and resulted in an average based k_S-value of 2.52 ± 0.98 $mg \cdot L^{-1}$, which is in good agreement with reported literature values (Lindner et al., 2011; Graf et al., 2020). A closer look reveals that sl-MSCC has a high potential, especially for the precise estimation of k_S-values at limiting substrate conditions and to obtain insights into growth heterogeneity and adaptation of cells growing at limiting substrate concentrations.

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eP292

Direct-geneFISH to link antibiotic resistance gene presence and phylogeny in microbial populations

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Linking antibiotic resistance genes (ARGs) presence with cell phylogeny can provide direct proofs on the antibiotic resistance potential of a specific bacterial group and can be used to trace the abundance and spread of such genes in microbial populations. Here we applied a modified direct-geneFISH protocol (Barrero-Canosa and Moraru, 2021), combined rRNA-targeted catalyzed reporter deposition - fluorescence *in situ* hybridization (CARD-FISH) and *in situ* gene detection, for the visualization and identification of sulfamethoxazole resistance gene (*sul1*) containing cells. With this technique, the phylogenetic identification and cell abundance can be obtained by hybridization of rRNA-targeted oligonucleotide probes, and the gene presence and abundance can be visualized after hybridization of gene-targeted polynucleotide probes. Three polynucleotide probes, each with a length of 273 bp, targeting *sul1* and a non-sense probe (NonPoly302) were designed using PolyPro (Moraru et al., 2011) and Geneious Prime software (https://www.geneious.com). As model organisms, we used *Citrobacter EC35* strain that contains the *sul1* gene, and *Pseudomonas Putida KT2440* and *Desulfosarcina BuS5* strains that do not contain this gene. Repetitive hybridization events using the singular and mixed strains were performed. The *sul1* gene was detected in *Citrobacter EC35* strain, but not in the other two strains, as expected. The *sul1* gene number in *Citrobacter EC35* ranged from 1 to 7 copies per cell. Hybridizations with the nonsense gene-probe resulted in no false-positive signals. In summary, our proof of principle results shows the potential of the direct-geneFISH approach to detect and trace the antibiotic resistance genes in mixed microbial populations, and open the door for detection of ARG in environmental samples.

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Comparing the growth behaviour of industrial microbes under nutrient oscillation using dynamic microfluidic single-cell cultivation

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Microbes have a central role in industrial biotechnology, where they act as biocatalysts in the production of fine and bulk chemicals from renewable resources [1]. For industrial production scales, large-scale bioreactors are needed for a profitable process. Mixing the fermentation broth becomes increasingly difficult with larger scales, leading to gradients in process parameter like nutrients or dissolved oxygen across the bioreactor [2]. As a microbe moves through the bioreactor, it experiences the spatial gradients as a change in its environment, to which it needs to adapt. It has been shown, that gradients in bioreactors can lead to reduced growth and yield [2] and population heterogeneity [3] in a bioprocess. Testing a microbe's reaction to large-scale gradients during strain development could contribute to improving scale-up.

The novel technique of dynamic microfluidic single-cell cultivation (dMSCC) is perfectly suited to investigate a microbe's reaction to precise and frequent environmental changes [4]. In combination with live cell imaging, growth of single-cells and micro-colonies, production of fluorescent proteins and cell-to-cell heterogeneity can be observed. In dMSCC, microbes can be subjected to oscillations ranging from seconds to hours. In one oscillation interval, the medium condition can be switched from, for example, carbon source to non-carbon source medium.

In this study, dMSCC was used to apply symmetric glucose oscillations to industrially relevant model systems like *Corynebacterium glutamicum*, which is used for the production of various amino acids. Although the total time spent in each medium condition stayed constant, different oscillation frequencies led to alterations of the specific growth rate of *C. glutamicum*. Latest results will be shown.

Using dMSCC, microbes can be exposed to changing environments. This works discusses future application fields for dMSCC in microbiology and biotechnology.

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eP294

Visualization of cell heterogeneity in *Staphylococcus aureus*: at the single cell level *cap* transcription does not correlate with polysaccharide synthesis.

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Phenotypic variability within a genetically homogeneous population is now recognised as important to prepare the bacterial population for better survival under changing environmental conditions. In *Staphylococcus aureus*, the capsular polysaccharide (CP) protects against phagocytosis but also has the drawback of impeding adherence to host cells. CP synthesis was shown to be highly heterogeneous and growth phase dependent. The biosynthetic enzymes responsible for CP synthesis are encoded by the *capA-P* operon with the principal promotor (P*cap*) located upstream *capA*. These observations were achieved using single-cell assays such as promotor-fluorescent protein fusion (P*cap-cfp/yfp*) and CP immunofluorescence. We also established an *in situ* hybridization method to detect individual mRNA molecules within single *S. aureus* cells (mRNA-FISH). All methods verified the growth phase dependency and high cell variability of *cap*/CP expression. However, *cap* transcription proceeded CP synthesis: promotor activity and mRNA FISH signals were found at mid-exponential growth phase, while CP formation could only be detected in stationary phase bacteria. Interestingly, by combining the different methods we found that P*cap* driven *cfp/yfp* expression (fluorescence) does not correlate with the native *cap* mRNA level (mRNA-FISH) or CP synthesis (immunofluorescence) within the same bacterial cells. The same was evident when *cap* and *cfp/yfp* mRNA species, both resulting from P*cap* activity, were correlated using dual mRNA-FISH. Thus, mRNA structure/stability and other post-transcriptional mechanisms are likely detrimental for the timely CP synthesis in a sub-population of non-growing bacterial cells.

George SE. et al (2015) Mol Microbiol.98:1973; Keinhörster D. et al (2019) Mol Microbiol. 112:1083

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eP295

Integrated oxygen control and imaging in microbial single-cell analysis

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Microfluidics and live-cell microscopy provide great opportunities for emerging microbial single-cell analyses (SCA). New insights deriving from SCA revised our understanding of cellular interactions, growth, morphology and many other fields. Various microorganisms have been cultivated and investigated on a single-cell level already. Despite the huge potential of microfluidics, most cultivation devices lack any form of O_2 control and monitoring. In most cases, O_2 transport still relies on diffusion through a permeable chip material. Nevertheless, O_2 plays a major role in microbial life and laboratory cultivations. In our contribution, we present an integrated microfluidic approach for O_2 control and imaging during SCA. The set-up for our system consists of three main components:

1) A 3D-printed mini incubator shielding the microfluidic device from the surrounding atmosphere and undesired flux of gasses through the gas permeable chip material. Additionally, the incubator includes conventional online O_2 sensing.

2) An innovative polydimethylsiloxane (PDMS) based microfluidic device incorporating gas channels adjacent to the fluid-filled cultivation channel, enabling on-chip gassing and control. This miniaturized approach facilitates dynamic oxygen levels and steady state O_2 gradients across the cultivation chambers. External mass flow controllers allow to perfuse defined gas mixtures of O_2, N_2 and CO_2.

3) An inverted live-cell imaging microscope including a fluorescence-lifetime imaging (FLIM) system in combination

with the O_2 sensitive indicator Tris(2,2′-bipyridyl)dichlororuthenium(II)-hexahydrate. This camera-based approach is used to image and quantify O_2 with a spatio-temporal resolution.

We intensively characterised our microfluidic cultivation system for O_2 control. Dynamic O_2 levels as well as anaerobic conditions can be applied during cultivations. Furthermore, O_2 gradients and cultivation habitats with different concentrations are feasibly. Our approach allows dynamic changes within several minutes and hours. Furthermore, FLIM imaging is a great tool to online monitor O_2 concentrations with spatial and temporal resolution. Among other cultivations, we investigated the model organism *Pseudomonas fluorescens* Pf-5 at the single-cell level under various O_2 concentrations. By deep-learning based image analysis, growth rates and morphology were derived. We will present technical functionally as well as single-cell studies of relevant microorganisms.

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eP297

Improving Multiple Displacement Amplification for Single-Cell Omics using Droplet Microarrays

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Bacterial cells typically only contain a few femtograms of DNA and RNA per cell, which is much less than what current library preparation methods require for input. To overcome this limitation, several amplification methods have been developed for single-cell sequencing. Multiple Displacement Amplification (MDA) has become the most widely used method for single-cell studies of bacteria since it creates large amplicons, uses a high fidelity φ29 DNA polymerase with a low error rate, and amplifies isothermally. However, there remain many pitfalls of MDA including the high costs of the reaction, amplification bias against high GC% templates, chimera formations, uneven amplification, and contamination from exogenous DNA and/or endogenous non-template synthesis. Previous studies have shown that reaction volume reduction from microliters to nanoliters and the reduction of surface contact within microfluidic systems and/or planar substrates, works to minimize many of MDA"s current problems. However, it is often found that previously published microfluidic devices have limited accessibility and face issues with cross-contamination, and that examples of high-throughput applications for microorganisms are lacking. Here, we used a commercially available droplet microarray (DMA) chip consisting of hydrophilic spots separated by superhydrophobic barriers, in combination with a non-contact liquid dispenser, to perform high-throughput MDA reactions in nanoliter droplets with *E. coli* DNA and single cells. Tests show that DNA can be successfully amplified on the DMA. This technology will advance the field of microbial single-cell genomics and transcriptomics by providing a high-throughput method to obtain cheaper and less biased amplification of single-cell DNA and RNA.

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eP298

High abundances of transposases shape the genome of the fish pathogen *Piscirickettsia salmonis*

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The fish pathogenic Gammaproteobacterium *Piscirickettsia salmonis* is the causative agent of the multi-systemic infection piscirickettsiosis. The disease can lead to high mortalities in different salmonids and causes considerable commercial losses in the Chilean salmon farming industry. So far, major aspects of its biology and evolution are still only poorly understood. Detailed comparative genomic analysis of over 70 high quality *P. salmonis* genomes, 55 of them newly sequenced in the course of this study, uncovered an abundance of insertion sequence elements rarely seen in bacteria. Transposase genes constitute up to 25% of coding sequences on *P. salmonis* chromosomes. They were grouped into over 100 sequence clusters, ranging from singletons to sequence types with more than 250 copies in single chromosomes. Detailed analyses of their genomic localization revealed that the transposases of *P. salmonis* likely were mostly active in the past, and caused the formation of high numbers of pseudogenes, thereby contributing to the divergence of distinct genogroups. In contrast, evidence for recent transposition activity is relatively scarce. The *P. salmonis* genomes are not yet markedly reduced in size, but appear to be in initial stages of adaption to host dependency and niche adaptation. BLAST searches for the most similar non-*Piscirickettsia* sequences available in GenBank resulted in low identity hits in closely related bacteria for some clusters, but revealed high identity hits in phylogenetically distant organisms, like Cyanobacteria, for others. This points to both, ancient acquisitions that have diverged together with their host genome during Gammaproteobacteria evolution and recent acquisitions, even across phylum borders.

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eP299

Culturomics of prokaryotic bacteria and archaea from hypersaline soils

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Introduction: Most microbiological studies on hypersaline habitats have been carried out on aquatic environments, especially saline lakes and solar salterns. In recent years, hypersaline soils have received more attention as they seem to harbour a more diverse population of microorganisms. In order to gain more insight about hypersaline terrestrial systems, we carried out extensive metagenomic studies of saline soils located at the saltmarshes of the Odiel Natural Park in Huelva, Southwest Spain, that revealed a large diversity of archaea and bacteria, but not a predominant group of microbes, in contrast to hypersaline aquatic habitats.

Objectives: The aim of this study was the determination of the prokaryotic diversity on hypersaline solis using a culturomics approach, based on culture-dependent methods, and the later comparison with previous and ongoing metagenomic studies on these habitats.

Materials and Methods: Four hypersaline soil areas from the Odiel saltmarshes area (Southwest Spain) were selected for sampling. The samples were used for culturing microorganisms on different media and growth conditions, based on previously developped methodologies, for a period of three months. Representative isolates were studied in detail. Additionally, community DNA was extracted and purified from these habitats for future comparative metagenomic-culturomics analysis.

Results and Conclusion: Around 3,000 strains have been isolated so far from different media and salinity concentrations. To date, 465 of them have been analysed based on the partial or almost complete 16S rRNA gene sequence comparisons. Most abundant groups were revealed using EzBioCloud prokaryotic databases. Within the domain *Bacteria*, we highlight representatives of the genera *Halomonas*, *Marinobacter*, *Aquibacillus*, *Bacillus* and *Thalassobacillus*, while on the domain *Archaea*, members of the genera *Halorubrum*, *Haloarcula* and *Halogeometricum* were mostly represented. Besides, of the total strains studied, 40 isolates showed 16S rRNA gene sequence percentages lower than 98.7 % with respect to current validly published species names. From those selected ones, we carried out taxogenomic studies to determine if they could constitute new species, as well as futher comparative genomic analysis, such as functional annotation, osmoregulation mechanisms, fragment recruitments or the production of compounds with biological activities, that could be relevant for future studies on these new taxa.

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eP300

Genome Sequencing of *S. aureus* SG511 Berlin reveals an accumulation of mutations in the fine-tuned regulatory system

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Infections caused by *S. aureus* still play an important role in human and animal diseases because many older antibiotics are no longer effective due to increasing resistance [1]. The large number of complete genomes now available for *S. aureus* provides a detailed insight into the evolutionary processes leading to strains of differing virulence and drug-resistance potential [2]. Here we present the sequence of the 2.74 Mbp genome of *S. aureus* SG511-Berlin and the antibiotic susceptibility profile of this strain, which is frequently used for antibiotic lead discovery. The genome of *S. aureus* SG511 Berlin was sequenced using Illumina techniques, annotated via the NCBI Prokaryotic Genome Annotation Pipeline and compared with the related MRSA252 and the MSSA *S. aureus* HG001. The susceptibility profile of *S. aureus* SG511 Berlin towards inhibitors of protein, DNA/RNA and folic acid biosynthesis was comparable to *S. aureus* HG001. Beyond that, *S. aureus* SG511 turned out to be hyper-susceptible towards cell wall and cell membrane targeting agents with exception of fosfomycin. In depth comparative genome analysis revealed that, in addition to the loss of function mutation in the antibiotic sensor histidine kinase gene *graS*, further mutations had occurred in the lysyltransferase gene *mprF*, the structural giant protein gene *ebh*, and the regulator genes *codY* and *saeR*, which might contribute to antibiotic susceptibility. In addition, an insertion element in *agrC* abolishes Agr-activity in *S. aureus* SG511, and the *spa* and *sarS* genes, which encode protein A and its transcriptional regulator, were deleted. In conclusion, *S. aureus* SG511 lacks mobile resistance genes and comprises several mutations compared to the related, antibiotic-resistant MRSA252, which individually or in a synergistic manner may contribute to the extraordinary susceptibility of this standard test strain against cell wall targeting antibiotics.

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eP301

The lytic *Shewanella* phage Phonos reproduces successfully without a phage-dependent decline of the host population

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Bacteriophages are viruses specialised in infecting bacterial cells. These viruses influence bacterial populations in various ecosystems. The effect of phages on their host cells and a bacterial community as a whole depends mainly on the reproduction cycle of the phage. Here, we distinguish between lysogenic and lytic phages. Whereas lysogenic phages are able to integrate into the host genome and are passively reproduced by the host cell cycle, lytic phages only reproduce in the host cell by separate replication using host enzymes. Typically the final step of a lytic reproduction is the release of virions through lysis of the host cells, which is clearly indicated by decreased growth of an infected culture.

To study bacteriophages and their influence on bacterial communities like biofilms, we isolated phages from the environment, which are capable of infecting our model organism *Shewanella oneidensis* MR-1. Among others, the newly isolated phage Phonos was characterized. Phonos is a member of the family *Myoviridae* and a lytic phage, which seems to reproduce very successful in a chronic way without a phage-dependent decline of the bacterial population. One potential explanation for the slow reproduction time of Phonos could be its adsorption receptor, the major pilin subunit of the MSHA pilus. The activity of this pilus depends on the second messenger c-di-GMP which varies in amount between different growth phases and bacterial lifestyles. In particular, the activity of the MSHA pilus is important for the initial attachment step of biofilm formation. However, first biofilm experiments showed that Phonos promotes biofilm formation when added at initial time points.

Taken together, the current results show that the social biology of phages is very complex and not only based on simply killing the host.

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eP302

Analysis of lupine seed-based moromi microbiota reveals a novel salt tolerant Chromohalobacter species

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Seasoning sauce fermentations are known for their complex microbiota and chronological occurrence of specialized species. In this study, a novel species was isolated from a lupine-based moromi fermentation containing 20% salt. During the mid-term of the fermentation (week 2-12), it was highly abundant (up to 1.9×10^9 CFU/mL) and may have a great impact on the quality of the final seasoning sauce. Hitherto, the genus *Chromohalobacter* contains eight

species. Former species were isolated from salted foods or saline waters and only three of those were fully genome sequenced. As they revealed the ability to form extracellular polymeric substances, pigments and biogenic amines, this genus should be considered in high-salt food fermentations. Based on phylogenetic data including 16S rDNA analysis and genome to genome comparison we propose a novel species with the closest relatives *C. japonicus* and *C. beijerinckii*.

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eP303

Bac*Dive*, Bac*Media*, LPSN and TYGS: building a microbial research data and analysis infrastructure

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Information on microorganisms not only is scattered throughout the scientific literature, databases and other sources such as unpublished lab notes but is also growing exponentially. For a deeper understanding of microbial evolution and ecology, genomic, taxonomic and phenotypic data needs to be better aggregated, standardized and interlinked. DSMZ provides access to web services and databases offering well-structured, large-scale scientific information, and analysis of high-quality microbial research data.

The bacterial metadatabase Bac*Dive* (https://bacdive.dsmz.de) has become the leading database for standardized prokaryotic phenotypic data. As of release 07/2021 the database offers information for 82,892 bacterial and archaeal strains covering taxonomy, morphology, cultivation, metabolism, origin, and sequence information within 1,048 data fields. Based on these structured data, the *advanced search* enables a unique feature, a profile search, which allows finding strains based on their attributes (e.g. enzyme activity, antibiotic resistance, isolation source).

The recently introduced culture media database Bac*Media* (https://bacmedia.dsmz.de) consists of 3,200 standardized culture media for more than 40,000 bacteria, archaea, yeasts and fungi. Bac*Media* includes various search and comparison tools, adjustments of the final volume, growth data for associated strains, and PDF export of recipes. This database is continuously extended and curated by DSMZ experts.

The List of Prokaryotic names with Standing in Nomenclature (LPSN, https://lpsn.dsmz.de) provides comprehensive information on names of prokaryotes, authorities, publications, nomenclatural types, synonyms, emendations, nomenclatural status, taxonomic status, etymologies, culture collections. Sequence data for type strains can directly be transferred to tools for 16S rRNA gene and genome sequence analysis available in its sister database TYGS.

The Type (Strain) Genome Server (TYGS, https://tygs.dsmz.de) is a user-friendly, digital high-throughput platform for genome-based prokaryote taxonomy, connected to a continuously growing database of cross-checked genomic and nomenclatural information. TYGS infers phylogenies and state-of-the-art estimates for (sub-)species boundaries from user-provided genomic data sets automatically linked to the closest type-strain genomes. TYGS results provide comprehensive access to nomenclatural information and associated taxonomic literature.

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eP304

Identification of a novel family of *Pseudomonas* cyclic-β-glucan synthases

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Bacteria produce a variety of polysaccharides with functional roles in cell surface coating, antibiotic resistance, host interactions and biofilms. However, functional identification of synthases is becoming increasingly reliant on homology with a relatively few well characterised enzymes. Recently, we began a bioinformatics investigation of an "Orphan" bacterial cellulose synthase catalytic subunit (BcsA)-like protein found in three model pseudomonads, *P. fluorescens* SBW25, *P. putida* KT2440 and *P. syringae* DC3000. These Orphans were not associated with the fully functional *bcs* operons but might enhance cellulose production by providing additional copies of the subunit to the holoenzyme. However, pair-wise alignments indicated that they were not recent duplications of the operon gene and with less than 41% identity were unlikely to have a similar function. We identified over 112 Orphan orthologues among the pseudomonads suggesting these highly conserved proteins have some other adaptive role. A combination of HMMER searches, Proteus2 and Protter secondary structure predictions suggest a two-domain structure for the Orphan protein including a periplasmic ß-glucanase domain and a cytoplasmic glycosyltransferase (GT)-like family domain with eight transmembrane helices, with conserved catalytic residues and domains identified by comparison to enzymatically characterised orthologs. This structure was further supported by SWISS-Model template searches which allowed us to model the Orphan using a fungal ß-1,3-glucanosyltransferase for the first domain and a bacterial BcsA subunit for the second with reasonable GMQE estimates. Based on these findings, we suggest the Orphans are a novel family of branched glucan synthases. However, we noted rather belatedly that the *P. aeruginosa* PA14 Orphan (NdvB) is required for cyclic β-(1→3)-glucan (CβG) synthesis which is involved in biofilm-associated antibiotic resistance, and in Rhizobium and Xanthomonas CβGs are involved in osmoregulation, plant infection and induced systemic suppression. It is therefore tempting to suggest a more specific function for the Orphans as CβG synthases that provide similar adaptive responses for a range of pseudomonads. Our Covid-lockdown inspired bioinformatics research illustrates how important it is to look more carefully at BLAST analyses before accepting functional annotations, and how many more uncharacterised polysaccharide synthases might be found in the genomes of other bacteria.

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eP305

The (super)phylum Chloroflexi – Where, what, and how are they related?

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The Chloroflexi phylum has mainly been studied with a focus on functional metabolic traits such as the ability of reductive dehalogenation. Less emphasis was put on more global approaches to describe the phylum and its systematics. Where are Chloroflexi to be found? What is the relation of these findings? How diverse are they? What is the genomic potential and how does it define the individual clades? What

can we learn from the pan genome and the clade-specific core genomes? Can metagenomic and single-cell approaches fill the knowledge gaps? And finally: Do Chloroflexi and closely branching phyla indeed form one single superphylum?

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eP306

Camaraderie among cyanobacteria from marine habitats: mat-forming *Coleofasciculus* strains and their heterotrophic housemates

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Introduction

The filamentous cyanobacterium *Coleofasciculus chthonoplastes* is an ecological keyplayer of microbial mats in tidal flats. In the coastal environment it lives in close relationship with different heterotrophic bacteria. Many isolates from the North and Baltic Sea have been deposited at the DSMZ, which provides the promising opportunity to compare this bacterial composition.

Objectives

16S-rRNA gene sequences of *C. chthonoplastes* and the associated heterotrophic bacteria should be established and analysed based on a novel 16S-ITS-amplicon PacBio sequencing approach. Complete 16S-rDNA and ITS sequences will allow to resolve the phylogenetic relationship of the cyanobacteria and to classify the associated heterotrophs. The metagenomes of the four most promising strains should be sequenced.

Material and Methods

16S-ITS-amplicon and metagenome sequencing was performed on the PacBio and Illumina NovaSeq platform, respectively. For both sequencing methods, newly established bioinformatic pipelines with filtering, clustering/binning and quality checks were used (Marter et al., 2021).

Results

(1) The established 16S-ITS-sequences allowed to validate the authenticity of 23 *Coleofasciculus* strains. Five initially misclassified strains represent other cyanobacterial genera such as *Microcoleus*. Most cultures were uni-cyanobacterial, but six ones comprised up to nine additional cyanobacteria. (2) 349 unique 16S-ITS sequence variants of heterotrophic bacteria were identified in the marine cyanosphere. Most of these sequences represent *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes*. The genus *Marinovum* of the roseobacter group is the most common housemate of *Coleofasciculus*. Based on the 16S-rDNA sequence identities two putative new bacterial orders and eight new families could be identified. (3) Metagenome sequencing and binning allowed us to establish high quality metagenome-assembled genomes (MAGs) of four *Coleofasciculus* strains and 132 MAGs of associated heterotrophs. (4) Phylogenetic analysis of the 16S-rRNA gene clearly shows that the sequences are located in two clades. 16S and digital DNA-DNA-hybridisation documents that strain WW12 represents a second species in the genus *Coleofasciculus*.

Conclusion

Metagenome sequencing of the phototrophic lead organism *Coleofasciculus* provided first comprehensive insights into the composition of the crucial microbial mats in tidal flats.

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eP307

Quantification of metabolic impacts driven by recent lateral gene transfer (LGT) using network expansion

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Introduction

Lateral gene transfer (LGT) plays a major role in prokaryotic evolution enabling the incorporation of beneficial genes for the recipient by bypassing vertical evolution. These genes are often coding metabolic enzymes that allows to establish new pathways or to increase the efficiency of existing ones. The metabolic network expansion algorithm allows to quantify the topological impact of enzymes and their respective reactions on metabolic networks.

Aims and Objectives

Today, the impact of enzymes acquired via LGT on the recipients metabolism is not fully clarified, as well as the influence on donor and recipient metabolism. In this study we want to describe the impact of recently acquired metabolic genes on the recipient metabolism on a large scale. Further we want to understand if the impact magnitude of the acquired genes is different regarding the transfer mechanisms and taxonomic distances.

Methods

The identification of recent lateral gene transfers has been performed using a combination of phylogenetic analyses with statistical investigations of the amelioration processes of the GC-contents of different genes. Genetic sequences with significantly different GC content compared to their genome and aberrant phylogenetic structure were considered as putative LGTs. 2,382 prokaryotic genomes were used for the analysis, all of them having metabolic network information in the BioCyc Database.

Results

Metabolic impacts of acquired enzymes are different between donor and recipient metabolism. On average, same enzyme impacts are higher on the recipient metabolism compared to the donor. This suggests a risky willingness of the acquiring organism for a putative increased metabolic fitness. Further, metabolic genes encoded in prophages, that may be the result of a transduction event and putatively beneficial for phage escape by boosting the host metabolism, have significantly lower metabolic impact than expected. This observation suggests a strong purifying selection on metabolically valuable genes encoded on prophages.

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eP308

Comparative Genomics of Chromosomally-Encoded Pesticidal Genes Reveals a Novel Prophage-Associated *cry* Cassette

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Introduction: *Bacillus thuringiensis* (Bt) is known for the production of a large variety of toxic proteins, which are widely used as biopesticides in crop protection strategies. These pesticidal toxins are usually encoded on conjugative plasmids and exposed to various recombination events that can lead to improved host adaptation and increased fitness. Nevertheless, some studies have identified rare instances of chromosomally encoded toxins.

Objectives: In this comparative genomics study, chromosomally encoded three-domain Cry toxins of Bt genomes were systematically investigated in order to elucidate how and why were they established within the chromosome.

Methods: The analysis of all Bt genomes from the NCBI was done using IDOPS, a software designed to identify bacterial pesticidal proteins and compare their genetic environments.

Results: We identified (i) a novel genetic cassette consisting of two toxin-associated genes that surround a coding sequence of a three-domain Cry toxin, (ii) evidence of recombination events resulting in various three-domain Cry toxins encoded by either one or two separated genes, and (iii) cassette-associated *Siphoviridae*-like prophage regions.

Conclusion: A conserved arrangement of a prophage-associated *cry* cassette consisting of three components was discovered in *Bt* genomes. The chromosomally encoded cassette consists of highly conserved non-pesticidal components and a variable *cry* toxin. Comparative genomics indicates a contribution of gene exchange by recombination to the evolution of Cry toxins. The discovery of a chromosomal prophage-associated *cry* cassette opens the door to further research on non-plasmid vectors for the horizontal acquisition of *cry* genes by Bt. These observations might hold the key for understanding the evolution of chromosomally encoded Cry toxins.

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eP309

Tree species specific controls on phyllosphere bacterial community composition increase from spring to autumn in a temperate floodplain forest

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Phyllosphere microbiomes undergo substantial changes during different developmental stages of plants. While these changes are well understood for herbaceous plants, we know little about phenology-associated changes of forest canopy microbiomes and their key drivers. We assessed phyllosphere bacterial community composition in May, July and October across four common tree species (*Acer pseudoplatanus*, *Quercus robur*, *Tilia cordata* and *Fraxinus excelsior*) and two positions within the canopy (top and mid) of a floodplain hardwood forest in central Germany. Bacterial 16S rRNA gene targeted amplicon sequencing and quantitative PCR were combined to follow changes in estimated absolute abundances of individual taxa. Sampling month explained about 40% of overall bacterial community variation, while the effect of tree species identity increased from 10 % in May to 20 % in October. May phyllosphere communities showed high variability across tree species and canopy positions and were characterized by high abundances of potential plant pathogens such as *Erwiniaceae,* and taxa commonly associated with pollen and insects. In contrast, variability was strongly reduced in October, and typical phyllosphere genera such as *Hymenobacter*, *Sphingomonas*, *Deinococcus*, and *Massilia* increased by up to three orders of magnitude compared to May. Higher abundances of these taxa were linked to decreased nitrogen content and increased C/N ratio of leaf tissue representative of conditions of early senescence, which was further modulated by canopy position. Our results suggest that tree species specific controls on phyllosphere communities of temperate forest canopies increase during the vegetation period, with drivers of community composition shifting from a presumably high relevance of random colonization to strong controls mediated by the senescent stages of the leaves.

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eP310

Key iron cycle-associated organisms in rice paddy soil impacting phosphorous availbility

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Phosphate is important for plant nutrition including rice, an essential component of mineral fertilizers, and becomes more and more limiting due to the depletion of global deposits. Phosphate adsorbs to or forms diverse iron minerals. Thus, the microbial iron cycle that is driven by various iron-reducing and oxidizing microorganisms impacts iron mineral formation and dissolution and thus phosphate availability. Rice plants transport oxygen via their roots into the soil through their aerenchymas leading to oxic-anoxic gradients. Organic root exudates and oxygen provide an excellent environment stimulating Fe(II) oxidation to Fe(III) and iron hydroxide plaque formation around the roots. Towards the anoxic soil, Fe(III) reduction and dissolution of

the iron hydroxides predominate. Identification of iron cycling microbes is thus of interest to understand phosphorous availability in the root zone of rice plants. Thus, we identified and quantified iron cycling microbial key players in a phytobox experiment.

The experiment described here corresponds to a simulation of agricultural rice cultivation by cultivating phytoboxes with rice soil fertilized with a phosphorus concentration of 60 mg / kg soil and a 7-day-old rice seedling for 60 days. Furthermore, a mesh bag filled with goethite or ferryhydrite labeled quartz sand was placed in the ground. Finally, soil material was removed from different areas of the soil at various points in time of the experiment. The sample materials were cultivated in various iron-containing media and quantified using the MPN approach. In addition, the bacterial community of the various sample materials was analyzed by amplicon sequencing of the 16S rRNA gene and key organisms in the iron cycle were identified.

The evaluation of the MPNs showed that the rhizoplane and rhizosphere contained high concentrations of cultivable iron bacteria, whereas goethite- and ferryhydrate-sand only showed a low number of cultivable iron bacteria. The results of the amplicon sequencing revealed the iron oxidizer genus *Sideroxydans*, *Rhodopseudomonas*, *Acidovorax* and *Thiobacillus*, as well as the iron reducers *Geobacter*, *Magnetospirillum*, *Rhodoferax*, *Desulfovibrio* and *Geotrhrix* as the most abundant key organisms of the iron cycle in rice soil. The abundances of the iron bacteria in rhizoplane and rhizosphere shows a dynamic relationship between iron oxidizers and reducers which shifts towards the iron reducer with increasing distance from the root.

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eP312

Emergence of multidrug-resistant *Vibrio parahaemolyticus* in imported seafood in Germany: Genetic basis and transmissibility of resistance plasmids

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In European countries, multidrug-resistant (MDR) *Vibrio parahaemolyticus* are increasingly reported from imported fish/seafood intended for human consumption. As a natural inhabitant of aquatic environments, *V. parahaemolyticus* in fish/seafood poses a public health risk, as contaminated food products may cause gastrointestinal infections and/or septicaemia, especially in immunocompromised people. The global commodity flow seems to force the dissemination of MDR isolates and will limit the therapeutic options for the treatment of *Vibrio* infections in Europe. Antimicrobial resistance-testing in *V. parahaemolyticus* was conducted according to 2013/652/EU by broth microdilution. MIC data were interpreted using ECOFFs of EUCAST. S1-PFGE, MiSeq-sequencing and bioinformatics were performed to characterize the isolates in detail. The transferability of ESBL- and carbapenemase-producing was assessed by *in vitro* filter mating experiments. Antimicrobial resistance-testing of *V. parahaemolyticus* spp. isolates from imported seafood (n=144) revealed several ESBL- (n=11) and one carbapenemase-producing isolate (n=1). MiSeq-sequencing showed a broad genetic diversity of the isolates regarding their sequence types and resistance determinants (i.e., *bla*CTX-M, *bla*CMY). The carbapenem resistance of one isolate was caused by a plasmid-associated *bla*NDM-1 gene that could be efficiently transmitted to several clinically relevant *Enterobacteriaceae*. The transmissibility, genetic composition of the individual ESBL- plasmids will be also shown in detail. As the number of reports on MDR *Vibrio* from imported fish/seafood of the South-East Asian increases, questions on the safety of food products from this region arises. Our findings underline that antibiotic resistance surveillance need to be extended to the environment close to human activities and foods of aquatic origin.

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eP313

Interkingdom Interactions during Biofilm Initiation in Saliva

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Question: The fungal-bacterial partnership in human oral microbiome has been associated with many biofilm-related diseases, including severe early childhood caries. We investigated the functional benefits that *Candida albicans* and *Streptococcus mutans* derive from forming multicellular coaggregates in saliva on apatitic surfaces.

Methods: We developed a coaggregation model of *Candida albicans* and *Streptococcus mutans* using pooled human saliva and saliva-coated hydroxyapatite (sHA). Super-resolution time-lapse imaging coupled with flow-cell microfluidics was used to investigate the growth dynamics and functional benefits of coaggregates. The software BiofilmQ was used for spatiotemporal image quantification.

Results: We observed that *C. albicans* and *S. mutans* self-assemble into spatially structured coaggregates comprised of bacterial clusters, a network of fungal hyphae and extracellular polysaccharide (EPS) matrices. The coaggregates showed high binding affinity to sHA, displaying several functions that were not observed for single-species aggregates, particularly regarding the tolerance to external stresses and collective spreading across surfaces. Among the multicellular functions of the coaggregates was an unexpected "hitch-hiking" growth behavior, whereby *S. mutans* aggregates were found attached and "riding" onto elongating fungal hyphae, promoting bacterial spreading across the apatitic surface and causing more severe tooth-enamel decay. Notably, the functional benefits were diminished when either the bacterial or fungal counterpart was individually inactivated *via* specific antimicrobials or disruptive agents, indicating a highly interdependent partnership.

Conclusion: Taken together, our results indicate that *C. albicans* and *S. mutans* can form a self-organizing coaggregate in human saliva and initiate biofilms as a single unit with enhanced functionalities, promoting oral disease.

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eP314

Random genetic mutations in Local *A. thaliana* ecotype leads to altered phyllosphere microbiota recruitment

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Introduction

Arabidopsis thaliana is a winter annual whose leaves become colonized by diverse leaf microbiota each

season. Leaves are critical for plant function and their health is ensured by a synergy between plant innate immunity and colonizers that modulate plant defenses, directly eliminate threats, and shape microbiomes. We study diverse wild *A. thaliana* plants and their microbes in Jena, Germany. Our work suggests that colonization of these plants early in their life cycle is important, but that the impact of colonization differs between the populations. However, how leaf microbiota are recruited in general and how recruitment is affected by these different genetic landscapes remains largely unknown.

Objectives

Understanding how balance is achieved in the phyllosphere microbiome of a wild *A. thaliana* population

Materials and Methods

We generated mutants in a local wild type *A. thaliana* genotype using chemical mutagenesis. Resultant M2 generation plants were used for analyzing endophytic bacterial load by CFU counting and amplicon sequencing. Selected plants are being further characterized for altered microbiome recruitment by amplicon sequencing and phenotype analysis in M3 generation.

Results

Surprisingly, many of the resultant mutant plants exhibited a high bacterial load in the M2 generation compared to the wild type, indicating altered microbiota recruitment. This was confirmed for many of the mutant lines in the mostly homozygous M3 generation. One mutant with a dwarf and short petiole phenotype, as well as delayed flowering is being further studied to better understand factors influencing early bacterial colonization, including arisal of microbial interactions.

Conclusion

Our results suggest that genetic mutations in plants influences phyllosphere microbiota recruitment and assembly drastically. Ongoing works in characteristic mutants will give further insight into how altered microbiota recruitment affects plant health and resilience of microbial communities.

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eP315

Improvement of atopic dermatitis by synbiotic baths

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Atopic dermatitis (AD) is a widespread chronic inflammatory dermatologic disorder. This randomized, double-blind study aims to evaluate the effect of synbiotic baths with a defined mixture of six viable lactic acid bacteria (LAB) and prebiotics, without bacteria and prebiotics and placebo baths without prebiotics and bacteria to treat AD patients over a period of 14 days. Therefore, AD patients were randomly assigned into three groups using synbiotic (n = 7), prebiotics (n = 8) or placebo baths (n = 7). Severity of AD was evaluated over time by using severity scoring of atopic dermatitis (SCORAD) and by patient questionnaires. In addition, microbiome on eczematous skin surface was sampled by swaps from each patient before the bath treatment, and after 9, 11 and 14 days of bath treatment. Thereafter, nucleic acids were extracted and the bacterial 16S rRNA gene was amplified via PCR for subsequent amplicon sequencing. Results showed a significantly reduced SCORAD over time of AD patients after daily synbiotic or prebiotic baths. Moreover, AD patients after daily synbiotic baths had a significantly improved pruritus and skin dryness and their bacterial microbiome was enriched by LAB. Taken together, a synbiotic bath is a promising topical skin application to alleviate AD.

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eP316

Establishment of a synthetic microbiome to improve heat tolerance of the sea anemone *Nematostella vectensis*

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Mostly all living multicellular organism are considered as metaorganisms, composed of a host and its diverse microbiome. The microbiome plays an important role in the development, function and fitness of the host. Thus, the host-microbe interactions have been of great importance for the host to evolve, diversify and adapt during time. The sea anemone *Nematostella vectensis* has emerged as a marine model organism for studies on host-microbe interactions. Living in estuarine ecosystems, highly variable aquatic environments, *N. vectensis* has evolved the capability of surviving in a wide range of temperatures and salinities (Darling et al., 2005). Its microbiota is specific for its three developmental life stages (Mortzfeld et al., 2016) and contributes to thermal resistance (Baldassarre et al., 2021). Interestingly, recolonization dynamics of adult polyps recapitulates ontogenetic colonization events, while early colonizing bacteria are capable to mono-associate with germ-free animals, in contrast to most late colonizers (Domin et al., unpublished). However, most of the mechanisms underlying the complexity of the natural microbiome form and function remain unknown. Here, we aim to establish a synthetic microbiome to study the mechanisms of microbiome establishment and its consequences for thermal adaptations with reduced complexity. We made use of an collection of bacterial isolates (611 isolates), retrieved from different developmental stages (Domin et al., 2018) and from animals long-term acclimated to different temperatures (Baldassarre et al., 2021). First, we aim to characterize the ability of early and late colonizers to colonize Nematostella in mono-association. Second, based on metabolic network reconstruction, di-association experiments combining early and late colonizer aim to identify early colonizers enhancing colonization of late colonizers. Third, we aim to design a reduced synthetic microbiome recapitulating the complexity of ontogenetic colonization. Finally, we will assemble a synthetic microbiome, including isolates from acclimated animals, to study the contribution of individual microbial colonizers to the heat tolerance of the animals. Here, single isolates of interest will be tested previously assemble for temperature resistance and recolonization ability.

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eP317

Systematic cross-biospecimen evaluation of DNA extraction kits for long- and short-reaad multi-metagenomic sequencing studies

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In the past decade, microbiome research has become a trending topic with an exponential increase of available data [1]. High quality DNA is a crucial step in metagenomic studies. Bias by different isolation kits impairs the comparison across data sets. A trending topic is, however, the analysis of multiple metagenomes from the same patients to draw a holistic picture of microbiota associated with health and diseases. We thus collected bile, stool, saliva, interdental plaque, sputum and conjunctival swab samples and performed DNA extraction with three different commercially available kits. For each combination of specimen type and DNA extraction, 20 GB metagenomic data were generated using short-read sequencing. While profiles of the specimen type showed closest proximity to each other, we observed notable differences in the alpha diversity and composition of the microbiota depending on the DNA extraction kit. For different sample types, different kits yielded the best results. We reached consistently good results using the Qiagen QiAamp Microbiome DNA Kit. Depending on the specimen, our data indicates that over 10 GB sequencing data are required to have sufficient resolution, but DNA based identification was superior to identification by mass spectrometry. Finally, long-read nanopore sequencing confirmed the results (correlatin>0.98). Our results thus suggest using a strategy with only one kit for studies aiming for a direct comparison of multiple microbiotas from the same patient.

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eP318
Influence of host-microbiome interactions on physiology of the Pacific oyster in extreme habitats

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Host-microbiome interactions are of certain importance in stressful and highly fluctuating environments (such as intertidal zone) where the prompt response from the microbial community might amplify the much slower mechanisms of phenotypic plasticity and evolutionary adaptation of multicellular hosts. Intertidal species in general, and invasive species, in particular, are characterized by high-stress tolerance, and one such recent invasion of the Pacific oyster *Crassostrea gigas* in the hotspot of the Wadden Sea was probably the most dramatic. Recent studies have recognized several fundamental molecular mechanisms involved in the broad tolerance of *C. gigas* to environmental stressors, along with oyster-microbe studies that are primarily focused on defense systems and immune responses of *C. gigas*. However, environmental stress or disease can lead to a shift of microbial balance in *C. gigas*, but it remains unknown whether the changes in the host-microbiota have any functional implications and whether native microbiome structure is vital for the performance and survival of the host in the naturally extreme fluctuating environment.

In our study, we will assess associations between the taxonomic and functional diversity of oyster microbiota, identify the potential functional links between the host and microbiome transcriptomic responses, and test the effects of microbiome taxonomic and functional changes on host physiology and survival along the natural stress gradient in the Wadden Sea.

The stress-induced shifts in microbial diversity and functional activity will be determined with 16S metabarcoding and metatranscriptomics and also gain insights into the functional reorganization of the *C. gigas* microbiota during holobiont acclimatization to different microhabitats.

The stress-induced experiments will determine whether exposure to different environmental stressors results in distinctive shifts of the microbial community along with its functional consequences. Whereas the transplantation study will help to determine the long-term shifts in taxonomic and functional diversity of the microbe assemblages and assess the potential linkages of the host and microbe stress responses during transplantation into the non-native habitat.

Integration of both studies would offer links between host physiology and microbiome diversity and function, and its potential interplay in the holobiont"s adaptation to the stressful intertidal environments of the Wadden Sea.

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eP319
Influence of soil microbiome composition and functionality on plants vegetative processes

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One of the most significant and important sectors in the global context is agriculture. Soil fertility and agricultural production is highly dependent on what plant protection and growth promotion substance is used. Currently, the world focuses on special attention to ecological farming. This is a viable alternative to traditional agriculture. Agricultural biostimulants is a bioproducts containing substances of natural origin and beneficial microorganisms which can be used to activate seeds, plants and soil. Biostimulants used in plants improve the nutritional efficiency of plants, causes changes in vital and structural processes to affect plant growth, improves an abiotic and biotic stresses tolerance and increases the yield and quality of products. The object of this study was to explain influence of soil microbiome composition and functionality on plants vegetative processes. The study was conducted with three different soils of Lithuania agricultural fields. Samples of each soil were collected to identify the composition of the soil microbiome and isolate microorganisms which have a constructive effect on plants and soil. After determination of each microorganism growth conditions of fermentation process was created a microbial consortium – a mixture of microorganisms which works synergistically on plants growth promotion and soil improvement. Experiments with this microbial consortium were performed in plant growth chambers under different conditions to ascertain the best microbial consortium composition. The results helped to identify a biostimulant which demonstrated the strong

application potential for future agricultural and biotechnological application.

