JAHRESTAGUNG

ANNUAL CONFERENCE

OF THE ASSOCIATION FOR GENERAL AND APPLIED MICROBIOLOGY **2025**

23–26 MARCH 2025

BOCHUM | GERMANY

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ABSTRACTS

Content

Plenary Lecture

PL-04
Enzyme Discovery, Engineering and Applications in Biocatalysis
*U. Bornscheuer[1]
[1]*University of Greifswald, Institute of Biochemistry, Dept. of Biotechnology & Enzyme Catalysis, Greifswald, Germany*

This lecture will cover our recent achievements in the discovery, protein engineering and application of enzymes in biocatalysis [1].

For the conversion of complex polysaccharides from marine algae, we have discovered a new class of P450 monooxygenases from marine bacteria, which play a central role in the demethylation of porphyran [2]. Furthermore, we identified the entire degradation pathway of the complex algal carbohydrate ulvan involving >13 different enzymes from marine bacteria [3] and we could show that *Bacillus licheniformis* can grow on these ulvan hydrolysates [4].

For the recycling of plastics, we have initially studied PET and created different improved esterases [5]. Recently, we established a protocol enabling a fair comparison of different PETases reported in literature [6]. Moreover, we have identified the first three urethanases in a metagenomic library able to depolymerize polyurethanes [7]. We determined their X-rays structures and improved them by enzyme engineering [8]. We also established enzyme cascade reactions to degrade poly(vinylalcohols) [9] and more recently also the challenging polyethylene [10].

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Invited Lecture – Fachgruppen

Evolution of regulation

IL-FG-R-03
The role of genetic and environmental factors in driving gene regulatory rewiring patterns
*T. Taylor[1]
[1]*University of Bath, Life Sciences, Bath, United Kingdom*

How does novelty arise in evolution? Is the process solely based on chance or might we be able to discern rules for the evolution of innovation? Are there genetic or environmental factors that might predispose particular adaptive routes over others to the evolution of novelty? Work from my lab addresses these questions using a combination of molecular genetic manipulations and experimental evolution, within the context of gene regulatory networks, using a bacterial model system. We have shown that pre-existing gene regulatory network structures bias repurposing of certain transcription factors by facilitating promiscuity. And that, transcription factor activity (and by extension the environment) has the potential to drive adaptive potential for regulatory rewiring and innovation. These insights advance our understanding of the evolutionary accessibility of particular transcription factors for innovation, with broad implications for understanding and predicting bacterial adaptation to novel environments.

Symbiotic interactions

IL-FG-SI-07
Form and function of arbuscular mycorrhiza symbiosis
*C. Gutjahr[1]
[1]*MPI of Molecular Plant Physiology, Root Biology and Symbiosys, Potsdam, Germany*

Most land plants form symbioses with *Glomeromycotina* fungi to acquire mineral nutrients from the soil. This so-called arbuscular mycorrhiza (AM) symbiosis is evolutionarily ancient and found in the oldest fossils of land plants, sparking speculations about its importance in the colonization of the land by originally aquatic plants during evolution, at a time when plants had not yet evolved complex root systems for nutrient uptake. The fungi form extended hyphal networks in the soil to scavenge mineral nutrients. These are transported into the root and released via beautifully-shaped, highly branched hyphal structures, the arbuscules inside inner root cells. In return they receive up to 20% of photosynthetically fixed carbon from their host in the form of sugars and lipids. As a consequence, AM contributes significantly to plant nutrition and to global carbon cycles.

For symbiosis establishment, AM fungi colonize the root interior and the inside of plant cells. Symbiotic infection of single, already differentiated cells within the tissue context requires a poorly understood cellular remodeling program that is intertwined with mechanisms that control plant development and physiology. In my presentation, I will provide examples of how we investigate the molecular mechanisms underlying development and functioning of this fascinating symbiosis.

IL-FG-SI-08
Structural and metabolic connection in planktonic photosymbiosis
*J. Decelle[1]
[1]*CNRS, Cell and Plant Physiology, Grenoble, France*

Single-celled marine plankton can establish various kinds of symbioses to gain energy. Plastid symbiosis, whereby host cells temporarily integrate microalgal cells (photosymbiosis) or just their photosynthetic plastids (kleptoplastidy) as intracellular solar-powered carbon factories, is a key interaction in worldwide surface oceans. Plastid symbiosis was at the origin of a major evolutionary innovation that spread photosynthesis across eukaryotes, transforming the biosphere. Despite this ecological and evolutionary importance, very little is known about how a photosynthetic machinery is structurally and metabolically integrated into a host cell and what mechanisms allow cells to transport carbohydrates, the main photosynthetic product and energetic currency. During this presentaiton, I will share the subcellular imaging and molecular approaches we are using to decipher this complex interaction at different scales. We will focus on the morphological changes of the photosynthetic machinery as well as the transportome remodeling of the symbiotic microalga within a host. Crossing boundaries between structural biology, eco-physiology and evolution, we have the ambition to resolve fundamental mechanisms in widespread planktonic symbioses.

IL-FG-SI-09
How do zombie fungi adaptively hijack insect host behaviour? A molecular ecology perspective
*C. de Bekker[1]
[1]*Utrecht University, Biology, Utrecht, Netherlands*

The evolutionary arms race between parasites and their hosts can culminate into complex extended phenotypes that further disease progression and transmission. The fungus-adaptive changes in behaviour as seen in *Ophiocordyceps*-infected carpenter ants are a prime example. These "zombie ants" demonstrate a suite of behaviours that are thought to circumvent the colony's social immune responses. Subsequently, the hijacked ant climbs and attaches itself to an elevated position that benefits fungal spore development and dispersal, and does so with a precise daily timing. These fungus-induced behaviours are not unique to *Ophiocordyceps* infections. Parallel behaviours have also been observed in invertebrates infected by other fungi, viruses and trematodes. The precise mechanisms involved in these behavioural manipulations are unknown. To unravel these mechanisms, we have developed the *Ophiocordyceps*-ant interaction into an integrative model system that allows us to study parasitic behavioural manipulation in greater detail in the lab. By combining fungal culturing and lab infections with behavioural assays and multiple omics approaches, we propose several comprehensive mechanistic hypotheses about the fungal proteins and ant receptors involved in this phenomenon. These hypotheses include specific fungal "manipulation" effectors of interest and their potential binding to ant proteins involved in light perception, biogenic amine binding and daily rhythms. To test these hypotheses, we are currently, for the first time in this model, integrating functional genetics assays to determine the function of presumed fungal "manipulation" effectors, the host behaviours they elicit, and the host pathways underlying those phenotypes. Our results will provide detailed insights into fungus-insect interactions in general while advancing our understanding of parasitic hijacking of animal behaviour in particular. Moreover, the evident role of biological clocks in these interactions paves the way to study antagonistic organismic interactions at their intersection with the field of chronobiology. We expect our findings to have a translational impact on more sustainable insect pest control practices while our investigations into fungal bioactive proteins and metabolites and their host targets might give rise to the discovery of novel drugs.

IL-FG-SI-10
Enzyme donation by widespread *Rickettsiales* in protists and simple aquatic invertebrates
*H. Gruber-Vodicka[1]
[1]*Christian-Albrechts-University, Zoological Institute, Kiel, Germany*

Rickettsiales are intracellular bacteria that depend on their eukaryotic hosts" resources for their metabolism. One clade of aquatic *Rickettsiales* of the family *Midichloriaceae* is among the most ubiquitous bacterial phylotypes in aquatic samples, occurring in almost 10% of all aquatic samples. With a large host diversity of protists and early branching animals such as sponges, placozoans, and cnidarians, these Midichloriaceae are regarded mainly as parasites or pathogens and were linked to coral disease outbreaks.

Here, I will present evidence for a surprising and highly beneficial role for these intracellular symbionts that inhabit the endoplasmic reticulum in most of their hosts - digestive enzyme donation. Across all host groups, data from genomic, expression, enzyme characterization, and imaging experiments show that these ubiquitous aquatic *Midichloriaceae* provide enzymes to break down sugar-based macromolecules. Based on the characterization of heterologously expressed enzymes, the symbionts provide digestive enzymes for laminarin, one of the most abundant macromolecules in aquatic systems. In addition, our data show an anti-microbial role, as one broadly-expressed enzyme can attack the cell walls of bacteria. Using in-depth tree-reconciliation analyses, I will discuss the evolutionary chains of events that likely led to the taming of notorious parasites into important intracellular symbionts that can turn dietary fibers into efficient metabolic resources for sugars. These insights into the beneficial roles of a group of symbionts labeled parasitic because of their intracellular lifestyle will provide a better understanding of these common symbioses that can alter physiology, ecology, and gene flow from within host cells. Given their broad host range and geographic distribution, these aquatic Midichloriaceae are at the center of many current frontiers of aquatic sciences on a changing planet, such as biomonitoring and conservation efforts, aquaculture, and the aquarium trade.

Environmental microbiology

IL-FG-EM-04
Genomics-based insights into evolution and ecology of marine microbes and their viruses
*C. Rinke[1]
[1]*University of Innsbruck, Mikrobiologie, Innsbruck, Austria*

The wealth of microbial genomes recovered over the last decade has had profound implications for our understanding of microbial evolution, classification, and ecology, in the marine environment. For example, we used evolutionary relationships to develop a rank normalised taxonomy, the

Genome Taxonomy Database (GTDB), that provides objective taxonomic assignments for bacterial and archaeal genomes. With genome sequences becoming the main currency of microbial classifications, changes in nomenclature were required as well, leading to the launch of the SeqCode based on genome sequences as nomenclatural types. Genomes of uncultured lineages have enabled deep phylogeny and the proposal of many new lineages, including the phylum Asgardarchaeota, that has changed our view of the domains of life. Microbial genomics has also advanced microbial ecology, e.g., in combination with machine learning approaches, we recovered microbial indicator taxa and could infer their ecological roles. Finally, the wealth of microbial genomes has improved the identification of relationships between viruses and their archaeal and bacterial hosts, leading to the discovery and characterisation of novel viral taxa.

Bioinformatics meets microbiomes

IL-FG-FGM-05
Providing access to MGnify for microbiome data discovery and interpretation: challenges, successes, and future directions
*L. Richardson[1]
[1]EMBL-EBI, Coordinator for EMBL-EBI's Microbiome Resources, Hinxton, United Kingdom

The MGnify resource hosts one of the largest collections of publicly available assembled metagenomes, and an associated database containing billions of derived proteins. In recent years, MGnify has expanded its offerings to include biome-specific catalogues of metagenome-assembled genomes (MAGs), derived from these assemblies as well as community-contributed MAGs. These catalogues are providing valuable insights into microbial diversity across various environments and host-associated ecosystems, including those of humans.
In this presentation, I will discuss the scientific considerations, technical advancements, and challenges encountered in making these data and services accessible to the research community. Particular focus will be placed on the efforts to ensure the provenance and transparency of our data products. Finally, I will explore the evolving landscape of microbiome research and the pivotal role MGnify will continue to play in advancing scientific discovery and collaboration.

Quality management

IL-FG-QM-15
Antimicrobial efficacy and application of disinfectants
*K. Steinhauer[1,2,3]
[1]bactologicum GmbH, Itzehoe, Germany
[2]University of Applied Sciences Kiel, Kiel, Germany
[3]VAAM-Fachgruppe Qualitätsmanagement, Frankfurt a.M., Germany

In clinical set-ups as well as in the production environment, e.g. in pharmaceutical or food production, hygiene is of outmost importansce.When confronted with pathogens or spoilage agents, especially if they are not yet well studied, effective hygiene protocols are needed. In the medical field, effective preventive measures are key to prevent vulnerable patients from infections. In production areas, effective hygiene measures are needed to protect goods from spoilage or microbial contamination.

The broad portfolio of standards/laboratory tests, to substantiate activity claims for chemical disinfectants addressing the special features of applications of antimicrobial formulations will be explained. Furthermore, the concept of using standardized surrogate test organisms is illustrated, and the European standardized test approach to claim microbicidal and virucidal efficacy, the specificity of claims and their relevance for hygiene measures are introduced.

IL-FG-QM-16
Pulsed Light: An important advance in Pre-fill Sanitization
*P. Schullerer[1]
[1]Polytec GmbH, Vertrieb Photonik, Waldbronn, Germany

Safety is important in all food industries, and that"s especially true in the dairy business. Yogurt, as example, is highly susceptible to contamination by microorganisms that can come from various sources, including the equipment used in production. This makes pre-fill sanitization of yogurt cups a crucial step in a safe production process. It eliminates potential contaminants in the yogurt cup right before filling, ensuring that the yogurt remains safe for consumption and complies with safety standards.

The talk will shed light on the sanitization of surfaces using pulsed Xenon light. It will illuminate the advantages and discuss some limitations.

IL-FG-QM-17
Plasma sterilization of thermolabile materials
*P. Awakowicz[1]
[1]Ruhr-Universität Bochum, Lehrstuhl für Angewandte Elektrodynamik und Plasmatechnik AEPT, Bochum, Germany

The motivation for new sterilization methods is based on the treatment of thermolabile materials like food packing materials, specific implant materials like UHMWPE, pharmaceutical tubs for syringe packing and much more. Autoclaving is not always possible, due to heat limitations or time restrictions. Ethylene oxide is toxic, mutagenic and carcinogenic, furthermore it is even explosive. Beside the fact that there is a large variety of different methods, there is no one which is well suited for a fast and reliable treatment of heat sensitive materials. This talk will show that low pressure plasma sterilization offers a well investigated and understood method that is able to exactly fulfill the above mentioned restrictions: it is fast, cold, and its mechanisms are quantified.
As test samples, B. subtilis spores are investigated. Contamination procedure has been standardized, a defined monolayer on sample carrier is realized by an in-house made spray coater. The plasma system used for all spore treatments is based on an inductively coupled rf-plasma reactor with large volume. Typically filled with a mixture of argon, nitrogen and oxygen and operated at 5 Pa, the plasma itself is carefully and quantitatively characterized. Thereby delivering its fundamental parameters like the gas temperature, the electron density, electron energy distribution function (EEDF) an many others. Most importantly, the light emission in the VUV/UV range (116-280 nm), is determined absolutely and relatively calibrated. In this

wavelength region absorption of the spore DNA is largest. It is shown that an inactivation of spores is strongly based on the gas mixture filled in the plasma chamber. The reason for this behavior strongly relates on the individual excited levels of each atom or molecule thereby emitting at different wavelengths with different intensities. Further on, the inactivation is correlated to a well-known wavelength dependent formula from Munakata et al (Photochem. Photobiol. 54 (1991)). Finally, plasma etching of spores is measured and the ICP sterilization is shown for an industrial system for pharmaceutical filling of syringes.

Microbial pathogenicity

IL-FG-MP-11
Unravelling *E. coli* pathogenesis in the human gut by employing advanced intestinal cell and tissue-based model systems
*S. Schuller[1]
[1]*UEA, Norwich Medical School, Norwich, United Kingdom*

Pathogenic *E. coli* are a major cause of intestinal disease worldwide including acute and chronic diarrhoea, inflammatory bowel disease and colorectal cancer. Research has shown that bacterial pathogenesis is governed by environmental cues which tightly regulate virulence gene expression. In the gut, these signals comprise oxygen levels, fluid shear, pH, digestive enzymes and metabolites produced by the intestinal epithelium and resident microbiota. In addition, pathogens are highly adapted to their host and use specific binding receptors and nutrient resources to establish their niche. Therefore, it is important to take these factors into account when investigating how pathogenic bacteria cause disease. In this talk, I will give an overview of human in vitro and ex vivo intestinal model systems applied in our lab to better understand *E. coli* pathogenesis and contribute to the development of targeted treatment strategies.

Synthetic microbiology

IL-FG-SM-12
Engineering growth-coupled biosynthesis of animal pigments in bacteria
*P. I. Nikel[1]
[1]*DTU Biosustain, DTU Biosustain, Copenhagen, Denmark*

The mining of genomes across life has unearthed a bounty of biosynthetic potential for diverse molecules essential to build a bio-based future. While the heterologous expression of metabolic pathways has been adopted with broad success, most approaches face a common challenge: low initial production levels that require extensive, resource-intensive iterative strain engineering refinement. In this talk, I will discuss a growth-coupled biosynthesis strategy that links microbial growth with the production of specialized compounds—some of which, such as animal pigments, have not been produced in microbes before. The plug-and-play versatility of this strategy is showcased by producing the structurally-complex animal biopigment xanthommatin, a color-shifting ommochrome with promising material and cosmetic applications. In this framework, xanthommatin biosynthesis drives the growth of an engineered *Pseudomonas putida* 5,10-methylenetetrahydrofolate auxotroph (PUMA). With the support of genome-scale metabolic modeling, PUMA was engineered to be regulated by endogenous formate, a co-product generated in the multi-step conversion of tryptophan to xanthommatin. Adaptive laboratory evolution further streamlined gram-scale xanthommatin bioproduction through growth-rate selection, positioning this strategy as a solid approach for initiating and optimizing microbial production of high-value molecules.

Fungal biology and biotechnology

IL-FG-FBB-02
Sensing and Sugar Transporting in *Trichoderma reesei*
*R. Silva[1]
[1]*University of Sao Paulo, Ribeirão Preto Medical School, Department of Biochemistry and Immunology, São Paulo, Brazil*

The filamentous fungus *Trichoderma reesei* is extensively used in the biofuel industry due to its remarkable ability to deconstruct lignocellulosic biomass, a critical process in bioethanol production. This fungus employs various sugar transporters, such as Tr44175 and Tr69957, to facilitate the uptake of key carbohydrates like cellobiose and sophorose, which are essential for regulating the expression of cellulolytic enzymes and thereby enhancing cellulose hydrolysis. Moreover, studies on membrane proteins with CFEM domains, such as Tr111205, have shown that they not only improve sugar uptake but also play roles in osmotic stress response and cell wall integrity. Molecular docking analyses indicate that these proteins have a high specificity for binding sugars, optimizing metabolic efficiency across diverse lignocellulosic substrates, including sugarcane bagasse. These findings expand our knowledge of nutrient sensing in *T. reesei* and suggest strategies for strain engineering to boost enzyme production, which could significantly reduce costs in industrial bioprocessing.

Others

SFB session / Research center session Ruhr

IL-SFBR-14
Cellular and Molecular Mechanisms of Stress Response in the Thermoacidophilic Archaeon *Sulfolobus acidocaldarius*
*B. Siebers[1]
[1]*University of Duisburg Essen, Molecular Enzyme Technology and Biochemistry (MEB), Faculty of Chemistry, Essen, Germany*

The thermoacidophilic archaeon *Sulfolobus acidocaldarius* employs diverse strategies to thrive in extreme environments. At the cellular level, stress responses include increased motility and DNA uptake upon UV irradiation 1, enhanced biofilm formation under organic solvent exposure 2, and specific responses to nutrient starvation 3, showcasing its adaptability to various challenges.

At the molecular level, trehalose and polyphosphate (polyP) metabolism play critical roles in stress resilience. Trehalose acts as a compatible solute stabilizing macromolecules and membranes, synthesized via three distinct pathways 4. A triple mutant lacking all pathways failed to grow under salt stress, while wild-type cells showed increased trehalose

levels under salt stress, emphasizing its role in osmotic stress protection.

PolyP functions as an energy and phosphate reserve, contributing to homeostasis under stress. We identified a novel archaeal polyphosphate kinase, *Sa*PPK3, with reversible activity that drives ATP formation under low energy charge 5. With a combined experimental modelling approach using quantitative 31P NMR, we demonstrated that *Sa*PPK3 buffers energy under stress, synthesizing polyP only when ATP/ADP ratios are high. Phylogenetic analyses place *Sa*PPK3 in a new PPK3 family, patchily distributed across Archaea and Bacteria.

Together, these findings provide a deeper understanding of the molecular strategies employed by *S. acidocaldarius* to cope with extreme conditions, contributing to our broader knowledge of archaeal stress physiology and offering potential applications in biotechnology and industrial microbiology.

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SL-SFBR-125

A tale of two habitats: Diverging microbial stress-responses in water and sediment

*D. Bludau[1,2,3], L. Boden[1], G. Sieber[1,2], A. Deep[1,3], D. Baikova[4], G. M. David[5], U. Hadžiomerović[2,4], T. L. Stach[2,6], J. Boenigk[1,2]

[1]University of Duisburg-Essen, Biodiversity, Essen, Germany

[2]University of Duisburg-Essen, Center for Water and Environmental Research, Essen, Germany

[3]Westphalian University of Applied Sciences, Engineering and Natural Sciences, Recklinghausen, Germany

[4]University of Duisburg-Essen, Environmental Microbiology and Biotechnology, Essen, Germany

[5]Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Plankton and Microbial Ecology, Stechlin, Germany

[6]University of Duisburg-Essen, Environmental Metagenomics, Essen, Germany

Aquatic environments are increasingly impacted by a variety of stressors, such as temperature fluctuations and rising salinity, often driven by climate change, human activities, and land-use changes. These stressors can influence microbial communities in different ways across water and sediment habitats. Alterations in microbial community composition can disrupt nutrient cycling, food web dynamics, and overall ecosystem functioning, with potential consequences for biodiversity and water quality. Understanding how microbial communities in both habitats respond to these stressors is crucial for assessing ecosystem resilience and the broader impacts of environmental stressors on aquatic ecosystems. This study investigates the effects of salt and temperature stress, both individually and in combination, on microbial communities in freshwater environments. We focused on assessing differences between water and sediment communities using 16S and 18S rRNA gene sequencing. A controlled mesocosm experiment was conducted using water and sediment samples exposed to four treatments: control, salt stress, temperature stress, and combined salt-temperature stress. Samples were taken following a 10-day acclimatization phase over a 10-day period, with stressors applied on day 2 and removed on day 6. Microbial community composition was analyzed using amplicon sequencing (16S for prokaryotes, 18S for eukaryotes). Our results demonstrated that microbial communities in water and sediment remained largely distinct, reflecting strong habitat specificity. Water communities were significantly impacted by salt and combined treatments, whereas temperature stress had negligible effects. A significant, yet much weaker, effect of the various stressors on community composition was observed in the sediment samples, compared to the more pronounced changes in the water samples. Eukaryotic communities (18S) were more sensitive to stressors than prokaryotic communities (16S), highlighting differential responses to environmental changes. This study demonstrates that microbial communities in freshwater ecosystems exhibit distinct responses to environmental stressors, with water habitats being more vulnerable to salt. The heightened sensitivity of eukaryotes suggests they may serve as early indicators of ecosystem perturbation. These insights are crucial for developing targeted conservation strategies to mitigate the impacts of stressors on aquatic biodiversity.

SL-SFBR-126

Unraveling microbial roles in stream ecosystem functioning: Insights into stressor impacts and community interactions through metatranscriptomics and metabarcoding

*D. Beisser[1], A. Deep[1,2], D. Bludau[1,2], M. Shah[1,2], G. Sieber[2], T. L. Stach[3], J. Starke[3], A. Probst[3], J. Boenigk[2]
[1]Westphalian University of Applied Sciences, Engineering and Natural Sciences, Recklinghausen, Germany
[2]University of Duisburg-Essen, Biodiversity, Essen, Germany
[3]University of Duisburg-Essen, Environmental Metagenomics, Essen, Germany

Streams are subjected to multiple stressors that interact in complex ways, significantly impacting biodiversity and ecosystem functions. These interactions make it challenging to predict changes in these systems. A comprehensive mechanistic understanding of ecosystem degradation and recovery is still lacking, which hinders our ability to predict stressor impacts on biodiversity and ecosystem functioning and to manage the restoration of degraded freshwater systems.

In our recent work, we have investigated diverse stressor effects, including changes in temperature, salinity, hydrology, inundations, and treated wastewater discharge, on prokaryotic and eukaryotic microbes. Further, we are currently investigating a key ecosystem function: leaf litter degradation in streams, mediated by bacterial and fungal heterotrophs. The specific taxa involved, their interactions within microbial decomposer communities, and the enzymatic and metabolic pathways driving these processes remain largely unexplored and are not yet well understood, potentially varying with environmental conditions. This project aims to elucidate the roles of different fungal and bacterial groups in the enzymatic decomposition of coarse particulate organic matter (CPOM) under single and multiple stressor scenarios. By employing metatranscriptomics, metabarcoding, and DNA-SIP, we seek to uncover the contributions and interactions of these microbial groups. Specifically, we investigate functional redundancy and interrelationships between taxonomic groups to better understand ecosystem resilience and functionality.

SL-SFBR-127

Degradation and recovery of microbial ecosystem functions in rivers exposed to multiple stressors

*V. Brauer[1], D. Baikova[1], U. Hadžiomerović[1], I. Madge Pimentel[2], P. Rehsen[2], D. Buchner[2], R. U. Meckenstock[1]
[1]University of Duisburg-Essen, Environmental Microbiology and Biotechnology, Essen, Germany
[2]University of Duisburg-Essen, Aquatic Ecosystem Research Group, Essen, Germany

Microorganisms in sediments are key players for the functioning of rivers, yet, we do not understand how they respond to and recover from multiple anthropogenic stressors, which are increasingly threatening river ecosystems worldwide. We therefore investigated the effects of two globally relevant stressors, temperature increase and salinization, on two microbe-driven ecosystem processes, which are degradation of dissolved organic carbon (DOC) and degradation of bacterial dead biomass (necromass). Using controlled lab experiments with isolated sediment microbial communities, we found that temperature increase had clear positive and salinization clear negative effects on the degradation of DOC and necromass, even at rather low stressor levels. Using outdoor mesocosm experiments where sediment microbial communities are integrated into the riverine food web, we found that the same low stressor levels had no effect on DOC degradation but on necromass degradation, whereas stressor release increased DOC degradation but not necromass degradation. The results indicate that the effects of multiple stressor increase and release on sediment microbial processes depend on the concentration and the bioavailability of the carbon source, and that changes in energy availability may mask multiple stressor effects.

SL-SFBR-173

Flow Velocity and Anthropogenic Stressors Drive Microbial Community Dynamics in Riverine Ecosystems

*J. Starke[1], C. Moraru[1], T. L. Stach[1,2], A. Soares[1,2], A. M. Kelly[3], N. Batucan[4], I. Madge Pimentel[5], M. R. Penk[3,6], C. Matthaei[4], J. J.-Piggott[6], F. Leese[5], A. Probst[1,2]
[1]University of Duisburg-Essen, Environmental Metagenomics, Research Center One Health Ruhr of the University Alliance Ruhr, Faculty of Chemistry, Essen, Germany
[2]University of Duisburg-Essen, Centre of Water and Environmental Research (ZWU), Essen, Germany
[3]University College Dublin, School of Biology and Environmental Science, Dublin, Ireland
[4]University of Otago, Department of Zoology, Dunedin, New Zealand
[5]University of Duisburg-Essen, Aquatic Ecosystem Research, Faculty of Biology, Essen, Germany
[6]Trinity College Dublin, Zoology, School of Natural Sciences, Dublin, Ireland

Microbial communities in riverine ecosystems are essential drivers of biogeochemical cycling, mediating nutrient transformation and energy flow. However, they are substantially affected by anthropogenic stressors such as pollution, alteration in flow regime and land-use change and, which can alter their composition and functionality, leading to ecosystem degradation. Here, we performed metagenomic analyses of sediment samples from four different flow-through stream mesocosm setups in Germany, Ireland and New Zealand encompassing a total of 254 mesocosms. Our results revealed differential responses of microbial communities to altered flow velocity, an important stressor identified in previous research. Microbial communities from two riverine ecosystems showed significant compositional shifts at slower flow in the mesocosms, whereas the other two showed only minor changes, suggesting a resilience that we infer to be linked to higher microbial diversity. We also identified significant differences in encoded metabolic gene profiles between the microbial communities of the European and New Zealand experiments, particularly in genes associated with nitrogen, methane and sulfur cycling. To further investigate this difference, we selected one stream ecosystem substantially affected by reduced flow velocity and performed in-depth metatranscriptomic analyses revealing up-regulation of, *e.g.*, heat-shock proteins across multiple community members. Notably, a combination of elevated water temperature and flow velocity reduction showed an antagonistic effect at transcription level, highlighting the complexity of stressor interactions associated with river ecosystems. This study stresses the importance of understanding anthropogenic impacts on river microbial communities to mitigate the consequences of these stressors.

SFB Session / HHU/FZ Jülich

IL-SFBJ-06

Mutational hotspots drive rapid evolution of bacterial defenses against protistan predation

*J. van Gestel[1]

[1]*European Molecular Biology Laboratory, Heidelberg, Germany*

Soils are a hotspot of microbial predation, with numerous bacterivorous protists scavenging for bacterial prey. Despite this predation pressure, most functional genomic studies on soil bacteria are performed in the absence of trophic interaction partners. This biases our view on the selection pressures that shape bacterial evolution. Here, we aim to overcome this bias by studying how, under predation, selection affects *B. subtilis*" growth and survival. By screening thousands of genetic mutants with and without predation, we reveal that tens of different genes affect predation resistance, most of which have never been associated with predation before. We also show that mutations can rapidly emerge *de novo* and mostly occur in three loci only. These mutational hotspots either cause filamentation or biofilm formation and prevent protists from engulfing bacterial cells. Resistance however comes with a major cost. Mutants grow slower than susceptible cells and are rapidly replaced in the absence of predation. This strong antagonistic selection favors genetic regulation. Indeed, in one of the loci, we discovered a putative genetic switch that allows cells to switch back-and-forth between a slow-growing resistant state and a fast-grow susceptible state. We speculate that other cases of phase variation in *B. subtilis* can be linked to predation as well. Free-living bacteria might therefore evade predation much like pathogens use phase variation for immune evasion.

SL-SFBJ-071

Establishment of a conditional knockout system in the endosymbiont-harboring trypanosomatid *Angomonas deanei*

*L. Kröninger[1], Z. Kim[1], E. Nowack[1]
[1]*Heinrich Heine University, Institute of Microbial Cell Biology, Düsseldorf, Germany*

Introduction: Mitochondria and plastids originate from endosymbiotic events more than one billion years ago in which free-living bacteria were taken up by a host cell and subsequently evolved into organelles that are now fully integrated into the cellular networks of the host. Since the acquisition and evolution of these organelles date far back in time, we use the trypanosomatid *Angomonas deanei,* which acquired a bacterial endosymbiont (ES) more recently, to study early steps of endosymbiosis. *A. deanei* harbors a single β-proteobacterial ES whose cell cycle is synchronized with and likely controlled by the host by so-called endosymbiont-targeted proteins (ETPs) that are encoded by the host but transported to the ES.

Goal: Preliminary work revealed that homozygous deletions of several ETP-encoding genes cannot be obtained, suggesting that these genes are essential for survival of the symbiosis. Thus, to study how the ETPs effect the host-ES interaction, we aimed to develop genetic tools to generate inducible homozygous knockout (or knockdown) mutants.

Methods & results: To this end, we generated an *A. deanei* strain that produces the T7 RNA polymerase (T7RNAP) from a highly expressed locus. To avoid interference with the endogenous expression machinery we identified at least seven potentially silent loci that appeared suitable for transgene insertion and were expected to be transcriptionally inactive under native conditions. Using a blasticidin resistance cassette with T7 promoter as a reporter gene in the T7RNAP background, we demonstrated T7RNAP-dependent gene expression from one of these silent loci. Finally, implementation of the transcriptional regulator TetR enabled us to induce expression in a ligand-depended manner. Furthermore, we are exploring the integration of a blue light-inducible T7RNAP in *A. deanei*.

Summary: *A. deanei* is an emerging model system to study early steps of organellogenesis. The conditional knockout system, that enables now inducible perturbations of the system, will be key for the in-depth exploration of the host-ES interaction, and, in particular, to observe effects of ETP losses.

SL-SFBJ-072

Intracellular networking: coupling of mitochondrial energy metabolism with microtubule-dependent mRNA logistics

*L. van Wijlick[1], S. K. Devan[1], P. Künzel[1], J. Postma[1], M. Feldbrügge[1]
[1]*Heinrich-Heine-University, Institut of Microbiology, Düsseldorf, Germany*

Eukaryotic cells are particularly characterized by the integration of an endosymbiont, which has gradually led to the development of highly specialized organelles. During the adaptation process, almost the entire genetic material of the endosymbiont was transferred into the nucleus of the host, significantly increasing the complexity of cellular logistics. Consequently, not only complex protein translocation systems had to evolve in the envelope membranes of the organelles, but also intensive bidirectional communication between the organelle and the cell nucleus in order to fine-tune the availability and demand for specific compounds in the organelle and the surrounding cell at any given time.

In the polar-growing fungus *Ustilago maydis*, which has emerged as a new model system for cell biology, we discovered a novel link between endosomal long-distance transport of mRNA and mitochondrial energy metabolism. The key RNA-binding protein Rrm4 binds numerous nuclear-encoded mitochondrial mRNAs and loss of *rrm4* causes defects in the formation of mitochondrial respiratory chain complexes. We used genetic engineering coupled with life-cell imaging to track the distribution of Atp3, the γ subunit of ATP synthase, in polar-growing cells with regulatable Rrm4. Fusion of Atp3 with photoconvertible fluorescent protein (tdEosFP), which can be converted from green to red emission upon exposure to violet light, enabled us to demonstrate that mitochondrial localization of newly synthesized Atp3 is dependent on long-distance transport in polar hyphae. Genome-wide transcript analysis indicates that *U. maydis* hyphae with defective microtubule dependent long-distance transport undergo a significant metabolic shift, suggesting a compensatory response to impaired mitochondrial function. To assess metabolic changes on a temporal-quantitative scale we are implementing genetically encoded, ratio metric Matryoshka biosensors for ATP and NADH in our life-cell imaging coupled with sophisticated metabolomics.

Overall, we show that in polar cells such as fungal hyphae or neurons, mRNA logistics post-transcriptionally regulate mitochondrial energy metabolism, revealing key insights into host-organelle networking. However, our understanding of the principles underlying the establishment, maintenance and evolution of intracellular microbial associations, such as endosymbiont-derived organelles, remains in its very early stage.

SL-SFBJ-073

Race for Iron: Modulating siderophore-based social interactions via light

*S. H. Paik[1], A. Krüger[2,3], M. Bund[2,3], J. Wiechert[2,3], D. Kohlheyer[2,3], J. Frunzke[2,3], T. Drepper[1]
[1]Heinrich Heine University, IMET, Jülich, Germany
[2]Heinrich Heine University, Düsseldorf, Germany
[3]Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany

Microbial communities are essential to various global ecosystems, participating in crucial biological processes. The intricate social interactions within these communities, characterized by intra- and interspecies communication, significantly shape their behaviour and composition. In this context, exchanging various secondary metabolites is pivotal, influencing cooperation, competition, and survival strategies. Among these metabolites, siderophores stand out because of their importance in microbial iron acquisition and their involvement in mediating interactions that affect community dynamics and ecological balance.

Although siderophore-driven interactions have been the subject of extensive research, the spatiotemporal dynamics of these processes remain poorly understood. To bridge this gap, we employ new technologies such as optogenetics and microfluidics, which allow noninvasive control of cellular functions with high spatiotemporal resolution and high precision in studying microbial interactions at the single-cell level.

For instance, by applying light-responsive switches, we can dynamically alter the production of siderophores within microbial communities. This approach allowed a dynamic transition from siderophore production to non-production states and even to overproduction. Using this strain in co-cultures, we could show how different illumination conditions that modulate pyoverdine production impact community composition, revealing shifts in community structure linked to siderophore output.

Ultimately, leveraging these technologies will shed light on how metabolite-based interactions influence community structure and function over time and space, providing new insights into microbial cooperation, competition, and resilience.

SL-SFBJ-074

Let it sense! Biosensor creation using computational and structural approaches

*A. Papadopoulos[1], M. Anlauf[2], S. Weidtkamp-Peters[2], S. Smits[1]
[1]Heinrich Heine University, Center for Structural Studies, Düsseldorf, Germany
[2]Heinrich Heine University, Center for Advanced Imaging, Düsseldorf, Germany

Gaining insight into the physiology of individual microbes and their interactions within intricate networks necessitates a profound understanding of dynamic metabolic processes. Deciphering and quantifying these processes hinges on key factors, including pathways of metabolite transport, nutrient exchange, and signaling. High temporal and spatial resolution of metabolic pathways can be achieved through the use of genetically encoded biosensors in combination with advanced imaging techniques.

Here, we establish a pipeline for biosensor development based on rational design, leveraging state-of-the-art molecular techniques integrated with computational structural biology to create novel biosensors for various metabolites, such as ATP, ferrous iron, and sucrose. This pipeline currently employs two distinct biosensor designs: the FRET-based and Matryoshka biosensor cassettes. FRET-based biosensors are relatively straightforward and largely empirical in their design, in contrast to the Matryoshka biosensors, which require a more sophisticated design process. In Matryoshka biosensors, fluorescent proteins must be embedded within the recognition protein in a way that enables them to report metabolite binding through changes in fluorescence intensity. Additionally, ratiometric Matryoshka biosensors not only track metabolite binding but also monitor the biosensor"s expression levels within cells, facilitating direct comparisons across experiments and various bacterial growth phases. Our design pipeline has significantly accelerated the development of biosensors, achieving high success rates, whereas previous empirical design approaches were often time-consuming and heavily reliant on trial and error approaches.

Here we will highlight the crucial steps in the design and show how computional structural biology can help to change from emperical to rational sensor design. As an example, we developed a novel biosensor for ferrous iron, validated both *in vitro* and *in vivo*, which senses within the micromolar range, that is based on the DtxR recognition protein.

SL-SFBJ-173

Unravelling the genomic complexity of *Peltigera* cyanolichens: Insights into a complex cross-kingdom community

*V. Joisten-Rosenthal[1], M. Heinen[2], L. Hüttebräuker[3], C. Kelly[4], K. Krooß[5], S. Zander[1], S. Weidtkamp-Peters[5], F. Altegoer[2], M. Pauly[3], B. Thomma[4], M. Feldbrügge[2], B. Usadel[1,6]
[1]Heinrich Heine University, Düsseldorf, Germany
[2]Heinrich Heine University, Düsseldorf, Germany
[3]Heinrich Heine University, Düsseldorf, Germany
[4]University of Cologne, Cologne, Germany
[5]Heinrich Heine University, Düsseldorf, Germany
[6]Forschungszentrum Jülich GmbH, Jülich, Germany

Many fungi engage in diverse symbiotic relationships, ranging from pathogenic and commensal to mutualistic interactions. Lichens exemplify a highly successful mutualistic system, typically formed between a fungus (mycobiont) and one or more photosynthetic organisms (photobionts), including green algae and/or cyanobacteria. In addition to this core partnership, lichens host a variety of associated fungal and bacterial communities.

The genus *Peltigera* is widespread across multiple continents and serves as a representative of cyanolichens due to its symbiotic relationship with the cyanobacterial photobiont *Nostoc*. Despite more than two centuries of lichen research, many aspects of lichen biology remain unresolved. However, recent advances in genomics are beginning to fill these gaps, providing new insights into their complex biology. This includes the discovery of a carbohydrate-active-enzyme from the glycoside hydrolase 8 (GH8) family, likely acquired through horizontal gene transfer, as a candidate for facilitating lichen symbiosis in Trebouxiophyceae [1].

To contribute to this growing knowledge, we present over 30 metagenome assemblies from *Peltigera* lichens collected across Germany, the Netherlands, Switzerland, and Lithuania. These assemblies, which capture both photobiont and mycobiont genomes, were produced using state-of-the-art long-read sequencing. Our analyses include interspecies comparisons among *Peltigera* mycobionts and investigations

of structural variations between symbiotic *Nostoc* and its free-living counterparts. Additionally, we analyze the metagenome assembled genomes (MAGs) of the *Peltigera* microbiome to identify core microbial members across species and sampling locations. To gain insights into the spatial organization of these core microbes within the lichen thallus, we aim to integrate our omics data with microscopy. For this, we established the LichenMetaImage Use Case, a collaborative effort among CRC 1535 MibiNet and the NFDIs NFDI4Microbiota, DataPLANT, and NFDI4BIOIMAGE.

This study offers an in-depth analysis of the complex genomic structure of *Peltigera* lichens, comparing their primary components (myco- and photobiont) and examining the core members of the microbiome within the lichen community, contributing valuable insights to the growing field of lichen research.

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Hochschulnetzwerk / HAW workshop

SL-HAW-091
Engineering of *Synechococcus* sp. strain PCC 7002 for the photoautotrophic production of light-sensitive riboflavin (vitamin B2)
*M. Mack[1], B. Kachel[1]
[1]*Hochschule Mannheim, Biotechnology, Mannheim, Germany*

Due to their capability of photosynthesis and autotrophic growth, cyanobacteria are currently investigated with regard to the sustainable production of a wide variety of chemicals. So far, however, no attempt has been undertaken to engineer cyanobacteria for the biotechnological production of vitamins, which is probably due to the light-sensitivity of many of these compounds. We now describe a photoautotrophic bioprocess to synthesize riboflavin, a vitamin used as a supplement in the feed and food industry. By overexpressing the riboflavin biosynthesis genes *ribDGEABHT* from *Bacillus subtilis* in the marine cyanobacterium *Synechococcus* sp. PCC 7002 riboflavin levels in the supernatant of the corresponding recombinant strain increased 56-fold compared to the wild-type. Introduction of a second promoter region upstream of the heterologous *ribAB* gene – coding for rate-limiting enzymatic functions in the riboflavin biosynthesis pathway – led to a further increase of riboflavin levels (211-fold compared to the wild-type). Degradation of the light-sensitive product riboflavin was prevented by culturing the genetically engineered *Synechococcus* sp. PCC 7002 strains in the presence of dichromatic light generated by red light-emitting diodes (λ = 630 and 700 nm). *Synechococcus* sp. PCC 7002 naturally is resistant to the toxic riboflavin analog roseoflavin. Expression of the flavin transporter *pnuX* from *Corynebacterium glutamicum* in *Synechococcus* sp. PCC 7002 resulted in roseoflavin-sensitive recombinant strains which in turn could be employed to select roseoflavin-resistant, riboflavin-overproducing strains as a chassis for further improvement.

Kachel, B., & Mack, M. (2020). Engineering of *Synechococcus* sp. strain PCC 7002 for the photoautotrophic production of light-sensitive riboflavin (vitamin B2). *Metabolic Engineering*, *62*, 275-286.

SL-HAW-092
Plant-microbe interactions in sustainable crop production
*K. Burow[1], R. Proma[1,2], N. Neetu[1], D. Gaber[1,3], S. Sabu[1], A. Alonso[1], J. Brandes[1], P. Franken[1,3]
[1]*Erfurt University of Applied Sciences, Research Centre for Horticultural Crops (FGK), Erfurt, Germany*
[2]*Friedrich Schiller University, Microbiology, Jena, Germany*
[3]*Assiut University, Department of Botany and Microbiology, Assiut, Egypt*

The research group "Plant-Microbe Interactions in Sustainable Crop Production", conducted at the Research Centre of Horticultural Crops at the University of Applied Sciences Erfurt (Thuringia), focused on the role of microorganisms in horticultural crop production and their contributions to sustainable horticultural practices. Microbial communities, including bacteria and fungi, colonize plant roots, enhancing nutrient and water uptake while bolstering resistance to biotic and abiotic stresses. These microorganisms can significantly reduce the reliance on synthetic pesticides and growth regulators, positioning them as essential components of sustainable management systems.

The performance of plants is significantly influenced by the composition of microbial communities that colonize various plant tissues. Understanding whether plants affect the structure of these communities is crucial. Comparing microbial communities across different cultivars, their wild relatives, and genetically engineered genotypes can yield valuable insights. Through sequencing and network analyses, along with parallel isolation and cultivation, specific bacteria and fungi that contribute to the stability of these microbial communities can be identified. Future phases of the research will introduce diverse beneficial microorganisms, such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), to enhance substrate quality and plant growth in peat-free substrates improving nutrient mobilization and plant stress tolerance.

While plant resistance to pathogens is often emphasized in horticultural crop breeding, the responses of plants to microorganisms are rarely considered. However, molecular analyses indicate that many genes are involved in these interactions. Key traits such as enhanced water and nutrient uptake, which lead to improved growth, are quantifiable and can be assessed across different genotypes. Consequently, analyzing diverse plant populations could identify molecular markers useful for breeding new cultivars tailored for sustainable production systems.

SL-HAW-093
Façade eluates affect active and total soil microbiome
F. Reiß[1], N. Kiefer[1], S. Kalkhof[1], *M. Noll[1]
[1]*Hochschule Coburg, Institut für Bioanalytik, Coburg, Germany*

The application of biocides in building materials has become a prevalent practice to mitigate the growth of microorganisms such as algae, fungi, and bacteria on the façades. These can leach out from the material and reach the nearby soil environment. This study aimed to characterize the effect of façade eluates generated within different leaching experiments on total and metabolic active soil microbial community composition and functions. Façade eluates were produced by immersion testing DIN EN 16105 and a natural

weathering experiment. Afterward, soil microcosms were treated with the respective façade eluate and incubated for 29 days. Subsequently, the active and total soil microbial community compositions were investigated.

Fungal internal transcribed spacer region gene and bacterial 16S rRNA gene were sequenced for active (bromodeoxyuridine labeled DNA) microbial community and total community. Façade eluates reduced total bacterial and fungal gene copy numbers. Overall, active bacterial and fungal richness was reduced and altered in community composition in comparison to the total richness and composition, respectively. Façade eluates retrieved of façade samples without biocides did alter the soil microbial communities to the same extent as façade eluates with biocides. Additionally, members of the active microbiome that benefit from the presence of façade eluates and omitted ones could be identified. Our result demonstrated that façade eluates affect active and total soil microbial community composition and function regardless of the leaching procedure and biocides addition.

SL-HAW-094
Developing a fungivorous amoeba into a biological control agent of plant pathogenic fungi
*F. Hillmann[1], V. Gobba[1], M. Gruben[1,2], A. Peters[2]
[1]Hochschule Wismar University of Applied Sciences, Biochemistry and Biotechnology, Faculty of Engineering, Wismar, Germany
[2]e-nema, Gesellschaft für Biotechnologie und biologischen Pflanzenschutz mbH, Schwentinental, Germany

Crop diseases caused by fungi result in billions of euros in annual losses for agriculture. The widespread use of chemical fungicides remains the primary solution, but its extensive use has been questioned due to potential risks for ecology and health. In the PIAMBIZID project we aim to develop a biological alternative based on a fungus-eating amoeba, which could replace or reduce the use of harmful chemical fungicides. The project involves a collaboration between academia and industry to bring this innovation from lab research to practical application in the field of biological plant protection.
The main objective of the project is to qualify scientific research on the fungus-eating amoeba for innovative applications in biological fungicides, including the scale up of the cultivation and production of amoeba cysts for field trials and developing biotechnological processes for long-term storage and formulation. The project further aims to evaluating the efficacy of the amoebae against at least two plant fungal infections, identify the regulatory requirements for the biofungicide and preparing it for market entry. In the first phase, we have developed a methodology to cultivate the amoeba in liquid cultures and bioreactors, while collaboration with e-nema GmbH is providing the necessary infrastructure and knowledge for upscaling and testing. Initial laboratory results show that the amoeba can be cultured efficiently, achieving comparably high cell densities in controlled environments. The development of storage and reactivation techniques has also been successful, allowing the amoeba cysts to remain viable after long-term storage. Preliminary tests have demonstrated the amoeba"s effectiveness against two different plant fungal pathogens. The scale-up of production to pilot-scale volumes has been achieved, ensuring sufficient quantities of the amoeba for upcoming field trials. In summary, PIAMBIZID is a combined effort of academic and industrial expertise to provide a viable and environmentally friendly alternative for controlling fungal crop diseases.