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eP320

Keep an eye on me! - Compositional analysis of the slit lamp bacteriota

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Regularly touched surfaces represent fomites. Bacteria on these surfaces can easily be transferred from person to person, promoting the spread of infectious diseases. The same applies to ophthalmologically relevant surfaces, such as spectacles[1] or microscope oculars[2], which are frequently touched, sometimes by different persons, and positioned close to the human eye. In medical environments, slit lamps are widely and frequently used devices for eye examinations. Previous, cultivation-based studies reported a notable contamination of slit lamps with bacteria, mostly coagulase-negative staphylococci, followed by micrococci, bacilli, but also *Staphylococcus aureus*[3].

Our study[4] aimed at obtaining a comprehensive, cultivation-independent overview of the slit lamp bacteriota to better evaluate its hygienic relevance.

We performed massive 16S rRNA gene sequencing to analyse the bacteriota of 46 slit lamps from two tertiary care centers at two sampling sites, respectively, during routine patients examinations within an unannounced audit. In addition, MRSA (multi-resistant *Staphylococcus aureus*) was searched for by means of qPCR.

82 samples yielded sufficient sequences for downstream analyses and revealed contaminations with bacteria of mostly human skin, mucosa and probably eye origin, predominantly cutibacteria, staphylococci and corynebacteria. The taxonomic assignment of 3369 ASVs revealed 19 bacterial phyla and 468 genera across all samples. No MRSA signals above the detection limit were detected. However, Procrustes analysis suggested an exchange of bacteria between the patient´s and doctor´s sites.

Our study provides comprehensive insight into the slit lamp microbiota. It underlines that slit lamps carry a highly diverse, skin-like bacterial microbiota and that thorough cleaning and disinfection after use are highly recommendable to prevent eye and skin infections. Currently we work on establishing a metagenomic approach, based on whole genome shotgun sequencing (WGS), to more comprehensively detect bacterial resistant genes and virulence factors on ophthalmologically relevant surfaces. Furthermore, WGS can provide a broader and more accurate resolution of microbial diversity. However, harvesting sufficient template DNA for such analyses is challenging.

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2) Fritz et al. (2020b). J Clin Med, 9 (5) 1572
3) Olcerst (1987). Am Ind Hyg Assoc J, 48. 425
4) Fritz et al. (2021). Front Cell Infect Microbiol, In press.

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eP321

Nanopore basecalling effects on genome recovery quality for bacterial isolates and environmental samples

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Oxford Nanopore offers practical devices for the sequencing of microbial genomes. The MinION is the entry level device and it is more and more applied in microbiological laboratories. It is small, flexible, easy to handle and cost efficient. However, the quality of recovered genomes is highly dependent on signal and data processing downstream the actual sequencing. A critical step for the quality is the basecalling, i.e. the transformation of the raw signal to the nucleotide sequence of the reads. Basecalling can be done by different programs and with different accuracy [1, 2]. Some procedures are simple and fast, others require the application of state-of-the-art neural networks and require specialized hardware for efficient execution [3]. We found high accuracy basecalling to have major effects on the quality of recovered genomes. To follow the route of quality, we compared basecalled read, assembled contigs and finally generated genomes to systematically analyze the effects of basecalling on the different levels in the genome recovery process. We showed that high accuracy reads generate substantially better genomes for bacterial isolated and environmental samples. In addition, the crucial steps during processing are highlighted to justify that it is worth performing high accuracy basecalling.

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[2] Zhang, Yz., Akdemir, A., Tremmel, G. *et al.* Nanopore basecalling from a perspective of instance segmentation. *BMC Bioinformatics* 21, 136 (2020). https://doi.org/10.1186/s12859-020-3459-0

[3] Perešíni, P., Boža, V., Brejová, B. *et al.* Nanopore base calling on the edge, *Bioinformatics*, 2021;, btab528, https://doi.org/10.1093/bioinformatics/btab528

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eP322

qPCR based detection of three beneficial bacterial strains colonizing F. excelsior leaves

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Microbe-caused plant diseases are a big threat to agricultural and forestry ecosystems and production. The application of pesticides leads to severe environmental problems by contaminating the environment with harmful xenobiotics. An application of pesticides in forest ecosystems is not possible due to their complexity. A positive effect of specific bacteria of the plant-associated microbiome on their health has been revealed previously, e.g. some of them act as antagonists against microbial pathogens. In central Europe occurs the massive ash dieback caused by the invading fungal pathogen *Hymenoscyphus fraxineus*. We develop an approach based on the inoculation of ash plants with bacterial strains that are antagonistic or able to establish a

colonization resistance against the fungal pathogen. To assess the long-term success of such an inoculation, the objective is to monitor the inoculated bacterial strains and measure their persistence on or in the plant leaves, which is the major infections site of the fungal pathogen. We will establish an efficient way to measure the inoculation success by a specific quantification of the inoculated bacterial strains by quantitative PCR. We have screened the genomes of three bacterial strains for strain-specific sequence fragments and designed primer-probe-systems to be used for a qPCR detection. We created genome databases for each of the bacterial strains *Bacillus veleszensis* A4P130, *Luteimonas* sp. D4P002 and *Aureimonas altamirensis* C2P003 with the closest related strains and species. The genomes were then compared by the software Neptune to find specific sequence fragments of the genome only present in the reference genome. After securing the uniqueness of the found genome fragment by a BLAST search against the total nucleotide database, primer-probe-systems were designed with OligoArchitect. After optimization of the PCR conditions in the laboratory, a high sensitivity and specifity were achieved for all three qPCR assays by testing an extended set of phylogenetically related plant-associated bacteria. Based on the developed qPCR assay, we will evaluate the success of inoculation of bacterial strains on ash trees and the effect on the ash dieback in ongoing plant experiments.

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eP324
Standardized microbial mock community against pandemic threats

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Microbial monitoring has become an increasing and essential research interest particularly due to the global SARS-CoV-2 pandemic. Predominantly affected was public transportation, which is a mean of transportation used by millions of people on a daily basis. Mass transit systems represent confined rooms, where transmission of infectious diseases through bioaerosols and surfaces is facilitated.

To this date, no authentic, microbial enriched bioaerosol has been used and tested for aerosol distribution research. In this study, an artificial microbial mock community was developed, that consists of surrogates from human-associated bacteria that have been found in the airplane cabin (based on Weiss *et al.* 2019). For technically safe usage in field studies, it was inactivated by high dose X-Ray irradiation. We aimed it to be highly reproducible; for this reason it consists of commercially available bacterial type strains.

Among others, low temperature shelf life and desiccation stress testing was performed as with both X-Ray irradiated and unirradiated mock communities. For analysis, 16S amplicon rRNA sequencing was performed by two independent facilities.

The obtained results from amplicon sequencing conducted in two individual sequencing facilities showed that the abundances within the mock communities were similarly distributed, but did not completely match. That observation was made for almost all treatments, which proves that significant abundance shifts can be well detected by 16S rRNA sequencing. After desiccation, the mock community was still detectable with similar abundances as the control. Moreover, testing the stability of the mock community at 4°C over one week, a good shelf life was observed.

The findings build a foundation for future studies in interdisciplinary aerosol research, using the developed microbial mock community as a biological tool for various studies in applied bioaerosol research as well as for technical improvement studies. It represents a realistic, yet safe and reproducible product used in bioaerosol research for pandemic research, contributing to the development of optimized ventilation systems, or as reference for the evaluation of decontamination methods.

Weiss, Howard, Vicki Stover Hertzberg, Chris Dupont, Josh L. Espinoza, Shawn Levy, Karen Nelson, and Sharon Norris. 2019. "The Airplane Cabin Microbiome." Microbial Ecology 77 (1): 87–95. https://doi.org/10.1007/s00248-018-1191-3.

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eP325
Comparative Genomic Analysis of Phages in Three Common Gnotobiotic Mouse Models

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Gnotobiotic murine models harboring microflora are important tools in microbiome research as they provide evidence of how bacterial consortia influence the host system and trigger health and disease. However, so far, no data has been generated, which clarifies the diversity of phages and viruses in various gnotobiotic models, which may strongly modulate the effects of the introduced microflora affecting their abundance and activity as well as their genome composition. In the frame of this study, we investigated the virome of two gnotobiotic murine models, namely Oligo-MM12 (12 bacterial species) and reduced Altered Schaedler Flora (ASF, 3 bacterial species). For comparison, the virome of specific Pathogen Free (SPF) mice was determined. We used a metagenomics approach to assess viruses, prophages, and bacteriophages from the ileum and colon of 6 weeks old female mice. We found in all samples reads, which could be linked to eukaryotic viruses of the family *Retroviridae*, which were not affected by the different bacterial diversity in the investigated murine models. In contrast, for prophages and bacteriophages, we could identify a positive correlation between bacterial diversity in the gut of the mice and diversity of associated reads for bacteriophages and prophages. Overall bacteriophages followed the expected pattern of their bacterial hosts. For example, bacteriophages associated with *Lachnospiraceae* and *Muribaculaceae* prevailed in SPF colon and ileum, while bacteriophages linked to *Akkermansiaceae* were prominent in Oligo-MM12 ileum, whereas bacteriophages assigned to *Clostridicaceae* dominated ASF colon. Only bacteriophages of the families *Myoviridae* and *Siphoviridae* were present in all samples. For prophages, a similar pattern was observed. Prophages linked to *Clostridicaceae* were most prominent in Oligo-MM12 and ASF while prophages linked to *Lactobacillaceae* were specific to ASF. Interestingly for both SPF and ASF, a higher number of prophages were present in the colon than the ileum, whereas this was not observed for the MM12 mice. We found that virus-associated AMGs contributed mainly to amino acid metabolism, and their distribution correlated with microbiota complexity, indicating that differences in the virome strongly impact horizontal gene transfer.

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eP326

Hydrogeological controls and genomics of biogeochemically-relevant bacterial consortia in subsurface mine waters

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Pyrite oxidation drives iron (Fe) and sulfur (S) availability across Earth"s biomes and is partly microbially mediated. *In situ* microbial communities can thus either enhance pyrite weathering or act as a biogeochemical bottleneck in the iron and sulfur cycles. However, our understanding of community structure dynamics and associated biogeochemical reactions in Fe- and S-rich lithologies, such as pyritic coal, is limited. Here we present the first comprehensive regional and seasonal survey of genus-level structure of bacterial groundwater communities in a coal-based aquifer (South Wales Coalfield (SWC)), using 16S rRNA gene amplicon and shotgun metagenomics sequencing.

Seasonal changes in community structure were limited as revealed by 16S rRNA gene amplicon sequencing, suggesting limited influence of surface water and surface-derived microbial communities on subsurface communities. Instead, hydrogeologically distinct mine water blocks largely explained bacterial community structure variation across sites. Two Fe(II)-oxidising Betaproteobacteriales genera, *Gallionella* and *Sideroxydans*, dominated the bacterial communities across 12 sites and seven mine water blocks, while three sites in a single mine water block were dominated by two S-oxidisers, *Sulfuricurvum* and *Sulfurovum*.

A total of 159 high-quality metagenome-assembled genomes (MAGs) representative of coalfield microbiomes were recovered to further interrogate the co-occurrence and metaboic potential of these bacterial genera. *Gallionella* and *Syderoxydans* presented metabolic pathways to support microaerophilic pyrite oxidation. It was posed that under anaerobic conditions only *Gallionella* could continue to do so - *Syderoxydans* putatively making use of a T6SS to predate or parasitize. Genomic evidence was found to support an anaerobic lifestyle for *Sulfuricurvum* that could further metabolise a panoply of sulphur species. Surprisingly, Sulfurovum was inferred to follow a sessile lifestyle, not presenting typical anaerobic metabolic pathways.

The co-occurrence of pairs of Fe(II)- and S-oxidising bacterial genera suggests functional redundancy coupled with genus-specific morphologies and life strategies in SWC groundwater, indicating the importance of distinct environmental and ecological niches on biogeochemistry at seasonal and regional scales.

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eP327

Comparing the maize rhizosphere microbiome of old landraces and modern varieties in the context of drought tolerance

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Plant-associated microbial communities are well known to affect health and agronomic performance of crop plants. Within the BMBF-funded project RhizoTraits, we investigate how the microbiome of modern, yield optimized varieties of *Zea mays* differs compared to that of old landraces. We hypothesize that plant traits fostering the formation of a beneficial microbial community have been compromised in the breeding of modern varieties. Focusing on drought stress mediation as one important function of the microbial community, we are performing a systematic characterization of the rhizosphere and root microbiome of plants grown in two large-scale experiments. In the first experiment, we screened 48 different maize varieties under greenhouse conditions. Subsequently, we analyzed a selection of these varieties in detail under field conditions. Our currently ongoing analysis include methods for the quantitative, qualitative and functional characterization of the rhizosphere bacterial and microeukaryote community. Microbial abundances and activities are assessed using basic indicators such as root-associated extracellular enzyme activities. Community composition is investigated via SSU rRNA amplicon sequencing. By linking our findings with data on root morphology, rhizodeposition, plant physiology and edaphic properties, we aim to contribute to the development of a holistic understanding of the mechanisms and functions within the plant-microbe system under water limitation. This will provide a valuable basis for considering the rhizosphere microbiome in the development of future crop varieties and agricultural practices.

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eP328

Understanding the functional roles of within-host-evolution of gut microbiota

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The mammalian gut is a highly dynamic microbial ecosystem that impacts host fitness. The metabolic networks established between intestinal bacteria affect several microbiome functions including colonization resistance (CR) against enteric pathogens, which is in part mediated by competing for nutrients that are essential for the invading pathogens such as *Salmonella enterica* serovar Typhimurium (*S.*Tm). One of the factors that strongly influence these metabolic networks and bacterial interactions is within-host evolution. While community composition and function have been extensively studied, it remains unclear how these are affected by within host evolution. In this study, we use experimental evolution as a tool to get insights into the adaptation of a defined microbial consortium to the gut environment and its functional relevance for CR against *S.* Tm. Our preliminary results show that during colonization of the mouse gut key strains involved in CR adaptively evolve and accumulate extensive, non-synonymous mutations in functional gene classes. We also show that this community evolution translates into an increased colonization resistance to *S.* Tm enteric infection. Further understanding the

mechanisms driving evolution will allow engineering microbial communities towards a healthy state of the host.

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eP329

Getting me a good reference – A pipeline to create a customized reference sequence set to analyse the oral microbiome

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Polymicrobial biofilm-associated infections still represent a major risk of developing peri-implantitis. This can lead to the loss of the dental implant. It is not yet sufficiently known how genotypes and phenotypes of the complex oral microbiome are linked in this process.

To identify biomarkers that enable prediction of disease progression, the integration of genomics and omics data is a key method. However available datasets do not sufficiently combine the high-quality data available from reference strains with the genomic diversity covered in metagenome sequencing projects.

Here we present a pipeline to incorporate several publically available sequence repositories, related to the oral microbiome, in a customized comprehensive reference data set. As a result we combine the advantages of well curated databases like the Human Oral Microbiome Database (HOMD) with the broader diversity of hard to culture microbial strains covered by metagenome studies. In addition to an increased mappability of transcriptomic reads, we gain a more comprehensive view on functional characterisation and annotation of the human oral microbiome, creating a sufficient basis for biomarker detection.

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eP330

Resolving low-abundance microbiomes from swabs in DNA protectant

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Bacterial communities for sequencing-based metagenomics analyses are regularly collected with swabs and stored in DNA/RNA protectant. However, this affects and limits the options for further sample processing. Especially samples with low bacterial cell numbers, such as throat swabs, require well-adapted DNA extraction procedures. In addition to low bacterial DNA content, bacterial adherence to the swab and possible incompatibility of the DNA/RNA protectant with the DNA extraction protocol need to be taken into account. We established a protocol to resolve the bacterial community to the highest possible depth under these conditions, while maintaining the community composition as good as possible, and at the same time to minimize the influence of DNA contaminations.

Therefore, we compared different DNA extraction protocols, including two different kits (QIAamp DNA Mini kit (Qiagen) and FastDNA SPIN Kit for Soil (MP Biomedicals)) and two different procedures each. These included the swab as well as the complete DNA protectant solution to minimize the loss of parts of the bacterial community. A procedure for amplicon sequencing based on complete 16S rRNA-gene sequencing with HiFi reads (PacBio) was established to optimize the resolution of the microbiomes under these conditions. As representative for low abundance microbiomes found on throat swabs, we used a standardized, commercially available mock community. This comprised 10 different bacterial and yeast species, which were diluted to the extremely low bacterial numbers as they were typically observed on such swabs. To evaluate bacterial numbers and also potential DNA contaminants, bacterial spike-ins were added to samples as well as extraction negative controls.

Since many human oral samples come with low bacterial numbers, special laboratory pipelines maximizing output and integrating sufficient controls are necessary to resolve these communities.

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eP331

The Role of Soil Microbiome in the Response of two Potato cultivars towards Short-term Drought

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Soil microbes play an important role in plant health and fitness. Despite potato being the 4th most important food crop on the global scale, most extensively farmed potato cultivars are highly sensitive to drought. Understanding the role of the microbiome in modulating drought tolerance could provide an additional tool to address this issue. In this study, we investigated the correlation between the rhizobiome and the performance of two potato cultivars, MONI, known to be drought-tolerant and HERBSTFREUDE, which was considered as more drought sensitive. Considering this, we expected (H1) that a reduction of soil microbe diversity (autoclaved soil) would increase the sensitivity of MONI to drought. Also, we hypothesized (H2) that the rhizosphere microbiome of MONI is more diverse than HERBSTFREUDE. Moreover, under stress conditions, monoderm phyla, such as Actinobacteria (streptomyces), Chloroflexi and Firmicutes, will be more enriched in MONI when compared to HERBSTFREUDE (H3). We conducted a greenhouse trial with in vitro potato plantlets, grown for 28 days in natural (NSM) or autoclaved soil (DSM) and subjected to drought stress for 14 days. Plant parameters confirmed that MONI is drought tolerant, regardless of the soil type (as expected in H1) and the bacterial community structure of the rhizobiome was not affected by the stress. However, HERBSTFREUDE below-ground parts were adversely affected by drought, especially in DSM, where we observed an increase in the bacterial species diversity. For both cultivars, fungal communities were less affected by drought but showed a strong response towards the soil microbiome manipulation.

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eP332

Targeted use of microbial consortia to support plants in sustainable cultivation

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The development of sustainable products, optimized processes and methods for the conservation of natural resources is currently a broad field of research. Especially in practical horticulture, the development and improvement of peat-free substrates becomes increasingly important. One field of research of the Erfurt Research Centre for Horticultural Crops (FGK) concerns plant-beneficial microorganisms for sustainable crop production. In frame of this research, defined microbial consortia are selected to replace the functional properties of peat.

In the first step of this project, the influence of the microbiota of compost of plants and of the greenhouse environment on microbial substrate and plant root communities in dependence of the genotype and the developmental stage of the model plant petunia (*Petunia axillaris* and *Petunia exserta*) is investigated by next generation sequencing (NGS). During the culture period, plants were phenotyped, the physical and chemical properties of substrates and the microbiome of substrates and roots were analyzed and rhizosphere-associated/potential endophytic microorganisms were isolated, cultured, characterized and identified in parallel.

The results of the microbiome analysis indicated that Proteobacteria dominate the bacterial endophytic community and that representatives of Basidiomycota from compost prefer to colonize the roots of *P. exserta* during time. Comparing the different stages of plant development, the relative abundance of Verrucomicrobiota and Basidiomycota seems to be higher at later stages. Furthermore, an increase of Verrucomicrobiota and Sumerlaeota in substrates could be observed over time. Microorganisms from the greenhouse surrounding establish a complex microbial community in the substrates and in the roots, which differs substantially from the input from the compost. Approximately 350 bacterial and fungal isolates were cultured and are currently characterized with respect to their physiological properties to assemble a functional microbial community. Network analyses are intended to support the identification of microbial key players and communities within the ecosystem with the aim of the compilation of microbial consortia for application in peat-free substrates.

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eP333
Associated microbiota present during the *Aurelia aurita* polyp life stage is essential for polyp to jellyfish transition

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All multicellular organisms are associated with a microbiota, which is mostly unique for each host. This microbiota forms a common functional unit with their host through multiple forms of interaction, a so-called metaorganism. It is already known that this host-microbe symbiosis plays a significant role in health and fitness of a host. In particular, it was recently shown that the associated microbiota of the moon jellyfish *Aurelia aurita* is crucial for reproduction output and moreover protects against pathogenic-bacteria.

Due to the demonstrated lack of asexual reproduction in the absence of a native microbiota (germ-free animals) (Weiland-Bräuer *et al*., 2020), our current studies focus on deciphering the crucial time point(s) of microbial presence during the life cycle of *A. aurita*, which leads to a typical reproduction output.

For this purpose, a re-colonization experiment of sterile *A. aurita* polyps was conducted implementing the re-colonization at different time points in the life cycle. This clearly demonstrated that the reproduction can exclusively be restored by re-colonization of the polyp life stage before entering the strobilation process. Those morphological observations were further verified by transcript analysis of selected developmental genes of the host by quantitative (q)RT-PCR. Here the absence of the native microbiota in general resulted in decreased transcript levels of strobilation genes. In addition, similar transcript patterns as observed for the native polyp were exclusively revealed for the re-colonization of the polyp life stage and not for the early and late strobila stages. These findings strongly indicate a host-microbe communication at the polyp stage, which is crucial for the polyp-strobila transition.

Our findings pave the way for studying the functions of microbial community members and their underlying interactions in more detail. For instance, we are currently underway to verify the microbial key players within the highly complex and diverse native microbiota crucial for an intact reproduction and aim to decipher the molecules underlying this function.

Reference: Weiland-Bräuer N, Pinnow N, Langfeldt D, Roik A, Güllert S, Chibani CM, Reusch TBH, Schmitz RA. 2020. The native microbiome is crucial for offspring generation and fitness of *Aurelia aurita*. mBio 11:e02336-20. https://doi.org/10.1128/mBio.02336-20

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eP334
***Hydra* and *Curvibacter* - inter-kingdom communication at the base of animal evolution**

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Every multicellular organism must be considered a metaorganism, a close association of viral, microbial and eukaryotic species. Using *Hydra* as a model we aim to get a deeper understanding of interkingdom signaling between a host and its microbiota. *Curvibacter sp.* represents the most abundant colonizer on a *Hydra* polyp (Minten & Fraune, 2020). Interestingly, *Hydra* and *Curvibacter* show a high degree of congruency in their phylogenetic trees, indicating co-speciation. *Curvibacter* uses quorum sensing (QS) for host colonization, while *Hydra* modifies QS signals that might help retaining *Curvibacter* colonization (Pietschke et al 2017). Moreover, host transcriptomic analysis revealed a strong response to *Curvibacter*-derived acyl homoserine lactone (AHL) that overlapped with the response of germ-free animals to *Curvibacter* recolonization. One of the responsive gene codes for Eco1 peptide, known to respond to bacterial colonization and temperature (Taubenheim et al, 2020). Eco1-KD animals revealed a disturbed foot-inhibition gradient, indicating a potent role of this peptide in response to symbionts and environment.

Here, we aim to understand the underlying inter-kingdom communications on a molecular level. On one hand we use a metatranscriptomic approach to identify *Curvibacter* genes necessary for host colonization. The data revealed that *Curvibacter* significantly upregulates genes that are also regulated by AHLs, indicating that QS plays an important role during host-colonization. These results were confirmed using

novel *Curvibacter* bioreporters in an *In vivo* experiment, showing that *Curvibacter* expresses these genes only in host-association. Interestingly, most of the strongest upregulated genes during host-colonization are involved in the detoxification of reactive oxygen species (ROS), indicating a major role of ROS in the symbiotic interactions. We also identified a small secreted peptide, exclusively expressed in *Curvibacter* during host association. Currently we generate *Curvibacter* knock-out mutants to investigate the function of these genes. On the host side we aim to identify the AHL receptor(s) and perform transcriptional knockdown to study the role of bacterial derived molecules on host development. These new insights will lead to a better understanding of signalling interactions between *Hydra* and *Curvibacter* and potentially unveil ancient mechanisms of host-microbe crosstalk.

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eP335

Mechanisms determining temporal bacterial colonization dynamics during early *Nematostella* development

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Organisms such as humans and animals are dependent on a coordinated unity and mutual communication with microorganisms residing within them. The starlet sea anemone *Nematostella vectensis,* and its associated microbiome provide a valuable platform to address questions related to the symbiosis between a host and its microbiome. A remarkable feature of *N. vectensis* are the three different microbiota compositions associated with the three life cycle stages (Mortzfeld et al., 2016). However, how this shift of microbial community arises is still unknown. In previous experiments, gnotobiotic adult *N. vectensis* polyps were recolonized with bacterial consortia of all life cycle stages. 16S rRNA sequencing revealed that independent of the bacterial inoculum all recolonization successions mimic the ontogenetic pattern (Domin et al., 2018). This indicates that the ontogeny is not the driving factor of the microbiome but other host-driven factors and bacteria-bacteria interactions. RNA-Seq experiments two days post recolonization revealed a large subset of receptor tyrosine kinase signaling pathway components differentially regulated in response to bacterial colonization. Combining this result with a tissue specific transcriptome exposed that these genes are also highly expressed in nematosomes (Babonis et al., 2016). Nematosomes are free-floating multicellular bodies insight the hosts cavity, which apparently carry the ability of phagocytosis. Therefore, we assume that nematosomes are an important host factor to regulate and maintain the microbiome composition. To investigate the role of nematosomes shaping initial colonization events, we are currently establishing *N. vectensis* strains containing genetically manipulated nematosomes. Therefore, we identified Adam and cJun as promising target genes, revealing high expression in nematosomes. We study these genes by genetic manipulation strategies including: (i) a fluorescent reporter line under the promoter of respective genes, (ii) inducible deletion of nematosomes by NTR system, and (iii) modification by CRISPR/Cas9 to obtain deficiency in the proper functionality of nematosomes. Our goal is to treat the aforementioned genetically modified *N. vectensis* strains with bacterial inoculates to study the phagocytotic activity and transcriptomic changes to ultimately identify the role of the host in the regulation of initial bacterial colonization events.

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eP336

Deep RNA sequencing identifies novel clues of microalgae and bacteria interactions

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Introduction. Tight associations between microalgae and bacteria caused the evolution of a complex network of cross-kingdom interactions. Orchestrated nutrient exchange, quorum sensing mediation and quorum quenching of the signals define a wide spectrum of associations at highly complex assemblages of unicellular microalgae and associated bacteria. However, it still remains mostly unknown if the associated microbiota is specific for the microalga and which role individual bacterial taxa play.

Recently, we isolated *Dyadobacter* sp. HH091, which strongly stimulated growth and photosynthesis of the alga *Scenedesmus quadricauda* and *Micrasterias radians*.

Objectives. Our goal is to identify key mechanisms and signals involved in the interaction of bacteria and microalga.

Methods. We sequenced the microbiome of the microalga *Scenedesmus quadricauda* isolated various bacterial strains and established a metatranscriptome.

Results. A metatranscriptome analysis in *Scenedesmus quadricauda* microbiome indicated that the transcriptionally most active bacteria belong to the phylum of the Proteobacteria and Bacteroidetes. The most strongly expressed genes were those linked to secretion systems, QQ mechanisms, polysaccharide utilizing gene clusters, unique signal transduction systems and different mechanisms of secondary metabolites. These data give first clues on the specificity of the Dyadobacter-alga interactions.

Conclusion. These data expand our understanding of species interactions within aquaculture and identify several genes involved in the molecular basis of bacteria-alga interactions that can serve as an established synthetic plant-bacteria system. Therefore, the genome and metabolic potential of *Dyadobacter* sp. HH091 is of particular interest in understanding bacteria-algae interactions.

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eP337

Unveiling a novel function for methylated metabolites in algal-bacterial crosstalk

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Marine heterotrophic bacteria are closely associated with phototrophic algae, and play crucial roles in global carbon cycling. These bacteria consume photosynthetically-fixed carbon, and redistribute algal-produced metabolites throughout Earth"s carbon pools. The bacterial consumers face a seasonally re-occurring problem: Algal carbon production is low in winter, but peaks with the onset of algal blooms in spring. Seasonal fluctuations in algal carbon production force bacteria to endure prolonged phases of starvation, and swiftly activate their metabolism in response to the onset of algal blooms. We found that minute amounts of *N*- and *S*-methylated compounds, which are abundantly produced by algae, kick-start the metabolism of bacteria

known to dominate algal blooms. Growth experiments revealed that algal-produced methylated compounds induce a marked shortening of the bacterial lag-phase. Transcriptomics, metabolomics, and biochemical analyses further indicate that methyl groups are a limiting resource for bacteria during the lag-phase. We hypothesize that algal-associated bacteria, which are adapted to efficiently harness methyl groups from phototrophic hosts, outgrow their competitors at the onset of algal blooms. Our findings reveal a novel function for methylated compounds in phototroph-heterotroph interactions.

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eP338

Breznakiellaceae fam. nov. and the evolutionary radiation of "termite gut treponemes" (_Spirochaetales_)

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The intestinal tracts of termites are abundantly colonized by a diverse assemblage of spirochetes. Most of them fall into two monophyletic groups within the radiation of the genus *Treponema* that occur exclusively in termites. A comprehensive phylogenomic analysis including 317 metagenome-assembled genomes (MAGs) obtained from 45 termite species revealed that members of the genus *Treponema* are extremely diverse and represent two separate, family-level lineages: the *Treponemataceae* sensu stricto, which comprise the majority of the validly described *Treponema* species, and *Breznakiellaceae* fam. nov., which comprise the remaining members of the genus, including the majority of "termite gut treponemes" and the recently isolated *Breznakiella homolactica* from cockroaches. The new family accommodates the misplaced *Treponema* spp. as new combinations (*Leadbetteria azotonutricia*, *Gracilinema caldarium*, *Helmutkoenigia isoptericolens*, and *Zuelzeria stenostrepta*). In addition, we propose *Rectinemataceae* fam. nov. to include the genus *Rectinema* in order to avoid paraphyly of *Treponemataceae*. A reclassification of previously obtained 16S-rRNA-based amplicon libraries from termite guts with the new phylogenomic framework of *Spirochaetales* helped to identify MAGs from so-far uncultured, flagellate-associated lineages and sheds new light on the evolutionary radiation of "termite gut treponemes" and their acquisition of reductive acetogenesis by lateral gene transfer.

References:

Song, Y., Hervé, V., Radek, R., Pfeiffer, F., Zheng, H., Brune, A. (2021) Characterization and phylogenomic analysis of *Breznakiella homolactica* gen. nov. sp. nov. indicates that termite gut treponemes evolved from non-acetogenic spirochetes in cockroaches. *Environ. Microbiol.* **23:** 4228–4245.

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eP339

A novel family of short DNA-binding proteins might be involved in providing nuclear control over the genetic system of the evolutionary-early-stage photosynthetic organelle in the amoeba _Paulinella chromatophora_

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The cercozoan amoeba *Paulinella chromatophora* contains a photosynthetic organelle (the "chromatophore") that evolved ~100 million years ago from a cyanobacterium – independently from and much more recently than the primary endosymbiosis that resulted in plastids in plants and algae. Previous work has shown that despite its more recent origin, the chromatophore is genetically integrated into the host cell depending on the import of hundreds of nucleus-encoded proteins. As a result, the chromatophore contains chimeric metabolic pathways and multiprotein complexes composed of proteins of dual genetic origin. How gene expression in nucleus and chromatophore is coordinated to assemble multiprotein complexes and chimeric pathways at stochiometric amounts is currently unknown.

Nucleus-encoded chromatophore-targeted proteins include an expanded family (~200 predicted members) of small proteins of around 7 kDa of unknown function. Interestingly, homologous sequences are also present as a domain of unknown function in a number of DNA-interacting proteins from other species. By using X-ray crystallography and 3D structure modeling software we were able to determine the 3D structure of members of this group of short proteins demonstrating similarities to different DNA-binding proteins including the C-terminal domain of the bacterial xenogenic silencer MvaT. By using electrophoretic mobility shift assays (EMSA) and bio-layer interferometry (BLI), we show that members of this group of proteins indeed bind to DNA with physiologically relevant affinities. Furthermore, we show that binding affinity is much higher to a chromatophore-derived promotor region compared to a random DNA fragment from a commercial plasmid.

Based on the collected data, we hypothesize that this expanded family of short chromatophore-targeted proteins binds to specific regions in the chromatophore genome and provides nuclear control over chromatophore gene expression, genome replication or genome segregation.

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eP340

Three endosymbiont-targeted proteins are likely involved in coordinating the cell cycle of the trypanosomatid _Angomonas deanei_ and its b-proteobacterial endosymbiont

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Introduction

The trypanosomatid *Angomonas deanei* harbors an obligate, nutritional β-proteobacterial endosymbiont that lies free in the host cell cytoplasm. Cell cycle of host and endosymbiont are tightly synchronized indicating a high level of cellular integration. Interestingly, a number of proteins described as essential components of the cell division machinery in free-living proteobacteria are missing in the endosymbiont.

Objectives and Approach

To gain insights into host-symbiont interaction in general and in particular to test whether host-encoded proteins might complement for cell division genes lost from the endosymbiont genome, here we aimed to identify host-encoded proteins that localize at the endosymbiont division site and characterize them functionally. To this end, we employed a combination of mass-spectrometry based proteome analysis, expression of fluorescent marker protein fusions in *A. deanei,* and in vitro protein activity assays.

Results

Proteome analyses of purified endosymbionts identified 7 host-encoded **e**ndosymbiont **t**argeted **p**roteins (ETPs). Fluorescent marker protein fusions of three of these ETPs (ETP2, ETP7, and ETP9) localize in a putative division ring surrounding the endosymbiont division site. While ETP7 and ETP9 seem to be restricted to the division ring, ETP2 can be found at the poles of the endosymbiont too. Null-mutants of ETP2 and ETP7 could not be obtained suggesting an essential function of these proteins. Interestingly, ETP7 is annotated as a putative peptidoglycan (PG) hydrolase suggesting a possible complementation of a PG-binding protein (PBP) with PG hydrolase activity lost during endosymbiont genome reduction. Specific binding of a fluorescent Penicillin derivate to ETP7 supports its PG-binding ability. Currently, ETP7 is tested rigorously for PG-hydrolase activity by different in vitro assays.

Conclusion

As PBPs are known for their role in bacterial division, ETP7 provided by the host might allow for the restructuring of the PG-layer of the endosymbiont at the division site which is required for endosymbiont division. Our study demonstrates that host-symbiont interactions can go far beyond the exchange of metabolites and specific host proteins can be targeted to an endosymbiont where they likely control key aspects of endosymbiont biology such as cell division.

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eP341
Symbiont rejuvenation in an ancient nutritional symbiosis? Clade specific evolution of the chemosynthetic Ca. Riegeria symbionts is linked to rare host switching in the mouthless marine flatworm Paracatenula

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Without the influx of new genetic material, the genomes of obligate and vertically transmitted symbionts ultimately disintegrate. Symbiosis breakdown often is avoided via complementation from secondary symbionts or via host encoded functions. Mouthless *Paracatenula* flatworms obligately depend on their intracellular *Cand*. Riegeria symbionts for all aspects of nutrition. Here we show that in this hundreds of millions of year old chemosynthetic association, strong stabilizing selection for the symbiont shields all essential biosynthetic pathways from disruption. The symbiont genomes for a large diversity of host species show no signs of disintegration, are between 1.2 and 1.5 Mb and code for complete host nutrition and efficient chemoautotrophy. The symbionts provide all building blocks for macromolecules, all vitamins and co-factors and a variety of storage options for the holobiont. Despite the stable vertical transmission for the majority of host clades, we observe rare symbiont switching events that are linked to patterns of genome rejuvenation such as multiple gene gains and a maximum genome size of 2.0 Mb. Apparently, rare rebirth fuels the evolutionary ecology of one of the oldest animal-microbe interactions.

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eP342
Potential roles for lipids and lipases in nutrition and immunity during symbiotic plant-microbe interactions

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In plants, the root endophytic fungus *Serendipita indica* enhances growth, nutrition and conveys protection against pathogens. It follows a biphasic colonization strategy comprising a biotrophic and a cell-death associated stage. The latter is accompanied by induction of fungal hydrolytic enzymes and transporters. However, it remains unclear why *S. indica* shows this biphasic growth style.

Lipids are essential cell components that make up membranes and serve as energy and carbon storages. They also function during signal transduction and stress signaling. As synthesis of lipids is energy-demanding, plant-derived lipids are taken up by both pathogenic and mutualistic fungi as nutrition. Lipids, among other molecules, can be perceived by plants as microbe-associated molecular patterns (MAMPs), leading to Ca^{2+}-influx, production of reactive oxygen species (ROS) and induction of Pathogenesis-related genes. Recently, the role of lipids during plant immune signaling is being unraveled.

Here, the function of secreted hydrolytic enzymes during the cell-death associated growth phase of Sebacinales is investigated. Besides, perception mechanisms of lipids derived from Sebacinales and how this is integrated with host immune signaling are of interest.

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eP343
Adaptive laboratory evolution of *Corynebacterium glutamicum* provides mechanistic insights into the interaction of heme-responsive two-component systems

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Heme is a multifaceted molecule. While serving as a prosthetic group for essential cellular processes, elevated intracellular levels are toxic to cells. The complexity of this stimulus has shaped the evolution of regulatory networks. Interestingly, several corynebacterial species employ two paralogous, heme-responsive two-component systems (TCS), HrrSA and ChrSA, to cope with heme stress and to maintain intracellular heme homeostasis. Cross-talk at the level of phosphorylation was previously shown to occur between the heme-responsive TCS, while phosphatase activity appeared to be specific for the respective cognate kinase (1).

In this study, an adaptive laboratory evolution (ALE) of *Corynebacterium glutamicum* on increasing heme concentrations led to the identification of beneficial mutations in the histidine kinase ChrS. While heme concentrations above 5 µM are already toxic to wild type cells, evolved clones were able to grow even in the presence of more than 100 µM. The most efficient mutant showed a frameshift mutation downstream of the DHp (dimerization and histidine

phosphotransfer) domain of the histidine kinase thereby abolishing the activity of the catalytic domain. Transcriptome analysis as well as reporter studies highlighted the importance of the ChrA-regulated heme exporter HrtBA for the cellular adaptation to heme stress conditions. Further mutational analysis provided insights into heme-responsive TCS signaling. Remarkably, our study also revealed an important role of heme-binding proteins for coping with elevated heme levels. Altogether, this study gives insights into the key strategies employed by *C. glutamicum* to adapt to heme stress.

1. Hentschel E, Mack C, Gätgens C, Bott M, Brocker M, Frunzke J. 2014. Phosphatase activity of the histidine kinases ensures pathway specificity of the ChrSA and HrrSA two-component systems in *Corynebacterium glutamicum*. Mol Microbiol 92:1326-42 doi:10.1111/mmi.12633.

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Transcriptional analysis of *disA,* the gene coding for diadenylate cyclase in *C. glutamicum*

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The second messenger cyclic di-AMP is an essential signaling molecule in bacteria which is involved in many diverse aspects of bacterial physiology, including cell wall metabolism, potassium uptake, osmotic stress, and detection of DNA damage. The Gram-positive, non-pathogenic *Corynebacterium glutamicum* is not only used for the industrial production of amino acids but also serves as a model organism for pathogenic *Mycobacterium tuberculosis.* In *C. glutamicum*, c-di-AMP is synthesized by the sole diadenylate cyclase DisA and its coding gene is located downstream of the DNA repair gene *radA*[1]. These genes are considered to form a bicistronic operon, with only three bases in between both genes.

We here show the genetic organization as well as the expression profile of the hypothetical *radA-disA* operon. Previously, it was reported that *radA* is upregulated by DNA damage and thereby we tested *C. glutamicum* WT with different DNA stresses. Surprisingly, in stress conditions, increased transcript amount was observed for *radA* but *disA* levels were not affected. Although RT-PCR showed that *radA-disA* is transcribed as an operon, we also identified and verified an additional promoter for *disA*. Further comparative promoter studies and investigation of a putative antisense transcript as a potential regulating element revealed independent expression patterns for *radA* and *disA*, suggesting only involvement of RadA but not DisA in DNA damage response.

[1] Pfeifer-Sancar *et al.* Comprehensive analysis of the *Corynebacterium glutamicum* transcriptome using an improved RNAseq technique. *BMC Genomics* 14, 888 (2013)

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eP345

The role of the DUF1127 protein family in phosphate and carbon metabolism in *Agrobacterium tumefaciens*

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In any given organism, the function of one-third of all proteins is unknown. One domain of unknown function is DUF1127. This domain contains arginine-rich proteins which are found in approximately 17,000 proteins in 4,000 bacteria, e.g. in Rhodobacter. sphaeroides [1]. In the plant pathogen *A. tumefaciens* we identified seven proteins containing the DUF1127 domain: three small proteins (47 and 48 amino acids) and four long proteins (72 to 101 amino acids) [2]. The respective genes are differently regulated by the transcriptional regulator LsrB and expressed under different conditions [2]. Our current study focusses on the small DUF1127 proteins, which promoted us to construct and characterize a triple deletion-mutant.

The small DUF deletion mutant display a variety of different phenotypes, including a growth defect in stationary phase, increased cell aggregation and increased biofilm formation. Additionally the deletion mutant displays an increased phosphate uptake compared to wildtype *A. tumefaciens*. Transcriptome sequencing (RNA-seq) of the mutant revealed a large number of differentially regulated genes in late exponential and stationary growth phase. The most affected genes are involved in phosphate uptake, glycine/serine homeostasis, and nitrate respiration. The results suggest an important function of the small DUF1127 proteins in phosphate metabolism, nutrient acquisition and carbon metabolism of *A. tumefaciens.*Ongoing studies aimed to understanding the molecular mechanism of DUF1127 action.

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[2] Kraus, A., Weskamp M., Zierles, J., Balzer, M., Busch, R., Eisfeld, J., Lambertz J., Nowaczyk, M., Narberhaus, F. (2020). Arginine-Rich small proteins with a domain of unknown function, DUF1127, play a role in phosphate and carbon metabolism of *Agrobacterium tumefaciens*. Journal of Bacteriology 202(22).

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eP346

Regulation of small regulatory RNAs by a LysR-type transcription factor in *Agrobacterium tumefaciens*

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Small regulatory RNAs (sRNAs) are ubiquitous riboregulators found in all three domains of life. They are key factors in the adaptation to changing environments. In the phytopathogen *A. tumefaciens,* more than 1500 sRNAs have been identified in the past years, indicating a crucial role for sRNA mediated regulation [1-3]. At present, only a few of these sRNAs have been functionally characterized, e.g. AbcR1, which regulates multiple ABC-transporter genes. However, little is known about the mechanism of their transcriptional regulation.

In our current study, we report how the LysR-type transcription factor LsrB acts as superior regulator of various small RNA transcripts [4]. Transcriptome profiling via RNA-seq of a *lsrB*-deletion mutant revealed more than 1000 genes with altered expression, including 102 sRNAs. Deletion of *lsrB* causes pleiotropic effects regarding growth, virulence, antibiotic resistance and motility. This suggests a global regulatory role for the transcription factor by harnessing the regulatory power of sRNAs. Via *in vitro* binding assays, we identified AbcR1 and three, novel cuckoo sRNAs as direct targets of LsrB. We combine *in silico* motif

finding, *in vitro* binding assays and genome-wide binding profiling to analyze the regulatory network of LsrB.

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[2] 3. Lee, K., Huang, X., Yang, C., Lee, D., Ho, V., Nobuta, K., Fan, J. B. & Wang, K. (2013). A genome-wide survey of highly expressed non-coding RNAs and biological validation of selected candidates in *Agrobacterium tumefaciens*. PLoS One 8(8): e70720.

[3] Wilms, I., Voss, B., Hess, W.R., Leichert, L.I. & Narberhaus, F. (2011). Small RNA-mediated control of the *Agrobacterium tumefaciens* GABA binding protein. Mol Microbiol 80(2): 492–506.

[4] Eisfeld, J., Kraus, A., Ronge, C., Jagst, M., Brandenburg, V. B. & Narberhaus, F. (2021). A LysR-type transcriptional regulator controls the expression of numerous small RNAs in *Agrobacterium tumefaciens*. Mol Microbiol 116(1): 126–139.

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eP347

Elucidation of the role of Asp23 family proteins in fatty acid acquisition in *Bacillus subtilis*

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Fatty acids are essential precursors for the synthesis of phospholipids that serve as the main components in biological membranes. The acquisition of fatty acids is based on either essential *de novo* biosynthesis or the uptake of exogenous fatty acids. The highly specific acetyl-CoA-carboxylase (ACCase) complex catalyzes the conversion of acetyl-coA to malonyl-CoA to initiate the biosynthesis of fatty acids. Exogenous fatty acids are phosphorylated by a two-component fatty acid kinase (Fak) prior to incorporation as acyl-phosphates and further processing. The regulation of both committed steps in fatty acid acquisition, however, remains unknown.

We have identified the unknown and highly expressed proteins YqhY and YloU of the conserved Asp23 protein family as promising candidates to regulate fatty acid acquisition. The genes *yqhY* and *yloU* encoding the paralogous Asp23 family proteins share conserved operons with *accBC* and *fakA*, respectively. Based on the genomic co-localization, protein-protein interaction experiments revealed physical interactions between YqhY and AccBC, and YloU and FakA, respectively. This work aims to further characterize the putative regulatory effects of the Asp23 family proteins on fatty acid acquisition in *B. subtilis.*

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The oxidative stress response of *Yersinia pseudotuberculosis* is under thermoregulation

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Temperature-dependent gene expression during environmental (25°C) and host-body temperatures (37°C) plays an important role for the foodborne pathogen *Yersinia pseudotuberculosis*. Thermoregulation can affect the transcriptional and translational level. RNA thermometers (RNATs) are temperature-modulated RNA structures in the 5"-untranslated region (5"-UTR) of transcripts. At low temperatures, these structures prevent translation by occluding the ribosome binding site. At host-body temperature, the structure begins to melt, relieving translational repression. During infection of a warm-blooded host the bacteria encounter a wide range of reactive oxygen species (ROS). In previous transcriptome-wide RNA structure probing studies, we found evidence for the existence of such RNATs in the 5"-UTR of multiple transcripts of the oxidative stress response, suggesting a multi-layered regulation of these genes. Here, we support this hypothesis by translational reporter gene fusions and biochemical experiments. This translational control is complemented by transcriptional upregulation at 37°C compared to 25°C as shown by qRT-PCR. Accordingly, we observed a higher resistance against H2O2 in cells grown at 37°C compared to 25°C. Our findings provide insights into the regulation of the oxidative stress response as a mean of virulence-associated adaptation during infection.

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eP349

Role of ClpXP protease in regulation of photosynthesis genes: identification and verification of ClpXP substrates of *Dinoroseobacter shibae*

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Introduction: The marine bacterium *Dinoroseobacter shibae* is capable to perform aerobic anoxygenic photosynthesis and activates gene expression of the photosynthetic gene cluster (PGC) in the dark. Screening of a *D. shibae* transposon mutant library for loss of pigmentation and specific bacteriochlorophyll absorbance identified the *clpX* gene locus encoding the ATP-dependent protease subunit ClpX of the ClpXP protease. Transcriptome analysis revealed a role of ClpX in activating the expression of the PGC and a repressor function of PpsR. In a "trapping" assay with ClpP(S106A), PpsR, Dshi_1135 (potential blue light dependent LOV histidin kinase) and HemA (5-aminolevulinate synthase) were identified as potential targets of ClpXP.

Objectives: Determination of the role of the ClpX subunit of the ClpXP protease in regulation of the PGC of *D. shibae*. Identification and verification of ClpXP substrates.

Materials & methods: Proteome analyses were carried out under light, blue light and dark conditions comparing the wildtype strain and the *clpX*::Tn mutant strain. Co-purification experiments with an ATPase negative ClpX(E182Q)-Strep subunit, carrying a mutation in the Walker B motif, under light conditions were established to identify substrates and adapter proteins. The interaction partners were identified by LC-MS/MS. Interaction of ClpX with the potential substrates was tested using a bacterial two-hybrid system (BACTH). In addition, *in vivo* degradation experiments using western blots were done by detection of the potential target protein with specific antisera in extracts of the wild type and *clpX*::Tn, *clpA*::Tn, *clpP*::Tn mutant strains.

Results: Proteome data supported the results from the transcriptomic experiments that there is a light-dependent regulation of the PGC. Proteins involved in bacteriochlorophyll and pigment biosynthesis were only

found under dark conditions. The amount of photosynthetic proteins were found decreased comparing the clpX::Tn mutant and the wild type strain cultivated under dark conditions. Proteins identified in co-elution fractions of the ClpX walker B mutant are also found in the ClpP trapping experiments. BACTH experiments revealed first indications for an interaction between ClpX and PpsR as well as Dshi_1135. The *in vivo* degradation experiments with PpsR, Dshi_1135 and HemA discriminate between ClpXP and ClpAP protease activity.

Conclusion: In *D. shibae* ClpXP has a function in regulation of the PGC.

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Identification and verification of interaction partners of the Dshi_1135 LOV protein involved in the regulation of the photosynthetic gene cluster of *Dinoroseobacter shibae*

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Introduction:

The marine bacterium *Dinoroseobacter shibae* possesses a photosynthetic gene cluster (PGC), encoding all necessary components for bacteriochlorophyll a biosynthesis and aerobic anoxygenic photosynthesis. Screening of the transposon mutant library of *D. shibae* for loss of pigmentation and Bchl *a* absorbance identified the gene locus Dshi_1135, encoding a potential blue-light dependent LOV (light, oxygen, voltage) histidine kinase. Transcriptome analysis revealed a role of Dshi_1135 in activating the expression of the PGC. Moreover, a repressor function of PpsR and an activating role for PpaA were shown.

Objectives:

Identification and verification of interaction partners of Dshi_1135 LOV protein

Materials & methods:

After growth under blue light, light and dark conditions chemical cross-linking using formaldehyde was used to fix interaction partners. Fixed interaction partners of Dshi_1135-Strep were co-purified and subsequently identified by LC-MS/MS analysis or detected specifically via Western Blot. In addition, the bacterial two-hybrid system was used to analyze the interaction of Dshi_1135 with the potential interaction partners PpsR and PpaA.

Results:

LC-MS/MS analysis of the co-purified Dshi_1135-Strep interaction partners identified about 20 enriched proteins compared to proteome probes. Among them, the transcriptional regulators PpsR and CtrA, RNA polymerase subunits (RpoA, RpoB, RpoC, RpoD) and elongation factors (NusA, Tsf) were found. The transcriptional repressor of the PGC, PpsR, was identified under all light conditions, but was found enriched especially under dark conditions. A Western Blot of the elution fractions of the co-purification using a PpsR specific antiserum supported this result. In addition, results of the bacterial two-hybrid analysis also indicated an interaction of PpsR and Dshi_1135 under dark conditions. Moreover, the bacterial two-hybrid system showed the dimerization of Dshi_1135, PpsR and PpaA as well as a Vitamin B12-dependent interaction of the two regulators PpsR and PpaA, indicating an anti-repressor role of PpaA.

Conclusion:

The Dshi_1135 LOV protein of *D. shibae* is interacting with the transcriptional repressor PpsR under dark conditions presumably acting as an anti-repressor. A regulatory model of light-dependent regulation of the PGC is proposed.

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Characterizing the role of the small regulatory RNA S596 in the iron limitation response of *Staphylococcus aureus*

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Question: Small regulatory RNAs (sRNAs) play important roles in bacterial gene regulation. Iron-responsive sRNAs exist in many bacteria and mediate an "iron-sparing response" as described for RyhB of *E. coli* or FsrA of *B. subtilis*. Recently, a potential functional analog, the Fur-regulated sRNA S596, was identified in the human pathogen *S. aureus* [1]. Here, we aimed to determine the putative targetome of S596 to reveal its role in the adaptation of *S. aureus* to iron-limited conditions often encountered in the host. Since sRNAs bind to target mRNAs through short regions of imperfect complementarity, *in silico* prediction of targets is challenging. In addition, sRNAs can have direct and indirect effects on global gene expression. Therefore, experimental and bioinformatic approaches were combined in this study.
Methods: Mutants deficient in Fur, S596, or both were constructed in the *S. aureus* HG001 background. For experimental identification of putative S596 targets, proteome profiles were analyzed by mass spectrometry in data-independent acquisition (DIA) mode for the four strains grown under iron-limited and iron-rich conditions. *In silico* target prediction was performed using the CopraRNA2 tool [2].
Results: Comparison of the growth of the four strains revealed almost no difference between the *S. aureus* wild type and the *s596* mutant under both conditions, whereas the severe growth defect of the *fur* mutant was in part restored by deletion of *s596.* The proteome analysis, covering 1772 proteins, showed substantial differences between all strains. As potential S596 targets we identified 73 proteins exhibiting significantly different abundances between the *fur* and *fur s596* mutant as well as the wild type and the *s596* mutant under iron limitation. Of these 73 potential targets, 29 are supported by *in silico* analysis. The putative S596 targetome includes several iron-sulfur cluster or heme containing proteins such as CitB, FdhA, KatA and MiaB.
Conclusion: A putative S596 targetome was identified, supporting the hypothesis that S596 is an iron-sparing response mediating sRNA. In agreement with FsrA and RyhB targets, S596 affects the expression of TCA cycle and heme biosynthesis enzymes. Currently, selected targets are being investigated to gain further insights into underlying mechanisms and physiological consequences of S596-mediated regulation in *S. aureus*.
[1] Mäder *et al.* PLoS Genet. 2016, 12:e1005962 [2] Wright *et al.* Proc Natl Acad Sci USA. 2013, 110:E348796

eP352

Exploring the iron-starvation stimulon of *Staphylococcus aureus* using a combination of bioinformatics and experimental approaches

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Question: Pathogenic bacteria cope with host-mediated iron limitation known as nutritional immunity, and therefore the iron stimulon is of special interest for understanding *S. aureus* physiology. Fur is the main regulator of iron homeostasis, classically acting as repressor of iron-uptake systems under iron-rich conditions. A recent global analysis of *S. aureus* gene expression revealed new aspects of iron regulation by predicting an updated Fur regulon and identifying an iron-responsive sRNA (named S596) [1]. Additionally, modes of action differing from the classical Fur regulation mechanism are known for various organisms and iron-regulated genes clearly outweigh direct Fur targets. We therefore aimed to experimentally and computationally validate predicted Fur regulon members and to derive models of accessory iron-responsive regulatory mechanisms in *S. aureus*.
Methods: Analyses were largely performed on a proteomic data set covering *S. aureus* HG001 and isogenic mutants deficient in Fur, S596, or both cultivated in iron-rich and iron-limited medium. Experimental candidates of Fur-regulon members were determined by statistical analyses and protein patterns were grouped by hierarchical clustering. Further, 21 staphylococcal genomes were searched for conserved Fur boxes upstream of orthologous genes.
Results: Based on the proteomic profiles, 340 proteins were identified as potential Fur-regulon members, of which 49 proteins represent previously predicted Fur regulon members. However, this number most likely underestimates the true regulon size, due to challenging membrane protein detection and atypical protein patterns. Additional analysis of protein profiles revealed primarily two distinct regulation patterns of Fur-repressed genes, suggesting further regulatory mechanisms. Combination of these data sets with the analysis of conserved Fur boxes suggested a positive role of Fur in regulation of *sdhCAB* and *citB*. This finding is supported by experimental data including Northern blot and extracellular metabolome analyses.
Conclusion: The modes of action of Fur in *S. aureus* are more diverse than the canonical Fur regulation. By acting as a positive regulator of the TCA cycle, Fur is directly linked to the central metabolism. The idea of accessory iron-responsive regulatory mechanisms is supported by the detection of 25 i-modulon-like components [2].
[1] Mäder *et al.* PLoS Genet. 2016;12:e1005962 [2] Poudel *et al.* Proc Natl Acad Sci USA. 2020;117:17228-17239

eP353

Physiological significance of the EF-P paralog EfpL

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Translation of the genetic code is a defining feature of living systems, a mechanism that does not always work problem-free. Insertion of consecutive prolines into the polypeptide chain causes ribosomal arrest. In bacteria, the stalling is alleviated by translation elongation factor P (EF-P). In addition to EF-P approximately 12% of all eubacteria, including the Gram-negative model organism *Escherichia coli*, encode a paralogous protein, which we termed EfpL. Despite significant structural differences in the catalytic loop region, both EF-P and EfpL facilitate the translation of polyprolines. Further, EfpL overproduction compensates for the Δ*efp* mutant growth phenotype. However, the compensatory effect is incomplete, suggesting an accessory role of EfpL during translation of consecutive prolines. Thus, using ribosome profiling, we are currently investigating the arrest motif spectrum addressed by the factor. The resulting data will allow us to decipher the physiological significance and evolutionary advantage of encoding EF-P and EfpL simultaneously.

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A novel signal transduction cascade tuning OdhI function in *Corynebacterium glutamicum*

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Introduction: The activity of the corynebacterial 2-oxoglutarate dehydrogenase complex (ODH) is regulated by the small FHA (forkhead-associated) domain-containing protein OdhI. OdhI binds with nM affinity to the E1 subunit OdhA of ODH and thereby inhibits ODH activity [1-3]. Phosphorylation of OdhI by the soluble serine/threonine protein kinase PknG triggers a conformational change and prevents binding to OdhA [4]. The inhibition of ODH activity by OdhI enables a shift of the carbon flux from the TCA cycle towards L-glutamate synthesis and thus nitrogen assimilation. The *pknG* gene is located in a putative operon with *glnH* and *glnX* encoding a putative glutamine-binding lipoprotein and a membrane protein, respectively.

Objectives: As PknG plays a key role for the regulation of this important branch point in central metabolism, the question arises how the activity of PknG itself is regulated. The genomic organisation of *pknG* and further data suggest a signal transduction cascade with PknG acting as a modulator while GlnX operates as a transmitter transferring information about extracellular nutrient concentrations, sensed by GlnH, to PknG. An important aim of our research is to gain a detailed understanding of this signal transduction cascade.

Materials & methods: The established markers for periplasmic and cytoplasmic localisation, alkaline phosphatase or β-galactosidase, were fused to GlnX variants of different length to experimentally analyse the topology of the membrane protein. Furthermore, we purified His-tagged GlnH and performed binding studies with potential ligands using isothermal titration calorimetry (ITC).

Results and conclusion: GlnX fusions with either alkaline phosphatase or β-galactosidase enabled the establishment of an experimentally supported topology model that allows a better understanding how the membrane-integral protein GlnX interacts with PknG in the cytoplasm and with the extracytoplasmic protein GlnH. ITC binding studies with purified GlnH indicated that L-aspartate and L-glutamate rather than L-glutamine are the ligands sensed by GlnH.

[1] Niebisch et al. (2006) J Biol Chem 281: 12300-12307

[2] Krawczyk et al. (2010) FEBS Lett. 584: 1463-1468

[3] Raasch et al. (2014) J Biotechnol 191: 99–105

[4] Barthe et al. (2009) Structure 17: 568-578.

eP355

Signal input into the *E. coli* biofilm matrix control network via the diguanylate cyclase DgcE and a GTPase partner system

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The ubiquitous second messenger c-di-GMP promotes bacterial biofilm formation by playing diverse roles in the underlying regulatory networks. This is reflected in the multiplicity of diguanylate cyclases (DGC) and phosphodiesterases (PDE) that synthesize and degrade c-di-GMP, respectively, in most bacterial species (1). One of the 12 DGCs encoded in the genome of *Escherichia coli* K-12, DgcE, serves as the top-level trigger for extracellular matrix production during macrocolony biofilm formation (2). Its multi-domain architecture – a N-terminal membrane-inserted MASE1 domain followed by three PAS, a GGDEF and a degenerate EAL domains – suggested complex signal integration and transmission through DgcE.

Genetic dissection of DgcE revealed activating roles for the MASE1 domain and the dimerization-proficient PAS3 region, whereas the inhibitory EALdeg domain counteracts the formation of DgcE oligomers. It was found that the GTPase RdcA, together with its partner protein RdcB, are essential for the activation of DgcE, probably by aligning and thereby promotion dimerization of the PAS3 and GGDEF domains. The characterization of the RdcA, containing the conserved motifs needed for GTP hydrolysis at its N-terminus, revealed that the DgcE activation and RdcA/DgcE interaction depend on GTP hydrolysis, but are still possible with a RdcA variant not binding GTP or only the C-terminal domain of RdcA. These data suggest GTP as an inhibitor and the pronounced decrease of the cellular GTP pool during entry into stationary phase as a possible input signal sensed by RdcA. Further analysis of the role of RdcB indicated that it has an positive function in DgcE signaling, rather than being involved in removing the inhibitor GTP, e.g., as a GTPase activating or nucleotide exchange factor.

Taken together, our results led to the model of RdcA being a sensor of the cellular energy levels that together with RdcB controls the DgcE-dependent activation of matrix production when GTP levels decrease during the transition to stationary phase.

(1) Sarenko et al. (2017) mBio 8: e01639-17. (2) Lindenberg et al. (2013) EMBO J. 32: 2001-2014. (3) Pfiffer et al. (2019) PLoS Genetics 15: e1008059.

eP356

Similar but different – *Photorhabdus asymbiotica* interkingdom signaling via the LuxR solo SdiA

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LuxR solos are involved in quorum sensing (QS) based communication in bacterial populations. They are widespread and only found in proteobacteria. However, these LuxR family receptors lack a cognate LuxI synthase involved in signal production. One prominent member of these receptors is the SdiA LuxR solo found in *Escherichia* and *Salmonella*. SdiA harbors an AHL-signal binding domain similar to LuxR receptors binding acyl-homoserine lactones (AHL) but is also speculated to bind other signals derived from eukaryotes and can therefore be involved in interkingdom signaling between bacteria and their eukaryotic hosts. For some plant associated bacteria, such as *Pseudomonades*, SdiA-like receptors, were found to be involved in bacteria-plant interaction.

Photorhabdus spec. are Gram-negative entomopathogenic bacteria of the family of *Enterobacteriaceae*, harboring many LuxR solos sensing yet unknown signals. For entomopathogenic *Photorhabdus luminescens* we found a SdiA-like LuxR solo reacting on plant root exudates indicating a bacteria-plant interaction of *P. luminescens* via SdiA. Further, SPR analyses revealed binding of SdiA to its own promoter P*sdiA* as well as to the promoter of *adiA,* a gene also found to be involved in the interaction of bacteria with different hosts. *Photorhabdus spec.* are known to live in a dualistic lifecycle living in symbiosis with entomopathogenic nematodes and being pathogenic towards insects. However, in the last decades one strain, *P. asymbiotica*, emerged to be additionally pathogenic towards humans causing skin infection and traumata on wounds. Additionally, as the only member of this family it harbors two SidA-like LuxR solos, one homolog to SdiA of *P. luminescens* (SdiA2), the other slightly different (SdiA1). Both are also found to form a cluster with *aidA*, indicating its involvement in bacteria-host interaction. However, the less homologous *sdiA1*clusters not only with one but 5 *aidA* genes. Indeed, via SPR analyses, we identified selected *aidA* promoters of *P. asymbiotica* and the related P*sdiA1* to be specifically bound by SdiA1 with high affinity.

In conclusion, understanding the interkingdom signaling of *Photorhabdus* might elucidate the different fates of *Photorhabdus* strains in or on its different hosts, and especially the specificity of SdiA in sensing vertebrate hosts such as humans. Since SdiA is also found in other human pathogens this receptor could act as promising target for the development of novel antimicrobial drugs.

eP357

Unusual rocketeer-like mobility of *Agrobacterium tumefaciens* is dependent upon the capsule-forming, water-insoluble exopolysaccharide curdlan.

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Question: Curdlan is a water-insoluble homopolysaccharide produced by *Agrobacterium tumefaciens,* a common plant pathogen. The structure and gelling properties of this (1→3)-β-glucan makes it commercially valuable in the food industry and an increasing number of biomedicine applications. However, the role of curdlan in the lifestyle of the bacterium remains unknown.

Methods: Here, I have investigated the regulation of curdlan production as well as its deposition in relation to the cell surface. I used a variety of genetic and microscopic methods, as well as a novel automated setup using 96-well plates. This setup relied on the use of the fluorescent curdlan-specific dye, aniline blue, and mVenus reporter fusions.

Results: Curdlan is deposited as a discrete capsule. Encapsulated cells were confined to flocs (cell aggregates) in liquid cultures, whereas capsule-less cells were planktonic. Growth on a solid agar surface also resulted in capsule formation by most cells in the colony. Intriguingly, the curdlan

capsule occasionally promoted the escape of its cell. This de-capsulation action pushed the cell away from the colony, over a solid surface, in a "rocketeer-like" fashion. Replacing the native promoter of the curdlan biosynthetic gene cluster, *crdASC*, with a strong, constitutive promoter dramatically improved curdlan production by up to 50-fold, demonstrating excellent commercial potential for achieving high yields of curdlan during fermentation.

Conclusions: Regulation of curdlan production naturally occurs primarily at the level of transcription control, and ensures production strictly under the requirements of nitrogen limitation and good access to carbon and oxygen. This environment reflects the natural conditions typically found on or even within plant tissue. Capsule formation strengthens biofilm formation through cell-cell aggregation, and possibly also provides a novel form of escape from the biofilm during pathogenesis.

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eP358

Genome-wide high resolution identification of bacterial regulatory small RNAs based on hybrid transcriptome sequencing data.

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In bacteria, small RNAs (sRNA) are important post-transcriptional regulators, and a single small RNA can influence the activity of numerous mRNAs by a variety of mechanisms. Bacteria typically express hundreds of these regulatory small RNAs. While extensively studied in a few bacterial species, their abundance, evolution, and biological functions remain largely unknown in most bacteria despite their vital role. Here we describe the application of high-throughput sequencing approaches together with computational data integration for the genome-wide annotation of sRNAs in 20 bacterial species of the phylum Gammaproteobacteria. For this purpose, we have developed a generic workflow in which sequencing data of differential RNA-Seq (dRNA-Seq) and Term-Seq are jointly analyzed to precisely call the 5" ends and 3" ends of the small RNAs, and confidently produce high resolution annotations. The approach includes a scoring and ranking method to quantify the confidence in predicted sRNAs. The method was successfully benchmarked against manually-curated sRNAs and is capable of detecting sRNAs encoded in various locations including those overlapping with open reading frames.

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eP359

A novel locally c-di-GMP-controlled exopolysaccharide synthase required for bacteriophage N4 infection of *E. coli*

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A major target of c-di-GMP signaling is the production of biofilm-associated extracellular polymeric substances (EPS), which in *Escherichia coli* K-12 include amyloid curli fibres, phosphoethanolamine-modified (pEtN-)cellulose and poly-N-acetyl-glucosamine (PGA). However, the characterized c-di-GMP-binding effector systems are largely outnumbered by the 12 diguanylate cyclases (DGCs) and 13 phosphodiesterases (PDEs), which synthetize and degrade c-di-GMP, respectively. *E. coli* possesses a single protein with a potentially c-di-GMP-binding MshEN domain, NfrB, which – together with the outer membrane protein NfrA – is known to serve as a receptor system for bacteriophage N4.

Here, we show that NfrB not only binds c-di-GMP with high affinity, but as a novel c-di-GMP-controlled glycosyltransferase synthesizes a secreted EPS, which can impede motility and is required for N4 infection. In addition, a systematic screening of the 12 DGCs of *E. coli* K-12 revealed that specifically DgcJ is required for the infection with phage N4 and interacts directly with NfrB. This is in line with local signaling models, where specific DGCs and/or PDEs form protein complexes with particular c-di-GMP effector/target systems.

Our present study highlights a novel example of how specificity in c-di-GMP signaling can be achieved by showing NfrB as a novel c-di-GMP binding effector in *E. coli*, which is controlled in a local manner specifically by DgcJ. We further show that NfrB is involved in the production of a novel exopolysaccharide. Finally, our data shine new light on host interaction of phage N4, which uses this exopolysaccharide as an initial receptor for adsorption.

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eP360

Classification of uncharacterized RNAs with unknown function in *Bacillus subtilis* and identification of interaction partners of small novel proteins

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Question: Global transcriptome data allow high-resolution detection of specific transcription units and provide clues about transcription start sites, terminators and even degradation mechanisms. They further led to the observation of short transcripts or mRNA extensions potentially encoding very small proteins or regulatory RNAs. In the model organism studied here, *Bacillus subtilis*, these are about 1500 transcription units [1]. These small effector molecules might be a missing link in understanding fundamental aspects of *B. subtilis* physiology.

Methods: To functionally study these uncharacterized RNAs, we first established an *in vivo* test system to provide a basic classification into coding, non-coding or regulatory RNAs. Therefore, we created the plasmid-based screening system pMAX_TREP for the controlled expression of transcribed genomic loci in *B. subtilis*. The pMAX_TREP system has been created to allow *(i)* efficient and fast cloning of desired features, *(ii)* stable plasmid propagation, *(iii)* very tight control of the target promoter for toxic gene products and *(iv)* easy *in vivo* screening, real time quantification and localization studies by FACSAria™ monitoring and microscopy of the translational fusion to the green fluorescent protein reporter. Finally, *(v)* very quick purification of fusion proteins is achieved by a C-terminal TWIN-STREP-tag (IBA) for subsequent identification by mass spectrometry in one workflow. To obtain information on the physiological function of the newly identified proteins, they are subjected to a combination of SPINE (strep-protein interaction experiment) [2] and SILAC (stable isotope labeling) to reliably identify *in vivo* protein:protein interaction partners by mass spectrometry.

Results & Conclusion: The pMAX_TREP system was successfully tested on 5 uncharacterized RNAs of *B. subtilis* with unknown function. The first pilot experiments already provided phenotypic data as well as explicit allocation into

coding (small proteins) and non-coding RNAs. Additionally, potential interaction partners were found for a newly identified small protein.

[1] Nicolas et al. "Condition-dependent transcriptome reveals high-level regulatory architecture in Bacillus subtilis." *Science* 335 (2012): 1103-6. doi:10.1126/science.1206848

[2] Herzberg et al. "SPINE: a method for the rapid detection and analysis of protein-protein interactions in vivo." *Proteomics* 7 (2007): 4032-5. doi:10.1002/pmic.200700491

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eP361

Characterization of the structure of the oxidative stress sub-regulon within the general stress response of *Bacillus subtilis*

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Question: The control of the activity of the alternative sigma factor SigB is the key component within the general stress response in *Bacillus subtilis* which mediates the global induction of stress gene expression. Even though the activation of SigB is the essential prerequisite for general stress gene expression, this is not sufficient for an effective expression of all general stress regulon members or the development of their protective function. SigB induced secondary regulators facilitate the modulation of gene expression levels creating stress specific sub-regulons. One of the sub-regulons is the adaptation to oxidative stress involving the secondary regulators MgsR and Spx. Differential binding to specific promoter elements located upstream of the core promoters enhances the transcription efficiency of their target genes significantly. Deciphering the contribution of different promoter elements to overall promoter strength is tedious. Therefore, the plasmid-based screening system pHIS was constructed to easily address aspects like easy and quantitative monitoring of promoter activity via fluorescence reporter gene expression and *in vivo* analysis of all desired promoter constructs in real time. By using the pHIS plasmid, we characterized specific regulatory groups of the oxidative stress sub-regulons within the general stress response in *B. subtilis*.

Methods: Transcript levels of different promoters of *Bacillus subtilis* wildtype, *ΔmgsR* and *Δspx* mutants were visualized and quantified by Northern blot experiments. Detailed functional analysis of promoter upstream elements was carried out using the pHIS plasmid by measuring the GFP fluorescence with a FACS Aria™ after 4 % (v/v) ethanol induction.

Results & Conclusion: By using the pHIS plasmid a conserved upstream sequence for the transcriptional activator MgsR could be identified, which has a significant effect on promoter activity. Furthermore, we found evidence that both regulators, MgsR and Spx, are not solely collaborating with SigB at SigB-type promoters, but also enhance gene expression levels from SigA type promoters. These findings add a new level of complexity to the SigB regulation network and assign new interesting physiological and mechanistic functions to both regulators MgsR and Spx.

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eP362

Activation of cryptic genes by counter-acting xenogeneic silencing

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Xenogeneic silencing proteins (XS) facilitate the acquisition of novel genetic material into the host genome by silencing foreign gene expression. In a previous study, we identified the Lsr2-like XS protein CgpS of *Corynebacterium glutamicum*, which was found to be crucial for maintaining the lysogenic state of the large CGP3 prophage (1). Bioinformatic analysis of CgpS ChAP-seq (chromatin affinity purification and sequencing) data and in vivo reporter studies of target promoter fusions revealed a clear preference of CgpS towards AT-rich regions containing multiple TpA steps (2). The comparison of the binding pattern with transcriptomics and proteomics revealed that CgpS only partly dissociated from the CGP3 region during prophage induction. These results emphasized remodeling of the silencer nucleoprotein complex at the prophage together with a partial reallocation of CgpS binding to further host genome targets.

Binding of specific transcription factors (TFs) to silenced promoter regions may oppose XS activity leading to counter-silencing, thereby providing access to horizontally acquired DNA. In this study, we followed a synthetic counter-silencing approach to analyze how the binding of a specific transcription factor may counteract XS. For this purpose, CgpS target promoters with and without additional TF operator sites were fused to reporter genes (e.g. *eyfp*) to systematically assess the interference between the XS protein and the specific TF. 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. Operator sequence as well as its position showed a significant impact on the dynamic range and maximal promoter output (2).

Interestingly, we achieved TF-independent counter-silencing by expressing sRNAs complementary to promoter regions, which are silenced by CgpS. In contrast to the TF-based counter-silencing approach, sRNA-mediated counter-silencing is independent of promoter sequence modifications but also allowed for modulated activation of a wide range of CgpS target promoters.

Overall, this study enhances our understanding of how XS and counter-silencing facilitates evolutionary network expansion in bacteria.

(1) Pfeifer E. et al. (2016) *Nucleic Acids Res.*, 44(21):10117-10131

(2) Wiechert J. et al. (2020) mbio, 11, e02273-02219

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eP363

New insights into acid stress adaption in γ-proteobacteria using various Next-Generation Sequencing (NGS) techniques

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Bacteria have various sensing and signaling mechanisms to withstand environmental stress. For pathogens which infect the human host, it is especially crucial to rapidly adjust to the low pH of the stomach, which acts as a bactericidal barrier. To survive in highly acidic environments, many bacteria have evolved acid-inducible amino acid decarboxylase-antiporter systems (ARs). These systems contribute to a relief of acid stress by H^+ consumption through decarboxylation of their cognate amino acids and export of the more alkaline reaction products. The aim of this project is to better understand the acid resistance network in γ-proteobacteria and to discover new proteins that counteract acid stress.

Escherichia coli requires very few cells to colonize the host. A major reason for this is that *E. coli* is equipped with a variety of ARs, including the Gad, Adi and Cad system, all of which are heterogeneously distributed among the population[1]. To discover potential interconnecting elements between the different ARs and to asses acid-depended global changes in protein copy numbers, we performed ribosome profiling (Ribo-Seq). In parallel, RNA-Seq was conducted to asses differential transcription and translation of cells exposed to mild and severe acid stress. Using this approach, we identified numerous previously undiscovered adaptations of *E. coli* to acidic environments.

The marine bacterium *Vibrio campbellii* is not as resistant to acid stress as *E. coli*. Only one AR system, the Cad system, is available[2]. To further analyze the impact of this system, a ChIP-Seq analysis was performed to detect CadC-binding sites, and an RNA-Seq analysis using a *cadC* mutant was conducted to discover directly and indirectly regulated genes involved in acid stress adaption. We found that CadC in *V. campbellii* directly binds to the *cadBA* promoter and upregulates the downstream genes. In addition, we identified other indirectly regulated genes.

Overall, we were able to uncover additional, previously unknown adaptations to acid stress in a highly acid-resistant and a less resistant model organism. In addition, we found one operon involved in acid resistance in both *V. campbellii* and *E. coli*.

1. Brameyer, S. *et al.* Division of labor and collective functionality in *Escherichia coli* under acid stress, submitted.
2. Brameyer, S. *et al.* Molecular design of a signaling system influences noise in protein abundance under acid stress in different gammaproteobacteria. *J. Bacteriol.* **202**, (2020).

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eP364

RNA thermometers control the assembly and functionality of type III secretion system in *Yersinia pseudotuberculosis*

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The foodborne pathogen *Yersinia pseudotuberculosis* uses a type III secretion system (T3SS) as a molecular syringe to inject effector proteins into the host cell that suppress the host immune response. Synthesis and assembly of T3SS components and secretion of effector proteins rely on host body temperature, which induces RNA thermometer (RNAT)-controlled translation of several T3SS components. RNATs are located in untranslated regions of temperature-regulated transcripts and regulate the expression of the downstream coding region by reversible structural rearrangements. The conformational change in response to an increasing temperature allows ribosome binding to the liberated Shine-Dalgarno sequence, which permits translation only at an infection-relevant temperature of 37 °C.

In RNA structuromics approaches, we identified three putative RNATs associated with the structural components YscT and YscJ essential for the initial assembly of T3SS, and the gatekeeper YopN. Post-transcriptional thermoregulation of these RNAT candidates was validated by reporter gene studies. Point mutations in the structural area of the SD sequence causing closed or more open RNA structures resulted in altered translation efficiencies in comparison to the wildtype. RNAT functionality was confirmed by *in vitro* structure probing and ribosome binding studies.

To study the physiological relevance of RNAT-controlled T3SS assembly and effector protein secretion, *yersinia* deletion mutants were equipped with plasmids expressing the respective genes either with their natural or with point-mutated RNAT variants. Growth experiments and effector protein translocation assays with eukaryotic host cells confirmed that T3SS assembly and functionality depend on RNATs in a temperature-dependent manner, making this an efficient and energy-saving strategy for bacterial pathogens during infection.

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eP365

Identification of novel ribosome binding proteins in *Bacillus subtilis*

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Ribosomes are responsible for protein synthesis in the cell by translating mRNA into polypeptide chains. Since this function is vital for survival of any organism, the macromolecule has been the subject of many structural and functional studies. It consists of a large and a small subunit, which both consist of ribosomal RNA (rRNA) molecules as well as numerous ribosomal proteins. Since ribosomes is largely made up by the rRNA and the enzymatic activity is also mainly attributed to the nucleic acid they are classified as ribozymes. Over the past years, it has become known that many factors interact with, and bind to the ribosome keeping its activity under a tight control by the cell. A recent proteome-wide crosslinking study performed with *B. subtilis* revealed that two proteins of unknown function bound to the small subunit of the ribosome. The proteins have a sequence similarity of 51 % and are highly conserved in Gram-positive bacteria. They also both contain an RNA binding domain. This work aims to validate the potential interaction of these proteins with the ribosome as well as to shed a light on the functions of the two proteins

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eP366

Thermo-responsive regulation of the small RNA OmrA in *Yersinia pseudotuberculosis*

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Yersinia pseudotuberculosis is a food-borne pathogen that requires the ability to adapt flexibly to sudden changes in its environment. The thermal shift from ambient environmental conditions to host entry demands fine-tuned responses by regulatory networks for successful pathogenesis. Part of this

complex network are small RNAs (sRNAs) that allow bacteria to properly respond to the transition from environment to host entry. Another layer of post-transcriptional regulation revealed through global RNA structuromics are transcripts that respond to temperature changes, known as RNA thermometers.

Building on these two concepts, this study aims to identify sRNAs that structurally breathe with the changes in temperature. Based on our existing datasets of RNA structures in *Yersinia pseudotuberculosis*, we focus on OmrA which shows compelling structural changes at 25°C and 37°C. Various *in silico* analyses were used to predict the seed region along with putative targets of OmrA as well as its conservation compared to other enterobacterial sequences. Point mutations were introduced at structures predicted to respond to thermal fluctuations. The expression of OmrA as well as its stability compared to its stable variant at different temperatures were examined by northern blot analysis.

Our results show a highly conserved seed region in *E. coli, Salmonella sp.,* and *Y. pseudotuberculosis.* However, we found a stretch of nucleotides unique to *Yersina sp.* after the seed region up until the rho-independent terminator hairpin. RNA stability assays show a drastically reduced half-life of OmrA at 37°C compared to 25°C, whereas the half-life of point-mutated variant was significantly extended at 37°C.

In conclusion, the stretch of sequences found to be unique to *Yersinia sp.* seems to contribute to the formation of a stem-loop structure which obscures a U-rich motif at lower temperatures which then melts as the temperature rises, akin to host entry. An increase in temperature seems to reduce the half-life of this specific sRNA which suggests an increased turn-over rate as sRNA-mRNA complexes are rapidly degraded.

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eP367

Dissecting the binding behavior of the multi-RRM protein Rrm4 during endosomal mRNA transport in *Ustilago maydis*

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Transport of mRNAs is crucial to regulate protein expression at the spatio-temporal level. The localization of mRNA is coupled to the intracellular transport machinery by RNA-binding proteins (RBPs). A well-studied translocation mechanism is the endosomal mRNA transport along microtubules in infectious hyphae of the plant pathogen *Ustilago maydis.* The key component of endosomal mRNA transport is the RBP Rrm4, which is important for the unipolar growth of fungal hyphae. Rrm4 contains three RNA Recognition Motifs (RRM) and three MademoiseLLE (MLLE) domains for protein-protein interactions, which is necessary to anchor the mRNP complex on early endosomes.

Rrm4 binds predominantly to the 3'UTR of target transcripts and the landmarks of translation, such as start and stop codons - this supports the current hypothesis of local translation on endosomes. Furthermore, Rrm4 binds the sequence motif UAUG, which is present in one third of all identified binding sites. The motif occurs mostly in binding sites mapping to the Open Reading Frame (ORF) and start codons and is bound by the third RRM domain (RRM3). Mutations within RRM3 however do not interfere with hyphal growth, whereas mutating the first RRM (RRM1) domain leads to a loss of function of Rrm4. This indicates that the RRM domains differentially contribute to RNA binding and may selectively affect distinct mRNA target sets. However, the principles of RNA recognition by multi-domain RBPs are not well studied. Therefore, we started - to dissect the binding behavior of the different RRM domains of Rrm4 by applying *in vivo* UV-crosslinking to alleles of Rrm4 carrying mutations in the RRM1 domain (Rrm4mR1). Recently, we obtained a transcriptome-wide view of Rrm4 and Rrm4mR1 by using a comparative *Individual-nucleotide resolution UV CrossLinking and ImmunoPrecipitation* (iCLIP2) approach. This should enable us to further characterize the roles of the different RRMs of Rrm4 during endosomal mRNA transport.

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eP368

Genome-wide profiling of regulatory RNAs in the human pathogen *Klebsiella pneumoniae*

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The enterobacterium *Klebsiella pneumoniae* is a human pathogen that causes a wide range of hospital- and community-acquired infections. The continuous evolution of *K. pneumoniae* through the integration of horizontally-acquired genetic elements has resulted in the emergence and spread of hypervirulent isolates as well as strains with resistances against multiple antibiotics. However, not all phenotypes can be explained by genome sequences alone and the regulatory networks employed by *K. pneumoniae* to optimize expression of virulence determinants and stress responses are barely understood. Specifically, regulation at the post-transcriptional level - hence the contribution of regulatory RNAs - which has been identified as a central component of gene expression control in other bacteria has not been carefully addressed in *K. pneumoniae* yet. This pillar of gene regulation may play a key role in *K. pneumoniae* virulence since multiple determinants of its pathogenicity – including iron metabolism, outer membrane protein synthesis or LPS modification – have been shown to be at least partially controlled through regulatory RNAs in other organisms.

To fill this gap, we have employed differential RNA sequencing (dRNA-seq) to globally determine the transcriptional start sites (TSSs) and to annotate sRNAs of four different *K. pneumoniae* strains, representing one classical and one hypervirulent reference strain as well as two clinical isolates. In addition, we have analyzed the association of RNA with the major RNA-binding proteins, Hfq and ProQ, by co-immunoprecipitation studies. Overall, our datasets provide a comprehensive resource to study the impact of post-transcriptional gene expression control in the human pathogen *K. pneumoniae*.

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eP369

Functional motif analysis of the endosomal mRNA transport protein Upa2 in *Ustilago maydis*

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Spatiotemporal gene expression is sharply regulated by post-transcriptional modifications as well as dynamic processes such as mRNA localization and transport. In highly polarized cells, long-distance transport of mRNAs along microtubules is carried out by early endosomes, through ribonucleoprotein (RNP) complexes containing cargo

mRNAs, RNA-binding proteins, and accessory proteins. One of the core components of endosomal mRNA transport in the phytopathogen *Ustilago maydis* is the protein Upa2. Loss of Upa2 impairs endosomal mRNA transport by altering the distribution of the poly(A)-binding protein Pab1. Upa2 contains four N-terminal PAM2 domains that interact with the MLLE domain of Pab1. In addition, the C-terminus harbors a short SMHAP motif of unknown function and a GWW motif important for endosomal localization. Furthermore, the GWW motif has been reported to be involved in protein-protein interactions in other organisms. However, the interaction partner of this motif is still unknown. To identify proteins that specifically interact with the GWW motif of Upa2, we will use the yeast two-hybrid system to screen a cDNA library of *U. maydis*. For this screen, the C-terminus of Upa2 fused to a Gfp is used as a bait protein to monitor expression as well as subcellular localization. To achieve homogeneous expression of the bait protein, we modified the system by integrating the bait into the *Saccharomyces cerevisiae* genome instead of using an expression bait plasmid. Microscopic analysis revealed homogeneous expression of the bait protein in the yeast cells. Surprisingly, the hybrid protein was localized not only in the nucleus but also in patches at the cell membrane. Similar localization has been described for proteins associated with the formation of eisosomes in yeast. The localization of Upa2 on the cell membrane suggests a novel, unknown function of Upa2 in addition to endosomal mRNA transport in *U. maydis*. Colocalization studies of Upa2 and eisosomal proteins, as well as identification of Upa2 interaction partners, may help to decipher the function of Upa2.

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eP370
The RNA-binding protein RibR and its role in the regulation of FMN riboswitch activity in *Bacillus* species
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The RNA-binding protein RibR plays a role in regulating the riboflavin levels in *Bacillus subtilis* cells by targeting the flavin mononucleotide (FMN) riboswitch. FMN itself is an important cofactor for flavoenzymes and is recognized by the corresponding riboswitch in the 5"-untranslated region of riboflavin synthesis and transport genes. Specific binding of FMN to the aptamer domain of the riboswitch leads to a conformational change of the RNA structure, which in turn represses expression of the downstream genes. The regulatory mechanism was now found to be even more elaborate, in so far as the protein RibR in *B. subtilis* additionally targets the complex RNA secondary structure and thereby counteracts the effect of the ligand FMN. Strikingly, *B. subtilis* RibR not only possesses a second domain with flavokinase activity, its induction is also connected to the presence of certain sulfur compounds. However, the specific physiological function of the two RibR domains and how this protein is associated with the sulfur metabolism of the cell still remains unknown.

Therefore, we intend to further characterize the two domains of RibR. We want to show, why these two different domains are connected and whether RibR fulfills similar purposes in different *Bacillus* species. Additionally, we are interested in the physiological importance of its regulatory function and the link to the cells sulfur metabolism.

We apply a newly established reporter gene assay, using a dual-luciferase system, to report the effects of RibR-like proteins from various *Bacillus* species on the respective riboswitch and the expression of downstream genes. The CRISPR/Cas9 system is employed to create *B. subtilis* strains with markerless deletions of *ribR* or either one of its distinct domains. The formation of riboflavin is then used as an internal reporter to measure the effect of RibR induction on the expression of riboflavin synthesis genes.

In this setting we observed that the effect of RibR was visible when methionine and taurine were the sole sulfur sources present. Furthermore, preliminary experiments showed that not only RibR from *B. subtilis*, but also a truncated version of RibR from *Bacillus amyloliquefaciens* – lacking the second flavokinase domain – offsets the effect of FMN on FMN riboswitch activity.

Our findings give promising insights into a presumed new group of RNA-binding proteins that modulate riboswitches and are part of an intricate regulatory system for gene expression.