MiCon session

SL-MiCon-162
Viral Take-over of Host Pigment Biosynthesis: Understanding the Role of Cyanophage-Encoded Tetrapyrrole Biosynthesis
*N. Frankenberg-Dinkel[1]
[1]Rheinland-Pfälzische Technische Universität, Abteilung Mikrobiologie, Kaiserslautern, Germany

Viruses and bacteriophages (viruses that infect bacteria) are the most abundant biological entities on our planet. In the marine environment, cyanophages (viruses infecting cyanobacteria of the genera *Prochlorococcus* and *Synechococcus*) have significant impact on ecology, evolution, and biogeochemical processes. Their self-replication is dependent on the molecular machinery of a host bacterium to generate viral progeny. Bacteriophages may have a lysogenic or lytic cycle, with the latter ultimately resulting in the lysis of the host cell. Infection by a lytic phage transforms the host bacterium into a so-called virocell. The virocell represents the intracellular state of the phage"s life cycle whose sole function is to produce virions. Phage infection induces a dramatic change in various host metabolic pathways, which is further expanded by the introduction of auxiliary metabolic genes (AMGs)1,2. Cyanophage encoded AMGs are often related to light harvesting, pigment biosynthesis and photosynthesis and are suggested to modulate and supplement the host bacterium"s metabolism to satisfy the elevated metabolic demand. Over the years, our research has focused on AMGs associated with tetrapyrrole pigment biosynthesis3,4. We have shown that phage-encoded homologs often mimic the activities of their cyanobacterial counterparts, with one notable exception: the phage's ferredoxin-dependent bilin reductase phycoerythrobilin synthase (PebS)3. This enzyme independently performs a reaction that normally requires two sequential enzymes in uninfected host cells. With the recently established recombinant phage technology5 we hope to gain insights into the role of cyanophage-encoded PebS, its importance for phage progeny production and to elucidate how cyanophage proteins interact with host metabolism.

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SL-MiCon-163
From NMR to function: Mechanistic Insight into the Interaction between Ferredoxin and the Ferredoxin Dependent Bilin Reductase PebS

*F. Zeqiri[1], Y. Miyanoiri[2], Y. Misumi[2], A. C. Ngo[3], N. Frankenberg-Dinkel[4], G. Kurisu[2], E. Hofmann[1]
[1]*Ruhr University Bochum, Protein crystallography, Bochum, Germany*
[2]*Osaka University, Institute for Protein Research, Osaka, Japan*
[3]*Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany*
[4]*University of Kaiserslautern-Landau, Department of Microbiology, Kaiserslautern, Germany*

Ferredoxin-dependent bilin reductases (FDBRs) play a critical role in photosynthetic organisms by catalyzing the reduction of biliverdin (BV) to different open-chain tetrapyrroles, including phytochromobilin (PΦB), phycocyanobilin (PCB), and phycoerythrobilin (PEB)1. These open-chain tetrapyrroles serve as chromophores that facilitate light-depended processes, e.g. in development or regulation of photosynthesis. Different representatives of the class of FDBRs are very distinct in their stereo- and regioselective reduction of BV, yet they all rely on the efficient interaction with the electron donor Ferredoxin (Fd) during catalysis.

In this study we investigated the interaction between the phycoerythrobilin synthase PebS1, encoded by a phage, and its host Fd. To determine the molecular interaction between PebS and Fd, NMR spectroscopy and computational docking were employed. Using [15N]-labelled gallium-substituted Fd from *Thermosynechococcus elongatus,* chemical shift perturbations were observed, allowing the identification of residues critical for protein-protein interactions2. Using these data, we performed HADDOCK docking simulations to predict the contact interface between both proteins and guide the design of PebS variants potentially disrupting the interaction. These variants lead to inhibition of protein activity and therefore support the location of the proposed interface. Based on a phylogenetic analysis, we expand this to the phage Fd and other Fd-FDBR interaction pairs to obtain a better understanding of this essential protein-protein interaction.

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SL-MiCon-164
Actinobacterial Glutathione *S*-transferases: Catalysts for racemic kinetic resolution of Epoxides?
*M. Haarmann[1], M. Scholz[1], D. Eggerichs[1], A. Maier[1], D. Tischler[1]
[1]*Ruhr University Bochum, Faculty of Biology and Biotechnology, Bochum, Germany*

Epoxides are important platform chemicals in industry but also present in nature. Particularly chiral epoxides hold significant industrial interest due to their application in agrochemical or drug synthesis. A major challenge in organic chemistry is achieving high-yield production while maintaining regio- and enantioselectivity. Enzyme-catalyzed epoxidation offers these features but is limited by their selectivity, restricting its universal applicability. Some enzymes such as epoxide hydrolases (EHs), CoM-transferases (CoMTs) or glutathione *S*-transferases (GSTs) have shown epoxide conversion with certain selectivity. As the selectivity of GSTs is not well studied, these enzymes became our focus.

Lately, the bacterial GST *Gr*StyI was found in the styrene degradation pathway of *Gordonia rubripertincta* CWB2. Unlike other styrene degraders, CWB2 can metabolize various styrene-related compounds, leading to the co-metabolic formation of ibuprofen for example. The presence of GSTs in bacterial styrene degradation is unusual but appears to broaden the substrate spectrum of this pathway. Similar gene clusters were found in styrene and isoprene-degrading bacteria, encoding GSTs *styI* and *isoI*. Consequently, we heterologously produced 5 GSTs in *E. coli* for characterization of substrate selectivity and enantiomeric preference. Herein, *Gr*StyI appeared as the most promising enzyme for enzymatic kinetic resolution.

To explore *Gr*StyIs potential for enantioselective epoxide opening, chiral GC-FID analysis was conducted on various aromatic and aliphatic epoxides. For styrene oxide derivatives, *Gr*StyI exhibited high selectivity for (*S*)-enantiomers, while the enantiomeric preference of more diverse structures like aliphatic epoxides showed reduced enantioselectivity. Given its high enantioselectivity towards (*S*)-epoxides, we performed enzymatic kinetic resolution. To proof the feasibility, the reaction volume was subsequently increased to 150 ml for large-scale production, yielding approx. 14 mg (*R*)-styrene oxide, validated by NMR.

Summarizing, we identified *Gr*StyI as highly enantioselective for (*S*)-styrene oxide and structural analogous. Our study highlights *Gr*StyIs potential for large-scale production of enantiopure epoxides like styrene oxide. Moreover, GSTs from isoprene degradation appear as promising candidates for the production of enantiopure aliphatic epoxides.

SL-MiCon-165
Interrogating a PKS Ketosynthase Domain via Site-Directed Active-site Mutations
*J. Hu[1], M. Grote[1], S. Kushnir[1], F. Schulz[1]
[1]*Ruhr University Bochum, Faculty of Biology and Biotchnology, Bochum, Germany*

Polyketides, known for their diverse structures and potent pharmaceutical activities, underpin a wide array of clinical applications, including antibiotics, anticancer agents, and immunosuppressants. Their structural diversity arises from polyketide synthases (PKSs), modular enzymatic assembly lines that offer significant potential for genetic engineering to produce novel derivatives with improved therapeutic properties [1]. However, engineered PKSs often exhibit low activity, resulting in poor yields of the desired derivatives. In our previous work, we deactivated the reduction domain in module 4 of the monensin PKS, which enabled the detection of oxidized derivatives but revealed a biosynthetic bottleneck [2]. High intermediate accumulation suggested a substrate transfer inefficiency between modules 4 and 5, potentially linked to the substrate specificity of the ketosynthase (KS) domain in module 5.

To address this, we inspected the KS domain sequences of monensin PKS in comparison to those of other polyether-forming PKSs, identifying fingerprint motifs likely influencing substrate specificity. Guided by multiple sequence alignments, we performed site-directed mutagenesis on the KS5 domain and introduced point mutations into two engineered mutants: the KR40-null mutant (with the ketoreductase domain in module 4 deactivated) and the DH40-null mutant (with the dehydratase domain in module 4 deactivated). The same mutations were introduced in the A495-WT mutant as a control [3]. Targeted LC-MS-based metabolomics was employed to evaluate the impact on product spectrum.

Our findings highlight the critical role of KS domains in PKS assembly line efficiency. Strategic alterations at key active-site residues can improve overall productivity, modify substrate preference, or influence stereospecificity, paving the way for more efficient production of diverse polyketide derivatives.

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SL-MiCon-166
Exploring the antibacterial properties of natural products from a soil-derived *Streptomyces* isolate: A comprehensive metabolomic and proteomic approach

*L. S. Kaufmann[1], L. Sagurna[1], J. E. Bandow[1]
[1]Ruhr University Bochum, Microbiology, Bochum, Germany

Natural products are known for their diverse chemical structures and potent bioactivities. Thus, delving into natural product exploration has emerged as one of the most promising sources for drug discovery. Actinomycetes, particularly *Streptomyces* species, were identified as prolific producers of antibiotics and continue to offer significant potential. Recent studies revealed that the full biosynthetic potential of many *Streptomyces* strains has not yet been fully exploited1,2.

In this study, we investigate the antibacterial potential of natural products produced by the soil-isolate CS39. Whole genome sequencing confirmed that this isolate belongs to the genus *Streptomyces*. By cultivating the isolate under various conditions, extracts were obtained, which were then assed for their antibacterial activity against model organisms and ESKAPE pathogens. Furthermore, metabolomic studies of active extracts were performed by LC-MS/MS. Using the GNPS workflow, a molecular network was created and detected masses annotated3. Selected natural products were isolated using mass spectrometry-guided purification. Interested in their unexplored mechanisms of action, biological characterization and mechanism of action studies supported by global proteomic analysis of the response of *B. subtilis* 168 were performed.

Our results showed that the crude extracts from *Streptomyces* CS39 exhibit antibacterial activity against both gram-positive and gram-negative bacteria. Antibiotic compounds known for their efficacy against gram-positive bacteria were successfully annotated and three of them purified. Proteomic analysis of *B. subtilis* 168 to these natural products showed upregulation of marker proteins some of which related to energy metabolism, oxidative stress, and protein folding stress. Future research will focus on further characterization of the mechanisms of action of the natural products, as well as investigation of further compounds that may explain the observed activity against gram-negative bacteria.

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SL-MiCon-167
The anti-tubercular callyaerins target the *Mycobacterium tuberculosis*-specific non-essential membrane protein Rv2113

*D. Podlesainski[1], E. Adeniyi[2], Y. Gröner[2], F. Schulz[1], V. Krisilia[2], N. Rehberg[2], T. Richter[1], D. Sehr[1], H. Xie[1], V. Simons[2], A. L. Kiffe-Delf[2], F. Kaschani[1], T. Ioerger[3], M. Kaiser[1], R. Kalscheuer[2]
[1]University Duisburg-Essen, Fakultät Biologie, ZMB, Chemische Biologie, AG Kaiser, Essen, Germany
[2]Heinrich Heine University, Düsseldorf, Germany
[3]Texas A&M University, College Station, TX, United States

Tuberculosis remains one of the most threatening bacterial infections, particularly due to the increasing prevalence of antibiotic-resistant strains of the pathogen *Mycobacterium tuberculosis*. Therefore, alternative antibiotics with divergent mechanisms of action are urgently required. Recently, callyaerin A and B, two derivatives of the callyaerins, a group of cyclic peptides bearing an unusual (*Z*)-2,3-diaminoacrylic acid unit, were identified as promising anti-tubercular natural products. Through a combination of spontaneous resistant mutant screening and affinity-based protein profiling (A*f*BPP) the non-essential but *Mycobacterium tuberculosis*-specific membrane protein Rv2113 was identified as the biological target of the callyaerins. However, the function of Rv2113 has not been fully resolved. Fluorescent microscopy experiments showed that Rv2113 is not involved in the cellular uptake of the callyaerins. Instead, our data indicate that Rv2113 is involved in mycobacterial lipid metabolism.

Basics of hand disinfection

WS-HD-01
Workshop basics of hand disinfection

*K. Steinhauer[1], F. Gugsch[2], C. K. Tan[2]
[1]VAAM-Fachgruppe Qualitätsmanagement, Frankfurt a.M., Germany
[2]bactologicum GmbH, Itzehoe, Germany

Effective hand hygiene is an undisputed and essential part of infection prevention in the medical field. In production processes, personal hygiene is of great importance and hand hygiene is particularly important in critical production

processes such as sterile production under GMP (Good Manufacturing Practice).

This workshop on the basics of hand disinfection focuses in particular on the importance of hands as a vector in the transmission of infectious agents or contamination in production processes. Various efficacy spectra (e.g. bactericidal, levurocidal, limited virucidal, virucidal, etc.) of hand disinfectants against relevant microorganisms and viral pathogens will be presented. The effectiveness of hand washing and hand disinfection as well as the various applications of hand hygiene in practice will be discussed.

The aim of this workshop is to give a brief insight into the essential aspects of effective hand hygiene and to work out the historical discovery of hand hygiene in a short case study in small groups.

Short Lecture - Fachgruppen

Biotransformation

SL-FG-BT-022
BPP Bioportides – How to standardize a protein based platform for gene delivery
*C. Kutzner[1]
[1]*Badische Peptide und Proteine (BPP) GmbH, Mannheim, Germany*

Introduction: Gene delivery is a topic of utmost importance that we are constantly confronted with in all fields of microbiology. Genetic modifications may take all kinds of forms. At times, it is necessary to insert a gene for recombinant expression and sometimes we want to silence certain pathways. BPP Bioportides promise to implement one tool for various such applications in a broad variety of different organisms. The nucleic acid binding domain of the BPP Bioportide has been observed to show affinity for different types of nucleic acids. The cell penetrating domain can be adapted to the target membrane. This capability was successfully employed in projects involving the transfection of plants but also the transformation of filamentous microorganisms.

Project Goals: BPP Bioportides are a novel tool. Hence, it is necessary to establish a standard procedure to assess the quality of a certain batch but also to investigate the suitability of a certain Bioportide with respect to a certain nucleic acid and organsism. E. coli is used as a standard organism that is transformed with standard plasmids using different Bioportides and heat shock as a reference method in order to establish a relative scale and method to compare BPP Bioportide suitability for different organisms.

Materials and methods: A series of different Bioportides is used for transformation in E.coli and compared with heat shock transformation. The read out is assessed using two methods. The classical approach is two count CFUs on selection agar plates and evaluate transformation efficiency and frequency. The second method employs analysis of culture growth in a fluid selection medium. The culture growth is investigated using light scattering and oxygen concentration as readouts.

Results: The data of both methods are in line and show that the investigated Bioportides lead to higher transformation efficiencies than the heat shock method in log phase E. coli cells. The data collected in fluid culture add information regarding the vitality of the cells. Both methods are also useful to highlight differences in performance between the BPP Bioportides in use.

Summary: BPP Bioportides are novel platform for gene delivery in various microorganisms. The study at hand provides a tool for the comparison and standardization of Bioportide utilization in unknown organisms. This provides new opportunities to improve and optimize gene delivery.

SL-FG-BT-019
From Host Selection to Process Innovation: Aspergillus niger and Its Alternatives in Enzyme and Protein Production
*J. E. Domeyer[1]
[1]*BRAIN Biotech AG, -, Zwingenberg, Germany*

The selection of an optimal host organism for heterologous protein production is a critical and challenging aspect of industrial biotechnology. Each host offers unique advantages and limitations that must be carefully considered for efficient enzyme or protein production. To address this challenge, BRAIN Biotech has developed a suite of producer strains, including *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Komagataella phaffii*, and *Aspergillus niger*. Among these, *A. niger* has emerged as one of the most productive hosts in terms of final product titers, making it an attractive candidate for industrial applications.

This presentation will focus on the strain and process optimization strategies employed to enhance *A. niger* as a production host. Key advancements include the use of proprietary CRISPR nucleases for targeted genome editing to facilitate the insertion of expression cassettes and the deletion of protease-encoding genes to reduce protein degradation. Additional modifications, such as the deletion of genes influencing viscosity in the fermentation broth, have been implemented to improve process efficiency. Furthermore, process optimization and downstream processing enhancements have been explored to maximize yields and reduce production costs.

By combining cutting-edge genetic engineering techniques with process engineering innovations, we have significantly improved the utility of *A. niger* as a robust and efficient production platform for industrial enzymes and proteins. This talk will give insights into how BRAIN Biotech utilizes *A. niger* for the biotechnological production of enzymes and other proteins.

SL-FG-BT-020
Accelerating the discovery of biocatalysts through cell-free protein synthesis: Advances and applications
*K. Rosenthal[1]
[1]*School of Science, Biotechnology, Bremen, Germany*

Cell-free protein synthesis (CFPS) is a well-established but constantly evolving method that enables protein synthesis *in vitro* without the need for living cells. This method has proven its effectiveness as a robust platform for screening biocatalysts and enables the rapid discovery and optimization of enzymes for various biotechnological applications. By separating protein synthesis from cell growth, CFPS has significant advantages compared to conventional heterologous protein synthesis *in vivo*, e.g.

shorter production times and the ability to synthesize proteins that are toxic to cells.

In recent years, we have developed and utilized various CFPS systems for the screening of a wide range of enzymes. Mainly based on *Escherichia coli* extracts, a total of seven proprietary CFPS systems have been developed and more than 30 different enzymes have been successfully synthesized across five enzyme classes. The presentation will provide insights into the application of CFPS systems based on *E. coli* extracts as a screening tool using various examples. These applications encompass the screening of enzyme homologs and variants, evaluation of various expression hosts, investigation of (co-)substrate ranges, and assessment of gene expressibility, such as genes from higher organisms in standard prokaryotic systems [1-3]. In addition, protein synthesis under non-natural conditions [4] and the synthesis of complex proteins such as membrane proteins using CFPS are discussed. In addition to the fast reaction, the openness of the system, which means that it can be easily modified, if necessary, is a major advantage in order to create highly defined environmental conditions for protein synthesis.

Overall, CFPS has been demonstrated to serve as a flexible platform for biocatalyst development, with promising applications spanning areas such as industrial biotechnology and drug discovery.

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SL-FG-BT-021
Heterologous expression in the eukaryotic micro alga *Chlamydomonas reinhardtii*

*N. Jacobebbinghaus[1], A. Einhaus[1], T. Baier[1], O. Kruse[1]
[1]*Bielefeld University, Center for Biotechnology, Bielefeld, Germany*

Green microalgae harbour great potential as sustainable light-driven hosts for engineered bioproduction of high-value products. Their capacity for efficient photosynthesis fuels a strong metabolic flux towards various hydrocarbons, which can be tapped for the production of valuable hydrocarbon based chemicals such as Terpenoids and Carotenoids.

The microalga *Chlamydomonas reinhardtii* stands out as a well-developed model organism: established transformation methods allow feasible transformation and straightforward mutant screening is provided by a large set of reporter genes. However, advanced genetic and metabolic engineering driven from the nuclear genome is generally hindered by low transgene transcription rates and limited knowledge of underlying eukaryotic regulation.

In the last decade, a great molecular toolkit has been developed, which is still expanding. Here we present some of the most striking strategies to enhance gene expression in the microalga including promotor engineering, intron mediated enhancement and targeted integration. These and further strategies allow a more efficient use of the microalgas potential for protein expression and bioproduction.

Viruses of microbes

SL-FG-VM-146
Integration of chemical defense in the context of the bacterial immune system

*L. Kever[1], B. Rackow[1], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1, Jülich, Germany*

Under the constant threat of predation by (bacterio-)phages, bacteria evolved a vast array of anti-phage defense mechanism. Besides protein- and RNA-based defense systems, recent studies have highlighted the role of *Streptomyces*-derived secondary metabolites, such as the DNA-intercalating anthracyclines1 as well as several aminoglycoside antibiotics as further facet of bacterial immunity*2*-3. Molecules of both classes have been shown to inhibit infection of various dsDNA phages by interfering with an early stage of the phage life cycle*3*.

Here, we focus on the influence of antibiotic resistance genes on the molecular versatility of aminoglycosides. Remarkably, we showed that drug modification via different acetyltransferases as well as target site modification via 16S rRNA methylation enables an uncoupling of the antibacterial and antiviral properties of these compounds, providing the prerequisite for community-wide antiphage defense. Inhibition of a *Streptomyces* phage was also achieved using the supernatants from natural aminoglycoside producers, hinting at a broad physiological significance of the antiviral properties of aminoglycosides.4

Additionally, we aim to assess specific phage characteristics that render them susceptible to chemical defense mechanisms. To this end, a diverse set of *Streptomyces* phages as well as the BASEL phage collection, comprising 69 fully characterized *E. coli* phages5, was screened against known antiphage small molecules. This screening revealed distinct, taxonomically-related clusters of phages, which are particularly sensitive to certain compounds. Moreover, we intend to explore the integration of chemical defense strategies within the broader context of the bacterial immunity by investigating potential synergistic interactions with other defense mechanisms.

We expect the outcomes of this project to deepen our understanding of multicellular defense via small molecules and provide a more comprehensive view of bacterial antiphage immunity.

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SL-FG-VM-147
The role of a phage defense island in enterohemorrhagic *Escherichia coli*

*H. Li[1], S. Himpich[2,3], L. Grass[2,3], V. V. Loi[1], C. Borelli[1], T. P. T. Nguyen[1], L. Nowara[1], U. Dobrindt[4], M. Wahl[2,3], H. Antelmann[1]

[1]*Free University of Berlin, Institute of Biology-Microbiology, Berlin, Germany*
[2]*Free University of Berlin, Laboratory of Structural Biochemistry, Berlin, Germany*
[3]*Helmholtz-Zentrum Berlin für Materialien und Energie, Macromolecular Crystallography, Berlin, Germany*
[4]*University Hospital Münster, Institut für Hygiene, Münster, Germany*

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) O175:H7 strain EDL933 is a foodborne pathogen that can cause life-threatening infections, such as bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). We found that EDL933 is highly resistant to most phages that efficiently lyse *E. coli* K12 strains. Using DefenseFinder, three nuclease/helicase-based phage defense systems were predicted to be encoded on an O-island in EHEC strains, including Zorya-II, Druantia-III and Serket-I. Goals: We hypothesize that this phage defense island contributes to the strong phage resistance of EHEC and that the three systems act synergistically in the anti-phage defense. Thus, we aimed to determine the phage spectrum protected by each defense system and to study their synergistic interactions. We are further interested in the molecular mechanisms of the anti-phage defense. Materials & Methods: We used EOP and growth assays under phage infections to analyze the phage spectrum and synergy of the defense systems. Biochemical and structural analyses were applied to study the molecular mechanism of the Druantia-III and Serket-I antiphage defense. Results: EOP assays with *druHE*, *zorABE* and *serABCD*-deficient strains revealed that the three defense systems protect to different extents against a broad spectrum of restriction-sensitive phages, but not against hypermodified *Tevenvirinae*. Using double system mutants, we found that Zorya-II, Druantia-III and Serket-I provide additive resistance and synergy towards different phages. Biochemical and structural analysis established that DruE functions as 3"-5"-directional DNA helicase and nuclease containing unique molecular tools, which affect its molecular activities. Using *in vivo* complementation studies with DruE variants, we established that the DUF1998 domain with its conserved 4Cys-Zn2+ cluster is essential for the anti-phage defense, while the PLD1 and PLD2 domains are dispensable for functionality. Summary: Overall, we hypothesize that the three defense systems cooperate in phage DNA sensing, discriminate phage and host DNA by methylation, cleave non-methylated phage DNA at specific restriction sites and subsequently degrade phage DNA.

SL-FG-VM-148
Molecular sensing mechanisms of phage infection mediated through the Zorya anti-bacteriophage defense system
*P. Popp[1], M. Erhardt[1]
[1]*Humboldt University, Lebenswissenschaftliche Fakultät, Berlin, Germany*

In the dynamic battle between bacteriophages (phages) and their bacterial hosts, sophisticated anti-phage defense systems are pivotal for bacterial survival. Among these, the recently discovered Zorya system is notable for its unique approach to phage deterrence. The Zorya type I system in Escherichia coli consists of the membrane proteins ZorAB, which are homologous to the MotAB stator units of the bacterial flagellum, and the cytoplasmic effector proteins ZorCD. In this project, we aim to elucidate the molecular mechanisms that enable the Zorya system to detect and counteract phage infections. Utilizing a combination of structure-based mutagenesis, functional assays and live-cell total-internal reflection fluorescence (TIRF) microscopy, we investigate how the Zorya system responds to phage infection. Further, we explore the activation mechanism of the Zorya system and monitor the recruitment of Zorya components to the site of infection, which is crucial for inhibiting phage replication. Our research anticipates revealing detailed insights into the molecular sensing mechanisms and interactions within the Zorya system, thus contributing significantly to our understanding of bacterial immune strategies and potentially guiding the development of novel antibacterial therapies.

SL-FG-VM-149
Chimalliviruses bend membranes and translation
*M. Gerovac[1]
[1]*Helmholtz Centre for Infection Research – HZI, Braunschweig, Germany*

On first contact, bacterial viruses called phages inject their genetic payload into the host. Strikingly, some jumbo phages of the *Chimalliviridae* family form a self-made early phage infection (EPI) vesicle, creating an additional cellular boundary for their injected genome together with an encapsulated virion RNA polymerase. This EPI vesicle protects the genome and compartmentalises transcription. Transcripts are produced from the EPI vesicle and released into the cytosol for immediate translation. Previously, we identified and structurally characterised the phage factor ΦKZ014, which is immediately produced and occupies translating ribosomes. ΦKZ014 is reported to be recruited to the EPI vesicle together with ribosomes and is thought to play a role in the host stress response. It remains unclear how *Chimalliviridae* organise membrane structures and orchestrate localised translation events at the molecular level. To elucidate the intricate coupling between transcription and translation at the interface of the EPI vesicle membrane, we imaged snapshots shortly after infection using cryo-ET. In ongoing work, we are inferring the internal organisation of phage-infected cells at the interface of cellular boundaries. Our goal is to understand how phages can create cellular structures and compartments inside bacteria and how translating ribosomes are coupled to interfaces. Insights into the EPI vesicle-ribosome interplay may pave the way for novel delivery approaches and the design of artificial compartments for biotechnological and medical applications.

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Evolution of regulation

SL-FG-R-049
Placing and counting – spatial regulation of flagellation in bacteria
*K. Thormann[1]
[1]*Justus Liebig University Giessen, Gießen, Germany*

Many bacteria possess flagella, helical proteinaceous rotating filaments, which enable active motility in various environments. The flagellar apparatus is an intricate nanomachine, which is synthesized from a range of different building blocks of various stoichiometries. A complex regulation pattern underlies the stepwise formation of

flagellar subunits, resulting in an inside-out assembly of the flagellar apparatus.

Notably, flagellation occurs in species-specific pattern. In many environments, the monopolar flagellation (a single flagellum at one cell pole) is the predominant pattern. In these cells, flagellar synthesis has to be targeted to the appropriate cell pole, and, additionally, the number of flagella has to be restricted to a single one. For many bacterial species, this flagellation pattern is governed by the SRP-type GTPase FlhF as the polarity factor and the MinD-like ATPase FlhG as the flagella counting factor. In recent studies, we developed a model describing how onset of flagella synthesis is spatially controlled and how flagella formation is shut down to restrict the number of flagella to a single one. This model along with the implications and many still open questions will be presented here.

SL-FG-R-050
Two tales of transcriptional regulator evolution
L. M. Schulz[1], F. Dreier[1], L. Hahn[1], J. Petrovac[1], N. Sievers[1], *J. Rismondo[1]
[1]*Georg-August University Göttingen, Göttingen, Germany*

Bacteria need to adapt constantly to changing environmental conditions such as temperature, pH or nutrient availability. In addition, they may encounter toxic compounds, which are either produced by co-habitants or used in industrial or clinical setting to eliminate bacterial contaminations and infections. Transcriptional regulators, which can either repress or activate the expression of their cognate regulon, play an important role for these adaptation processes in bacteria. The human pathogen *Listeria monocytogenes* is ubiquitously found in nature and can easily adapt to changing environmental conditions. It can also easily enter food-processing facilities, which results in the production of contaminated food products. Disinfectants that usually contain quaternary ammonium compounds are used to eliminate bacterial contaminations in food-processing facilities. We could show that *L. monocytogenes* can easily become tolerant to these compounds by loss-of-function mutations in transcriptional repressors, which regulate the expression of efflux systems. Upon ingestion of contaminated food products, *L. monocytogenes* can switch to a pathogenic lifestyle and cause mild or severe infections depending on the immune status of the patient. This switch is dependent on the activity of the main virulence regulator PrfA, which controls the expression of most virulence genes of *L. monocytogenes*. Virulence gene expression is a costly process for the bacterium, thus expression and activity of PrfA needs to be tightly regulated and adapted in response to environmental factors. Regulation of PrfA takes place on transcriptional, posttranscriptional and posttranslational level to ensure that PrfA is only active, when *L. monocytogenes* enters its host. However, under certain conditions, *L. monocytogenes* can acquire gain-of-function mutations in PrfA leading to an enhanced virulence gene expression independent of the environmental conditions. All together, bacteria can easily adapt to changes in the environment by alterations in the gene expression due to mutations in regulatory systems.

SL-FG-R-051
Investigating signaling network evolution phenomena in the fungus *Magnaporthe oryzae*
*S. Jacob[1]
[1]*Institute of Biotechnology and Drug Research gGmbH, Molecular Biology & Fungal Genetics, Mainz, Germany*

Research in evolutionary dynamics has demonstrated that – apart from bacteria – also eukaryotic organisms like fungi can undergo rapid evolutionary changes within a "very short" timeframe. The molecular mechanisms driving these rapid adaptations remain poorly understood, largely due to their dependence on rare and unpredictable changes. Adaptive evolution in microorganisms typically involves advantageous genetic, transcriptomic, or proteomic changes that persist through natural selection. Understanding these mechanisms is crucial for addressing global challenges such as the emergence of new pathogens, the spread of invasive species, and the development of multi-drug resistance.

Within this work the rice blast pathogen *Magnaporthe oryzae* is used to highlight the complexity of evolution in regulatory networks of physiological and biochemical processes. The High Osmolarity Glycerol (HOG) pathway regulates cellular adaptation to environmental osmolarity. Loss-of-function (lof) mutants of the HOG pathway are osmosensitive and fail to produce the main critical osmotic stress response solute arabitol as it is in the wildtype strain. Interestingly, when these lof mutants are exposed to constant osmotic pressure, stable suppressor strains emerge that produce high amounts of the osmolyte glycerol instead of arabitol.

This study aimed to identify the genes responsible for adaptation to long-term stress and the shift from arabitol to glycerol production. Candidate genes were identified and their roles in primary metabolite production. Within this work an effective bioinformatic pipeline for label-free quantification of proteome and phosphoproteome data was developed. Using *M. oryzae* as a model, a data-independent acquisition (DIA) approach was developed, significantly improving the quality and completeness of the data. This method reduced the LC-MS/MS analysis time and increased the identification of phosphosites, establishing a refined methodology and a comprehensive basis for studying signaling processes in filamentous fungi. Within this method, a new set of candidate genes involved in the adaptation process was identified. These findings contribute to a deeper understanding of the complex evolutionary mechanisms in *M. oryzae* and underscore the need for continued research to unravel the molecular basis of rapid evolutionary adaptations in microorganisms.

Environmental microbiology

SL-FG-EM-052
Microbial iron corrosion and possible corrosion protection measures in monopiles of offshore wind power systems
*N. Adam-Beyer[1], C. Skottke[2], M. Schmidt[3], C. H. Solterbeck[4], C. Niepel[5], U. Niepel[5], G. Haake[6], J. Schloesser[4], M. Perner[7]
[1]*GEOMAR Helmholtz Centre for Ocean Research, Geomicrobiology, Kiel, Germany*
[2]*University of Applied Sciences Kiel, Kiel, Germany*
[3]*GEOMAR Helmholtz Centre for Ocean Research, Marine Geosystems, Kiel, Germany*
[4]*University of Applied Sciences Kiel, Kiel, Germany*
[5]*Krebs Korrosionsschutz GmbH, Rostock, Germany*
[6]*JÖRSS-BLUNCK-ORDEMANN GmbH, Hamburg, Germany*
[7]*GEOMAR Helmholtz Centre for Ocean Research, Geomicrobiology, Kiel, Germany*

Microbially influenced corrosion of iron (MIC) is a widespread problem in the maritime industry, especially for support

structures of offshore wind turbines, including monopiles. The steel-made, cylindrical hollow piles are driven into the seabed, trapping seawater, and thus exhibit only a limited exchange with the environment. Microbial activity and biogeochemical processes inside the monopile, which influence the MIC, are highly impacted by these special conditions. So far, in-depth studies of MIC in monopiles are lacking and commonly used corrosion protection measures only address electrochemical corrosion.

In an interdisciplinary project we aim at developing effective, environmentally friendly and economically feasible protection measures against MIC in offshore monopiles. For this purpose, a solid understanding of the environmental conditions, microbial communities and mechanisms involved in MIC inside the monopiles is necessary. Thus, we carried out in situ incubations inside a monopile in the North Sea, incubating commonly used mild steel at 6 and 21 m water depth, accompanied by measurements of biogeochemical parameters. First results show a strong seasonal stratification of the water column inside the monopile with the absence of oxygen and accumulation of biogenic methane and hydrogen sulfide (up to millimolar concentrations) in bottom waters at certain times of the year. Despite storm-related ventilation events of the water column, RNA-based taxonomic profiles of biofilms from steel samples incubated at 21 m exhibited an enrichment in sulfate reducing Bacteria and methanogenic Archaea, hypothesized to be relevant players in MIC under anaerobic conditions. Steel-associated communities at 6 m water depth, hallmarked by oxic conditions, were enriched in Fe-oxidizing Bacteria. Current and future work involves ex situ and further in situ incubations of suitable coated steel and steel alternatives as well as the development of local inhibition measures to identify the most efficient measures for MIC inhibition inside monopiles.

SL-FG-EM-053
Sea doggo go blub? Placing new prokaryotic lineages on the tree of life

G. Kolyfetis[1,2,3], T. Bornemann[3,4], A. Soares[3,4], K. P. Kaur[3,4], A. Probst[3,4], M. Strejček[5], C. Vorgias[6], *P. Adam[7,3]
[1]University of Konstanz, Neurobiology, Department of Biology, Konstanz, Germany
[2]University of Konstanz, Centre for the Advanced Study of Collective Behaviour, Konstanz, Germany
[3]University of Duisburg-Essen, Environmental Metagenomics, Faculty of Chemistry, Research Center One Health of the University Alliance Ruhr, Essen, Germany
[4]University of Duisburg-Essen, Centre of Water and Environmental Research (ZWU), Essen, Germany
[5]University of Chemistry and Technology, Department of Biochemistry and Microbiology, Prague, Czech Republic
[6]National and Kapodistrian University of Athens, Department of Biochemistry and Molecular Biology, Athens, Greece
[7]Kiel University, Institute for General Microbiology, Kiel, Germany

In about two decades since metagenomics and single cell genomics became common practice in microbial ecology, a deluge of genomes from uncultivated taxa with immense taxonomic and metabolic novelty has become available. The genomic data also brought about taxonomic classification approaches that use phylogenomics with concatenations of tens to hundreds of marker proteins. However, with deeper phylogenies and more divergent lineages included in the tree of life comes a host of phylogenetic systematic errors (e.g., long branch attraction, compositional bias). Most microbiologists lack the experience to perform the advanced phylogenomic analyses required to address these errors and instead rely on automated tools for the taxonomic assignment of their new lineages. This in turn creates issues, if these phylogenies are used to formulate evolutionary hypotheses.

To address these issues, we have created WhereDoGGo (Where Does my Genome Go?), a phylogenomics pipeline that aims to address different sources of error in the placement of prokaryotic lineages on the tree of life, while being user-friendly enough to be used by researchers with minimal bioinformatics experience.

WhereDoGGo leverages the normalized prokaryotic taxonomy system of the Genome Taxonomy Database to pick genomes with a balanced and diverse taxonomic sampling. It can create concatenated alignments of several published market sets and employs IQ-TREE as a framework to perform a series of analyses aiming to address the different sources of systematic error in phylogenies.

In this talk, we will first present the structure and explain the use of WhereDoGGo. Then, we will demonstrate its utility through a series of test cases, including the phylogenomic placement of novel lineages from coastal hot springs in the South Aegean Volcanic Arc.

SL-FG-EM-054
Machine learning-driven analysis of metabarcoding data to identify anthropogenic trace substances in aquatic ecosystems

*E. Zschaubitz[1], T. Sperlea[1], C. C. Glackin[1], L. Vogel[1], C. Nietz[1], H. Schröder[2], C. Hassenrück[1], M. Kanwischer[3], M. Labrenz[1]
[1]Leibniz Institute for Baltic Sea Research, Biological Oceanography, Rostock, Germany
[2]Planet AI GmbH, Rostock, Germany
[3]Leibniz Institute for Baltic Sea Research, Marine Chemistry, Rostock, Germany

Aquatic microbial communities play crucial roles in ecosystem functioning, providing a more comprehensive reflection of biological systems than single organisms. This makes them especially promising for evaluating ecosystem health. Although phytoplankton has been used in biomonitoring, bacterial communities offer additional insights. Despite their potential, bacterial communities and other microbial taxa, such as those revealed through 18S rRNA metabarcoding data, have not yet been fully integrated into official biomonitoring programs.

In this study, we demonstrated that using 16S and 18S rDNA metabarcoding data combined with machine learning enables the prediction of anthropogenic trace substances. Over one year, eDNA samples for 16S and 18S rRNA gene metabarcoding were collected twice weekly from 14 locations along the Warnow Estuary and Baltic Sea coast, with measurements of over 40 anthropogenic trace substances, such as pharmaceuticals, herbicides, and UV filters.

We trained Random Forest models to predict and quantify pollutants from the metabarcoding data, achieving high accuracy for a subgroup of contaminants. Using further regression analyses, we identified taxa that could be used as early warning indicators of substance concentrations exceeding ecotoxicological thresholds. Because the datasets used in this study span fresh water and marine habitats over the entire annual cycle, we expect the results to generalize easily to other locations.

Based on our findings, we propose that the integration of eDNA metabarcoding and machine learning will become a

vital component of official biomonitoring programs, providing a scalable and efficient method for assessing ecosystem health and impacts of anthropogenic pressures in aquatic environments.

SL-FG-EM-055

Microbiome reconstruction of *in situ* incubations reveals key players in polymer degradation in Arctic deep-sea hydrothermal sediments

*K. Saß[1,2], H. Arsin[2], A. E. Fedøy[2], R. Schmitz-Streit[1], W. Streit[3], I. H. Steen[2], R. Stokke[2]
[1]*Christian-Albrechts-University of Kiel, Institute for General Microbiology, Kiel, Germany*
[2]*Bergen University, Department for Biological Sciences, Bergen, Norway*
[3]*Hamburg University, Institut für Mikrobiologie und Biotechnologie, Hamburg, Germany*

Deep-sea hydrothermal vents are among the most extreme habitats on Earth and represent interesting targets for marine bioprospecting and biodiscovery. However, the full extent of the microbial heterotrophic capabilities at these environments is still being explored. Metagenomic studies already found that previously uncultured heterotrophic microorganisms control the carbon flow in hydrothermal deep-sea sediments through organic matter decomposition. Therefore, these habitats constitute important environments 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.

Here, we report on the development and the enrichment strategy of novel *in situ* incubators to investigate the degradation processes of the natural polymer chitin and artificial plastic polymers, like PET and HDPE. Sequence data of *in situ* enrichments with sediment samples from the Jan Mayen and the newly discovered Jøtul vent field show changes in the microbial community structure and indicate responsible microorganisms involved in the polymer degradation processes.

In addition to typical heterotrophic microorganisms, we identified potential novel chitin degraders within the uncultivated candidate phylum KSB1. These microorganisms are of particular interest, because they could neither be cultivated before, nor their role in carbon metabolism was described. By using different bioinformatical approaches we identified various chitin degrading enzymes of the KSB1 phylum. After heterologous expression and purification, the respective enzymes showed high chitin-degrading activity, demonstrating a significant role in the carbon cycle for KSB1 bacteria. The combined *in situ* incubation of chitin and plastic polymers also indicated an accelerated degradation of plastic polymers. By sequencing the transcriptomes, it was possible to reconstruct the microbiomes and uncover the underlying metabolic processes.

These results indicate that polymer 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. Furthermore, 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.

SL-FG-EM-056

Marine *Verrucomicrobiota* specialize in complex organic matter degradation

*L. H. Orellana Retamal[1], I. Wilkie[1], N. von Possel[1], T. Sauma Sánchez[1]
[1]*Max Planck Institute, Molecular Ecology, Bremen, Germany*

Verrucomicrobiota bacteria are commonly found in soil, marine, and gut environments. Depending on their environment, members of this phylum specialize in breaking down a variety of polysaccharides. In the marine ecosystem, *Verrucomicrobiota* are considered important consumers of complex algae-derived sulfated polysaccharides containing methyl pentoses, implicating them in organic matter turnover.

Here, we used visualization and multi-omic approaches to examine *Verrucomicrobiota* MAGs recovered from different-sized fractions (as a proxy for lifestyle) obtained during spring blooms in the North Sea.

We found that *Verrucomicrobiota* populations rely on specific adaptations to consume hard-to-degrade organic matter such as fucosidases, sulfatases, and bacterial microcompartments. We also detected these specialized pathways in metaproteomes recovered from the same samples. Genomes belonging to the family *Akkermansiaceae* were more prevalent in metagenomic samples from higher-sized fractions, unlike members of the DSM-45221 or MB11C04 families, indicating an apparent niche differentiation. The visualization of *Akkermansiaceae* cells attached to particles indicated a likely alternating free-living and particle-associated lifestyle, unlike the free-living lifestyle of DSM-45221 and MB11C04 members.

Comparing these results to known *Verrucomicrobiota* diversity further reinforces the highly specialized genomic features for organic matter degradation in marine members of this phylum. Thus, our results suggest that specialized *Verrucomicrobiota* populations subsisting in different niches could determine the fate of complex polysaccharides consumed during algae blooms and impact marine carbon cycling.

Synthetic microbiology

SL-FG-SM-122

Automating the DBTL-cycle for E. coli: Integration of modular cloning, CRISPRCas9 and Proteomics for advanced strain engineering

*T. Stoltmann[1], J. Tenhaef[1], T. Redeker[1], L. Wachtendonk[1], J. Marienhagen[1], S. Noack[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

Introduction: The current process for developing microbial strains is both time-consuming and costly, mainly due to the many manual steps involved. Through miniaturization, parallelization, automation and digitalization, there is great potential for a significant acceleration of strain engineering.

Methods: Here, we present automated workflows tailored to E. coli based on the seamless integration of modular cloning techniques (MoClo), genome editing tools (CRISPR-Cas9) and quantitative omics methods (proteomics).

Results: MoClo enables the rapid screening of libraries with various DNA building blocks such as promoters or ribosomal

binding sites. The plasmid-based workflow includes the entire Golden Gate reaction up to the transformation and plating of 96 assemblies on one plate. The additional integration of an automated CRISPR-Cas9 module enables the precise and targeted modification of the E. coli genome, which is crucial for the further metabolic engineering of producer strains. The latter covers the entire process from the generation of single guide RNA plasmids to the actual knock-out and curing step. Finally, to accelerate the subsequent phenotypic characterization of the genetically modified strains, an automated high-throughput module for untargeted proteomics was set up. The entire workflow, spanning from cultivation through bead mill processing to the Bradford assay, can be represented on the platform.

Conclusion: In summary, the automated MoClo, CRISPR-Cas9 and proteomics workflows provide a comprehensive toolset for the rapid engineering of E. coli strains. Each module can be operated with minimal human interaction and high robustness, also facilitating the exploration of the genetic diversity of this important model organism.

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ACS Synthetic Biology **2024** *13* (7), 2227-2237

DOI: 10.1021/acssynbio.4c00298

SL-FG-SM-123

Analysis of the homeostatic control of glutamate in *Bacillus subtilis*

*L. Araujo da Silva Amorim[1,2], L. Ferro Tavares[2], V. F. Bertolazzi Zocca[2], B. Blombach[1], D. Biscaro Pedrolli[2]

[1]Technical University Munich, Microbial Biotechnology, Straubing, Germany

[2]São Paulo State University (UNESP), Department of Bioprocess Engineering and Biotechnology, Araraquara, Brazil

Glutamate plays an essential role in cellular metabolism, representing a crucial metabolic intersection linking carbon and nitrogen metabolism[1]. Therefore, this study aimed to analyze the export and consumption of glutamate in strains of *Bacillus subtilis*. To this end, *B. subtilis* was used as a chassis for deleting the *pbuE* gene, whose deletion can increase the intracellular concentrations of NAD^+/NADH[2]. The engineered and parental strains were cultivated in 500 mL of mineral medium enriched with 60 g/L glucose for 50 h. During cultivation, samples were taken to evaluate cell growth spectrophotometrically and the concentration of glucose and glutamate in the medium by HPLC. *B. subtilis* wt exported glutamate to the medium during the exponential growth phase, reaching the peak between 20h and 30h. *B. subtilis* has been described to maintain high levels of intracellular glutamate during this phase, which may lead to the export of excess glutamate to sustain homeostatic levels[3]. *B. subtilis* Δ*pbuE* exported 0.15 g/L more glutamate than the parental strain. This indicates that increased concentrations of NAD^+/NADH may be a determining factor in the availability of the amino acid in the cell. In *B. subtilis*, the only reaction that directly connects glutamate to NAD^+/NADH is the conversion of glutamate into 2-ketoglutarate catalyzed by the RocG, which requires NAD^{+4}. Once the glucose concentration dropped and became limiting to growth, cells started to consume the glutamate from the medium. *B. subtilis* has a complex regulatory system related to glutamate, which can assess the availability of carbon and nitrogen in the medium and thus adjust glutamate homeostasis accordingly[3]. Therefore, the data provided in this study contribute to understanding the complex dynamics of glutamate synthesis and export in different metabolic contexts in *B. subtilis* during the fermentation process.

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SL-FG-SM-124

Mining of *Vibrionacea* genomes for plasmid origins as to obtain stable single-copy backbones for applications in Vibrio natriegens

*D. Stukenberg[1], L. Abendroth[1], M. Zick[1], T. Waldminghaus[1]

[1]TU-Darmstadt, Darmstadt, Germany

Plasmids are extrachromosomal replicons which are ubiquitous in many families of bacteria archaea. This is also the case for the *Vibrionacea*, a family with many famous members, e.g. the human pathogens *Vibrio cholerae* and *Vibrio parahaemolytics*. Previous studies have shown that approximately a third of *Vibrionacea* isolates harbor plasmids and it is likely that these plasmids carry genes for niche adaptation and pathogenicity. Additionally, plasmids have a long history in biotechnological applications, like the production of insulin, and more recently, the synthesis of mRNA vaccines.

Plasmids can be present in copy numbers from a single copy per cell, up to several hundreds. Especially single- and low-copy plasmids carry dedicated plasmid segregation systems. One such example is the ParABS machinery, consisting of the *parS* DNA sequence, typically located close to the origin of replication. *parS* is recognized by the CTPase ParB. The third component is ParA, a Walker-type ATPase, which binds unspecifically to DNA and interacts with ParB. Through a Brownian-ratchet mechanism caused by unbinding of ParA from the nucleoid, both copies of the ParB-bound plasmids are translocated to opposite cell poles, thereby ensuring stable inheritance of the plasmids to both daughter cells.

We are interested in identifying ParABS systems present in *Vibrionacea* to investigate the diversity of these segregation mechanisms and to harness them as novel tools.

Through a Hidden-Markov-Model-based genome mining approach, we identified almost 50 ParABS systems on plasmids from *Vibrionacea* isolates, with a replication protein

in close proximity. Through this bioinformatic analysis, we identified sequences, which are likely responsible for replication and segregation of these plasmids. We proceeded by cloning these sequences and evaluated their performance in the fast-growing bacterium *Vibrio natriegens*. Our results indicate that the resulting plasmids have a single or low-copy number and varying degrees of stability. In some cases, the resulting plasmids are highly stable and persist in the population even in absence of antibiotic selection.

In summary, we performed genome mining of *Vibrionacea* genomes to identify ParABS systems with accompanying replication proteins as plasmid origins. We experimentally characterized these sequences in *V. natriegens* to obtain stable single-copy origins which might allow the construction of synthetic chromosome in future projects.