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eP371
Role of the class 2 type VI CRISPR-Cas system in *Rhodobacter capsulatus* in stress response
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CRISPR-Cas systems are also referred to as adaptive immune system in archaea and bacteria. Mobile genetic elements for example of invading viruses are integrated into the CRISPR locus and prevent, together with corresponding Cas-proteins, further infection of the same virus by degrading the viral genome. Over the last few years CRISPR-Cas systems were found to have more alternative functions than just being an adaptive immune system. CRISPR-Cas systems were found to participate in internal gene regulation and regulation of developmental programs, as well as taking part in stress response in different bacteria.

This project targets the class 2 type VI CRISPR-Cas system of the alphaproteobacterium *Rhodobacter capsulatus* and elucidates whether it is functional beyond adaptive immunity. The main protein in this system Cas13a inherits RNA degradation activity. This CRISPR-Cas system was found through RNA-Sequencing data comparing non stressed with photooxidative stressed cultures, indicating a function in stress response to photooxidative stress, since the amount of crRNAs was increased in the stressed cells. Besides from the indication of participation in photooxidative stress response, different crRNA amounts were shown to strongly increase in stationary phase. In order to investigate the function of the CRISPR-Cas system, different stressors were applied and RNA was extracted and analysed via Northern Blot Analysis. Aside from different stressors the amount of crRNAs was also monitored in different growth phases. Together with transcriptional fusion data these results give an overview under which circumstances the system is activated. Furthermore, the main Cas protein Cas13a was removed or overexpressed to investigate the function of the protein in *R. capsulatus in vivo*. Unfortunately, there was not yet a clear phenotype found comparing strains expressing Cas13a or not. Since Cas13a degrades RNA, a new RNA-Sequencing experiment was performed with the strains expressing Cas13a compared to strains without Cas13a to find the target RNAs that get degraded through Cas13a. The results are currently validated.

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eP372
Analysis of translation-independent RNA localization in Cyanobacteria

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RNAs are known to localize heterogeneously throughout eukaryotic cells. Here, different mechanisms involved in RNA-trafficking were discovered over the past few years. Special *cis*-acting sequential motifs determine the final RNA localization and further provide binding sites for RNA-binding proteins, necessary for RNA-transport. Although those processes were thought to solely occur in eukaryotes, recent evidence suggest that translation-independent, directed RNA-trafficking could be important for local regulation of gene expression in prokaryotes as well.

In this study, we investigate translation-independent RNA-transport in the cyanobacterial strain *Synechocystis* sp. PCC 6803 using two independent experimental approaches. The RNA-sequencing technique "Rloc-sequencing" combining cell fractionation and RNA-sequencing and the visualization technique fluorescence in situ hybridization (FISH) combined with high-resolution microscopy are used to analyze spatial RNA organization. Using Rloc-sequencing we further aim to identify different sequential motifs and corresponding RNA-binding proteins responsible for RNA-transportation. Recent results show that different transcripts encoding proteins involved in photosynthesis accumulate at the thylakoid membrane in a translation-independent manner.

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eP374
Analysis of the substrate affinity of endoribonuclease RNase E in the cyanobacterium *Synechocystis* sp. PCC 6803

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RNA degradation and maturation are central for RNA metabolism. The three ribonucleases RNase E, RNase Y and RNase J play central roles in these processes. They were mainly investigated in *Escherichia coli* (*E. coli*), encoding exclusively RNase E, and *Bacillus subtilis* (*B. subtilis*), encoding RNase Y and J, but no RNase E homologue. Thus far, it is not understood how RNase E and J interact when being present in the same organism. Cyanobacteria encode homologues of both RNase E and RNase J, but lack a homologue of RNase Y. RNase E is an endoribonuclease targeting single-stranded, adenine and uracil rich regions. Furthermore, it cleaves preferentially 5"-monophosphorylated RNA fragments and recognizes them by a mechanism termed "5" sensing". To elucidate the substrate specificity and biological functions of RNase E in cyanobacteria, we created RNase E mutant strains of the model cyanobacterium *Synechocystis* sp. PCC 6803. These strains were used for the transcriptome-wide mapping of RNase-E-dependent processing sites by RNA-seq.

Using a temperature-sensitive RNase E mutant strain and the "transient inactivation of an essential ribonuclease followed by RNA-seq" (TIER-seq) approach, we mapped 1,472 RNase-E-dependent cleavage sites. These revealed a similar, but slightly different cleavage signature for cyanobacterial RNase E compared to gammaproteobacterial homologues. The TIER-seq analysis was complemented by the comparison of strains encoding either wild-type or 5"-sensing-deficient RNase E. Interestingly, only a small proportion of RNase-E-dependent processing sites was dependent on 5" sensing, indicating an important role of other targeting mechanisms. This is in line with findings for E. coli RNase E.

The resulting data sets provide a transcriptome-wide overview of targets of RNase E. They illustrate the role of RNase E in the maturation of several RNA species such as rRNA, tRNA and potential 3" end-derived sRNAs. Furthermore, they hint towards a role of RNase E in the regulation of copy numbers of plasmids pSYSA and pSYSM. Examples of mRNAs likely regulated jointly by RNase E and an sRNA substantiate the enzyme"s role in post-transcriptional regulation. Regarding general RNA degradation via the 5"-end dependent pathway, the data sets suggest a redundant or concerted action of RNase E and RNase J.

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eP375
Regulation of genes with SAM-II riboswitch-containing 5"-UTRs in *Sinorhizobium meliloti*

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Methionine is used together with ATP to synthesize S-adenosylmethionine (SAM), the main methyl donor in the cell. In bacteria, the SAM biosynthesis is posttranscriptionally regulated by SAM-binding riboswitches, which are located in the 5"-UTRs. In the nitrogen fixing plant symbiont *Sinorhizobium meliloti* two genes encoding enzymes for methionine biosynthesis harbor SAM-II riboswitches, *metA* and *metZ* (1). To address their regulation, reporter *egfp* plasmids were used. Furthermore, *metA* and *metZ* mRNA levels were analyzed by qRT-PCR and the corresponding liberated SAM riboswitches (riboswitch-containing small RNAs) by Northern blot hybridization. The results revealed that both *metZ* and *metA* are regulated at the level of transcription and posttranscriptionally. The posttranscriptional *metA* regulation was found to be complex, including a translation-regulating riboswitch followed by a transcription terminator, the read-through of which is necessary for *metA* expression. This premature transcription termination of *metA* results into an approximately 100 nt long sRNA RA1, which processed to an approximately 70 nt long sRNA RA2 containing the SAM-II riboswitch. This processing depends on *metK* expression and thus probably on SAM. The shorter RA2 sRNA is strongly stabilized in rich medium. Using coimmunoprecipitation, we tested whether MetK, which was proposed to be an RNA binding protein (2), specifically binds RA2. However, our results suggest rather unspecific RNA binding by MetK, which is significantly decreased in the presence of SAM.

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eP376
Central carbon metabolism, sodium-motive electron transfer and ammonium formation by the vaginal pathogen *Prevotella bivia*

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Prevotella spp. are part of the healthy human microbiome, being the predominant species in the nasopharynx, respiratory system, oral cavity and gut system. This genus does not contain obligate pathogenic species, but some strains have been implicated in multiple diseases, like inflammatory disorders, opportunistic infections, oral biofilm diseases or bacterial vaginosis (BV). Several studies indicate that a change of *Prevotella* abundance in the microbiome can trigger such diseases. One example is *P. bivia*, which inhabits the vaginal milieu and is involved in BV. BV is the most commonly reported microbiological syndrome among women in reproductive age and it is associated with a variety of health issues. The factors triggering BV are unclear, but it is characterized by the loss of the healthy *Lactobacillus*-rich vaginal microbiome, replaced by strictly anaerobic microorganisms, such as *P. bivia* and *Gardnerella vaginalis*. Here, *P. bivia* is an important source of ammonia, lipopolysaccharides and sialidase activity in the vaginal mucus. In this study, the energy metabolism of *P. bivia* was characterized with a special focus on the Na^+ -translocating NADH:quinone oxidoreductase (NQR), quinol:fumarate reductase (QFR) and ammonia formation.

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eP377

Characterization of *nqrM*, a component of the *nqr* operon coding for the sodium-translocating NADH:quinone oxidoreductase of *Vibrio cholerae*

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Abstract

The Na+ -translocating NADH:quinone oxidoreductase (NQR) is a membrane bound respiratory enzyme which harbors flavins and Fe-S clusters as redox centers. The NQR from *Vibrio cholerae* is the main producer of the sodium motive force (SMF) and drives energy-dissipating processes such as flagellar rotation, substrate uptake, ATP synthesis and cation-proton antiport (Toulouse et al. 2018). It was shown that the *nqr* operon harbors, beside the six structural *nqrABCDEF* genes, the downstream genes *apbE* and *nqrM*, encoding a flavin insertase (ApbE) and the putative maturation factor (NqrM) of the NQR. A putative role of NqrM in FeS assembly was considered. We previously demonstrated an induction of the expression of the *nqr* operon by iron in wt *V. cholerae*. In contrast, expression of the *nqrM* gene in *Vibrio cholerae Δnqr* was repressed by iron (Agarwal et al. 2020). Here we investigated the physiological role of NqrM and its functional relation to the NQR. Divergent phenotypes were observed in growth studies of *Vibrio cholerae* O395 N1 wild-type compared to different deletion mutants under various conditions. The impact of NqrM on the expression of genes involved in metabolism and stress response was analyzed by RT-qPCR studies.

Literature

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eP378

Methanogenic archaea use a bacteria-like methyltransferase system to convert methoxylated aromatic compounds

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Methoxylated aromatic compounds are components of lignin and coal and are very abundant on Earth. However, the conversion of these compounds has previously only been described for bacteria and not for archaea. Only recently the methanogenic archaeon *Methermicoccus shengliensis* was shown to be capable to convert methoxylated aromatic compounds, also called methoxydotrophic growth [1]. In a recent study we showed that *M. shengliensis* uses an *O*-demethylation/methyl transfer (Mto) system that is more related to that of acetogenic bacteria than the methyl transferase system of methylotrophic archaea [2]. With biochemical approaches we were able to show that the methyl group is transferred from the methoxylated compound to the corrinoid protein MtoC by the *O*-demethylase MtoB. We further got strong evidence by activity assays and protein crystallization that MtoA transfers the methyl group to tetrahydromethanopterin instead of coenzyme M, which differs from the conventional methanogenic methyl-transfer systems. This most likely leads to an altered energy metabolism and redox (im)balance during methoxydotrophic growth. In summary, methoxydotrophic methanogenesis differs regarding its methyl transfer and C1 metabolism from other methanogenesis pathways and might play an important role in anoxic subsurface environments.

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eP379

Bac*Media*: a culture media database and its application for predicting cultivation conditions of so far uncultured microorganisms

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Even in this age of next generation sequencing and other high-throughput methods, growing microbial strains in laboratory media remains essential for a comprehensive characterization and therefore for a deeper understanding of microorganisms. Despite their importance, culture media recipes have been neglected in terms of standardization and availability.

We present Bac*Media* (bacmedia.dsmz.de), the world"s first expert-curated culture media database, comprising recipes and molecular compositions of more than 3,200 standardized culture media for more than 40,000 microbial strains from all domains of life. Culture media were extracted in an automated and manually curated approach from non-standardized recipe documents of the culture collections DSMZ and JCM. Bac*Media* offers a number of intuitive search and comparison tools, the possibility to identify media

from closely related taxonomical groups and the integration of strain-specific adjustments. The state-of-the-art website allows paperless use of media recipes on mobile devices; however, it is also possible to export the recipes to PDF for archiving and printing. An internal editor interface ensures continuous extension and curation of culture media by experts from the DSMZ.

Finding a suitable medium for an uncultured microorganism represents a frequent challenge. By connecting standardized culture media to genomes, phenotypes and ecological data, we develop large-scale machine learning models. These models aim to assist microbial researchers in finding cultivation conditions based e.g. on genome data. Development is step-wise, starting with proof-of-concept predictions for well-studied species and ultimately aims to predict comprehensive conditions for the majority of yet uncultured prokaryotes. Wet lab cultivation experiments are conducted to validate and improve the established models.

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eP380

Ruling-out of a cytoplasmic bypass for the energy-converting methyltransferase Mtr in *Methanosarcina acetivorans*

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Methanogenesis is a unique energy metabolism carried out by members of the domain *Archaea*. In contrast to most other methanogens, which reduce CO_2 to methane with hydrogen as electron donor, *Methanosarcina acetivorans* is able to grow on methylated compounds, acetate or carbon monoxide (CO), which are metabolized *via* distinct yet overlapping pathways. For the use of any single methanogenic substrate, the membrane-integral, energy-converting N^5-methyl-tetrahydrosarcinapterin (H_4SPT):coenzyme M (HS-CoM) methyltransferase (Mtr) is required. The enzyme is unique in energetically coupling the transfer of methyl groups between H_4SPT and HS-CoM with the translocation of sodium ions across the membrane. 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 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 Mts system is not involved in bypassing Mtr, thereby disproving a previous proposal. Alternative mechanisms for CO_2 formation from a methyl group donor were addressed through labeling experiments using ^{13}C-methanol. We found that *M. acetivorans* provides the reducing equivalents for methane formation, rather than by oxidizing methyl-S-CoM to CO_2, through oxidizing an intracellular storage compound. Thus, no *in vivo* Mtr bypass exists in *M. acetivorans*.

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eP381

Involvement of the porin MspD in *M. smegmatis* zinc homeostasis

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Uptake of metals such as zinc is essential for bacterial survival. Translocation across the membrane(s) occurs e.g. via specific transporters such as the widely distributed importer ZnuABC or unspecific ion-channels. The mycobacterial membrane is of extraordinary complexity with an inner (IM) and a lipid-rich outer membrane (OM), which is not comparable to most other prokaryotes. The saprophytic species *Mycobacterium smegmatis* (MSMEG) possesses two types of ZnuABC importers, which are assumed to be located in the IM. *Mycobacterium smegmatis* porin D (MspD) is part of a group of four highly similar paralogue transmembrane protein channels – MspA to MspD, which densely cover the OM of MSMEG. Even though, amongst other functions, low-affinity acquisition of metals such as iron and copper have been shown for MspA-C proteins, no function for MspD was described so far. In addition the transport of zinc across the OM is otherwise unknown. Expression of *msp*D was shown to be induced upon zinc starvation and upon deletion of the zinc uptake regulator Zur. Hence, we were interested to elucidate the role of MspD in zinc homeostasis of MSMEG.

We created single, double and triple combinations of MSMEG zinc transporter and MspD mutants, complemented them with *msp*D under its own promoter or under the promoter of *rpm*B, which is strongly induced upon zinc starvation, and analysed growth behavior as well as transcriptional changes. In growth experiments deletion of *msp*D (MSΔ3) or *msp*D and *znu*ABC (MSΔΔ5) in minimal Sauton"s medium (SM) and on MB agar plates did not lead to significant growth defects. However, qRT-PCR experiments revealed that the expression of zinc starvation marker *rpm*G was induced in MSΔΔ5, indicating a lower zinc status in this mutant. Complementation with *msp*D alone completely restored the starvation phenotype suggesting that MspD supports zinc passage through the OM. Deletion of both *znu*ABC importers and *msp*D (MSΔΔΔ7) resulted in highly induced *rpm*G expression and severe growth defects in SM, which could be restored upon zinc supplementation but not by complementation with *msp*D, presumably because zinc passage through the IM is not possible anymore. Together, these findings indicate that zinc balance in MSMEG is maintained by passive and active zinc transport through the OM and IM. Hence, zinc import by IM transporters is facilitated by zinc passage across the OM via MspD.

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eP382

The plant-derived naphthoquinone lapachol induces an oxidative stress response in *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* is a major human pathogen, which rapidly acquires resistance to multiple antibiotics. Thus, the discovery of new antimicrobial compounds is an urgent need to combat infections with drug resistant *S. aureus* isolates. The 1,4-naphthoquinone lapachol was previously shown to exert antimicrobial effects against Gram-negative and Gram-positive bacteria. **Objectives:** In this study, we were interested whether the antimicrobial activity of lapachol against *S. aureus* involves oxidative stress or the thiol *S*-alkylation chemistry [1]. **Materials & Methods:** To study the mode of action, we applied RNA-seq transcriptomics, redox biosensor measurements, *S*-bacillithiolation assays and mutant phenotype analyses. **Results:** In the transcriptome, lapachol

caused an oxidative and quinone stress response and protein damage as revealed by induction of the PerR, HypR, QsrR, MhqR, CtsR and HrcA regulons. Lapachol treatment further resulted in up-regulation of the SigB and GraRS regulons, which is indicative for cell wall and general stress responses. The redox-cycling mode of action of lapachol was supported by an elevated bacillithiol (BSH) redox potential, higher endogenous ROS levels, a faster H_2O_2 detoxification capacity and increased thiol-oxidation of GapDH and the HypR repressor. The ROS scavenger *N*-acetyl cysteine and microaerophilic growth conditions improved the survival of lapachol-treated *S. aureus* cells. Phenotype analyses revealed an involvement of the catalase KatA and the Brx/BSH/YpdA pathway in protection against lapachol-induced ROS formation in *S. aureus*. However, no evidence for irreversible protein alkylation and aggregation was found in lapachol-treated *S. aureus* cells. **Conclusion:** The naphthoquinone lapachol mainly causes ROS formation and an oxidative stress response as antimicrobial mode of action in *S. aureus*.

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eP384

Insights into tartrolon resistance mechanisms in *Listeria monocytogenes*

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The gram-positive bacterium *Listeria monocytogenes* is a member of the *Bacilli*. It occurs ubiquitously in the environment and can cause severe invasive diseases in humans upon ingestion. Like most bacteria, *L. monocytogenes* can deal with harmful substances, secreted by natural competitors such as other bacteria or plants by induction of gene expression of specific ATP binding cassette (ABC) transporters. Transporters for such natural compounds are usually highly specific. We recently identified the *lieAB* operon as essential for resistance against aurantimycin, a compound secreted by *Streptomyces aurantiacus* to fight competitors (1). After screening various promoters of potential genes encoding multiple drug resistance transporters (MDR transporters) fused to *lacZ* we identified a promoter that is being induced upon contact with tartrolon A and B, secondary metabolites produced by *Sorangium cellulosum* (2). We here show, that a mutant lacking the corresponding transporter shows a 40-fold lower minimal inhibitory concentration (MIC) to both tartrolons than the wild type. The MIC of tartrolon A and B has already been described for *Bacillus subtilis* (2). In good agreement with our observations, we confirmed that expression of the genes encoding the putative tartrolon transporter are sufficient to confer resistance to *B. subtilis*. Measurement of β-galactosidase activity of this promoter-*lacZ* fusion in the absence of the repressor protein led us to observe a stronger promoter activity than in the wild type, being a good indication that the repressor binds to its own promoter. A strain lacking the repressor also shows an increased resistance to tartrolon A and B. Our results provide insights into the function of a not yet characterized ABC transporter of *L. monocytogenes* and helps to further understand the survival mechanisms of this important human pathogen to survive within its environmental reservoir.

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eP385

Reduction of Aromatic Acids to Corresponding Alcohols by Coupled Enzyme Assays

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Aromatoleum aromaticum EbN1 and other species of the genus *Aromatoleum* have been sources for many enzymes catalyzing reactions with aromatic carbohydrates. Aromatic carboxylic acids can be found in various sources from crude oil to lignin. They have a broad range of applications. So far, industrial synthesis and applications rely on lithium/aluminium catalysts amongst others. Here we present a pathway way of biocatalytic reduction from aromatic acids to corresponding alcohols utilizing recombinant enzymes from *A. aromaticum* EbN1.

The tungsten cofactor containing aldehyde oxidoreductase (AOR) is an oxygen sensitive enzyme that is able to catalyze the oxidation of a broad variety of aromatic and aliphatic aldehydes to the respective acids in presence of an electron acceptor. [1] Recent results show that AOR is also catalyzing the reverse reaction, reduction of benzoate to benzaldehyde, albeit at very low rates and under conditions strongly favoring acid reduction, e.g., low pH. Reports of thermophilic AOR orthologs also demonstrate slow reduction of organic acids directly to the corresponding aldehydes, if the thermodynamic equilibrium is made favorable for this reaction by the presence of semicarbazide or alcohol dehydrogenases removing the aldehydes from equilibrium [2, 3] Therefore, introduction of a coupled system with a benzyl alcohol dehydrogenase (BADH) also from *A. aromaticum* EbN1 enabled us to study the kinetics for the acid reduction reactions of AOR.

It has been reported that BADH is produced in various growth conditions. [4] We established recombinant expression and purification of BADH to study its kinetics for reduction of aldehydes and oxidation of alcohols. Furthermore, the BADH itself has a broad substrate spectrum of mainly aromatic compounds and favors NAD(H) as cofactor, but works with NADP(H) as well. This allows multiple experimental settings, either including or excluding cofactor.

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eP387

Different amino acids – different strategies: How *Bacillus subtilis* deals with amino acid stress in a c-di-AMP free background

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Deleting all three diadenylate cyclase-encoding genes creates a *B. subtilis* strain that lacks the essential second messenger c-di-AMP (Δ*dac*). Such a strain is not viable on complex medium and is sensitive to several amino acids, including the most abundant amino acid glutamate. Glutamate shifts the affinity of the potassium-importer complex KtrCD from low affinity to high affinity, causing more potassium ions to be imported into the cell, which is toxic to the Δ*dac* strain[1]. Multiple amino acids degradation pathways generate glutamate, which explains suppressive mutations in KtrCD, when these normally toxic amino acids are added to the medium. However, not all mutations are related to potassium. The presence of glutamine and alanine resulted in the inactivation of the AimA protein. Follow-up experiments have identified AimA as alanine and glutamine transporter and confirmed its role as the major low affinity amino acid uptake transporter in *B. subtilis*. Stressing the cells with histidine provoked mutations in the gene encoding AzlB, the transcriptional repressor of the *azl* operon. In the absence of AzlB, the AzlCD exporter complex is constitutively expressed, which allows the export of histidine. The different types of suppressor mutations (importer vs. exporter) will be discussed.

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eP389

Proteins with potential importance for recovery from TisB-induced dormancy in *Escherichia coli*

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Introduction:

In *Escherichia coli*, the type I toxin-antitoxin system *tisB/istR-1* is part of the SOS response. Toxin gene *tisB* is strongly induced as soon as DNA damage occurs. TisB is a small hydrophobic protein located in the inner membrane, causing growth stasis due to depolarization and ATP depletion. TisB-induced dormancy favors formation of transiently multidrug-tolerant cells, so-called persisters.

Objectives:

The aim of this study was the identification of genes that influence TisB-induced dormancy.

Materials and methods:

We used an optimized, plasmid-based TisB expression system and RNA-Seq to identify genes with altered mRNA levels upon TisB-induced dormancy. RNA-Seq data was validated by either qRT-PCR or Northern blots. Deletion mutants were constructed and analyzed with respect to TisB-dependent dormancy as well as recovery from dormancy. TisB toxicity was analyzed by the use of fluorescent dyes. Protein aggregation was monitored using a fluorescent reporter construct and microscopy.

Results:

Among the up-regulated genes were many genes with functions related to stress (e.g., *soxS*: oxidative stress; *cpxR, cpxP*: inner membrane stress; *ibpB, spy*: protein aggregation), but also genes encoding poorly characterized inner membrane proteins (e.g., *azuC, ydjM, yqaE, yebE*). Preliminary data indicate that TisB expression causes protein aggregation. Deletion of *ibpB* and *spy*, encoding molecular chaperones, does not affect TisB toxicity but delays recovery from dormancy. We conclude that IbpB and Spy enable recovery by dissolving protein aggregates. We also observed that recovery from TisB-induced dormancy is strongly delayed when *azuC, ydjM* or *yqaE* are deleted. Whether the corresponding inner membrane proteins maintain membrane integrity upon TisB stress or directly interact with TisB and antagonize TisB toxicity remains an open question.

Conclusions:

Endogenous expression of type I toxins is a survival strategy in unfavorable environments, but also inflicts stress. Bacteria respond to toxin expression by inducing stress-related genes. The corresponding proteins either counteract toxin-dependent stress or might be involved in directly dampening toxin functioning.

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eP390

Cobalamin (Vitamin B_{12})-dependent methyl transfer: Filling a gap in the betaine catabolism of *Phaeobacter inhibens*

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Betaine (= N,N,N-trimethylglycine) is a small, zwitterionic and multimethylated osmolyte that is commonly produced by land plants and algae. Some heterotrophic bacteria present in the rhizosphere or in the phycosphere including members of the *Rhizobiales* as well as the *Rhodobacterales* can catabolize betaine via a series of demethylation reactions resulting in glycine. The enzyme catalyzing the first demethylation reaction remains enigmatic. A betaine-homocysteine methyltransferase is commonly suggested to fill this gap. However, here we present evidence that a cobalamin-dependent methyltransferase system is involved in the betaine catabolism of the marine heterotrophic alphaproteobacterium *Phaeobacter inhibens* (*Rhodobacterales*). The genomes of 40 members of the *Rhodobacterales* representing ten different genera were subjected to a pangenomic analysis. A functional enrichment analysis was performed to reveal the distribution of components of a cobalamin-dependent methyltransferase system across the genomes. The expression of identified

genes and the production of the cofactor cobalamin was analyzed in *P. inhibens* upon cultivation with betaine as well as glucose as control substrate. The transcriptional analysis supports an involvement of identified components in betaine catabolism. A 25-fold increased cobalamin production in *P. inhibens* upon cultivation with betaine in contrast to glucose further suggests an involvement of cobalamin as cofactor in the catabolic reactions of betaine. We conclude that the betaine catabolism comprises cobalamin-dependent methyl transfer that drives the cobalamin production in *P. inhibens*. Since many betaine-producing algae are cobalamin-auxotrophs, betaine could play a central role in algal-bacterial cross-feeding. This role will be investigated in the future.

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eP391

Cell death during nutrient starvation in *Staphylococcus aureus* cells lacking (p)ppGpp is linked to disturbed GTP homeostasis

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The stringent response is one way by which bacteria respond to changes in nutrient availability. Upon nutrient limitation or in response to other stresses the alarmones guanosine pentaphosphate and tetraphosphate, collectively named (p)ppGpp, are synthesized in *S. aureus* by three alarmone synthetases Rel_{Sau}, RelP and RelQ and play an important role bacterial stress response, virulence and persistence [1-3]. Upon synthesis of (p)ppGpp, GTP levels decrease sharply via consumption of GTP [1].

During growth experiments we could observe that *S. aureus* and an isogenic (p)ppGpp^0 mutant show similar growth but entering the stationary phase the (p)ppGpp^0 mutant shows significantly decreasing survival compared to wildtype. This indicates that induction of the stringent response due to nutrient starvation can prevent cell death in the wildtype. Accordingly, we observed a stringent response-like transcription profile (reduction in the expression of *rpsI* and an increase in *psmα*) [4] and increased *rsaD* expression, which is indirectly regulated by GTP levels, in the wildtype but not in the isogenic (p)ppGpp^0 mutant. In other firmicutes GTP dysregulation leads to failure to adapt to nutrient starvation [5]. When we compared the growth of guanine-auxotrophic mutants in wildtype and (p)ppGpp^0 background, no difference in survival in late stationary phase could be observed. This indicates that the uncontrolled increase of GTP in the (p)ppGpp^0 strain contributes to cell death under starving conditions. Furthermore, under anaerobic conditions no difference in survival between wildtype and the (p)ppGpp^0 strain can be observed indicating that increased susceptibility to reactive oxygen species might play a role in survival.

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eP392

Exploring the responses of *B. subtilis* to fosfomycin: an "omics" approach

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Introduction

The current increasing incidence of antimicrobial resistance and the lack of new antibiotics in development and discovery have led to a reassessment of the potential of already known antibiotics. One promising drug candidate for weaving combination therapies is fosfomycin.

The antibiotic FOS, which forms its own class of antibiotics: epoxide, is a well-known antibiotic that recently has re-attracted attention, since it shows synergistic effects in combination with many antibiotics of different drug families. FOS has broad-spectrum bactericidal activity against Gram-positive and Gram-negative bacteria. FOS covalently blocks the enzyme MurA which catalyzes the condensation of UDP-GlcNAc with PEP representing the first dedicated enzymatic step of peptidoglycan biosynthesis. Whereas Gram-negative bacteria possess a single, essential MurA, most Gram-positive bacteria have two MurA analogues which are both attacked by FOS.

Objectives

FOS acts synergistically in combination with many drugs due to unexplored cellular effects, such as oxidative stress or starvation response associated with ppGpp accumulations. In addition to investigating possible FOS off targets based on similar enzyme activity and specificity to MurA, the general cellular effects will also be investigated.

Materials & methods

These objectives were approached with a multi-omics approach. In addition to broad transcriptomic and proteomic analyses of the identical samples, targeted metabolomics investigations of various metabolic pathways and enzyme reactions were carried out. In advance, numerous growth experiments and MIC determinations were carried out under different conditions and media.

Results

It was concluded that a pure end-time MIC determination has no significance for the growth process, since cultures of *B. subtilis* recover from FOS treatment without gaining resistances and even grow to higher densities compared to control cultures.

Based on initial results, the link to oxidative stress has been confirmed. In addition, there are many indications that *B. subtilis* tries to overcome the emerging cell wall stress with the increased build-up of lipoteichoic acids and can thus stabilize the cell wall even without preferred peptidoglycan.

Conclusion

In order to better understand known antibiotics, the cellular effects need to be studied, especially for FOS, there are a number of yet unexplored cellular effects.

eP393

Genetic tools for the redirection of the central carbon flow towards the production of lactate in the human gut bacterium *Phocaeicola (Bacteroides) vulgatus*

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Species of the genera *Bacteroides* and *Phocaeicola* play an important role in the human colon. The organisms contribute to the degradation of complex heteropolysaccharides to small chain fatty acids, which are in part utilized by the human body. Furthermore, these organisms are involved in the synthesis of vitamins and other bioactive compounds. Of special interest is *Phocaeicola vulgatus*, originally classified as a *Bacteroides* species. We analyzed different tools for the genetic modification of this microorganism, with respect to homologous gene expression of the *ldh* gene encoding a D-lactate dehydrogenase (LDH). Therefore, the *ldh* gene was cloned into the integration vector pMM656 and the shuttle vector pG106 for homologous gene expression in *P. vulgatus*. We determined the *ldh* copy number, transcript abundance, and the enzyme activity of the wild type and the mutants. The strain containing the shuttle vector showed an approx. 1500-fold increase in the *ldh* transcript concentration and an enhanced LDH activity that was about 200-fold higher compared to the parental strain. Overall, the proportion of lactate in the general catabolic carbon flow increased from 2.9 % (wild type) to 28.5 % in the LDH-overproducing mutant. This approach is a proof of concept, verifying the genetic accessibility of *P. vulgatus* and could form the basis for targeted genetic optimization.

eP394

Selection for sugar uptake bypass routes in *Corynebacterium glutamicum*

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Introduction: An important feature in microbial production processes is the carbon source uptake rate. In the major industrial amino acid producer *C. glutamicum*, glucose, fructose and sucrose are taken up and phosphorylated by the permeases PtsG, PtsF and PtsS of the phosphotransferase system (PTS) [1]. Although the *myo*-inositol transporters IolT1 and IolT2 can take up glucose and fructose in an unphosphorylated state, intracellular phosphorylation was only reported for glucose by the kinases Glk and PpgK. Thus, the possibility of PTS-independent uptake and metabolization remains limited to glucose.

Objectives: To identify further transporters or kinases, which enable the PTS-independent utilization of glucose or fructose, *C. glutamicum* strains lacking the respective PTS permeases and glucose kinases should be constructed. By conducting growth experiments with these strains, the occurrence of suppressor mutants should provide further transporters and kinases to bypass PTS-mediated sugar uptake.

Materials & methods: The suicide plasmid pK19*mobsacB* was used to delete *ptsF*, *ptsG*, *glk* and *ppgK* in *C. glutamicum* and to achieve constitutive *iolT1* expression by promoter mutation. The deletion strains were cultivated in CGXII medium with either glucose or fructose as sole carbon source in a microbioreactor. Strains which revealed delayed growth in minimal medium were used for recultivation under similar conditions. If the result could be verified, the genomic DNA of single clones was isolated and sequenced.

Results: *C. glutamicum* Δ*ptsF*Δ*ptsG* showed no growth in CGXII medium with fructose. Additional overexpression of *iolT1* did not change this outcome and could not provoke the evolution of a fructose kinase within the time period tested. When *C. glutamicum* Δ*ptsF*Δ*ptsG* was cultivated in CGXII medium with glucose, a growth delay was observed, which could be overcome by expression of *iolT1*. Further deletion of *glk* and *ppgK* in this strain did not cause a complete growth inhibition, but resulted in a strong growth delay, suggestive for the occurrence of a suppressor mutant.

Conclusion: The occurrence of suppressor mutants of *C. glutamicum* Δ*ptsF*Δ*ptsG* and *C. glutamicum* Δ*ptsF*Δ*ptsG*Δ*glk*Δ*ppgK* P06-*iolT1* strains suggest that further yet unidentified glucose uptake transporters and kinases exist in *C. glutamicum*, which can be utilized PTS-independent metabolization of glucose.

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eP395

Evidence for an Alternative, Ferredoxin-Dependent Glutamate Synthase in *Escherichia coli*

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Glutamate is a major constituent of *Escherichia coli*"s protein inventory. When *E. coli* grows on a medium containing ammonium as the sole nitrogen source, about 80 % of the cell's nitrogen flows through glutamate[1]. Under nitrogen-limiting conditions, *E. coli* synthesizes a glutamate synthase, GltDB, that scavenges L-glutamine for L-glutamate synthesis and requires electrons from NAD(P)H.

Bioinformatic analysis revealed that *E. coli* encodes two GltD-homologous glutamate synthase domains within the AegA and YgfT proteins, which are predicted to harbour an additional ferredoxin-like domain[2]. This architecture is reminiscent of the plant-type glutamate synthases, which receive electrons from ferredoxin instead of NAD(P)H, suggesting an unexpected electron transfer mechanism for AegA or YgfT. However, these proteins apparently lack the trans- and deamidation functionality of GltB and thus their substrate specificity currently remains enigmatic. Here, we investigated electron transfer reactions and the physiological involvement in nitrogen metabolism of the two proteins.

We characterized expression of the *aegA* promoter and found it was strongly induced under anaerobic conditions in the mid-exponential growth phase. The expression was reduced in the presence of excess nitrate and induced in its absence, but was unaffected by addition of formate or fumarate.

Mutants lacking the *gltD* or *aegA* genes, or combinations thereof, were characterized for their growth behaviour under ammonium-limited conditions. The double mutant was auxotrophic for ammonium under aerobic and anaerobic growth conditions and lost the ability to use glutamine as nitrogen source. The *aegA* single mutant had no 'fixed-nitrogen' phenotype.

Bacterial two-hybrid studies revealed an interaction between AegA and a previously uncharacterized ferredoxin-like protein YsaA and the formate dehydrogenase FdhH[3].

Unfortunately, the identified interactions proved too transient for *in vitro* confirmation. Nevertheless, purification of the AegA and YgfT enzymes identified a FMN cofactor and confirmed the presence of approximately five [4Fe-4S]-clusters. These proteins can transfer electrons from or to NAD(P)H, but their physiological redox partners remain to be identified.

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eP396

LiaF and its Role within the Cell Envelope Stress Response of *Bacillus subtilis*

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The cell envelope is one of the most crucial structures in bacteria, considering its function as a barrier to the harsh and competitive environment *Bacillus subtilis* is living in. Therefore, maintaining the cell envelope's integrity is of utmost importance for the survival of bacteria. The intactness of the cell envelope is ensured by a highly regulated and well-orchestrated cell envelope stress response (CESR) network. The LiaRS-LiaF-Three-Component-System (3CS) represents a significant part of the CESR network. The 3CS perceives changes within the cell membrane via the histidine kinase LiaS. Subsequently, LiaS passes the signal through the response regulator LiaR, which binds the *lial* promoter and activates the expression of the effector genes *lial* and *liaH* [1]. The transmembrane protein LiaF represents the third part of the 3CS and acts as a negative regulator of the LiaS activity. Considering that a deletion of the encoding gene *liaF* leads to a phenotypic "locked-ON" state, resulting in a permanent response of the *Lia*-system through unstopped activation of the promoter P*lial* [2], it seems that LiaF takes a vital role within the 3CS. To further unravel the function of LiaF, an epitope tag was N-terminally cloned to the protein of interest (POI), enabling analysis through immunoprecipitation, protein purification, and Western blotting after expression of the recombinant FLAG-tag fusion protein. Overexpression was successfully conducted using an IPTG-inducible system. The FLAG-tag has proven sufficient for the purification of the transmembrane protein using affinity precipitation. Additionally, Western blot analysis indicated the interaction of our POI with members of the *Lia*-system and provided new insights into the relationship between LiaS and LiaF. Furthermore, these experiments proved the use of the FLAG-Tag as an effective tool for co-immunoprecipitation and interaction studies.

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eP397

H_2 partial pressure switches autotrophic pathways in an anaerobic bacterium

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Although the co-occurence of two different autotrophic CO_2 fixation pathways in one organism has been proposed in several cases, it has never been unequivocally confirmed experimentally. Usually, the possible co-occurrence of the reductive tricarboxylic acid cycle and the Calvin-Benson (CB) cycle has been discussed. Here, we study another example of the co-existence of two different autotrophic pathways. The nitrate reducing bacterium *Ammonifex degensii* possesses genes for both the Wood-Ljungdahl (WL) pathway and the CB cycle in its genome. We show that both pathways are used by *A. degensii* for autotrophic carbon fixation. *A. degensii* balances its metabolism depending on the H_2 partial pressure, governing the availability of the reduced ferredoxin for the cell. At 80% of H_2, favoring ferredoxin reduction, it uses the ferredoxin-dependent WL pathway but switches to the less efficient but ferredoxin-independent CB cycle during growth at 10% H_2. Correspondingly, the growth yield of *A. degensii* (in g carbon/mol produced NH_4^+) is higher at 80% H_2 than at 10% H_2. As the presence of key genes of different autotrophic pathways in one organism is not a rare trait among prokaryotes, this type of metabolic adjustment may be widespread in a microbial world in natural environment.

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eP398

A mass spectrometry-based screening method for PDF inhibitors in *Bacillus subtilis*

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Cotranslational deformylation of the initial methionine of a nascent polypeptide is an essential process in prokaryotes that is conducted by the peptide deformylase (PDF). Deformylation is required for correct protein maturation and function. Actinonin is a potent inhibitor of the bacterial PDF and is therefore being investigated as antibiotic for the treatment of a variety of infectious diseases. Actinonin was found to also inhibit eukaryotic PDFs, including human PDF (HsPDF). For treatment of bacterial infections, inhibition of the human PDF presents an undesired activity. The molecular structure of actinonin has been modified with the aim to enhance antibacterial activity and selectivity for bacterial PDF over HsPDF.

In this work, we established a mass spectrometry-based method that allows to measure *in vivo* PDF inhibition by actinonin derivatives. In a proteome analysis, for FbaA we found a well measurable increase of formyl-methionine retaining N-terminal peptides upon actinonin treatment. To asses PDF inhibition, mass spectrometry is performed with lysate of a genetically modified *Bacillus subtilis* strain, harboring a plasmid for overexpression of the corresponding gene *fbaA*.

The workflow comprises the assessment of the antimicrobial activity of putative PDF inhibitors in standard MIC assays and the subsequent cultivation and treatment of bacteria with nonlethal concentrations during gene overexpression. The trypsin-digested lysate was subjected to mass spectrometrical analysis regarding the formylation state of N-terminal peptides of the overproduced protein FbaA. The

screening method was established and optimized using the lead compound actinonin as reference.

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eP399

DMSO reduction by the acetogenic bacterium *Moorella thermoacetica*

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Introduction: Acetogenic bacteria are an ecophysiologically important group of strictly anaerobic bacteria. Their characteristic feature is to oxidize organic as well as inorganic electron donors, coupled to the reduction of CO_2 *via* the Wood-Ljungdahl pathway. In addition to CO_2, acetogens can use alternative electron acceptors such as nitrate, fumarate, inorganic sulfur compounds or aromatic acrylates[1]. The genome sequencing of *Moorella thermoacetica*, the cytochrome- and quinone-containing acetogenic model organism, revealed the presence of a potential membrane-bound DMSO reductase[2]. Since DMSO reduction as terminal electron acceptor has not been studied in acetogens we stated to address the question whether and how DMSO is reduced.

Objectives: The objective of this work was to elucidate DMSO as alternative electron acceptor for the acetogen *M. thermoacetica* DSM 521.

Materials & methods: Growth experiments, enzyme assays and genome-wide expression analyses were performed.

Results: *M. thermoacetica* was able to reduce DMSO during heterotrophic growth with glucose and autotrophic growth with H_2 + CO_2. DMSO reduction was accompanied with an increase in biomass yields. Under HCO_3^-/CO_2-free conditions DMSO could replace CO_2 as terminal acceptor. The biochemical characterization of the DMSO reduction revealed a membrane-bound DMSO reductase, capable to use the artificial quinone AQDS as well as methyl- and benzyl viologen as electron donor. The pH optimum was at pH 5 and the temperature optimum at 60°C. Transcriptome analysis revealed the genes encoding for the DMSO reductase. Furthermore, major genes related to redox balancing proteins like the bifurcating hydrogenase and menaquinone were downregulated.

Conclusion: *M. thermoacetica* can use DMSO as terminal electron acceptor using a membrane-bound DMSO reductase.

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[2] Pierce E. *et al*., *Environ. Microbiol*. 10:2550-2573 (2008).

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eP400

Gut Bacteria for Biotechnology: Developing a cultivation method for *Bacteroidetes* towards propionate production from polysaccharides

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Bacteroidetes are one of the most abundant phyla found in the human gut. They have a positive effect on the health of their host, for example by releasing short chain fatty acids, including propionate [1]. As precursor for many products, propionate is a valuable compound in a variety of industries, produced in large quantities via petrochemical processes [2]. The objective of this work is to explore *Bacteroidetes* for biotechnological propionate production from polysaccharides.

On the road towards the development of platform production organisms, we selected ten species known to produce propionate from the *Bacteroidetes* phylum. The strains were initially grown in complex media, and fermentation products were measured by HPLC-analysis. Two promising strains were then cultivated in defined minimal medium with glucose (DMMG). Finally, different media compositions were examined for their influence on growth and propionate production of those two.

From the ten examined strains, *Parabacteroides johnsonii*, which produced on average 11 mM propionate in complex medium and *Bacteroides graminisolvens*, which achieved the highest propionate/acetate ratio (1,7), were chosen to be further investigated. During growth on DMMG, the growth rate and final propionate concentration of *P. johnsonii* cultures significantly decreased to 0,1 h^{-1} and 3.8 mM respectively. Although growth of *B. graminisolvens* in DMMG was not affected, no propionate was formed. Addition of B_{12} improved growth of *P. johnsonii* to a growth rate of 0,22 h^{-1} and enhanced final propionate concentration of *B. graminisolvens* cultures to 8,96 mM.

Additionally, new *Bacteroidetes* isolates were obtained on substrates like xylan and cellulose. These isolates were also screened for their potential to produce propionate, and the most promising propionate producers among them will be included in further experiments to increase product yield.

Our results show, that media composition has a significant effect on growth and product formation, depending on the strain tested. Finding appropriate culture conditions, which result in high propionate yield in a range of *Bacteroidetes* is therefore a crucial step for the selection of model strains to further examine propionate production of *Bacteroidetes*. With this work, first steps have been made for such a cultivation method.

[1] Louis *et al.* 2017, *Environmental microbiology* 19: 29-41

[2] Gonzalez-Garcia *et al.* 2017, *Fermentation* 3: 21

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eP401

***Escherichia coli* cellular response to fosfomycin: novel insights into the drug transport and its bacteriolytic effects**

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Introduction:

Fosfomycin is an old broad-spectrum bactericidal antibiotic, used to treat lower urinary tract infections. Recently it has been re-evaluated, showing efficiency in combinatorial therapy against drug resistant bacteria. FOS inhibits the

MurA enzyme, blocking the peptidoglycan synthesis in the first step, but little is known about other cellular effects.

Objectives:

This pivotal project wants to investigate the cellular effects of FOS in *E. coli*. We focused on the drug transport, assessing the role of UhpT and a possible involvement of OmpC; we also investigated the bacteriolytic effect of FOS, by developing a solid protocol to follow FOS cellular effects in liquid medium and under the microscope. This helped us optimizing conditions for next omics studies. The better understanding of FOS cellular effects will hopefully lead to a rationalisation of its medical usage.

Materials and methods:

The *E. coli* BW25113 strain and relative mutants of the Keio collection were used. Their susceptibility to FOS was optimised on solid medium (E-tests and disc-diffusion tests) and in liquid culture (in a microplate reader and in 500 mL flasks). A protocol for the time-lapse monitoring of cells" growth and FOS effects was developed in LB liquid medium, using a microplate reader, and with phase-contrast microscopy. Liquid cultures were also optimized for transcriptomics and metabolomics experiments (currently conducted).

Results:

MIC values were determined not only on solid LB, but also in liquid culture. It was possible to hypothesise a role of OmpC for FOS outer membrane transport, unknown so far. UhpT was showed to be the main FOS importer on solid medium and responsible for the first lysis of cells, detected in liquid medium; here the cells could grow again after an adaptation period and further cycles of lysis and regrowth were detected. This behaviour correlated with microscopy observations, were we saw L-forms-like spheroplasts appearing and eventually lead to regrowth of longer rod-shaped cells.

Conclusions:

The transport of FOS into *E. coli* was better investigated and growth curves were assessed as a valuable tool to understand FOS cellular effects, revealing different lysis events. Surviving cells changed their morphology into L-forms and reverted to rods; this could be a target to avoid FOS resistance. This observation suggested a new hypothesis for the intracellular action of FOS.

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eP402
Isolation and characterization of laughing gas reducing bacteria from various environments

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1. Introduction

Nitrous oxide (N2O; laughing gas) is a powerful greenhouse gas and a major cause of ozone layer depletion. During the last century, human activities severely increased N2O emissions, mainly from agriculture, livestock farming and wastewater treatment. N2O is produced by nitrifiers, denitrifiers and microorganisms catalyzing dissimilatory nitrate/nitrite reduction to ammonia (DNRA). However, only one type of nitrous oxide reductase (NosZ) has been described. This enzyme is present in bacterial and archaeal species from different phyla including Proteobacteria and Firmicutes. Many of these organisms are able to grow by N2O respiration.

2. Objectives

It was aimed to isolate and characterize microorganisms that are highly efficient in N2O respiration and N2O turnover.

3. Materials and methods

Formate and acetate were used as electron donors to isolate laughing gas reducing bacteria under an atmosphere of pure N2O. Samples included anoxic digestates, sludge and soil. Obtained strains were tested for their ability to grow by N2O respiration. Furthermore, the specific N2O reduction rate and apparent affinity for N2O was determined by micro-respirometry.

4. Results

More than 150 isolates were phylogenetically assigned based on partial 16S rRNA gene sequences. The most prominent classes were Beta- and Gammaproteobacteria as well as Bacilli. Growth experiments indicated the presence of many fast-growing N2O-respiring species while micro-respirometry revealed efficient cellular N2O turnover rates as well as high-affinity N2O reduction. In many cases, partial *nosZ* genes were amplified by PCR and sequenced.

5. Conclusion

The applied enrichment conditions enabled fast and straightforward isolation of novel N2O reducing bacteria. It remains to be seen whether these isolates are applicable to mitigate N2O emissions from their original habitats.

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eP403
Structural Insights into Enzymatic Benzene Ring Reduction

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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-carbonyl-CoA to a stoichiometric ATP hydrolysis to ADP and P_i[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 with an unprecedentedly wide substrate spectrum including methylated and chlorinated substrates[3]. We succeeded in heterologous production of the enzyme in *Escherichia coli* and solved the crystal structure of the catalytic subunits at 1.9 Å. The result provides evidence for the proposed radical-based mechanism at an active site [4Fe-4S]-cluster.

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eP404

Substrate pre-selection by CofC and CofD enzymes govern the biosynthetic pathway of coenzyme F_{420} and derivatives

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Introduction: F_{420} is a specialized deazaflavin redox cofactor that promotes various metabolic pathways in a range of bacteria and archaea. It contributes to bacterial persistence, antibiotic biosynthesis, pro-drug activation and archaeal methanogenesis. Moreover, it has emerged as a potential tool for biocatalysis. We have recently discovered 3PG-F420, a new derivative of coenzyme F_{420} in the endofungal bacterium *Mycetohabitans rhizoxinica* and provided insights into its biosynthetic pathway.[1] Specifically, we showed that 3PG-F_{420} has evolved from F_{420} by a substrate specificity switch in the 2-phospholactate guanylyltransferase (CofC). In general, CofC enzyme guanylates the metabolites 2-phospholactate (2-PL), phosphoenolpyruvate (PEP) or 3-D-phosphoglycerate (3-PG) to yield reactive intermediates. The phosphotransferase CofD mediates the transfer of 2-PL, PEP or 3-PG moiety to redox active core Fo (7,8-didemethyl-8-hydroxy-5-deazariboflavin), for their entry into either F_{420}-0, DF_{420}-0 or 3PG-F_{420}-0 biosynthesis, respectively.

Objective: It remained elusive which amino acid residues within the CofC enzyme conferred the specificity switch towards 3-PG and how genetic mutation might have led to the evolution of 3PG-F_{420} biosynthesis. Another open question concerned the role of 2-PL in the biosynthesis of F_{420} in archaea.

Methods & Results: Substrate preference profiles of various CofC homologs were assayed biochemically to obtain an overview about the structure-specificity relationship. These data combined with structural investigations (modelling and X-ray crystallography) enabled the design of a site-directed mutagenesis study revealing residues which are critical for 3-PG activation. The identical residues then allowed for the engineering of FbiD (CofC) from *Mycolicibacterium smegmatis,* a known 2-PL/PEP activating enzyme into a 3-PG activating variant. Moreover, we investigated several homologs of CofD for their influence on CofC specificity, thus revealing that CofD further controls the biosynthesis of F_{420}-derivatives. [2]

Conclusion: These results helped to resolve an ongoing debate in the literature about the starting point of the F_{420} biosynthesis in archaea and bacteria. Moreover, our work inspires future efforts to discover additional rare cofactors and to further improve their applicability in biotechnology.

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eP405

Modifications of the glycosyl-chain of mycofactocin – a novel cofactor of mycobacteria

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Introduction

We recently discovered mycofactocin[1], a novel redox cofactor that was predicted in a bioinformatics study[2]. It belongs to the natural product class of ribosomally synthesized and post-translationally modified peptides, is synthesized by enzymes of the biosynthetic gene cluster (BGC) *mftABCDEF*[4] and is involved in the utilization of alcohols as carbon and energy sources[3]. In *Mycolicibacterium smegmatis* we found that the redox-active core molecule premycofactocin can be glycosylated by the glycosyltransferase MftF and that the resulting glycosides can act as a cofactor for carveol dehydrogenase. Structure elucidation further showed that the second sugar moiety is singly *O*-methylated[1]. However, the biochemistry of these modifications and their physiological role were yet to be elucidated.

Objectives

Since the modifications of mycofactocin appear to be non-essential for its redox activity *in vitro*[5] but MftF is an integral part of a mycofactocin BGC[2], the goal of this study was to investigate the biosynthetic origin and function of the structural modifications of mycofactocin in *M. smegmatis*.

Material and Methods

To reach the objectives of this study, a state-of-the-art multi-omics approach was used in conjunction with genetic modifications of *M. smegmatis*, classical biochemical methods and bioinformatic analyses.

Results

We could obtain further knowledge on the biosynthetic pathway of mycofactocin *in vivo*, elucidate the biochemistry of mycofactocin modification reactions and gain information on how these unusual modifications affect the biochemical characteristics and physiological role of this cofactor in *M. smegmatis*.

Conclusion

As a novel redox cofactor with curious modifications, clarification of the structure-function relationship presents an ongoing challenge for microbial biochemistry and physiology. However, using an interdisciplinary approach we were able to gain valuable knowledge on how the structure and biosynthesis of the natural product define its physiological function.

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eP406

Purification and characterization of heterologously-produced aldehyde:ferredoxin oxidoreductase (AOR) from *Thermoanaerobacter* sp. X514

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With the increasing need for alternative liquid fuels, production of alcohols from biomass and from synthesis gas (mainly H_2, CO, CO_2) becomes important. Promising whole-cell biocatalysts are anaerobic microorganisms that utilize a pathway involving aldehyde:ferredoxin oxidoreductase (AOR). This enzyme catalyzes the reduction of organic acids to aldehydes with reduced ferredoxin as electron donor. The aldehydes are then reduced to the corresponding alcohol by an alcohol dehydrogenase (ADH). AORs are oxygen-sensitive proteins containing tungsten and they have wide substrate spectra reducing aliphatic, branched-chained aliphatic and aromatic acids.

Our goal is the biochemical characterization of AOR from *Thermoanaerobacter* sp. X514. *Thermoanaerobacter* spp. are thermophilic sugar fermenters and well known for their high ethanol yields; with one known exception: the acetogen *Thermoanaerobacter kivui*. Since *T. kivui* is genetically available and has no *aor* in its genome, the *aor* gene from *Thermoanaerobacter* sp. strain X514 was cloned into *T. kivui* for heterologous expression. For biochemical characterization, the enzyme was produced in *T. kivui*, purified under anoxic conditions, and different anaerobic enzyme assays were used to determine its biochemical properties. AOR activity was measured by acetaldehyde oxidation to acetate with benzyl viologen as artificial electron acceptor as reduction of benzyl viologen can be monitored photometrically at 600 nm.

Heterologous AOR-His purification from *T. kivui* was successful, as we showed by SDS-PAGE, with specific antibodies and enzyme assays. AOR-His purified from *T. kivui* grown under standard cultivation conditions only had specific activity of less than 1 U mg^{-1}. But after optimizing of the growth conditions, we found that the addition of 120 nM tungsten instead of 12 nM tungsten increases the AOR activity of the purified enzyme about ten times (up to 10 U mg^{-1}) without impacting *T. kivui* growth. Highest specific activity yet was measured when adding 12 µM tungsten (up to 20 U mg^{-1}).

Fortunately, the use of a protein production system in a related anaerobe allows for the production of AOR enzyme variants for the deeper understanding of the enzyme properties. The role of AOR in biological alcohol production is important, and therefore it is vital to learn more about the biochemical properties of this tungstoenzyme and its structure, also in regard to possible applications in biotechnological engineering.

eP407

Mechanisms of activation of organic acids in the sulfur-reducing deltaproteobacterium *Desulfurella acetivorans*.

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Desulfurella acetivorans is an obligate anaerobic deltaproteobacterium capable of growing heterotrophically by acetate oxidation via the oxidative tricarboxylic acid cycle or autotrophically by forming acetyl-CoA from inorganic carbon via the reverse oxidative tricarboxylic acid cycle [1]. The activation of organic acids to the corresponding CoA thioesters represents a critical reaction of the central metabolism. In 1990, it was suggested that acetate kinase and phosphate acetyltransferase were responsible for acetate activation to acetyl-CoA in acetate-grown *D. acetivorans*, whereas succinyl-CoA conversion to succinate in the TCA cycle was catalysed by succinyl-CoA synthetase [2]. Nevertheless, four genes annotated as CoA-transferases were found in the genome of this organism, and the activity of acetate:succinyl-CoA CoA-transferase (ASCT) was measured in cell extracts of acetate-grown *D. acetivorans* (0.17 µmol min^{-1} mg^{-1} protein). We were able to identify the class I CoA-transferase AHF96498 as the enzyme responsible for acetate activation via CoA-transfer from succinyl-CoA (acetate + succinyl-CoA ↔ acetyl-CoA + succinate). The heterologously produced protein was highly active with acetate and succinyl-CoA (K_m for acetate of 0.4 mM and V_{max} of 9.4 µmol min^{-1} mg^{-1} protein), while it was also active with propionate, butyrate and formate as CoA acceptors. Phylogenetic analysis of different ASCTs identified previously shows that the acetate activation via CoA-transfer from succinyl-CoA evolved many times independently in different bacterial groups.

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eP408

Regulation and Structural Characterization of the Pyruvate:Quinone Oxidoreductase (PQO) from *Corynebacterium glutamicum*

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The Gram-positive *Corynebacterium glutamicum* grows on a variety of substrates and is widely used to produce amino acids. The phosphoenolpyruvate-pyruvate node in *C. glutamicum*, a metabolic link between glycolysis and citric acid-cycle, is important for carbon flux distribution and thus an important target for metabolic engineering. Part of this node is Pyruvate:Quinone Oxidoreductase (PQO), catalyzing pyruvate oxidation to acetate and identified in prior work as tetrameric flavoprotein, activated by lipids and detergents[1]. The aim of this study was to further characterize PQO and to gain insights into its so far unknown physiological role.

Enzyme activity patterns in cell-free extracts of *C. glutamicum* wildtype and of a strain deficient of the chromosomal *pqo* gene and expressing a plasmid-bound

pqo gene were determined under different cultivation conditions. The enzyme was purified natively from the PQO-overproducer with a purification protocol exploiting the intrinsic behavior of the cytosolic enzyme to act as a membrane protein in presence of its substrate and cofactors. LC-MS analysis of PQO was performed to reveal post-translational modifications (PTMs) and based on these experimental data, site-directed mutagenesis was used to create mutated PQO variants. Protein crystals originating from purified PQO enabled to solve its 3D-structure.

The activity patterns of the wildtype and the PQO-overproducer strongly indicated PTMs. LC-MS analysis proved that PQO is significantly succinylated at least at two lysine residues. One of the lysine residues, K325, was substituted with alanine or glutamate, respectively. The activity pattern of the respective strains revealed a pivotal role of K325 for the enzyme activity. The structure of PQO, solved both in an open form and in a ternary complex with FAD and thiamine pyrophosphate, shows that the enzyme is an homotetramer, with an overall fold close to *Escherichia coli* pyruvate oxidase but the C-terminal amphipathic α-helix in a very different conformation. It also shows that K325 is located at the surface close to the C-terminus, suggesting a role in stabilizing the helix position.

Overall, PQO is structurally unique and is modified via PTMs. The influence of these properties on the physiological role of the enzyme remains to be elucidated.

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eP409

Dissection of the Determinants Required for ATPase Activity of the [NiFe]-Hydrogenase Accessory Protein HypD

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[NiFe]-hydrogenases are enzymes that harbour an unusual bimetallic $NiFe(CN)_2CO$ cofactor in their large, catalytic subunit. Assembly of this cofactor requires the functions of six highly conserved Hyp proteins (HypA-F). While the HypEF proteins generate the CN^- ligands of the cofactor from carbamoylphosphate, the origin of the CO ligand remains to be determined. The CO ligand might originate from endogenously produced CO_2 already bound to an Fe ion on a HypD-HypC complex[1]. The reduction of CO_2 to CO requires a redox potential of approximately $E^{0'}$= -535 mV (in solution); however, the redox potential of the FeS-cluster in HypD ($E^{0'}$= -260 mV)[2] is too positive to be able to reduce CO_2 directly. The reduction step could be achieved if it were coupled to ATP hydrolysis. In a previous study, our group could show that HypC-HypD and HybG-HypD scaffold complexes indeed have an ATP-hydrolyzing activity[3], despite HypD having no obvious ATP-binding site. This current project aims to identify which residues in HypD are important for the ATPase activity. We wish to identify the ATP-binding site on HypD and to determine whether the ATPase activity is coupled to its redox activity. To accomplish this, amino acid-exchange variants of two highly conserved motifs found in HypD were generated. While the $GC_{69}PVC_{72}$ motif is part of the proposed electron-transfer route through HypD and is thus a strong candidate for having a role in ATPase function, we also investigated whether the G_{146}FETT motif might be required for ATP binding.

In vivo and *in vitro* hydrogenase and ATPase activity measurements of purified HypG-HypD complexes with exchanges in the GFETT-motif showed that these amino acid residues have a potential function in the interaction with HypC and HybG during maturation of the $Fe(CN)_2CO$ group. We could, however, rule out that the GFETT motif is part of the ATP-binding site.

Construction of HypD-variants in which the conserved Cys69 or Cys72 residues were changed to alanine resulted in a reduction of the ATPase activity by 75 %. Moreover, *N*-ethylmaleimide (NEM), which inhibits disulfide bond formation, decreased the ATP-hydrolyzing activity of native HypC-HypD complexes. Together, these results suggest that the ATPase activity might be coupled to electron transfer from the [4Fe4S]-cluster in HypD.

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eP410

A novel protein lipoylation pathway in sulfur oxidizers involves radical SAM proteins with unusual iron-sulfur cluster-binding regions

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Many sulfur-oxidizing bacteria and archaea pursue a sulfur oxidation pathway involving not only a heterodisulfide-reductase like enzyme system but also a 15-kDa lipoylated protein, LbpA [1]. The LbpA proteins from sulfur oxidizers do not serve as substrates for the canonical lipoate-binding protein biosynthetic machineries of *Bacillus subtilis* and *E. coli*, i.e. they are not lipoylated via LplA protein ligase-mediated lipoate scavenging nor modified by *de novo* synthesis via octanoyl transferase (LipB) and radical S-adenosyl domain (RadSAM)-containing lipoate synthase, LipA [1]. Instead, a set of potential lipoylation proteins is encoded in immediate vicinity of *shdr-lbpA* clusters. Among these is a lipoate ligase acting specifically on LbpA but not on closely related GcvH proteins [1]. The other encoded proteins include two unusual radical SAM proteins resembling LipS1 and LipS2 from the archaeon *Thermococcus kodakarensis*, which have recently been shown to act together as lipoyl synthase *in vitro* [2]. In addition, a gene for a geranylgeranyl reductase-like FAD-binding protein is usually found in the lipoylation gene cluster in sulfur oxidizers. We produced this protein as well as LipS1 and LipS2 from the Alphaproteobacterium *Hyphomicrobium denitrificans* heterologously in *E. coli*. Lipoate synthases perform one of the most chemically challenging reactions known in enzymology by activating C-H bonds in a reaction mechanism involving the formation of a radical. All canonical lipoate synthases share the typical [4Fe-4S](2+/1+) cluster of radical SAM enzymes (RS cluster) bound by a CX3CX2C motif and are also known for harboring a second essential auxiliary Fe-S cluster ligated by an N-terminal CX_4CX_5C motif. In contrast, LipS1 and LipS2 contain cysteine arrangements appearing suited for RS cluster binding but no signature sequence for binding an auxiliary Fe-S cluster. Instead, other cysteines in unprecedented arrangements are conserved in LipS1 and LipS2. We purified the recombinant proteins under anoxic conditions and reconstituted their iron-sulfur clusters. In both cases, brown proteins exhibiting UV-vis spectra characteristic for FeS-clusters were obtained. Quantification of iron and sulfur indicated the presence of two FeS clusters in both proteins one of which must be coordinated in a novel fashion. These experiments are complemented by gene inactivation studies.

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[2] Jin et al 2020 Appl Environ Microbiol 86, e01359

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eP411
ATP synthesis in an ancient ATP synthase at low driving forces
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Introduction: Hyperthermophilic archaea are close to the origin of life. Some anaerobic archaea live under strong energy limitation and have to make a living at the thermodynamic equilibrium, often allowing for the synthesis of less than a mol of ATP per mol of substrate. Obviously, this requires adaptations of the energy-conserving machinery to harness small energy increments. The ATP synthases of many anaerobic archaea have an unusual motor subunit *c* that otherwise is only found in eukaryotic V_1V_O ATPases. Eukaryal V_1V_O ATPases are incapable of ATP synthesis and are designed by nature to energize intracellular membranes by ATP hydrolysis. The evolutionary switch from synthase to hydrolase is thought to be caused by a doubling of the rotor subunit *c*, followed by a loss of the ion binding site.

Objective: To provide biochemical evidence that ATP synthesis is possible under physiological conditions despite the unusual *c*-subunit and to investigate whether ATP synthases with this unusual *c*-subunit have a different energetic threshold and require more energy than bacterial F_1F_O ATP synthases.

Materials & methods: The A_1A_O ATP synthase from *Eubacterium callanderi* with a V-type *c*-subunit as well as the hybrid F_1F_O ATP synthase from *Acetobacterium woodii* and the F_1F_O ATP synthases from *Escherichia coli* and *Propionigenium modestum* were purified, reconstituted into proteoliposomes and the energetic thresholds for ATP synthesis were determined.

Results: The A_1A_O ATP synthase from *E. callanderi* indeed synthesized ATP at physiological membrane potentials, despite its unusual *c*-subunit. The minimal driving force for ATP synthesis was about 90 mV and found to be lower than in other F1FO ATP synthases. Only the energetic threshold for ATP synthesis in the enzyme from *A. woodii* was similar, whereas the threshold values for ATP synthesis in the ATP synthases from *P. modestum* (120 mV) and *E. coli* (150 mV) were significantly higher.

Conclusions: We have unequivocally demonstrated the capability of an ATP synthase with a V-type *c*-subunit to synthesize ATP with physiological relevant driving forces of 90 – 150 mV. This is the long-awaited answer to an eminent question in microbial energetics and physiology, especially for life at the thermodynamic limit of ATP synthesis. Along those notions is the finding that even smaller potentials are sufficient to drive ATP synthesis. The structural or energetic basis for this phenomenon remains elusive.

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eP412
Fermentation of itaconate in *Pelosinus* sp.
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Itaconic acid (methylene succinic acid), a C_5-dicarboxylic acid, serves as an antimicrobial agent and controls inflammatory response in mammalian macrophages (Michelucci *et al*., 2013). It is also produced by a broad variety of soil fungi which results in the ubiquitous occurrence of itaconate in significant amounts (Cordes *et al*., 2015). Many soil bacteria and various pathogens are capable of using itaconate as a carbon source under aerobic conditions, and the corresponding itaconate degradation pathway is well understood (Sasikaran *et al*., 2014), whereas anaerobic metabolism of itaconate has never been studied. Here we isolated an anaerobic soil bacterium belonging to the genus *Pelosinus* that was capable to ferment itaconate and studied the corresponding metabolic pathway. While aerobic bacteria degrade itaconate via its activation to itaconyl-CoA, hydration to (*S*)-citramalyl-CoA and cleavage to acetyl-CoA and pyruvate, itaconate fermentation started with its isomerization to citraconate, which was further hydrated to (*R*)-citramalate, activated to (*R*)-citramalyl-CoA and finally cleaved to acetyl-CoA and pyruvate. We identified the *Pelosinus* sp. itaconate fermentation gene cluster, heterologously produced its gens and characterized the corresponding enzymes.

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eP413
The function of the energy converting hydrogenase Ech2 in *Thermoanaerobacter kivui*.
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Thermoanaerobacter kivui is one of few promising thermophilic acetogenic microorganism for syngas (H_2, CO_2 and CO) conversion into bulk chemicals. The reduction of CO_2 and CO occurs *via* the Wood-Ljungdahl pathway as terminal electron accepting reactions. Two membrane energy conserving hydrogenases (Ech1 and Ech2) are part of the energy and redox metabolism of *T. kivui*. Their assumed function is the reduction of protons to molecular hydrogen (H_2) with electrons derived from the oxidation of reduced ferredoxin (Fd_{red}) during growth on H_2/CO_2. Since Ech1 and Ech2 are the only putative coupling sites (1), the redox potential difference is utilized to build up an electrochemical membrane potential - which can be converted into ATP by ATP synthase (2). In heterotrophic metabolism, the complexes may exploit the membrane potential to provide Fd_{red} to the cells.

Here, we addressed the question why *T. kivui* has two Ech complexes, and whether they have distinct physiological functions. Therefore, we generated a deletion mutant of the Ech2 gene cluster (Δ*ech2*) based on a recently developed genetic toolbox for *T. kivui* (3).

To generate the deletion mutant Δ*ech2*, the non-replicating plasmid pTKvMB0022 was redesigned with fragments of upstream and downstream region of the *ech2* gene cluster. The *ech2* complex (TKV_RS09580- TKV_RS09615) was deleted, using the gene encoding orotate

phosphoribosyltranserase (*pyre*) as a selective marker via two single homologous recombination events.

Growth experiments with *T. kivui* wild type and the ΔEch2 mutant show a similar growth rate on glucose (0.42 h^{-1} and 0.44 h^{-1}, respectively), mannitol (0.36 h^{-1} and 0.36 h^{-1}, respectively) and H_2/CO_2 (0.53 h^{-1} and 0.50 h^{-1}, respectively). Resting cell experiments show that the ΔEch2 does not produce acetate, formate and H_2 when incubated with CO/N_2 (4), and we were not able to adapt Δ*ech2* to CO.

In conclusion, the Ech2 complex of *T. kivui* is essential for CO metabolism but not for the growth on glucose, mannitol and H_2/CO_2. qPCR analyses will provide further inside the gene regulation of its H_2 metabolism. We are currently investigating the role of Ech1 in *T. kivui* metabolism.

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eP415

A HMM-based predictor for sulfur metabolism-related genes discloses a novel pathway for protein lipoylation in Bacteria and Archaea

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Lipoate-binding proteins (LbpA) resembling classical glycine cleavage system H proteins are indispensable components of the sulfur-oxidizing heterodisulfide reductase-like enzyme system (sHdr) that occurs in a large number of chemolithotrophic sulfur-oxidizing prokaryotes [1,2]. In the model organisms *Hyphomicrobium denitrificans* and *Thioalkalivibrio* sp. K90 mix, the *shdr-lbpA* genes are linked with genes for enzymes possibly involved in maturation of the lipoate-binding proteins. These include lipoate-protein ligase(s) and two different radical SAM domain proteins although these organisms contain at least one of the established lipoate maturation pathways, i.e. lipoate scavenging or *de novo* synthesis involving the radical SAM domain protein LipA [1]. Indeed, we showed that LbpAs are not modified by the canonical lipoyl attachment machineries but that the LplA-like lipoate-protein ligases encoded in immediate vicinity of *shdr-lbpA* gene clusters act specifically on these protein substrates [1].

Here, we posed the question whether the novel maturation pathway for lipoate-binding proteins is confined to sulfur-oxidizing Bacteria and Archaea pursuing the sHdr pathway or whether it is of more general importance. However, publicly available annotation pipelines are not able to reliably distinguish between genes encoding the novel versus the canonical lipoylation pathways. Thus, we developed a graphical user interface supported tool based on Hidden Markov Models (HMMs) that specifically finds sulfur metabolism-related proteins and corresponding genes in assemblies. These are then assigned to operon structures named according to putative operon function. The precision and recall of the HMMs was validated by cross-validation and separately on a test set. Performance was also compared to methods serving similar purposes. When applied to the NCBI refseq representative set of genomes, the tool revealed an unexpectedly broad distribution of the novel lipoate biosynthesis pathway. Among the Bacteria it is not only present in sulfur oxidizers but also in a number of sulfate-reducing Firmicutes and Deltaproteobacteria not encoding LbpA as a lipoylation substrate. In Archaea, the novel pathway appears to be even more wide-spread, coinciding with recently published biochemical analyses [3].

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[2] Koch and Dahl 2018 ISME J 10, 2479

[3] Jin et al 2020 Appl Environ Microbiol 86, e01359

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eP416

ThermoSynCon: Isolating thermophilic acetogens and developing genetic tools for Syngas conversion to biobased chemicals

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Syngas, which contains various ratios of CO_2, CO and H_2, is generated by industrial processes such as gasification of biomass, or as a waste-gas from steel mills. Acetogenic bacteria are capable of converting Syngas into value-added carbon-based chemicals. Given the high temperatures of these industrial gas-streams, "ThermoSynCon" aims to identify thermophilic acetogens capable of Syngas fermentation at temperatures between 50 and 80°C. This work involves isolation of novel strains from the environment, and development of improved genetics tools for *Thermoanaerobacter kivui* (T_{opt} = 65°C), the current best thermophilic Syngas fermenter.

Samples for isolation of novel thermophilic acetogens were taken from high temperature environments including compost and biogas plants. These samples were enriched for acetogens by culturing under H_2/CO_2 atmosphere with BES to inhibit methanogens. The microbial diversity of the enrichments was analyzed by metagenomics, and in some cases supplemental carbon sources, including methanol, betaine and lactate, were added to select for diverse metabolic capabilities. If acetate was detected, the enrichment was further passaged and single colonies isolated by plating, serial dilution, or the roll-tube technique. This yielded several pure isolates capable of acetogenic growth on CO2/H2 above 60°C. The 16S rRNA sequences indicate isolates are related at the genus or species level to known thermophilic acetogens, with some exhibiting previously unreported growth phenotypes. Further characterization of the new isolates will determine which are most appropriate for Syngas fermentation.

To identify new promoters, sugar inducible and repressible genes were investigated in *T. kivui* by culturing cells on various sugar mixtures and analyzing transcriptional responses by qPCR and RNAseq. Mannitol appears to be a less favored growth substrate, since the operon for mannitol uptake was repressed by the presence of other sugars. In contrast, the fructose uptake operon was dramatically up-regulated by the presence of fructose, regardless of which other sugars were present. The promoter regions from these genes should allow for carefully controlled expression of heterologous genes in *T. kivui*.

The novel isolates and genetic tools generated in this project are important steps towards the application of thermophilic

acetogens as Syngas fermenting industrial platform organisms.

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eP417

Transformation of bile acids by *Clostridioides difficile*

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Introduction

Bile acids are important signaling molecules for the maintenance of a healthy intestinal tract. Microbial transformations of primary bile acids in the gut lead to a large diversity of secondary bile acids. From these, especially the 7-α-dehydroxylation products deoxycholic acid (DCA) and lithocholic acid are known to inhibit vegetative growth of *Clostridioides difficile*, one of the most important intestinal pathogens. While the effect of bile acids on *C. difficile* endospore formation and vegetative growth is well established, only little is known about transformation of bile acids by *C. difficile* itself.

Objectives

The presented study aimed to analyze the potential of bile acid biotransformation by different strains of *C. difficile* and to compare the pattern of formed products to those of several probiotic bacteria including *C. scindens*, which is known for its protective role against *C. difficile* infections.

Material and Methods

Strains were cultivated for 24 h in medium containing either chenodeoxycholic acid (CDCA), cholic acid (CA), glycocholic acid (GCA), taurocholic acid (TCA) or 7-oxodeoxycholic acid (7-oxo-DCA). Bile acids were extracted from supernatants and analyzed by HPLC-HRMS.

Results

Strains of *C. difficile* converted CDCA and CA to their corresponding oxo-derivatives (7-oxo-LCA and 7-oxo-DCA). TCA and 7-oxo-DCA were further metabolized to CA. Transformation of GCA was not observed. In contrast, strains of *C. scindens* could not convert taurine-conjugated bile acids, but metabolize GCA to CA and further to DCA. The latter is also the main product of the conversion of CA and 7-oxo-DCA. Utilization of CDCA is similar to those observed for *C. difficile.*

Conclusion

Conversion of bile acids by *C. difficile* primarily lead to the formation of 7-oxo-DCA, which could be further metabolized to DCA by *C. scindens*. Thus, accumulation of DCA may mediate the protective effect of *C. scindens* and other 7-dehydroxylating bacteria against *C. difficile* infections.

For deconjugation of TCA or GCA activity of a bile salt hydrolase (BSH) is required, but no BSH enzymes are described in *C. difficile* so far. However, a glutaminase A homolog has been shown to possess BSH activity for taurine-conjugated bile acids in *Phocaeicola vulgatus* [1] and for this enzyme also a homolog in *C. difficile* is found.

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eP418

Addressing the resilience of *Bacillus subtilis* biofilms to hydrogen peroxide

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Introduction

Biofilms are highly resistant microbial communities, that show protection and resistance mechanisms against extreme environmental conditions and aggressive disinfectants. These surface-associated consortia are embedded in extracellular polymeric substances (EPS), a self-built matrix made up of different biopolymers. Due to the difficulty of removal and increased resistances biofilms provoke serious problems in almost all man made settings. Hence, there is an urgent need for reliable sterilization approaches. Plasma, ionized gas can provide effective inactivation of microorganisms. Its antimicrobial properties are achieved by a mixture of reactive oxygen and nitrogen species (RONS), excited atoms/molecules, charged particles and UV-photons. Although plasmas are already used as therapeutic care, knowledge about the interaction between plasma components and microorganisms, especially biofilms, is sparse.

Objectives

This project aims to improve the inactivation efficiency of microbial biofilms by plasma. Therefore, it's crucial to uncover underlying protection mechanisms and understand interactions between individual plasma elements and biofilm components.

Materials & methods

Since biofilms are highly heterogeneous communities changing over time, various maturation states were treated with hydrogen peroxide (H_2O_2). Here, H_2O_2 represents a source for ROS and one of the major biocidal parts formed during plasma gas discharge. One of the endpoints for inactivation assessment was determined by colony forming units (CFU) of spores as well as vegetative cells. In addition, the impact of matrix components in ROS resistance was investigated by using an EPS deficient strain (lacking *epsA-O*) which was compared to the wildtype (*B. subtilis* NCIB3610).

Results

Treated wildtype biofilms, including spores and vegetative cells show enhanced survival with increasing age. On the other hand, in EPS deficient biofilms, only spores survive better at high maturation and long H_2O_2 exposure. Furthermore, spores that are isolated from young EPS deficient biofilms show lower survival with prolonged H_2O_2 incubation compared to matured biofilms.

Conclusion

This study shows that high maturation, EPS and sporulation of biofilms are essential for resistance to ROS. In order to reveal complex interactions between a multicomponent system as plasma and biological structures, further single stress stimuli will be tested and supported by transcriptomics data.

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eP419

The transcriptomic response of *Wolinella succinogenes* cells exposed to nitrate, nitric oxide and nitrous oxide

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1. Introduction

The biogeochemical nitrogen cycle includes a variety of microbial metabolic pathways, among them the reduction of nitrate via nitrite to ammonium (nitrate ammonification), nitric oxide (NO) reduction to nitrous oxide (N_2O, laughing gas) and N_2O reduction to dinitrogen. The nitrate-ammonifying Epsilonproteobacterium *Wolinella succinogenes* respires nitrate and N_2O, among other electron acceptors such as fumarate or sulfite, and produces well-characterized respiratory enzyme systems reducing nitrate (Nap), nitrite (Nrf) or nitrous oxide (Nos). When exposed to a suitable electron acceptor, the cells are envisaged to sense the respective substrate and to regulate the transcription of relevant genes accordingly.

2. Objectives

It was aimed to investigate the transcriptomic response of *W. succinogenes* to nitrate, NO or N_2O and to analyze the potential regulons.

3. Materials & methods

The nitrogen compounds mentioned above were added to fumarate-respiring *W. succinogenes* cultures. Cells were harvested immediately before and at several time points after the addition of nitrate (10 mM), dissolved NO (1 µM) or an atmosphere of pure N_2O. RNA isolation and transcriptome analysis (RNA-seq) was carried out in collaboration with the NGS Competence Center Tuebingen (NCCT). Transcriptome alignment, quantification and statistical evaluation was performed using the programming language R.

4. Results

The evaluation of transcriptome dynamics allowed the identification of many genes whose expression was significantly up- or downregulated after a pulse of nitrate or NO. The upregulated genes comprised the clusters encoding the respiratory Nap, Nrf and Nos systems as well as genes encoding proteins putatively involved in NO detoxification (nitrosative stress defense) or sensing of nitrate and NO, respectively. Furthermore, the expression of various other genes was affected whose precise function is currently not known. In contrast, the exposure to an N_2O atmosphere did not result in a comparable regulation of gene expression.

5. Conclusion

The results indicate the presence of individual regulons that respond to the presence of nitrate and NO, respectively. In contrast, the data do not support the presence of a nitrous oxide stimulon.

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eP421

Thiosulfate oxidation and sulfur transport in the Alphaproteobacterium *Hyphomicrobium denitrificans*

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In many sulfur-oxidizing prokaryotes, a heterodisulfide reductase-like metallo-enzyme complex (sHdr) and a dedicated lipoate-binding protein (LbpA) act together in the oxidation of sulfane sulfur to sulfite in the cytoplasm [1,2]. The sHdr-LbpA system is not only found in lithoautotrophs but also present in bacteria like *Hyphomicrobium denitrificans*, that use inorganic sulfur compounds such as thiosulfate as supplemental electron donor while growing on organic carbon sources. Here, we gathered information on how exactly thiosulfate oxidation is initiated in the periplasm of *H. denitrificans* and how sulfur is transferred into the cytoplasm for further oxidation by sHdr-LbpA.

Two thiosulfate-oxidizing pathways are present: Thiosulfate dehydrogenase catalyzes oxidative condensation to tetrathionate which is a dead-end product [1]. The genes for the second pathway are situated in the same genomic island as the *shdr-lbpA* operon. SoxXA catalyzes the oxidative fusion of thiosulfate to a conserved cysteine of the sulfur carrier protein SoxYZ. Then, the sulfone group is hydrolytically released by SoxB. Strains carrying markerless deletions of *soxYZ* or *soxXA* indeed proved thiosulfate oxidation negative. Thus, the other four SoxYZ homologs encoded in *H. denitrificans* cannot functionally replace the protein encoded in immediate vicinity of *shdr-lbpA*. Just as in other organisms lacking the periplasmic sulfane dehydrogenase SoxCD, sulfane sulfur still bound to SoxYZ cannot be oxidized in the periplasm but has to be transferred into the cytoplasm. Two potential transporters are encoded in the sulfur oxidation island. Both exhibit significant similarity to the recently structurally characterized YeeE/YedE-family thiosulfate transporter from *Spirochaeta thermophila* [3], and indeed *H. denitrificans* strains lacking these genes have a thiosulfate oxidation negative phenotype. A chromosomally complemented *H. denitrificans yedE* deletion strain regained thiosulfate oxidation capability unless either one of three conserved cysteines was exchanged to serine. Information about the actual sulfur species transported by the *Hyphomicrobium* YeeE/YedE proteins is collected by complementation of *E. coli yedE* strains [3], that cannot import thiosulfate as a source for sulfur assimilation.

[1] Cao et al. 2018 eLife 7, e37439 [2] Koch and Dahl 2018 ISME J 10, 2479 [3] Tanaka et al 2020 Sci Adv 6, eaba7637

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eP423

A metabolic puzzle: chemolithoheterotrophy in *Hyphomicrobium denitrificans*

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The Alphaproteobacterium *Hyphomicrobium denitrificans* is not restricted to chemoorganoheterotrophy during aerobic or anaerobic growth on methanol or other C1 and C2 compounds but can switch into a mixed metabolic mode in which it also oxidizes thiosulfate. In contrast to expectations that the availability of an additional inorganic electron donor

should result in growth advantages due to supplementation of respiration and thus increased proton motive force and ATP production, a pronounced negative effect on growth rate is observed when the organism is cultured in the simultaneous presence of methanol and thiosulfate [1,2]. This effect is particularly impressive when cultures are pre-induced with thiosulfate.

We set out to explain this puzzling observation and first collected information about the regulation of the sulfur-oxidizing heterodisulfide reductase-like system (sHdr) operating in *H. denitrificans* [1]. The *shdr* genes are preceded by a gene for an ArsR-type transcriptional repressor, sHdrR. Phylogenetic analyses place sHdrR on a distinct branch clearly separated from all its characterized homologs. Recombinant sHdrR specifically binds to a 362-bp DNA fragment between its own and the neighboring upstream gene. A markerless deletion strain lacking the regulator produces the sHdr system constitutively as proven by immunoblot analysis. When exposed to thiosulfate, the Δ*shdrR* strain exhibits a strongly reduced growth rate even without induction of pre-cultures. As soon as thiosulfate is consumed, the growth rate increases substantially. Fully in line with these observations, the negative effect of thiosulfate on growth rate is released in strains lacking a functional sHdr system and thus being unable to consume thiosulfate [1,2]. These results further confirm that thiosulfate negatively influences biomass production from methanol in laboratory batch culture. We propose that this is due to an over-reduction of the cellular nicotinamide dinucleotide and cytochrome *c* pools caused by thiosulfate oxidation. This over-reduction prevents effective assimilation of methanol into biomass, as in depth genomic analysis of metabolic routes in *H. denitrificans* revealed that methanol must first be oxidized all the way to formate before it can be hooked up to tetrahydofolate and re-reduced up to the level of formaldehyde and delivered into the serine pathway for assimilation.

[1] Cao et al. 2018 eLife 7, e37439 [2] Koch and Dahl 2018 ISME J 10, 2479

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eP424

Biosynthesis of various methylmenaquinone derivatives

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1. Introduction

Menaquinone (MK) serves as an essential membranous redox mediator in various electron transport chains of aerobic and anaerobic respiration. In addition, the composition of the quinone/quinol pool has been widely used as a biomarker in microbial taxonomy. The HemN-like class C radical SAM methyltransferases (RSMTs) MqnK, MenK and MenK2 have recently been shown to facilitate specific menaquinone methylation reactions at position C-8 (MqnK/MenK) or C-7 (MenK2) to synthesize 8-methylmenaquinone, 7-methylmenaquinone and 7,8-dimethylmenaquinone. Primary structure motifs of the MqnK/MenK/MenK2 family allowed a functional categorization.

2. Objectives

It was aimed to characterize selected purified MK methyltransferases in order to verify functional predictions from *in silico* analyses.

3. Materials & methods

Selected putative MenK and MenK2 menaquinone methyltransferases were produced in *Escherichia coli* BL21 (DE3) and investigated in an *in vitro* MK methylation assay. Menaquinone species were separated and identified by UV/Vis spectroscopy and mass spectrometry.

4. Results

Using cluster and phylogenetic analyses, 828 protein sequences from the MqnK/MenK/MenK2 family were characterized. The functional differentiation between MenK and MenK2 enzymes was proposed on the basis of the primary structure motifs QxTxYPLM and QxTxSPLY, respectively. The specificity of several isolated representative MenK and MenK2 enzymes in menaquinone methylation was examined and confirmed the bioinformatic predictions.

5. Conclusion

The results indicate that many microbial cells have the potential to synthesize different methylated MK derivatives and that this capacity can be predicted from a given genome sequence. This knowledge may contribute to the future design of microbial quinone/quinol pools in a synthetic biology approach.

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eP425

Characterization of the three CobW proteins sheds new light on the metal pools of *Cupriavidus metallidurans* CH34

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Zinc is an essential trace element due to its occurrence in a high number of metalloenzymes and metalloproteins with functions in all cellular pathways. In the beta-proteobacterium *Cupriavidus metallidurans* CH34 a zinc proteome has been described accommodating 120.000 zinc-binding proteins. This number is higher than the amount of around 70.000 zinc ions per cell in TMM and allows the cell to buffer incoming zinc. Also, the correct allocation of this trace element is of uttermost importance, both when in scarcity or excess. As a consequence, a prerequisite condition is to maintain zinc homeostasis. Central to all these processes is the Zur regulon comprising three members: Zur as transcriptional regulator (Zinc Uptake Regulator), ZupT, a zinc importer of the ZIP family, and three recently characterized members belonging to the COG0523 family of the G3E superfamily of P-loop signal-recognition-associated SIMIBI class GTPases, named $CobW_1$, $CobW_2$ and $CobW_3$.

Our aim is to understand the contribution of the three CobW proteins in buffering and allocation of zinc ions between the loosely- and tightly-bound zinc pool of the cell. Furthermore, the roles they play in the low-specificity pool of other transition and heavy metals is of interest in the high metal resistance model organism *Cupriavidus metallidurans* CH34.

In this endeavour, a multi-branched approach is undertaken by combining physiological characterization with molecular techniques, a state-of-the art proteomic study and

development of a HPLC-ICP-MS analytical system for the separation of metalloprotein pools.

Although all three genes of CobW members are part of the Zur regulon, only *cobW*1 is strictly regulated and repressed by sufficient zinc conditions and strong induced under zinc scarcity. On the other hand, *cobW*2 and *cobW*3 show high constitutively expressed levels. The proteomic approach allows a quantification of these gene products under several conditions and separation of metalloprotein pools gives us an insight into the metal protein ratio and allows a qualitatively assignment. Moreover, the loss of CobW2 and CobW3 influences these metal pools.