SL-FG-SM-133
Multi-omic characterization of the enzyme turnover numbers in *Vibrio natriegens* at the genome-scale
*S. E. Wilken[1]
[1]Institute for Quantitative and Theoretical Biology, Biology, Düsseldorf, Germany

Vibrio natriegens is a halophilic, Gram-negative marine bacterium that is increasingly used in metabolic engineering applications due to its high growth rate. In sparse, defined media the organism has a doubling time of ~25 minutes. Previous work has shown that *V. natriegens* produces less protein per ribosome than *Escherichia coli*, suggesting that its ribosomal translation rate is not faster than that of *E. coli*. However, to achieve faster growth, *V. natriegens* increases its number of ribosomes per cell far beyond that of *E. coli* at the same growth rate. Given that its protein density is similarly constrained to that of *E. coli*, this necessitates that its metabolic enzymes are more efficient, and able to catalyze flux at a higher rate to sustain its metabolism. In this work, we investigate the apparent turnover (kcat) numbers of all its metabolically active enzymes under a variety of growth conditions.

The apparent turnover number of *V. natriegens* enzymes were measured *in vivo* by conducting quantitative proteomics and 13C metabolic flux analyses, under ten different conditions. These conditions included growth using six different carbon sources, and four stress conditions, in defined media. A high quality genome-scale metabolic model was constructed and curated using additional experimental data gathered during the project. This model was constrained using measured data, and used to gap fill missing flux measurements. Combining the 13C measured and imputed fluxes with the proteomic data, allowed us to estimate the apparent turnover number of ~800 metabolically active reactions (kcat := flux/enzyme).

Our results reveal that the metabolic enzymes of *V. natriegens* are on average three to ten times faster than those of *E. coli* under similar conditions. The enhanced turnover numbers partially explain why the organism is able to grow faster than *E. coli*. The turnover numbers can be used to parameterize an enzyme constrained flux balance analysis model of *V. natriegens*, and quantitatively predict the onset of overflow metabolism, which we validated with a chemostat run.

The combined experimental/computational approach employed here shed light on mechanism *V. natriegens* uses to accelerate its growth. This approach can be extended to other bacteria, increasing the availability of *in vivo* measured enzyme turnover numbers, and improving the predictive accuracy of enzyme constrained metabolic models.

SL-FG-SM-168
Psychrophilic limits of survival – what are the implications for icy moon exploration?
*T. Zaccaria[1,2], K. Beblo-Vranesevic[2], M. I. de Jonge[3,4], M. G. Netea[1,3,5], P. Rettberg[2]
[1]Radboud University Medical Center, Department of Internal Medicine, Nijmegen, Netherlands
[2]German Aerospace Center (DLR), Radiation Biology, Cologne, Germany
[3]Radboud University Medical Center, Radboud Center for Infectious Diseases, Nijmegen, Netherlands
[4]Radboud University Medical Center, Department of Laboratory Medicine, Nijmegen, Netherlands
[5]Rheinische Friedrich-Wilhelms-Universität Bonn, Department for Immunology and Metabolism, Bonn, Germany

Exposing microorganisms to extreme environmental conditions can help us understand what their survival limits are. On Earth, microorganisms can be found in almost all environments, and are capable of tolerating extreme physical and chemical stressors. Investigating how these microbial extremophiles tolerate and thrive with these stressors might give us an indication of how life might survive on other planets. Several environments on Earth classified as extreme are valuable analogues which replicate the locations found on other bodies of the solar system. An interesting astrobiology location in the solar system are the icy moons Europa and Enceladus. These moons could support life due to their peculiar environment having a subsurface ocean covered by a thick ice layer. By making use of psychrophilic microorganisms which are already adapted to grow at low temperatures we can investigate whether they could hypothetical endure on the icy moons. This information might also shed light on how life could develop on the icy moons. Additionally, making use of Earth extremophiles in laboratory simulations, we can evaluate which ones might pose a threat of contamination if found to be hitchhiking on a spacecraft bound for the icy moons.

The experiments initially focused on selecting four psychrophilic microbial extremophiles candidates from icy moon-analogue environments on Earth. The selected organisms *Chromohalobacter sarecensis*, *Planococcus halocryophilus*, *Rhodotorula frigidalcoholis* and *Rhodotorula mucilaginosa*, and were grown in minimal media supplemented with a single carbon source in order to represent more accurately the nutrient-limiting conditions in the subsurface ocean of the icy-moons. Following growth in minimal media we exposed the organisms to laboratory simulated extreme conditions in order to evaluate their survival limits. These conditions include: desiccation at subzero temperatures, freezing at -80°C and freezing and thawing, exposure to UV-C and polychromatic UV radiation as well as exposure to X-ray radiation. Our results highlight how all organisms can tolerate to an extent the extreme conditions, however, the yeasts are the ones which tolerate the most the conditions of desiccation and freezing and thawing. Additionally, current experiments also include the analysis of RNA extracted from yeast samples after exposure to extreme conditions to evaluate the mechanisms of repair.

SL-FG-SM-169

Planetary Protection of Planet Mars – Potential risks of contamination by sulphate-reducing organisms from Earth?

*F. Gibis[1,2], K. Beblo-Vranesevic[3], P. Rettberg[3], W. Liebl[1], A. Ehrenreich[3]
[1]*Technical University Munich, Chair of Microbiology, School of Life Sciences, Freising, Germany*
[2]*Technical University Munich, Chair of Aquatic Systems Biology, School of Life Sciences, Iffeldorf, Germany*
[3]*German Aerospace Center (DLR), Astrobiology research group, Radiation Biology Department, Institute of Aerospace Medicine, Cologne, Germany*

Mars, commonly referred to as the Red Planet due to the red color of iron oxides present in Martian regolith, is considered the most Earth-like planet in our solar system and has fascinated humanity from for centuries. One of the key questions in Mars exploration is whether life exists or has existed on the planet. To answer this question, evidence for present or past microbial life on Mars is being sought. With the increasing number of research missions reaching Mars, planetary protection has gained significant importance to prevent contamination by terrestrial microorganisms. Endospore-forming sulfate-reducing bacteria of the family *Peptococcaceae* represent a group of obligate anaerobic organisms that can be found on Earth under extreme conditions in various subsurface environments including freshwater and marine sediments, mines, oil reservoirs, and aquifers at depths of up to 3,000 meters. In addition to their ability to form highly resistant endospores, they are capable of chemolithoautotrophic growth using sulfate, H_2, and CO_2. This characteristic, combined with their adaptability to extreme conditions, theoretically positions them as potential candidates to become stowaways to Mars and survive the harsh environment in the form of spores.

In this study, two representatives of the *Peptococcaceae* family were investigated regarding their ability for autotrophic growth in the presence of Martian regolith and their tolerance to desiccation and radiation as present on Mars. The experiments demonstrate that the two species, *Desulforamulus putei* TH-11^T and *Desulfosporosinus lacus* STP12^T, can easily grow autotrophically in an H_2/CO_2 atmosphere using the sulfate present in the Mars regolith simulant MGS-1S as an electron acceptor. Since CO_2 is part of the Martian atmosphere, water could be present occasionally and H_2 could be formed through serpentinization of iron oxides where there might be a possibility that endospores who reach Mars could germinate and grow. Furthermore, we demonstrate that spores formed during growth on MGS-1S showed high resistance to an exposure to elevated temperatures, oxygen, polychromatic UV radiation and desiccation and could potentially survive dormant on Mars and may reach Mars as highly resistant contaminants of space probes. The results provide a first hint to the potential risk of contaminating Mars by spore-forming sulphate-reducing bacteria regarding topics like planetary protection and search for possible life on Mars.

SL-FG-SM-170
Fungal biomining – In-situ resource utilization in spaceUtilizing fungi to help establish resource – efficient space missions

*J. Piepjohn[1], K. Steinacker[1,2], K. Beblo-Vranesevic[1], A. Cowley[2], S. Leuko[1]
[1]*German Aerospace Centre, Applied Aerospace Biology, Köln, Germany*
[2]*European Astronaut Centre (EAC), Linder Hoehe, Germany*

Filamentous fungi are powerful microorganisms for biotechnological applications. They are well-established cell factories for the production of organic acids, enzymes, and antibiotics. With the prospect of human colonization on celestial bodies such as on the Moon and Mars, the sustainability of space missions is of greater relevance and immediacy. As fungi take little demand and are tolerant towards extreme environmental conditions, it is only logical to consider them for in-situ resource utilization applications for space research. For future missions to terrestrial-like bodies such as Mars and the Moon, with surfaces that are covered abundantly with regolith, it is of high importance to establish sustainable mining applications since these bodies are great sources for ore retrieval, which is especially important since resupply missions are expensive. This study used Aspergillus simplicissimum as a model organism to determine the suitability of filamentous fungi for biomining approaches. We were able to show that our fungal species was capable of growing under simulated micro gravity conditions, as well as in the presence of Lunar Highland Simulant 1 (LHS-1) and the European Astronaut Centre Lunar Regolith Simulant (EAC-1A). Moreover, we were able to show that fungi are capable of extracting minerals and metals from lunar regolith simulants. The analysis of precipitated metals, as well as the ICP-MS analysis of the leachate, showed that several metals were dissolved in the liquid phase. The suitability of microorganisms for biomining is well known. However, to date, only applications for bacteria have been studied extensively and industrially established. This study shows that A. simplicissimum and other filamentous fungi are very promising candidates for ISRU applications in space.

SL-FG-SM-171
Brine Beneath: The hidden microbial world of Lüneburg's salt

*K. Runzheimer[1,2], L. Schwab[3], J. Gamez Rössler[2], F. Miele[2], S. Leuko[2]
[1]*Carl von Ossietzky University, Oldenburg, Germany*
[2]*German Aerospace Center (DLR), Cologne, Germany*
[3]*Friedrich Schiller University, Jena, Germany*

Environments with extremely high salt concentrations serve as analogue sites for astrobiological research. Despite their seemingly inhospitable and harsh conditions, these environments support a diverse array of extremophilic organisms. The saturated Lüneburg brine forms at a depth of 60 m below the surface through dissolution of Zechstein halite. It has been used commercially for salt extraction for over 1000 years until it was closed in 1980 and although it remains easily accessible for sampling, it had not been investigated before. Studying extremophiles from these saline habitats is important, as it offers valuable insights into a hypersaline habitat which cannot benefit from primary production through sunlight and resembles the boundaries of life on Earth and the possibility of life beyond our planet. This work provides a habitat description of the Lüneburg brine, using hydrochemical, cultivation-dependent and independent methods.

The analyses included sequencing of variable regions V1-V2 and V3-V4 of the 16S rRNA gene, isolation of culturable aerobic microorganisms, enrichment of a heterotrophic and an autotrophic anaerobic microbial community as well as chemical profiling of the brine. Selected microbial isolates were tested for their resistance to astrobiological-relevant stressors. The 16S rRNA amplicon analysis identified the presence of halophilic organisms: *Desulfohalobiaceae* (18%), *Thiohalorhabdaceae* (11%) and three families from

the order *Halobacterales* (16%). A high abundance of yet-uncultivated *Nanosalinaceae* (15%) were detected and recognized as promising candidates for pure culture isolation. Anaerobic enrichments demonstrated the presence of *Nanosalinaceae*, contingent upon substantial co-enrichment of *Halobacteriaceae*. Microscopic analysis indicated notable morphological diversity, with isolates displaying pleomorphism, encapsulation and biofilm production during growth. The isolates displayed tolerance towards high radiation (>500 Gy) and NaCl concentrations up to 30%, as well as enhanced growth in typically toxic perchlorates, with concentrations that are also present on Mars.

The Lüneburg brine represents an aphotic habitat and thus a representative subsurface analog for other planets in outer space. It hosts a range of halophilic microorganisms capable of tolerating high radiation and salt concentrations. This study highlights the potential of this accessible habitat for astrobiological research.

SL-FG-SM-172
Microbial monitoring and microbial diversity in the EDEN-ISS greenhouse, a test facility for plant-cultivation in space

*L. Gaiser[1], K. Beblo-Vranesevic[1], D. Engel[1], J. Fahrion[2], R. Van Houdt[2], L. G. de Chalonge[3], J. Bunchek[4], P. Zabel[4], D. Schubert[4], P. Rettberg[1]
[1]*Deutsches Zentrum für Luft- und Raumfahrt (DLR), Institut für Luft- und Raumfahrtmedizin, Bonn, Germany*
[2]*Belgian Nuclear Research Centre (SCK CEN), Microbiology Unit, Mol, Belgium*
[3]*Dublin City University, Life Sciences Institute, School of Chemical Science, Dublin, Ireland*
[4]*German Aerospace Center (DLR) Institute for Space Systems, Bremen, Germany*

The limited storage space for consumable goods like food and medicine is one of the major factors currently limiting the duration and distance of crewed spaceflight missions. To overcome these limitations research into bio-regenerative life support systems (BLSS) is being conducted. BLSS utilize the regenerative abilities of plants and microorganisms to provide food, clean water, breathable air and potentially other goods like medicine. Before these technologies can be incorporated they need to be extensively tested and validated on Earth. TheEDEN-ISS project aimed to test plant cultivation technologies for BLSS in a space analogue environment. These technologies were tested in a greenhouse situated at the Neumayer III station in Antarctica for multiple years, to secure an isolated test environment. To ensure the continued operation of a BLSS, it is vital that plants remain healthy, which necessitates the monitoring of the greenhouse microbiome to ensure that pathogens are detected early and counter-measures can be engaged.

During different campaigns, swab surface samples were taken from various locations in the greenhouse before/after cleaning and samples taken at regular intervals over an entire growing season. The bioburden of each sample was estimated via viable count and the isolates were identified via partial 16S rRNA gene sequencing. Additionally, 16S amplicon sequencing was performed on DNA extracted directly from the swab-samples to characterize the microbiome.

The results revealed that the bioburden of the different sampling positions was not significantly reduced by the cleaning, indicating that the employed cleaning regime was unsuited in its current form to adequately lower the bioburden.

Identification of the isolates as well as the full microbiome revealed mostly environmental genera. However, in both cases genera containing potential pathogens were identified. The two sets of sequencing data had little overlap and emphasized different aspects of the greenhouse microbiome, highlighting the advantages of using a combined approach to obtain a more complete picture of the microbiome composition.

SL-FG-SM-173
From planetary safety to cultural heritage: an interdisciplinary exploration of microorganisms on inanimate surfaces

*C. Flocco[1], R. Pukall[1], I. Schober[1], C. Moissl-Eichinger[2], P. Rettberg[3], J. Overmann[1]
[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany*
[2]*Medical University of Graz, Graz, Austria*
[3]*German Aerospace Center, DLR, Köln, Germany*

Interdisciplinary approaches at the intersection of microbiology, cultural heritage, material sciences, and biosafety broaden the frontiers of knowledge by offering enriched perspectives on microbial communities and their interactions with the objects and materials they inhabit. Along these lines, we have investigated the microbial communities present on cultural heritage objects, such as ancient medieval books[a] housed at the Leipzig University Library, and those associated with the bioburden controls of spacecraft and cleanroom facilities of the European Space Agency (ESA)[b] mandated by planetary protection policies. Although these isolation sources might initially appear unrelated, both datasets share common taxa known for their capacity to survive under extreme conditions. These include endospore-forming genera *Bacillus*, *Paenibacillus*, *Peribacillus*, and *Priestia*, but also other genera including *Micrococcus*, *Kocuria*, and *Staphylococcus.* A current project moves beyond cataloguing efforts to employ targeted microbial cultivation combined with phylogenomic and functional analyses to investigate specific survival strategies of staphylococcal taxa. This work has broader implications, given the potential pathogenicity of the target genus. Samples were retrieved from 13th-century medieval manuscripts and from spaceflight hardware and assembly facilities at several ESA locations. Selected *Staphylococcus epidermidis* isolates were fully sequenced using a combination of long- and short-read sequencing technologies, followed by bioinformatic assembly. We obtained, twelve high-quality, closed *S. epidermidis* genomes from the medieval manuscripts and twenty-three from the ESA collection. Ongoing phylogenetic and functional analyses aim to elucidate evolutionary relationships and explore the mechanisms of persistence under extreme conditions. Our interdisciplinary and in-depth profiling of the ESA collection and cultural heritage isolates contributes not only to bioburden control strategies for space missions but also hold relevance for terrestrial facilities, such as hospitals and food production sites, where stringent contamination controls are critical, as well as for the preservation of cultural heritage.[a]Flocco *et al.* 2023. Touching the (almost) untouchable: a minimally invasive workflow [..] DOI: 10.3389/fmicb.2023.1197837.

[b]The ESA Microbial Collection at the DSMZ. www.dsmz.de/collection/catalogue/microorganisms/special-groups-of-organisms/esa-strains

SL-FG-SM-174
Rhodococcus for bioeconomy through synthetic biology
*A. C. Ngo[1]
[1]*Ruhr-Universität Bochum, Microbial Biotechnology, Faculty of Biology and Biotechnology, Bochum, Germany*

Environmental pollution has been the biggest trade-off caused by modernization and industrial revolution. Several efforts are now being channelled to circumvent this issue on environmental pollution and its repercussions such as global warming. The use of biological agents like exploiting the machineries of microorganisms has now emerged as a promising option. Microorganisms such as bacteria can adapt and evolve given the right amount of selective pressure. Therefore, it can be promising to exploit their inherent tools and machineries.

Actinobacteria such as *Streptomyces*, *Gordonia*, and *Rhodococcus* are considered metabolic powerhouses that can degrade and transform different compounds such as styrene, rubber, phenols, and nitroaromatics. Several studies have shown and explored the enzymes responsible in degrading these pollutants. But unlike model organisms such as *Pseudomonas putida* and *Escherichia coli*, we have yet to understand on how to use an actinobacteria such as *Rhodococcus* for the purpose of a microbial cell factory through synthetic approaches. Nonetheless, *Rhodococcus* presents a more robust system as it can withstand harsher conditions compared to other model organisms. It is also easier to cultivate than other actinobacteria. Here, we present available information for *Rhodococcus* such as a tailored genetic toolbox and how to utilize them for protein production. Moreover, the current progress of *Rhodococcus* towards the route of bioeconomy will be discussed such as its potential for upcycling. This includes conversion of lignin-derived compounds like vanillin or polyethylene for industrially valuable products. This highlights how engineering *Rhodococcus* for bioeconomy can be promising.

Functional genomics / microbiome

SL-FG-FGM-065
Discovering the hidden function of long non-coding RNAs of *Aspergilli*
*M. L. Fabre[1], A. E. Barber[1]
[1]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*

Fungal pathogens of the *Aspergillus* genus pose significant threats to human health and food production. Despite progress in understanding pathogenicity mechanisms, a crucial knowledge gap remains regarding the role of long non-coding RNAs (lncRNAs) in the evolution of fungi into successful pathogens. LncRNAs represent a vital yet often overlooked aspect of fungal biology, particularly in how *Aspergillus* species withstand stress to endure and thrive under adverse conditions. For instance, in *Candida auris*, the lncRNA DINOR regulates fungal virulence by modulating stress responses and hyphal growth, suggesting that lncRNAs in *Aspergillus* may similarly influence pathogenic mechanisms.

In our study, we aimed to unravel the role of lncRNAs in responding to changing conditions using a transcriptome analysis of >1000 samples *A. flavus, A. fumigatus, A. nidulans,* and *A. niger* available in public databases. Using two different machine learning algorithms we successfully predicted 4022, 1482, 3098, and 3129 potential lncRNAs for *A. flavus, A. fumigatus, A. nidulans*, and *A. niger,* respectively. Comparative analysis revealed that lncRNAs in these species share common characteristics, such as shorter length, lower expression levels, and reduced GC content compared to protein-coding genes. Notably, only *A. fumigatus*, the main pathogenic species, showed a lower proportion of lncRNAs relative to its genome size and a unique enrichment in subtelomeric regions. This finding suggests that the spatial organization of lncRNAs in *A. fumigatus* may play a critical role in regulating key processes, such as metabolism, contributing to its success as an opportunistic pathogen. We also identified lncRNAs that exhibited differential expression in response to stress and explored their potential functions by analyzing the nearby protein-coding genes. By employing this cis-regulation strategy, we identified diverse Gene Ontology (GO) terms and the biological functions impacted by lncRNAs across the four *Aspergillus* species.

In conclusion, our approach to identifying lncRNAs in *Aspergillus* species offers new insights into fungal pathogenicity and potential strategies to combat infections.

SL-FG-FGM-066
Infection-induced glycosaminoglycan release promotes microbiota expansion in *Drosophila* gut
A. Arias-Rojas[1], *I. Iatsenko[2]
[1]*Max Planck Institute, Genetics of Host-Microbe Interactions, Berlin, Germany*
[2]*Max Planck Institute, Genetics of host-microbe interactions, Berlin, Germany*

While the host genetics is known to affect the composition of intestinal microbial communities, whether the host genotype influences the abundance of intestinal commensals remains uninvestigated. To close this knowledge gap, we performed a *genome-wide association study* (*GWAS*) in the fruit fly *Drosophila melanogaster* to identify host genetic variants linked to the abundance of *Lactiplantibacillus plantarum* – a major gut commensal of fruit flies. Our GWA study uncovered a statistically significant association between polymorphisms in genes involved in *heparan sulfate synthesis* and *L. plantarum* load. RNAi-mediated knockdown of some of these genes resulted in reduced *heparan sulfate* synthesis and reduced abundance of *L. plantarum*. Thus, *heparan sulfate* mediates *Drosophila* gut colonization by *L. plantarum*. Mechanistically, *heparan sulfate* facilitates the adhesion of *L. plantarum* to host epithelium and promotes biofilm formation. We further showed that intestinal infection induces *heparan sulfate* synthesis by the host via activation of the Nf-kB immune signaling cascade. Increased availability of *heparan sulfate* during infection results in the expansion of *L. plantarum* population in the gut. Importantly, such commensal expansion is also beneficial for the host as *L. plantarum* protects the host from intestinal pathogens via colonization resistance. This work reports an intriguing case where commensal not only survives the inflammatory environment caused by the infection but also benefits from it by utilizing the additional resources made available during infection.

SL-FG-FGM-067
Uncovering synthetic steroid biotransformations in gut and environmental bacteria
*M. Beliaeva[1], M. Gross[1], M. Zimmermann[1]
[1]European Molecular Biology Laboratory, Molecular Systems Biology, Heidelberg, Germany

Synthetic steroids are widely used in both pharmaceuticals and veterinary medicine. Designed for increased potency and resistance to metabolism, these compounds tend to persist in the environment, raising ecological concerns such as disruption of aquatic ecosystems and impacts on plant growth. While several environmental bacteria have shown the ability to degrade natural steroids, such as testosterone and estrone, the degradation of synthetic analogs remains poorly understood. In this study, we screened four environmental species and eight gut bacteria to investigate the biotransformation of 20 steroids and steroid prodrugs, including synthetic estrogens, progestogens, and corticosteroids. Through the development of a tandem mass spectrometry-based pipeline, we identified key steroid biotransformation intermediates and products, revealing distinct steroid metabolizing pathways for the different bacteria tested. These findings illustrate the specific roles in steroid metabolism that environmental and gut bacteria play and suggest that prior steroid exposure to gut microbiota may affect the fate of synthetic steroids in the environment. To uncover the enzymes and enzyme pathways involved, we employed genetic screens and homology searches. Our developed approach provides a robust platform to identify steroid metabolites and select enzymes of specific biocatalytic interest for further characterization, as well as allows for mapping the network of genes, proteins, and species capable of steroid biotransformations within specific ecosystems, such as the human gut and environmental habitats.

SL-FG-FGM-068
From gut to data: An interactive platform for exploring human microbiome fingerprints via AutoML-Driven bioindicator discovery
*J. C. Kasmanas[1], M. Schloter[1], A. C.P.L.F. de Carvalho[1], P. Stadler[1], U. Nunes da Rocha[1]
[1]Helmholtz Centre for Environmental Research - UFZ, Department of Applied Microbial Ecology (AME), Leipzig, Germany

Despite sequencing advances, characterized profiles of the human gut microbiome remain limited, hindering microbial community classification. Using dense deep clustering, we standardized 509,610 metagenome-assembled genomes (MAGs) to create comprehensive microbiome fingerprints. Our resulting interactive platform comes with AutoML-driven bioindicator detection, which can scan functional potential patterns across countries and disease conditions.

We selected 14,082 metagenomic runs (>20MM depth) from HumanMetagenomeDB (https://web.app.ufz.de/hmgdb/) and recovered 302,781 MAGs using MuDoGeR. Following this, we included and harmonized 154,736 MAGs from Pasolli et al. (Cell, 2019) and 60,675 from Nayfach et al. (Nature, 2019) to create a unified, standardized dataset. After dereplication, we identified 6,794 species. All MAGs underwent gene annotation using the ISfinder, NCBI-RefSeq, UniProt, and HMM databases available via Prokka. Next, we created a presence/absence gene profile for each MAG in adult gut samples (>18 years), yielding 426,648 profiles with 40,424 non-redundant genes. Next, we implemented an autoencoder with dense layers and a rectified linear unit (ReLU) activation function, followed by DenMune clustering to capture metagenomic fingerprints. We analyzed MAG distribution across taxonomy, geography, and host medical conditions. Finally, we developed an interactive platform with AutoML-driven bioindicator detection to identify potential key microbiome elements from the embedded space using the HumanMetagenomeDB curated metadata.

The embedded space revealed distinct microbiome profiles, showing significant differences between control and colorectal cancerous samples and separate libraries by country (PERMANOVA < 0.05). Additionally, the MAGs clusters based on functional potential identified taxonomical groups diverging from their majority cluster, which could signal relevant functional shifts in specific strains. Our AutoML bioindicator detection system uncovered geographical markers, with *Lachnospira eligens*, for instance, emerging as a promising bioindicator for differentiating Chinese and USA gut microbiomes. This study provided a standardized dataset, downloadable genome collection, and interactive webapp, expanding previously published resources for future microbiome research.

SL-FG-FGM-069
Can perennial wheat influence the root-rhizosphere axis to enhance rhizomicrobiome diversity?
*S. Issifu[1,2], R. C. Wilhem[2], F. Rasche[1,3], A. Viji Elango[2]
[1]University of Hohenheim, 490E, Stuttgart, Germany
[2]Purdue University, Agronomy, West Lafeyette, United States
[3]International Institute of Tropical Agriculture, Nairobi, Kenya

The plant-associated microbiome plays a crucial role in enhancing agricultural productivity and ecosystem health. *Thinopyrum intermedium*, a perennial wheatgrass, offers potential ecosystem services but information about its microbiome. In the frame of the EU co-funded Biodiversa project NAPERDIV, we investigated the rhizomicrobiome of *T. intermedium* to assess biodiversity benefits compared to annual winter wheat.

Using a metagenomic approach, rhizosphere soil samples were collected from *T. intermedium* and annual wheat fields across Sweden, Belgium, and France, spanning a North-South agro-climatic gradient over two years (2021–2022). Bacterial 16S rRNA genes were amplified, and bioinformatics analyses were conducted. We integrated our data with an agroecological database based on four indicators (rhizosphere, grassland, organic matter, no-tillage) to understand the generalizability of our findings. Also, we tested the potential of *T. intermedium* to ensure year-on-year stability of the rhizomicrobiome using Bray Curtis dissimilarity.

Results indicated no significant differences in alpha diversity between the two crops, although *T. intermedium* hosted more amplicon sequence variants (ASVs). Beta diversity was more influenced by country, sampling depth, and sampling year than by crop type. Comparison with root endophytes showed that in both plants, the rhizomicrobiome was more diverse, affirming existing understanding that the endophytes are a subset of the rhizomicrobiome. Comaprison with the agroecological database revealed that the rhizomicrobiome of *T. intermedium* hosted more taxa associated with grasslands, organic matter, rhizosphere, and no-till practices. We confirmed that the rhizomicrobiome associated with *T. intermedium* was significantly stable compared to annual wheat based on year-on-year assessment.

Overall, we found considerable similarities in the rhizomicrobiome of *T. intermedium* and annual wheat, suggesting minor influence of perenniality on the rhizomicrobiome. This study is the first to analyze the rhizomicrobiome of *T. intermedium* in a European context, providing additional insights into understanding the influence of perennialization on rhizomicrobiome.

SL-FG-FGM-070
SaxA-mediated degradation of ITC plant toxin functions as a public good in a bacterial community
*M. Mauri[1], K. Unger[1], R. Allen[1], M. Agler[1]
[1]*Friedrich Schiller University, Jena, Germany*

Plant leaves host diverse microbial communities of beneficial commensals and opportunistic pathogens, whose balance is crucial for plant health. Brassicaceae plants like *Arabidopsis thaliana* defend against pathogens by producing glucosinolates (GLS), which are enzymatically converted into antimicrobial isothiocyanates (ITCs) upon tissue damage by bacteria. ITCs inhibit bacterial growth and are a key element of the plant defense. However, pathogenic *Pseudomonas* spp. can detoxify ITCs via the hydrolase SaxA, while commensals are typically unable to detoxify ITCs. Understanding the effects of ITC degradation dynamics on the leaf microbiome is essential for advancing knowledge of disease resistance in ecologically relevant plants.

In this study, we test whether *Pseudomonas viridiflava* 3D9 (*Ps* 3D9)"s ability to degrade 4-methylsulfinylbutyl (4MSOB - ITC) can shield the entire bacterial leaf community from plant defenses, even when other commensals cannot. We explored this by constructing an artificial community of five commensals which were isolated along with *Ps* 3D9 from healthy wild *A. thaliana* leaves. We either added the wildtype *Ps* 3D9 or *Ps* 3D9Δ*saxA* which does not degrade ITCs.

We first examined the effects of 4MSOB-ITC on the growth rates of axenic cultures of *Pseudomonas* 3D9, its ΔsaxA mutant, and commensals. To assess the impact of detoxification, we further developed a mathematical model based on extended Lotka-Volterra equations, incorporating ITC toxicity and the ability of *Pseudomonas* to degrade it. We then extended the model to include interactions between ITC-degrading *Pseudomonas* and non-degrading commensals, predicting community composition at varying degrader concentrations. Our results identify the threshold where the degrader provides communal protection from ITC toxicity. Model predictions align well with experimental data, confirming the protective role of the degrader strain.

Studying the GLS defense system in *A. thaliana* enhances our understanding of plant-microbe interactions and offers valuable insights for modeling microbial communities.

Microbial pathogenicity

SL-FG-MP-101
Insights from a novel commensal 3D melanoma skin model: Advanced tumorigenesis due to bacterial colonization
*A. Rosin[1], H. Sprenger[1], M. Kissner[1], K. Neuhaus[2], T. Tralau[1], T. Höper[1], L. Lemoine[1]
[1]*German Federal Institute for Risk Assessment, Berlin, Germany*
[2]*Technical University Munich, Core Facility Microbiome, Freising, Germany*

The skin microbiome, which comprises bacteria, fungi, mites, and viruses, has been demonstrated to play a critical role in skin homeostasis and disorders. The contribution of the skin microbiome to various skin cancer types, including melanoma, gained increasing attention, as current studies revealed an altered composition of bacterial communities at melanoma sites compared to healthy skin. However, the underlying mechanisms of the complex interplay between the skin microbiome and melanoma progression remain elusive.

We established a co-culture system capable to study host microbiome interactions during melanoma progression *in situ*. The system is based on the commercially available Melanoma model from MatTek colonized with skin bacteria obtained from skin swabs from a healthy volunteer.

The melanoma models showed a stable co-colonization over the cultivation period of 12 days, with no transcriptional activation of bacterial defense pathways. 16S rRNA gene amplicon sequencing indicated reduced bacterial diversity with *Streptococcus* being the most abundant genera on the last day of cultivation. Pathways related to melanoma progression, such as RAF/MAP and PI3K kinase, were induced in the transcriptome of colonized models. Those findings correlate with the increased release of cytokines GM-CSF, VEGF, and PIGF, which promote angiogenesis, and melanoma markers MIA and S100B. Additionally, epithelial-mesenchymal transition was active in colonized skin models.

As a proof of concept, the results indicate a clear impact of microbial skin colonization on melanoma tumorigenesis, thereby providing an elegant method to elucidate the microbiome"s impact on cancer development. As a first approach, we colonized 3D melanoma models with a defined bacterial community that included both typical commensal bacteria and pathogens such as *Staphylococcus aureus* and *Corynebacterium striatum*, which were found to be abundant on melanoma sites. The initial results indicated more stable models regarding bacterial cell count in the presence of the bacterial mixture containing the pathogens compared to the mixture without, which might affect the progression of melanoma.

SL-FG-MP-102
Establishment of a 3D lung infection models to study *A. fumigatus* infection
*K. Volkmar[1], L. Radosa[1], L. Nikitashina[1], M. Strassburger[2], A. Montesano[3], T. Heinekamp[1], I. Jacobsen[3,4], O. Kniemeyer[1], A. Brakhage[1,4]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Molecular and Applied Microbiology, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology, Transfer Group Anti-Infectives, Jena, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology, Microbial Immunology, Jena, Germany*
[4]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*

Aspergillus fumigatus is an opportunistic fungal pathogen and one of the most common causes of invasive pulmonary aspergillosis in solid-organ transplant (SOT) and allogenic hematopoietic stem cell (HSC) recipients as well as leukemia patients. The prognosis is poor with mortality rates of 40-50 %. While neutrophils have been identified as the most important effector cell, the role of the epithelium in *A. fumigatus* infection is poorly understood. The reason for this is the use of 2D cell culture approaches, which do not recapitulate several important characteristics of the lung, like

cell-to-cell interactions, the heterogeneity of cell types or proximal to distal patterning. On the other hand, the translation of animal models to humans is often affected by species-specific differences between mice and men and the use of laboratory animals raises ethical concerns. To address these limitations and reduce the number of used laboratory animals, in accordance with the 3R principles (refine, reduce, replace), we sought to establish the use of murine precision-cut lung slices (PCLS) and a human epithelial lung organoid model to investigate the interaction of *A. fumigatus* with the epithelium. PCLS were viable for up to seven days and retained the complex 3D architecture of the lung, irrespective of the experimental treatment. Furthermore, PCLS were immunocompetent and showed a strong cytokine production upon treatment with different TLR agonists and viable *A. fumigatus* conidia. The immune response to *A. fumigatus* was dependent on fungal burden and was abrogated by voriconazole treatment, highlighting their potential as a tool for the screening of antifungal compounds. In summary, murine PCLS represent a scalable tool that retain the complex 3D architecture cellular heterogeneity of the lung. This makes them suitable to analyze the spatial interaction of pathogens and host cells in a near-native environment.

SL-FG-MP-103
3D tissue models for *Neisseria gonorrhoeae* infection research

L. M. Kafuri Cifuentes[1], M. Heydarian[1], N. Knetzger[2], C. Lotz[2], R. Rawal[1], T. Rudel[1], *V. Kozjak-Pavlovic[1]
[1]University of Würzburg, Microbiology, Würzburg, Germany
[2]Fraunhofer-Gesellschaft, Translational Center Regenerative Therapies TLC-RT, Würzburg, Germany

Neisseria gonorrhoeae is an obligate human pathogen and a causative agent of a sexually transmitted disease gonorrhea. Apart from the infections of the urogenital tract, gonococci cause eye infection in newborns, who get infected during delivery. Newborn conjunctivitis or ophthalmia neonatorum was one of the most common causes of blindness until the introduction of routine prophylaxis. Urogenital infections are treatable with antibiotics but are becoming a growing health concern due to the increase in the antimicrobial resistance among *N. gonorrhoeae* strains.

Our work focuses on development and testing of 3D tissue models that can be applied in the research of *N. gonorrhoeae* infections. In one approach, we developed tissue models using cell lines that were based on a small intestinal submucosa (SIS) scaffold derived from pigs. These models initially contained fibroblast and epithelial cells and were later refined by the addition of an endothelial cell layer, as well as neutrophils. Further on, we established a procedure to retrieve primary endometrial cells and are currently testing them for the 3D model generation. In a second approach, we used already established cornea models (collaboration with the group of Christian Lotz, Fraunhofer TLC-RT) to study the role of pilus in the eye infection with *N. gonorrhoeae*. Both approaches demonstrate the advantages of 3D-tissue models over 2D culture and open new possibilities for the application of such models in infection research.

Identification & systematics

SL-FG-IS-023
Microbial Identification in the Environment
*J. Pernthaler[1]
[1]*University of Zurich, -, Zurich, Switzerland*

Historically, bacterial identification has relied on phenotypic characteristics observed in pure culture, and phenotypic diagnostics remain a cornerstone of routine bacteriological monitoring, particularly in hygiene assessments. At the same time, classic environmental microbiology put a particular focus on bacteria in their respective biogeochemical roles as drivers of element cycles or biotransformation processes, whereas taxonomic identification of environmental microbes remained conditional to prior isolation. The need to differentiate bacterial genotypes directly in field samples probably emerged from the study of environmental pathogens but has gained widespread adoption with rRNA-based phylogenetics: Barcoding by next generation sequencing has revolutionized microbial community ecology, and fluorescence in situ hybridisation with rRNA-targeted probes allowed for environmental quantification of genotypically defined pro- and eukaryotic populations while preserving aspects of their phenotype. However, phylogenetic resolution based on rRNA genes often proved insufficient for the distinction of ecologically unique genotypes within microbiomes. Genome-resolved metagenomics offers higher taxonomic resolution and additionally also provides insights into the "physiological potential" of individual populations. While invaluable, this approache also introduces a 'caterpillar - butterfly' dilemma when inferring phenotypes from genomic data. In my presentation, I will explore strategies for achieving sufficient genotypic resolution in microbial identification while integrating ecophysiological insights essential for ecological interpretation.

SL-FG-IS-024
Raman spectroscopy as a comprehensive tool for microbial identification
*T. Tewes[1], D. Bockmühl[1]
[1]*Hochschule Rhein Waal, Life Sciences, Kleve, Germany*

Raman spectroscopy has become a powerful tool for the rapid, precise identification of microorganisms in diverse microbiological applications. Over recent years, our research has focused on developing predictive models to classify fungal and bacterial species using their unique Raman signatures, optimizing identification processes for environmental and clinical microbiology.

A key challenge in microbial identification is differentiating similar species in complex environments. Initial studies on entomopathogenic fungi demonstrated that Raman microspectroscopy effectively distinguishes *Metarhizium* and *Beauveria* species in conidial form, achieving over 98% prediction accuracy across varying growth conditions. Expanding into bacteria, we developed models based on bulk Raman spectra of 21 species grown under standardized conditions, achieving high theoretical accuracies through machine learning, particularly support vector machines (SVM). Despite substrate differences, such as stainless steel versus silver, practical accuracy remained robust at ~80% when applied to independent spectra.

We also addressed biofilm detection, focusing on dry-surface biofilms (DSBs) prevalent in healthcare settings. Raman spectroscopy successfully differentiated between vegetative cells and spores within these biofilms, advancing disinfection strategies. Incorporating convolutional neural networks (CNNs) further enhanced predictive models, especially for single-cell analysis. While bulk data models showed promise for single-cell predictions, challenges like fluorescence and photodegradation persist. This work contributes to creating adaptable Raman databases for microbial identification down to the single-cell level.

Beyond applications, we investigated why Raman spectroscopy works so effectively. By analyzing spectral variations and baseline effects, we moved beyond peak analysis to biochemical interpretations, gaining insights into factors driving classification accuracy.

Our research underscores Raman spectroscopy"s versatility in microbial systematics. From fungi in environmental studies to bacteria in biofilms and clinical diagnostics, this method offers a fast, reliable, and scalable solution for microbial identification, paving the way for broader adoption across disciplines.

SL-FG-IS-025
Large-scale, community-based description of prokaryotes
*T. Hitch[1], C. Pauvert[1], V. Scharf[2], K. Förstner[2], T. Clavel[1]
[1]*RWTH Aachen University, AG Clavel, Aachen, Germany*
[2]*ZB MED, Cologne, Germany*

A large fraction of prokaryotes remain poorly described, although genomes from isolates or metagenomes exist for many of them. This genomic information can be used to describe existing taxa and name new ones. The latter can now be done according to the rules of the SeqCode. Descriptions of taxa are essential to provide concise information to others unfamiliar with them, which is classically done in the form of protologues. As any single method of analysis provides only one piece of information at a time, accurate bacterial descriptions require multi-layered analysis to capture comprehensive information on the taxonomy, ecology and functions of the taxon under study. This requires sophisticated bioinformatic tools, which, combined with the large number of unknown taxa, makes the process of describing prokaryotes tedious and slow. Our bioinformatic tool Protologger (www.protologger.de) provides all the elements needed to describe novel prokaryote isolates or metagenomic-assembled genomes from their 16S rRNA gene and genome sequences within a few hours. It is hosted at de.NBI for stable deployment and accessible via a Galaxy interface for ease of use. Protologger has also been updated to use POCPu to determine genus delineation, analyse additional functional pathways, and include archaea. To speed up the process of prokaryote description by orders of magnitude, we propose a community-based approach. Protologger is being used to process all genomes available in the Genome Taxonomy Database (GTDB) (1,650 processed so far). All results are available via a Wikibase instance, allowing the community to contribute comprehensive descriptions. An API will be implemented to interface with the SeqCode registry, allowing the association of high-quality descriptions generated in this project with proposed name. Training videos have been produced to help users navigate the system and workshops will be organised by NFDI4Microbiota (https://nfdi4microbiota.de) to facilitate community participation.

Fungal biology and biotechnology

SL-FG-FBB-031
Insights into the Localization and Structural Composition of Lipid Rafts in Schizophyllum commune
*B. F. Porsche[1], R. Jesse[1], K. Krause[1], E. Kothe[1]
[1]*Friedrich Schiller University, Jena, Germany*

Lipid rafts are dynamic microdomains within the cell membrane. They contain high levels of sterols, sphingolipids, and specific proteins associated with lipid rafts. These domains act as platforms for signal transduction, membrane trafficking, protein sorting, and polarized growth (Alvarez et al., 2007). The filamentous basidiomycete Schizophyllum commune requires polarized growth, which depends on the transport of proteins and lipids to the hyphal tip through vesicle trafficking. Rafts contain specific proteins like stomatin and striatin. They play a crucial role in cell signalling and signal transduction. Further striatin is known for its protein-protein interactions with caveolin, a well-known raft related protein.
The aim of this work is to investigate the composition of raft domains and the localization and function of rafts in S. commune using two raft-associated proteins: stomatin and striatin. To analyse the overall membrane composition, we performed LC-MS measurements. Sterol-enriched raft domains were visualized by Filipin staining. Further, stomatin and striatin were labelled with the fluorescence proteins dTomato and eGFP, respectively, and visualized by laser scanning microscopy. To investigate the role of stomatin and striatin, we performed a knockout followed by RNA sequencing. The deletion of either stomatin and striatin in S. commune impacted the organism regarding morphological and genetic changes. The RNAseq results of S. commune Δsto showed an upregulation of several cell signalling groups like GPI-anchored proteins, MAPKs, and GPCRs. Further results showed a high effect on membrane-related genes and several membrane transporter groups. The deletion of striatin in S. commune seems to play a significant role in S. commune, especially in the dikaryotic life stage. The study has shown that raft-associated proteins of S. commune play an important role in hyphal growth, cell signalling, membrane stabilization, and membrane-related components. Enhancing our understanding of the structure and functionality of membrane microdomains in microorganisms could potentially provide valuable insights into mating, the interaction of organisms, and the pathogenesis process.
Alvarez, F. J., Douglas, L. M., & Konopka, J. B. (2007). Sterol-Rich Plasma Membrane Domains in Fungi. Eukaryotic Cell, 6(5).

SL-FG-FBB-032
Fungal Argonaute proteins mediate bidirectional cross-kingdom RNA interference during host infection
*A. P. Cheng[1], L. Huang[1], L. Oberkofler[1], N. Johnson[2], A. Weiberg[1]
[1]*Ludwig Maximilians University, Genetics, Faculty of Biology, Munich, Germany*
[2]*Pontificia Universidad Católica de Chile Santiago, Santiago, Chile*

Cross-kingdom RNA interference (ck-RNAi) is an emerging topic in host-pathogen interaction studies. Previously, it was shown that the fungal pathogen Botrytis cinerea transfers small RNAs into its host cells to suppress plant immunity genes for infection1, 2. However, the molecular factors that are involved in ck-RNAi remain largely unknown. We recently developed a GFP ckRNAi "switch-on" reporter tool in transgenic plants to track for the first time RNA communication in real-time during infection3. With this tool, we discovered Botrytis Argonaute (BcAGO)1 and BcAGO2 proteins are required for small RNA delivery into plants during fungal-induced ck-RNAi. Moreover, BcAGO co-immunopurification and small RNA sequencing revealed that tomato plants also send small RNAs into B. cinerea that bind to the Botrytis AGOs. These tomato small RNAs are active in silencing fungal virulence gene expression as a defence response mechanism. In conclusion, we provide strong evidence for bidirectional cross-kingdom RNA communication during fungal-plant interaction, and AGOs are key factors in this communication mechanism.

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2. Wang, M., Weiberg, A., Dellota, E., Jr., Yamane, D. & Jin, H. Botrytis small RNA Bc-siR37 suppresses plant defense genes by cross-kingdom RNAi. RNA Biol 14, 421-428 (2017).
3. Cheng, A. P. et al. A fungal RNA-dependent RNA polymerase is a novel player in plant infection and cross-kingdom RNA interference. PLoS Pathog 19, e1011885 (2023).

SL-FG-FBB-033
Understanding and harnessing the carbon catabolic potential of Fomes fomentarius for industrial applications
*A. S. Birke[1], T. Cairns[1], C. Freidank-Pohl[1], C. Regner[1], S. Jung[1], V. Meyer[1]
[1]*TU Berlin, Applied and Molecular Microbiology, Institute of Biotechnology, Berlin, Germany*

Fomes fomentarius is a promising candidate for fungal mycelium-based composite material production. However, its application in industry is hampered by a limited knowledge of its carbon catabolism. To accelerate the development of an industrially feasible composite production process based on F. fomentarius, we have grown it under 24 cultivation conditions, analysed gene expression patterns during substrate degradation, and constructed gene co-expression networks. Our analyses suggest that genes encoding putative lignocellulolytic CAZymes have been the subject of gene family expansion followed by transcriptional divergence, which will have contributed to niche specificity and metabolic robustness. Transcriptional regulation might be directed by two putative regulators, CalA and CalB, that we have identified using gene co-expression networks. A subset of CAZyme-encoding genes were found to be arranged in a novel type of metabolic gene cluster, termed contiguous co-expression clusters, that have not been described previously in fungi. This discovery has given us further insight into the transcriptional organisation and regulation of F. fomentarius genes. Lastly, we identified 35 sugar transporters in silico. Applying SPOT, a state-of-the-art model, we predicted potential substrates and analysed their respective gene expression patterns, the results of which indicate that sugar transporters in F. fomentarius might be promiscuous towards more than one substrate. By carrying out gene expression analysis and constructing co-expression networks focused on F. fomentarius carbon catabolism, we have been able to single out target genes for future studies aiming to optimise substrate utilisation. Ultimately, this insight will facilitate strain improvement approaches of F. fomentarius for industrial applications.

SL-FG-FBB-034

G-protein coupled receptors regulate morphological development in the plant pathogen Ustilago maydis

*P. Nakonz[1], F. Altegoer[2]
[1]*Heinrich Heine University, Mikrobiologie, Düsseldorf, Germany*
[2]*Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany*

G-protein-coupled receptors (GPCRs) are the largest class of eukaryotic receptor proteins that regulate physiology and are important drug targets in humans. Although GPCRs are present in fungi and might be similarly druggable, they are far less understood than their human counterparts. This is of particular interest, as pathogenic fungi are gaining increasing attention, as human-, animal- and plant diseases caused by fungi are on the rise. Therefore, a mechanistic understanding of fungal GPCRs is crucially lacking. In my project, we investigate the contribution of GPCRs to the pathogenicity of the basidiomycete fungus Ustilago maydis. In a first step, more than 30 previously undescribed proteins that share structural similarity with 7-transmembrane GPCRs were discovered on the genome of U. maydis. These potential GPCRs are highly diverse on the sequence level and many are not conserved in well-studied ascomycete fungi and might be an adaptation to its specific plant-pathogenic lifestyle. A closer inspection of the expression profiles of the GPCR candidates showed that many are differentially expressed during infection of the host Zea mays. We therefore hypothesize that these receptors enable the fungus to detect host-specific signals but likely also metabolites. Some of these stimuli might also be important for regulating the dimorphic switch from yeast-like sporidia to infectious hyphae in U. maydis. To first gain more knowledge on their potential functions, we not only performed a structure-guided categorization of the receptors but also used structure prediction tools to predict interactions between the receptors and the four Gα-subunits of U. maydis. We are currently implementing a yeast reporter system that allows us to identify different ligands recognized by the receptors and understand the importance of these signals for pathogenic development of U. maydis. Since GPCR signal transduction is linked to the secondary messenger cAMP, we also determined cAMP levels revealing that different GPCR deletion backgrounds are decreased in cAMP. These results further suggest that some of the studied receptors are indeed GPCRs and linked to the cAMP signaling pathway of U. maydis. Our research will contribute to a better understanding of fungal GPCRs in terms of signal transduction, its link to cAMP levels and overall pathogenicity in U. maydis. These findings will provide a basis for detailed GPCR-ligand interactions and enhance our understanding of signal perception in pathogenic basidiomycete fungi.