The CobW proteins are part of the zinc repository by acting as metal buffering compartments and are involved in allocating metals efficiently to metal-dependent proteins.

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eP426
Pyruvate uptake in Gamma-proteobacteria and its biological relevance

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Pyruvate is a key metabolite in every living cell. It not only acts as the hub between aerobic and anaerobic metabolism, but it also serves as a scavenger of reactive oxygen species, is important for the resuscitation of viable but nonculturable cells and has been found as crucial for the virulence of several bacterial pathogens (1-6).

Gamma-proteobacteria excrete and reuptake pyruvate to varying degrees during growth (7). We identified the responsible pyruvate transporters in three model organisms, *Escherichia coli*, *Salmonella enterica* and *Vibrio campbellii*, and investigated the impact of pyruvate uptake in these bacteria (8, 9).

Our studies reveal that the three model organisms not only possess different numbers and types of pyruvate uptake systems, but also show deviating phenotypes when pyruvate uptake is abolished by deletion of the responsible transporter genes. Interestingly, all mutants lost their chemotactic response to pyruvate, indicating that intracellular pyruvate is required to activate chemotaxis. Moreover, we found that in both *E. coli* and *V. campbellii* pyruvate uptake is important for resuscitation from the viable but nonculturable state. *S. enterica* deletion mutants showed only small disadvantages *in vivo* in various infection models, whereas the virulence of *V. campbellii* deletion mutants towards gnotobiotic brine shrimp larvae was strongly reduced.

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eP427
Characterization of *Neobacillus vireti* laughing gas respiration

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1. Introduction

Biologically catalyzed nitrous oxide (N_2O, laughing gas) reduction to dinitrogen gas is a desirable process in the light of ever-increasing atmospheric concentrations of this important greenhouse gas and ozone depleting substance. A diverse range of bacterial species produce the copper cluster-containing enzyme N_2O reductase (NosZ). Based on phylogenetic analyses, NosZ enzymes have been classified into clade I or clade II and the corresponding organisms are expected to differ significantly with regard to electron transport processes to NosZ as well as NosZ active site maintenance and repair. In many organisms N_2O reduction contributes to a mode of anaerobic respiration in its own right, called N_2O respiration. Here, the Gram-positive nitrate-ammonifying model organism *Neobacillus vireti* was investigated towards N_2O respiration. This bacterium contains a clade II NosZ enzyme and two individual *nos* gene clusters (*nosC-Z-B-D-Y-F* and *nosD-L-Y-F*).

2. Objectives

It was aimed to characterize the ability of *N. vireti* wild-type cells and *nos* mutants to grow by N_2O respiration and to determine specific N_2O reduction rates and apparent N_2O affinities.

3. Materials and methods

N. vireti wild-type cells as well as cells of the non-polar Δ*nosZ* and Δ*nosC* mutants were tested for growth at the expense of N_2O reduction in anoxic TSB medium containing 5 mM nitrate under an atmosphere of 10% N_2O/90% N_2. Specific N_2O reduction rates and apparent affinities for N_2O were determined by micro-respirometry.

4. Results

Cultures of wild-type *N. vireti* cells showed a growth advantage in the presence of N_2O, in contrast to the Δ*nosZ* and Δ*nosC* mutants. Wild-type cells exhibited a high N_2O turnover rate and an apparent affinity for N_2O in the low micromolar range. While cells of the Δ*nosZ* mutant failed to reduce N_2O, a residual activity was found in the Δ*nosC* mutant.

5. Conclusion

A refined model of *nos* cluster-dependent N_2O respiration in Gram-positive bacteria containing a clade II NosZ enzyme is presented. It appears that this type of N_2O respiration is less complex compared to that of many Proteobacteria using clade II NosZ.

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eP428
The community metabolic network of the OMM[12] model community: An in *vitro* exploration

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The Oligo-Mouse-Microbiota (OMM^{12}) community is a synthetic model community for the murine gut microbiome. It comprises twelve genome-sequenced strains from the five dominant eubacterial phyla of the mouse gastrointestinal tract that are culturable *in vitro* both individually and as a consortium. Colonization of mice with the OMM^{12} confers colonization resistance against *Salmonella enterica* serovar Typhimurium.

The OMM^{12} is openly available and has been used in multiple preclinical studies, but the functional basis of the bacterial network has only started to be elucidated. A first systematic analysis of pairwise interactions has been published by Weiss et al.; and Eberl et al. identified galactitol as the crucial nutrient on which colonization resistance is based.

Here, we aim to analyse the metabolic interplay not just in pairwise cultures but in the whole community. We use continuous *in vitro* culture of the OMM^{12} community paired with single strain and community proteome analysis to illuminate not only the metabolic potential of the single strains but their concrete metabolic niche in the OMM^{12} community as well as their reaction to community disturbances such as the elimination of an important community driver or the addition of bile salts in the media.

This first functional insight into the community metabolism of the OMM^{12} community provides a basis for the deeper exploration into community coexistence of bacterial strains that share a metabolic space.

Eberl, C., A. S. Weiss, L. M. Jochum, A. C. Durai Raj, D. Ring, S. Hussain, S. Herp, C. Meng, K. Kleigrewe, M. Gigl, M. Basic and B. Stecher "*E. coli* enhance colonization resistance against *Salmonella* Typhimurium by competing for galactitol, a context-dependent limiting carbon source." Cell Host & Microbe.

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eP429

Enrichment of a new *Nitrospira* species in a continuous bioreactor

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In nature and conventional nitrogen removing wastewater treatment plants, nitrite-oxidizing bacteria (NOB) carry out the second step of the nitrification process, oxidizing nitrite to nitrate. Consequently, understanding the physiological properties of these microorganisms is of paramount importance for wastewater treatment plant design and better understanding their role in the global nitrogen cycle. Both in natural and engineered ecosystems, these microorganisms live under low substrate concentrations, however cultivation attempts for these microorganisms under substrate limitation is scarce. Here, we used samples originating from the municipal wastewater treatment plant in Seehausen, Germany, to cultivate NOB. For over 780 days, NOB were enriched in a continuous bioreactor and fed with limiting amounts of nitrite and low oxygen concentrations (<5 µM). The enrichment culture converted nitrite to nitrate with the expected 1:1 stoichiometry. Metagenomic analyses revealed that a member of the *Nitrospira* genus was the only NOB in the bioreactor. The 16S rRNA gene sequence of the enriched microorganism exhibited high sequence similarity with *N. defluvii* and *N.* sp. ND1 (>99%). However, ANI and AAI values below 90% showed that the enriched *Nitrospira* was a new species. CARD-FISH analyses corroborated the high abundance of the novel *Nitrospira* in the culture and showed cell aggregation typical of *Nitrospira* species. The initial physiological characterization of the *Nitrospira* enrichment culture indicated that atmospheric concentration of oxygen and temperatures between 28 and 37 °C were optimal for the nitrite oxidation activity of the culture. Isolation of the novel *Nitrospira* species is being conducted and will be followed by further physiological and genomic characterization to determine its metabolic capacity.

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eP430

Weaving together a model for microtubular sheath synthesis in the metal oxidizing bacterium *Leptothrix cholodnii* SP-6

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Metal-oxidizing bacteria generate an extracellular polysaccharide (EPS) structure that binds to metals to avoid becoming entombed within an iron or manganese crust and to remain suspended in the water column. *Leptothrix cholodnii* SP-6 and other *Leptothrix* spp. are freshwater, filamentous, metal-oxidizing bacteria that build a microtubular sheath that binds metals. The sheath is a highly resilient structure that includes fibrils with sulfhydryl and amino groups that bind various metals, but the genetic basis for the biosynthesis, assembly, and secretion of the fibrils remains unknown.

The goal of our reseach was to build a model for sheath biosynthesis, assembly, polymerization, and export. We performed random Tn5 mutagenesis on *L. cholodnii* SP-6, characterized the sheathless mutant phenotypes, and determined the sheath-associated gene clusters. Of 1,200 sheathless colonies, 45% of the colony morphologies were most similar to a sheathless *Leptothrix* spp. variant, *L. mobilis*. The Tn5 insertion site was identified for 68 mutants, with 40 grouping into 3 statistically significant clusters. Two clusters included multiple sugar and polysaccharide associated genes, one of which had 18 Tn5 insertions and at least 33 flanking putative polysaccharide-associated genes. Mutants with Tn5 insertions in a biosynthesis or a glycosyl-transferase gene had a marked decrease in cell filament length, with chains of 3 cells or more, making up only 16 % of the population vs 68 % for wild type. Fluorescent-conjugated maleimide bound to wildtype sheaths, but not to mutants from either of the 2 sheathless clusters or to *L. mobilis.* When a Tn5-disrupted glycosyl-transferase in the polysaccharide-rich gene cluster was complemented, the sheathed phenotype was restored.

The genes present in the polysaccharide-rich gene clusters are similar to those in prolific EPS producers. We propose that *Leptothrix cholodnii* SP-6 uses the Wzy/Wzx machinery to generate the fibril that forms the microtubular sheath. Future questions include whether this model can be applied to the EPS structures made by other metal oxidizing bacteria.

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eP431
Deep2bac: Automating microbial trait prediction with deep learning
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Background

In the era of high-throughput sequencing technologies, scalable and distributed approaches are required to derive information from the flood of multidimensional molecular data. One subfield of machine learning called deep learning has been shown to handle large volumes of data efficiently and successfully, even outperforming older algorithms.

We introduce (Deep2bac,) a deep learning framework which can semi-automatically derive features from annotated bacterial genomes, tune hyperparameters of an artificial neural network and train and save the best model. Supported feature types implemented so far are protein clusters, protein domains or k-mers.

Methods

Deep2bac performance was assessed based on complete bacterial genomes obtained form NCBI RefSeq. Metadata about phenotypes was acquired from the Genomes Online Database (GOLD). Gene coding sequences were mapped onto NCBI protein clusters and Pfam protein domains and used as feature space for the neural network. Feature generation is provided based on a versatile and efficient workflow implemented for the scalable NextFlow workflow management system. Tuning and training is implemented in TensorFlow with the Keras API.

Results

Deep2bac was validated predicting four different phenotypes: gram-property, oxygen requirement, motility and sporulation of bacteria. Finished models can be acquired with an identifier. It successfully created the features, tuned the hyperparameters and trained models with chosen input. Deep2bac-light is a standalone version that can be used to compute predictions based on pre-trained models, which are centrally offered for download

Conclusion

Deep2bac is a framework which has a broad applicability to recent biological and medical challenges, with the possibility to easily train novel models, or utilize existing models. Employing Deep2bac, automating the prediction of bacterial phenotypes and their properties is significantly eased.

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eP432
Acclimatization of *Methylocystis* sp. strain SC2 to high NH_4^+ load by multiple proteomic and metabolic response mechanisms
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Proteobacterial methane-oxidizing bacteria (MOB) are able to utilize CH_4 as their sole source of carbon and energy. Their key enzyme is particulate methane monooxygenase (pMMO) that oxidizes methane to methanol. Ammonium (NH_4^+) acts not only as an important N source for methanotroph growth but can also have an inhibitory effect on CH_4 oxidation and growth, in particular at a high NH_4^+ load. The competition between NH_3 and CH_4 for the active site of the pMMO leads to the formation of toxic hydroxylamine. In particular, the intracellular acclimatization of alphaproteobacterial MOB to increasing NH_4^+ load is not yet well studied. Here, we applied a combination of physiological growth experiments, global proteomics, profiling of intracellular amino acids, and measurement of NOx compounds to elucidate the acclimatization response of the alphaproteobacterial *Methylocystis* strain SC2 to increasing NH_4^+ concentrations.

Under CH_4 replete conditions, NH_4^+ concentrations higher than 30 mM triggered an extended lag phase and strongly diminished the growth rate of strain SC2. However, regardless of the ammonium concentration (1 to 75 mM NH_4^+), SC2 growth always yielded the nearly same biomass after prolonged incubation. The addition of 100 mM NH_4^+ had a complete inhibition effect on SC2 growth. Increasing NH_4^+ load induced multiple acclimatization mechanisms. The ionic stress effect of high NH_4^+ loads triggered the activity of the "salt-in" strategy of potassium (K^+) accumulation, production of various stress-responsive genes, and synthesis of proline as an osmoprotectant. A total of 438 proteins were differentially regulated (DRPs), with a strong relationship between the number of DRPs and the increase in NH_4^+ load. The change in apparent *Km* value for methane oxidation to 2.72 µM (30 mM NH_4^+) and 3.40 µM (50 mM NH_4^+) relative to 0.34 µM under standard growth conditions (1 mM NH_4^+) mirrors the increased inhibition effect of excess NH_4^+ on CH_4 oxidation. The latter triggered a significant upregulation of the hydroxylamine detoxification pathways, thereby resulting in a greatly increased production and cellular release of nitrite (NO_2^-) and nitrous oxide (N_2O).

In conclusion, the acclimation limit of strain SC2 to 75 mM NH_4^+ is presumably due to the cellular need to combat both osmotic stress and the increasing production of toxic hydroxylamine.

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eP433
Investigation of various phosphoglucomutases from different conserved domain families
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Phosphoglucomutases play a crucial role in glycogen metabolism and mobilization (Whitehouse et al., 1998). These enzymes belong to the α-D-phosphohexomutase (αPHM) superfamily, which is widespread in all domains of life. Based on phylogeny the NCBI conserved domain database (CDD) provides a differentiated classification, which separates αPHM enzymes into 11 conserved domain subfamilies (Lu et al., 2020).

The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis*) possesses two α-Pgm-like enzymes encoded by *sll0726* and *slr1334*, belonging to the cd5801 and cd5800 CDD subfamilies respectively (Lu et al., 2020).

The PGM sll0726 has been characterized in detail. It catalyzes the interconversion of α-D-glucose-1P (Glc-1P) and α-D-glucose-6P (Glc-6P) and plays a key role in glycogen utilization during nitrogen starvation (Doello et al., 2021). While Sll0726 accounts for 97 % of this PGM activity, very little is known about Slr1334, which despite its low PGM activity appears to be essential for *Synechocystis* (Liu et al., 2013). Therefore, we aimed to further characterize this enzyme to gain novel insights into the function of Slr1334. For this purpose, enzymatic assays measuring the PGM activity and the influence of different effectors were performed with both *Synechocystis* PGMs. The results revealed, that glucose-1,6-bisphosphate (Glc-1,6-BP) acts as an activator for both Sll0726 and Slr1334. This corresponds to previous studies which showed that in mammals Glc-1,6-BP is a key regulator of enzymes in carbohydrate metabolism including the PGM (Carreras et al., 1986). Subsequently, it was observed that while fructose-1,6-bisphosphate (Frc-1,6-BP) inhibits Sll0726, it activates Slr1334. Further enzymatic assays revealed that Slr1334 can form Glc-1,6-BP from Glc-1P and Frc-1,6-BP. Therefore, by investigating a cryptic PGM we were able to identify an essential enzyme with crucial function: the first bacterial glucose-1,6-bisphosphate synthase. To elucidate the broader biological relevance of PGMs for the bacterial metabolism, various PGMs from phylogenetically diverse bacteria belonging to different CDD subfamilies are being analyzed and characterized.

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eP434

Biochemical and molecular characterization of a universally distributed protein with yet unknown function, CutA

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As living organisms, we share a lot with our bacterial relatives. Among those commonalities are orthologous proteins with often still undiscovered functions, such as the family of CutA proteins which are found in all domains of life. While the human CutA protein is specifically expressed in the brain where it is expected to play a substantial role in Alzheimer pathology [1], the bacterial CutA was assumed to confer tolerance against copper. However, a recent study could not support this assumption [2]. Therefore, there is still very little sound information about the cellular role of CutA.

CutA structurally resembles the prominent trimeric signal transduction proteins of the PII superfamily, which indicates a possibly similar function. Due to high conservation of CutA proteins among different lineages, these ancient proteins are expected to play a crucial role in bacterial physiology. Our aim is to investigate biochemical and molecular properties of CutA within different bacterial species to detect a possible common function.

To achieve this goal, we created knockout mutants of the *cutA* encoding gene in the phylogenetically distinct proteobacterial or cyanobacterial strains *E. coli* BW25113 and *Synechococcus elongatus* PCC 7942. We aim to characterize common phenotypes of these strains under different growth conditions. Using recombinant proteins, we studied the biophysical properties of the CutA proteins and found that they show remarkable heat stability, implying that CutA may be a key factor in the ability of bacteria to adjust to their environment under severe stress conditions.

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eP436

Distinct bacterial communities associated with *Aurelia aurita* shift due to species-specific viral communities

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The importance of the microbiota associated with the metaorganism (i.e. a collection of microorganisms including microbial eukaryotes, archaea, bacteria, and viruses) cnidarian moon jellyfish *Aurelia aurita* at each stage of the life cycle, has already been recognized. It has been stated, in previous research, that the microbiota is essential to ensure the health and survival of the metaorganism in each life stage.This study focuses on the polyp life stage of the *Aurelia aurita*, analysing the delicate balance between the bacterial community and the impact that bacteriophages can have on it.

An experiment was conducted introducing two novel bacteriophages isolated from marine environment samples. The fitness traits were taken into examination for the duration of the experiment and it was evident how the introduction of obligate parasites changed the homeostasis of the organism. The impact of introducing bacteriophages has changed massively the microbial balance, which was detected by amplicon 16S rRNA V1-V2 sequencing during the entire period of the investigation. Confirming the fundamental role of certain bacteria species on the host fitness traits, which were deeply impaired.

Therefore the delicate bacterial community has been modified to such an extent that the survival of the metaorganism is compromised. To confirm the impact of the bacteriophages, an experiment was conducted with sterile animals for the same period of time, with the significant result that the fitness traits of the polyps were not at all affected. As a result, some bacterial species play a crucial and essential role in maintaining an environment advantageous to the survival of the metaorganism.

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eP437

Chemical defense against phages: inhibition of phage infection by aminoglycosides

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Introduction

Bacteriophages are viruses predating on bacteria, and as such pose a threat to bacterial communities. In response, bacteria have evolved a wide range of defense mechanisms,

which rely mostly on proteins acting at the cellular level. Interestingly, early studies showed potential antiphage properties of aminoglycosides, a major class of antibiotics produced by *Streptomyces*[1]. However, the biological significance of these observations was not explored, and the underlying mechanism of action remains unclear.

Objectives

We aim at investigating the antiphage properties of aminoglycosides, with a focus on the mechanism of action and the dual antibacterial and antiphage function of these antibiotics.

Methods

We first conducted an extensive screening, both on plates in liquid cultures, to assess the extent of phage inhibition by aminoglycosides. To gain insights into the step of the lifecycle inhibited by aminoglycosides, we performed qPCR, RNA-seq and fluorescence microscopy to follow phage replication and transcription. Finally, relationships between antibacterial and antiviral properties of aminoglycosides were studied by using aminoglycoside enzymatically modified *in vitro*.

Results

We show that aminoglycosides are potent inhibitors of phage infection in diverse bacterial species[2]. We demonstrate that aminoglycosides do not prevent the injection of phage DNA into bacterial cells, but instead block an early step of the viral life cycle, before genome replication. Importantly, phage inhibition was also achieved using supernatants from natural aminoglycoside producers, hinting at a broad physiological significance of the antiviral properties of aminoglycosides. Strikingly, we show that acetylation of the aminoglycoside antibiotic apramycin abolishes its antibacterial effect, but maintains its antiviral properties.

Conclusion

This study expands the known functions of aminoglycosides in bacterial communities. It further suggests that aminoglycosides are not only used by their producers as toxic molecules against their bacterial competitors, but could also provide community-wide protection against phage predation.

References

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eP438

Streptomyces venezuelae development during phage infection

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Bacteria of the genus *Streptomyces* undergo a complex life cycle starting from single spores developing into a branched mycelium that is followed by the formation of aerial hyphae under unfavorable conditions. From aerial hyphae, spore chains are being generated and dispersed. This multicellular development is controlled by a complex regulatory network. Part of this network are *bld* and *whi* genes encoding regulators which control each other or directly activate and inhibit further genes necessary for key developmental steps. The so-called "master regulator" BldD controls more than 160 different targets in the *Streptomyces venezuelae* genome. It inhibits for example expression of *bldN*, which activates genes encoding rodlins and chaplins necessary for erecting aerial hyphae, and *whiB* which, together with WhiA, stops tip growth of aerial hyphae and activates septation, segregation and cell division in order to form spore chains.

In the environment, *Streptomyces* are under the constant threat of infection by bacteriophages. In this study, we focused on investigating the multicellular development in the context of phage infection using the model species *Streptomyces venezuelae*. Genome analysis showed that WhiB-like proteins represent the most abundant transcriptional regulator in phages infecting Actinobacteria (actinobacteriophages). These finding suggest that actinobacteriophages modulate the developmental programme during infection. Stereomicroscopic investigation of *S. venezuelae* infection by phage Alderaan revealed enhanced mycelial differentiation at the interface between the lysis zone and the bacterial lawn. During plaque formation, we first observed an enlargement of the lysis zone on wild type lawns, which was followed by a significant decrease upon progressing infection. Furthermore, the comparative analysis of *S. venezuelae* WT and different mutants with defects in cellular development revealed the importance of cellular differentiation for the development of a phage tolerance phenotype of *Streptomyces*.

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eP439

Expanding CRISPR-Cas technology for bacteriophage T4 mutagenesis

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Bacteria are the most abundant cellular organisms and play crucial roles for almost all life on earth. Nevertheless, they are under immense evolutionary pressure from viral invaders — bacteriophages. Like all viruses, bacteriophages are very species-specific with regard to their hosts. Bacteriophage research focuses on several model phages, which primarily infect *Escherichia coli*, such as bacteriophage T4. T4 is one of the most complexly built viruses that infect *E. coli*. Research on T4 phage strongly contributed to understanding fundamental biological processes. However, molecular mechanisms for the controlled takeover of *E. coli* by T4 phage are poorly understood. The genome of the T4 phage is composed of 169 kbp and encodes 273 proteins. To date, only 55% of T4 proteins are linked to a specific biological function. Thus, the functional role of the majority of T4 proteins remains unknown.

First attempts to elucidate the functional role of T4 phage proteins were based on the generation of knockout mutants by introducing deletion of large DNA regions into the T4 genome. However, the deleted regions often included several genes, which makes the identification of the functional role of the encoded protein difficult. Thus, an efficient site-specific mutagenesis approach of the phage is required to understand the functional role of T4 phage proteins during the process of infection. Novel technologies

such as CRISPR/Cas revealed exciting possibilities for targeted gene editing in viruses. However, the T4 phage genome is highly modified, thereby escaping the host restriction systems including Cas nucleases.

Here, we demonstrate strategies to overcome this challenge to enable the site-specific mutagenesis of the T4 phage genome in vivo. We apply CRISPR/Cas based approaches for the site-specific mutation of T4 phage proteins to elucidate their impact on the infection cycle, both at phenotype and molecular level. In this study, we use an optimized CRISPR/Cas approach to generate T4 phage mutants that carry inactive versions of T4 phage enzymes described to be essential for T4 phage infection. Thereby, we aim to complete the overall picture of the T4 phage infection process and to identify the function of uncharacterized T4 phage proteins. Moreover, we think that our improved CRISPR/Cas technology is a powerful tool that can be transferred to study other phages.

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eP440

Molecular cross-talk between Sa3int phages and their *Staphylococcus aureus* host

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As a major opportunistic pathogen of human and animals *Staphylococcus aureus* asymptomatically colonizes the nasal cavity, but is also a leading cause of life-threatening acute and chronic infections. *S. aureus* strains can carry up to four temperate phages, many of which possess accessory genes coding for staphylococcal virulence factors. More than 90% of the human nasal isolates of *S. aureus* were found to carry Sa3int phages, which integrate as prophages into the bacterial *hlb* gene thus disrupting the expression of the sphingomyelinase Hlb, an important virulence factor under certain infection conditions. The virulence factors-encoding genes carried by the Sa3-phages are all highly human-specific and probably essential for bacterial survival in the human host. Thus, both insertion of the prophages into and excision from the bacterial genome have the potential to confer a fitness advantage to *S. aureus*. However, how the *S. aureus* host modulates the life cycle of its temperate phages remains largely unknown (1). Our data suggest that the bacterial factors supposedly involved in the interaction of the bacterial host with its phages are strain specific, with certain *S. aureus* strains being more prone than others to support either a lysogenic or a lytic life cycle (2). We constructed and integrated Sa3int phages into different phage-cured *S. aureus* strains and found significant differences in phage transfer rates between different strains. Based on this finding, strains were grouped into low and high transfer strains. The transfer rate correlated with phage replication. In the low transfer strains, the phages are more directed towards lysogeny. To get a more precise picture of the regulatory circuits we constructed replication deficient mutants, performed differential RNAseq to determine the transcriptional units and analysed a set of mutant strains. By this means some bacterial and phage genes were identified which are likely to control the strain dependent phage life cycle.

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eP441

Understanding the dynamics of Plant-Bacteria-Bacteriophage interactions

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Plant pathogenic bacteria are a big threat for food production and cause an annual yield loss of 10 %. In recent years the problem got even worse since many of these plant pathogenic bacteria became resistant to classical treatments like copper or antibiotics. Phages as specialized viruses of bacteria with a narrow host range could provide an effective and sustainable solution. For this reason, we isolated novel phages for different plant pathogenic bacteria. Many of those plant pathogenic bacteria target one of the most vulnerable part of the plant in terms of nutrient and water uptake - the root. Not only does the root function as uptake organ, its carbon-rich exudations into the soil make it a desirable place for bacteria also.

Many of the bacteria in the rhizosphere can be pathogenic for the plants, and the role of viruses shaping the plant microbiome along the root is not well understood. In our project, we aim to enlighten those dark area in the plant-bacteria-phage interaction along the root on a molecular and physiological level.

To accomplish this, we are using a sterile fabricated Ecosystem the EcoFAB. The gnotobiotic environment in the EcoFAB is achieved by fusing a fluidic camber on a microscope slide, both enclosed in a sterile container. This enables the investigation of the plant-pathogen interaction, as well as plant-phenotypic changes over time. Further, we used, plaque assays, TEM, sequencing, and functional annotation of phage genes as well as growth curves to validate our potential phage candidates.

Here, we present the isolation and phenotypic characterization of phage *Alfirin* infecting *A. tumefaciens,* phage *Pfeifenkraut* infecting *X. translucens* and phage *Athelas* infecting *P. syringae*. All phages show a lytic lifestyle, which is supported by genome sequencing and phage infection curves, making them suitable candidates to test their potential *in planta*. Furthermore, we present first results on phenotypic changes in Arabidopsis root length during interaction with plant pathogenic bacteria.

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eP442

Reconstitution of phage T4 lysis and lysis inhibition in *Escherichia coli*

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Lytic phages tightly regulate the lysis of their host cells by controlling the access of phage-encoded muralytic endolysins to the bacterial cell wall. Endolysins can be released *via* holes formed by membrane proteins that are termed canonical holins. The regulation of lysis timing occurs by controlling the activity of the holins. Timing of lysis can be achieved by the regulated formation of holins and their accumulation in the cytoplasmic membrane until a critical density is reached for hole formation. Many holins are regulated by specific antiholins that sense superinfections and delay lysis. T4 is the prototype for studies on this lysis inhibition (LIN), in which two antiholins, RI and RIII, are involved in binding the periplasmic and cytoplasmic domains of the holin T, respectively. The cytoplasmic RIII alone

cannot establish a stable LIN and stabilizes the inhibitory effect of the periplasmic RI, whereas RI alone is sufficient for the establishment of LIN. Recently we could demonstrate that, in contrast to previous reports, RI has a cleavable Sec signal peptide. This simplified the current view of phage lysis regulation and implied a fundamentally different interpretation of the recently published structure of the soluble domains of the RI–T complex [1]. We now have functionally reconstituted the regulated lysis system with the required components in *Escherichia coli*, which permits analyses of specific roles of proteins, protein domains, or single residues in lysis or lysis inhibition. We already successfully used the system for the functional assessment of conserved residues of the system, and showed that R34 in holin T is important for holin function and interaction. We also demonstrated that signal peptide cleavage of RI is essential for its antiholin function. These assays support our recently published model of the T–RI complex, in which the RI-N-termini point away from the cytoplasmic membrane surface.

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eP443

Persistent viral infections of uncultivated subsurface archaea are associated with drastic morphological changes of the host

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Introduction: The deep biosphere is populated by a great diversity and abundance of Archaea and Bacteria. We recently challenged the long-standing paradigm that these microorganisms are mainly infected by lysogenic viruses, by demonstrating active lytic infections of *Candidatus* Altiarchaeum (Ca. Altiarchaeum) in deep aquifers. Moreover, previous studies have shown that *Ca.* Altiarchaeum uses CRISPR-Cas immune system to protect against viral infections.

Objectives: Here, we studied the persistence of virial infections of *Ca.* Altiarchaeum by lytic viruses and performed correlative microscopy to identify potential morphological changes of the host during infection using virus-targeted direct-geneFISH (virusFISH) and atomic force microscopy (AFM), challenging the current standing paradigm that lysogeny dominate in the subsurface.

Methods: VirusFISH was applied to groundwater biofilms that were dominated by *Ca.* Altiarchaeum to enumerate viral infection stages (adsorption of virus, advanced infection, and viral cell lysis). This technique was also combined with AFM in a correlative approach for structural visualizations.

Results: By comparing the biofilm samples collected from a terrestrial aquifer in 2019, 2020 and 2021, we found 2,111 viral infections detected in 62,072 counted archaeal cells, most were in the advanced infection phase (early viral replication to maturation), with intracellular virus signals (71.18%), and lower proportions some in the virus adsorption phase (12.03%), or in the cell lysis phase (16.78%). Our correlative virusFISH-AFM approach revealed morphological changes, i.e., increase in size and shape, of *Ca.* Altiarchaeum cells due to viral infections.

Conclusion: Our results demonstrate that lytic viral infections of *Ca.* Altiarchaeum persist in the studied aquifer over years, irrespective of the increase in resistance of *Ca.* Altiarchaeum via CRISPR-Cas immunity. Moreover, we demonstrate that *Ca.* Altiarchaeum undergoes drastic morphological changes during viral infection *in situ*.

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eP444

Volatile Fungal Compounds as Possible Triggers for Prophage Induction

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Introduction: Lysogenic bacteria (prophage containing bacteria) are widespread in soil. They release temperate phages upon prophage induction under stress conditions. In soil, lysogens share their habitat with other soil organisms such as fungi. Though fungal metabolites are known for their antibiotic effect, little is known about a possible impact on prophage induction as an alternative effect on bacteria.

Objectives: We aim to identify possible influences of fungal metabolites on prophage induction.

Methods: Using controlled laboratory microcosms we exposed the lysogenic soil bacterium *Arthrobacter* spp. B_2A_2-9 and a λ-phage containing model lysogen (*E. coli* K124 F^-) to volatile or soluble metabolites of four different soil fungi (*Marasmius rotula, Agrocybe praecox*, two *Trichoderma* species). A qPCR method was established to measure the change of phage numbers in presence and absence of fungal induction. The impact of fungal metabolites on bacterial growth was determined by a cell counter.

Results: Enhanced phage numbers were observed in the presence of fungal volatile compounds (VOCs). VOCs of soil fungi led up to 34- and 3.5-fold increased phage numbers in cultures of *E. coli* K124 F^- and *Arthrobacter* spp. B_2A_2-9, respectively, while bacterial growth remained uninfluenced. In contrast, soluble fungal compounds of *Marasmius rotula* and both *Trichoderma* species inhibited the growth of both bacteria whereas phage numbers were decreased by all fungi tested.

Conclusion: Our data point at the potential of fungal VOCs to mediate prophage induction. As fungal VOCs can easily spread in vadose environments, fungi may exert long-distance influences on prophage induction thereby affecting microbial community structure and nutrient cycling.

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eP445

A Novel Euryarchaeal Virus-Host System

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All domains of life must contend with viral infections. These can have considerable impacts on entire ecosystems and often outnumber their hosts by a considerable margin. Archaea are likewise preyed upon by their corresponding viruses, however, despite several novel discoveries about the infection mechanisms of crenarchaeal viruses in recent years, archaeal viruses remain poorly understood. Euryarchaeal viruses and their infection mechanisms are even less well understood, partly due to a lack of genetically accessible virus-host systems [1].

Recently, a new virus-host system was isolated from a hypersaline lake. These lakes are a rich source of both halophile euryarchaea and their viruses. This system consists of the siphovirus-like Haloferax tailed virus 1 (HFTV1) and its host, *Haloferax gibbonsii* LR2-5 [2], [3]. *Hfx. gibbonsii* LR2-5 grows well in defined media and is highly motile. Its genome has recently been sequenced an annotated [2]. On the other hand, HFTV1 is a highly specific virus, which cannot infect *Hfx. volcanii* H26 nor the *Hfx. gibbonsii* strain Ma2.38. Since the infection mechanism of this virus is not yet fully understood, the reason for this specificity is not known. However, bioinformatic analysis of *Hfx. gibbonsii* LR2-5 has shown that its surface differs from closely related strains [2]. Thus, HFTV1 host specificity may be related to early steps of infection.

Since *Hfx. gibbonsii* LR2-5 is closely related to the established model organism *Hfx. volcanii*, has a known lytic virus and a lack of obvious viral defense systems, it is a promising new model organism for euryarchaeal virus-host studies. Further, transformation and gene expression protocols have recently been established for this organism. Here, progress in establishing a genetic system for *Hfx. gibbonsii* LR2-5 as well as transforming the strain with HFTV1 gDNA will be described.

Together this new system will allow us to shed new light on the infection mechanisms of euryarchaeal viruses.

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eP446

Targeted design and manipulation of defined microbial consortia by bacteriophages

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The human gut is a complex ecosystem, consisting of eukaryotic cells, bacteria and viruses and alteration of this intestinal microbiota is associated with an increasing number of human diseases. Bacteriophages and viruses of Archea are important effectors and indicators of human health and disease by managing specific bacterial population structures and by interacting with the mucosal immune system.

To obtain functional insights into the gastrointestinal microbiome and its function in health and disease, we aim to establish a model to investigate the interaction of bacteriophages and cognate host bacteria in the mammalian gut. Therefore, we isolated specific phages from environmental samples for members of a minimal bacterial consortium, the Oligo-MM14, which consists of 14 well-characterized bacterial strains that colonize gnotobiotic mice in a stable and reproducible manner. These phages are used to analyze their effect on the stable community in the murine gut with respect to compositional and functional alterations as well as phage-host bacterial interaction over time.

We show, that phages lead to initial depletion of the target population and thereafter coexist with the bacteria over long periods of time. In summary, our work yields insights into phage-bacterial interactions in the gut and the effect of phages on fundamental microbiome functions, which will be important for evaluating the future use of phages for targeted microbiome manipulation.

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eP447

Exploring the periplasmic space of Pseudomonas for phenazine reduction and improved current generation in a bioelectrochemical system

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Phenazines are redox active compounds produced naturally by *Pseudomonas aeruginosa* under limited-oxygen conditions to help offset electrons to distant oxygen or other extracellular electron acceptors [1]. By utilizing the technology of Bioelectrochemical System (BES), phenazines are promising for developing oxygen-limited bioprocesses where they serve as electron mediators to the anode, which results to electrical current generation [2]. However, the efficient usage of phenazines in this technology is being undermined by lack of a good understanding on how phenazines are reduced in the cell and the cellular components they interact with. While some cytoplasmic enzymes have been shown to reduce phenazines [3], we recently showed that the periplasmic glucose dehydrogenase of both *Pseudomonas aeruginosa* and *Pseudomonas putida* could equally reduce phenazines and that improved electrical current could be generated in a BES by engineering the over-expression of these enzymes [4]. Nevertheless, the proportion of the electrons coming from the substrate which are channeled into making electric current (coulombic efficiency) is still not sufficient. This challenges the prospects of introducing this technology into main-stream biotechnology as a means of driving oxygen-limited bioprocesses. At this end, we are currently interrogating what the consequences of the interactions of the phenazines with the periplasmic components of the respiratory chain could be with a view of improving current generation in BES.

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Angicin, a bacteriocin of *Streptococcus anginosus*

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Introduction: To kill competitors many bacteria produce antimicrobial peptides, called bacteriocins. Bacteriocin production is a common trait of Lactic acid bacteria. To this group belongs the opportunistic pathogen *Streptococcus anginosus*, a commensal of mucosal membranes that can also cause severe invasive infections. So far, bacteriocin production of *S. anginosus* has not been systemically investigated.

Objectives: The aim of this study was to analyze bacteriocin production of *S. anginosus* and to characterize the identified bacteriocins.

Material & Methods: By a Radial Diffusion Assay 95 *S. anginous* isolates were screened for bacteriocin production and the spectrum of activity was determined. In subsequent steps, characteristics like heat, pH and degradation stability of the produced bacteriocin were investigated. To identify the genetic basis of bacteriocin production targeted mutagenesis was carried out and the putative bacteriocin was synthesized.

Results: *S. anginosus* strain BSU 1211 was identified as a potent bacteriocin producer and selected for further study. The produced bacteriocin was labeled Angicin and showed a high activity against closely related streptococci, listeria and Vancomycin-resistant *Enterococcus faecium.* Angicin was active in pH ranging from 2-10 and no loss of antimicrobial activity was visible after heat treatment (90 °C for 1 h). *S. anginosus* harbors a bacteriocin-like-peptide locus (*blp3*), which is adjacent to a quorum sensing system, controlling Angicin production. The bacteriocin is encoded by the gene *blp3.4*, which is part of the blp3 locus of *S. anginosus.*

Conclusion: Angicin is the first identified and described bacteriocin of *S. anginosus.* It shows activity against the important food pathogen *L. monocytogenes* and the clinically relevant VRE, making it an interesting compound for a potential use in food preservation or in a clinical setting. Its high resistance towards heat and pH changes supports a wide variety of commercial and clinical applications.

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Pulcherrimin characterization across Staphylococcus

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Antimicrobial compounds are a key bacterial strategy for competing microbes, and they are found in 80% of the cultivable Staphylococcus species from nasal isolates. Numerous biosynthetic gene clusters (BGCs), that produce such antimicrobial compounds, have been identified in Staphylococcus aureus, and evidence suggests they are frequently transferred between lineages. To better understand how widespread these clusters are and how often they are transferred between Staphylococcus, we searched the NCBI public database or one such BGC, pulcherrimin. We found the cluster in a handful of Staphylococcus species, usually on a plasmid. The cluster was most often found in its entirety, with the exception of S. aureus where nearly 40% of assemblies had only the transport (immunity) genes. A phylogenetic analysis did not show evidence of the cluster jumping from one species to another, although S. epidermidis promiscuously accepts various cluster compositions.

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Evaluation of The Effect of Some Medicinal Plants against Quorum Sensing Regulated Virulence Factors in *Staphylococcus aureus*

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Due to the rising cases of antibiotic resistance among pathogenic bacteria worldwide, there is increasing need for alternative medicine such as medicinal plants and the search for new drug targets. Quorum sensing, a cell density-dependent regulation of virulent bacterial gene expression which regulates pathogenesis of bacterial infection, has become a good drug target against pathogenic organisms and a means to overcome the challenge of resistance. Thus, the effect of some commonly used medicinal plants in Southeast Nigeria namely *Carica papaya, Psidium gujava* and *Citrus cinensis* against quorum sensing regulated virulence factor such as twitching motility, Biofilm formation, proteolytic activity and cell adhesion in *Staphylococcus aureus* was evaluated in this study using spectrophotometric methods. Result showed that *Psidium gujava* plant extract had the best inhibition of twitching motility for *Staphylococcus aureus* with diameter of stained growth 0.10cm. *Carica papaya* plant extract showed greater inhibition on Biofilm formation with a reduction in optical density (OD) from 0.137 to 0.044 and cell adhesion from 0.67 to 0.18 while the inhibition of proteolytic activity was lowest in *Psidium gujava* with an increase in optical density (OD) from117.6 to 0.801 and highest in *Carica papaya* with a reduction in optical density (OD) from 0.779 to 0.398. Overall, the plant extract showed variable effects with *Carica papaya* plant extract showing the best effect against quorum sensing regulated virulence factors in *Staphylococcus aureus*. This study shows the potential use of these plant extracts in the treatment of microbial infections by inhibiting bacterial virulent factors and its associated antibiotics resistance capabilities.

Keywords: Anti-qourom sensing, Bacterial infections, Biofilm, *Staphylococcus aureus*, Plant extracts

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Discovery of four new monoterpene synthases from Actinobacteria

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Terpenes are one of the largest classes of secondary metabolites. It is surprising that from >80.000 terpenes/terpenoids known today only ca. 1.3 % are

produced by bacteria with monoterpenes being particularly underrepresented (0.04 %). Considering the vast number of bacteria estimated to exist on earth (5x1030) the lack of knowledge concerning bacterial terpene biosynthesis becomes obvious. Recent advances showed that bacteria use the canonical as well as a newly discovered non-canonical terpene biosynthesis pathway where modified, i.e. methylated substrates are used.

The aim of our project is to find new terpene synthases in bacteria with special focus on non-canonical terpene synthases and to identify their substrate and product spectra.

Since 2-methylisoborneol (2-MIB) biosynthesis relies on coupled methyltransferase (MT) & terpene synthase (TPS) reactions, we were looking for MT/TPS gene pairs with HMMsearch using the EnsemblBacteria database. Four bacterial strains were selected: i) *Amycolatopsis japonica* (*A.j.*), ii) *Kitasatospora cheerisanensis* (*K.ch.*), iii) *Saccharopolyspora erythraea* (*S.e.*), and iv) *Streptomyces noursei* (*S.n.*) and the corresponding MT/TPS genes were cloned. After overexpression followed by Ni-NTA purification, MT/TPS enzymes were tested *in vitro* for their substrate & product spectra using enzyme assays and GC/MS analysis.

As expected, MT/TPS pairs of all four strains resulted in production of 2-MIB (*A.j.*, *K.ch.*, *S.n.*) and 2-methyl-2-bornene (*S.e.*), respectively. To our surprise, all TPS enzymes proved to be multi-substrate as well as multi-product enzymes. Hence, they were able to use 2-methyl-GPP and GPP as substrates, and finally lead to the production of non-canonical terpenes 2-MIB/2-methyl-2-bornene and of canonical monoterpenes, the *A.j.*, *K.ch.*, & *S.n.* TPS was characterized as α-pinene synthases whereas *S.e.* TPS was a sabinene synthase.

In summary, with this genome mining approach we were able to identify four 2-MIB/2-methyl-2-bornene producing bacteria. Additionally, we succeeded in the identification of four new monoterpene synthases with different but specific product spectra. This result is remarkable since so far only a linalool and cineole monoterpene synthase were biochemically characterized in bacteria.

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Genome Sequence-based Screening for novel phosphonate producers

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Phosphonates are a unique class of natural products with diverse chemical structures and bioactivities. Numerous phosphonate natural compounds found there way into the market as for example the herbicide bialaphos, the antimalarial agent fosmidomycin and the antibiotic fosfomycin.The structural unity of all phosphonates is the characteristic C-P bond, which is formed in an initial biosynthetic reaction step catalysed by the enzyme phosphoenolpyruvate phosphomutase (PepM), which converts phosphoenolpyruvate (PEP) to phosphonopyruvate (PnPy) [1]. Due to the conservation of the PepM enzymatic reaction in the vast majority of phosphonate producers, the respective biosynthetic gene *pepM* is well suited to be used as a molecular marker to screen for potential phosphonate producer strains.