Short Lecture

Anaerobic metabolism

SL-AM-075

Gut bacteria metabolize natural and synthetic steroid hormones via the reductive OsrABC Pathway

*C. Jacoby[1], S. Light[1]
[1]*University of Chicago, Microbiology, Chicago, IL, United States*

Steroid hormones, critical regulators of various physiological processes, are metabolized by both the host and gut bacteria, with profound implications for mammalian health. Due to their diverse effects, many natural and synthetic steroids are widely used to treat autoimmune diseases, inflammatory disorders, hormone deficiencies, and cancer. In this study, we isolated a novel gut bacterium, *Clostridium steroidoreducens* strain HCS.1, and identified a unique steroid metabolism pathway, OsrABC, capable of reducing a broad range of steroid hormones, including glucocorticoids, mineralocorticoids, androgens, and progestins.[1] The OsrABC pathway consists of three key enzymes: OsrA, a 3-oxo-Δ1-steroid reductase that targets synthetic corticosteroids; OsrB, a versatile 3-oxo-Δ4-steroid reductase that produces 5β-dihydrosteroid intermediates; and OsrC, which further reduces these intermediates to 3β,5β-tetrahydrosteroid products. Our results suggest that this pathway may contribute to chronic inflammation by potentially interfering with glucocorticoid therapies and affecting drug bioavailability. Notably, *osrA* and *osrB* homologs were found to be highly enriched in active Crohn's disease patients, particularly in microbes such as *Clostridium tertium*, *Clostridium_AQ innocuum*, and *Ruminococcus_B gnavus*, the latter two of which have previously been associated with the disease. These microbes may exploit steroid metabolism to thrive in an inflamed environment, further perpetuating inflammation. In conclusion, our study highlights how microbial metabolism may influence the efficacy of steroid-based therapies in patients with inflammatory diseases, underscoring the need for personalized treatment approaches.

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SL-AM-076

Unraveling chloromethane conversion in the acetogen *Acetobacterium dehalogenans*

*J. Bernhardt[1,2], L. Hofmann[1,2], P. Klemm[3], O. Lemaire[4], T. Wagner[4], S. Vuilleumier[5], J. Kurth[1,2,3]
[1]*Microcosm Earth Center, AG Kurth, Marburg, Germany*
[2]*Philipps-Universität, Microbial Physiology Lab, Marburg, Germany*
[3]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*
[4]*Institut de Biologie Structurale, Grenoble, France*
[5]*Génétique Moléculaire, Génomique, Microbiologie, Strasbourg, France*

Chloromethane (CM) is an abundant environmental pollutant that contributes significantly to ozone depletion [1]. *Acetobacterium dehalogenans* is one of the few isolated anaerobic bacteria shown to utilize CM as its carbon and energy source [2]. The enzyme system responsible for its CM demethylation/dehalogenation has not been identified so far. We aim to identify and characterize this system and to understand the metabolic changes of *A. dehalogenans* during growth on CM versus the methoxylated aromatic syringate. We analyzed the growth and substrate conversion of *A. dehalogenans* grown on CM, syringate and both substrates and used comparative transcriptomics to analyze the gene expression pattern of *A. dehalogenans* grown under these conditions. We identified a corrinoid-dependent methyltransferase system with three adjacent genes *mtcABC* which were highly upregulated during growth on CM in comparison to syringate. We heterologously produced and purified the corresponding proteins in *E. coli*, subsequently performed activity assays to determine their substrate specificity, and conducted X-ray crystallography. We discovered that this Mtc methyltransferase system demethylates methyl halides such as chloromethane and iodomethane. X-ray crystallography of the MtcB methyltransferase revealed a hydrophobic internal channelling system for methyl halides. Phylogenetic analysis showed that homologous proteins are encoded by several

other anaerobic bacteria, especially Bacillota. Such organisms may also have the potential to convert methyl halides such as CM. The Mtc system is most closely related to the archaeal Mto system, which converts methoxylated aromatic compounds. In summary, we identified and characterized a novel enzyme system used for methyl halide demethylation in anaerobic microorganisms.

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SL-AM-077

Two distinct ferredoxins are essential for nitrogen fixation by the iron nitrogenase in *Rhodobacter capsulatus*

*H. Addison[1], P. Pfister[2], T. Glatter[2], J. Rebelein[1]
[1]*Max Planck Institute, Microbial Metalloenzymes, Marburg, Germany*
[2]*Max Planck Institute, Marburg, Germany*

Nitrogenases are the only enzymes able to "fix" atmospheric N2 into bioavailable NH3 and hence are essential for sustaining biological life. Nitrogenase catalysis is dependent on an ample supply of both ATP and electrons, the latter provided by low-potential redox proteins, often ferredoxins (Fds). Regarding this electron transport to nitrogenase proteins, many open questions remain; specifically electron transport to the iron (Fe-) nitrogenase had hardly been investigated. Our goal was to identify which Fds are important for nitrogen fixation by the Fe-nitrogenase and try to elucidate their functions.

Highlighted in our recent study, we identified two essential Fds for nitrogen fixation by the Fe-nitrogenase in *Rhodobacter capsulatus* (*R. capsulatus*)[1]. Genetic deletions of the Fd genes (*fdx)* in *R. capsulatus* were constructed, in a molybdenum nitrogenase deletion strain, and characterised via diazotrophic growth rates and *in vivo* nitrogenase activity. Deletion of two *fdx* genes, *fdxC* and *fdxN*, slowed diazotrophic growth and lowered Fe-nitrogenase activities. The Δ*fdxN* and Δ*fdxC* deletion strains were further studied via whole cell proteomics and plasmid complementation experiments. Proteomics revealed both strains had an upregulation of proteins essential for electron delivery to nitrogenases, indicating an interruption in electron transport to the Fe-nitrogenase. Plasmid complementation patterns differed greatly for Δ*fdxN* and Δ*fdxC*, revealing the two proteins were likely not redundant in function, but instead performed distinct roles.

To further explore the functions of FdN and FdC, our current research has focused on studying FdN and FdC *in vitro*. Specifically, FdN and FdC have been characterised spectroscopically, structurally and biochemically. Both Fds have been purified with redox-active FeS centres, shown by metal content analysis and UV-Vis spectroscopy, under reductive and oxidative conditions. Additionally, we have obtained an atomic resolution structure of one of the Fds, which has provided new details about the FeS cluster coordination, protein architecture and hydrogen bonding network. Overall, our findings have provided two key protein targets for the further study and bioengineering of nitrogen fixation systems, specifically those focussed on increasing the electron flux to nitrogenases for increased product formation.

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SL-AM-078

O_2-independent C-H activation by novel molybdenum-containing water dependent alkyl hydroxylases

*R. Weßbecher[1], T. Wittemann[2], P. Becker[3], T. Bick[4], T. Seifermann[1], P. Wendler[4], T. Klüner[2], R. Rabus[3], M. Boll[5]
[1]*University of Freiburg, Microbiology, Freiburg i.Br., Germany*
[2]*Carl von Ossietzky University, Chemistry, Oldenburg, Germany*
[3]*Carl von Ossietzky University, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany*
[4]*University of Potsdam, Institute for Biology and Biochemistry, Potsdam, Germany*
[5]*University of Freiburg, Microbiology, Freiburg i.Br., Germany*

Hydrocarbons are essential in numerous natural processes and represent the main components of crude oil.[1] The conversion of hydrocarbons into downstream products represents the basis for the petrochemical, pharmaceutical and plastic industries. Made up primarily of hydrogen and carbon atoms, hydrocarbons exist in various forms, including alkanes, alkenes and aromatic compounds. Their degradation is essential for the bioremediation of polluted environments, as they can be toxic and persistent in nature[1]. On the other side, the initial steps of bacterial alkane degradation may be important for the functionalization of alkanes into chiral alcohols as building blocks for value-added products. The activation of non-polar C-H bonds in hydrocarbons is a major challenge in both chemical and enzymatic syntheses due to high activation energies. In aerobic environments, bacteria use O_2 for the hydroxylation of C-H bonds catalysed by oxygenases or peroxygenases. In contrast, anaerobic bacteria employ alternative strategies for the initial hydrocarbon activation. Here, molybdenum-dependent hydroxylases of the type II DMSO reductase family have been postulated as key enzymes that use water to hydroxylate unactivated C-H bonds.

In this study, we isolated and characterized a p-cymene dehydrogenase (CmdABC) from denitrifying *Aromatoleum aromaticum* pCyN1[2] as well as a putative alkane hydroxylase (AhyABC) from the sulfate-reducing bacterium *Desulfococcus oleovorans* Hxd3[3,4] after heterologous production in the β-proteobacterium *Thauera aromatica* K172. Metal analyses and UV-vis spectroscopy of the heterotrimeric enzyme complexes indicate the presence of one molybdenum cofactor, five FeS clusters and one heme b, respectively. We used cryo-electron microscopy to obtain structural insights and combined these findings with biochemical studies and quantum mechanics/molecular mechanics (QM/MM) modelling to explore the limits of C-H bond activation by molybdenum-dependent hydroxylases. Taken together, the studies performed on both enzymes provide further insights into the mechanisms of water-dependent hydrocarbon activation in anoxic environments.

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Biotechnology

SL-BT-041

Biocatalytic synthesis of heterocycles using condensing amidohydrolases

L. Lernoud[1], R. Jakob[1], L. Warnick[1], M. L. Marx[1], *L. Winand[1]
[1]*TU Dortmund, Department of Biochemical and Chemical Engineering, Dortmund, Germany*

Heterocycles are essential structural motifs of many pharmaceuticals and agrochemicals. As chemical synthesis of heterocycles typically requires harsh reaction conditions and the use of hazardous agents, the synthetic utility of heterocycle-forming enzymes is increasingly investigated. In recent years, several unusual members of the amidohydrolase superfamily were described that catalyze intramolecular condensation reactions to form heterocycles in natural product biosynthesis pathways. The first such enzyme that was characterized in detail was the condensing amidohydrolase MxcM. MxcM was discovered in the biosynthetic pathway of the siderophore pseudochelin A, where it generates an imidazoline moiety via intramolecular condensation of a β-aminoethyl amide group.[1,2,3] In *Streptomyces* sp. Tü 6176, a homolog of this condensing amidohydrolase produces a benzoxazole during the biosynthesis of nataxazole.[4] A small enzyme cascade consisting of an AMP ligase and the amidohydrolase is sufficient to synthesize benzoxazoles from aryl carboxylic acids. The AMP ligase first catalyzes the dimerization of two building blocks via ester bond formation. Then, the condensing amidohydrolase catalyzes the biosynthesis of the benzoxazole. Heterologous expression of these two enzymes in *M. xanthus* led to the production of various benzoxazole derivatives in a combinatorial precursor-directed biosynthesis approach.[5] A phylogenetic analysis based on already described condensing amidohydrolases revealed a large variety of homologous enzymes in different bacterial phyla (unpublished data). This finding suggests that other condensing amidohydrolases involved in heterocycle formation exist and that the synthetic potential of this enzyme group has not yet been fully exploited. In this study, a new condensing amidohydrolase originating from a myxobacterium was investigated. Interestingly, our data indicate that the reaction mechanism of this enzyme differs from the one previously described.

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SL-BT-042

Process developement for heterologous Aurachin production

*J. Korb[1], M. Nett[1]
[1]*TU Dortmund, Technical Biology, Dortmund, Germany*

Introduction

Aurachins are a family of prenylated quinoline antibiotics [1], which possess potent antiprotozoal properties [2]. In the natural producer *Stigmatella aurantiaca* Sg a15 a farnesyl moiety is added to 2-methyl-4-quinolone (MQO) by the farnesyltransferase AuaA to produce aurachin D. The latter is then *N*-hydroxylated to aurachin C by the Rieske type oxygenase AuaF [3]. Recently, a method for the biocatalytic production of aurachin D from MQO using recombinant *E. coli* cells was described [4].

Goals

Due to their biological properties, the aurachins have gained attention as biochemical tool compounds and as drug candidates. Therefore, there is some interest in their cost-efficient production. The aim of this work was to intensify the biosynthesis of aurachin D in the heterologous host *E. coli* and to endow this bacterium with the *auaF* gene so that it can produce aurachin C.

Results

To simplify the downstream processing we switched from a complex fermentation medium to a defined minimal medium, which initially decreased the aurachin D titer in *E. coli* from 63.6 mg L-1 to 30.5 mg L-1. By increasing the glucose concentration in the minimal medium to 15 g L-1 and the NH4Cl concentration to 2 g L-1, aurachin D titers comparable to the complex medium were reached. With the increase of the substrate concentration from 90 mg L-1 to 180 mg L-1 an aurachin D titer of 104.2 mg L-1 could be achieved. Another rise of the product titer to 117.2 3 mg L-1 was possible after extending the cultivation time from 24 h to 48 h.

Plasmid-based co-expression of AuaA and AuaF enabled the production of aurachin C (6.6 mg L-1). Moreover, by lowering the precursor concentrations and *auaF* expression levels, an aurachin C titer of 23.5 mg L-1 was achieved.

Summary

The intensification of aurachin D production was successful and the product titer was almost doubled. Moreover, a system for aurachin C production was established. In the future aurachin C production can be optimized and new aurachin C derivatives can be produced by precursor-directed biosynthesis and screened for their antiprotozoal properties.

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SL-BT-043

Development of *Bacillus subtilis* as a production platform for bioactive secondary metabolites

*L. Lilge[1], O. Kuipers[2], R. Hausmann[1]
[1]*University of Hohenheim, Bioprocess Engineering, Stuttgart, Germany*
[2]*Univertsity of Groningen, Molecular Genetics, Groningen, Netherlands*

Bacillus subtilis is considered a workhorse in biotechnology. In this context, the *Bacillus* family demonstrates a remarkable biosynthetic potential for the production of bioactive metabolites. Here, bacteriocins and lipopeptides are important representatives involved in intercellular interactions as well as showing promising application potentials due to their structural properties. However, in order to use the potential of bioactive metabolites in *B. subtilis*, molecular regulatory circuits in production strains have to be studied and adapted by molecular strain engineering and tailored bioproduction processes. In this way, surfactin as a representative of the lipopeptide family and mersacidin as a member of the bacteriocin class have already been successfully introduced into adapted *B. subtilis* production strains by understanding the associated regulatory networks, followed by rational strain engineering.

In order to understand molecular regulatory networks within the biosynthetic pathways of surfactin and mersacidin, both the domesticated laboratory *B. subtilis* strain 168 and the sporulation-deficient strain 3NA were selected. Using reporter strains together with the integration of combinatorial gene knockouts, the expression of biosynthetic machineries could be evaluated and the influence of certain regulatory circuits could be studied. In this context, the cell differentiation associated with Spo0A-derived sporulation initiation and the regulation of the global transcriptional regulator AbrB were found to have significant relevance. This regulatory network plays a crucial role in the productivity of engineered *B. subtilis* production strains, which has been applied in adapted high cell-density fed-batch bioreactor cultivation processes. Targeted strain engineering allowed an increase in the production of surfactin to about 40 g/L, which is a record in scientific literature, whereas a mersacidin titer of about 100 mg/L could be reached, a 10-fold increase compared to the wild-type production strain.

In sum, molecular strain engineering based on understanding molecular regulatory networks provides the opportunity to realize the biosynthetic potential of *B. subtilis* as a production platform for bioactive metabolites. In addition, the use of tailored fed-batch bioreactor production processes with high cell-density enables the production of the target products mentioned in high quantities to enable future applications.

SL-BT-044

MyxoTech: harnessing the power of microbial evolutionary diversity

*T. Hesterkamp[1,2]
[1]*Helmholtz Centre for Infection Research – HZI, Braunschweig, Germany*
[2]*MyxoTech GmbH, Saarbrücken, Germany*

Myxobacteria (phylum *Myxococcota*) are environmentally distributed, Gram-negative bacteria described as prolific producers of bioactive secondary ("special") metabolites. These potently engage a variety of cellular mechanisms-of-action in pro- and eukaryotes. MyxoTech re-purposes the myxobacterial drug discovery platform technology developed at HZI-HIPS for commercial application in indication areas outside the institute"s core mission, infectious disease. Under license we access collections of myxobacterial isolates and associated know-how. Our first collection follows the conceptual framework established by Hoffmann et al. (2018) to maximize chemical diversity, hence discovery potential, as a function of taxonomic diversity. The collection comprises isolates from 21 genera, 119 operational taxonomic units (OTU) of myxobacteria, with 196 isolates genome-sequenced, cultivated, extracted and prefractionated for biological screening at third party labs. MyxoTech provides this collection preplated and assay-ready for convenient screening. Downstream activities such as activity-directed dereplication, preparation of neat compound samples and structure elucidation can be accessed as fee-for-service or collaboration. We are committed to contributing to discoveries in a number of application and indication areas including hematology/oncology, immunity and inflammation. We plan assay applications for cell aging, mitochondrial function as well as payloads with novel, differentiated mechanism-of-action. We also plan to venture into non-pharmaceutical applications and seek scientific dialogue on these topics with the VAAM conference attendants.

SL-BT-045

Optimization of biofilm-based production of platform chemicals using microscale membrane reactors

*C. Mandel[1], J. Weiler[1], J. Gescher[1], M. Edel[1]
[1]*Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany*

1. Introduction

In the transition to a circular economy, alternatives to conventional chemical production routes must be developed based on renewable carbon or atmospheric CO_2 to restore the balance of the global carbon cycle. We propose the production of platform chemicals using a genetically engineered *Cupriavidus necator* H16 strain thriving as biofilm on gas diffusion membranes. Due to its metabolic versatility, *C. necator* is able to utilize organic acids resulting from dark fermentations of biogenic waste and, as Knallgasbacterium, can fix CO_2 using H_2 as energy source. The targeted products can be entered into synthesis routes, e.g., for sustainable jet fuel.

2. Goals

The multiphase substrates of the bioconversion demand a suitable setup for efficient mass transfer and biomass retention, which can be realized in a membrane biofilm reactor (MBR). However, insights into biofilm formation, productivity per biomass, and influence of process parameters remain limited in such a setup. We established a microfluidic flow cell with an incorporated membrane that allows in-depth characterization and serves as a screening platform for a lab-scale MBR and robust process conditions.

3. Methods

C. necator was optimized for acetoin and 2,3-BDO production under availability of inorganic and organic carbon. The engineered strain was characterized regarding carbon efficiency and cultivation conditions. Cultivation as biofilm under autotrophic and heterotrophic conditions was performed in microfluidic reactors. Biofilm growth was

monitored using optical coherence tomography and limitations were elucidated.

4. Results

The engineered strain reaches a carbon efficiency of 86% for the production of acetoin and 2,3-BDO from organic acids and CO_2 (ratio 2:1). Cultivation as a membrane-bound biofilm allows a substantially higher cell density compared to planktonic systems, while downstream processing is simplified through cell retention. By fine-tuning the gas flow rate, growth can be limited to the membrane surface and the biofilm height controlled.

5. Summary

Genetically engineered *C. necator* is employed as biofilm on gas diffusion membranes to produce valuable chemicals from renewable carbon and CO_2. A microfluidic flow cell was developed for detailed analysis, serving as a screening platform to optimize conditions in a MBR. This approach demonstrates high carbon efficiency and cell densities, facilitating robust production suitable for large-scale applications.

SL-BT-046

Contaminant transformation and greywater treatment in redox-differentiated green roof model systems

*S. Derado[1], Y. Hammoudeh[1,2], L. Adrian[1,2], C. Ding[1]
[1]Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany
[2]TU Berlin, Berlin, Germany

Introduction: Green roofs have great potential to treat domestic greywater on-site while improving the urban microclimate and biodiversity. Preliminary experiments have shown that wetland roof systems are able to efficiently treat greywater which can be used as service water after appropriate hygienization (Rahman, 2023). However, further research is needed to understand underlying removal mechanisms to optimize green roof systems for wastewater treatment.

Objectives: We are investigating which redox conditions are beneficial for the treatment of greywater and the microbial transformation of poorly degradable contaminants.

Methods: Transformation of four wastewater chemicals of concern (namely benzotriazole, diclofenac, sulfamethoxazole and perfluorooctanoic acid) as well as the treatment of synthetic greywater were investigated. For this, two polypropylene boxes (each 37 cm × 26 cm) equipped with helophyte mats planted with *Carex acutiformis* served as down-scaled model systems under defined conditions. Redox-conditions were changed in the systems *via* permeation of nitrogen gas or compressed air and monitored using sensitive dissolved-oxygen sensors. Treatment of synthetic greywater was evaluated by measuring standard parameters for wastewater analysis. Transformation of the model chemicals was investigated by using LC-MS/MS technology. *De novo* proteomics were used to study the microbial community.

Results: We were able to establish different oxic and anoxic subzones within the model systems. Despite the small model size and low amount of plants (three plants per system), the synthetic greywater was treated efficiently with high removal of BOD, COD, TOC, total nitrogen and ammonium – all parameters showing higher removal efficiencies under aeration with compressed air. The transformation of the four recalcitrant model chemicals was highly redox-dependent. Especially benzotriazole and diclofenac showed significantly higher removal when introducing oxic zones, while sulfamethoxazole also showed high removal efficiencies under very anoxic conditions. Identification of transformation products and *de novo* proteomics gave further insights, highlighting once more the importance of redox-conditions in microbial processes.

Conclusion: This study shows the great potential of redox-dependent microbial transformation for the treatment of greywater in green roof systems.

Rahman, K.Z. et al., *Water* **2023**, *15*, 3375. doi: 10.3390/w15193375

SL-BT-047

Towards high atom economy in whole-cell redox biocatalysis: Up-scaling light-driven cyanobacterial ene-reductions in a flat panel photobioreactor

*P. Erlsbacher[1], H. C. Grimm[1,2], H. Medipally[1,3], L. Malihan-Yap[1], L. Sovic[1], J. Zöhrer[1], S. N. Kosourov[4], Y. Allahverdiyeva[4], C. E. Paul[5], R. Kourist[1]
[1]TU Graz, Institute of Molecular Biotechnology, Graz, Austria
[2]Leibniz University Hannover, Hannover, Germany
[3]KTH – Royal Institute of Technology, School of Engineering Sciences in Chemistry, Biotechnology and Health, Stockholm, Sweden
[4]University of Turku, Molecular Plant Biology, Department of Life Technologies, Turku, Finland
[5]Delft Univerity of Technology, Department of Biotechnology, Delft, Netherlands

Light-driven biotransformations in recombinant cyanobacteria benefit from the atom-efficient regeneration of reaction equivalents, such as NADPH, from water and light via oxygenic photosynthesis. However, effective light distribution is hindered by self-shading effects within photosynthetic cells and extended light paths, significantly limiting scalability.

We introduced a flat-panel photobioreactor with a 1-cm optical path length as a scalable system to provide efficient light distribution at high cell densities. Genes encoding five distinct classes of ene-reductases were heterologously expressed in *Synechocystis sp.* PCC 6803. The resulting strains were evaluated for their efficacy in light-driven ene-reduction reactions across a range of prochiral substrates.

Under standard small-scale reaction conditions, the recombinant strains expressing the ene-reductases OYE3 and mutated TsOYE demonstrated specific activities of up to 150 U g_{CDW}^{-1}, marking the highest activities recorded for photobiotransformations to date. These strains were chosen for scale-up in a 120-mL flat-panel photobioreactor. The strains achieved volumetric productivities up to 1 g L^{-1} h^{-1}, surpassing current photosynthesis-driven processes. This setup enabled the conversion of 50 mM 2-methyl maleimide in a fed-batch within approximately 8 hours. The atom economy of this process reached 88% outperforming traditional processes relying on sacrificial cosubstrates such as glucose and formic acid. An E-Factor of 203 indicates that volumetric yield and water consumption for cell cultivation are critical parameters impacting process sustainability.

In summary, we highlight essential factors influencing the sustainability of light-driven whole-cell biotransformations,

establishing a robust foundation for future optimization and scale-up efforts in photosynthesis-driven bioproduction

SL-BT-048

Computationally assisted design of *in situ* activatable recombinant microbial transglutaminase variants

*J. Müller[1], N. Zeno Alarake[1], L. Meschke[1], B. Moritz[1], F. Seifert[1], M. Pietzsch[1]
[1]*Martin-Luther-University Halle-Wittenberg, Institute of Pharmacy, Downstream Processing, Halle (Saale), Germany*

Microbial transglutaminase (MTG, EC: 2.3.2.13) from *Streptomyces mobaraense* catalyzes the formation of isopeptide bonds between proteinaceous glutamine and lysine residues. In addition, MTG catalyzes the conjugation of various amines to glutamine residues. These characteristics make it a valuable tool for usage in different industrial applications from medicine to food production. Native MTG is secreted as an inactive zymogen by *Streptomyces mobaraense* and proteolytically activated in the extracellular space. The zymogen consists of the mature polypeptide and an N-terminal pro-peptide, which is crucial for correct folding and keeping MTG inactive until its activity is needed. The expression as an inactive zymogen presents some difficulties for the recombinant production of highly active MTG, as the removal of the pro-peptide to activate MTG is a neccessary processing step. This extra step complicates purification, increases costs and can introduce unwanted side-effects, such as overdigestion of MTG by non-native proteases. To overcome these challenges, various approaches, e.g., the introduction of self-cleaving pro-peptide variants or the introduction of destabilizing mutations in the pro-peptide have been published. To simplify the production of active recombinant MTG, we systematically searched for mutations in the pro-peptide sequence, that would lead to protease-independent MTG-activation. By *in silico* site-saturation mutagenesis of selected pro-peptide residues and comparison of Rosetta ΔΔG predictions, we obtained destabilizing and stabilizing mutations. Several of these pro-peptide mutations were selected and the pro-peptide sequence was combined with the highly active and thermostable mature MTG variants TG16 and TG2A via Golden Gate Cloning. The resulting twelve single and two double mutants per MTG variant were expressed, purified and tested for protein stability, activity at a broad range of temperatures and ability to crosslink β-casein. Moreover, we present several MTG variants, that can be readily produced, easily purified and activated, as well as deactivated *in situ*.

SL-BT-086

Modeling of intravenous caspofungin administration using an intestine-on-chip reveals altered Candida albicans microcolonies and pathogenicity

*T. Kaden[1,2], R. Alonso-Román[3,4], P. Akbarimoghaddam[4,5,6], S. Besser[1], A. S. Mosig[2,4], K. Graf[1], M. Raasch[1], B. Hoffmann[5], M. T. Figge[4,5,7], B. Hube[3,4,7], M. Gresnigt[3,8]
[1]*University Hospital Jena, Jena, Germany*
[2]*University Hospital Jena, Jena, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology, Department of Microbial Pathogenicity Mechanisms, Jena, Germany*
[4]*Friedrich Schiller University, Cluster of Excellence Balance of the Microverse, Jena, Germany*
[5]*Leibniz Institute for Natural Product Research and Infection Biology, Applied Systems Biology, HKI-Center for Systems Biology of Infection, Jena, Germany*
[6]*Friedrich Schiller University, Biology, Jena, Germany*
[7]*Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University, Jena, Germany*
[8]*Leibniz Institute for Natural Product Research and Infection Biology, Junior Research Group Adaptive Pathogenicity Strategies, Jena, Germany*

The human intestine is a major reservoir of the commensal fungus Candida albicans and the main source for life-threatening systemic candidiasis. To dissect the fungal-host interactions, conventional 2D in vitro models are commonly used. However, these models lack biological complexity and are less suitable for evaluating antifungal drug administration routes and the drug concentration gradients across multilayered tissue barriers as observed in vivo. Therefore, a 3D intestine-on-chip model was leveraged to investigate fungal-host interactions and evaluate the antifungal activity of caspofungin.

This intestinal model includes a vascular compartment with endothelial and immune cells, an intestinal compartment with villus- and crypt-like structures, and peristaltic flow in both compartments. The model was infected with either a wild-type (SC5314) or caspofungin-resistant (110.12) strain of C. albicans. Caspofungin was vascularly perfused to simulate the intravenous treatment of candidemia. Complementary to biomolecular analyses, we developed an automated image analysis pipeline that provides 3D morphological characterization of fungal objects. Additionally, this pipeline provides quantification of the epithelial tissue architecture, the interaction between fungal objects and tissue structures, as well as the level of fungal invasion and translocation.

We demonstrated that C. albicans invades the epithelial tissue and translocates to the vascular compartment. Moreover, C. albicans induced severe host tissue damage by disrupting cell-cell contacts, increasing intestinal permeability, and promoting inflammation. Caspofungin treatment restored vascular and intestinal host tissue integrity. Further, fungal burden, tissue invasion, and vascular translocation were concentration-dependently reduced and accompanied by decreased inflammation. Advanced image-based analysis revealed a reduction in fungal biomass associated with more compact microcolonies and a diminished surface-to-volume ratio, which indicated a decreased exposure to the epithelial tissue after caspofungin treatment. In contrast, caspofungin showed limited effects in the case of infection with the caspofungin-resistant clinical isolate.

Collectively, the intestinal candidiasis-on-chip-model can be leveraged for in-depth in situ characterization of antifungal treatment efficacy, emerging fungal drug resistances, and associated changes in host-pathogen interactions.

SL-BT-089

Towards net zero land biotechnology – a potential assessment for selected bioprocesses in Germany

*A. Tüllinghoff[1], F. Harnisch[1], K. Bühler[1], D. Thrän[2]
[1]*Helmholtz Centre for Environmental Research - UFZ, Microbial Biotechnology, Leipzig, Germany*
[2]*Helmholtz Centre for Environmental Research - UFZ, Bioenergy, Leipzig, Germany*

To stay within the planetary boundaries transformation to a more circular economy is key, necessitating the consequent utilization of residues as resources. The circular material use (CMU) in Germany is only 12.2% that is below EU average, with biomass CMU of 7.6% being particularly low. On the other hand, Germany has about 240 Mio Mg a-1 dry mass of biogenic residues accruing, which are not well valorized yet [1]. Biotechnological processes will play a crucial role to

leverage biogenic residues for providing value-added products in a circular economy [2]. To accelerate increasing CMU the feasibility of biogenic residues for the wide range of biotechnological processes needs to be assessed.

Biogenic residues are heterogeneous while established bioprocesses typically demand specific compounds as substrates. To bring together resource quality with the dedicated demand, we categorized the residues compiled in the DE biomass monitor regarding their biochemical composition, and thus feedstock potential. 95% of biogenic residues were organized into a resource matrix, detailing their fractions and the quality of available data. Three biotechnological processes, making use of lignin, non-fibrous carbohydrates, and oil, respectively, served as model processes to assess the biogenic production potential. Material flows based on state-of-the-art conversion routes will be presented, depicting that residue-based production via all three biotechnology examples can meet national demands of specific polymer precursors, medium chain carboxylates, and platform chemicals, respectively, when only 20-30% of possible raw materials is mobilized. The high share of (unused) by-product, underlines the importance of production networks and cluster approaches as early as possible in biotechnological process development. Specific challenges to fully exploiting the potential of biogenic residues, including legal and acceptance issues, the need for intelligent biomass decomposition in networked production, and residue availability and management will be discussed.

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2. Naegeli de Torres, F. et al., 2023, https://doi.org/10.5281/zenodo.10370137

SL-BT-095

Renewable energy-driven O-Demethylation - the path to sustainable lignin valorization

*D. Calabrese[1], G. Lim[1], P. Cordero[1], L. Lauterbach[1]

[1]RWTH Aachen University, Microbiology, Aachen, Germany

Introduction: Lignin, a key component of lignocellulosic biomass, is an abundant and renewable resource that remains vastly underexploited. Selective depolymerization of lignin yields valuable lignin-derived aromatic compounds (LDACs) like alkylguaiacols and vanillate, which can serve as precursors for high-value platform chemicals. Enzymatic valorization of these LDACs encounters critical bottlenecks. Key enzymes, such as cytochrome P450s and Rieske oxygenases, enable efficient O-demethylation but are inherently unstable and depend on costly, stoichiometric amounts of cofactors, limiting both scalability and economic feasibility. Using H_2-driven cofactor regeneration via hydrogenases (SH) offers an effective solution.

Goals: (1) Develop high-performance, safe H_2-driven O-demethylation by coupling SH with cytochrome P450s and Rieske oxygenases; (2) Establish a scalable platform for H_2-driven reactions, linking LDACs valorization to renewable energy through a continuous flow chemistry system with in-situ H2 and O2 generation.

Methods: AgcA/AgcB, PbdA/HaPuX/HaPuR (P450s), and VanA/VanB (Rieske-type) have been expressed in *E. coli*, with SH produced homologously in *C. necator*; all were purified. Systems were tested in gas-tight batch reactions with varied NAD^+ levels and H2 held below 4%. A flow chemistry setup with PEM electrolysis enabled in situ H_2 production and scaled reaction volumes. Product yield and purity were assessed via real-time gas monitoring, HPLC, NMR.

Results: Batch reactions achieved high turnover numbers (TTNs) and >99% yield, effectively balancing cofactor cost and efficiency with minimized NAD^+ levels. Gas studies confirmed that maintaining hydrogen concentrations below 4% ensured safe operation without compromising performance. The flow system sustained high conversions (>99%) at an efficient Faradaic rate of 27.1%, with a final product yield of 82.3% after extraction. Together, these metrics underscore the system's scalability and efficiency, highlighting its potential as a viable option for sustainable, industrial-scale LDAC valorization.

Summary: This study presents a scalable platform for LDACs valorization, integrating H_2- and electro-driven cofactor regeneration in continuous flow. With efficient enzymes, safe gas handling, and renewable PEM technology, our approach offers a sustainable model for industrial biocatalysis and expands the potential for NAD(P)H-dependent oxidoreductase applications beyond LDACs valorization.

SL-BT-096

Co-utilization of CO_2 and lignin-related compounds by employing cocultivations of anaerobic and aerobic bacteria

*E. Meriläinen[1], E. Efimova[1], V. Santala[1], S. Santala[1]

[1]Tampere University, Faculty of Engineering and Natural Sciences, Tampere, Finland

Acetogens have an important role in utilizing C1 sources of carbon that are often unavailable for aerobic bacteria. These C1 carbon sources also include methyl groups of aromatic compounds that are found in plant materials, such as lignin. Lignin is an abundant biopolymer that has the potential to become a new renewable raw material for the production of green chemicals. These methyl groups are utilized by acetogens for CO_2 reduction yielding acetic acid, while the aromatic ring of the substrate stays unchanged. In contrast, most aerobic bacteria can break down and metabolize the aromatic ring, but they cannot utilize the carbon from the methyl groups. Notably, the aerobic *O*-demethylation required for the removal of the methyl groups is typically energy-limited, causes growth inhibition, and results in a loss of carbon in the form of CO_2.

To circumvent these problems and to develop a process to upgrade simultaneously CO_2 and lignin-related compounds, we synergistically integrated anaerobic and aerobic bacterial metabolisms through cocultivations. In practice, we first employed *Acetobacterium woodii* to anaerobically *O*-demethylate lignin-modelling substrates, producing acetate and demethylated aromatic compounds as metabolites. These metabolites were then used to cultivate aerobic production host *Acinetobacter baylyi* ADP1, which naturally cannot utilize carbons from either CO_2 or the methyl groups of aromatic compounds. By utilizing this approach, we demonstrated an upgrading of lignin-related guaiacol into a plastic precursor, muconate, with nearly equimolar yields [1]. This study highlights the possibilities of synergistic integration of distinctive bacterial metabolisms in upgrading challenging feedstocks.

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Anaerobic and Aerobic Bacteria." Biotechnology for Biofuels, vol. 17, no. 1, 2024, pp. 78–14, https://doi.org/10.1186/s13068-024-02526-0.

SL-BT-097

The iron nitrogenase reduces carbon dioxide to hydrocarbons and formate – unlocking new pathways for biological carbon capture and conversion

*F. Schmidt[1], J. Rebelein[1]
[1]*Max Planck Institute, Microbial Metalloenzymes, Marburg, Germany*

Nitrogenases are best known for catalyzing the reduction of dinitrogen (N_2) to ammonia at a complex metallic cofactor. However, recent advancements in nitrogenase research have revealed numerous promiscuous activities of nitrogenase, which have challenged the conventional perception of nitrogenases as solely N_2-converting enzymes. Remarkably, these include the reduction of carbon dioxide (CO_2), a reaction that offers novel perspectives for the recycling of carbon waste through direct conversion of CO_2 into short-chain hydrocarbons and formate. Among the three nitrogenase isozymes, the iron (Fe) nitrogenase shows the highest wild-type activity for the reduction of CO_2, but the molecular mechanisms facilitating this activity remain elusive. Our research aims to unravel the catalytic principles of the Fe nitrogenase and explore its biocatalytic potential for the conversion of CO_2 into value added products.

Over the past three years, we have pioneered the anaerobic purification of the Fe nitrogenase from *Rhodobacter capsulatus*, which allowed us to study its reactivity *in vitro*. By monitoring CO_2 reduction activity in the presence of N_2, we found the Fe nitrogenase to be highly promiscuous for the reduction of CO_2, which results in the formation of formate and methane. Intriguingly, these effects translate *in vivo*, where we observed an extracellular accumulation of formate and methane by Fe nitrogenase expressing strains upon exposure to CO_2 (Schmidt & Oehlmann *et al*., Science Advances, 2024). Intrigued by these unique reactivities, we sought to understand the molecular mechanism of the Fe nitrogenase, which lead us to solve a 2.35-Å cryogenic electron microscopy structure of the Fe nitrogenase complex (Schmidt & Schulz *et al*., Nature Structural & Molecular Biology, 2024). The structure reveals a [Fe_8S_9C-(*R*)-homocitrate] cluster in the active site and highlights a Fe nitrogenase specific complex architecture.

In conclusion, our results establish the Fe nitrogenase as a CO_2-reducing enzyme and show up new pathways for the utilization of CO_2 as a feedstock chemical. Our structural and biochemical analysis of the Fe nitrogenase provides a solid framework for further investigations on the molecular mechanisms of Fe nitrogenase catalysis. Eventually, we aim to utilize these insights to rationally design, test and build Fe nitrogenase based biocatalysts for the sustainable conversion of CO_2 into value-added products.

SL-BT-098

Conversion of plant polysaccharides to organic acids by a genetically accessible *Bacteroidia* isolate

*S. E. Kurrer[1], J. Schwarzbauer[1], H. K. Trost[1], L. O. Kirstein[1], A. Poehlein[2], R. Daniel[2], R. J. Fischer[1], M. Basen[1]
[1]*University of Rostock, Microbiology, Rostock, Germany*
[2]*Georg-August University Göttingen, Department of Genomic and Applied Microbiology, Göttingen, Germany*

Bacteria of the class *Bacteroidia* are abundant members of the gut microbiome and are well-known for their ability to degrade a variety of polysaccharides [1]. Besides, they also produce organic acids like propionate, which is an important precursor chemical in industry [2]. While the degradation of dietary fibers by *Bacteroidia* is extensively studied with regard to host health, their biotechnological potential has not yet been investigated. In this study, we aim to identify strains that degrade plant polymers and convert them to organic acids with regard to sustainable propionate production.

Isolates have been obtained using selective conditions for polymer using *Bacteroidia*. Subsequently, we compared the ability of 13 isolates and 14 type strains to produce organic acids with xylan and other polysaccharides as substrate. Isolate *Dysgonomonas gadei* BGG-A1 was able to use all tested substrates and, in addition, formed propionate as the main product, therefore this strain was chosen for further investigation. We checked the genome of BGG-A1 for polysaccharide-degrading enzymes, where we identified two gene loci that contain all essential genes for xylan degradation including a GH10 xylanase. The purified enzyme showed endo-xylanase activity. To test the isolate"s fermentation characteristics, it was grown in a pH-controlled batch culture with a high xylan concentration (36 g/l), where a shift in product composition was observed: Now lactate was the main product (106 mM) instead of propionate (43 mM). To increase propionate yield we aim to reduce unwanted side products by genetic engineering. As a first step, we successfully introduced an integrative plasmid into the genome, which is an important step on the way towards establishing a gene knockout system in BGG-A1.

In conclusion, we identified isolate *D. gadei* BGG-A1 as a propionate producing *Bacteroidia* strain with a large substrate spectrum. We investigated its capability to convert polysaccharides to fermentation products and could identify genes of the energy metabolism and gene clusters that are potentially involved in the degradation process. Currently we are pursuing a promising genetic approach that will enable to identify key functions of genes involved in polysaccharide conversion and may allow for engineering towards an increased propionate yield.

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2. Döring and Basen (2024) *Biotechnol Biofuels Bioprod.*

SL-BT-099

Developing a syngas fermentation process with *Clostridium ljungdahlii* for cyanophycin production

*C. Schöne[1], J. N. Ntihuga[2], C. M. Klask[2], K. Forchhammer[2], L. T. Angenent[2], B. Molitor[1]
[1]*Leipzig University, Leipzig, Germany*
[2]*Eberhard Karls University Tübingen, Tübingen, Germany*

Cyanophycin is a biopolymer consisting of a poly aspartate chain with arginine that is bound to the side groups of the aspartate chain. The biosynthesis of cyanophycin requires only a single enzyme: cyanophycin synthetase (encoded by *cphA*). It was first discovered in cyanobacteria, and later, it was found that many other bacteria, including Clostridia, possess the genetic capability to produce cyanophycin. Cyanophycin has many potential applications, such as its use as bioplastic in medical approaches. This triggered the development of biotechnological production routes with cyanobacteria and *Escherichia coli*, both of which have certain limitations, such as difficult scalability as well as low quantity and/or quality of the biopolymer. This study aims to establish *Clostridium ljungdahlii,* which is a model microbe

for syngas fermentation, as an alternative production platform *via* genetic engineering.

To achieve efficient cyanophycin production from syngas with *C. ljungdahlii*, several cyanophycin synthetases from various microbes will be investigated under different growth conditions. Additionally, metabolic engineering will be performed to enhance aspartate and arginine production from syngas. The resulting strains will be transferred to bioreactors, and the production process will be further optimized to achieve larger amounts of cyanophycin with high quality, subsequently enabling research to enhance the properties of the biopolymer for use in different applications. The planned strategies for the genetic modification of *C. ljungdahlii* and first results will be presented.

SL-BT-100

Transcriptome and proteome analysis of *Clostridium ljungdahlii* reveals key stress response pathways in microbial electrosynthesis

*S. Al Sbei[1,2], A. Kuchenbuch[3], S. Boto[1,2], F. Harnisch[3], M. A. Rosenbaum[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Bio Pilot Plant, Jena, Germany*
[2]*Friedrich Schiller University, Biology, Jena, Germany*
[3]*Helmholtz Centre for Environmental Research - UFZ, Microbial Biotechnology, Leipzig, Germany*

Microbial electrosynthesis (MES) allows microorganisms to utilize electrical energy (electrons) to produce value-added compounds. MES from CO_2 is based on anaerobic, electroautotrophic microorganisms that take up electrons from the cathode for CO_2 fixation and produce mainly acetate, through the Wood-Ljungdahl pathway (WLP)[1]. While similar to gas fermentation, the key difference is the direct feeding of electrons rather than H_2 gas. However, the performance of acetogenic microorganisms in MES is lower, with optical densities (OD) less than 0.4, compared to higher OD in gas fermentation. MES is typically conducted in H-type bioreactors, where a cation exchange membrane separates the anode and cathode compartments. This membrane is crucial to prevent O_2 that results from the anodic water-splitting reaction from reaching the cathode. However, O_2 still intrudes through the membrane, limiting the performance of the biocatalysts [2]. Therefore, we hypothesize that certain stress factors, such as O_2 intrusion or electron donor limitation cause the low performance of the biocatalyst when performing MES. To elucidate and identify these stress factors, we investigated the MES and gas fermentation performance of *Clostridium ljungdahlii*, grown in H_2/CO_2 autotrophic gas fermenters and H-type electrobioreactors. We compared growth and acetogenic performance under both conditions, using transcriptome and proteome profiling for physiological insights. Additionally, we used transmission electron microscopy to better understand the cellular responses to the different conditions.

The contribution will present the results of this comparative analysis showing changes in the expression of the WLP, upregulation in genes of microcompartment formation, and expression of an alternative ATP-generating pathway as a stress response during MES.

Acknowledgments: The authors acknowledge support from the Deutsche Forschungsgemeinschaft (priority program SPP2240).

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SL-BT-128

Methanol metabolism in the cytochrome- and quinone-containing acetogen *Moorella thermoacetica*

*F. Rosenbaum[1], A. Poehlein[2], R. Daniel[2], V. Müller[3]
[1]*Goethe University, Frankfurt a.M., Germany*
[2]*Georg-August University Göttingen, Institute of Microbiology and Genetics, Göttingen, Germany*
[3]*Goethe University, Institute of Molecular Biosciences, Molecular Microbiology & Bioenergetics, Frankfurt a.M., Germany*

Introduction: Acetogenic bacteria are an ecophysiologically important group of strictly anaerobic bacteria. Their characteristic feature is to oxidize organic and inorganic electron donors, coupled to the reduction of CO2 *via* the Wood-Ljungdahl pathway. Methylated compounds such as methanol are a common source of carbon and energy for acetogens. The cytochrome- and quinone-containing acetogenic model organism *M. thermoacetica* can metabolize methanol, but the physiology and biochemistry has not been investigated. An outstanding feature of *M. thermoacetica* is the ability to conserve additional energy by reducing a wide range of alternative electron acceptors including DMSO[1]. This additional energy can be used to achieve greater cell titers or to produce energy demanding products.

Goals: Here, we addressed the biochemistry and bioenergetics of methanol metabolism in *M. thermoacetica*. Moreover, the effect of DMSO on methanol oxidation was analyzed.

Materials & Methods: Growth experiments, genome-wide expression analyses and bioinformatic analysis were performed.

Results: Methanol supports the growth of *M. thermoacetica*. In growing cells, methanol was metabolized to acetate. In resting cells, a methanol to acetate ratio of 4:3 was observed. External CO2 was essential for methanol metabolism unless the alternative electron acceptor DMSO was available. In the presence of DMSO, growth was stimulated and significantly less acetate was formed; in resting cells, methanol was completely oxidized and reducing equivalents were completely used to reduce DMSO without producing acetate. Genome-wide expression analysis revealed the genes that encode for proteins that are important for methanol metabolism, redox balancing and DMSO reduction.

Summary: We identified the pathway of acetogenesis from methanol and its bioenergetics. DMSO and CO2 were used simultaneously as electron acceptors. The pathway of sulfidogenesis from methanol and its bioenergetics was also identified.