In our study, we aim to screen for novel phosphonate producer strains from the DSMZ strain collection based on genome-sequenced actinomycetes. The DSMZ strain collection harbours >3.600 actinomycetes, many of which have already been genome-sequenced. A bioinformatic search of all genome sequences revealed 33 strains containing a *pepM* gene and thus a potential phophonate biosynthetic gene cluster (BGC). Out of these 33 potential phosphonate producers, 15 showed antimicrobial activity in antibacterial bioassays with a phosphonate-sensitive *E.coli* strain WM6242 as a test organism [2]. Gene cluster networking analysis with biosynthetic gene clusters encoding for known phosphonates allowed for the selection of strains with unique and novel phosphonate BGCs. Phylogenetic analysis of PepM amino acid sequences put the potential phosponate producers in distinct clades [3]. These results were used to further dereplicate strains with a relatedness to known phosphonate producers. The biosynthetic gene cluster of selected hits will now be verified and characterised by mutagenesis. In addition, chemical elucidation and verification of phosphonates is undertaken by our partners, AG Chambers Hughes (Universität Tübingen) by means of 31P NMR spectroscopy and mass spectronomy.

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eP453

An optimized *Ustilago maydis* for itaconic acid production at maximal theoretical yield

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Introduction: Itaconic acid and its derivatives are found in many application fields, such as the production of paper, paints, and fibers, or in waste water treatment providing a stable market for this bio-based chemical. *Ustilago maydis*, member of the Ustilaginaceae family, is a promising host for the production of this dicarboxylic acid. Several itaconate overproducing *Ustilago* strains have been generated by metabolic and morphology engineering. This yielded stabilized unicellular morphology through *fuz7* deletion, reduction of by-product formation through deletion of genes responsible for itaconate oxidation and (glyco)lipid production, and the overexpression of the regulator of the itaconate cluster *ria1* and the mitochondrial tricarboxylate transporter encoded by *mttA* from *Aspergillus terreus*.

Objectives: The objective of this study was to further increase the itaconate production with *Ustilago maydis* by consolidating these different optimizations into one strain as well as to assess the performance of this strain under industrially relevant fed-batch fermentations.

Methods: Metabolic engineering, biochemical engineering.

Results: In this study, itaconate production was further optimized by consolidating different genetic optimizations into one strain designated as *U. maydis* K14. The combined modifications resulted in itaconic acid production at theoretical maximal yield, which was achieved under biotechnologically relevant fed-batch fermentations with

continuous feed. In a low-cell-density fermentation, 75.7 g L^{-1} itaconate was produced by *U. maydis* strain K14 within 312 h with an overall productivity of 0.24 g L^{-1} h^{-1} and a yield of 0.66 g_{ITA} g_{glu}^{-1}. This is the highest yield ever reported for *U. maydis*, and it is also higher than most reported yields achieved with *A. terreus*. In fact, this fermentation achieved the theoretical maximal yield during the production phase. When disregarding the glucose consumed during the first 24 h in the growth phase, the yield was 0.72 g_{ITA} g_{glu}^{-1}, or 1.00 mol_{ITA} mol_{glu}^{-1}.

Conclusion: This study explored the limits of microbial itaconic acid production with *U. maydis* by combinatorial metabolic, morphological, and biochemical engineering. The strain modifications combined with a continuous glucose feeding strategy enabled itaconate production at 100% of the theoretical maximum yield during the production phase in a low-density fermentation.

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eP454

Garvicin Q – Characterization of biosynthesis and mode of action

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Bacteriocins are ribosomally synthesized antimicrobial peptides, that either kill target bacteria or inhibit their growth. These natural compounds are widely used in food preservation and of increasing interest as potential alternatives to antibiotics. Garvicin Q is a broad spectrum bacteriocin targeting the mannose phosphotransferase system (Man-PTS) of e.g. *Listeria* sp. and *Lactococcus* sp. [1]. The aim of the study was to further characterize biosynthesis and mode of action of garvicin Q.

A *Lactococcus garvieae* isolate was shown to produce the heat-stable and protease-sensitive bacteriocin garvicin Q. Based on the genome sequence of the *L. garvieae* isolate, the garvicin Q gene cluster was predicted *in silico*. It consists of *garQ* (peptide gene), *garI* (immunity gene), *garC* and *garD* (transporter genes). Suitable growth conditions for bacteriocin production by *L. garvieae* were determined and it was shown that garvicin Q is mainly produced and secreted during the exponential growth phase. A *Listeria monocytogenes* mutant expressing a non-functional Man-PTS was highly resistant to supernatants of the *L. garvieae* isolate and purified garvicin Q suggesting that the Man-PTS serves as a receptor for garvicin Q. Further experiments using live, fluorescence-based *Listeria innocua* and *Lactococcus lactis* biosensors indicate that garvicin Q activity is mediated by formation of pores in the membrane of target organisms. To demonstrate that the genes *garQ*, *garC* and *garD* are responsible for biosynthesis of garvicin Q, a synthetic operon with codon-optimized gene sequences was expressed in *Corynebacterium glutamicum*. Cell-free culture supernatants of the recombinant *C. glutamicum* strain showed activity against the fluorescent biosensors indicating recombinant garvicin Q production.

In conclusion, our data shows that garvicin Q is a bacteriocin with membrane-damaging activity targeting the Man-PTS. Furthermore, recombinant production of garvicin Q was established in *C. glutamicum*.

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eP455

Tropolone natural product biosynthesis in *Streptomyces sp.*

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Tropolones are a group of natural products with potent metal-chelating properties that exhibit antibacterial, antiviral and antitumoral activity.[1] These compounds are hydroxylated derivatives of tropone that consists of a seven-membered, non-benzenoid, aromatic carbon-ring with an additional keto-group. In bacteria the precursor for these compounds surprisingly originates from primary metabolism, i.e. the CoA-dependent catabolism of phenylacetic acid (paa). However, depending on the producing strain, different sets of enzymes are used to modify this precursor. For the Gram-positive *Streptomyces* sp., the gene cluster encoding the dihydroxytropolone biosynthetic machinery was identified by gene-knockout studies.[2]

We now investigated dihydroxytropolone formation in *Streptomyces* sp. by *in vitro* reconstitution of the biosynthetic pathway using heterologously produced enzymes, which allowed us to gain insight into the individual biosynthetic steps and reaction mechanisms of the partaking enzymes. Conducted assays were analyzed via HPLC and GC-MS, accumulating products were compared to chemically synthesized standards.

Accordingly, the thioesterase TrlF cleaves the CoA-ester bond from the precursor molecule originating from phenylacetic acid catabolism. The flavoprotein monooxygenase TrlE then mediates the unanticipated series of reactions comprising hydroxylation, decarboxylation and ring oxidation. Finally, the two-component flavoprotein monooxygenase TrlCD catalyzes two consecutive ring-hydroxylations to yield dihydroxytropolone. The role of TrlA, an enoyl-CoA hydratase homologue encoded in the same gene cluster is still subject to investigation.

Taken together, the discovered enzyme functionalities substantially differ from the previously proposed roles that were based on gene knock out studies. Currently, the structure of the key enzyme TrlE is also studied by X-ray crystallography in order to gain further insights into the unusual reaction mechanism.

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A Novel Widespread Strategy for Chloramphenicol Inactivation in Bacteria

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Chloramphenicol (CAP) was isolated from *Streptomyces venezuelae* in 1947 and acts as broad-spectrum antibiotic, which binds to the 50S ribosomal subunit to prevent peptide bond formation and therefore inhibit protein biosynthesis in Gram-positive and Gram-negative bacteria. However, bacteria have developed numerous mechanisms to resist CAP, most frequently by enzymatic inactivation, e.g., via O-acetylation or O-phosphorylation. Beyond these mechanisms, there are also known efflux systems and permeability barriers [1]. In our study, we identified a novel strategy for enzymatic CAP modification and inactivation in *Nocardiopsis* sp. Isolation and characterization of the modified CAP revealed that the C-3 primary hydroxyl group is oxidized to the respective carboxylic acid, which renders the compound inactive. Through activity-guided chromatography, an enzyme could be purified from the cell-free lysate and identified as distinct member of the flavoprotein family. Detailed biochemical investigation of the heterologously produced enzyme confirmed its functionality and showed a stepwise dual oxidation of the C3-hydroxyl group. Subsequent protein BLAST searches suggested that this resistance mechanism is more widespread in Gram-positive bacteria including *Streptomyces* sp., which was confirmed by examining several of these functional homologues. In the course of our investigation, the same CAP derivative was reported in a separate study in Gram-negative bacteria as an early intermediate in a CAP catabolic pathway, while the responsible enzymes for the oxidation steps remained elusive [2]. We could now identify and also characterize the involved enzyme, showing that it belongs to the same enzyme family. However, phylogenetic analysis suggests that this newly discovered enzyme is only distantly related to the *Nocardiopsis* type despite catalysing the same reactions, suggesting that it evolved independently. Currently, the underlying enzyme mechanisms are further investigated by protein crystallography.

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eP457

Exploring the Diversity of Natural Products using Molecular Networks

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Natural products are known for their diverse and complex chemical scaffolds. Thus, natural product exploration has proven to be one of the most promising sources for drug discovery1. In an ongoing search for new bioactive compounds, recent studies revealed that the biosynthetic potential of many organisms has not yet been fully realized2. One reason for this is that the production of most natural products is highly dependent on a number of external factors3 which are often not met under laboratory conditions.

Here we describe an approach identifying novel natural product derivatives using untargeted liquid chromatography-coupled tandem MS (LC-MS/MS) and molecular networking. The natural gyrase inhibitor novobiocin produced by *Streptomyces niveus* was selected as a proof of concept study.

By cultivation of *S. niveus* in systematically varied media, we developed an optimized medium for novobiocin production. Extracts of those and other cultivations were obtained by liquid-liquid extraction and tested for their antimicrobial activity. Furthermore, whole metabolome analysis was performed by acquiring LC-MS/MS fragmentation spectra. Using the GNPS4 workflow, the acquired spectra were compared and clustered based on their similarity.

The molecular network analysis then revealed several known novobiocin precursors grouped within the novobiocin cluster, indicating that this approach is suitable for identifying structural analogs from a molecule of interest. Interestingly, we also identified molecules with similar fragmentation spectra and masses, which are not known to be precursors and therefore might be novel novobiocin derivatives. To increase the yield of these compounds, the cultivation conditions were further optimized. Future efforts will focus on their isolation, structural elucidation, and biological characterization.

The presented workflow is well suited for the identification of precursors and derivatives of molecules of interest and can also be applied to unknown bioactive compounds in natural extracts.

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eP458

Isolation and Characterization of the antibiotic-producing *Actinomadura* sp. from meerkat feces

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Introduction: In view of the rapid spread of antibiotic resistance and the resulting need for new therapeutics, the discovery and development of new antimicrobial compounds are the central focus of current research. One potential direction could be the study of secondary metabolites from strains, e.g. of the actinomycetes, which were isolated from unexplored habitats, in order to gain access to new or endemic species.

Objectives: Here, we study new actinobacterial isolates from faeces sample of a meerkat (*Suricata suricatta*) with regard to their antimicrobial activity.

Methods: Diluted faeces samples were plated on MYM agar. Potential *Actinobacteria* were isolated based on their typical colony morphology. A pure culture of the isolate was phylogenetic classified based on the 16S rRNA gene sequence. Mature colonies on MYM agar plates were overlayed with soft agar containing different Gram-negative, Gram-positive or fungal species as indicator strains. Cell

extracts from liquid cultures as well as colonies were further analyzed regarding their antibiotic spectrum by applying a panel of whole cell biosensors. Such indicator strains, in which an antibiotic-inducible promoter is fused to the luciferase cassette, provide an easily quantifiable and specific luminescence output in response to exposure to a specific antimicrobial compound. Active cell extracts are currently further analyzed for isolating and biochemically characterizing the corresponding antibiotic compounds.

Results: Colony and cell morphology indicated that the isolated strain belonged to the phylum *Actinobacteria.* 16S rDNA sequencing identified the isolate as *Actinomadura coerulea*. The isolate showed a strong and broad range of antibacterial activities against Gram-positive bacteria, including pathogens, as well as an antifungal effect on the growth of *Penicillium chrysogenum.* The secondary metabolites of the isolate potentially inhibit replication, block translation, and interfere with bacterial cell wall integrity. Detailed follow-up studies are currently ongoing.

Conclusion: Our approach demonstrates that combining new sources for isolating actinomycetes with whole cell biosensor screens provide a promising strategy for identifying novel antimicrobial compounds.

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Uptake studies of ADEP into prokaryotic and eukaryotic cells

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The rise of antibiotic resistance against recently applied antibiotics urgently calls for the discovery and evaluation of novel antibiotic classes with unique targets to dodge cross resistances.

One promising group of candidates are acyldepsipeptide antibiotics (ADEPs). Early research on ADEP compounds in the 2000s revealed a unique mode of action by interacting with an up to then undescribed target for antibiotics, the caseinolytic protease ClpP. ADEPs are known to dysregulate the proteolytic core ClpP towards destructive proteolysis in bacterial cells, while in parallel blocking its native functions. Thereby ADEP leads to the degradation of nascent and loosely folded proteins, as well as the accumulation of native ClpP substrates [1].

ADEP has proven to be effective against a variety of pathogenic bacteria *in vivo* including multi drug resistant *Staphylococcus aureus*. Against most Gram-negative species ADEP is less effective, despite its capacity to dysregulate Gram-negative ClpP homologs *in vitro* [2].

Furthermore, ADEP seems to be only mildly toxic to most eukaryotic cell lines, resulting in a comparably broad therapeutic window. However, it has been shown, that ADEP is able to dysregulate the human ClpP homolog, which is localized in the mitochondrial matrix [3].

Why is ADEP more toxic against bacteria than against eukaryotic cells? One possible reason for this behavior could be a differential capacity of ADEP to cross the physio-chemically different membranes in prokaryotic and eukaryotic cells. In my PhD project I am conducting uptake studies to investigate this hypothesis.

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The role of secondary metabolites in bacterial communities

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Plants represent the most important nutrient source for humans. Their health is of great interest for agriculture and therefore for our food supply. The plant microbiome is essential for the health and growth of its host. Several positive effects on plant growth, pathogen resistance and nutrient uptake have been shown to be caused by microbes culturing the plant phyllosphere. [1] The organisms not only interact with the plant but furthermore affect each other strongly. Interaction networks of the *Arabidopsis thaliana* leaf microbiome discovered a big number of inhibiting interactions taking place within the community. [2] Since secondary metabolites are fundamental units with which bacteria sense and respond their environment, we hypothesized, that these compounds play a major role within interactions of plant leaf microbiomes. With our study we aim to gain insides into the importance of specific strains and compounds for complex microbial communities on plants and therefore exploring ways to improve plant health and growth promotion. For this purpose, we use a synthetic community (SynCom) assembled from *Arabidophis thaliana* leaves. The SynCom grows stabile in the lab as well as on plants, representing an effective testing system.

To identify secondary metabolites, which play a role in SynCom interactions, we investigated the potential for the production of secondary metabolites by bacterial SynCom members *in sillico* and *in vivo*. Using co-cultivation experiments and HPLC-analysis of culture extracts, we discovered two major players within the SynCom bacteria being *Pseudomonas koreensis* and *Bacillus altitudinis*.

We identified, that *Pseudomonas koreensis* is able to produce the siderophore Pseudobactin. In growth experiments we saw, that Pseudobactin effects other SynCom bacteria by growth inhibition. In addition, *Bacillus altitudinis* was identified as the producer of an inhibiting compound, effecting almost all SynCom bacteria. The findings indicate the importance of the strains for the whole community. In further experiments, we aim to identify the inhibiting compound produced by *Bacillus* and use it as well as Pseudobactin to manipulate the *Arabidopsis* leaf model system.

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eP461

Heterologous Production of Aurachins in *Escherichia coli*

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The aurachins are a family of prenylated quinolone antibiotics, which were first discovered in the myxobacterium *Stigmatella aurantiaca*.[1] Bioactivity testing revealed that these natural products possess potent antiplasmodial activities, but also high cytotoxicity.[2,3] Therefore, there is a great interest in the generation of unnatural aurachin derivatives with a larger therapeutic window. While several research groups employ only chemical synthesis in the derivatization process,[4] we aim to redirect the biosynthetic pathway and to produce the desired compounds in a heterologous host. The key enzyme of the aurachin biosynthesis is the farnesyltransferase AuaA. It catalyzes the prenylation of a quinoline precursor with a farnesyl moiety to give the aurachin scaffold.[5] For the biosynthesis of new unnatural aurachin derivatives, the overexpression of AuaA is therefore essential. *E. coli* was selected as a host because the membrane-bound AuaA had already been functionally expressed in this organism for *in vitro* studies.[5] However, for the *in vivo* production of aurachins additional optimization steps are required, such as the improvement of the metabolic flux towards farnesyl-diphosphate. Furthermore, the ideal strain and expression system for the expression of the membrane protein AuaA needs to be identified.[6] Here, we will present our recent findings from this project.

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eP462

The small molecule chaperone AzeG is involved in secondary metabolite biosynthesis in *Pseudomonas aeruginosa*

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Introduction

The notorious opportunistic pathogen *Pseudomonas aeruginosa* orchestrates collective gene-expression programs by culture-density dependent chemical signaling called *quorum sensing* (QS). Many virulence-related behaviors, including biofilm formation and production of phenazines and alkyl quinolones, are influenced by QS. Recently, QS-controlled synthesis of azetidine-containing alkaloids (azetidomonamide A and B) involving the biosynthetic *aze* gene cluster was reported in *Pseudomonas aeruginosa*[1,2]. Although a biosynthetic route for azetidomonamides A and B has been proposed, the function of several genes within the *aze* gene cluster remains unknown.

Objectives

We aim to elucidate the role of the hypothetical protein AzeG, which exhibits similarities to epoxide hydrolases and polyketide cyclases, and the putative MFS transporter AzeK for the production of *aze* metabolites in *P. aeruginosa*.

Materials & Methods

We heterologously produced AzeG and assessed binding affinities to several intermediates and products of the *aze* biosynthetic pathway by thermal shift assays. In parallel, we addressed the effects of chromosomal deletions of *azeG* and *azeK* on the production of azetidomonamides.

Results

Within this project, we developed a *P. aeruginosa* strain for inducible overexpression of *aze* genes and bioproduction of the respective metabolites, which serves as chassis for the analysis of the biosynthetic gene cluster. Deletion of *azeG* drastically reduced the production of *aze* metabolites, and deletion of *azeK* resulted in a similar phenotype. Interestingly, enzyme assays suggested that AzeG does not fulfill a catalytic role within the biosynthetic pathway, but nevertheless interacts with pathway intermediates.

Conclusion

We propose that AzeG acts as a small molecule chaperone, which binds *aze* metabolites and thereby affects biosynthetic equilibria and/or contributes to metabolite export. Preliminary data moreover support the notion that AzeK acts as pathway-specific transmembrane exporter.

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eP463

Investigation of RiPPs originating from two-domain precursors

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Introduction

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a diverse class of secondary metabolites with a wide range of bioactivities. They all share a ribosomal origin, as the peptide sequence is encoded in a

so-called precursor gene. The precursor peptide usually consists of a leader sequence followed by the core sequence. Different enzymes recognize the leader sequence and install post-translation modifications on the core peptide, which after proteolytic cleavage is then released as the mature natural product. Often, precursor peptides are short and lack clear structural features. As an exception, the family of the "nitrile hydratase leader peptides" (NHLP) are characterized by unusually long leader sequences that show similarity to the enzyme nitrile hydratase [1]. In certain species of the order Burkholderiales, these NHLP precursors also appear as tandem genes, i.e. two copies of the precursor are present in a row. Intriguingly, in a few strains these genes are fused into a single double-leader precursor.

Objectives

Here, we investigated these so-far uncharacterized RiPP clusters in order to identify the produced metabolites, characterize the biosynthetic enzymes, and explore the role of the two leader domains in the precursors.

Materials & methods

Following a bottom-up approach, we heterologously expressed the biosynthetic gene cluster containing the fused double-leader precursor in *Escherichia coli* and analyzed the modifications in the His-tagged precursor peptide using intact protein LC-MS and proteomics approaches.

Results

The activity of the cyclodehydratase and methyltransferase present in the gene cluster could be successfully reconstituted in *E. coli*. The resulting modifications were localized at the C-terminus of the precursor peptide, as a dehydrated cysteine and a methylated threonine were observed. Surprisingly, expression and purification of the precursor peptides from a strain that carried a gene cluster containing two separate precursor genes revealed that the two precursors form a stable dimer. We are currently investigating, if these dimeric precursors harbor one or two core peptides.

Conclusion

Future research aims for the characterization of another enzyme in the gene cluster, a large kinase/cyclase protein, as well as the isolation of the RiPPs from the native producers and determining their bioactivities.

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eP464
Biosynthetic Studies on potent G protein inhibitor natural products
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The potent and selective Gq protein inhibitor depsipeptide FR900359 (FR), originally discovered as the product of the uncultivable bacterial plant leaf endosymbiont "*Candidatus* Burkholderia crenata", is member of a small and specialized natural product family, the chromodepsins [1]. FR is synthesized by a complex biosynthetic system comprising two nonribosomal peptide synthetase (NRPS) assembly lines [2]. Due to its unique mode and effective mode of action, FR is used as a pharmacological tool and shows promise for drug development against Gq-related diseases.

We have recently characterized a cultivable bacterial FR producer, *Chromobacterium vaccinii*, enabling detailed investigations into biosynthesis and attachment of the functionally important FR side chain [3]. We reconstituted side chain assembly by the monomodular NRPS FrsA and the non-heme monooxygenase FrsH, and characterize intermolecular side chain transesterification to the final macrocyclic intermediate FR-Core, mediated by the FrsA thioesterase domain. We furthermore harnessed FrsA substrate promiscuity to generate FR analogs with altered side chains and demonstrate indispensability of the FR side chain for efficient Gq inhibition by comparative bioactivity, toxicity and docking studies. Evolution of the specialized natural product FR and its side chain biosynthesis is discussed based on bioinformatics analyses. Finally, our investigations concerning the ecological role of FR900359 in soil and plant leaves will be presented.

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eP465
Investigating the catalytic potential of a putative TPS-MT fusion enzyme from *Burkholderia* sp.
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Terpene biosynthesis is highly conserved among different realms of life. In a first step, the universal building blocks IPP & DMAPP are synthesized either via the MVA or MEP pathway. Following this, isoprenyl pyrophosphates (e.g., GPP, FPP, GGPP) of different chain lengths are formed by condensation of these building blocks which are then converted to the final terpenes by terpene synthases (TPS). Optionally, these terpene hydrocarbons can be further modified by tailoring reactions (e.g., oxygenation, cleavage). Considering the large number of bacteria already known today it is even more surprising that until now only 62 bacterial TPS enzymes have been biochemically characterized.

Furthermore, it is interesting to note, that bacteria established an additional strategy of terpene biosynthesis, in which the well-known prenyldiphosphates (GPP, FPP) are methylated by a specific methyltransferase (MT) prior to the TPS reaction. In the few cases known so far both enzymes appear in gene clusters and produce terpenes of irregular length (C11 or C12, or C16, respectively).

Using HMM analyses we identified a potential fusion protein of a TPS with a MT from a *Burkholderia* species. The aim of this study is to investigate which enzyme activities are present in this fusion protein. Since the MT part almost doubles the size of a regular bacterial TPS alone, the MT sequence may influence the TPS activity and *vice versa*. Furthermore, we want to assess whether the TPS part is still catalytically active after truncation of the MT section and in

what way this affects the TPS product spectrum. For this purpose, the protein (or parts of it) will be heterologously overexpressed, purified and tested in enzyme assays with final GC/MS analysis of the resulting products to determine the potential TPS/MT enzyme activities.

Finally, this study will help us understand how bacteria evolved the non-canonical terpene biosynthetic pathway.

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eP466
Spatial and temporal cellular assembly of the glycopeptide biosynthetic complex
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Glycopeptide antibiotics (GPAs), like vancomycin or teicoplanin, are clinically important natural compounds, produced by various actinomycetes. They consist of highly crosslinked and diversely modified heptapeptides.

The biosynthesis of the vancomycin-type GPA balhimycin synthesized by *Amycolatopsis balhimycina,* has been the most widely studied GPA assembly line *in vivo*. The individual biosynthetic steps have been elucidated in great detail resulting in a model describing the reaction sequence. Following the supply of secondary-metabolite-specific precursors, the peptide backbone is assembled by non-ribosomal peptide synthetases (NRPS). Halogenation and the interconnection of the aromatic side chains by cytochrome P450 monooxygenases occur at the NRPS-linked intermediates in a defined order, suggesting the formation of a multi-enzyme complex consisting of three NRPS, three cytochrome P450 monooxygenases, and a halogenase. Subsequently, the completed aglycon is glycosylated in a specific sequence and finally secreted by an ABC-transporter. Whereas the chronology of the reactions could be defined, the dynamic, spatial and temporal aspect of the multi-enzyme complex still remains unclear. Therefore, the goal of this project is to determine interaction partners that may play a role in the formation of the complex.

(I) Using the bacterial two-hybrid system we investigated the interaction between the NRPSs and a MbtH-like protein (MLP1), encoded in the balhimycin biosynthetic gene cluster. MLPs are required for the activation of adenylation domains in NRP biosynthesis. MLP1 was shown to interact specifically with the first module of the three multi-modular NRPS enzymes.

(II) Construction of deletion mutants lacking the genetic information for the docking domains, responsible for the interaction between the NRPS enzymes, resulted in a strongly decreased balhimycin production. This highlights an important but not essential role of these domains in forming the biosynthetic complex.

(III) An MS analysis-based approach using Blue-native-PAGE to separate intact protein complexes from *A. balhimycina* provided preliminary evidence that the three NRPSs interact in the cytoplasm. To determine the possible interaction with other enzymes, the purification conditions which may influence the complex stability will be optimized further.

The obtained results will help to embed the biosynthetic process into the general cellular processes.

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eP468
Identification and characterization of ClpP targeting molecules
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The number of pathogens that have become resistant to antibiotics is rising worldwide and therefore the discovery of novel antibacterial agents is urgent. Natural products derived from microorganisms provide a promising and substantial source for new antibiotics.

Streptomyces hawaiiensis NRRL 15010 is the producer of the natural product ADEP (acyldepsipeptide 1, "factor A"). ADEP and ADEP derivatives represent a promising new class of antibiotics which have potent antibacterial activity against Gram-positive bacteria by deregulating ClpP, the proteolytic core of the bacterial caseinolytic protease [1,2]. The identification and a following characterization of the ADEP biosynthetic gene cluster (BGC) reveals a cluster-associated *clpP* homologous gene ($clpP_{ADEP}$). Heterologous expression of $clpP_{ADEP}$ in different ADEP-sensitive *Streptomyces* species confirmed its role in conferring ADEP resistance and revealed a novel type of antibiotic resistance [3].

In this project, we aim to identify and characterize novel ClpP-targeted natural products. Based on the localization of the resistance gene $clpP_{ADEP}$ in the ADEP BGC, a bioinformatic screening procedure was established. By combining antiSMASH and ARTS, the bacterial NCBI database was screened for BGCs in actinomycetes harboring potential *clpP* homologous genes. 13 interesting BGCs were selected encoding the synthesis of different types of natural products. The corresponding strains were grown in different media. The culture extracts were used in bioassays against *B. subtilis* 168 WT and *B. subtilis* 168 Δ*clpP* mutant to investigate the potential to target ClpP. One of the culture extracts exhibited differential activities against the two test strains. HPLC/MS analyses and comparisons of the masses with the masses in the natural product database suggests a new compound is being produced.

The localization of ClpP in the ADEP BGC and its characterization as a resistance mechanism allowed the establishment of a promising bioinformatic approach for the discovery of further ClpP-targeting antibiotics.

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eP469
Biofilm and pigment regulation in *Pseudomonas aeruginosa* – A new class of signal molecules involved?
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Introduction

Pseudomonas aeruginosa uses a complex network of chemical communication to coordinate strategies for virulence and survival. The most prominent signal molecules are acyl homoserine lactones (AHLs) and alkyl quinolones (AQs), both of which are part of quorum sensing (QS) systems and, as such, regulated by cell density. Recently, two new secondary metabolites of the pyrrolizidine-class were identified to be produced by a QS-controlled gene cluster of *P. aeruginosa* in very low amounts [1,2]. Homologs of the biosynthetic genes are widespread among bacteria[3].

Objectives

We aimed at analyzing the functions of the metabolites produced by the *aze* biosynthetic gene cluster.

Methods

Genetic manipulation, engineered bioproduction and purification of metabolites, enzyme assays, analysis of virulence factors and biofilm characterization.

Results

We developed inducible system for expression of the *Pseudomonas aeruginosa* PAO1 *aze* gene cluster that codes for the synthesis of azetidine containing metabolites enables production of over 50 mg purified material per liter culture supernatant. High level production allowed the identification of at least two new compounds produced by the biosynthetic pathway. While non-toxic to all tested microbial species, *aze* metabolites had specific physiological effects when supplemented to *P. aeruginosa*. Carbamate-type azetidyl compounds strongly induced biofilm formation while causing overproduction of red pigments. In suspension cultures, supplementation led to severalfold increase of pyocyanin production and PQS quorum sensing.

Conclusion

We propose that metabolites of the *aze* gene cluster function as signal molecules for the coordination of transitions in lifestyle and/or virulence factor production. With prevalence of homologous gene clusters in gamma-proteobacterial and actinobacterial species, the compound class could have a general role in signaling or cell differentiation.

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eP472

Inter-kingdom co-cultivation as targeted approach to induce silent fungal gene clusters and algal multicellularity

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Microorganisms produce numerous natural products (NPs) with important biological activities. However, their encoding gene clusters are often silent under standard laboratory conditions where they lack the ecological context, such as the presence of other microorganisms [1]. In many cases, the true ecological function of NPs remains obscure, despite being a crucial element for understanding functional interactions in microbiomes. Here, we report the targeted induction of an as-yet uncharacterized silent gene cluster of the fungus *Aspergillus fumigatus*, which is activated by the bacterium *Streptomyces rapamycinicus* during the bacterial-fungal interaction. The resulting NP is the novel fungal metabolite fumigermin, which inhibits the germination of spores of the inducing bacterium *S. rapamycinicus,* and thus helps the fungus to defend resources in the shared habitat against a bacterial competitor [2]. Furthermore, in the more complex, lichen-like association between *Aspergillus* spp., *Streptomyces* spp. and the green alga *Chlamydomonas reinhardtii*, we found that the fungus protects *C. reinhardtii* from the streptomycete-derived algicidal NP azalomycin F [3]. However, in the absence of the fungus, *i.e.* in a co-culture of the streptomycete and *C. reinhardtii*, the alga employs a different strategy to survive harmful azalomycin F through formation of a novel protective multicellular structure named gloeocapsoid. This finding suggests multicellularity may have evolved as a protection mechanism against harmful competing bacteria [4]. Collectively, our work illustrates the importance of revealing the ecological roles of NPs to understand how they shape the complex interactions of microorganisms.

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eP473

Construction of an optimized host for heterologous glycopeptide antibiotic production

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Glycopeptide antibiotics (GPAs) are remarkable in their chemical structures, consisting of

highly crosslinked and diversely modified core heptapeptides. Modifications on GPAs include

glycosylation, halogenation, methylation, acetylation or sulfonation.

In our lab we have extensively elucidated the complex biosynthesis of GPAs of balhimycin

produced by *Amycolatopsis balhimycina* and ristomycin produced by *Amycolatopsis*

japonicum. We could show that the biosynthesis can be categorized in three parts: precursor

supply, linking of the peptide backbone and modification reactions. The key steps of the

biosynthesis are carried out at a multi-enzyme complex consisting of NRPSs, oxygenases and

a halogenase. In addition to the Biosynthetic Gene Clusters (BGCs) encoding the "classical"

GPAs, unusual GPAs BGCs could recently be identified in the genome of actinomycetes.

However, in the most cases, these remain "silent" also under GPA production conditions. One

approach to activate gene expression is heterologous expression in a suitable host. For this

purpose, we optimized *A. japonicum*. The strain contains all the genes needed for precursor

synthesis and for resistance, it grows fast and is genetically easy to manipulate. We deleted

the NRPS genes of the ristomycin BGC and introduced regulator and tailoring genes, enabling

the heterologous production of all types of GPAs.

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eP474

Biosynthetic pathways of volatile organic compounds in the medicinal mushroom *Wolfiporia cocos*

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Studies on the brown-rot fungus *Wolfiporia cocos* often focuses on anti-inflammatory or antitumor effects of compounds produced by its sclerotium, which is traditionally used in Chinese medicine. However, the vegetative mycelium of this fungus produces a variety of aroma active compounds that are of interest for the aroma industry, such as linalool or methyl anthranilate produced by *W. cocos* during fermentation on black current pomace.[1] However, the biosynthetic pathways for most of the volatile organic compounds (VOCs) have not been elucidated yet.

Taking on this topic, the volatile compounds produced by vegetative mycelium of *W. cocos* have been analysed by means of gas chromatography-mass spectrometry. In parallel, a transcriptomic study was performed at time points with high presence of linalool and methyl anthranilate. To map the RNAseq data, a whole genome sequencing of *W. cocos* was performed as the already published genome is of insufficient quality.

Genes coding for the enzymes of the shikimate pathway, which produces the necessary precursors for the biosynthesis of methyl anthranilate, and the mevalonate pathway, which provides the precursors for linalool, have been identified and annotated in the *W. cocos* genome. The transcriptomic data has been mapped to the newly annotated *W. cocos* genome and expression levels of all relevant genes have been determined. As a result, the biosynthesis of methyl anthranilate and its precursors are proposed based on *in silico* experiments.

Further studies will address the validation of enzymes involved in both biosynthetic pathways.

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eP476

Microbial carbon dynamics in cryogenic environments of western Greenland Ice Sheet

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Microbiological activity on glacier and ice sheet surfaces can be a major factor responsible for their darkening. Among microbes, pigmented snow- and glacial ice algal blooms magnify surface melting due to increased light absorption. The related decrease in albedo and the enhanced melting further contribute to sea level rise. Additionally, the metabolic activity of cryogenic microbes in such blooms will, invariably, both release and consume dissolved organic carbon compounds. Understanding the dynamic of organic carbon is thus crucial to assess its potential role in sustaining algal blooms. To investigate microbial carbon dynamics and variability due to autotrophic and heterotrophic activity, we performed *in situ* experiments with algae-dominated surface samples collected from the Greenland Ice Sheet in the summer of 2021. Pigmented algae-rich snow and ice samples were incubated for four weeks in vented culture bottles on the snow/ice surface and under light and dark conditions. At set periods of time, dissolved and particulate organic carbon (DOC/POC) from replicate bottles were separated and preserved for carbon content and molecular composition analyses via Fourier transform ion cyclotron mass spectrometry. With increasing time, we observed an increase in DOC contents in all glacial ice and snow algae-dominated systems. In the glacial ice algae experiments, under light conditions DOC increased by more than one order of magnitude (from 80 to 950 µM), while in dark conditions the increase in DOC was smaller (up to 550 µM). In the snow algae experiment, the same trend with high DOC being produced under light conditions relative to dark was observed, albeit with a far lower range. The magnitude of DOC increase in light conditions reached 270 µM from an initial 40 µM, while it reached ~ 75µM under dark conditions. The metabolic processes in our experiments mirrored the changes in autotrophic and heterotrophic microbial community composition and activity as was documented in the differences in DOC compositions for the glacial ice or snow-algae (and light *vs.* dark) systems. This work provides first insights into the molecular composition of glacial ice- and snow algae dominated cryogenic systems, and evaluates the role of these algae on the supply of organic species on the Greenland Ice Sheet.

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eP477

Insights into the structure and function of a key phosphatase in the synthesis of the antimicrobial compound roseoflavin

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Roseoflavin is an analog of the B-vitamin riboflavin with anti-microbial properties. The roseoflavin biosynthesis pathway is comprised of four enzymatic reactions facilitated by three enzymes. The second step of the pathway is a dephosphorylation step catalyzed by the phosphatase, RosC that converts AFP (8-demethyl-8-amino-riboflavin-5"-phosphate) to AF (8-demethyl-8-amino-riboflavin). This phosphatase is promiscuous in nature and also carries out the conversion of FMN (Flavin mononucleotide) to riboflavin. Due to the nature of the substrates catalyzed by the enzyme it can be classified under haloacid dehalogenases, which are a class of enzymes hydrolyzing a variety of substrates. We aim to get further insights into the wide array of substrates catalyzed by this enzyme and want to investigate whether the enzyme is able to hydrolyze important substrates like FMN or pyridoxal phosphate, rendering it toxic for the host cell. The *rosC* gene was codon-adapted, expressed in *E. coli* and purified using affinity chromatography. The RosC protein was concentrated and crystallized in presence of AF, which is the product of the dephosphorylation reaction. We identified the important residues in the enzyme which constitute the substrate binding pocket as well as residues in the catalytic pocket. We also observed that a single amino acid mutation at Aspartate166 to Leucine imparts highly toxic properties to the protein. We aim to uncover important compounds that are additionally hydrolyzed in the cell by this protein to get a better overview on this class of enzymes.

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eP478
A timed off-switch for dynamic control of gene expression in *Corynebacterium glutamicum*

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Introduction

Dynamic control of gene expression mainly relies on inducible systems, which require supplementation of (costly) inducer molecules. In contrast, synthetic regulatory circuits, which allow the timed shutdown of gene expression, are rarely available and represent highly attractive tools for metabolic engineering. *Corynebacterium glutamicum* uses the cheap and lignin-derived compounds ferulic acid (FA), vanillin (Van) and vanillic acid (VA) as carbon and energy sources via expression of the *vanABK* operon [1]. This operon is activated by the transcriptional regulator VanR, *in vitro* only by VA, but *in vivo* as well by the pathway precursors FA and Van [2]. Since these compounds are co-metabolized with glucose, this regulatory circuit represents an elegant system to turn-off gene expression when FA, Van or VA are depleted.

Methods and Results

The pyruvate dehydrogenase complex (PDHC) is essential for growth of *C. glutamicum* on glucose and was shown to be an attractive target to improve production of pyruvate-derived compounds [3]. To control expression of PDHC in a VA-dependent manner, we replaced the native promoter of the *aceE* gene, which encodes the E1p enzyme of the PDHC, by *vanR* and a modified P*vanABK* promotor, yielding *C. glutamicum* ΔP*aceE*::*vanR*-P*vanABK** (Cg-Pace). The biomass yield of this strain increased linearly with the added amount of effector. After consumption of the phenolic compounds growth ceased, however, Cg-Pace continued to utilize the residual glucose to produce significant amounts of pyruvate, l-alanine and l-valine. Interestingly, equimolar concentrations of the three phenolic compounds resulted in different biomass yields and increasing effector concentrations shifted the product spectrum from pyruvate over l-alanine to l-valine. To further test the suitability of the VanR/P*vanABK** system, we overexpressed the l-valine biosynthesis genes *ilvBNCE* [4] in Cg-Pace which resulted in efficient l-valine production with a yield of about 0.36 mol l-valine per mol glucose.

Conclusions

As shown in our study, the VanR/P*vanABK** system is a valuable tool to control gene expression in *C. glutamicum* in a timed manner by one of the cheap and abundant phenolic compounds FA, Van and VA.

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eP479
Communities of Niche-optimized Strains (CoNoS) for production of value-added compounds

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Current bioprocesses for production of value-added compounds are mainly based on pure cultures of rationally engineered strains. These strains often possess vast overcapacities of specific central metabolic enzymes, a natural mechanism to cope with rapidly changing environmental conditions. In the comparably well-defined environment of a bioreactor, this additional enzymatic capacity is not required and represents a loss of carbon and energy for production of the desired product [1]. To make these overcapacities available for the production of small molecules, we designed synthetic communities of niche-optimized strains (CoNoS) [2] of *Corynebacterium glutamicum*. A CoNoS consists of at least two strains of the same species, each auxotrophic for one or more amino acids, which save carbon and energy by sharing the available enzymatic capacity for the required amino acids.

Our aim is to generate, evolve and study a stable CoNoS, yielding both knowledge about community evolution and production of the cross-fed amino acids, helping us ultimately to set up a CoNoS that produces a target amino acid more efficiently than pure cultures

Using the pK19*mobsacB* system, five different amino acid-auxotrophic strains of *C. glutamicum* were constructed, set up as synthetic communities, and further studied in a

BioLector microcultivation device and in a microfluidic chamber. Adaptive laboratory evolution (ALE) was performed via a liquid handling robotic system to evolve this community.

By combining two complementary auxotrophic strains, we successfully obtained several functional CoNoS for further characterization and optimization. The optimization includes an iterative process of ALE, evaluation of potentially beneficial mutations, metabolic engineering and process engineering techniques. Additionally, modelling of metabolic networks of co-culture systems helps to identify growth-limiting bottlenecks. We identified beneficial mutations by genome sequencing of evolved strains and are now investigating them further to understand the underlying mechanism.

Our project already demonstrated that it is possible to set up and evolve stable synthetic communities based on the CoNoS principle. Besides analysing relevant mutations future work will concentrate on strain and process optimization to obtain competitive production cultures.

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eP480

Conversion of the solvent producer *Clostridium saccharoperbutylacetonicum* into a butyrate producer

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Introduction & Objectives

For large scale production, butyrate is extracted from animal fats, produced from crude oil, or obtained by microbial fermentation. Using anaerobic non-pathogenic bacteria for fermentation can be considered as the most environmentally friendly and favorable solution as sustainable resources or waste can be used as substrates for highly selective butyrate production without carry-over of harmful chemicals. The hyper-butanol producer *Clostridium saccharoperbutylacetonicum* N1-4(HMT) was used as chassis strain for butyrate production. The aim of this study is to increase butyrate production by *C. saccharoperbutylacetonicum* using carbon neutral substrate containing lignocellulosic sugars.

Materials & Methods

The genes encoding phosphotransacetylase (Cspa_c13010) and butyraldehyde dehydrogenase (Cspa_c56880) were deleted to reduce acetate and alcohol formation. The genes located in the butyryl-CoA synthesis (*bcs*) operon encoding crotonase, butyryl-CoA dehydrogenase with electron-transferring protein subunits α and β, and 3-hydroxybutyryl-CoA dehydrogenase (Cspa_c04330 to Cspa_c04370), as well as the genes in the *ptb-buk* operon encoding phosphotransbutyrylase and butyrate kinase (Cspa_c02520 and Cspa_c02530) were overexpressed to channel the carbon flux further towards butyrate formation. In addition, glucose and ExcelloTM, a substrate yielded from lignocellulosic (i.e. spruce) biomass, were tested for further increase of butyrate production. Deletion of genes were carried out using Biocleave"s proprietary CLEAVETM method. Growth experiments were carried out in serum bottles, whereas fermentations were performed in 1-L fermenters with pH control using either glucose or ExcelloTM.

Results & Conclusion

The native hyper-butanol producer *C. saccharoperbutylacetonicum* was converted into the butyrate producer *C. saccharoperbutylacetonicum* Δ*bld*Δ*pta* [pMTL83151_BCS_P*bgaL*], overexpressing only the genes of the *bcs* operon. Butyrate was found to be the main product with maximum butyrate concentrations of 133 mM and 132 mM, respectively. Meager amounts of by-products were detected. Hence, the hyper-butanol producer *C. saccharoperbutylacetonicum* was successfully converted into a hyper-butyrate producer. By pH control, the butyrate yield was further increased in the fermentations compared to serum bottle batch growth experiments.

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eP482

Metabolic engineering of *Lactiplantibacillus plantarum* as an aroma-adding microorganism in beverage fermentation

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Lactic acid bacteria (LAB) are a group of Gram-positive microorganisms that produce lactic acid as the main product of carbohydrate fermentation. Due to their generally recognized as safe (GRAS) status, LAB are widely used in food industry and production of pharmaceuticals. In beverage production, *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) is used for sour beer fermentation. Previous studies found that the major compounds that contribute to the flavor of beer are the two monoterpenoids linalool and geraniol present in hops. These compounds can also be found in lavender, rose and other aromatic plants, but both the raw material and the extraction process are costly. In this study, we aim to genetically engineer *L. plantarum* WCFS1 as an efficient aroma producer. In contrast to most other bacteria, this organism possesses the mevalonate (MVA) pathway which could produce terpenoid precursors from acetyl-CoA. First, we developed a modular and standardized Golden Gate Assembly-based toolbox for the *de novo* assembly of shuttle vectors from *Escherichia coli* to LAB. The toolbox consists of a collection of the most relevant genetic parts for LAB, including different origins of replication, resistance cassettes for selection and a range of promoters with different strengths. Each genetic part of a plasmid can be exchanged conveniently due to their standardized fusion sites. Next, we introduced four plant-derived linalool and geraniol synthase genes that were expressed under the control of inducible promoters. The production of these two volatiles in *L. plantarum* WCFS1 culture was verified by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS). To boost the supply of monoterpenoid precursors, genes of two rate-limiting enzymes from the MVA pathway of *Saccharomyces cerevisiae* (3-hydroxy-3-methylglutaryl-coenzyme A reductase and farnesyl pyrophosphate synthase) were integrated into the toolbox and coexpressed with linalool/geraniol synthases driven by constitutive promoters. In summary, we have developed a

highly efficient and flexible cloning toolbox for engineering LAB as promising probiotics and biofactories. By fine-tuning their metabolism such as the MVA pathway in *L. plantarum* WCFS1, we hope to engineer LAB as flavor-adding microorganisms in fermented beverages on a laboratory and pilot scale.