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SL-BT-129
Metabolic engineering of *Pseudomonas taiwanensis* VLB120 as chassis for the production of chorismate-derived bulk and fine chemicals
*F. Kofler[1], B. Wynands[1], N. Wierckx[1]
[1]*Forschungszentrum Jülich GmbH, microbial catalysis, Jülich, Germany*

Increasing depletion of fossil resources requires a shift of industry towards a more sustainable production. This led to a focus on bio-based production of both bulk and fine chemicals.
Due to the robustness of *Pseudomonas taiwanensis* VBL120 as well as its highly versatile metabolism, this bacterium displays a suitable candidate for the production of a broad spectrum of compounds, especially aromatics. Chorismate is a key precursor for many aromatics, and we therefore modified *P. taiwanensis* to increase chorismate availability in the cell. This was achieved by targeting the bifunctional enzyme *pheA*, which catalyzes the first two steps from chorismate to the aromatic amino acids phenylalanine and tyrosine. Overall, metabolic engineering approaches could be applied to increase productivity up to 20.3±0.1% (Cmol/Cmol) from glucose and up to 25.4±2.1% (Cmol/Cmol) from glycerol, depending on the respective aromatic compound.
To demonstrate its applicability, production of different chorismate-derived hydroxybenzoates was shown. These compounds can serve either as plastic building blocks, food additives, or as precursor for more complex secondary metabolites. In the latter context, we enabled the conversion of 2,3-dihydroxybenzoate (DHB) into myxochelin. In this metal-chelating siderophore, two DHB molecules are coupled via a lysine molecule. Like many other non-ribosomal peptides, native myxochelin biosynthesis results in low titers with hosts that are difficult to handle. Transfer of its synthesis to *P. taiwanensis* enables more efficient metabolic engineering, while also opening up process engineering options like supplementation of different carbon sources or addition of a second phase extractant.

SL-BT-130
Two routes for tyrosol production by metabolic engineering of *Corynebacterium glutamicum*
*N. Junker[1], S. S. Poethe[1], V. F. Wendisch[1]
[1]*Bielefeld University, Genetics of Prokaryotes, Faculty of Biology and Center for Biotechnology (CeBiTec), Bielefeld, Germany*

The phenolic compound tyrosol is widely used in the pharmaceutical industry, owing to its beneficial effects on human health and its use as a precursor for key pharmaceuticals, including β1 receptor blockers. Tyrosol can be found in olive oil, but despite its natural synthesis in plants, low extraction efficiencies render microbial production a more viable alternative.

Here, we engineered the L-tyrosine overproducing *Corynebacterium glutamicum* strain AROM3 for the *de novo* production of tyrosol. Two routes were compared: one via 4-OH-phenylpyruvate as intermediate and the other via tyramine. Contrary to our expectation for the first route, the heterologous expression of the prephenate dehydrogenase gene from *E. coli* was not required for 4-OH-phenylpyruvate synthesis, although *C. glutamicum* lacks this enzymatic function. Instead, our findings revealed that 4-OH-phenylpyruvate is released by native aminotransferases upon deamination of L-tyrosine. Its further decarboxylation was achieved by heterologous expression of *ARO10* from *Saccharomyces cerevisiae* encoding a pyruvate decarboxylase. Given the instability of 4-OH-phenylpyruvate, the synthesis of tyrosol via the stable intermediate tyramine was pursued via the second route. Decarboxylation of L-tyrosine followed by deamination was accomplished by overexpression of the L-tyrosine decarboxylase gene *tdc* from *Levilactobacillus brevis* and the tyramine oxidase gene *tyo* from *Kocuria rhizophila*. Both routes converge by the synthesis of 4-OH-phenylacetaldehyde, which is subsequently reduced to tyrosol by native alcohol dehydrogenases. We identified the furfural dehydrogenase FudC to be one of the enzymes involved in this process, as its gene deletion reduced tyrosol production by 75 %. Via both routes, strain AROM3 overexpressing either *ARO10Sc* or *tdcLb* together with *tyoKr* produced more than 9 mM (1.2 g/L) of tyrosol from 40 g/L glucose in shake flask experiments. This proof-of-concept of fermentative tyrosol production will be followed up further.

SL-BT-131
Modular and standardized strain engineering of *Corynebacterium glutamicum* using Modular Cloning (MoClo)-based CRISPR/Cas12a workflow
*D. Kösters[1,2], J. Marienhagen[1,2]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*
[2]*RWTH Aachen University, Biotechnology, Aachen, Germany*

Automated strain construction facilities (biofoundries) leverage advanced molecular cloning strategies to economically construct a large number of genetically diverse production strains. Common molecular cloning tasks include synthesizing operons with varying induction strengths, constructing novel pathways, or engineering genetic circuits, all encoded episomally or functionally integrated into the host genome. A more recent concept addressing the demand for large libraries of genetic constructs is the standardization and modularization of genetic elements utilizing the Modular Cloning (MoClo) - principle.

This study presents a MoClo-based toolkit for rapid plasmid construction in *Corynebacterium glutamicum*, supporting both episomal gene expression and CRISPR/Cas12a-mediated genome modifications. A library of basic genetic parts (promoters, ribosome binding sites, coding sequences, and terminators) for the assembly of transcription units was established, and functionally validated by experiments through the expression of a fluorescent reporter gene. To address more complex cloning demands, the *3G Assembly* developed in *E.* coli, used to construct synthetic operons from pre-assembled transcription units was adapted for *C. glutamicum* and quantitatively validated.

The MoClo principle was then utilized for genome editing, yielding plasmid pJYS3_MoClo as a versatile genetic vehicle for CRISPR/Cas12a-based engineering of *C. glutamicum*. Due to limited knowledge about validated genomic integration sites in this organism, the standardization and modularity of pJYS3_MoClo was used to generate a data set of suitable genomic integration loci in *C. glutamicum* ATCC 13032. A number of different genomic locations could be validated for genomic integrations with editing efficiencies ranging from 2-10%. The lab-scale knowledge was then utilized to perform automated genome engineering by employing the AutoBioTech biofoundry.

Taken together, this work introduces a versatile molecular toolkit for *C. glutamicum* strain engineering, paving the way

for the automated metabolic engineering of this industrially-relevant organism.

SL-BT-132
Iterative *in vivo* "cut'n'paste" of functional genomic loci in bacteria
*S. Gude[1], A. B. Bertelsen[1], M. H. H. Nørholm[1]
[1]*DTU, Lyngby, Denmark*

Introduction:

Systematic reengineering of bacterial genomes is still in its infancy. Advances in genome engineering may enable novel applications in green biotechnology while simultaneously providing fundamental insights into the interplay between biological function and genome architecture.

Goals:

Here, we present a scalable *in vivo* method to iteratively relocate ("cut'n'paste") functional genomic loci onto episomes in bacteria. Our method aims to facilitate the creation of auxiliary functional modules, such as the recently described iModulons[1], that can be transferred and used in a plug-and-play fashion to add specific biological functionality to a minimal cell chassis when desired.

Methods:

We combine CRISPR-Cas9 counter-selection and lambda red recombineering with standardised components and an antibiotic marker recycling scheme to drastically reduce the need for *in vitro* manipulations and locus-specific workflow optimization.

Results:

We successfully relocate operons onto an episome and assay their performance in the novel genomic context.

Summary:

In vivo "cut'n'paste" promises to overcome the stark limitations imposed by *in vitro* techniques such as PCR or restriction enzyme cloning by letting CRISPR-Cas9, homologous recombineering, and the native bacterial DNA polymerase do the heavy lifting inside the cell.

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Archaea

SL-AR-079
Enhancing Methanogenesis performance in the Power-to-Methane process: Addressing H_2 and CO_2 intermittency
*J. Xue[1], W. Gu[1]
[1]*EPFL, Environmental Engineering Institute IIE, Lausanne, Switzerland*

Question

Power-to-Methane (PtM) technology stands at the forefront as a promising solution for the storage of surplus renewable energy aligned with CO_2 reduction. In the biological PtM process, methanogens utilize H_2 generated from water electrolysis powered by renewable energy and CO_2 from industrial waste gas to produce CH_4, namely methanogenesis. Our work aims to enhance the robustness of PtM processes by optimizing this methanogenesis step, addressing challenges related to intermittent gas feeding.

Methods

The model strain *Methanococcus maripaludis* MM901 was cultured in batch cultures to study its starvation-revival dynamics under H_2- *vs.* CO_2- starvation by monitoring OD_{600nm} and specific CH_4 production rate. To obtain mechanistic understanding of the observed difference, we also (1) measured NAD^+ and NADH in the cells during starvation using bioluminescent assays, as well as (2) performed oxygen exposure experiments, where cultures were exposed to air for 1h during starvation followed by monitoring their revival dynamics after O_2 removal.

Results

M. maripaludis MM901 exhibited a shorter lag time and higher metabolic activity after H_2 starvation compared to CO_2 starvation, and an increase in starvation time amplified the difference. We hypothesize that cells experience a reductive stress under CO_2 starvation. We measured NAD^+ and NADH in cells during starvation and observed significantly higher $NADH/NAD^+$ ratio in CO_2 starved cells indicating accumulation of reducing equivalence. O_2 exposure also exerts greater inhibition on the revival of CO_2-starved cultures over H_2-starved cultures shown by a significantly longer lag phase.

Conclusions

In CO_2-starved cells where there is H_2 leftover, it is possible that O_2 reacts with accumulated reducing equivalence, resulting in the production of highly oxidative reactive oxygen species, and subsequently damaged enzymes and DNA in cells. To ensure the process stability and gas product quality, where H_2 leftover is preferred over CO_2 leftover, further efforts are needed to adapt methanogens to starvation under CO_2 limitation.

SL-AR-080
Studies on the substrate depending iron reduction and reductive bioleaching of *Metallosphaera sedula*^{T}
*M. Hofmann[1], V. Speck[1], S. Hedrich[1]
[1]*Technical University Bergakademie Freiberg, AG Mikrobiologie & Biohydrometallurgie, Freiberg, Germany*

Since conventional leaching of nickel and copper from limonitic laterites via pyro- and hydrometallurgical processes requires large amounts of energy and results in high CO2 emissions, environmentally friendly alternatives like bioleaching are becoming increasingly important. The reductive bioleaching of limonitic laterites showed promising results using bacteria of the genera *Acidithiobacillus* and

Sulfobacillus [1]. Thermoacidophilic archaea of the genus *Metallosphaera* are known to oxidize sulfur and iron as well as reduce iron and they are suggested to have a high potential for reductive bioleaching of laterites [2].

The goal of this study was to investigate the influence of different substrates on the iron reduction of the thermoacidophilic archaeon *Metallosphaera sedulaT* and to evaluate its bioleaching potential in an initial leaching experiment.

M. sedula was incubated under anaerobic conditions at 70°C in Norris Medium with 50 mM Fe(III) and one of the following substrates: (i) 0.1 % complex organics (e.g. yeast extract, casamino acids), (ii) 10 mM amino acids (e.g. L-alanine, L-cysteine), (iii) 10 mM D-mannose, (iv) 1% elemental sulfur. In total 11 different substrates were tested. Bioleaching experiments were carried out anaerobically with 1% (w/v) goethite, hematite, and the laterites BaSt and BaC (from a site in Brazil). Iron reduction efficiency was monitored using the Ferrozine assay in all experiments. Fe, Ni and Co concentrations in the leach solution from laterite bioleaching were detected using ICP-OES.

The experiments revealed significant differences in iron reduction efficiency depending on the substrate used. Peptone showed the best results followed by S0 with a complete iron reduction after 5 and 7 days. The other substrates showed either a very slow reduction (L-alanine), incomplete reduction (yeast extract) and/or precipitation of iron(III) (yeast extract, glutamic acid). For the other amino acids and D-mannose, iron reduction was also observed in the negative controls. The bioleaching experiments revealed, that laterite and goethite were successfully leached by *M. sedula*, which will be explored for further application in reductive bioleaching.

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SL-AR-081

Identifying the missing steps of the autotrophic 3-hydroxypropionate/4-hydroxybutyrate CO_2 fixation cycle in *Nitrosopumilus maritimus*

*J. Liu[1], L. Bösing[1], E. Huang[1], S. König[2], M. Könneke[3], I. A. Berg[1]

[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

[2]*University of Münster, Graduate School of Natural Products, Münster, Germany*

[3]*Carl von Ossietzky University, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany*

Ammonia-oxidizing archaea (AOA) of the phylum Nitrososphaerota are the predominant nitrifiers in the ocean and in various soils. Most organisms in this group are autotrophs that use a modified 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle. In this cycle, acetyl-CoA is carboxylated to succinyl-CoA, which is then converted to two acetyl-CoA molecules with 4-hydroxybutyrate as the key intermediate. Compared to the HP/HB cycle operating in Thermoproteota, the AOA variant saves two ATP equivalents per turn, making it the most energy-efficient aerobic pathway for inorganic carbon fixation. These two variants of the HP/HB cycle evolved convergently, with a number of enzymes catalyzing the specific reactions of the cycle being non-homologous, as judged by the absence of homologs of the Thermoproteota enzymes in the genomes of autotrophic AOA. Here we identify all the missing enzymes of the cycle in the model AOA *Nitrosopumilus maritumus*, i.e. malonyl-CoA reductase, succinyl-CoA reductase and succinic semialdehyde reductase, and characterize ketothiolase that catalyzes the final step of the pathway, the formation of acetyl-CoA. The characterization of these enzymes elucidates the properties of the HP/HB cycle operating in Nitrososphaerota and clarifies the function of F_{420} in this group of archaea.

SL-AR-082

Fatty acid metabolism in *Sulfolobus acidocaldarius*

*C. Schmerling[1], X. Zhou[1], P. E. Görs[2], S. Köstlbacher[3], T. Kessenbrock[4], D. Podlesainski[4], D. Sybers[5], K. Wang[6], A. C. Lindås[6], J. L. Snoep[7], E. Peeters[5], M. Kaiser[4], T. J. G. Ettema[3], S. W. Meckelmann[2], C. Bräsen[1], B. Siebers[1]

[1]*University of Duisburg-Essen, Molecular Enzyme Technology and Biochemistry, Essen, Germany*

[2]*University of Duisburg-Essen, Applied Analytical Chemistry, Essen, Germany*

[3]*Wageningen University, Wageningen, Netherlands*

[4]*University of Duisburg-Essen, Chemical Biology, Essen, Germany*

[5]*Vrije Universiteit Brussel, Research Group of Microbiology, Brussels, Belgium*

[6]*Stockholm University, Department of Molecular Biosciences, Stockholm, Sweden*

[7]*Stellenbosch University, Institute of Biochemistry, Stellenbosch, South Africa*

Archaea synthesize membranes using isoprenoid-based ether lipids, while Bacteria and Eukarya use *fatty* acid-based ester lipids. Despite these differences, some studies have suggested that fatty acids are present in a few Archaea, such as Sulfolobus spp. (1, 2). Furthermore, several archaeal genomes encode complete sets of bacterial-like β oxidation homologues and few archaeal species were found to degrade fatty acids (1, 3). However, no studies to date have been able to fully elucidate the fatty acid metabolism in archaeal species. Here, we address this open question by investigating fatty acid degradation in the archaeal model organisms *Sulfolobus acidocaldarius by* biochemical characterization and *in vitro* pathway reconstitution. This work shows that a set of β oxidation homologues present *in S. acidocaldarius* constitute a functional fatty acid degradation pathway. This pathway involves the sequential activities of an acyl-CoA dehydrogenase (Saci_1123), a bifunctional 3(S)-hydroxyacyl-CoA dehydrogenase/enoyl-CoA hydratase fusion protein (Saci_1109) and β-ketothiolase (Saci_1114) in addition to an unusual fused archaeal electron transfer flavoprotein (Saci_0315). All four proteins were reconstituted *in vitro* to a complete and fully functional β oxidation cascade that catalyzed the complete degradation of medium chain fatty acid to acetyl-CoA with a pronounced specificity for NAD^+ and the (S)-hydroxyacyl-CoA stereoisomers. Importantly, a detailed characterization of these enzymes also supports that several mechanistic, specificity and thermodynamic constraints prevent this pathway from functioning in the reverse direction to support fatty acid synthesis. Instead, we propose the presence of a novel fatty acid synthesis pathway in *S. acidocaldarius* consisting of a β-ketothiolase (Saci_1121), ketoacyl-CoA reductase (Saci_1104), and hydroxyacyl-CoA dehydratase (Saci_1085) that form a stable assembly mediated by a DUF35 domain protein (Saci_1120), while the final step is catalysed by an NADPH-dependent enoyl-CoA reductase (Saci_1115). In summary our study demonstrates that Archaea are capable of synthesizing and degrading fatty acids and elucidates the molecular mechanisms involved in these processes.

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Biology of yeast and fungi

SL-BYF-116
Flotillin-containing lipid rafts in *Aspergillus nidulans* are linked to calcium
*M. Kawashima[1], T. Krüger[1], A. Brakhage[1]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Institute of Microbiology, Jena, Germany

Lipid rafts are tight assemblies of proteins and lipids in a biological membrane and are thought to be involved in many physiological processes such as immune signalling and host-pathogen interactions. However, due to their small sizes exceeding the resolution limit of conventional light microscopy, direct measurement and characterisation of lipid rafts in living membranes remains to be a challenge. While most studies on lipid rafts have been carried out on mammalian cells, here we use the genetic model fungus *Aspergillus nidulans* to allow a more versatile characterisation of lipid rafts at both molecular and organismic levels. In particular we investigate the role of flotillin (FloA), a lipid raft marker conserved across many organisms. Using a nanoluciferase reporter strain, we demonstrate that the *A. nidulans* flotillin FloA is highly expressed when the fungus is confronted with high calcium stress. Transcriptomic analysis further reveals that repression of FloA under these conditions leads to the upregulation of numerous mitochondrial genes, suggesting a functional connection between FloA and mitochondrial activity. In addition, in vivo protein-proximity labelling is performed to assess the physical interaction partner proteins of FloA. We also explore the role of FloA in microbial communication through co-cultivation experiments with the soil bacterium *Streptomyces iranensis* and show that the bacterium is able to induce the high expression of FloA, most likely by secreting a natural product. These findings contribute to a deeper understanding of lipid raft dynamics and the organisation of eukaryotic membranes.

SL-BYF-117
Probing *Aspergillus niger* spore dynamics: A droplet – based microfluidic approach for single-spore analysis and stressor evaluation
*S. M. Timofeev[1], C. Engwer[2], F. Schulze - Bockeloh[2], K. Brix[3], H. Kleine - Brüggeney[2], V. Göhre[4], R. Kautenburger[3], S. Leuko[1]
[1]German Aerospace Center (DLR), Applied Biology, Cologne, Germany
[2]Evorion Biotechnologies GmbH, Münster, Germany
[3]Saarland University, Department of Inorganic Solid-State Chemistry, Saarbrücken, Germany
[4]TU Darmstadt, Department of Chemical Technology and Biotechnology, Darmstadt, Germany

Aspergillus niger spores demonstrate complex and heterogeneous responses to environmental stressors, which complicates efforts to control fungal growth and resistance. In this study, we used a droplet – based microfluidic system to encapsulate individual *A. niger* spores in hydrogel beads, creating a 3D microenvironment conducive to controlled, high-resolution monitoring. This platform facilitates single-cell time-lapse microscopy, enabling detailed observation of germination patterns and branching behaviors across up to 1500 spores in a single experiment.

To evaluate the effects of copper ions (Cu^{2+}, delivered as $CuSO_4$ solution) and the antifungal agent caspofungin, we measured parameters such as swelling events, swelling size, hyphal area and main hyphae length. Initial findings revealed a pronounced heterogeneity in germination and outgrowth behavior under copper stress, even in a species known for its robust copper homeostasis mechanisms. The treatment with caspofungin in comparison to led to a 175% increase in swelling size and elevated the fraction of swollen spores in wild-type strains, while exposure to a 10 mM $CuSO_4$ solution resulted in a reduction across all growth metrics. ICP-MS measurements of copper content before and after incubatio over 24h on chip further indicated potential uptake of Cu^{2+} ions by spores within the hydrogel matrix. Viability and cell wall integrity assessments via LIVE/DEAD fluorescence and calcofluor white staining confirmed the spores' responses to these treatments.

This research highlights the nuanced, stress-induced heterogeneity in *A. niger"s* physiological responses and underscores the potential of microfluidic systems as powerful tools for real-time, high-throughput fungal analysis. This approach offers new insights for antifungal development and fungal behavior under metal-induced stress.

SL-BYF-118
The lipid transfer protein PDR16 of the filamentous ascomycete *Sordaria macrospora*
*S. Pöggeler[1], M. Nater[1], D. Stastny[2], S. Erb[1], A. Werner[1,3], G. Stahlhut[1], P. Griac[2]
[1]Georg-August University Göttingen, Genetik eukaryotischer Mikroorganismen, Göttingen, Germany
[2]Heinrich Heine University, Institute of Animal Biochemistry and Genetics, Bratislava, Slovakia
[3]Georg-August University Göttingen, Molekulare Strukturbiologie, Göttingen, Germany

Pulldown experiments with the Striatin interaction phosphatase and kinase (STRIPAK) component SCI1 identified an orthologue of the yeast PDR16 phosphatidylinositol (PtdIns) transfer protein as a putative target of the STRIPAK complex in *S. macrospora*.

The *S. cerevisiae* homolog of the *S. macrospora* PDR16 protein is predominantly associated with lipid droplets or is localized either to the plasma membrane (PM) or the PM-associated ER. The role of the Pdr16 protein in yeast metabolism is not fully understood. However, the absence of Pdr16p leads to increased susceptibility of yeast cells to azole antifungals, indicating its role in sterol biosynthesis. By regulating the phospholipid/sterol composition of plasma- and endo-membranes, *S. macrospora* PDR16 may be involved in membrane recruiting of the STRIPAK signaling complex required for hyphal fusion.

Deletion of *Smpdr16* in *S. macrospora* resulted in no obvious changes in growth, fruiting-body morphology or sexual reproduction under regular conditions. Only when grown at decreased temperatures the ΔSmpdr16 mutant displayed a reduced growth rate in comparison to the wt. SmPDR16 is localized at septa and endo-membranes, but localization at the plasma membrane cannot be ruled out.

To examine the functional conservation of PDR16 proteins, we heterologously expressed the *S. macrospora pdr16*

cDNA in a yeast *Δpdr16* mutant and verified that the *S. macrospora* gene can substitute for the absence of the yeast protein. To address the lipid/sterol binding capacity of PDR16, we will overexpress SmPDR16 in *E. coli* followed by purification to perform a lipid binding assay in HL60 cells.

SL-BYF-119
Sculpting fungal fate: the importance of RNA editing for developmental morphogenesis in filamentous fungi
*S. Herzog[1], M. Kutter[1], A. Somarajan[1], M. Skendrou[2], J. Grygosch[2], L. Bleeken[1], F. Krekel[1], U. Lipka[1], I. Teichert[1]
[1]Georg-August University Göttingen, Forest Botany and Tree Physiology, Göttingen, Germany
[2]Ruhr University Bochum, Allgemeine und Molekulare Botanik, Bochum, Germany

RNA editing, the post-transcriptional selective insertion, substitution or deletion of nucleotides plays a crucial role in functional diversity of gene expression within all domains of life. In ascomycetes, a diverse group of filamentous fungi RNA editing primarily involves adenosine-to-inosine (A-to-I) conversions. In contrast to animals the A-to-I editing occur predominantly within coding regions of transcripts involved in sexual reproduction. Interestingly, not only amino acid codons but also stop codons tend to be affected by editing leading to a change of TAG or TGA codons to TGG tryptophan codons. The precise mechanisms regulating the conversion of the codons still need further investigation. However, A-to-I RNA editing is suggested to play a crucial role in the formation of sexual structures like ascospores and fruiting bodies and might also provide proteome diversity that benefits the progeny.

To unravel the biological importance of A-to-I editing during development morphogenesis in filamentous fungi we analyzed so called *efd* (edited in fruiting body development) genes in the ascomycete *Sordaria macrospora*. Intriguingly, several deletion mutants of *efd* genes like *efd4* and *efd7* showed severe defects in their ascospore formation and/or discharge. Interestingly, complementation studies with constructs expressing either the edited version (TGG) or non-edited version (TAA) revealed putative functions for the editing sites during reproduction. Very recent results suggest a role for *efd16* in cellular structure and physical properties of sexual reproduction structures. To investigate this further we are applying a novel method measuring structural integrity of ascospores. These data will provide better insight of the impact of A-to-I editing during developmental processes and its role for the adaptive capabilities and morphogenesis of fungi.

SL-BYF-120
Structure-function analysis of Ubp3, Bre5 and the Ubp3/Bre5 complex
*L. Bostelmann-Arp[1], L. Schmitt[1]
[1]Heinrich Heine University, Institute of Biochemistry, Düsseldorf, Germany

Ubiquitination is a post-translational protein modification in eukaryotes, with broad signaling functions. In this process, the small molecule ubiquitin is covalently attached to target proteins through a complex three-step enzymatic cascade, which can result in the formation of polyubiquitin chains. Ubiquitin-mediated signaling is involved in a multitude of cellular functions and quality control mechanisms, requiring precise regulation. These quality control pathways include different types of selective autophagy such as ribophagy or mitophagy. To maintain a balance between various quality control mechanisms, deubiquitinating enzymes can remove or modify ubiquitin signals by cleaving ubiquitin or polyubiquitin chains. In *Saccharomyces cerevisiae*, one such deubiquitinating enzyme is Ubp3, which relies on its cofactor Bre5 for specific activity. The Ubp3-Bre5 complex plays a vital role in various cellular processes, particularly in stress response. Interestingly, it exhibits a dual role in cellular functions. For instance, it protects mitochondria from mitophagy while simultaneously promoting other forms of general autophagy. This investigation aims to elucidate how these distinct functions are coordinated and to explore the nature of the interaction between Ubp3 and Bre5. We demonstrate the establishment of a heterologous expression and purification strategy, to obtain protein for detailed structure-function analyses. With this *in vitro* approach, isolation of individual full-size proteins and their complex is targeted. This is achieved through a series of chromatography steps utilizing different affinity tags. Isolated proteins are investigated regarding their activity, structure and interactions. With this approach, it should be possible to elucidate the molecular mechanisms that govern their natural function and the subsequent hypotheses can then be verified *in vivo*. This study should therefore help to entangle the complex regulatory network in which Ubp3 is involved and advance our understanding of deubiquitinases.

SL-BYF-121
Basidiomycete model fungi and their communication in soil
*E. Kothe[1], E. Iyamu[1], L. Petersen[1], K. Krause[1]
[1]Friedrich Schiller University, Institute of Microbiology, Jena, Germany

For their life is soil, fungi are well-equipped. The filamentous lifestyle allows them to interact with different soil particles as well as plant roots. The hyphae spanning different niches in soil provide a route by which soil bacteria like *Bacillus* can access new environments. To delve into such interactions, different molecular signals are produced, among them volatiles. With respect to ectomycorrhizae, the involvement of fungal VOCs in modulating plant response is discussed.

For soil attachment and growth in soil, a model using glass surfaces is presented that also allows to assess thigmotropic clues that lead hyphae to attach to surface veins and into crevices. This goes to show that basidiomycete fungi like *Schizophyllum commune* can sense surface structures and respond with specific gene expression profiles to different surfaces.

Molecules involved in bacteria-fungi interaction are explored using *Bacillus subtilis* as a single celled bacterium as well as streptomycetes representing filamentous bacteria in interaction with fungi. Surface interactions of fungi enable bacterial movement, and the inhibition of swarming may indicate that the access of different niches is controlled by the fungus. In interaction with *Schizophyllum commune*, and in response to structured surfaces, bacterial signaling molecules are shown and the involvement of *B* pheromone receptor-like proteins, Brls, are discussed.

The specific ectomycorrhizal symbiosis of *Tricholoma vaccinum* with its compatible host spruce and its low-compatibility host pine is used to show the involvement of volatile terpenoid molecules in host communication. The trees respond with different phytohormone profiles suggesting recognition of beneficial versus pathogenic soil

microbes upon terpenes exuded by the fungus in a volatile-only chemical communication setting. Since ectomycorrhizal fungi determine the bacterial microbiome in forest soils, this specific volatile signaling prior to contact between the symbionts is seen as a first row of communication molecules in basidiomycete-host tree symbiosis.

Computational microbiology

SL-CM-150

Towards predicting significant changes in wastewater microbial communities: A machine learning approach using time series data

*A. K. Dörr[1], S. Imangaliyev[1], U. Karadeniz[2], F. Meyer[1], I. Kraiselburd[1]
[1]*University Hospital Essen, Institute for Artificial Intelligence in Medicine, Essen, Germany*
[2]*University of Duisburg-Essen, Computer Science, Essen, Germany*

During the Covid-19 pandemic, wastewater-based surveillance has emerged as a pivotal technology for monitoring health-related targets in the public sector [1]. We seek to support the monitoring of the various bacterial genera present in wastewater by developing a machine learning-based early warning system capable of detecting possible changes posing a threat to human health. To this end, the prediction performance of different machine learning architectures for bacterial time series data with and without additional metadata was tested. While the prediction of bacterial abundances returned satisfactory results, it became evident that the added metadata needed to be carefully selected depending on the sampling environment. Thus far, Long Short-Term Memory (LSTM) [2] models have demonstrated the most promising prediction performance. A prediction interval was constructed to encompass typical fluctuations in the data. Feature importance analysis was conducted using Shapley Additive Explanations (SHAP) [3]. In the subsequent stages, we intend to integrate outlier detection to develop an early warning system and investigate the potential of transfer or ensemble learning to enhance the model's performance and its applicability to other datasets.

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SL-CM-151

Assessment and prediction of spatio-temporal dynamics of *Vibrio vulnificus* in the coastal Baltic Sea

*C. C. Glackin[1], D. Riedinger[1], E. Zschaubitz[1], L. Vogel[1], T. Sperlea[1], H. Benterbusch[1], C. Nietz[1], M. Labrenz[1]
[1]*Leibniz Institute for Baltic Sea Research, Biological Oceanography, Rostock, Germany*

With rising infection rates in recent years *Vibrio vulnificus* pose an increasing threat to public safety in the coastal brackish Baltic Sea. *V. vulnificus*, associated with high mortality, can thrive in the Baltic Sea during summer months due to optimal conditions and can cause severe infections to humans through open-wound infections. Pinpointing a timeframe of increased risk of infection is the next step in curbing the increasing numbers of infections and also has wide-ranging ecological and economic effects. Routine monitoring of this bacterium in the Baltic Sea is critical to provide a warning system for the public when the risk of infection is potentially high. As part of an extensive twice weekly sampling campaign that included 14 locations in the coastal Baltic Sea across a one-year period between 2022-2023, we investigated the abundance of *V. vulnificus* using a multi-method approach, including droplet digital PCR (ddPCR) results targeting the *vvhA* gene region, agar cultivation, and species level classification of 16S rRNA gene sequencing. Physico-, biological- and hydrochemical parameters were measured concurrently and variables explaining *V. vulnificus* occurrence were identified by machine learning. In addition, numerous machine learning techniques were applied in order to predict *V. vulnificus* gene concentrations using ddPCR, with the objective of establishing models that accurately predict when *V. vulnificus* is most abundant in the coastal Baltic Sea. Time-series analysis was performed using variables from previous timepoints as predictors, with the goal of pinpointing the most important markers for creating an early warning system and highlighting the importance of improved coastal monitoring. First results will be presented at the conference.

SL-CM-152

Toxin-Antitoxin Systems – How does the double-edged sword look like?

*M. Schreiber[1,2], E. Barth[1,2], M. Marz[1,2,3,4]
[1]*Friedrich Schiller University, RNA Bioinformatics & High-Throughput Analysis, Jena, Germany*
[2]*Friedrich Schiller University, Bioinformatics Core Facility Jena, Jena, Germany*
[3]*Fritz Lipmann Institute (FLI), Jena, Germany*
[4]*Michael Stifel Center Jena, Jena, Germany*

Antibiotic resistant bacteria are an immediate threat to public health, animal husbandry and agriculture. Toxin-antitoxin (TA) systems in multidrug-resistant bacteria are promising approaches to combat them, but further research is needed to fully understand their distribution, functioning, regulation and evolution. TA systems are genetic modules consisting of a toxin and its antitoxin, which are proteins or rnas, and are involved in various cellular processes, including cell death and stress response. Eight different types, with type I and II being the most investigated, are known.

This project aims to computationally identify and characterize all TAS in all bacteria, archaea and phages from NCBI to gain more insights by using sequence similarity searches and hidden markov models and in the case of ncRNA component, we use RNAalifold[1], Infernal[2] and R-scape[3]. In contrast to a simple search, which is limited to direct sequence and structural similarity, our iterative approach allows us to identify more evolutionary divergence TA systems and enhances both sensitivity and accuracy. Furthermore, we intend to employ a single-plasmid-based approach to experimental verify novel TA systems of interest.

Through sequence alignment and secondary structure prediction, we identify conserved motifs and structural elements within these RNA components. Additionally, we explore potential RNA-RNA and protein-RNA interactions. Our findings provide insights into the structural and functional characteristics of these systems on a global level, paving the way for further experimental validation. We'll also expand the RFAM database to aid future research.

A deeper understanding of TA systems is an essential part of the search for alternatives to antibiotic treatment and future applications in medicine, animal husbandry, and agriculture. A comprehensive search shall reveal their whereabouts and appearance.

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SL-CM-153

Resolving challenges in Nanopore-Based sequencing and assembly of bacteriophage genomes

*S. Doijad[1], R. Beeloo[2], T. Schubert[1], S. Studenik[1], J. Van Cauwenberghe[1], X. Jia[1], A. Zomer[3], B. E. Dutilh[1]

[1]Friedrich Schiller University, Viral Ecology and Omics (VEO) Group, Institute of Biodiversity, Cluster of Excellence 'Balance of the Microverse', Jena, Germany

[2]Utrecht University, Theoretical Biology and Bioinformatics, Utrecht, Netherlands

[3]Utrecht University, Division of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht, Netherlands

Genome sequencing and assembly present challenges, with short-read methods often leading to fragmented assemblies, while long-read methods, though capable of providing contiguous reads, can lack accuracy. Recent advances in Nanopore's "duplex" technology improved per-base quality for long-reads, making it promising for genome assembly. To evaluate this for smaller genomes, we resequenced 182 bacteriophages, ranging from 6 to 79 Kb, and attempted assembly using state-of-the-art tools and best practices available. Only up to 15.4% (29 out of 182) of the initial assemblies matched the expected genome size (±5% bp). Our analysis revealed three key obstacles: (1) inefficient demultiplexing resulting in fewer reads binned, (2) barcode bleeding introducing contaminating reads from different samples, and (3) genomic concatemers generating reads exceeding the actual genome size. To address these, we developed two tools, "Barbell" and "NanoPrepPhage"; the former improves demultiplexing, and the latter removes contaminated reads and reduces the impact of concatemers. Unlike existing tools, Barbell does not rely on the edit distance but instead uses the expected Nanopore current to demultiplex reads. NanoPrepPhage filters contaminant reads using K-mer profiling, and trims reads to a shorter length (either 100, 250, or 500 bps), reducing the impact of concatemers. Combining both tools, all genomes were assembled with their expected sizes, highlighting the robustness and effectiveness of our protocol. I will explain the implementation of the tools, present our findings in bacteriophage sequencing, and detail the limitations we encountered. Combining Barbell with NanoPrepPhage together provides a new best practice that fully exploits the benefits of Nanopore "duplex" reads for reliable genome assembly.

SL-CM-154

PhyloNaP: a tool for phylogeny-based function prediction of enzymes involved in biosynthesis of natural products

*A. Korenskaia[1], J. Szenei[2], K. Blin[2], N. Ziemert[1]

[1]University of Tübingen, Translational Genome Mining for Natural Products, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), Interfaculty Institute for Biomedical Informatics (IBMI), Tübingen, Germany

[2]Technical University of Denmark, The Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark

Introduction: The rise of antibiotic resistance underscores the urgent need for novel antibiotics derived from natural products (NPs). Genome mining has revealed diverse biosynthetic gene clusters (BGCs) that encode these compounds. Accurate structural knowledge of NPs is essential for extraction and other experimental procedures; however, structure prediction is challenging due to limited functional annotations for tailoring enzymes in NP biosynthesis.

Goals: This project aims to develop PhyloNaP, a database and tool for the functional annotation of enzymes involved in NP biosynthesis, with the focus on tailoring enzymes using phylogenetic analysis. By consolidating fragmented phylogenetic and functional data, PhyloNaP enables more accurate NP structure predictions, ultimately aiding in the discovery of new antibiotics and other therapeutics.

Materials & Methods: PhyloNaP integrates functional annotations from literature and public repositories such as MiBiG, SwissProt, and AntiSMASHdb. The database stores phylogenetic trees, sequence alignments, and functional annotations gathered from publications and generated specifically for this project. Currently, PhyloNaP includes enzyme families like methyltransferases, P450s, and halogenases, with each tree annotated with experimentally validated data on substrates and products.

Results: Using PhyloNaP, researchers can explore annotated enzymes, classify new enzymes, and position them within existing phylogenetic trees. This mapping allows users to examine the functional characteristics of closely related enzymes. PhyloNaP also demonstrate substrate features and potential modification sites, enhancing the reliability of NP structure predictions. Additionally, PhyloNaP incorporates information on the taxonomy of the enzyme"s source organism and the main characteristics of its BGC, with links to corresponding NPs in the MiBIG database.

Summary: PhyloNaP fills a critical gap in NP research by providing a centralized repository of annotated phylogenetic data. Researchers are encouraged to contribute new trees, thereby enriching this shared resource and increasing its utility across the scientific community. By integrating both phylogenetic and functional data, PhyloNaP has the potential to accelerate the discovery of valuable natural products.

Acknowledgment: This project has received funding from the European Union"s Horizon Europe program under the Marie Skłodowska-Curie grant agreement No 101072485.

SL-CM-155

The de.NBI resource center for microbial bioinformatics

*J. Blom[1], M. Pfister[2], L. Fenske[2], J. M. Hahnfeld[3], O. Schwengers[2], A. Goesmann[2]

[1]Justus Liebig University Giessen, Bioinformatics and Systems Biology, Gießen, Germany

[2]Justus Liebig University Giessen, Bioinformatics and Systems Biology, Gießen, Germany

[3]Justus Liebig University Giessen, Bioinformatics and Systems Biology, Gießen, Germany

The German Network for Bioinformatics Infrastructure – de.NBI is a national, academic, and non-profit research

infrastructure initiated in 2015 by the Federal Ministry of Education and Research. Within de.NBI, the Bielefeld-Gießen Resource Center for Microbial Bioinformatics (BiGi) supports scientists with bioinformatics software tools, pipelines and resources for microbial research with a focus on the analysis of microbial genomes, metagenomes, and related datasets.

In the last decade, various software tools, pipelines and platforms for annotation, characterization and comparative analysis of genomic as well as metagenomic sequencing data have been maintained and further developed based on feedback from our growing user community. For the comprehensive annotation of microbial genomes, the fully-automated annotation software tool **Bakta** was developed. Bakta is available as command line tool as well as via a user-friendly web version. Bakta quickly emerged to be one of the most-actively used annotation tools worldwide. For the comparative analysis of bacterial genomes, we maintain the de.NBI flagship tool **EDGAR** which provides public as well as custom, password protected databases with more than 100,000 included genomes. Furthermore, a broad range of more specialized software tools were developed and maintained, e.g. **Platon** for the detection of plasmid-borne contigs within draft genomes, **ReferenceSeeker** for the taxonomic classification of microbial genomes, and **MGX2** for the processing and analysis of metagenomic data. A further important project is the large-scale bacterial genome repository **BakRep**, which provides access to a comprehensive assembly and annotation data, enriched with taxonomic information, for a total of ~2.4 million genomes.

Besides the development and maintenance of analysis software and data resources, BiGi also hosts a branch of the federated de.NBI cloud, which provides large-scale compute resources to the scientific community on demand. Additionally, BiGi offers training programs, workshops and user support to help the scientific community to use the various bioinformatics solutions effectively in their research.

In summary, the microbial researcher community can strongly benefit from the triplet of services provided by BiGi:

- A broad set of well-maintained and constantly improved software solutions
- An accessible powerful and growing cloud-computing infrastructure
- A regular offer of accompanying workshops and training courses

Environmental microbiology & ecology

SL-EME-001

Adaptation of *Novosphingobium aromaticivorans* for growing with aromatic compounds adsorbed to granular activated carbon as a potential cause for the stable occurrence of *Sphingomonadaceae* in fourth clarification stages of wastewater treatment plants

*Y. Abdullaeva[1], S. Rochau[1], M. Löwe[1], J. Holert[1], *B. Philipp[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

Activated carbon filters are increasingly being installed in wastewater treatment plants (WWTPs) to remove organic micropollutants from wastewater effluent through a physico-chemical adsorption process. Microbial biodegradation also contributes to the removal of micropollutants in those filters but the microbiomes and the underlying processes are mainly unexplored. Particularly, it is unknown whether active microbial desorption of micropollutants takes place.

In this study, the prokaryotic microbiome of an activated carbon filter newly installed as the fourth clarification stage of a municipal WWTP was analyzed over 12 months. Amplicon sequencing of genes for *16S rRNA* revealed a large diversity of bacteria. Families with relative abundancies above 1% stabilized after 2-6 months and included potential micropollutant degraders *Hyphomonadaceae* and *Sphingomonadaceae*. Among the latter, members of the genus *Novosphingobium* made up around 45% and were closely related to the type strain *N. aromaticivorans* DSM 12444, which is known to degrade synthetic chemicals. Members of the genus *Pseudomonas* were not detected in the microbiome but enrichment cultures with filter material yielded *Pseudomonas* sp. strains Teo15 and Salic. Laboratory experiments with strains DSM12444 and the pseudomonads showed that strain DSM12444 reached a significantly higher final optical densities with benzoic and salicylic acid that was pre-adsorbed to granular activated carbon (GAC) than strains Teo15 and Salic, respectively. Extraction of residual benzoic and salicylic acid from activated carbon granules indicated that more than 95 % of the aromatic compounds had been depleted by strain DSM12444 compared to about 70 % by strains Teo15 and Salic. Transcriptomic analysis of strain DSM1244 during growth with benzoic acid showed differential gene expression in the presence and absence of GAC. In particular, a different pathway for degrading aromatic compounds and two genes for hemerythrin-domain containing proteins, which can serve as redox sensors, were upregulated in the presence of GAC.

This study indicates that strain DSM12444 can specifically adapt to utilize aromatic compounds adsorbed to GAC which might be a selective advantage colonizing activated carbon filters. This property calls also attention to the potential use of this organism for bioaugmentation of fourth clarification stages of WWTPs.

SL-EME-003

Enhancing proteome analysis of environmental microorganisms: FISH-FACS and label-free quantitative proteomics from ultra-low cell numbers

*V. Kale[1], G. Ho[2], S. Maaß[1], A. Trautwein-Schult[1], D. Bartosik[3,4], T. Schweder[3,4], B. M. Fuchs[2], D. Becher[1]
[1]*University of Greifswald, Greifswald, Germany*
[2]*Max Planck Institute, Bremen, Germany*
[3]*University of Greifswald, Greifswald, Germany*
[4]*Institue of Marine Biotechnology e.V., Greifswald, Germany*

Metaproteomics is an essential approach to investigate microbial metabolic activity in diverse environments. However, functional analysis of specific microorganisms is often underexplored by the protein inference problem due to sequence homologies among closely related species. This challenge limits our understanding of the role of particular microbes in complex environmental samples. In this study, we have developed a workflow that combines fluorescence in situ hybridisation (FISH) and fluorescence-activated cell sorting (FACS) with mass spectrometry-based proteomics to analyse proteins from non-culturable bacteria directly from environmental samples. The workflow was first optimised using the culturable model bacterium *Polaribacter* sp. KT25b, isolated from the North Sea. This allowed for assessment of cell number requirements for robust protein identification and quantification following FISH and FACS. We determined that

samples containing $1x10^5$ cells are sufficient for reliable qualitative protein identification, while samples with $5x10^5$ to $1x10^6$ cells enable reproducible protein quantification. Furthermore, the use of a taxon-specific database improves data analysis by significantly reducing the size of protein groups compared to metaproteomics data. Additionally, we tested this workflow to gain deeper insights into the activity of key bacterioplankton species involved in North Sea phytoplankton blooms. Overall, this workflow addresses a major methodological gap, providing a broad application for directly enriching non-culturable microbes from their habitat and enabling downstream proteome analysis.

SL-EME-004

Survival of dormant (hyper)thermophilic sulfate reducing microorganisms at high temperatures over geologic timescales

*J. Kallmeyer[1], S. Bernsee[1], T. Nagakura[1]
[1]*GFZ German Research Centre for Geosciences, Potsdam, Germany*

Seepage of warm fluids from subsurface petroleum reservoirs and ocean crust releases a constant flux of thermophilic microorganisms into the cold deep sea, where some of them form spores and settle on the sea floor. Previous studies showed that spores from sulfate reducing *Firmicutes* can be revived upon heating to temperatures >50°C. As these experiments were restricted to surface sediments, the long-term survival of these spores remained unclear. We measured biological sulfate reduction rates (SRR) using highly sensitive radiotracer incubations in samples from a drill core from Guaymas Basin, a marginal ocean basin off the Pacific coast of Mexico with active sea floor spreading and a very high geothermal gradient of 950°C km^{-1}. The samples covered in-situ temperatures between 6 and 61°C and ages up to ~200 ky. Samples were incubated under in-situ pressure (25 MPa) and temperature and under increasingly higher temperatures, up to 50°C above in-situ values. Maximum incubation temperature was 111°C.

Samples from all depths revealed SRR increasing with temperature, SRR was detectable up to maximum incubation temperature, albeit at extremely low rates. The SRR data reveal two broad peaks of activity at 45 to 60°C and 75 to 95°C, respectively, with a distinct gap in between. We interpret this gap as a shift in microbial community composition from mesophiles/thermophiles to hyperthermophiles. As we can exclude any delivery of spores through advective transport from below or laterally, we postulate that these sulfate reducing microorganism were embedded at the time of deposition and survived over hundreds of thousands of years, even at elevated temperatures.

SL-EME-005

Enhanced benthic weathering as a CO2 removal strategy in seasonal hypoxic coastal waters of the Baltic Sea is influenced by cable bacteria activity after re-ventilation of bottom waters

R. Bährle[1], T. Bornemann[2], A. Soares[2], S. Boehnke[1], M. Fuhr[1], A. Dale[1], A. Probst[2], S. Geilert[3], *M. Perner[1]
[1]*GEOMAR Helmholtz Centre for Ocean Research, Marine Biogeochemistry, Kiel, Germany*
[2]*University of Duisburg-Essen, Duisburg, Germany*
[3]*Utrecht University, Utrecht, Netherlands*

Since the industrial revolution, atmospheric CO2 has increased dramatically resulting in warming of Earth"s climate. The oceans are fundamental for mitigating global climate change as they absorb heat and CO2. Consequently, the oceans have changed, affecting marine life and biogeochemical cycles. To slow down climate change, different CO2 removal (CDR) strategies are being tested such as enhanced benthic weathering (EBW). In EBW, alkaline minerals are added to the seafloor, accelerating weathering and releasing alkalinity to the water body. In the Baltic Sea, sediments exposed to seasonal hypoxic bottom waters appear ideal settings for EBW; yet, feedbacks of microbial metabolisms to EBW remain unknown. We addressed this knowledge gap by performing sediment core incubations adding alkaline minerals to Baltic Sea surface sediments, naturally exposed to seasonal hypoxia. Sediment cores were incubated under oxygen limited conditions and shifts in geochemistry and transcriptomes were monitored over time. Calcite amended incubations indicated the highest CDR potential. The combination of calcite dissolution related bicarbonate bioavailability and limited oxygen availability in bottom waters were likely the main drivers of *Cand.* Electrothrix (cable bacteria) enrichment. *Cand.* Electrothrix"s acidifying activity promotes carbonate- and FeS-mineral dissolution, as evidenced by bicarbonate, manganese, iron and sulfide accumulation in sediment porewaters. In line, transcriptomics suggested stimulation of overall microbial sulfur-, iron- and manganese oxidation. Moreso, transcript abundances posited shifts towards denitrification. Carbonic anhydrases (bicarbonate <-> CO2) were upregulated suggesting a calcite dissolution related bicarbonate source, coinciding with upregulated genes of autotrophic CO2-fixation. *Cand.* Electrothrix appeared to promote EBW. In turn, artificial calcite addition also increased buffering capacity of the sediments, affecting relative FeS-mineral dissolution. Relatively less available ferrous iron may restrict some formation of iron-oxides, known to act as barrier to sulfide emissions from sediments during phases of anoxia. Conclusively, according to shifts in transcript abundances, feedbacks may include stimulated EBW but also loss of dinitrogen from sediments as well as reduced retention of sulfide during anoxia. Metabolic rate measurements are urgently required to scrutinize the holistic effects of EBW on such coastal systems.

SL-EME-006

***BioMetArchive*: Deciphering the genetic content of metagenome-assembled genomes along the 1 Ma sedimentary sequence of ferruginous Lake Towuti, Indonesia**

*A. Vuillemin[1], F. Ruiz Blas[1], A. Bartholomäus[1], S. Yang[1], C. Henny[2], J. M. Russell[3], J. Kallmeyer[1]
[1]*GFZ German Research Centre for Geosciences, Section Geomicrobiology, Potsdam, Germany*
[2]*National Research and Innovation Agency (BRIN), Research Center for Limnology and Water Resources, Bogor, Indonesia*
[3]*Brown University, Department of Earth, Environmental, and Planetary Sciences, Providence, RI, United States*

Ferruginous (iron-rich, sulfate-poor) conditions prevailed in the oceans through much of Earth's history. Yet ancient biogeochemical cycles inferred from the rock record remain elusive in terms of microbial enzymatic processes, as modern analogues in which to study an active subsurface biosphere are scarce. Lake Towuti, Indonesia, features ferruginous bottom waters and sediments that offer conditions similar to those of Earth's early oceans. In spring 2015, the International Continental scientific Drilling Program recovered a 100-m-long sediment core in 156 m water depth just below the oxycline, documenting 1 million years of redox

changes at the sediment-water interface (SWI). The project *BioMetArchive* aims to characterize the presence of a subsurface biosphere in terms of microbial density, taxonomic and functional diversity, and comprehensively integrate metagenomic with geochemistry data.

Discrete water column samples resulted in 408 metagenome-assembled genomes (MAGs) that reflected a series of endogenic processes driven by planktonic populations, namely primary and secondary organic matter production alongside iron and ammonia oxidation with subsequent iron and sulfate reduction across water layers. Down to 1 m below lake floor (mblf), terminal electron acceptors became depleted, resulting in a drastic decrease in cell densities and microbial diversity. In the fermentative zone, sedimentary geochemical conditions selected for specific microorganisms, mainly Chloroflexota and Bathyarchaeia. Metagenomic sequencing from minute DNA amounts down to 68 mblf (~750 ka) resulted in 190 MAGs. Their genetic content revealed that Anaerolineae have metabolic potential to cope with oxygen fluctuations, inhabiting ferruginous bottom waters and the SWI. In sediments, Dehalococcoidia perform organic acid secondary fermentations combined with an acetogenic Wood-Ljungdahl pathway. Below the sulfate reduction zone, Bathyarchaeia prevailed as they harbour specific enzymes to harness reactive sulfur species from hydrogen sulfide. They exhibit metabolic potential for complex carbohydrate fermentation combined with a complete (homo)acetogenic Wood-Ljungdahl pathway. This shows that Bathyarchaeia rule carbon mineralization alongside methylotrophy in deep ferruginous sediments. Our findings suggest that the deep lacustrine biosphere persisting under ferruginous conditions requires energy conservation processes that show clear analogies to those of primeval microbial life.

SL-EME-060

Bacterial degradation of plasticizers on the example of diethyl phthalate (DEP)

*S. Bertoldi[1], A. Marsans[1], N. Sharma[1], D. Schlosser[2], N. Marcel[3], H. J. Heipieper[1], C. Eberlein[1]
[1]Helmholtz Centre for Environmental Research - UFZ, Environmental Biotechnology, Leipzig, Germany
[2]Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany
[3]Helmholtz Centre for Environmental Research - UFZ, Microbial Biotechnology, Leipzig, Germany

The continuing reports of plastic pollution in various ecosystems highlight the threat posed by the ever-increasing consumption of synthetic polymers. Plastics are frequently associated with additives such as phthalic acid esters. Such compounds are used as plasticizers to provide flexibility to plastic products and as common additives in various consumer products, and suspect to cause endocrine disruption in animals.

The bacterial degradation of diethyl phthalate (DEP) as a model compound for plasticizers was studied. In order to isolate bacterial DEP degraders, a biofilm present on a polyurethane tubing was scratched off. From this biofilm, a microbial consortium containing two strains *Microbacterium ginsengisoli* and *Pseudomonas canadensis* was isolated. These strains were further characterized and found to be capable to grow in mineral medium with DEP as sole carbon energy source. The complete degradation of up to 4 mM DEP was confirmed by UPLC analysis. Furthermore, the substrate spectrum of the microbial consortium was assessed by testing different carbon sources like phthalic acid, 3,4-Dihydroxybenzoic acid, and other phthalate esters. Based on that and on the metagenome of the bacterial consortium, a degradation pathway of DEP was suggested.

SL-EME-061

Natural oil slicks serve as microbial seedbank for accelerated hydrocarbon degradation in anthropogenic oil slick

*R. Walter[1], L. Voskuhl[1], L. Willemsen[1]
[1]University of Duisburg-Essen, Environmental Microbiology and Biotechnology, Microbiology of Ecotones, Essen, Germany

Crude oil can naturally seep to the Earth's surface, releasing oil into various ecosystems and forming oil slicks. Although oil is toxic to most life, some bacteria can use oil as carbon source and degrade it. Natural and anthropogenic oil slicks differ in their biological, chemical, and physical properties, yet natural seeps remain understudied from a microbiological perspective. Oil spills contribute largely to global hydrocarbon pollution and natural weathering is often the best choice for oil spill countermeasures in sensitive ecosystems like marshes and coral reefs, where physical cleanup could cause more damage. Accelerating weathering of anthropogenic oil spills before they reach vulnerable habitats could ultimately minimize ecosystem damages.

We hypothesized, that oil degradation is faster in natural oil films compared to recently introduced oil films and that adapted microorganisms, isolated from natural oil seeps, can accelerate anthropogenic oil slick weathering.

To study our hypotheses, water and heavy oil from a natural oil seep in Germany and from oil-unaffected river water were sampled, microorganisms are considered as adapted and naïve, respectively. Microcosm experiments were conducted to compare oil degradation rates between adapted, naïve and mixed microbes, treated with light and heavy oil. Oil biodegradation was monitored over 153 days by pH-measurements, cell counting, CO2-deveolopment by reverse stable isotope labeling (RSIL) and changes in the microbial community composition via 16S rRNA gene amplicon sequencing. A pH decrease was observed for all test conditions and aligned with the amount of produced CO2. Adapted microbes demonstrated up to 5x faster degradation rates of light oil compared to the naïve microbes. In mixed oil incubations, simulating the application of adapted microbes to anthropogenic spills, degradation rates were 0.26 ± 0.01 mM/day with microbes from the adapted water and 0.15 ± 0.02 mM/day in naïve water microcosms, resulting in 58% higher degradation rates.

Natural oil seeps can serve as a valuable source of oil degrading microbial communities that can be used as seedbanks for accelerated hydrocarbon degradation, especially in anthropogenic oil spill scenarios where oxygen is not a limiting factor.

SL-EME-063

Stress response of ISS and school isolates for assessment of survival mechanisms across different habitats

*C. L. Krämer[1,2], F. Arndt[2,3], K. Siems[2], A. L. Boschert[2,3,4], S. Leuko[5]
[1]University of Applied Sciences Bonn-Rhein-Sieg, Rheinbach, Germany
[2]German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne, Germany
[3]University Hospital Cologne, Institute of Medical Microbiology,

Immunology and Hygiene, Cologne, Germany
[4]MVZ Laboratory Dr. Limbach & Colleagues eGbR, Heidelberg, Germany
[5]German Aerospace Center (DLR), Department of Radiation Biology, Cologne, Germany

Microorganisms exposed to space-relevant stressors, such as microgravity, high levels of ionizing radiation, and desiccation, face distinctive survival challenges. These stressors have the potential to induce physiological and genetic adaptations, which can impact microbial behavior, growth rates, and pathogenicity. For example, a compromised immune system in microgravity environments may facilitate certain microorganisms to become more virulent, thereby posing health risks for astronauts on long-duration missions. Furthermore, space radiation can cause DNA damage, potentially driving the evolution of more resilient strains that could influence microbial communities in spacecraft habitats. Understanding these adaptive responses is critical for developing effective microbial control strategies and ensuring the safety of space missions, especially as humanity plans for prolonged space travel and habitation on extraterrestrial surfaces. Moreover, these insights could inform biotechnology applications on Earth, such as the engineering of stress-resistant microorganisms for industrial or medical use.

Within the study "Touching Surfaces", which tested antimicrobial surfaces in space and on Earth, several microbial isolates were retrieved. The isolates will be tested for their tolerance to key environmental stressors relevant to spaceflight. Stress resistance assays involve exposing microbial samples to X-ray doses simulating cosmic radiation, followed by assessment of cell viability. Desiccation resistance is evaluated by drying the samples under controlled conditions and monitoring recovery upon rehydration. Additionally, the susceptibility of the isolates to antibiotics will be tested.

This study focuses on microbial isolates collected in the "Touching Surfaces" project in both confined environments such as the International Space Station, and everyday settings like German schools. The project aims to evaluate whether these distinct environments yield microbial isolates with differing stress resistance profiles. The central hypothesis being tested is whether isolates from confined and extreme habitats display enhanced resistance towards spaceflight relevant stressors compared to those from everyday environments such as schools.

SL-EME-084
Baseline estimates for cell-to-cell interactions in marine and terrestrial habitats
*J. W. Fink[1], Y. M. Bar-On[1], R. Phillips[1], V. J. Orphan[1]
[1]California Institute of Technology, Pasadena, CA, United States

Microbial taxa in the environment do not exist in isolation but are highly connected to other microbial cells in the exchange of nutrients, toxins, and mobile genetic elements. The presence of auxotrophy in many taxa means it is essential that cells can bridge the spatial distances between them, but the exact distances are largely unknown. What is the number of cells that a microbe can realistically expect to interact with in natural environments? Here we use order-of-magnitude calculations to estimate a typical distance between cells, based on empirical estimates of cell abundance for terrestrial and marine habitats. Our estimates reveal that 30%-80% of the global microbial biomass occurs in spatially structured habitats, with expected cell-to-cell distances ranging from 10s of microns in the soil to ~200 microns for planktonic cells in the ocean water column. Despite these short distances, only a small fraction of the molecules secreted by one cell reaches the other cell due to the three-dimensional diffusion of molecules. In a careful comparison, we estimate the spatial distances that cells can bridge purely by the diffusion of nutrients and with additional cell motility. Overall, our work provides an estimate for cell distances that helps to better contrast surface and subsurface habitats and formulate a baseline expectation for the conditions that microbial communities are experiencing in nature.

SL-EME-087
Enhancing cultivable microbial diversity from mouse gut microbiome using droplet microfluidics
*S. Hengoju[1], K. Abdissa[1], S. Boto[1], K. Martin[1], A. Samimi[1], I. Jacobsen[1,2], M. A. Rosenbaum[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Biopilot Plant, Jena, Germany
[2]Friedrich Schiller University, Biology, Jena, Germany

To comprehend the complex dynamics of the gut microbiome and its influence on host health, it is essential to cultivate and isolate gut microorganisms. However, traditional culturing methods often struggle to capture the full diversity of gut microbiota, especially when it comes to rare and slow-growing species (*1*, *2*). In this study, we introduce a droplet microfluidic platform as an efficient and high-throughput approach for cultivating and isolating microorganisms from mouse gut. Miniaturized droplets with volume ranging from few picoliters to nanoliters are generated at kilohertz frequencies targeting confinement of single gut bacteria per droplet (*3*–*5*). Millions of these droplets are incubated in parallel, with homogenous incubation conditions. We cultivated and isolated a diverse range of gut microorganisms from mouse fecal pellets. A comparative analysis with conventional agar plating techniques showed that our droplet cultivation method yielded a greater number of unique isolates, highlighting its superior ability to capture the cultivable fraction of the gut microbiome. Additionally, 16S rRNA amplicon sequencing demonstrated that our system reflects changes in microbial diversity. Droplet microfluidics serves as an innovative solution for expanding the cultivable fraction of the gut microbiota.

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SL-EME-088
Unveiling the carbon fixation potential of marine Prokaryotes using MarMAGs, a novel user-friendly database with over 130 thousand metagenome assemble genomes
*M. Kabiru Nata'ala[1,2], A. P. Avilaa Santos[1], J. C. Kasmanas[1], E. Borchert[1,3], J. Zarzycki[4], N. C. M. Gomes[5], T. Keller-Costa[6], R. Costa[6], M. Perner[3], M. Nikolausz[7,8], S. Kleinsteuber[7,8], I. A. Berg[7,8,9], T. J. Erb[4], H. Harms[1], P. Stadler[2], *U. Nunes da Rocha[7]

[1]Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany
[2]Leipzig University, Department of Computer Science and Interdisciplinary Centre of Bioinformatics, Leipzig, Germany
[3]GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany
[4]Max Planck Institute, Marburg, Germany
[5]Centre for Environmental and Marine Studies (CESAM), Biology, Aveiro, Portugal
[6]University of Lisbon, Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Lisbon, Poland
[7]Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany
[8]Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany
[9]University of Münster, Münster, Germany

Our team designed the MarMAGs, a user-friendly database containing over 130 thousand marine metagenome-assembled genomes (MAGs). Users can access this data from coordinates in a map or by filtering it based on its metadata (e.g., marine biome, biogeographic features, and sequencing platform). Further, we showcased carbon fixation as a use case. We recovered metagenome-assembled genomes (MAGs) from 3405 marine metagenomes selected from MarineMetagenomeDB (https://web.app.ufz.de/marmdb/) and added 52162 MAGs from the OceanDNA dataset and 8558 marine MAGs from the GEM catalog. We evaluated genome quality, taxonomy, and assembly. After metadata harmonization, we identified the genes for prokaryotes' carbon fixation pathways that included the Reductive pentose phosphate cycle (CBB), Reverse Citrate cycle, 3-Hydroxypropionate bicycle (3HPB), Dicarboxylate-hydroxybutyrate cycle (DCHB), Hydroxypropionate Hydroxybutyrate cycle, and Reducive Acetyl-CoA pathway. Due to the complexity of reversible enzymatic reactions and the versatility of the involved enzymes, the Reductive Glycine pathway and reverse oxidative TCA cycle were not annotated in our analysis. We recovered 73,944 novel MAGs that, together with the other two MAG datasets, comprised the MarMAGs with 134,644 MAGs. The MarMAGs contain 31.2%(42047) high-quality and 68.78%(92617) medium-quality MAGs. Bacteria dominate the taxonomic composition of the MAGs at 89.89% (121046), and archaea at 10.11% (13618) spread across 135 phyla. Our analyses revealed 3.96% (5333) of the MAGs with genetic potential for at least one carbon fixation pathway, with CBB comprising 79.75% (4253), while 3HPB 0.02% (1) and DCHB 0.08% (4) have the least of them. Also, 28 MAGs affiliated to 5 phyla have the genetic potential for two pathways. Additionally, we are conducting phylogenetic analyses to explore the relatedness of MAGs based on their genetic capacities for carbon fixation and provide insights into the evolutionary adaptations of marine prokaryotes. The MarMAGs database is a powerful tool for understanding marine prokaryotes' genetic potential. For example, investigating the genetic potential of carbon fixation within MarMAGs provided a detailed view of marine prokaryotes' diverse carbon fixation strategies. Our interactive web platform is a step forward in democratizing access to this valuable genome resource for researchers interested in microbial ecology, carbon cycling, and evolutionary biology in the marine environment.

SL-EME-105

Towards informed antimicrobial use in aquaculture: How fish age, species and climate shape gut microbiome responses to florfenicol and peracetic acid

*L. M. Streb[1], P. Cholewinska[2], S. Gschwendtner[1], J. Geist[3], G. Freitas Pereira de Souza[4], S. Rath[4], M. Schloter[1,5]
[1]Helmholtz Centre Munich, Research Unit Comparative Microbiome Analysis, Munich, Germany
[2]Ludwig Maximilians University, Chair for Fish Diseases and Fisheries Biology, Munich, Germany
[3]Technical University Munich, Chair of Aquatic Systems Biology, Munich, Germany
[4]University of Campinas, Chemistry, Campinas, Brazil
[5]Technical University Munich, Environmental Microbiology, Munich, Germany

Introduction

Antibiotics and antiparasitics are essential for managing infectious diseases in commercial aquaculture, particularly in high-density rearing systems like hatcheries. While antibiotics have been linked to increased abundance of antibiotic resistance genes and persistent shifts in the intestinal microbiome of farmed fish, the influence of underlying host factors (age, species), environmental determinants (climate) and co-application with antiparasitics on microbiome dynamics remains unclear.

Goals

This study focused on the effects of antimicrobial treatment on the gut microbiome of brown trout (*Salmo trutta*) and nile tilapia (*Oreochromis niloticus*), exploring the co-selective pressure of combined florfenicol (FF) and peracetic acid (PAA) on microbial composition and how host age, climate, and species shape the microbiome"s response to antimicrobial exposure.

Methods

In a freshwater recirculating system (9 – 11 °C), adult and juvenile brown trout were subjected to three treatments (10 mg/kg bw FF, FF+PAA, 0.005% PAA) compared to an untreated control group. Adult nile tilapia in a pond system (24 – 28 °C) received 10 mg/kg bw FF. Fecal samples were collected before treatment, on the last day of treatment (day 10) and four times during the post-treatment phase, followed by 16S rRNA gene amplicon sequencing on the Illumina MiSeq platform.

Results

Fish age and species significantly shaped the gut microbiome response to FF and PAA treatment, with juvenile trout showing greater microbiome volatility than adults. Changes included declines in beneficial commensals (*Cetobacterium, Lactococcus*), whose network positions remained altered despite recovered abundance by day 28. FF impacts were enhanced in warmer climates, with shifts persisting up to day 30 and reduced bacterial diversity in tilapia. *Aeromonas*, *Streptococcus* and *Cetobacterium* were identified as common responders following FF treatment.

Summary

Addressing a critical knowledge gap regarding the extent to which the response to antimicrobials is dependent on the developmental stage of the fish and rearing climate, our study emphasizes the importance of integrating gut microbiome characteristics related to fish age and species in aquaculture management practices. This study also provides the first evidence that external PAA application disrupts gut microbial communities, highlighting potential long-term consequences of combined use of antibiotics and antiparasitics.

SL-EME-106

Management of *Lasiodiplodia theobromae*, a mango dieback disease, in the UAE with the application of endophytic actinobacteria

*K. El-Tarabily[1], S. AbuQamar[1], A. Sulaiman[1], S. Alnuaimi[1], N. Alhemeiri[1], S. Alhosani[1], F. Husain[1], B. Mathew[1]
[1]*United Arab Emirates University, Biology, Al-Ain, United Arab Emirates*

Dieback induced by the fungus *Lasiodiplodia theobromae* is a significant affliction in mango farms inside the United Arab Emirates (UAE). This study identified 36 actinobacterial isolates from mango roots in the UAE, with 24 categorized as streptomycetes (SA) and 12 as non-streptomycetes (NSA). Eleven isolates (8 SA and 4 NSA) exhibited antagonistic activity against *L. theobromae*, attributed to the formation of diffusible antifungal metabolites, extracellular cell-wall-degrading enzymes (CWDEs), or both. Employing a "novel" mango fruit bioassay, all isolates were evaluated *in vivo* for their capacity to diminish lesion severity on fruits infected with *L. theobromae*. Three isolates, two from *Streptomyces* and one from *Actinoplanes* spp., had the most potent inhibitory action against this pathogen *in vitro* and were consequently chosen for testing on mango seedlings. Our findings indicated that the antifungal efficacy of *Streptomyces* UAE1 was linked to antibiosis, as well as the synthesis of CWDEs (namely, chitinase) and siderophores; conversely, *Streptomyces* UAE2 and *Actinoplanes* UAE1 were identified as being connected to antibiotic- and CWDE-production, respectively. Pre-inoculation in greenhouse studies with the most promising actinobacterial isolates yielded significant disease protection in mango seedlings later inoculated with the pathogen. The significant decrease in the estimated disease severity indices of mango dieback following individual biocontrol agent (BCA) treatments, in contrast to the pathogen alone, substantiates their efficacy in managing mango dieback disease. Mango seedlings infected with *L. theobromae* and treated with *Streptomyces* UAE1 exhibited a considerable reduction in both the number of defoliated leaves and the conidia counts of *L. theobromae*, decreasing by 3- and 6-fold, respectively, compared to the other two biocontrol agent treatments. This suggests that the synergistic antifungal properties of *Streptomyces* UAE1, employing several mechanisms, impeded the *in planta* invasion of *L. theobromae*. This is the first study on the effects of endophytic BCA against *L. theobromae* in mango seedlings utilizing endophytic microbial antagonists. This is the inaugural report of endophytic actinobacteria naturally present in UAE or elsewhere, demonstrating the capacity to inhibit mango dieback disease.

SL-EME-109

Antimicrobial-resistant *Escherichia coli* in irrigation water of different quality, and leaves of irrigated cilantro and alfalfa plants from the Mezquital Valley, Mexico

*D. Pulami[1], A. Hidri[1], I. M. Axmann[2], G. Bierbaum[2], B. J. Heyde[3], J. Siemens[3], C. Siebe[4], S. P. Glaeser[1]
[1]*Justus Liebig University Giessen, Institut für Angewandte Mikrobiologie, Gießen, Germany*
[2]*University Hospital Bonn, Institut für Medizinische Mikrobiologie, Immunologie und Parasitologie, Bonn, Germany*
[3]*Justus Liebig University Giessen, Institute for Soil Science and Soil Conservation, Gießen, Germany*
[4]*Universidad Nacional Autónoma de México, Instituto de Geología, Mexico City, Mexico*

Background:

Water scarcity has increased the use of wastewater (WW) for irrigation, especially in low/middle-income countries. However, WW irrigation poses risks, such as the spread of antimicrobial-resistant bacteria (ARBs) and pathogens like coliforms into the food chain. Humans are exposed to ARBs through cilantro consumption or indirectly via contact with alfalfa-fed livestock.

Hypothesis and Material/Method:

This study examined the presence of coliform ARBs in irrigation waters, including untreated WW (UWW), treated WW (TWW), groundwater (GW), spring water (SW), and irrigated crops (cilantro, alfalfa) collected in the Mezquital Valley, Mexico. We focused on the cultivation of bacteria colonizing the endosphere or strongly attached to the surface of leaves. We hypothesized that, (I) irrigation water quality influences the spread of coliform ARBs to plants, and (II) plants irrigated with TWW may harbour ARBs with a higher degree of multi-resistance due to potential horizontal gene transfer during WW treatment, compared to those irrigated with UWW, GW and SW.

Result and Conclusion:

Extended spectrum β-lactamase producing (ESBL) and carbapenem-resistant (CR) *E. coli* were cultivated by selective pre-enrichment and subsequent isolation on selective *E. coli* media. *E. coli* were identified by *uidA* PCR and MALDI-TOF MS. A total of 210 ESBL (130 alfalfa, 24 cilantro, 20 UWW, 18 per TWWs) and 40 CR *E. coli* (22 alfalfa, 14 UWW, 4 TWW) were isolated. ESBL/CR *E. coli* were not cultured from GW and SW. So far, WW treatment showed no significant effect on the multi-resistance of *E. coli*. Multi-resistance of *E. coli* isolated from alfalfa and cilantro was slightly lower compared to WW *E. coli* (without significant differences), depending on irrigation water quality or manure application[Office1] . Both, WW derived irrigation water and manure were determined as sources of multi-resistant *E. coli* in the studied crops. The *E. coli* strains will be characterized in more detail by genome sequencing.

SL-EME-134

Effects of metal and salt stress on mycorrhizal interaction

*K. Krause[1], M. Östreicher[1], O. Abdulsalam[1], C. Henke[1], L. Petersen[1], W. Boland[2], E. Kothe[1]
[1]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*
[2]*Max Planck Institute, Jena, Germany*

Mycorrhiza is the mutualistic symbiosis of fungi and plant and known to stabilize plant health. Here, we focus on ectomycorrhiza of shrubs and trees, which is important in times of climate change and forest dieback, especially for reforestation of disturbed and/or contaminated area.

We investigated the ectomycorrhizal interaction of the basidiomycete *Tricholoma vaccinum* with spruce (*Picea abies*) adding different metals and seepage water from a former uranium mining site in Ronneburg, Thuringia, Germany. These treatments were used to mimick metal and salt stress of contaminated habitats, and to study its effect on the exchange of nutrients and signals between both mycorrhizal partners.

Our study was based on growth experiments, followed by expression analyses using RNAseq experiments, the analyses of volatiles by SPME fibers and GC-MS, the investigation phytohormones and their biosynthesis.

Cultivation on metal containing media showed reduced growth of mycorrhizal partners followed by slower mycorrhiza formation. Further the volatile profile of mycorrhized plants grown on medium treated with metal changed, those with mycorrhiza emitted tricyclene, a- and b-pinene, camphene, myrcene, limonene, terpinolene, camphor and bornyl acetate. Expression analyses comparing pure culture of the fungus, mycorrhiza and metal and salt stressed fungus and mycorrhiza revealed strong gene expression changes, e.g., an upregulation of transporters. These changes indicate a reduced plant defense, a rearranged secondary metabolite production, an upregulated water supply and energy production, as well as an increased detoxification in metal and salt stress conditions.

Mycorrhiza known to be beneficial for plant and fungi can be strongly affected by metal and salt stress. This might influence also other interacting organisms. In reforestation strategies with mycorrhizal fungi it has to be considered to select fungal strains showing abilities to manage different stresses occurring in the habitat.

SL-EME-135

Longitudinal variations in soil microbiome diversity and microbial activity in a grassland ecosystem under a climate change scenario

*D. Rosado Porto[1,2], V. Pfaff[1], D. Premalal Senarath[1], S. Ratering[1], C. Orozco Sánchez[1,2], G. Moser[3], M. Deppe[3], C. Müller[3,4], S. Schnell[1]

[1]Justus Liebig University Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany

[2]Simón Bolívar University, Faculty of Basic and Biomedical Sciences, Barranquilla, Colombia

[3]Justus Liebig University Giessen, Institute of Plant Ecology, Gießen, Germany

[4]University College Dublin, School of Biology and Environmental Science and Earth Institute, Dublin, Ireland

Several experiments have assessed how elevated CO_2 (eCO_2) affects the soil microbiome. Nonetheless, there are still gaps in our knowledge of how eCO_2 and warming combined influence soil microorganisms. The soil microbiome's responses to eCO_2 and warming are diverse, highly ecosystem/location specific, and also time dependent. Reductions in microbial biomass, fungal abundance, and shifts in microbial communities have been described.

In this context, this study aims to longitudinally determine the combined effects of eCO_2 and warming on the diversity of the soil microbiome and the microbial activity of a temperate grassland ecosystem.

To achieve the aforementioned, soil samples were taken in 2019, 2021, and 2023 at the Giessen Thermo FACE experiment on a permanent grassland site near Giessen, Germany, which combines eCO_2 and warming alongside a soil water gradient. RNA extraction and 16S rRNA (cDNA) metabarcoding sequencing were performed from bulk and rhizosphere soils, and data were processed with QIIME2 and R software. Physiochemical soil parameters, soil respiration, and greenhouse gas fluxes were determined. Machine Learning (ML) algorithms and Linear Mixed Models (LMM) were created to assess correlations among microbiome diversity, microbial metabolic activity, and experiment environmental conditions.

ML and LMM results demonstrated that soil microbiome and microbial activity are highly time-dependent. However, warming and soil water content are the main drivers of soil microbiome alpha diversity ($p<0.05$), whereas eCO_2 has little influence. Moreover, increments in soil microbial respiration rates positively correlated with increased CO_2 concentrations and elevated temperature ($p<0.05$). Besides, our results showed a negative correlation between alpha diversity and respiration rates. ML applied to taxa selection and correlation identified 30 bacterial ASVs positively associated with warming. Regarding soil greenhouse gas fluxes, LMM results demonstrated that higher CO_2 and N_2O emissions positively correlated with warming and eCO_2. In conclusion, our results indicate that the soil microbiome has been affected under a climate change scenario, mainly driven by warming and soil water content, and to a lesser extent, eCO_2, which was reflected in an increment in soil microbial alpha diversity and microbial activity, accompanied by higher emission levels of greenhouse gases such as CO_2 and N_2O.

SL-EME-137

Soil-on-a-Chip - Deciphering dynamics of root colonization in microfluidic habitats

*C. F. Kaiser[1,2], S. Egli[1,2], M. Keilhammer[1], C. Elizondo[3,4], S. Edrich[1], M. Zupunski[1], I. B. Bischofs[3,4], G. Grossmann[1,2]

[1]Heinrich Heine University, Institute of Cell and Interaction Biology, Düsseldorf, Germany

[2]Heinrich Heine University, Cluster of Excellence on Plant Science, Cologne, Germany

[3]University of Heidelberg, Complex Adaptive Traits (CATs) in beneficial Bacilli, Heidelberg, Germany

[4]Max Planck Institute, Complex Adaptive Traits, Marburg, Germany

Plant roots establish symbiotic interactions with soil microbes, enhancing nutrient uptake, pathogen resistance, and resilience to stress. To unravel the regulatory mechanisms behind these relationships, we study the interactions between *Arabidopsis thaliana* roots and *Bacillus* species, known for their growth-promoting and immunomodulatory effects.

We focus on the role of cell type differentiation in *Bacillus subtilis* during root colonization, hypothesizing that these traits support adaptive strategies under plant immune pressure, similarly to their pivotal role in biofilm formation and mainentance. By combining transcriptional reporter strains of *B. subtilis*, ROS-sensitive dyes, and immunity markers in *A. thaliana*, we visualize the spatiotemporal dynamics of colonization, assessing immune interference, ROS production, and bacterial cell state.

Adapted microfluidic RootChips allow dynamic, highly resolved and minimally invasive observations of the root microenvironments, enabling controlled studies of signaling processes. Additionally, structured microdevices simulating soil architecture let us dissect each phase of *B. subtilis* colonization and track the activation and modulation of plant immunity. This approach highlights the interference of plant defense and cell type differentiation in structuring root colonization by *Bacillus subtilis*.

SL-EME-138

Rhizomicrobiome of terra preta soil manipulates grapevine chemotype, resistance against trunk diseases, and endophytic community structure

*I. Khattab[1], A. K. Kaster[2], P. Nick[1]

[1]Karlsruhe Institute of Technology (KIT), Joseph Gottlieb Kölreuter Institute for Plant Sciences, Karlsruhe, Germany

[2]Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces 5 (IBG 5), Eggenstein-Leopoldshafen, Germany

Similar to human gut enterotypes, soil microbiota might harbour beneficial microorganisms maintaining plant health and resilience against environmental challenges. We searched for soil microorganisms promoting the grapevines resilience against trunk disease (GTD) which is one of the most destructive diseases for viticulture worldwide. We found that mixing the soil of the vineyard with compost and biochar promoted the plant defence mechanisms and controlled the disease development. Rhizomicrobiome and plant responses were studied using multi-omics techniques, e.g. shotgun metagenome sequencing as well as targeted and untargeted metabolomics. By reconstructing the bacterial genomes, mixed soil showed higher abundance of many bacterial taxa, e.g. g_Niastella, g_Chryseolinea of p_Bacteroidota with remarkable pathways, e.g. ubiquinone, iso-chorismate and chitinase synthesis pathways. Such pathways could promote the defence mechanisms in grapevines against biotic stress. Metabolomics analysis further revealed that mixing soil with compost and biochar influenced the chemotype of roots tissues. In grapevine roots grown in standard soil, GTD infection corresponded with higher levels of organic acids and sugar derivatives. On the other hand, compost and biochar significantly reduced sugar and organic acid concentrations in roots while increasing the abundance of twenty aromatic and phenolic compounds with antioxidant properties, such as stilbenes and flavonoids. Some bioactive stilbenes members such as resveratrol, piceatannol, and 2-Hydroxyiminostilbenes, which were significantly prevalent in plants of mixed soil, were classified as grapevine resistance factors against trunk diseases.

SL-EME-139

Positive interactions between plant-beneficial inoculants and members of the resident soil microbiome improve plant performance under stress

*I. Kampouris[1], J. H. Behr[2], L. Sommermann[3], D. Francioli[4], N. Naziri[1], L. O. Schulz[1], T. Kuhl-Nagel[2], S. Paul Chowdhury[5], N. Moradtalab[4], R. Zrenner[2], J. Geistlinger[3], U. Ludewig[4], G. Neumann[4], K. Smalla[1], R. Grosch[2], D. Babin[1]
[1]Julius Kühn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany
[2]Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Plant-Microbe Systems, Großbeeren, Germany
[3]Anhalt University of Applied Sciences, Department of Agriculture, Ecotrophology and Landscape Development, Bernburg, Germany
[4]University of Hohenheim, Institute of Crop Science (340h), Stuttgart, Germany
[5]Helmholtz Centre Munich, Network Biology, Munich, Germany

Inoculants containing plant-beneficial microorganisms (BMs) can assist plants in BM recruitment from the soil microbiome. BMs can improve plant growth, especially under stress. We hypothesized that stress disrupts BM recruitment from soil, which can be restored by BM inoculation. We performed drench-inoculation of field-grown maize in two consecutive years with a BM consortium consisting of *Bacillus atrophaeus* ABi03, *Pseudomonas* sp. RU47 and *Trichoderma harzianium* OMG16. Sampling five weeks after inoculation showed that the consortium increased plant biomass and iron uptake in 2020 during severe drought compared to non-inoculated control plants, but not in 2021 with average precipitation. Also, the consortium-dependent modulation of the resident rhizosphere microbiome, based on 16S rRNA gene and ITS amplicon sequencing, depended on the year. Several ASVs classified as *Comamonadaceae* spp. significantly increased in the rhizosphere due to BMc inoculation in 2020, but not in 2021, and were predictive for iron uptake. This suggests that plant-BMs improved plant growth only during stress exposure due to the recruitment of soil resident BMs. To test this second hypothesis, we compared the 16S rRNA gene of these *Comamonadaceae* ASVs to a bacterial strain collection from the rhizosphere of maize grown in the same field. The alignment showed that several isolates matched the sequences of ASVs enriched by the consortium inoculation. These isolates were classified as *Variovorax* spp. and *in vitro* functional characterization revealed their stress mitigation capacity due to degradation of 1-aminocyclopropane-1-carboxylic acid. *In vitro* co-cultivation assays showed that the consortium members boosted the biomass of several *Variovorax* isolates. Moreover, the inoculation of the consortium complemented with *Variovorax,* as representative of the resident soil microbiome, resulted in higher plant growth under drought in the greenhouse compared to the consortium alone. Consequently, our study indicates that positive interactions between inoculants and the resident microbiome are crucial for plant performance.

SL-EME-002

Fluid flow generates bacterial conjugation hotspots by increasing the rate of shear-driven cell-cell encounters

*M. Zbinden[1], J. Huisman[2], R. Stocker[1], J. Słomka[1]
[1]ETH Zurich, D-BAUG, Zurich, Switzerland
[2]Massachusetts Institute of Technology, Department of Physics, Cambridge, MA, United States

Conjugation accelerates bacterial evolution by enabling bacteria to acquire genes horizontally from their neighbors. Because plasmid donors must physically connect with recipients to allow plasmid transfer, environmental fluid flows may increase conjugation rates by increasing cell-cell encounters through mixing driven by fluid shear, which creates relative movement between donors and recipients. However, existing experimental assays do not directly control cell-cell encounters, which hinders the establishment of a connection between the population-level conjugation rate and the microscale mechanisms that bring cells together. Here, we performed conjugation experiments between E. coli bacteria, varying the shear flow to control the rate of cell-cell encounters. We discovered that the conjugation rate increases with shear until it peaks at an optimal shear rate of ≈ 100/s, reaching a value five-fold higher than the baseline set by diffusion-driven encounters. This optimum marks the transition from a regime in which shear promotes conjugation by increasing the rate of cell-cell encounters to a regime in which shear disrupts conjugation. Fluid flows are widely present in aquatic systems, gut, and soil, and our results indicate that fluid shear could induce hotspots of bacterial conjugation in the environment.

Microbial communities

SL-MC-057

Inside-out: Remodeling of gonococcal microcolonies due to changing pili interaction network

*S. Wimmi[1], I. Wielert[1], K. Zhou[2], M. Hennes[1], B. Sabass[2], B. Maier[1]
[1]University of Cologne, Biological Physics, Cologne, Germany
[2]Ludwig Maximilians University, Faculty of Veterinary Medicine and Faculty of Physics, Munich, Germany

Neisseria gonorrhoeae, the causative agent of one of the most common sexually transmitted diseases in humans, rapidly aggregates from single cells to microcolonies with thousands of cells. This process is facilitated by its type 4 pili (T4P). Driven by a tug-of-war between the T4P of neighboring cells, the bacteria are motile within the

microcolony and from an interaction network. In the colony center this motility is highly restricted.

In this study, we investigate how changes in the T4P interaction network lead to a global reconstruction of the whole microcolony and the dispersal of cells.

To do so, we combine flow chamber-based microscopy and single-cell tracking with an agent-based dissipative-particle-dynamics model that allows the simulation and prediction of bacterial microcolony behaviour. In addition, we apply a direct cysteine-maleimide-based pili visualization protocol to investigate the piliation level of single cells with fluorescent microscopy.

We show that after several hours of microcolony maturation, the inner motility-restricted core can become motile again. Like in a jet, highly motile cells flow from the center to the periphery and cause an eversion of the entire colony from the inside to the outside. Theoretical modeling predicts such spatio-temporal dynamics when the cell-cell interactions in the core are lower than interactions in the outer envelope of the colony. We argue that reduced growth resources in the center of the colony could result in fewer interactions between the cells. Indeed, direct visualization of T4P, shows that piliation is decreased in response to oxygen limitation, resulting in reduced T4P-mediated cell-cell interaction. Additionally, we find that dead cells that have accumulated in the center of the colony are expelled during the eversion and that large amounts of live and dead cells are shed throughout the eversion process into the environment.

Overall, this study shows how small changes in the active pili interaction network of microcolonies can trigger global colony remodeling and the dispersal of cells.

SL-MC-058

The periodontal pathogen *Tannerella forsythia* thrives in the oral microbial community by means of peptidoglycan salvage

M. Borisova-Mayer[1], I. Hottmann[1], T. Stehle[2], G. Zocher[2], *C. Mayer[1]
[1]*Eberhard Karls University Tübingen, IMIT - Organismic Interactions/Microbial Glycobiology, Tübingen, Germany*
[2]*Eberhard Karls University Tübingen, IFIB - Biochemistry II, Tübingen, Germany*

Periodontitis is an inflammatory disease leading to the destruction of the teeth-supporting tissue, ultimately causing tooth loss. *Tannerella forsythia,* an anaerobic, Gram-negative oral pathogen of the Bacteroidota phylum, is part of the red complex consortium of periodontal pathogens, strongly associated with the late stages of periodontitis progression. *T. forsythia* critically depends on co-habiting bacteria in the oral biofilm for the provision of nutrients [1]. Earlier axenic culturing studies revealed that the organism is auxotroph for *N*-acetylmuramic acid (MurNAc), which is an essential component of the bacterial peptidoglycan (PGN) cell walls. Later, whole genome sequencing revealed that the bacterium lacks the generally essential genes encoding the canonical MurA/MurB enzymes, required for the *de novo* synthesis of PGN precursors, thus rationalizing MurNAc auxotrophy. We showed that *T. forsythia* accepts soluble PGN-derived fragments as well as polymeric PGN as sources of exogenous MurNAc [2]. We discovered two, presumably periplasmic, unique exolytic β-*N*-acetylmuramidases (NamZ1 and NamZ2), which cleave exogenous PGN glycan sugar strands at the non-reducing ends, generating *N*-acetylglucosamine (GlcNAc)-MurNAc disaccharides and MurNAc, respectively [3]. Further, we identified two inner membrane transporters: AmpG, importing disaccharides generated by NamZ1 [3] and MurT, importing MurNAc generated by NamZ2 [4]. Metabolism of these sugars involves two intracellular MurNAc kinases, that were characterized and their crystal structures solved [5,6]. Our study unraveled a complex PGN salvage metabolism that is crucial for the oral pathogen *T. forsythia* to survive within the oral microbial community.

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SL-MC-059

Sequencing of the human lung microbiome – Methodical challenges, controls and first metagenome assembled genomes from a nearly sterile habitat

*L. Möller[1], J. Petersen[1], S. Huang[1], F. Burkart[1], A. Methner[1], C. Herzmann[2], K. I. Gaede[2], S. Krauss-Etschmann[2], M. Jarek[3], H. Gosh[1,4], W. Behrens[1], J. Sikorski[1], H. Brinkmann[1], B. Bunk[1], J. Overmann[1,5]
[1]*Leibniz Institute DSMZ, Microbial Ecology and Diversity, Braunschweig, Germany*
[2]*Research Center Borstel Leibniz Lung Center, Borstel, Germany*
[3]*Helmholtz Centre for Infection Research – HZI, Braunschweig, Germany*
[4]*University of Freiburg, Institute for Infection Prevention and Hospital Epidemiology, Freiburg i.Br., Germany*
[5]*Technical University of Braunschweig, Microbiology, Braunschweig, Germany*

Question: Microbial populations are usually present within many sites of the human body and in the past, it became increasingly clear that they play key roles in maintaining health, as well as causing diseases, when its natural composition is disrupted. While the healthy lung was considered traditionally sterile, an increasing number of studies, culture dependent and independent, indicate otherwise. A better understanding of the lung microbiome is therefore needed to assess its potential role in disease development and prevention. However, due to the low bacterial biomass nature of the lung, molecular research has remained challenging and to date was mostly restricted on 16S rRNA-gene amplicon analysis. In our study, we aimed to overcome these limitations and conduct a first metagenomic analysis of bacterial communities of the human lung.

Methods: By performing bronchoscopy on healthy individuals of smokers and non-smokers, bronchoalveolar lavage was collected and subjected to metagenomic analysis. To enable bacterial metagenomic analyses, samples had to be subjected to extensive clean up procedures, depleting human background DNA, while preserving the original microbial community.

Results & Conclusions: In the end, a robust method could be developed to create the first metagenome assembled

genomes from this habitat characterized by very low microbial abundances. This represents a key step forward in understanding of the human lung microbiome and provides the baseline for future microbiome analyses.

SL-MC-062

Race for iron: An exploiter's perspective on siderophore-mediated networking in bacterial communities

*A. Krüger[1], S. H. Paik[2], M. Bund[1], J. Wiechert[1], T. Drepper[2], D. Kohlheyer[1], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*
[2]*Heinrich Heine University, IMET: Institute of Molecular Enzyme Technology, Düsseldorf, Germany*

The trace element iron is a physiological requirement of almost every living organism as it plays important roles in numerous biological processes. Nevertheless, due to the extreme low solubility of Fe^{3+} in aerobic environments, microbes need to employ sophisticated strategies to manage homeostasis of this valuable resource. One strategy is the production and secretion of Fe^{3+}-complexing siderophores. Within this project, we assess competitive and cooperative strategies for the acquisition of iron through siderophores as public goods in defined microbial communities.

Corynebacterial species have been reported as frequently occurring natural siderophore exploiter in natural microbiomes, such as the nasal microbiome (1). Within this project, we investigate *Corynebacterium glutamicum* as model to explore the exploiter"s perspective on siderophore-mediated networking. To establish defined microbial communities, we screened for different siderophore producers allowing *C. glutamicum* to overcome iron-limitation. These result indicated that *C. glutamicum* is capable of utilizing a chemically diverse set of xenosiderophores including *Escherichia coli* enterobactin, *Bacillus subtilis* bacillibactin and *Ustilago maydis* ferrichrome. Adaptive laboratory evolution experiments with *C. glutamicum* and spent medium from different siderophore producers resulted in the isolation of *C. glutamicum* strains with superior xenosiderophore utilization capabilities. Establishment of synthetic co-cultures grown in microfluidic devices confirmed the dependency of *C. glutamicum* on enterobactin production of *E. coli* under iron limiting conditions. With this set-ups we are able to examine the impact of iron management strategies on the structure and dynamics of microbial communities, uncovering spatial variations and dynamics.

1. Zhao et al. (2024): *The ISME Journal*.

SL-MC-064

Sterol-degrading actinobacteria function as an unexpected sterol source for sterol-auxotrophic *Caenorhabditis* nematodes

C. Kaiser[1], M. Hörnschemeyer[1], D. Schmidt[1], B. Philipp[1,2], E. Liebau[3], *J. Holert[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*
[2]*Fraunhofer-Gesellschaft, Environmental Microbiology, Schmallenberg, Germany*
[3]*University of Münster, Institute of Integrative Cell Biology and Physiology, Münster, Germany*

During evolution the nematode *Caenorhabditis elegans* has lost the ability to synthesize steroids *de novo*, making its survival dependent on exogenous sterols for reproduction and life stage development. Strikingly, *C. elegans* feeds almost exclusively on bacteria, which typically do not synthesize steroids, raising the question of how *C. elegans* acquires sterols in nature. We recently found that sterol-degrading *Rhodococcus* and *Mycobacterium* bacteria accumulate sterols in intracellular lipid droplets under stress conditions such as nitrogen limitation and hypothesized that these bacteria may constitute an important sterol source for *C. elegans* in oligotrophic habitats.

To test this hypothesis, we compared *C. elegans* brood sizes with different sterol-accumulating and sterol-free bacteria as food sources on sterol-free medium. While *C. elegans* was not able to produce offspring with sterol-free *E. coli*, *M. smegmatis* or *M. aurum,* around 250 offspring were produced per worm when *M. smegmatis* and *M. aurum* had accumulated intracellular sterols or with sterol-free bacteria when cholesterol was supplied in the growth medium. To differentiate bacteria- and medium-derived sterol uptake in *C. elegans*, we performed feeding assays with fluorescently labelled cholesterol. Confocal microscopy showed that bacteria-derived cholesterol was taken up via the pharynx into the gut and across the gut wall. In contrast, medium-derived cholesterol localized primarily in the nerve ring and sensory dendrites but was almost absent from the intestine, indicating differences in sterol uptake and transport. Feeding assays with *C. elegans* sterol uptake mutants showed that the intestinal cholesterol transporter CHUP-1 was required for uptake of bacteria-derived cholesterol into the worm body, while deletion of the sphingomyelin synthase SMS-5, which is involved in cholesterol absorption in the pharynx, had no significant effect. Proteomic analyses are currently underway to further investigate different physiological responses in *C. elegans* to bacteria- and medium-derived sterols.

Our results show that the sterol-auxotrophic *C. elegans* can use intracellular bacterial sterol storage compounds to maintain its reproductive cycle, suggesting that sterol-degrading actinobacteria represent a natural and previously unstudied sterol source for bacterivorous animals in oligotrophic habitats. This opens a new field of research into sterol-dependent regulation and developmental processes in *C. elegans*

SL-MC-104

Collateral damage of antibiotic eradication targeting nasal Staphylococcus aureus requires alternative decolonization strategies

*D. Gerlach[1], S. Heilbronner[1]
[1]*Ludwig Maximilians University, Biology, Martinsried, Germany*

Question: *Staphylococcus aureus* is a major human pathogen which causes a wide variety of infections. *S. aureus* colonizes approximately the nares of 20% of the human population. Decolonization treatments prior to invasive medical inventions lead to a reduced risk in subsequent infections. The proven method for decolonization of *S. aureus* is a treatment with the antibiotic mupirocin, which leads to problems on the long run. Firstly *S. aureus* recolonizes usually after the treatment has stopped. Secondly antibiotic resistance to mupirocin in *S. aureus* is emerging. In addition, S*. aureus* is part of a complex microbiome in the human nares. Other bacterial species have promoting or suppressive effects on *S. aureus* nasal colonization. The effect of mupirocin treatment on the nasal microbiome is poorly understood on the species and strain

level. In this study we determine the global effect of nasal decolonization strategies on the nasal microbiome. Furthermore, we propose potential alternative methods with increased precision and selectivity.

Methods: We used a combination of *in vitro* techniques and *in silico* prediction to determine antibiotic susceptibility and colonization dynamics upon mupirocin treatment of a comprehensive collection of nasal commensals. Furthermore, we validated our results with *in vivo* colonization data.