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eP483
Isopropanol production using metabolically engineered strains of *Acetobacterium woodii*
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Introduction

Due to shrinking supply of fossil fuels an alternative pathway for production of platform chemicals such as acetone or isopropanol must be developed. Usually, isopropanol is produced from propylene. However, an alternative pathway is the isopropanol production by metabolically engineered strains of acetogens. Acetogens are autotrophic and anaerobic bacteria, which use greenhouse gases as an energy and carbon source. Acetogens form acetyl-CoA from CO2 or CO via the Wood-Ljundahl-Pathway, which can be further processed to products such as acetate or butyrate. The genes *thlA* (encoding thiolase A), *ctfA/ctfB* (encoding acetate/butyrate CoA transferase), and *adc* (encoding acetoacetate decarboxylase) are required to process acetyl-CoA to acetone. In a further step, acetone can be converted by the gene *sadh* (encoding secondary alcohol dehydrogenase) into isopropanol. In this study, the gene *sadh* from *Clostridium beijerinckii* DSM 6423 (CLOBI_40010) and from *Clostridium ljungdahlii* DSM 13528 (CLJU_c24860) were used to produce isopropanol with recombinant *Acetobacterium woodii* DSM 1030 strains. Another secondary alcohol dehydrogenase was found via BLAST analysis in *C. beijerinckii* DSM 15410.

Materials and methods

For isopropanol production, plasmids with the genes *thlA*, *ctfA/ctfB*, and *adc* combined with different secondary alcohol dehydrogenases were constructed. *A. woodii* was transformed with the resulting plasmids pJIR750_ac3t3s1 (containing *sadh* of *C. beijerinckii* DSM 6423), pJIR750_ac3t3s2 (containing *sadh* of *C. beijerinckii* DSM 15410), and pJIR750_ac3t3s3 (containing *sadh* of *C. ljundahlii*) via electroporation. The effect of the plasmids regarding isopropanol production by the newly constructed recombinant *A. woodii* strains was examined by performing heterotrophic and autotrophic growth experiments.

Results and conclusion

Heterotrophic production of isopropanol with recombinant *A. woodii* strains using fructose as substrate was successful. Highest amounts of isopropanol were achieved by *A. woodii* [pJIR750_ac3t3s2] producing 20.44 ± 0.16 mM isopropanol. Acetone was still produced as a byproduct (9.2 ± 0.1 mM). Its conversion to isopropanol would further improve the yield.

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eP484
Identification and conditional deletion of key genes involved in LPS biosynthesis in *Magnetospirillum gryphiswaldense*
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Magnetic nanoparticles are of increasing importance for many applications in the (bio)medical field. Examples include their usage as agents in magnetic imaging techniques or hyperthermia, in both cases requiring particles with stable magnetic moments and a narrow size distribution. However, controlling these parameters in chemical synthesis is still challenging. A promising alternative might be provided by so-called magnetosomes produced by magnetotactic bacteria. The alphaproteobacterium *Magnetospirillum gryphiswaldense* biomineralizes cuboctahedral magnetite nanoparticles enveloped by a phospholipid bilayer. Due to a strictly regulated biomineralization process, magnetosomes exhibit a variety of extraordinary properties, such as high crystallinity, strong magnetization and uniform shape and size [1].

However, due to their bacterial origin, the use of magnetosomes in (bio)medical applications has so far been limited by different toxicity factors. While in cell culture studies when incubated with mammalian cells the particles exhibited only low cytotoxicity and thus, were considered to be biocompatible [2], the endotoxicity might be a challenging issue. During magnetosome isolation, the particles unspecifically bind endotoxic lipopolysaccharides (LPS) of the outer membrane. In initial testings, values ranging from 2.000 to 12.000 endotoxin units (EU) per ml have been determined. Although particle displayed endotoxicity could be reduced to 10 to 100 EU/ml by removal of the magnetosome membrane, the non-enveloped magnetite nanoparticles tended to aggregate and lost their colloidal stability, unfavourable for medical applications [3].

To overcome these limitations, we therefore will first identify key genes involved in LPS biosynthesis in *M. gryphiswaldense*. Genetic engineering is subsequently used to generate (conditional) deletion mutants of selected LPS genes, which have previously been deleted in *E. coli* in order to reduce the endotoxicity [3]. Finally, viable strains as well as their isolated magnetosomes are analysed with regard to their endotoxin content.

Overall, our results will not only delineate a strategy for the generation of endotoxin-reduced *M. gryphiswaldense* strains but also contribute to a better understanding of LPS biosynthesis in a magnetotactic bacterium.

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eP485
Recombinant 1,3-propanediol production using *Clostridium acetobutylicum* ATCC 824
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Introduction

1,3-propanediol (1,3-PDO) can be used for polytrimethylene terephthalate (PTT) production, a biodegradable polyester, utilized e.g. as fibers for carpets or textiles. Thus, the demand of 1,3-PDO is increasing. A biobased production will be desirable. One possibility is the use of microorganisms such as *Clostridium pasteurianum* or *Clostridium beijerinckii*

which can naturally produce 1,3-PDO from crude glycerol. However, these clostridia are difficult to engineer genetically. An alternative is the use of recombinant *Clostridium acetobutylicum* strains. *C. acetobutylicum* is a well-studied organism with many modification tools available. Genes encoding a glycerol dehydratase and a 1,3-PDO dehydrogenase are necessary to produce 1,3-PDO. Since *C. acetobutylicum* lacks genes for those enzymes, genes from *C. beijerinckii* DSM 15410 were used. In *C. beijerinckii* DSM 15410, genes with the locus tags K684DRAFT_00976 and K684DRAFT_00977 encode the two subunits of the cobalamin-independent glycerol dehydratase. Downstream of these genes, the ATP:cobalamin transferase and 1,3-PDO dehydrogenase encoding genes (K684DRAFT_00978 and K684DRAFT_0099) are located.

Material and Methods

For recombinant 1,3-PDO production, the plasmid pMTL83251_Ppta_ack_1,3Diolis containing these genes was constructed using the Ppta_ack promoter from *Clostridium ljungdahlii* for constitutive gene expression. The effect of pMTL83251_Ppta_ack_1,3Diolis regarding 1,3-PDO production by the constructed recombinant *C. acetobutylicum* [pMTL83251_Ppta_ack_1,3Diolis] strain was investigated by performing growth experiments with minimal medium using 100 mM glycerol and 20 mM xylose as substrates. As control, the wild type *C. acetobutylicum* strain as well as the vector strain *C. acetobutylicum* [pMTL83251] were used.

Results and Conclusion

Throughout the growth experiment, glycerol was only consumed by *C. acetobutylicum* [pMTL83251_Ppta_ack_1,3Diolis]. In total, 73 mM glycerol were metabolized and 40 mM 1,3-PDO were produced, resulting in a yield of 0.54 mol mol^{-1}. This result shows that 1,3-PDO production using recombinant *C. acetobutylicum* strains is an alternative to the production with natural 1,3-PDO producers.

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eP486

New insights into the substrate determinants of benzylsuccinate synthase (BSS) by analysing mutated variants *Ammar Alhaj Zein, Kai Krämer, Johann Heider*

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In recent years, anaerobic toluene degradation has been shown to proceed by a common pathway in various unrelated groups of bacteria which couple toluene degradation to anaerobic respiration or fermentation in syntrophic cocultures [1]. The pathway is initiated by (*R*)-Benzylsuccinate Synthase (BSS), which belongs to the family of glycyl radical enzymes and contains a conserved glycine residue close to the C-terminus, which is converted to a glycyl radical moiety by an activating enzyme [1]. The enzyme is a heterohexamer of α2β2γ2 composition and catalyzes the addition of the methyl group of toluene to the double bond of a fumarate cosubstrate to form (*R*)-Benzylsuccinate as first intermediate of the degradation pathway [1] [2]. BSS can be regarded as a prototype for many other fumarate-adding enzymes (FAE) initialising anaerobic degradation of other hydrocarbons, such as xylenes, cresols, methylnaphthalene, or alkanes.

In our study, we established the first recombinant production system for activated BSS and used this system to construct several mutants affected in the active site in the alpha subunit of the enzyme. We show that a single mutation is sufficient to generate a variant with expanded substrate range that converts *m*-xylene in addition to the previously recognized substrates toluene and the cresol isomers [2] [3]. In accordance to the predicted effects, the constructed variant specifically turns over *m*-xylene, but does not convert *o*- or *p*-xylene. In our ongoing study, we are also creating and characterizing additional BSS variants to understand more of the reaction mechanism of BSS and to expand or alter its substrate range for potential applications. Moreover, we are also trying to recombinantly produce and purify other fumarate-adding enzymes for further biochemical and structural characterization.

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eP487

SporoBeads: using the inner and outer coat of *Bacillus subtilis* endospores as a protein displaying platform

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Many bacterial species differentiate into dormant cell types to survive adverse conditions. Upon starvation, the Gram-positive model organism *Bacillus subtilis* initiates the formation of highly resistant and dormant endospores. The core of these spores contain packed DNA, which is protected by the cell wall cortex and three different protein layers named inner coat, outer coat and crust.

Earlier experiments have demonstrated that the crust can be utilized for displaying proteins [1]. Here, we further exploit the potential of the spore envelope as a protein displaying platform by diving deeper into the spore surface and use proteins of the inner and outer coat as anchors. Previous work indicated the endospore proteins OxdD (inner coat) and CotB (outer coat) of *B. subtilis* as promising anchor protein candidates [2], which were therefore analyzed in this study as future targets for protein auto-immobilization inside the endospore coat.

Fluorescence microscopy is chosen to be the first detection method to determine putative anchor proteins on the isolated spores. Initially, the reporter gene *sfgfp* was fused N- and C-terminal to *oxdD* and *cotB* via Gibson Assembly and transformed into *B. subtilis.* These constructs were put under the transcriptional control of two different promoters. The already evaluated crust gene promoter P*cotYZ* , which is active during sporulation, and ensures a proper timing of fusion protein production. Additionally, the strong and constitutive promoter P*lepA* was also evaluated. Initial results reveal the suitability of both OxdD and CotB to act as anchor proteins for sfGFP display, with the former giving rise to a stronger fluorescence signal compared to the latter. In line with previous studies, N-terminal fusions generally resulted in a higher fluorescence intensity, which would make this the best promising fusion side for the putative anchor proteins. Currently we are working on improving the

fluorescence signal of sfGFP-CotB fusions and have initiated studies with additional candidate anchor proteins from the inner and outer coat, using the experience from CotB- and OxdD-fusions as design rules for subsequent constructs.

The results from this ongoing study will lead to a better understanding of the potential of using the endospore envelope as a protein auto-immobilization platform for biotechnological applications.

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eP488

Reactor design for gas-driven production of fine chemicals in *Cupriavidus necator*

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Introduction: Hydrogen gas can be sustainably produced by electrolysis and represents a carbon-free reductant. Knallgas bacteria like *Cupriavidus necator* oxidize H2 and use its electrons to regenerate common coenzymes like ATP and NADH, driving interesting enzymatic reactions. To date, there is only a limited gas fermentation strategy demonstrating this concept1. Chi.Bio's recent development of mini-fermenters2 enables monitoring and automatic control of small-scale fermentations. However, this system is not adequately equipped for gas fermentation.

Objective: We aim to update Chi.Bio to accommodate gas fermentation with predefined gas mixtures. As a proof-of-concept, we autotrophically cultivate *C. necator* to produce *N*-heterocycles from an imine substrate, sustainably driven by H2 gas. This is the first step in our SPP2240 project, which aims to power biosynthesis in whole cells.

Materials & Methods: Chi.Bio consists of a control computer, a pump board, and a 30 mL small-scale bioreactor equipped with various LEDs and spectroscopic sensors that allow fully automated growth and temperature control. Using a 3D-printed lid, we created two inlets and two outlets. Aside from the in- and outlet of media for OD-control, one inlet is used to continuously blow a predefined gas mixture through the media, and the remaining outlet is used as a pressure release.

The gas mixture is continuously supplied from a gas-mixing station, and the partial pressures of each gas added are controlled with special flow meters.

Conclusion: The development mentioned above of Chi.Bio facilitates the setup of a gas fermentation and enables the monitoring of multiple small-scale gas fermentations in tandem to determine strain-specific growth optima. As an outlook, H2 can be produced *in situ* employing an integrated electrolyzer without explosive compartments. Thus, advances in gas fermentation are contributing to advanced electro-powered bioreactors and -refineries.

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eP489

H2-driven production of *N*-heterocycles in *Cupriavidus necator*.

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Introduction

Transition to sustainable energy is one of the great challenges of our society. To integrate CO2 into a bioeconomy process, the MSCA project ConCO2rde aims to harness autotrophic microorganisms such as *Cupriavidus necator* to create efficient biorefineries and transform CO2 into fine chemicals. Under chemolithoautotrophic conditions, *C. necator* grows with CO2 as a carbon source, H2 as an electron donor and O2 as an electron acceptor representing a versatile system for biotransformation. Previously, we demonstrated the *in vitro* synthesis of piperidines via an enzymatic H2-driven cascade [1]. The pathway included an O2-dependent putrescine oxidase, a NAD(P)H dependent imine reductase (IRED) and an O2-tolerant NAD+-reducing hydrogenase for cofactor regeneration. However, to achieve robust autotrophic biotransformations in *C. necator*, expansion of heterologous enzyme production and genetic engineering strategies are necessary.

Objectives

Our objective is to develop *C. necator* as a platform organism, which contains the above described enzymatic cascade for fine chemicals production. For this purpose, heterologous enzyme production in *C. necator* is evaluated by diminishing protein degradation and improving protein folding.

Materials & methods

Strain development was carried out by disrupting the Lon protease with a suicide vector and compared to co-synthesizing the heat-shock GroESL chaperon [2]. Enzyme production and activity was evaluated using spectrophotometric assays and immunoblots [1].

Results

Preliminary results of strain development of *C. necator* and H2-driven whole cell biocatalysis will be presented.

Conclusion

Autotrophic biorefineries are promising sources for chemicals, contributing to a CO2-based bioeconomy. Our approach consists in developing *C. necator* strains for

heterologous enzyme production and in expanding the genetic toolbox towards the synthesis of high-value organic compounds.

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eP490

Bacterial cell size decrease by genetic knockouts

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Bacterial cell division is performed by the assembly of the FtsZ-ring, a process which also determines cell size, and which is coupled to nutrient availability in the environment, crucial for the survival under frequently changing and harsh conditions. From an evolutionary perspective, larger cells are favored as they seem less susceptible towards environmental stresses, which are known to strongly influence cell size and shape. In this study, we aimed for a reduction in cell size to examine effects on growth behavior and cell shape by introducing mutations involved in the synthesis of Uridine diphosphate glucose (UDP-glucose). UDP-glucose is a compound synthesized by the cell from the conversion of glucose to UDP-glucose by two enzymes, a pyrophosphorylase (galU) and a phosphoglucomutase (pgm), and it serves as a proxy for the cell"s nutrient status. The compound itself promotes the interaction of a glycosyltransferase (opgH) with FtsZ, resulting in a delay of the assembly of the FtsZ-ring and therefore larger cells. Under nutrient poor conditions however, opgH favors self-interaction, leaving the FtsZ monomers unhindered, which in turn results in smaller cells. Here, we tested different knockout mutants in the UDP-Glucose synthesis in Escherichia coli and determined changes in cell growth, shape and size. Cells were examined with the Coulter Counter for their size and they were simultaneously observed under the microscope to determine alterations in cell shape while also testing different growth media and growth phases. We found that a single knockout mutant *Δpgm* grown in M9 minimal medium resulted in significantly smaller cells and an altered cellular shape, for which we observed almost coccoid bacteria. We also found that the double knockout ∆*galU*∆*opgH* is unable to grow in M9 minimal medium. Altogether, these results show that single deletions in the UDP-Glucose synthesis pathway cause a decrease in cell size and create rounder cells, which are likely incapable of coordinating cell growth and division with their intracellular nutrient status, rendering these knockout mutants unable to adapt to their environment.

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eP491

Employing Enzyme Engineering in Synthetic One-Carbon Metabolism

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Introduction

The circular carbon economy envisions the assimilation of one-carbon (C1) compounds into value-added products. Of high interest is direct C1-C1 condensation, which arguably offers the most elegant solution for carbon fixation. However, the natural solution space for these reactions is limited, requiring highly complex enzymes.

Objectives

This study aimed to generate a low-complexity C1-C1 carboligase. *Methylorubrum extorquens* oxalyl-CoA decarboxylase (MeOXC) was engineered towards the ligation of the C1 units formaldehyde and formyl-CoA into glycolyl-CoA.

Methods

Variant libraries of MeOXC were generated using an iterative saturation mutagenesis approach. For the screening, a lysate-based high-throughput assay for the detection of glycolyl-CoA was established. Kinetics of the enzymes were determined using HPLC-MS. The final variant was evaluated for the condensation reaction of formyl-CoA with an array of aldehydes. Finaly, variants were tested in whole-cell biocatalysts for the conversion of formaldehyde into glycolate.

Results

The iterative saturation mutagenesis of MeOXC resulted in the final variant MeOXC4 after four rounds of optimization. Compared to MeOXC, MeOXC4 showed an improvement in carboligation by over three orders of magnitude. Condensation of formyl-CoA with all aldehyde substrates tested was improved up to 300-fold between MeOXC4 and wildtype. In a whole-cell system, MeOXC4 outperformed the wildtype and all previously tested alternatives.

Conclusion

By enabling C1-C1 condensation, MeOXC4 closes an important gap in C1 assimilation, allowing the creation of more efficient assimilation pathways. Recently, multiple studies have targeted the interconversion of formate, formyl-CoA and formaldehyde, as well as the downstream channeling of glycolyl-CoA towards central metabolism or value-added products. In this context, MeOXC4 is a versatile tool for applications in C1 metabolism that brings the field a step closer to actualizing novel synthetic C1 fixation pathways *in vivo*.

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eP492

Efficient formatotrophic growth for bulk chemical production in *Cupriavidus necator* via the reductive glycine pathway

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The use of engineered microorganisms can drive the transition to a true circular bio-economy. In fact, by rewiring the metabolism of microbial cell factories, we can propose new-to-industry biorefinery concepts which rely on the exclusive use of renewable energy and CO2 as feedstock. The reductive glycine pathway (rGP) was recently proposed and established in the bacterium *Cupriavidus necator* as the most efficient aerobic assimilation pathway for formate (electrochemically produced from CO2). Although the rGP theoretically outperforms the Calvin–Benson–Bassham (CBB) cycle in terms of biomass yield per formate consumed, the published rGP growing strains have failed to surpass the wild-type yields relying on the CBB cycle. Here, we aim at further increasing the rGP capacity for improved formatotrophic growth, followed by the production of poly-hydroxybutyrate (PHB) and crotonate as proof of principle. For this purpose, we removed the metabolic burden of 2 plasmids expressing the rGP from the *C. necator* strain capable of full growth via the rGP on formate. Then, we integrated the full pathway into the genome of *C. necator* using transposons and selection for pathway activity. Last, we employed adaptive laboratory evolution to improve the strains growth rates and biomass yields on formate. By comparing the gCDW yields per formate consumed of our various engineered rGP strains to the CBB control strain, we proved that our improved rGP strain reaches a biomass yield of 3.3 gCDW/mol formate consumed, while the WT reaches only 2.9 gCDW/mol. In parallel, we assessed the ability of *in vivo* production of PHB from formate, and we demonstrated heterologous production of the bulk chemical crotonate via a thioesterase encoded by *Escherichia coli*"s gene *ydiI*. Synthesis of these two biotechnologically relevant molecules will be first optimized under heterotrophic growth conditions by controlling gene expressions and tackling thermodynamic bottlenecks. Then, the best performing gene variants will be used for formatotrophic growth and production via the improved rGP. Altogether, we are creating an industrially relevant platform strain for the production of many value-added chemicals, and report for the first time the superiority of an "ex-synthetic" pathway over the natural counterpart.

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eP493

The role of Extracellular-Polymeric-Substances in symbiotic host-microbe interactions

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Extracellular-Polymeric-Substances (hereafter EPSs) are a wide range of compounds, which mainly consist of different types of complex carbohydrate-containing macromolecules. EPSs are synthesized and secreted by several strains of microorganisms. Microbial EPS fulfill diverse tasks, for example, adhesion to surfaces, biofilm formation or protection against environmental cues such as salinity and drought. Furthermore, they are key players in the interactions within microbial communities and in host-microbe associations. Studies have revealed the diverse role of EPS in these inter-species interactions. In the nitrogen-fixing symbiotic interaction of diverse *Legume* plant species with members of the *Rhizobia* genus, EPSs influence the establishment and maintenance of nodule formation and further symbiotic interactions. Lipopolysaccharides produced by the human pathogen *Neisseria meningitidis* contributes to severe symptoms of meningococcal disease, including induction of septic shock and mechanisms by which the bacterial cells can evade the host anti-inflammatory response. Various enzymes and proteins are necessary for the synthesis, polymerization, transport, and release of EPS. In the last decades, several studies revealed different molecular mechanisms for EPS synthesis and export.

In this project I will investigate the molecular mechanisms of EPS synthesis of the symbiotic bacterium *Curvibacter* sp. AEP1-3 (hereafter *Curvibacter*), which is the main bacterial colonizer of the freshwater polyp *Hydra vulgaris* (hereafter *Hydra*). *Curvibacter* is a rod-forming β-proteobacteria that homogeneously colonizes the outer ectodermal cell layer of *Hydra*, which consist of a loose meshwork of glycoproteins with properties like the mucus in the mammalian colon. Based on a reciprocal BLAST analysis with high quality genomes of the NCBI reference sequence database, I have identified a set of *Curvibacter* sequences which are orthologous to known genes relevant for synthesis and export of lipopolysaccharides. Interestingly, genes encoding the export and chain-length determination of lipopolysaccharides show a conserved syntenic pattern throughout different species of β-proteobacteria. These findings provide a great starting point for the design of *in vitro* and *in vivo* experiments for the analysis of the influence of EPS on symbiotic interactions between *Hydra* and *Curvibacter* and set the stage for synthetic projects aiming the establishment of artificial symbiosis.

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eP494

Metabolic burden in *P. putida*: trade-off between transcription and translation during heterologous protein production

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The genetic modification of microbial cells to harbor heterologous genes is an important driver for metabolic engineering. Yet, the overproduction of heterologous proteins is often limited by the cellular capacity to distribute resources. The resources, normally used by the host organism for cell growth and maintenance, are also needed for the heterologous gene expression. Once resources are heavily drained toward heterologous genes, the cell experiences additional stress, often reflected by decreased cell growth and increased mutation rates, known as *metabolic burden.* The gram-negative bacterium *Pseudomonas putida,* is considered a promising workhorse in synthetic biology and biotechnology, due to its highly versatile metabolism and tolerance to toxic compounds. We constructed a *P. putida* strain that experiences metabolic burden when the fusion protein MBPeGFP is produced. Additionally, we implemented genetic tools to inspect resource allocation, transcription and translation during metabolic stress. By varying the inducer concentration, introducing a stabilizing mRNA stem-loop upstream of the RBS sequence, and by quantifying mRNA via qPCR, we analyzed the cell"s response to metabolic burden from a transcriptional perspective. By varying RBS strength, we also had a look at the cell"s response from a translational perspective. Strains were grown in M9-Minimal medium and glucose, while optical density and fluorescence were measured *in vivo* in a microplate reader. Red fluorescence reflected the native expression via the genomically integrated *mCherry*, while green fluorescence reflected the heterologous expression via the plasmid based *eGFP.* The MBPeGFP producing strain showed a production rate of 491.17 ± 5 [RLU/(OD*h)]. Compared to controls, it showed a prolonged lag phase (13 h), a statistically significant decrease in growth rate (0.2 h^{-1}) and a 2.3 fold decrease in mCherry production rate; all indicative of metabolic burden and a resources shift. Inducer variation revealed the K_m to be at 16 ?M. The introduced stem-loop led to a 107 fold

increase in mRNA transcript in consequence to the prolonged mRNA lifetime. A stronger RBS did not increase the MBPeGFP production rate, indicating a threshold in the translation machinery. These genetic tools give us insight into the cellular resource distribution during metabolic burden and a means to tune heterologous gene expression at transcriptional and translational level in the future platform organism *P. putida.*

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eP495

Nanopore Sequencing workflow for quick and cost-efficient amplicon, plasmid, and library analysis

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Introduction:The increased complexity of DNA constructs and the ability to work with large, pooled libraries in Synthetic Biology workflows makes it challenging to perform sequencing validation in an inexpensive manner Sanger sequencing currently leads sequencing validation approaches, but is outperformed by short-read Next Generation Sequencing (NGS) in terms of cost. However, short-read based NGS struggles to analyze repetitive sequences and needs substantial investments into equipment. In contrast, long-read sequencing technologies have become prominent in recent years. Particularly, Nanopore Sequencing has the advantage of not relying on substantial investments into equipment, allowing users to perform these experiments in standard molecular biology laboratories – even remotely. Highly multiplexed approaches on single Nanopore Sequencing flow cells allow broad applications for sequencing of different types of DNAs in parallel, such as plasmids and PCR amplicons. We established multiplexing procedures coupled to laboratory automation to make the sequencing of amplicons and plasmids a cost-efficient routine application.

Methods: Multiplex dual-barcoded approaches are established for amplicon sequencing or transposition based experiments. We adapted our protocols to laboratory automation by performing reactions in ultra-low volumes. Barcoded DNA is subsequently used for library generation and sequencing on different types of flow cells using MinION devices. A downstream workflow for quick validation and data analysis is built based on existing tools.

Results: We have established a robust workflow for amplicon sequencing of hundreds of constructs based on a flexible dual-barcoding strategy. In parallel, we are adapting a transposase-based procedure for a highly multiplexed whole plasmid sequencing approach to sequencing hundreds of plasmids. Finally, we apply Nanopore Sequencing to routinely sequence microbial genomes in a cost-efficient manner, usually resulting in a single contig for each chromosome.

Conclusion: Synthetic biology creates large amounts of DNA constructs that need to be verified. However, standard Sanger Sequencing is not cost-efficient for high-throughput applications. Here, we show that highly multiplexed sequencing of amplicons and whole plasmids can be achieved *in house* with minor equipment and resource investment.

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eP496

Building DNA up to chromosome size with a modular, scarless cloning system

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Building synthetic chromosomes has so far focused on re-writing natural chromosomes instead of building them from scratch. Despite the drop in DNA synthesis costs the price of mega-base DNA fragments is still out of reach for most laboratories. This limits the explorative space to build large libraries of synthetic chromosomes, many of which may not be viable, but this data would bring us closer to understand life. A combination of laboratory automation and modular cloning would allow for the construction of many variations of synthetic chromosomes. The main drawback of most modular cloning systems is the four base-pair fusion sites resulting in little scars within transcription units, which ideally should be avoided.

Methods: We optimized a golden gate standard based on the SapI restriction-enzyme. This allowed scarless cloning within transcriptional units, setting ATG and TGA as CDS fusion sites for promoter and terminator sequences respectively. Additional fusion sites were tested to achieve precise and efficient transcriptional unit assembly. The cloning procedure was made compatible with laboratory automation to enable quick building of large DNA stretches based on domesticated parts.

Results: Testing was based on the successful assembly of a fluorescence reporter gene, allowing quick readouts to test protocol optimizations. Reducing the fusion site from four to three base-pairs, a result of the switch from standard type-IIs enzymes to SapI, potentially reduces assembly efficacy. However, in our standard 3-part transcriptional unit assembly the correct assembly rate was up to 100% and on average 62% in our 5-part assemblies. We coupled part verification to an *in house* Nanopore Sequencing workflow, which allowed to quickly verify hundreds of constructs at low cost. Laboratory automation was used to build libraries of standardized parts, as well as for their validation and subsequent assembly into transcriptional units and finally concatenation of these into large units.

Conclusion: Modular cloning may be one of the most suitable techniques to construct highly diverse sequence libraries for the construction of large DNA stretches and even synthetic chromosomes. In combination with laboratory automation this technique allows to study not a single, but hundreds of large DNA variations or synthetic chromosomes. Here we present our scarless cloning strategy and automation pipeline, which is scalable and applied for prokaryote and eukaryote organisms.

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eP497

Role of siderophores in public good-based interspecies communication within microbial communities

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Microbial communities exist in almost any habitat worldwide. They play a superior role in essential biological processes like detoxification, food production and pathogenesis. Social interaction and interspecies communication can strongly affect the dynamics and behavior of such a microbial community. For these processes, the exchange of beneficial compounds, also referred to as public goods, plays an important role. We established synthetic microbial model

consortia to gain deeper insights into the role of iron-scavenging siderophores as public goods for interspecies communication. One of these consortia consists of *Pseudomonas putida* and *Rhodobacter capsulatus*. When confronted with iron limitation, *P. putida* synthesizes the fluorescent siderophore pyoverdine, which can efficiently bind ferric ions (Fe^{3+}). When released to the environment, this public good enables access to ferric ions as a strictly limited resource for all community members that are capable to specifically import pyoverdine. As indicated in preliminary bioinformatics analysis, the genome of *R. capsulatus* harbors copies of putative pyoverdine receptor genes, however corresponding siderophore biosynthesis genes could not be identified. Thus, *P. putida* and *R. capsulatus* might be suitable candidates for analyzing the production and utilization of a public good. To determine the function of pyoverdine in interspecies communication, we first analyzed cell growth of *P. putida* and *R. capsulatus* in different single and co-cultivation setups. We could demonstrate that growth of *R. capsulatus* is strongly impaired in iron-deficient medium but could be restored either by adding cell-free pyoverdine-containing supernatant of *P. putida* wild-type cultures or in respective co-cultivation experiments. Our findings indicate that pyoverdine can act as a public good in this microbial model consortium. Based on these observations, we started to dynamically perturb different siderophore-based interactions in order to further study the underlying interorganismal iron signaling networks.

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eP498
The future of bacterial databases
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Introduction

The exponentially growing amount of biological data in recent years has posed the challenge of how to organize this data in a comprehensive way. As more and more information is amassed by high-throughput analyses, these data must be presented in an intuitive way to help scientists to develop novel hypotheses. This is especially true for the research on model organisms, which are intensely studied and representative for larger taxonomic groups. Model organism databases therefore aim to integrate data from various sources in a comprehensible manner. *Subti*Wiki is currently the most complete freely accessible database on *Bacillus subtilis*, the Gram-positive model bacterium [1]. It features up-to-date manually curated data, and its user-friendly presentation is well liked in the scientific community. Developed by our group in 2009, *Subti*Wiki has grown in data and features ever since, and covers the functional requirements of a general model organism database very well.

Objectives

The further development of *Subti*Wiki is currently held back in some ways by the underlying web framework with its inflexible structure and limited functionality. This impairs the development of new features. Here, we present a new framework to support the *Subti*Wiki website in the future. This framework aims to increase the ease of implementing completely new features such as interaction between different classes of biological molecules. With this, the novel implementation can serve as a general framework for databases on any bacteria.

Methods

Flask, a well-established backend framework, and several of its extensions were employed to replace the current *Subti*Wiki implementation.

Results

The fundamental changes in data organization and the structure of the backend provide a substantial advancement of maintainability which was a major goal of this work. Additionally, some elements of the user interface were enhanced, such as the editor used to modify data on gene/ protein pages. Its improved usability could encourage more community contribution in the future. We will make the framework available as open source. This will allow the scientific community to adapt the framework to their own needs. Our database framework thus provides a foundation for new model organism databases to come.

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eP499
Microbial production of indole via bacterial TSA and plant IGL
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Indole is a typical nitrogen heterocyclic aromatic compound widely present in food products and natural environment. Indole is of pharmaceutical industry relevance since indole moiety occurs in a wide variety of compounds that possess wide spectrum of biological activities such as anticancer, antiviral, antimicrobial, anti-inflammatory, anti-HIV, and antidiabetic (de Sa Alves et al., 2009). Microorganisms are able to produce indole by tryptophanase reaction with L-tryptophan as the substrate while several plants were shown to synthesize indole in the presence of indole-3-glycerol phosphate (IGP) by the activity of indole-3-glycerol phosphate lyase (IGL), an enzyme first discovered in maize (Frey et al., 1997). The L-tryptophan biosynthetic pathway is another source of indole in both microorganisms and plants. Tryptophan synthase (TS) encoded by *trpA* (TSA) and *trpB* (TSB) form a heterocomplex which catalyzes the hydrolysis of IGP to indole and the subsequent condensation of indole and L-serine to form L-tryptophan. The intermediate indole, however, is not released from the TS complex but instead is transferred from the α-subunit to the β-subunit sequentially. An efficient fermentative indole production from glucose by metabolically engineered *Corynebacterium glutamicum* harboring either TSA or IGL metabolic pathway is described in this study. Furthermore, since the TSA homologs, BXs and IGLs could directly catalyze indole production independent of TSB, complementation experiments were performed to determine if the said enzymes can complement L-tryptophan auxotrophy in a *trpA C. glutamicum* mutant. The metabolic engineering strategy developed here is helpful in the exploration of TSA enzymatic capability and can be useful for the production of indole analogues in the future.

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eP500
On the flexibility of the cellular amination network
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Ammonium is essential to make all nitrogenous cellular compounds. As ammonium assimilation requires concomitant glutamate formation, we created an *Escherichia coli* strain lacking glutamate biosynthesis enzymes as platform to study and engineer the amination network. This enabled us to systematically analyze which amino acids serve as amine source for glutamate production and hence rescue growth. We found that amino acids which either serve as substrate for glutamate producing transaminases or are converted to glutamate complement the phenotype. Additionally, we engineered utilization of the previously non-complementary amino acids β-alanine, l-alanine, glycine and l-serine as amine source by transaminase overexpressions and, if necessary, removal of intracellular amino acid sinks.

Finally, we successfully replaced canonical ammonium assimilation via glutamate with aspartate or leucine producing ammonium fixation routes. Our study thus reveals the high flexibility of the amination network, both in terms of transaminase promiscuity and adaptability to new connections and ammonium entry points.

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eP501
Metabolic characterisation of multiple strains of *Staphylococcus aureus*
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Staphylococcus aureus is a human commensal bacteria, living in up to 30% of the population harm-free. However, in a number of cases, this can lead to opportunistic pathogenic infection that causes serious harm. Among the diseases caused by *S. aureus* are pneumonia, bacteremia, toxic shock syndrome and meningitis. *S. aureus* has several antibiotic resistant strains, including methicillin resistant *Staphylococcus aureus* (MRSA), which causes around 10 000 deaths per year in the USA alone. We will use genome-scale metabolic models (GEMs) to better understand the mechanisms behind *S. aureus* virulence. In recent years, metabolic modelling has proven an effective tool for prediction of host-pathogen interactions, identification of novel drug targets, and analysis of synthetic lethality. With this in mind, we will build GEMs for a range of *S. aureus* strains which have readily available fully sequenced genomes. We will use the networks to calculate the metabolic scopes of different strains following a network expansion algorithm, as well as to determine gene essentiality. Further to this, characterising strains by similarities in their physiological properties, for example antibiotic resistance and virulence, and investigating whether this can be related to similarities in the metabolic capabilities will shine light on causes for highly virulent and resistant strains. Our work highlights the need for a broader understanding of the metabolic pathways of different strains of *S. aureus*, and provides a starting point for further inspection.

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eP502
The Pulse-Width Modulation Model of Periodic Gene Transcription
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Periodic transcript abundances of large gene groups are observed in a variety of contexts, for example as an output of circadian clocks throughout all domains of life or during ultradian oscillations in budding yeast continuous culture at periods ranging from 40 min to 12 h. However, in all these contexts recent proteomic data showed that these periodic dynamics on transcript level are not reflected on protein level due to long protein half-lives. This raises the question whether periodic transcript abundances play any functional role or are just an inconsequential side product of general circadian or ultradian physiology.

Here, I present a mathematical formulation of the pulse-width modulation (PWM) interpretation of the phenomenon. Varying the relative lengths of distinct phases of a circadian or ultradian cycle can continuously modulate steady-state protein abundances. In budding yeast this model can predict the observed non-linear dependence of oscillation period on growth rate, as well as the near-linear correlation of the ribosomal biomass fraction with growth rate (cf. growth laws, resource allocation). I suggest that PWM is an evolutionary ancient and global mechanism of gene regulation, where gene expression of two distinct groups of genes, e.g. GC-rich vs. AT-rich, can be tuned to adapt growth to seasonally changing diurnal conditions and nutrient supply.

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eP503
Computational combinatorial analysis of carbon fixation pathways
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Efficient C1 and CO2 fixation is a crucial step for establishing a circular bioeconomy. As such, improving existing carbon fixation pathways has become a major goal of synthetic biology, with several novel pathways engineered in recent years. This effort has yielded enzymes that catalyze new-to-nature reactions, expanding the available metabolic design space; so that combinations of existing pathways could lead to new cycles with favorable characteristics. Here, we systematically generated and analyzed *in silico* the pathway combinations of a set of natural and artificial aerobic carbon fixation pathways.

The analysis started from a manually curated model with kinetic and thermodynamic data. A network analysis, enumeration of elementary flux modes, was used with the goal of generating all possible pathways arising as a combination of reactions in the model. These combinatorial pathways were then evaluated *in silico*. First, they were filtered for thermodynamic feasibility using the Max-min Driving Force algorithm[1]. Based on previous work from our group[2], we then compared the pathways according to their

predicted product-to-substrate yield and specific activity, as calculated with the Enzyme Cost Minimization algorithm[3].

Most combinatorial pathways found were thermodynamically infeasible. Among the feasible solutions, we describe some variations of carbon fixation pathways with a predicted specific pathway activity comparable to existing natural cycles—the MCG-TaCo cycle and the MOG bicycles. This highlights the potential of "mix-and-match" approaches for searching the metabolic design space for new solutions, when used in conjunction with a systematic analysis approach for pathway comparison.

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eP504

A robot-assisted phenotypic screening for high-throughput studies on pH-homeostasis in *Escherichia coli* using the pH sensitive biosensor mCherryEA

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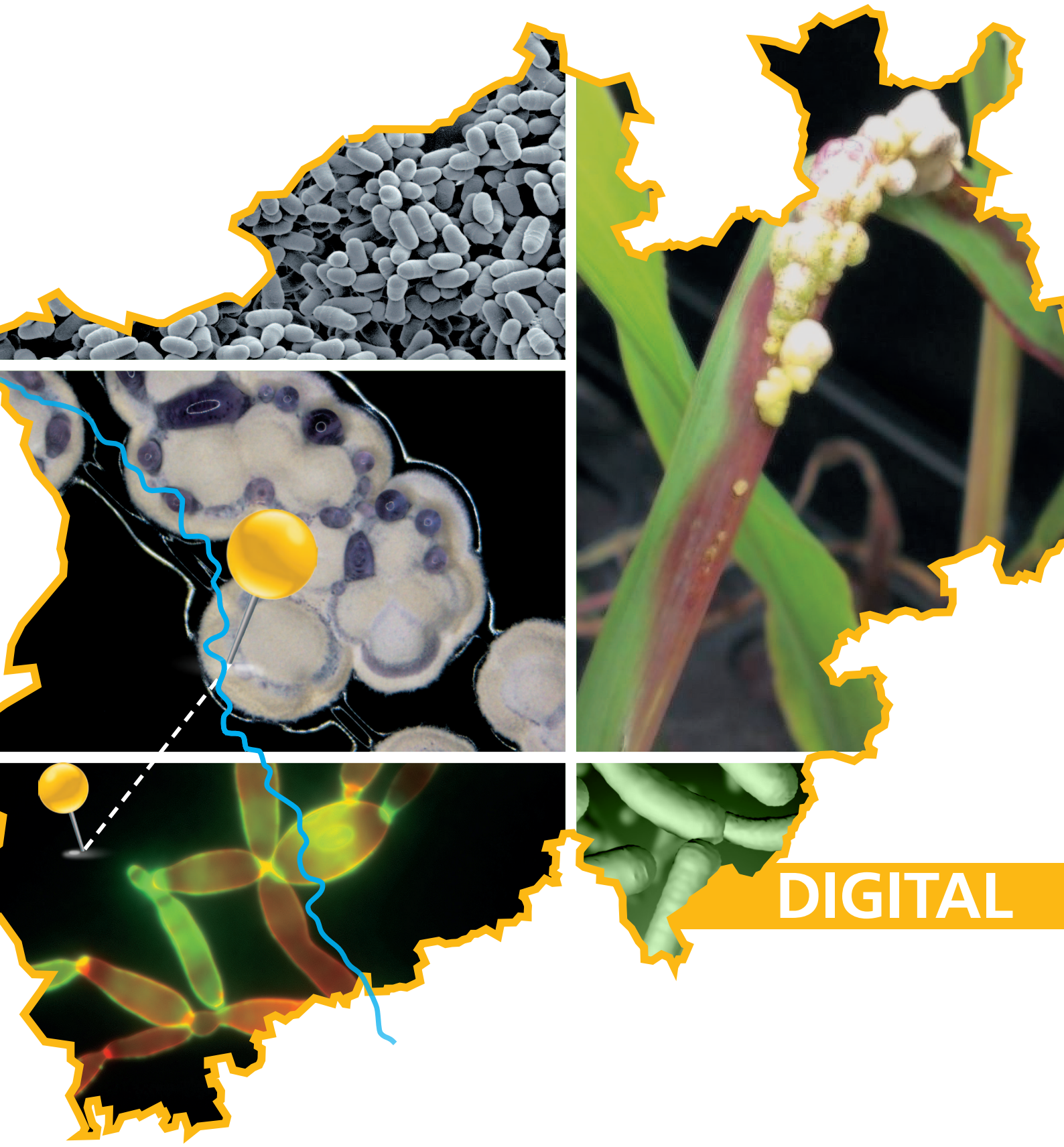
Phenotypic screening is a commonly used strategy in order to analyze particular variants from either rationally or randomly generated strain libraries. However, strain handling and analyses of these libraries can be laborious when conducted manually. For this, robot-assisted workflows are an adequate tool to screen in a high-throughput manner. Ideally, the trait of interest can be coupled to an easy detectable readout such as growth, colorimetric or fluorescence intensity changes. Genetically encoded biosensors enable to assess intracellular changes not only of target metabolites but also of more general parameters like oxidative stress, DNA damage or pH. Many processes and regulators underlying pH-homeostasis have been identified via phenotypic screening of strain libraries towards non-growth at low or high pH values. Nevertheless, direct screens with respect to changes of the internal pH in mutant strain collections are limited by laborious methods including fluorescent dyes or radioactive probes. In this study, we used the pH-sensitive mCherry variant mCherryEA as ratiometric pH biosensor. For our screening workflow, we combined imaging technologies with robot-assisted colony picking and spotting. We visualized the internal pH of *E.coli* mutants from a small transposon mutagenesis derived library via arrayed colonies on agar plates using a Gel-Doc Imaging System. The investigation of mutants, which showed an altered internal pH revealed that the transposon was inserted into genes known to be associated with pH-homeostasis and pH stress adaptation. This screening approach demonstrates that sensor-based analysis of arrayed colonies on agar plates is a sensitive approach for a fast identification of genes involved in the pH-homeostasis or pH stress adaption in *E. coli*.



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