Results: We show that presence of certain alleles of the mupirocin target explains antibiotic resistance across a wide variety of nasal commensals from very diverse phylogenetic clades. Presence of identified resistance determinants governs growth in a competitive *in vitro* system. Observed microbiome disturbances caused by mupirocin treatment correlate well with *in vivo* colonization data.

Conclusion: We show that mupirocin treatment has notable collateral side effects on nasal commensals perturbing the composition of nasal microbiomes. Side effects are especially pronounced on other- often harmless - commensal staphylococci, which are frequently antagonizing *S. aureus* colonization. Hence, we propose alternative antimicrobial approaches targeting molecular structures which are unique to pathogenic commensals. A promising alternative could be bacteriophages, microbial viruses. In contrast to frequently used antibiotics bacteriophages target distinct surface structures that are unique to *S. aureus* allowing potentially selective and controlled eradication of this pathogens from the human nose.

SL-MC-107
Cross-kingdom interactions of *Ustilago* and *Pseudomonas* via secondary metabolites
*P. Künzel[1], N. Wierckx[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

Many fungal strains in the family Ustilaginaceae secrete itaconic acid (ITA) along with its derivatives, 2-hydroxyparaconate (2-HP) and itatartarate (ITT) under nitrogen limitation. While the antimicrobial properties of ITA are well documented, the ecological role of its derivatives remains largely unexplored. Notably, the bacterium *Pseudomonas aeruginosa* possesses a specialized ITA metabolic operon with genes not directly related to ITA degradation (PA0879-PA0881), but essential for tolerance to the ITA derivatives, suggesting a molecular arms race within their shared habitat (de Witt et al., 2023). This study aims to investigate how fungal secondary metabolites shape cross-kingdom communities and elucidate their effects on bacterial metabolism and tolerance, using the non-pathogenic strain *P. putida* KT2440, engineered to heterologously express the ITA metabolic operon from *P. aeruginosa* PAO1. Inhibition studies using *Ustilago* supernatants and purified 2-HP and ITT on different engineered *Pseudomonas* strains identified the uncharacterized ring-cleaving dioxygenase Rdo_{PA} (PA0880) as a key mediator of tolerance to ITA derivatives. Interestingly, the ITA synthesis clusters of several fungal strains, such as *Ustilago maydis* and *Aspergillus terreus*, contain genes with high sequence similarity to rdo_{PA} (UMAG_12299, *rdo1*; ATEG_10557, *rdoA*). Complementation studies and biochemical characterization confirm that these evolutionarily distant enzymes share lactonase activity for the enantioselective conversion of (S)-2-HP to (S)-ITT. Combined with the finding that (S)-2-HP inhibits ITA degradation, this conversion clarifies Rdo_{PA}'s physiological role in *Pseudomonas*, while its role in fungi, remains unclear. Preliminary results with eGfp-tagged Rdo variants in *U. maydis* revealed its localization to the mitochondria, and we currently hypothesize a self-protection mechanism against the ITA derivatives, potentially due to toxic side activities of mitochondrial enzymes. With a clearer understanding of the mode of action and tolerance mechanisms, future studies will employ rational genetic modifications to fine-tune fungal production and bacterial degradation of ITA and its derivatives, incorporating transporters to regulate metabolic flux (battle of rates). These targeted modifications aim to mimic the dynamic evolutionary interplay within the ecological niche in a synthetic co-culture, advancing our understanding about the rules of interaction.

SL-MC-136
The soil-borne fungus *Verticillium dahliae* differentially employs molecular tools to establish interactions with phylogenetically distant terrestrial microalgae
*H. Rövenich[1], A. Kraege[1], E. Schnell[1], H. Haeweker[1], O. Nielsen[1], E. Chavarro-Carrero[1], B. Thomma[1,2]
[1]*University of Cologne, Institute of Plant Sciences, Cologne, Germany*
[2]*Cluster of Excellence on Plant Sciences, Cologne, Germany*

The ubiquitous soil-borne fungus *Verticillium dahliae* is a notorious pathogen of hundreds of vascular plant species. In absence of a suitable plant host, *V. dahliae* resides in the soil where it encounters, and interacts with, a rich soil microbial community. In addition to bacteria and other fungi, this soil microbial community contains significant numbers of unicellular microalgae. Considering the capacity of *V. dahliae* to interact with plant hosts, we set out to test if this fungus evolved strategies to interact with microalgae. To this end, we co-cultured *V. dahliae* with phylogenetically diverse terrestrial microalgal species, showing that it engages in stable interactions with microalgae, ranging from beneficial to detrimental depending on the algal lineage. These differential interactions are characterized by close physical associations, often resulting in the formation of interspecies biofilms. Transcriptomic analyses showed that *V. dahliae* mounts fundamentally different transcriptional programs, which depend on the microalgal partner. These findings show that the plant pathogenic fungus *V. dahliae* has the capability to establish symbiotic interactions with extremely divergent plant lineages through different lifestyles, ranging from mutualistic to pathogenic, and readily adapts to these different host niches by utilizing host-specific molecular toolkits.

Microbial diversity and evolution

SL-MDE-156
Enrichment of a parasitic member of *Cand*. Gracilibacteria along with its host from groundwater
*M. Herrmann[1,2], J. Bach[1], C. E. Wegner[3], M. Taubert[4,5], M. Westermann[6], M. A. Rahman[4], K. Küsel[1,2,5]
[1]*Friedrich Schiller University, Aquatic Geomicrobiology, Jena, Germany*
[2]*German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany*
[3]*Heinrich Heine University, Bioinorganic Chemistry, Düsseldorf, Germany*
[4]*Friedrich Schiller University, Institute of Biodiversity, Aquatic Geomicrobiology, Jena, Germany*
[5]*Friedrich Schiller University, Cluster of Excellence Balance of the*

Microverse, Jena, Germany
[6]University Hospital Jena, Electron microscopic center, Jena, Germany

The Candidate Phyla Radiation (CPR) superphylum harbors about 25% of the total bacterial diversity. The lifestyle and ecological role of its members are still poorly understood due to the lack of cultured representatives. We obtained a stable enrichment culture of a member of the *Cand*. Gracilibacteria (BD1-5) along with its host *Azonexus* sp. from groundwater of a sandstone aquifer. *Cand*. Gracilibacteria is a sister phylum of the *Cand*. Absconditabacteria, which is associated with a parasitic, host-associated lifestyle, raising the question if parasitism is a widespread trait among these lineages of the CPR. The culture was grown under anoxic, nitrate-reducing conditions with hydrogen or acetate as an electron donor. Each partner grew up to a density of about 10^8 cells per ml, together accounting for 50 to 95% of the total community. Scanning electron microscopy revealed that Gracilibacteria strain FG1 is about 0.4 µm in size and lives epibiotically on its host. Observations of host cell degeneration suggest a parasitic nature of the interaction. The parasite divides by forming stacks of up to eight cells attached perpendicular to the host cell which might also play an important role in the infection process. When transferred to a fresh host culture, Gracilibacteria strain FG1 showed a rapid infection and growth response, resulting in a 50 times increase in cell number within three days. Metagenomic analysis revealed that strain Gracilibacteria FG1 lacks complete pathways for amino acid biosynthesis and central carbon metabolism, indicating strong dependence on the host cell for basic metabolism and cell division. The presence of genes encoding proteins related to type IV pili and type II secretion systems pointed to mechanisms of establishing cell-to-cell interactions. Relative abundances of Gracilibacteria strain FG1 and *Azonexus* sp. in the in situ groundwater community varied from 0.01 – 36% and 0.4 - 18%, respectively, over a 4-years observation period, suggesting a high relevance of this parasitic interaction for groundwater community dynamics. Our findings expand previous observations of a parasitic lifestyle of *Cand*. Absconditabacteria to the sister phylum *Cand*. Gracilibacteria, suggesting that cell sizes larger than 0.2 µm, stack formation resulting from host-attached cell division, and fatal parasitism are a typical feature of these groups, contrasting with smaller cell sizes and potentially non-predatory lifestyles of other CPR lineages.

SL-MDE-157

BioMetArchive: Geomicrobiological insights into the 1 Million-Year sedimentary record of ferruginous lake Towuti

*F. Ruiz Blas[1], J. Kallmeyer[1], C. Henny[2], J. M. Russell[3], H. Vogel[4], A. Vuillemin[1]
[1]GFZ German Research Centre for Geosciences, 3.6 Geomicrobiology, Potsdam, Germany
[2]Research Center for Limnology and Water Resources, National Research and Innovation Agency (BRIN), Jawa Barat, Indonesia
[3]Brown University, Department of Earth, Environmental, and Planetary Sciences, Providence, RI, United States
[4]Institute of Geological Sciences and Oeschger Centre for Climate Change Research, University of Bern, Bern, Switzerland

Lake Towuti, Indonesia is a permanently stratified lake influenced by tropical weathering of an ultramafic catchment. This results in high iron but minimal sulfate inflows into the basin, creating anoxic ferruginous conditions in its bottom waters (>130 m). The International Continental Scientific Drilling Program (ICDP) retrieved a 100-m-long sediment core covering 1 million years of sedimentation history. Through taxonomic and metagenomic analyses, complemented with pre-existing environmental and geochemical datasets, the project *BioMetArchive* provides a comprehensive characterization of the lacustrine subsurface biosphere in terms of diversity, abundance, and metabolic functions.

As electron acceptors in the pore water became depleted within the upper 0.5 m below lake floor (mblf), cell counts decreased from 109 to 104 cells cm-3, concurrent to a shift in microbiome composition leading to a predominance of Bathyarchaeia. Metabolic features attributed to this class include sulfur transformation and (homo)acetogenic fermentation. Taxonomic profiling of 16S rRNA genes revealed a drastic decrease in microbial diversity inherent to substrate depletion during shallow burial. In contrast, increased compositional variability in sulfate-reducing bacterial populations was observed around tephra layers and diatom oozes, indicative of volcanic eruptions and primary productivity peaks, respectively. Microbial alpha and beta diversity also varied according to the different lithologies of stratigraphic units. In the upper subunit (20 mblf), an active and diverse microbial community involved in OM remineralization is driving effective turnover of volatile fatty acids and other solutes in the pore water. The second subunit (20-70 mblf) harbors a deep biosphere community adapted to a nutrient-depleted environment. In contrast, the lowermost subunit (70-100 mblf) is characterized by increased microbial diversity.

Thus, variations in 16S rRNA gene assemblages show that microbial diversity and composition are closely linked to the depositional history of Lake Towuti. We conclude that dynamic shifts in depositional conditions during the Late Pleistocene are tractable in the taxonomic and functional diversity of the subsurface biosphere. With increasing burial depth, sediment substrate depletion actively selects for acetogenic Bathyarchaeia, highlighting their capability to persist as main constituents of the deep subsurface microbial biosphere.

SL-MDE-158

New insights into the spacer diversity of CRISPR-Cas systems in natural environments

*K. Sures[1], S. P. Esser[1], T. Bornemann[1,2], C. J. Moore[1], A. Soares[1,2], J. Plewka[1], P. A. Figueroa-Gonzalez[1], S. E. Ruff[3,4], C. Moraru[1], A. Probst[1,2,5,6]
[1]University of Duisburg-Essen, Research Center One Health Ruhr, Essen, Germany
[2]University of Duisburg-Essen, Center of Water and Environmental Research, Essen, Germany
[3]Marine Biological Laboratory, The Ecosystems Center, Woods Hole, MA, United States
[4]Marine Biological Laboratory, Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Woods Hole, MA, United States
[5]University of Duisburg-Essen, Center of Medical Biotechnology, Essen, Germany
[6]Lawrence Berkeley National Laboratory, DOE Joint Genome Institute, Berkeley, CA, United States

CRISPR (clustered regularly interspaced short palindromic repeat) arrays and *cas* (CRISPR-associated) genes of prokaryotes form a unique immune system that confers adaptive immunity against invading mobile genetic elements (MGEs), such as viruses [1] and plasmids [2]. CRISPR-Cas systems contain short sequences, the so-called spacer sequences, that are incorporated into the CRISPR array following an invasion of a foreign nucleic acid [1]. Spacer sequences serve as a memory of previous infections, thereby allowing for sequence-specific targeting of the MGE

[3]. In addition, recent studies showed that CRISPR-Cas systems have potential regulatory functions [4] and mediate immunity of certain archaea against their archaeal episymbionts [5]. Despite all the mechanisms of spacer acquisition described so far, a matching protospacer can only be detected for a small proportion of all spacers in genomic databases [6]. Therefore, we tested the hypothesis that spacers originate from chromosomal DNA of prokaryotic genomes from the same environment. Using metagenomics, we analyzed the CRISPR-Cas systems of metagenome-assembled genomes from two subsurface environments. Spacer-to-protospacer matching revealed that spacers were frequently acquired from chromosomal DNA of different prokaryotes inhabiting the same environment. Further, our results suggest that the spacer acquisition from prokaryotic DNA occurs in almost all types of CRISPR-Cas systems. Analyses of the host genomes revealed a frequent presence of genes encoding proteins potentially involved in the uptake or breakdown of environmental DNA. Based on these findings, we propose that DNA uptake by the cell facilitates the acquisition of spacers from prokaryotic DNA, contributing to the diversity of spacers found in CRISPR arrays from natural environments. Taken together, our results provide a possible explanation for the origin of some of the many unmatched spacers in public databases.

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SL-MDE-159

Uncovering the evolutionary pathways of siderophores in bacteria

*B. Pourmohsenin[1], Z. Reitz[2], M. Medema[1,2], N. Ziemert[1]

[1]Eberhard Karls University Tübingen, IMIT - Organismic Interactions/Microbial Glycobiology, Tübingen, Germany

[2]Wageningen University, Bioinformatics Group, Wageningen, Netherlands

Siderophores are small organic molecules that bind and transport iron. These natural products have applications in medicine, agriculture, and environmental sciences. Bacteria are prominent producers of siderophores, which are essential for scavenging iron from their environment. Like other specialized metabolites, siderophore production is encoded within Biosynthetic Gene Clusters (BGCs). Studying siderophore-encoding BGCs leads to understanding their distribution and evolution, which is valuable for scientific research and design of novel molecules with desired properties.

To investigate the distribution and evolution of siderophore biosynthesis within the bacterial kingdom, we created an extensive database of experimentally validated siderophore BGCs. In addition, we are examining siderophore transport systems and their evolution across bacterial species to explore their potential use in developing Trojan horse drugs. By exploiting these transport pathways, it may be possible to deliver antimicrobial agents selectively to pathogenic bacteria.

Using Hidden Markov models, we conducted a comprehensive search to identify all potential siderophore producers within the GTDB tree of bacteria. We then performed a phylogenetic analysis on core genes involved in the biosynthesis of iron-chelating moieties. By reconciling the gene trees with their corresponding species tree, we traced evolutionary events—such as gene duplication, gene loss, and horizontal gene transfer—that have shaped their current distribution.

Our findings suggest that hydroxamates are likely the most ancient chelator group, with at least three distinct origins, predominantly found in Pseudomonadota and Actinobacteriota. Catecholates, also rather ancient, likely have a single origin in Actinobacteriota, with transfers to Pseudomonadota and Firmicutes. In contrast, pyoverdines appear to be the most recent siderophore pathway, found exclusively in Pseudomonads. Preliminary analysis of siderophore transport systems reveals conserved mechanisms that may be adapted for targeted drug delivery.

These insights into the evolutionary history of siderophores provide a foundation for future research, including the development of feasible mix-and-match methods and the selection of optimal heterologous hosts. Furthermore, understanding siderophore transport systems could inform the design of novel therapeutic approaches to deliver antimicrobial agents selectively to pathogens via Trojan horse strategies.

SL-MDE-160

Novel insights into the lipid divide – bacterial enzymes can synthesize lipids with archaeal lipid-backbone stereospecificity

*P. Rieche[1], M. Exterkate[1]

[1]Heinrich Heine University, Membrane Biogenesis and Lipidomics, Düsseldorf, Germany

One of the key events in early cellular evolution is the separation of the last universal common ancestor (LUCA) into Archaea and Bacteria. Although both are prokaryotes, these two domains of life differ in many ways, including the architecture of their lipid membranes. The textbook teaches us bacterial lipids exist of fatty acid lipid tails, that are ester-linked to the *sn*-glycerol 3-phosphate lipid backbone. On the other hand, archaeal lipids are made up of isoprenoid lipid tails, that are ether-linked to the stereochemical different *sn*-glycerol 1-phosphate backbone. This segregation, commonly referred to as the "Lipid divide", is however not as strict as long thought. Recent literature reports on the presence of both glycerol-phosphate backbones in the lipids from *Bacillus subtilis*. Here, we explored to what extent the observed stereochemical variety of the bacterial lipids can be attributed to their general biosynthesis. In short, the bacterial acyl-transferases that attach the lipid tails to the glycerol phosphate backbone, show no clear preference to the stereochemistry. In other words, the variety in lipid backbone is an intrinsic part of bacterial lipid biogenesis, suggesting a less strict role for stereochemistry in the lipid divide.

SL-MDE-161

Escherichia coli* resistance against predatory bacterium *Bdellovibrio bacteriovorus

S. Mridha[1,2,3], S. L. Loose[1], O. Mattmann[1,3], R. Kümmerli[3], *S. Huwiler[1]

[1]University of Zurich, Department of Plant and Microbial Biology, Zurich, Switzerland

[2]University of Pennsylvania, Microbiology, Philadelphia, PA, United States

[3]University of Zurich, Department of Quantitative Biomedicine, Zurich, Switzerland

The rise of antimicrobial resistance (AMR) in bacterial pathogens requires investigation of alternative treatments like predatory bacterium *Bdellovibrio bacteriovorus*. This predator kills and feeds on many AMR Gram-negative pathogens of the WHO priority pathogens list as prey. *B. bacteriovorus* has potential as a "living antibiotic" as it is non-pathogenic, has low cytotoxicity and elicits only a minimal immune response. To use this predator and to better understand its impact on microbial populations, it is essential to determine whether a bacterial prey could develop resistance to *B. bacteriovorus.* To address if and how fast bacterial prey resistance arises and determine its potential genetic determinants, we performed an experimental prey evolution study. We exposed *Escherichia coli* as prey intermittently to predator *B. bacteriovorus* in alternate cycles, involving a predation phase where prey was exposed to different predation pressures, followed by a recovery phase, where surviving prey was grown in absence of the predator. After the experimental prey evolution, we compared the growth of evolved prey lineages versus the ancestor. We found evidence for media adaptation under no-, partial resistance under low-, and strong resistance under high-predation pressure respectively. We found that lineages which evolved without predation pressure were susceptible to predation, suggesting that resistance adaptations are specific to predation and not media. When comparing the growth rate of evolved prey lines to ancestor in the absence of predator, we observed a trade-off between predation resistance and fitness. To investigate the genetic basis of resistance, we sequenced the genomes of evolved prey lineages and compared them to the ancestor prey. We identified resistance mutations primarily associated with the outer membrane. *E. coli* gene knockout mutants confirmed that a small subset of genes was mainly responsible for the resistant phenotypes. Altogether our study reveals predation resistance is associated with outer membrane changes in prey bacteria. While further studies are required to investigate predation resistance development of clinical AMR pathogen strains in differentiated cell culture, our study represents a basis for the development of an alternative, sustainable AMR treatment.

Infection biology

SL-IB-013

Antibiotics that do not cause Shiga-toxin mediated disease in *C. rodentium* φ*stx2d*-infected mice and their effect on the intestinal microbiota

*S. Mühlen[1,2], A. K. Heroven[3], M. Greweling-Pils[3], D. H. Pieper[3], P. Dersch[2]
[1]Ruhr University Bochum, Molecular Immunology, Bochum, Germany
[2]University of Münster, Münster, Germany
[3]Helmholtz Centre for Infection Research – HZI, Braunschweig, Germany

Enterohaemorrhagic *Escherichia coli* (EHEC) is a food-borne pathogen that causes disease ranging from watery diarrhoea to acute renal failure. Disease progression is attributed to the production of Shiga toxins (Stx). Treatment of EHEC infections remains supportive, and the use of antibiotics is highly controversial as they can induce Shiga toxin expression and may, therefore, increase the risk of haemolytic-uraemic syndrome development. While Stx induction has been assessed *in vitro*, the *in vivo* confirmation was hampered by the lack of an adequate mouse model. Introduction of a lysogenic phage encoding *stx2d* into the mouse pathogen *C. rodentium*, however, created a strain that mimics a human EHEC infection in mice.

Infecting mice with *C. rodentium φstx2d,* we investigated the effects of antibiotic treatment on host pathology. Using the fluoroquinolone enrofloxacin known to induce Shiga toxin production, we observed that the mice showed rapid weight loss after treatment onset even though the infection was cleared. Furthermore, the kidney damage in these mice was comparable to that observed for infected but untreated mice. Several tested antibiotics, however, could clear the infection without resulting in kidney damage. These included ampicillin, rifampicin, and tetracycline. Unfortunately, the antibiotics that most efficiently cleared the *C. rodentium φstx2d* infection also had the most detrimental effect on the faecal microbiota, causing a severe decrease in microbiota diversity.

Our data suggest that while some antibiotics increase Stx production and release, others may well be used as treatment options, especially during outbreaks. However, great care needs to be taken when using these antibiotics, as one has to weigh the harmful effects of both the infection and the antibiosis.

SL-PB-014

Potassium-dependent switching dynamics between persistence and antibiotic susceptibility in Mycobacterium

*A. Ulmer[1], V. Srinivasan[1], M. Mauri[1], R. Allen[1]
[1]Friedrich Schiller University, Institute of Microbiology, Jena, Germany

Mycobacterium tuberculosis, which causes tuberculosis, leads to over one million deaths annually. This bacterium can develop resistance to multiple antibiotics, making it essential to find more efficient treatment methods to protect human health. One way bacteria evade antibiotics is by entering an altered physiological, non-growing state that allows them to survive antibiotic exposure. Such cells are called persisters. Recent studies suggest that potassium may influence the switch between persistent and susceptible states. Understanding and quantifying the effect of potassium on switching is crucial, for example in the clinical treatment of *Mycobacterium* infections.

Mycobacterium smegmatis is a bacterium used as a non-pathogenic proxy for studying the virulent *M. tuberculosis*, with which it shares a large part of genetic homology, physiology, and cellular structure. We grew *M. smegmatis* cells to stationary phase with nutrient depletion for both short (1 day) and long durations (3 days). Following this, the cells were transferred to fresh medium with varying potassium concentrations, and their growth dynamics were observed. We found that stationary growth conditions and altered potassium concentrations affect population growth dynamics.

We developed a mathematical model that accounts for cells switching out of the persistent state, as well as for growing cells. This model allows us to quantify effects on the total population growth, such as altering cell growth, various initial amounts of persister cells, and different switching rates into active state. Using the experimental data, the model was used to predict the fraction of persister cells in the population over time, as well as to provide switching rates, lag times, and growth rates. Different scenarios were simulated with the

model to estimate the time until all persisters switched to susceptible state.

Our experiments quantify effects of various potassium concentrations on the switching from persistent to susceptible states in *M. smegmatis* and *M. tuberculosis*. By using mathematical modelling, we provide new insight into the contributions of persister cells and switching rates to the cell pool. These insights will refine our knowledge of *Mycobacterium* and pave the way for more detailed assays in the future, as well as streamline plausible strategies for effective antibiotic treatment.

SL-IB-015

Cross-Kingdom interactions: A commensal yeast promotes *Salmonella* typhimurium virulence

K. Jaswal[1], O. Todd[1], R. Flores Audelo[1], W. Santus[1], S. Paul[2], M. Singh[3], J. Miao[2], D. Underhill[4], B. Peters[2], *J. Behnsen[1]
[1]*University of Chicago, Microbiology and Immunology, Chicago, IL, United States*
[2]*University of Tennessee Health Science Center, Memphis, TN, United States*
[3]*University of Chicago, Department of Pathology, Chicago, IL, United States*
[4]*Cedars-Sinai Medical Center, Los Angeles, CA, United States*

Enteric pathogens engage in complex interactions with the host and the resident microbiota to establish gut colonization. Although mechanistic interactions between enteric pathogens and bacterial commensals have been extensively studied, whether and how commensal fungi affect pathogenesis of enteric infections remains largely unknown. Here we show that colonization with the common human gut commensal fungus *Candida albicans* worsened infections with the enteric pathogen *Salmonella enterica* serovar Typhimurium. Presence of *C. albicans* in the mouse gut increased *Salmonella* cecum colonization and systemic dissemination. We investigated the underlying mechanism and found that *Salmonella* binds to *C. albicans* via Type 1 fimbriae and uses its Type 3 Secretion System (T3SS) to deliver effector proteins into *C. albicans*. A specific effector, SopB, was sufficient to manipulate *C. albicans* metabolism, triggering increased arginine biosynthesis in *C. albicans* and the release of millimolar amounts of arginine into the extracellular environment. The released arginine, in turn, induced T3SS expression in *Salmonella*, increasing its invasion of epithelial cells. *C. albicans* deficient in arginine production was unable to increase *Salmonella* virulence *in vitro* or *in vivo*. In addition to modulating pathogen invasion, arginine also directly influenced the host response to infection. Arginine-producing *C. albicans* dampened the inflammatory response during *Salmonella* infection, whereas *C. albicans* deficient in arginine production did not. Arginine supplementation in the absence of *C. albicans* increased the systemic spread of *Salmonella* and decreased the inflammatory response, phenocopying the presence of *C. albicans*. In summary, we identified *C. albicans* colonization as a susceptibility factor for disseminated *Salmonella* infection, and arginine as a central metabolite in the cross-kingdom interaction between fungi, bacteria, and host.

SL-IB-140

The iron-responsive sRNA IsrR of *Staphylococcus aureus* is involved in the regulation of virulence gene expression, in particular *via* the Sae system

*L. M. Busch[1], A. Ganske[1], S. Reißel[1], L. Bleul[1,2], C. Hentschker[1], H. Wolfgramm[1], N. Atasayan[1], M. Gesell Salazar[3], M. Schaffer[3], A. Reder[1], S. Michalik[1], C. Wolz[2], K. Surmann[1], U. Völker[1], U. Mäder[1]
[1]*University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany*
[2]*Eberhard Karls University Tübingen, IMIT - Infectionbiology, Tübingen, Germany*
[3]*University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany*

The Gram-positive pathogen *Staphylococcus aureus* colonizes the nasal mucosa of ~30% of the human population, but also causes various diseases. Adaptation to changing host conditions, such as severe restriction of iron availability, requires precise regulation of genes involved in virulence and metabolic functions.

Therefore, *S. aureus* possesses various adaptive mechanisms, particularly iron acquisition systems, which are controlled by the global regulator Fur (ferric-uptake regulator) and induced upon iron limitation. In many bacteria, Fur-dependent regulatory RNAs (sRNAs) mediate an iron-sparing response by inhibiting the synthesis of non-essential iron-containing proteins. Recently, the sRNA IsrR (iron sparing response regulator; former S596) was identified [1,2] and its requirement for full *S. aureus* virulence in a mouse model was shown [2]. We identified the global IsrR targetome by combining proteomic and *in silico* prediction approaches [3], which, however, does not fully explain the underlying mechanism of this phenotype.

We noticed a positive association of *isrR* expression with α-hemolysin (Hla) activity of *S. aureus* HG001. Subsequently, we performed a mass spectrometry-based secretome analysis to investigate the influence of IsrR on virulence factor production. *IsrR*-expressing and non-expressing strains were compared under iron-limited and -sufficient conditions. To address differences in the amount of secreted proteins depending on the growth phase and the strain background, a normalization strategy based on an external 15N-labeled standard was employed for robust relative quantification. The SaeR regulon was positively influenced by IsrR and thus, we investigated the interplay of IsrR and the major virulence regulator SaeR. IsrR has no direct effect on the SaeRS protein levels but is likely involved in the activation of the Sae system.

Concluding, we demonstrate that IsrR positively influences expression of numerous secreted virulence factors particularly belonging to the SaeR regulon and the heme uptake system (e.g. IsdB). Thereby, IsrR establishes a link between the iron limitation response and virulence in *S. aureus*.

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SL-IB-141

Genomic and microbiological characterization of CTCL-associated *Staphylococcus aureus*: antibiotic resistance, virulence, and the search for novel antimicrobial drugs

P. Licht[1,2], A. Schüffler[3], R. Heermann[4,5], V. Mailänder[1,2], *N. Dominelli[4]
[1]*University Medical Center Mainz, Department of Dermatology, Mainz, Germany*
[2]*Max Planck Institute, Mainz, Germany*
[3]*Institute of Biotechnology and Drug Research gGmbH (IBWF), Mainz, Germany*
[4]*Johannes Gutenberg-University Mainz, Institut für Molekulare*

Physiologie, Mikrobiologie und Weinforschung, Mainz, Germany
[5]Institute of Biotechnology and Drug Research gGmbH (IBWF), Institute for Biotechnology and Drug research gGmbH [IBWF], Mainz, Germany

Mycosis fungoides (MF), the most common form of cutaneous T-cell lymphoma (CTCL), is a rare subtype of non-Hodgkin lymphoma primarily affecting skin tissue and causing various lesions (1). MF patients are classified as either SA-positive or SA-negative based on the predominant colonizing bacteria. In SA-positive patients, *Staphylococcus aureus* extensively colonizes lesions, leading to an altered skin microbiome. Conversely, lesions in SA-negative patients are colonized by other *Staphylococci* and *Cutibacterium* species. The presence of *S. aureus* is clinically significant, as SA-positive patients experience more complications than their SA-negative counterparts (2).

Multiple *S. aureus* strains have been isolated from MF plaques and patches. Microbiological and molecular analyses revealed an increased resistance to multiple antimicrobial peptides and antibiotics, including methicillin. Further genomic analyses indicated diverse *Staphylococcus* protein A (SpA) subtypes enriched with octapeptide repeat domains on MF lesions, some of which have not yet been characterized. Additionally, variations in the agr system's autoinducer peptide (AIP), the central molecule in *S. aureus* quorum sensing, were observed among these isolates, highlighting its potential as a target for novel therapeutic approaches.Whole genome sequencing also identified the presence of virulence factors, such as toxins like LLO and EntE, which may be of significant interest in pathogenesis. These data align with the HPLC analysis, where an additional metabolite was detected in the CTCL-associated *S. aureus* strains, which is not present in the control strains. Lastly, antifungal extracts and fractions were also investigated for their ability to inhibit growth and biofilm formation of MF-associated *S. aureus,* offering a promising avenue for novel therapeutic strategies.

In summary, the primary objective is to elucidate the role of Staphylococci, particularly *S. aureus*, in MF patients, with a focus on antibiotic resistance and virulence factors as potential therapeutic targets. This understanding could contribute to the development of novel treatments for MF-associated infections.

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SL-IB-142
New metal complex antibiotic kills bacteria by overactivating general proteolysis

*A. B. Schäfer[1,2], Ç. Özsan[3], A. Frei[3,4], M. Wenzel[1,2]
[1]Chalmers University of Technology, Department of Life Sciences, Gothenburg, Sweden
[2]University of Gothenburg, Gothenburg, Sweden
[3]University of York, Chemistry, York, United Kingdom
[4]University of Queensland, Community for Open Antimicroial Drug Discovery, Center for Superbug Solution, Institute for Molecular Bioscience, St. Lucia, Queensland, Australia

With the rise in antibiotic resistance the need for new antibiotics has increased. One group of new innovative compounds that provide unique structural properties are metal complexes. Being mostly known for their anticancer activity, they also offer broad antibacterial properties. In fact, Salvarsan, one of the first clinically applied antimicrobial compounds, is a metal complex. Yet, metal complexes are still underexplored as antibiotics, even though they offer unique activities and mechanisms worth further investigation.

Here, we report our findings on the mode of action of a unique new metal complex (ACMA) with potent antibacterial activity. Using spectroscopic, microscopic, and biochemical assays we show that ACMA does not form pores or depolarize the cell membrane but has a completely different target. Instead, ACMA caused rapid loss of fluorescence intensity of all tested protein reporter fusions. This was not due to fluorescence interference, leakage, or inhibition of protein synthesis. Indeed, SDS page and Western blot showed that ACMA decreased the overall protein content. In addition, kinetic measurements showed rapid onset of protein degradation. A preliminary mutant screen did not identify a single protease being responsible for this effect. Taken together our data indicate that ACMA activates protein degradation, probably by multiple quality control proteases. This is a unique mechanism of action, distinct from the specific interaction of acyldepsipeptides with the ClpP protease which deregulates regulated proteolysis.

SL-IB-143
HOCl forms lipid *N*-chloramines in cell membranes of bacteria and immune cells

*L. R. Knoke[1], S. Abad Herrera[2], S. Heinrich[3], F. M. L. Peeters[3], J. E. Bandow[3], T. Günther-Pomorski[2]
[1]Ruhr University Bochum, Microbial Biochemistry, Bochum, Germany
[2]Ruhr University Bochum, Molecular Enzyme Technology and Biochemistry, Bochum, Germany
[3]Ruhr University Bochum, Microbiology, Bochum, Germany

Neutrophils orchestrate a coordinated attack on bacteria, combining phagocytosis with a potent cocktail of oxidants, including the highly toxic hypochlorous acid (HOCl), renowned for its deleterious effects on proteins. *In vitro* studies have shown that, like in proteins, HOCl results in the chlorination of membrane lipid amino residues found in the head groups of some lipids.

Here, we examined the occurrence of lipid *N*-chloramines *in vivo*, their biological activity, and their neutralization. Using a chemical probe for *N*-chloramines, we demonstrate their formation in the membranes of bacteria and monocytic cells exposed to physiologically relevant concentrations of HOCl. *N*-chlorinated model membranes composed of phosphatidylethanolamine, the major membrane lipid in *Escherichia coli* and an important component of eukaryotic membranes, exhibited oxidative activity towards the redox-sensitive protein roGFP2, suggesting a role for lipid *N*-chloramines in protein oxidation. Conversely, glutathione a cellular antioxidant neutralized lipid *N*-chloramines by removing the chlorine moiety. In line with that, *N*-chloramine stability was drastically decreased in bacterial cells compared to model membranes. Using neutrophil like cells expressing roGFP2 we addressed the immune modulatory potential of lipid *N*-chloramines.

We propose that lipid *N*-chloramines, like protein *N*-chloramines, are involved in inflammation, modulation of immune cells and accelerate the host immune response.

SL-IB-144

Continuous exchange of the inner membrane ring component SctD is required for the assembly and function of the type III secretion system

*C. Brianceau[1,2], S. Wimmi[2,3], T. Kronenberger[4,5,6], A. Diepold[1,2]
[1]*Karlsruhe Institute of Technology (KIT), Applied Biology, Karlsruhe, Germany*
[2]*Max Planck Institute, Ecophysiology, Marburg, Germany*
[3]*University of Cologne, Cologne, Germany*
[4]*University of Tübingen, IMIT - Infectionbiology, Tübingen, Germany*
[5]*German Center for Infection Research (DZIF), Tübingen, Germany*
[6]*University of Eastern Finland, School of Pharmacy, Faculty of Health Sciences, Kuopio, Germany*

Infectious diseases continue to pose a significant burden on humanity, remaining a major cause of illness and death worldwide. Among the various mechanisms employed by infectious agents, the Type III Secretion System (T3SS) is a highly conserved and often essential virulence factor found in many Gram-negative bacterial pathogens. This molecular nanomachine assembles in the bacterial cell wall, forming a syringe-like structure with a needle-like extracellular appendage that enables pathogens to inject protein toxins directly into eukaryotic host cells. Once inside, these toxins disrupt host cellular functions to promote bacterial survival and replication. While a core of T3SS components remains static and tightly bound to the overall structure, its cytosolic components are dynamic, continuously exchanging subunits with a cytosolic pool under physiological. We recently found that unexpectedly, the core structural inner membrane (IM) ring component SctD reversibly partially dissociated from the T3SS under acidic conditions, challenging previous assumptions about its structural stability. We therefore investigated whether SctD, like the cytosolic components, also exchanges subunits at the T3SS under physiological conditions. Using functional assays and fluorescence recovery after photobleaching, we confirmed and further characterized that SctD indeed undergoes subunit exchange while the T3SS is actively secreting. To better understand the biological significance of this dynamic behavior, we engineered an SctD mutant containing two cysteine substitutions in its periplasmic domain. This allowed us to reversibly modulate SctD exchange through targeted crosslinking to neighboring SctD subunits in the IM ring, depending on the redox conditions in the periplasm. Using this engineered SctD mutant, we found that SctD subunit exchange is crucial for integrating the T3SS export apparatus within the IM ring of the T3SS basal body and for promoting protein secretion by the T3SS. Our results reveal an unexpected dynamic behavior of the central T3SS components SctD and, for the first time, directly link this exchange to the T3SS assembly and its function in secreting protein toxins.

SL-IB-145

Recruitment of the cellular lipid transport protein CERT to *C. psittaci* inclusions regulates the timing of bacterial egress

*J. Scholz[1], G. Holland[2], M. Laue[2], S. Banhart[1], D. Heuer[1]
[1]*Robert Koch Institute, Sexually Transmitted Bacterial Pathogens (STI) and HIV, Berlin, Germany*
[2]*Robert Koch Institute, Unit of Advanced Light and Electron Microscopy, Berlin, Germany*

Egress of intracellular pathogens is highly regulated and carefully timed. For the zoonotic bacterium *C. psittaci*, the predominant egress pathway is *Chlamydia*-containing sphere (CCS) formation, a calcium-dependent sequential mechanism including protease activity, inclusion membrane destabilization, intracellular calcium increase, and plasma membrane blebbing. How egress is regulated to ensure that it takes place only after *C. psittaci* intracellular development is thus far unknown. Here, we show that *C. psittaci* recruits the cellular ceramide transporter CERT to its inclusion during intracellular development, but this recruitment is reduced at late time points prior to egress. In addition, an early loss of CERT at the inclusion membrane induced by CERT-KO induces premature egress by CCS formation. Complementation of the CERT-KO with different CERT-GFP constructs prevents premature egress, except of complementation with a variant lacking the inclusion targeting PH domain, showing that the localization of CERT is critical for CCS formation. The CERT-KO induced premature CCS are formed by the sequential process described for mature CCS, but they contain mostly RBs and are predominantly non-infectious. Thus, our findings suggest that the timing of *C. psittaci* egress by CCS formation is regulated by the recruitment of CERT. We propose that CERT stabilizes the chlamydial inclusion by the formation of ER-inclusion membrane contact sites during intracellular development, and the loss of CERT recruitment facilitates inclusion membrane destabilization and CCS formation.

Phage biology

SL-PB-016

The Replication Cycle of ΦKZ depends on a Phage-encoded Nuclease

*L. Buhlmann[1], Y. Zhu[2], V. Rech[1], M. Gerovac[1], J. Vogel[1,2]
[1]*University of Würzburg, Institute of molecular infection biology, Würzburg, Germany*
[2]*Helmholtz Centre for Infection Research – HZI, Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*

Bacteriophages with genomes larger than 200 kBp, known as jumbo phages, interact with their hosts in complex ways. The *P. aeruginosa* phage ΦKZ, for example, encodes more than 300 genes, most of which are of unknown function. Using a novel mRNA-based knockdown approach, we screened for essential genes, leading to the identification of *ΦKZ155*. Knockdown of this gene abrogated phage replication and further analysis revealed that *ΦKZ155* encodes an RNase HI, which is crucial for the replication of the phage genome.

SL-PB-017

Impact of bacteriophages on plant-pathogen interactions in a tripartite system

*S. H. Erdrich[1,2,3], U. Schurr[2], G. Grossmann[1], J. Frunzke[3], B. Arsova[2]
[1]*Heinrich Heine University, Institute of Cell and Interaction Biology, Düsseldorf, Germany*
[2]*Forschungszentrum Jülich GmbH, IBG-2, Jülich, Germany*
[3]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

With the world population on the rise, it is crucial to optimize the utilization of arable resources and minimize food loss. Plant pathogenic bacteria which are estimated to account for 10% of our annual yield losses are gaining resistance against classical control strategies like antibiotics, which are a major problem. Bacteriophages as very specialized bacterial viruses hold great potential to overcome this challenge. Therefore, we investigate phages as a sustainable and targeted solution for biocontrol in agriculture with the potential to shape the plant microbiome.

Our research has led to the isolation and characterization of novel phages for the economically relevant plant pathogen

Xanthomonas (Erdrich et al., 2022). Among those, we recently isolated the strictly virulent phage Seregon, which proved to be suitable for biocontrol of the plant pathogen *Xanthomonas campestris*. However, the influence of bacteriophages on plant physiology, particularly during interactions with pathogenic bacteria in planta, remains a significant knowledge gap. Understanding the phage's impact on bacterial infection dynamics and plant defence responses is pivotal for successful application strategies.

Here, we present infection studies of *Arabidopsis thaliana* with the plant pathogen *Xanthomonas campestris*. The application of the *Xanthomonas* phage Seregon could successfully counteract the bacterial infection almost to the level of the uninfected control. A meta-transcriptome analysis provided unprecedented insights into expression dynamics in this tripartite system and showed decreased plant defence gene upregulation in the presence of the bacteriophage. While *X. campestris* infection led to the expression of several salicylic acid-responsive genes and activation of the glucosinolate biosynthetic pathway. Furthermore, we observed a significant reduction of virulence expression in the bacterial transcriptome in the presence of the phage.

In conclusion, we show that natural phage diversity can be harnessed for phage-based plant biocontrol. Furthermore, our findings indicate that the application of phages effectively protects *Arabidopsis thaliana* against the pathogen *Xanthomonas campestris*. These results highlight the promising role of phages in plant protection strategies and pave the way for further exploration of their use in agricultural practices.

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SL-PB-018

Anthropogenic impacts on the global groundwater viral ecosystem

*F. Borim Corrêa[1], S. Jurburg[1,2], C. Cantuarias[1], N. Ghanem[1], L. Wick[1], A. Chatzinotas[1,2,3]
[1]Helmholtz Centre for Environmental Research - UFZ, Department of Applied Microbial Ecology, Leipzig, Germany
[2]German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany
[3]Leipzig University, Biology, Leipzig, Germany

Urbanization profoundly impacts ecological systems, yet its effects on groundwater viral communities, particularly bacteriophages, remain poorly understood. Groundwater sustains Earth's ecosystems and human societies through drinking water, agriculture, and natural habitats. Bacteriophages play a crucial role by modulating bacterial communities through predation and gene transfer, influencing microbial processes that maintain groundwater quality. We investigated the relationship between anthropogenic land use and viral diversity in groundwater ecosystems, hypothesizing that urbanization and agriculture reduce viral diversity while enhancing stress-resistance metabolic gene diversity. We analyzed 373 groundwater metagenomes across five global biomes, reconstructing 30,046 high-confidence uncultivated viral genomes using a custom pipeline and MetaPhlAn for bacterial diversity estimation. We annotated 7,495 auxiliary metabolic genes using DRAM-v, analyzed viral lifestyle using PhaTYP, and integrated high-resolution land cover and urbanization datasets with machine learning models. Our initial findings supported our hypotheses: urbanization correlated with reduced viral diversity in groundwater systems through host depletion and altered environmental conditions. Metabolic gene analysis revealed distinct patterns across land uses, with specific signatures in built-up, semi-arid, forest land covers, suggesting unique viral adaptations. We found consistent associations between urbanization and stress-resistance genes, indicating shifts in host functional potential. Further analysis showed approximately 65% of identified viruses were virulent, suggesting frequent lytic interactions. To address sample autocorrelation, subsequent analyses will incorporate spatial sampling techniques. These findings contribute to our understanding of viral ecology in groundwater ecosystems and could inform approaches to water management, agriculture, and pollution control in urbanized landscapes. Our results indicate that anthropogenic land use substantially influences groundwater viral communities, providing insights relevant for urban development and ecosystem management strategies.

SL-PB-085

Heat shock coupled transduction: a fast and facile method to circumvent the staphylococcal restriction barrier

L. Schulze[1,2], *J. Mössner[1,2], B. Krismer[3,2,4], A. Peschel[3,2,4]
[1]University of Tübingen, IMIT - Infectionbiology, Tübingen, Germany
[2]Cluster of Excellence EXC 2124 Controlling Microbes to Fight Infections, Tübingen, Germany
[3]Eberhard Karls University Tübingen, IMIT - Infectionbiology, Tübingen, Germany
[4]German Center for Infection Research (DZIF), Tübingen, Germany

As common members of the human microbiota, staphylococcal species colonize humans at various body sites. While some of these staphylococci, like *Staphylococcus aureus,* have the potential to cause a variety of devastating diseases, most of them have adopted a commensal lifestyle. Many of these staphylococcal species have been shown to produce various secondary metabolites, excluding more pathogenic bacteria from their niches, or modulating the host immune system to overcome infections by different pathogens. However, studying staphylococci in the laboratory context remains challenging, as many strains possess a strong restriction barrier, which hinders genetic manipulation. Even though significant progress has been made in recent years, the ability to study these bacteria and their genetic potential is still severely limited.

In this work, we describe a novel method combining heat shock and transduction to enable the genetic manipulation of previously inaccessible staphylococcal strains. We present evidence that the method is applicable to different plasmids, species, and different phages. Our experimental data indicates that different species show a varying temperature optimum for the heat shock and that restriction-competence is recovered over time, suggesting a temporary inactivation of restriction enzymes. We propose that this method is generally applicable to different bacteria, for which transducing phages are available, and is not limited to staphylococci. Overall, this method will enable the study of previously genetically inaccessible bacteria in a fast, easy, and cost-effective way.

Membranes and transport

SL-MT-108
Antimicrobial peptide import in *E. coli* as a defense mechanism
*O. D. Caspari[1,2], S. Dubrac[2], T. Burdich[1], D. Tulow[1], N. Stahl[1], F. Barras[2]
[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*
[2]*Institut Pasteur, Microbiology, Paris, France*

Part of the innate immune system, antimicrobial peptides (AMPs) are produced by virtually all types of cells in a bid to kill or control microbial adversaries. AMPs are being discussed as a new treatment option that promises to engender less development of resistance than existing antibiotics. Yet a variety of prokaryotic mechanisms for resistance against AMPs exist. We followed up on the idea that AMP import into the bacterial cytoplasm could operate as a defense mechanism. Using E. coli, we studied existing mechanisms for AMP import, focusing on a well-characterized transporter called sbmA. SbmA was known to render bacteria susceptible to proline-rich AMPs that act on internal targets, which we confirm using Apidaecin 1B as a model. Using Magainin 2, we show that sbmA simultaneously provides resistance to helical-amphipathic, ribosomally-produced AMPs that target bacterial membranes. Whether the presence of sbmA helps or hurts is thus contingent on the nature of the attacking peptide, hinting at an evolutionary arms race between attack and defence.

Prokaryotic cell biology

SL-PCB-113
Spatial and temporal dynamics of pneumococcal cell wall under host immune response revealed by super-resolution microscopy
*C. Vilhena[1,2], Z. Cseresnyés[3], R. Gerst[3], A. Jost[4], M. T. Figge[3,5]
[1]*Friedrich-Alexander University, Pharmaceutical Biology - Bacterial Interface Dynamics lab, Erlangen, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology, Infection Biology, Jena, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology, Applied Systems Biology, Jena, Germany*
[4]*Friedrich Schiller University, Institute for Applied Optics, Jena, Germany*
[5]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*

Introduction: The cell wall of *Streptococcus pneumoniae* is a complex, multi-layered structure that surrounds the bacterial cell, providing rigidity, and protection. It also plays a crucial role in host-pathogen interactions by anchoring key proteins involved in immune evasion. The cell wall is composed of a thick peptidoglycan (PG) layer attached to teichoic acids, and lipoteichoic acids. The PG itself is formed of long chains of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) units, which are cross-linked by short peptide chains.

Objectives: Understanding the organization and synthesis of the PG layer and its interactions with cell wall anchored proteins is essential for comprehending how the organism is protected against external cues.

Methods: To explore the relationship between cell wall protein anchoring and the rate of cell wall synthesis, we examined the spatial and temporal distribution of the surface-exposed pneumococcal protein PspA on the reference strain D39 (WT). The subcellular localization was accessed by super-resolution structured illumination microscopy (SIM). The super-resolution 3D image stacks were processed using HuygensPro and Imaris, whereas the 2D images were analysed using custom-developed workflows written in the graphical image processing language JIPipe. We then compared the PG layer synthesis rates between the WT and a PspA mutant strain (Δ*pspA*). To spatially distinguish between new and old PG and thusly address the temporal resolution, we sequentially labelled new PG with three various fluorescent D-amino acid (FDAA) probes. Throughout the experiments, we grew bacteria in a regular media and under an immunological challenging condition (i.e., presence of serum).

Results: PspA showed a homogenous distribution along the cell surface with the exception of the septal area. Interestingly, when bacteria were exposed to human serum, the PspA relocated to a predominantly mid-cell, septal region. We observed that PG was asymmetrically synthesized. Marked differences occurred in the labelling pattern of Δ*pspA* when growing in the serum-containing media. There was minimal overlap between old and new PG in the WT strain but this was different in the Δ*pspA* strain.

Conclusions: We observed for the first time a dynamic spatial positioning of pneumococcal proteins during an infection scenario, thus opening new ways to reassess how pathogens exploit their protein machineries to evade the immune system.

SL-PCB-114
Functional characterization of the peptidoglycan recycling pathway in *Caulobacter crescentus*
*P. Richter[1], J. Biboy[2], N. Paczia[3], W. Vollmer[2,4], M. Thanbichler[1,3,5]
[1]*Philipps-University Marburg, Biology, Marburg, Germany*
[2]*Newcastle Univerisity, Cell and Molecular Bioscience, Newcastle, United Kingdom*
[3]*Max Planck Institute, Marburg, Germany*
[4]*University of Queensland, Department of Molecular Biosciences, Queensland, Australia*
[5]*LOEWE Center for Synthetic Microbiology, Marburg, Germany*

Most bacteria possess a peptidoglycan (PG) cell wall that is required to withstand the turgor pressure, maintain cell shape and establish a physical barrier against the environment. The PG layer needs to be constantly remodeled to enable the cells to elongate, grow and divide.

During this process, small PG fragments are released into the periplasm. As these fragments can be used as a source for new PG building blocks, many bacteria take great effort in recycling these degradation products. Enterobacteria, such as *Escherichia coli*, for instance, reuse up to 60 % of released PG fragments. To this end, the turnover products are transported into the cytoplasm and further degraded by a set of PG recycling-specific enzymes, which separate the two sugar moieties and degrade the peptide stem.

While *E. coli* and other Gammaproteobacteria have rather simple shapes, the class of Alphaproteobacteria shows a variety of different cell shapes. In these organisms, correct PG remodeling and therefore also the availability of PG precursors seems to be even more important to maintain the complex cell shapes. On organism belonging to the Alphaproteobacteria is *C. crescentus,* a crescent-shaped bacterium that is characterized by a biphasic life cycle

involving to morphological distinct cell types and asymmetric cell division.

Here, we identified that *C. crescentus* also has a functional PG recycling pathway, which is, although not necessary for general growth, essential to maintain proper cell shape. Additionally, we observed that PG recycling is needed to maintain the natural ß-lactam resistance of *C. crescentus*. By characterizing the so far unknown PG recycling mechanism in Alphaproteobacteria, identifying its relevance for the cells and understanding the connection between ß-lactam resistance and PG recycling, we aim to gain novel insights which can help uncovering potential treatments against pathogenic Alphaproteobacteria such as *Bartonella* or *Brucella* species.

SL-PCB-115
YhaM, a novel player of SOS response?

*R. Hinrichs[1], M. Meiser[1], J. Hanßmann[2], M. Girbig[3], M. Lechner[1], G. Madej[4], C. Ziegler[4], G. Hochberg[3], M. Thanbichler[2], G. Bange[1], J. Pané-Farré[1]
[1]Philipps-University Marburg, Synmikro, Marburg, Germany
[2]Philipps-University Marburg, Biology, Marburg, Germany
[3]Max Planck Institute, Marburg, Germany
[4]University of Regensburg, Biological Physics, Regensburg, Germany

A reliable response to DNA damage is essential for living organisms to survive [1]. The consequences of DNA damage range from reduced fitness to various diseases such as cancer [2,3].

Bacillus subtilis (B. subtilis) reacts to DNA damage by invoking the SOS response, which is controlled and directed by the protein RecA and the transcriptional repressor LexA[4,5].

A gen-operon that has been understudied concerning SOS response contains *yhaO-sbcE-yhaM*. YhaM (*yhaM*) has been characterized as a member of a family of 3'-exoribonucleases bearing hydrolytic ribonuclease activity. It is characterized by its unique composition and arrangement of domains. The protein consists of an N-terminal oligonucleotide domain (OB) and a C-terminal metal-dependent phosphohydrolase domain (HD). The involvement of YhaM could be demonstrated for different pathways of RNA degradation [6,7]. The specific function of YhaM is not clear [8].

Here, we introduce YhaM as a new member of the bacterial SOS response machinery.

Structural characterization revealed the formation of a hexamer ring where the individual monomers are assembled in a head-to-tail conformation. The hexamer interface is formed by the phosphohydrolase domain (HD) and the oligonucleotide binding domains decorate the opening of the resulting pore. Our biochemical data, in combination with *in vivo* experiments, led to a hypothesis where the OB domain acts as a scanner for hydrolysis identifying chromosomal locations activity. We observed that the loss of *yhaM* leads to a Mitomycin C (MMC) sensitive phenotype. Microscopy analyses showed that YhaM accumulates together with RecA during DNA damage, especially when double-strand breaks are formed by MMC, and has a role in the repair mechanism. This suggests that YhaM has its site of action on DNA, which is further corroborated by the finding that the overproduction of YhaM results in a replication block and highly condensed DNA.

Therefore, we introduce YhaM as a new member of bacterial SOS response machinery, based on its structural and catalytic properties.

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Prokaryotic physiology and metabolism

SL-PPM-007
Insights into guanidine assimilation, its regulation and utilization to drive synthetic processes in cyanobacteria

M. A. Itzenhäuser[1], R. Stauder[1], J. A. Dewald[2], C. E. Weinberg[2], *S. Klähn[1]
[1]Helmholtz Centre for Environmental Research - UFZ, Solar Materials Biotechnology, Leipzig, Germany
[2]Martin-Luther-University Halle-Wittenberg, General Biochemistry, Halle (Saale), Germany

Cyanobacteria receive increasing attention in bioeconomy research as they could be used as whole-cell biocatalysts for a light-driven, CO_2-neutral and hence, sustainable production of chemicals and fuel components. The realization of this potential requires deep understanding of cyanobacterial metabolism and its regulation as well as the simultaneous development of molecular tools for metabolic engineering. Here, we provide insights into the assimilation of guanidine via a recently identified guanidine hydrolase (GdmH) [1]. The corresponding *gdmH* gene is widely distributed among cyanobacteria and allows them to grow on guanidine as sole nitrogen source. Consistently, strains lacking *gdmH*, either naturally or by gene deletion, do not grow on guanidine. In the model strain *Synechocystis* sp. PCC 6803, the expression of *gdmH* is controlled at the transcriptional level by NtcA that mediates transcriptional activation in response to nitrogen limitation. Moreover, a conserved RNA motif is present in the 5"UTR that forms the aptamer of a guanidine I riboswitch. Via in-line probing we demonstrate specific and high affinity binding of guanidine to the aptamer. *In vivo*, the riboswitch enabes guanidine-specific and titratable induction of gene expression. In addition, the *gdmH* gene is frequently co-localized with ABC transporter genes. Their mutation also interferes with guanidine-dependent growth of *Synechocystis* pointing towards a guanidine-specific substrate binding protein in the periplasm. However, this interference can only be observed in presence of rather low guanidine concentrations between 0.1 and 0.5 mM. At concentrations >1 mM the mutants grow similar to WT indicating the presence of additional, yet to be identified guanidine uptake systems. In addition, we made use of the guanidine riboswitch to achieve precise and dynamic regulation of heterologous gene expression in cyanobacteria. As guanidine is quite cheap and the required guanidine concentrations are rather low, guanidine riboswitches enable gene expression control at large scale without significant costs for inducers. Altogether, our findings not only

contribute to the understanding of cyanobacterial metabolism, but also highlight the potential of guanidine riboswitches as valuable tool for synthetic biology applications in cyanobacteria.

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SL-PPM-008
Investigating the cryptic sulfur incorporation during biosynthesis of the marine antibiotic tropodithietic acid
*M. Rakhmanov[1], S. Sowa[1], T. Martelli[1], J. Felsch[1], R. Teufel[1]
[1]*University of Basel, Department of Pharmaceutical Sciences, Basel, Switzerland*

Question: The structurally unique antibiotic tropodithietic acid (TDA) is produced by various gram-negative marine bacteria like *Phaeobacter inhibens* and contains two sulfur atoms, which appear to be incorporated non-canonically. Focus of this work was the investigation of the cryptic sulfur incorporation during TDA biosynthesis.

Methods: Candidate enzymes were chosen based on bioinformatic predictions and knockout studies. These candidate enzymes were heterologously produced, and their biochemical functions assessed using *in vitro* assays as well as biophysical methods. To test biosynthetic hypotheses structural analogues of postulated intermediates were synthesized and used as surrogate substrates for these investigations. Complementary structural studies were conducted using X-ray crystallography to scrutinize the underlying enzymology.

Results: We were able to successfully reconstitute a three-enzyme cascade *in vitro*, leading to the integration of a thiol group into a model substrate. More precisely, it could be shown that a GSH moiety attached to an aromatic backbone *via* a C-S bond is sequentially degraded by a γ-glutamyl-cyclotransferase, metallopeptidase and cysteine-S-conjugate-β-lyase. Additionally, the structures of the γ-glutamyl-cyclotransferase aswell as the β-lyase could be elucidated using X-ray crystallography, granting insight into the catalytic mechanism behind GSH degradation and C-S bond cleavage.

Conclusion: These findings corroborate the notion of GSH as the source of sulfur during TDA biosynthesis and pave the way for the biotechnological production of analogous compounds. Interestingly, the degradation of GSH to a reactive thiol has been described in the biosynthesis of the infamous fungal epipolythiodiketopiperazine type toxins during formation of a transannular disulfide bridge, making this the second report of GSH involvement in microbial secondary metabolism in general and the first one specifically in bacteria [1].

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SL-PPM-009
Elucidating Polyhydroxybutyrate metabolism in Streptomycetes
*J. K. Schniete[1], N. Tschowri[1]
[1]*Leibniz University Hannover, IFMB, Hannover, Germany*

Question

Plastic pollution poses a significant environmental threat causing multiple problems harming life on earth. One potential approach to lessen plastic pollution is to replace conventional plastics with biodegradable plastics, such as polyhydroxybutyrate (PHB). PHB can be produced and broken down by various bacteria using PHB synthase (PhaC) and PHB depolymerase (PhaZ), making it an ideal candidate for a circular economy approach. The goal is to identify and characterise the enzymes involved in PHB metabolism in *Streptomyces* and to evaluate whether they can serve as biofactories for converting PHB into antibiotics, as they are known as prolific hydrolytic enzyme and antibiotic producers.

Methodology

S. venezuelae was utilised as a model strain and was tested in a growth assay for PHB degradation. Known PHB-metabolizing enzymes were used as queries to bioinformatically identify PHB-related proteins based on sequence or structural similarities. Knockout mutants were created for each of the putative candidates and were analysed phenotypically.

Results

S.venezuelae can grow in minimal media with PHB as the sole carbon source. We identified three potential enzymes involved in PHB metabolism in *S. venezuelae*: a putative PhaC enzyme (vnz_01345), an extracellular PHB depolymerase (PhaZ, vnz_35915), and a PHB dehydrogenase (BdhA, vnz_03225). Macrocolonies of the Δ*vnz_01345* strain (putative PhaC) and the *Δvnz_03225* strain (putative BdhA) exhibited more difficulty in sporulation compared to the wild type (WT) when grown on high salt sporulation medium. In contrast, the strain Δ*vnz_35915* (putative PhaZ) demonstrated better growth than the WT under the same conditions.

Conclusion

We showed that *S.venezuelae* can utilize PHB as a carbon source and identified several candidate enzymes responsible for PHB metabolism in *Streptomyces*. The phenotypic analysis suggests that PHB may have a protective role under osmotic stress conditions, as it has been shown for other bacteria. Further characterisation is necessary to understand these enzymes better and to prove their role in PHB metabolism. However, these results are promising and indicate *Streptomyces* as a suitable PHB upcycling platform.

SL-PPM-010
Regulatory mechanisms controlling the catabolism of glycogen in cyanobacteria
*S. Doello[1], N. Neumann[1], J. Sauerwein[1], B. Kauderer[1], K. Forchhammer[1]
[1]*Eberhard Karls University Tübingen, IMIT-Organismic Interactions/Microbial Glycobiology, Tübingen, Germany*

Glycogen is a wide-spread reserve polymer important for the survival to environmental stresses of all kinds of organisms. In the cyanobacterium *Synechocystis* sp. PCC 6803, glycogen is necessary to overcome dark and starvation periods. Given the important role of this polysaccharide, its metabolism is subjected to a complex regulation. The activity of the enzymes involved in glycogen synthesis and degradation must be tightly controlled to ensure rapid access to the sugar stores when required. Our research focuses on how such a responsive and fine control is achieved via post-translational modifications of the glycogen catabolic enzymes. We found that modulation of the activity of glycogen phosphorylase (GlgP), phosphoglucomutase (PGM), and glucose-6-phosphate dehydrogenase (G6PDH) through phosphorylation, redox-induced modifications, allosteric interactions and metabolite-level regulation is essential for survival under fluctuating environmental conditions. Interestingly, some of these mechanisms are not specific of cyanobacteria, but evolutionary conserved from bacteria to humans.

SL-PPM-011

Being lazy is not always bad: The effects of c-di-AMP signalling on glutamine toxicity in cyanobacteria

*A. M. Enkerlin[1], K. Selim[1]
[1]*Heinrich Heine University, Microbiology, Düsseldorf, Germany*

Cyanobacteria exhibit a distinctive lifestyle as photoautotrophic bacteria, necessitating rapid responses to environmental shifts that jeopardize their carbon/nitrogen (C/N) balance. To maintain homeostasis, cyanobacteria employ cyclic nucleotide second messengers, such as cyclic di-adenosine monophosphate (c-di-AMP)[1]. In cyanobacteria, c-di-AMP has now been implicated in a broader range of functions, including C/N balance regulation[2], day-night acclimation[3], and natural competence[4]. While mechanistic aspects of C-regulation have been elucidated[3], N-regulation remains unexplored. In the search for novel c-di-AMP targets, we identified the glutamine (Gln) transporter BgtAB as a c-di-AMP target. Gln was reported to be toxic for cyanobacteria[5], but its mode of action remained elusive. Surprisingly, the c-di-AMP-free mutant (Δ*dacA*) was able to grow on toxic Gln concentrations and use it as sole N-source, while wildtype (WT) died. We found that Δ*dacA* exhibited impaired Gln uptake compared to WT. Here, targeted metabolomics unveiled an overaccumulation of arginine and its intermediates in WT cells growing on Gln. In contrast, Gln metabolization was severely hampered in Δ*dacA*, highlighting a role for c-di-AMP in controlling N-metabolism. We further show that the high Gln uptake in WT induces *dacA* gene expression and c-di-AMP overproduction, which is known to be toxic for bacteria[6]. Overall, our findings suggest that c-di-AMP positively regulates BgtAB, and the resulting Gln uptake triggers c-di-AMP overproduction, establishing a lethal feedback loop. This work presents the first mechanistic explanation of Gln toxicity in cyanobacteria due to c-di-AMP signalling and showcases the role of c-di-AMP in N-metabolism.

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SL-PPM-012

Novel approaches in genetics and systems biology of marine *Roseobacter* group bacteria

C. Iacometti[1], S. Ketelaar[1], L. Wöhlbrand[2], R. Rabus[2], *L. Schada von Borzyskowski[1]
[1]*Leiden University, Institute of Biology, Leiden, Netherlands*
[2]*Carl von Ossietzky University, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany*

Introduction

Roseobacter group bacteria are a large and diverse group of Alphaproteobacteria that are abundant across marine ecosystems. They are known for their versatile metabolism and genome plasticity. Frequently, these bacteria interact with other marine organisms, especially algae. Therefore, they are thought to play an important role in mediating the marine carbon cycle. Since *Roseobacter* group bacteria have only been investigated in the past 30 years, many aspects of their physiology, ecology, and evolution are still unknown. Consequently, further studies on different *Roseobacter* group bacteria are required to better understand this important group of bacterioplankton.

Goals

Here, we aim to establish novel genetic tools and to investigate the metabolic network of three model strains from the *Roseobacter* group, with the dual goal of 1) enabling more sophisticated approaches for genetic modification and 2) improving our understanding of the physiology of these abundant marine bacteria.

Materials & methods

We focused our investigations on the three model strains *Ruegeria pomeroyi*, *Phaeobacter inhibens*, and *Dinoroseobacter shibae.* Using molecular cloning, transformation or conjugation, and high-throughput screening experiments, we tested different genetic constructs in all three bacteria. Furthermore, we generated comprehensive proteomics datasets for *D. shibae* grown under different conditions. Finally, we compared this dataset to proteomics datasets of *P. inhibens* to identify shared and divergent traits in the proteomes of these *Roseobacter* group bacteria.

Results

We generated a vector suite with different constitutive and inducible promoters that is functional across the three tested model strains. Furthermore, we characterized changes in the proteome of *D. shibae* on rich medium and minimal medium with different carbon sources. Finally, we identified the core and accessory proteomes of *P. inhibens* and report new insights on the regulation of catabolic and anabolic pathways in response to nutrient availability.

Summary

The establishment of novel genetic tools and the comparative analysis of comprehensive proteomics datasets highlight that *Roseobacter* group bacteria continue to be an exciting field of study and pave the way for further

investigations on the fundamental biology and the application potential of these marine microorganisms.

SL-PPM-090

Utilization of the small regulatory protein CP12 to engineer photosynthetic electron fluxes in cyanobacteria

*M. A. Itzenhäuser[1], F. Brandenburg[1], M. Theune[2], S. Timm[3], M. Hagemann[3], K. Gutekunst[2], S. Klähn[4]
[1]Helmholtz Centre for Environmental Research - UFZ, Solar Materials Biotechnology, Leipzig, Germany
[2]University of Kassel, Molecular Plant Physiology, Kassel, Germany
[3]University of Rostock, Department of Plant Physiology, Rostock, Germany
[4]Helmholtz Centre for Environmental Research - UFZ, Solar Materials Biotechnology, Leipzig, Germany

Photoautotrophic microorganisms like cyanobacteria hold great potential for sustainable production of chemicals and fuels from light, CO2, and water. However, their economical applicability is limited, in part due to low product yields achieved to date. Recent insights into their metabolism, energy and metabolite fluxes, and metabolic regulation provide new opportunities for interventions to enhance product yields. For conversions that only require electrons – such as redox catalysis or the formation of hydrogen by hydrogenases - the partitioning of electrons from the photosynthetic electron transport chain (PETC) determines the yield. In these processes the Calvin-Benson-Bassham (CBB) cycle is the main competitor for electrons. Here, the small regulatory protein CP12 was used to reduce electron flux into the CBB cycle and redirect them into valuable products in the model cyanobacterium *Synechocystis* sp. PCC 6803. In most phototrophic organisms, CP12 is used to control the CBB cycle in dark-night cycles by binding and inhibiting the enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and phosphofructokinase (PRK). The native CP12 of *Synechocystis* itself is redox-regulated, inhibiting the CBB cycle only in the dark and becoming deactivated by a reduced cell status when light is present and the PETC is running1,2. We therefore employed variants of CP12 either derived from cyanophages - which use them during infection in light as auxiliary metabolic genes yielding a more reduced status of their hosts3 - or rationally designed to be unaffected by the redox-status. By inducible expression of these CP12 variants, we were able to modulate photoautotrophic growth and shift the intracellular redox potential to a more reduced state. This altered metabolic state increased the electron flux from photosynthesis into hydrogen production and, in combination with disruption of cyclic electron flow and partial uncoupling of the proton-motif-force, enabled higher continuous hydrogen production. By utilizing CP12 variants to redirect electrons from the CBB cycle toward hydrogen production in cyanobacteria, we highlight the potential of small regulatory proteins for metabolic engineering to optimize resource allocation towards desired products.

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SL-PPM-110

EF-P and its paralog EfpL (YeiP) differentially control translation of proline-containing sequences

A. Sieber[1], M. Parr[2], J. von Ehr[3], K. Dhamotharan[3], P. Kielkowski[4], T. Brewer[1], A. Schäpers[1], R. Krafczyk[1], F. Qi[5], A. Schlundt[3,6], D. Frishman[2], *J. Lassak[1]
[1]Ludwig Maximilians University, Microbial Biochemistry, Planegg, Germany
[2]Technical University Munich, Munich, Germany
[3]Goethe University, Frankfurt a.M., Germany
[4]Ludwig Maximilians University, Chemistry, Munich, Germany
[5]Xiamen University, Xiamen, China
[6]University of Greifswald, Greifswald, Germany

Polyproline sequences are deleterious to cells because they stall ribosomes. In bacteria, EF-P plays an important role in overcoming such polyproline sequence-induced ribosome stalling. Additionally, numerous bacteria possess an EF-P paralog called EfpL (also known as YeiP) of unknown function. Here, we functionally and structurally characterize EfpL from Escherichia coli *and demonstrate its role in the translational stress response. Through ribosome profiling, we analyze the EfpL arrest motif spectrum and find additional sequences beyond the canonical polyproline motifs that both EF-P and EfpL can resolve. Notably, the two factors can also induce pauses. We further report that EfpL can sense the metabolic state of the cell via lysine acylation. Overall, our work characterizes the role of EfpL in ribosome rescue at proline-containing sequences, and provides evidence that co-occurrence of EF-P and EfpL is an evolutionary driver for higher bacterial growth rates.*

SL-PPM-111

Fundamental quantitative insights into bacterial spore electrophysiology

*R. Schira[1,2,3], Y. Gietz[1,2,3], M. Kilian[1,2,3], S. Schmelzer[1,2], C. Westhoven[1,2], R. Koch[1,2], E. Kaganovitch[4], B. Wollenhaupt[4], D. Kohlheyer[4], I. B. Bischofs[1,2]
[1]Max Planck Institute, Marburg, Germany
[2]University of Heidelberg, Heidelberg, Germany
[3]University of Heidelberg, Heidelberg, Germany
[4]Forschungszentrum Jülich GmbH, Jülich, Germany

Cells harness out-of-equilibrium electrochemical gradients to power bioenergetics, homeostasis and signaling. In contrast, electrophysiological features and functions in dormant bacterial spores remain obscure.

Here, we introduce and apply a non-invasive combined experimental-theoretical approach that overcomes limitations of classical techniques, thereby enabling fundamental biophysical insights into the electrophysiology of *Bacillus subtilis* spores. To this end, we monitor individual spore pH dynamics upon microfluidics-controlled environmental fluctuations using genetically encoded fluorescent reporters. Our observations indicate that external pH or salinity changes trigger transitions of internal spore pH to new equilibria within hours to days. Fitting a thermodynamic model of passive proton flux to time series data allowed pH sensor calibration and quantitation of spore pH and voltage changes. Our estimates suggest that ionic environmental changes can alter spore pH and electric potential slowly but substantially.

Thus, dormant spores are open passive systems responding and equilibrating to changing ionic environments on faster-than-lifetime timescales. Thereby, a spore's electrophysiological state is dynamic and relies on environmental factors.

SL-PPM-112
Environmental impacts on antibiotic resistance in *E. coli* strains Nissle, MG1655, and W
*S. Brameyer[1]
[1]*Ludwig Maximilians University, Microbiology, Planegg, Germany*

Question: Antibiotic resistance is a critical public health issue, with extensive resources devoted to discovering new methods to combat it. Recent studies indicate that antibiotic resistance observed in laboratory conditions may differ significantly from clinical scenarios. Additionally, cross-protection against stressors such as acidity may enhance antibiotic resistance and induce cross-sensitivity. Leveraging this phenomenon could optimize antibiotic treatment strategies to reduce resistance; however, differences between experimental and clinical conditions suggest that the clinical implications of cross-sensitivity warrant further investigation. This study aims to compare the antibiotic resistance profiles of *E. coli* strains Nissle, MG1655, and W under varied environmental conditions that mimic the human gastrointestinal tract, including acidic environments, oxygen and nutrient limitations. Alongside minimal inhibitory concentration (MIC) determination for different antibiotic classes, we investigate the promoter activity of key stress-response pathways in response to antibiotics. This integrated approach seeks to elucidate the relationship between stress pathway activation, at the single-cell level, and antibiotic efficacy across diverse strains.

Methods: MICs for antibiotics - such as ampicillin, chloramphenicol, nalidixic acid, trimethoprim, oxytetracycline, polymyxin B, and norfloxacin - were determined for each strain under various abiotic stress conditions. Additionally, we assessed promoter activity of selected stress-response pathways through fluorescence microscopy to investigate their heterogeneous activation and correlation between stress response and antibiotic resistance.

Results: Significant variability in MIC values was observed among strains and conditions. For instance, MG1655 and Nissle exhibited increased resistance to nalidixic acid under low-nutrient conditions, while pre-exposure to acidic conditions elevated resistance to ampicillin in MG1655 and *E. coli* strain W.

Conclusion: This study underscores the distinct antibiotic resistance and stress-response profiles of *E. coli* strains Nissle, MG1655, and W under diverse environmental conditions. The findings provide insights that may refine the clinical and experimental use of *E. coli* strains, support targeted antimicrobial strategies, and enhance understanding of bacterial stress responses.

RNA biology

SL-RNA-035
RNA-mediated adaptation to a new chromosome
*R. Ghandour[1], D. Mazel[2], M. E. Val[2], K. Papenfort[1]
[1]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*
[2]*Institut Pasteur, Bacterial Genome Plasticity Unit, CNRS UMR3525, Paris, France*

Approximately 10% of bacterial species possess more than one main chromosome, an architecture named multipartite genome. Such arrangement enables quicker adaptation to new environmental niches. *Vibrio cholerae*, for example, has two chromosomes: Chr1 and Chr2. While genes encoding for essential cell functions and pathogenicity are located on Chr1, Chr2 encodes genes involved in specialized metabolism and pathogenicity. A novel giant replicon (named hereafter Chr3) has been found in a recent isolate of *Vibrio cholerae*. Despite being present in various Vibrionaceae, the function of Chr3 remains elusive.

To shed light on the impact of Chr3 on its host, we performed transcriptional and post-transcriptional analysis. Comparative transcriptomics showed that Chr3 is conditionally expressed, influencing Chr1 and Chr2 gene expression mostly in the exponential growth phase. Global analysis of RNA duplex formation using RIL-Seq (RNA-interaction-by-ligation-and-sequencing) revealed a vast network of Hfq-assisted interactions between Chr3 and the other two chromosomes. In combination with RIP-Seq (RNA immunoprecipitation and sequencing) analysis, we found 74 sRNAs (small regulatory RNA) encoded on Chr3. Whilst the majority of the newly annotated sRNAs target genes encoded on Chr3, Vcr313 targets genes on primary chromosomes involved in tRNA metabolism. We further show that Chr3 impacts quorum sensing, possesses a functional typeI-F CRISPR-Cas, and contains a more specialized Hfq-like RNA-binding protein. Overall, our data shows ample interactions between all three chromosomes suggesting they are equally involved in post transcriptional gene control. Moreover, the new set of advantageous genes encoded on Chr3 makes it an important entity to expand the adaptation capacities of *Vibrio cholerae*.

Signal transduction & gene regulation

SL-STGR-036
Understanding the link between potassium homeostasis and c-di-AMP signalling in *Streptomyces*
*T. Holdgrewe[1], N. Tschowri[1]
[1]*Leibniz University Hannover, AG Tschowri, Hannover, Germany*

Question

Soil bacteria such as *Streptomyces* are often exposed to osmotic stress during rainfall and drought and thus have evolved diverse strategies for adaptation. In many Firmicutes, the nucleotide second messenger c-di-AMP controls ion and osmolyte transport to maintain osmotic homeostasis. While the synthesis and degradation of c-di-AMP by the deadenylate cyclase DisA and the phosphodiesterase AtaC, respectively, are well studied, our knowledge about c-di-AMP effectors and functions in these Actinobacteria is very limited. Our goal is to understand the link between c-di-AMP and ion homeostasis, specifically focussing on the Kdp system involving the sensor histidine kinase KdpD, the response regulator KdpE, and the high affinity potassium transporter KdpFABC.

Methods

Mutagenesis was used to generate a set of mutants carrying deletions in genes encoding the different components of the Kdp system, c-di-AMP metabolism, or a combination of both. The resulting mutants were used to investigate development and resistance towards potassium stress by phenotypical analysis. Complementation experiments with different KdpD variants were performed to further understand the KdpD dependent phenotypes. Using a bacterial-two-hybrid library, screens for novel KdpD interaction partners were initiated.

Results

Our phenotypical analysis revealed that disruption of the Kdp system in *S. venezuelae* wildtype has no effect on the development or resistance towards potassium stress. However, strikingly, double mutants carrying mutations in *kdpD* and either in *disA* or *ataC* showed a strong delay in development and a growth defect. Interestingly, the observed phenotypes were independent of KdpE and KdpFABC, which suggests that KdpD can act via a non-classical pathway. Complementation studies with different KdpD variants showed that kinase- and phosphatase-inactive versions of KdpD were not able to fully complement the developmental defects, supporting the hypothesis that in *Streptomyces* KdpD affects the phosphorylation status of a yet unknown interaction partner.

Conclusion

Our data demonstrate that KdpD is important for *Streptomyces* development and balanced growth, particularly when c-di-AMP homeostasis is disturbed. Since the observed phenotypes where independent of KdpE and KdpFABC, we hypothesize that KdpD controls the activity of response regulators other than KdpE and aim at identification of novel intraction partners of the histidine kinase.

SL-STGR-037
The role of polyphosphate in Pseudomonas aeruginosa mediated killing of Staphylococcus aureus
R. Shah[1], J. K. Narh[1], M. Urlaub[1], O. Jankiewicz[1], C. Johnson[1], *J. U. Dahl[1]
[1]Illinois State University, School of Biological Sciences, Normal, IL, United States

Pseudomonas aeruginosa and *Staphylococcus aureus* have frequently been co-isolated from patients with cystic fibrosis or burn/chronic wounds, fueling the interest in studying the mechanistic details of this interaction. It was reported that both pathogens generate molecules that modulate their interaction with significant impact on disease outcomes. *P. aeruginosa* outcompetes *S. aureus in vitro* and *in vivo,* which is mediated by several *P. aeruginosa* virulence factors. We recently discovered that polyphosphate (polyP), an efficient stress defense system and virulence factor in *P. aeruginosa*, plays a role for the pathogen's ability to inhibit and kill *S. aureus* in a contact-independent manner. PolyP is composed of long chains of phosphate (Pi) molecules linked via phosphoanhydrid bonds, is found in all three domains of life but only in bacteria have the enzymes of polyP metabolism been well studied. Microbial polyP synthesis is catalyzed by polyP kinase (Ppk) while polyP is degraded by exopolyphosphatase (Ppx) into Pi molecules. Bacteria lacking Ppk (i.e. ΔpolyP) are defective in virulence, biofilm formation, and oxidative stress response. We found that *P. aeruginosa* ΔpolyP cells are less detrimental to *S. aureus* growth and survival while the Gram-positive pathogen is significantly more compromised by the presence of *P. aeruginosa* cells that produce high levels of polyP. Moreover, *P. aeruginosa* polyP production is stimulated by *S. aureus* through a heat-sensitive secretion product. The polyP-dependent phenotype of *P. aeruginosa*-mediated killing of *S. aureus* could at least in part be direct, as we detected polyP in the spent media, which causes significant damage to the *S. aureus* cell envelope. However, more likely is that polyP's effects are indirect. We show that *P. aeruginosa* pyocyanin production is regulated by polyP on the transcriptional level, which harms *S. aureus* through membrane damage and potentially the generation of reactive oxygen species, resulting in the increased expression of antioxidant enzymes. In summary, our study adds a new component to the list of biomolecules that the Gram-negative pathogen *P. aeruginosa* generates to compete with *S. aureus* for resources. A more in-depth understanding of how polyP influences interspecies interactions is critical, as targeting polyP synthesis in bacteria such as *P. aeruginosa* may have a significant impact on other microorganisms and potentially result in dynamic changes in the microbial composition.

SL-STGR-038
PII signaling superfamily: a new paradigm for controlling cellular metabolism
*K. Selim[1,2]
[1]Heinrich Heine University, Düsseldorf, Germany
[2]Phototrophe Mikrobiologie, Düsseldorf, Germany

Because of their photosynthesis-dependent lifestyle, cyanobacteria evolved sophisticated regulatory mechanisms to adapt to oscillating day-night metabolic changes, which is very crucial for their multicellularity lifestyle. How they coordinate the metabolic switch between autotrophic and heterotrophic metabolism in light/dark cycles is poorly understood. Members of the PII signaling superfamily adeptly monitor and synchronize the cell's carbon, nitrogen, energy, redox, and diurnal states, primarily by binding interdependently to adenyl-nucleotides, including charged nucleotides (ATP, ADP, and AMP) and second messengers such as cAMP, and c-di-AMP. These proteins also undergo a variety of posttranslational modifications, such as phosphorylation, adenylation, uridylation, carboxylation, and disulfide bond formation, which further provide cues on the metabolic state of the cell. Serving as precise metabolic sensors, PII superfamily proteins transmit this information to diverse cellular targets, establishing dynamic regulatory assemblies that fine-tune cellular homeostasis. This highlights PII signaling superfamily as a central switch-point in cyanobacterial cell physiology.

SL-STGR-039
New insights in the transcriptional regulation of the Type IVa pilus machine of *Myxococcus xanthus*
A. Treuner-Lange[1], *R. Waßmuth[1], T. Glatter[1]
[1]Max Planck Institute, Abteilung für Ökophysiologie, Marburg, Germany

Type IVa pili (T4aP) are ubiquitous cell surface filaments important for a variety of processes including surface motility. T4aP-dependent motility depends on cycles of T4aP extension, surface adhesion, and retraction. Because the T4aP adheres strongly to the surfaces, cells are pulled forward during retractions.

T4aP-dependent motility is one of two distinct motility systems, which are used by *Myxococcus xanthus* to move across surfaces forming spreading, predatory colonies in the presence of nutrients and spore-filled fruiting bodies in the absence of nutrients. T4aP of *M. xanthus* are composed of thousands of copies of the major pilin PilA and tipped by a complex composed of four minor pilins and the PilY1 adhesins. Interestingly, *M. xanthus* encodes three sets of minor pilins and PilY1 adhesins, and based on high confidence AlphaFold-Multimer model of these tip complexes, the different N-terminal domains of the PilY1 proteins, make the ultimate tip. Thus, *M. xanthus* has the potential to assemble T4aP with three different tip complexes involved in adhesion.

Based on RNAseq analyses, cluster_1 and cluster_3 genes are expressed in presence of nutrients, while cluster_2 genes are not expressed. By contrast, during starvation on a surface, cluster_2 genes are expressed, expression of cluster_3 genes increases, while expression of cluster_1 genes decreases. These transcriptomic data indicate that expression of the three clusters is regulated in response to nutrients and/or surface attachment. This is in agreement with whole cell proteomics data, which show that (i) cells grown on an agar surface accumulate predominantly cluster_3 proteins, (ii) T4aP prepared from cells grown on an agar surface are predominantly composed of PilA and cluster_3 proteins, while (iii) cells grown in suspension accumulate similar amounts of cluster_1 and cluster_3 proteins.

We asked how the expression of the corresponding genes is regulated. To this end, we focused on the NtrC-like transcriptional regulators PilR and PilR2, which are essential for T4aP-formation and T4aP-dependent motility. Our whole-cell proteomics data suggest that PilR is important for expression of *pilA* while PilR2 is important for expression of cluster_1 & _3 genes. ChIP-qPCR experiments confirmed a specific enrichment of PilR-FLAG at the p*ilA*-promoter and of PilR2-FLAG at the promoter of cluster_1 and cluster_3. Using ChIP-Seq and RNA-Seq we now aim to explore their regulons in more detail.

SL-STGR-040

Conformational dynamics of the cytoplasmic acid-stress sensor AdiY of *Escherichia coli*

*G. Gallo[1], S. Brameyer[1], K. Jung[1]
[1]Ludwig Maximilians University, Microbiology, Martinsried, Germany

The arginine-dependent (Adi) system is a critical component of *Escherichia coli*'s enzyme-based acid resistance mechanisms, enabling the bacterium to survive in highly acidic environments such as the human digestive tract[1]. This system consists of the arginine decarboxylase AdiA and the arginine/agmatine antiporter AdiC, which work in tandem to combat intra- and extracellular acidification[2]. Central to the precise regulation of this system is AdiY, a transcriptional activator belonging to the AraC/XylS family. AdiY controls the expression of the *adiA* and *adiC* genes in response to acidic conditions, particularly during anaerobic growth[3]. In this study, we used an *in-silico* approach to identify a set of amino acids within the C-terminal DNA-binding domain of AdiY that appear to play a key role in regulating its activity. We have tested the activation profile of a series of AdiY variants with amino acid replacements at these positions. Various biochemical techniques were used, including the measurement of *in vitro* DNA-binding affinity by Surface Plasmon Resonance (SPR) spectroscopy and the assessment of promoter activation *in vivo*. Our observations revealed that wild-type AdiY exhibits increased DNA binding affinity to its target promoters at acidic pH (5.8 to 6.0), which is attributed to pH-dependent conformational changes. Conversely, AdiY is unable to bind its target promoters at physiological pH. These findings provide new insights into the functional dynamics of AdiY as a soluble cytoplasmic pH sensor and highlight its importance in the cellular response to intracellular pH fluctuations.

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SL-STGR-083

Illuminating protein interactions: Photo-Leucine crosslinking in *Bacillus subtilis*

*R. Warneke[1], F. Schildhauer[1], C. Herzberg[2], P. Neumann[3], R. Ficner[3], X. Xin[1], M. Mema[1], J. Rappsilber[1], J. Stülke[2]
[1]TU Berlin, Chair of Bioanalytics, Berlin, Germany
[2]Georg-August University Göttingen, Department of General Microbiology, Institute for Microbiology & Genetics, GZMB, Göttingen, Germany
[3]Georg-August University Göttingen, Department of Molecular Structural Biology, Institute for Microbiology & Genetics, GZMB, Göttingen, Germany

Understanding the intricate network of protein-protein interactions (PPIs) is essential for unraveling the complexities of cellular function. Photo-crosslinking has emerged as an exciting new method for probing PPIs in many organisms but has not yet found widespread application in bacteria. In this study, we aim to apply photo-crosslinking with photo-leucine in the well-studied gram-positive model organism *Bacillus subtilis*, laying the groundwork for its potential future applications.

We show that photo-leucine becomes highly toxic for *B. subtilis* and investigated the mechanisms of photo-leucine uptake. We provide evidence that the branched-chain amino acid importers BcaP and BraB are involved in the uptake of photo-leucine. Additionally, we unveil the pivotal role of the bipartite exporter AzlCD in facilitating the efficient export of photo-leucine from the cell. Furthermore, our investigation uncovers a previously uncharacterized exporter, AexB, belonging to the novel "sleeping beauty" group of the EamA family [1]. Notably, AexB is found to be positively regulated by the transcription factor AerB, a member of the AraC family. Additionally, we isolated a mutant highly resistant to photo-leucine and identified a single amino acid exchange in the leucine-tRNA synthetase LeuS responsible for this resistance. Using a molecular docking approach, we show that the mutation alters the binding cavity for leucine in a manner that only allows leucine, but not photo-leucine, to bind productively. Furthermore, incorporation rate studies in the suppressor mutant with the LeuS mutation revealed that the photo-leucine incorporation rate was below the detection limit. This work presents the homeostasis of photo-leucine in *B. subtilis*, laying the groundwork for future application of photo-crosslinking and thus the possible identification of novel protein-protein interactions as well as structure refinement.

1. Warneke et al. (2023) mBio 15:e03456-23.

Synthetic microbiology

SL-PHD-174
Development of CRISPR-Cas9-based tools for the fast-growing bacterium Vibrio natriegens
*D. Stukenberg[1], A. Faber[2], J. Hoff[2,3], A. Becker[2,3,4]
[1]*TU Darmstadt, Darmstadt, Germany*
[2]*University of Western Australia, Perth, Australia*
[3]*Technical University Munich, Straubing, Germany*
[4]*Philipps-University Marburg, Marburg, Germany*

Vibrio natriegens is the fastest-growing bacterium known to date with a doubling time of less than 10 min under optimal conditions. This exciting property together with the ability to utilize a wide range of nutrients suggests V. natriegens as a next-generation organism for biotechnology and synthetic biology. To fully harness the potential of V. natriegens in these fields, it is crucial to have efficient genetic tools at hand.

To improve our ability to perform efficient genome engineering in V. natriegens, we developed the "NT-CRISPR" method. NT-CRISPR takes advantage of the ability of V. natriegens to perform natural transformation. This allows bacteria to take up free DNA from the environment and integrate it into their genome through homologous recombination. Natural transformation was previously described to be very efficient in V. natriegens with up to 10% of cells in a population to be correctly modified. NT-CRISPR adds a CRISPR-Cas9 based counterselection step to increase the efficiency of genome engineering through natural transformation even further and to enable more challenging modifications. By targeting the wild type sequence with CRISPR-Cas9, non-modified cells are efficiently eliminated. We demonstrated the capabilities of NT-CRISPR by performing a range of deletions, integrations and point mutations with efficiencies approaching 100 % and additionally succeeded in performing three simultaneous deletions.

For applications, where a permanent modification of the genome is not desirable, e.g. for the study of essential genes, we developed "graded-CRISPRi". graded-CRISPRi uses a catalytically inactive Cas9 variant (dCas9) to block transcription of a target gene, thereby resulting in a knockdown of that gene. In a first step, we characterized the efficiency of this tool by targeting the sequence of the fluorescent reporter gene mScarlet-I. To allow graded knockdowns, we tested libraries with truncated gRNAs or gRNAs with mismatches to the target sequence. This resulted in a gradient of knockdown strengths from full repression to no measurable effect. We applied this concept by targeting essential genes and obtained a wide distribution of growth rates.

In summary, with NT-CRISPR and graded-CRISPRi, we expanded the set of genetic tools available for V. natriegens. This will enable ambitious projects in the future with this fascinating bacterium.

Poster

Anaerobic metabolism

P-AM-001
Characterization of the unique Type IV Nitrogenase Homolog from *Endomicrobium proavitum*
*M. Herzog[1], J. Rebelein[1]
[1]*Max Planck Institute, Department of Biochemistry and Synthetic Metabolism, RG Rebelein, Marburg, Germany*

Nitrogenases are a group of complex metalloenzymes that convert molecular nitrogen (N_2) to its bioavailable form ammonia (NH_3). Thus, they play a critical role in biological nitrogen fixation. In general, there are three nitrogenase isoforms in diazotrophic microorganisms. The most common/prevalent nitrogenase is the molybdenum (Mo) nitrogenase and the less abundant vanadium (V) and iron (Fe) nitrogenases, named after the metal composition of the active site cofactor. All three nitrogenase isoforms are known as *bona fide* nitrogenases. However, there are several nitrogen fixation-like (Nfl)-enzymes that share sequence similarities and conserved regions but lack the ability to fix dinitrogen. The distinct activities of all those enzymes are attributed to differences in their catalytic metal center. The activities of those Nfl-like enzymes range from bacteriochlorophyll synthesis (dark-operative protochlorophyllide oxidoreductase and chlorophyllide *a* oxidoreductase, reducing carbon-carbon double bonds of the tetrapyrrole rings) (Moser & Layer, Methods Mol Biol, 2019), F430 biosynthesis (Ni2+-sirohydrochlorin *a*,*c*-diamide reductase) (Vazquez Ramos *et al.*, FEBS J, 2024 / Moore *et al.*, Nature, 2017) and methylated sulfur compounds (North *et al.*, Science, 2020 / Lago-Maciel *et al.*, bioRxiv, 2024).

However, in contrast to the type Nfl-enzymes described above, another type IV homolog from *Endomicrobium proavitum* has recently been shown to reduce N_2 to NH_3 *in vivo* (Zheng *et al.*, Environ. Microbiol., 2016). Following up on this surprising finding, we set out to comprehensively characterize this enzyme. I am currently investigating its atomic structure using cryo-electron microscopy, determining the metal content of its cofactors by inductively coupled plasma optical emission spectroscopy, and exploring its substrate spectrum *in vitro*. Our exciting findings will provide new insights into the inner workings of Nfl-enzymes and nitrogenases, potentially revealing the fundamental principles of N_2-reduction and the different substrate specificity of those enzymes.

P-AM-002
Novel citramalate lyase with ATP cofactor in pathogenic enterobacteria
*L. Brocks[1], *L. Schäfer[1], E. A. Cassens[1], L. Schmidt[1], J. Eggers[1], S. A. Simon[2], T. Bornemann[2], A. Probst[2], U. Demmer[3], U. Ermler[3], I. A. Berg[1]
[1]*University of Münster, Münster, Germany*
[2]*University of Duisburg-Essen, Chemistry, Essen, Germany*
[3]*Max Planck Institute, Frankfurt a.M., Germany*

Successful expansion of pathogenic cells requires metabolic pathways that allow efficient utilization of nutrients provided in the intestines of the hosts. The genomes of 15 % of sequenced enterobacteria, all pathogens including *E. coli* O157:H7 (EHEC) [1,2], contain a gene cluster for the fermentation of glutamate, an amino acid abundant in the gut. This cluster encodes a novel citramalate lyase that requires either ATP or ADP as a cofactor. Structural analysis

of the enzyme revealed that although ATP is neither hydrolyzed nor does it directly involved in the citramalate lyase reaction, it is involved in the formation of the reaction site and in the coordination of substrate and products by coordinating Mg2+ ions that allow the formation of the substrate binding site in addition to some amino acid residues, all of which involved in the catalytic mechanism are conserved. We identified the corresponding amino acid residues involved in catalysis and in the binding of citramalate and ATP and confirmed their function via kinetic analysis of the respective exchange variants. Some residue substitutions resulted in loss of or reduced interaction with ATP or (*S*)-citramalate, as demonstrated by thermal shift assays.

This enzyme is the prototype of a novel and diverse family of C-C lyases found in 19 % of bacterial genomes and spanning over 82 bacterial phyla. The enzyme structure is simpler than that of conventional citrate lyases. In addition, the unusual cofactor role of ATP gives the enzyme its plasticity, allowing easy horizontal transfer and adaptation to novel functions. In conclusion, the novel family of enterobacterial C-C-lyases widely distributed in different bacterial groups.

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2. Kronen & Berg, *PLOS One* **12**:e0145098 (2015).

P-AM-003

Unraveling the conversion of methoxylated compounds in *Tepidanaerobacter acetatoxydans*

*L. Reuter[1,2], J. Kurth[1,3,2]

[1]*Philipps-University Marburg, Microbial Physiology Lab, Department of Chemistry, Marburg, Germany*

[2]*Microcosm Earth Center, Marburg, Germany*

[3]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*

Methoxylated aromatic compounds (MACs) are components of lignin and are therefore very abundant on Earth. However, their impact on the global carbon cycle is not well understood. To convert these compounds microorganisms use *O*-demethylase systems that comprise four different enzymes. So far, the O-demethylase systems of some bacteria and one archaeon [1] have been characterized. The anaerobic bacterium *Tepidanaerobacter acetatoxydans* is known to use different organic compounds as carbon and energy source such as organic acids, alcohols, sugars and amino acids as well as acetate in syntrophy with methanogens [2]. Although the conversion of MACs has not been previously observed, the organism harbors a genomic region with several genes encoding components of *O*-demethylase systems in its genome. Chemolithotrophic growth and acetogenesis have not yet been described for *T. acetatoxydans*, which encodes most of the acetogenic key enzymes, but lacks for formate dehydrogenase [3]. The question rises if the *O*-demethylase systems in *T. acetatoxydans* are functional and if the organism can produce acetate from MACs. To answer this we studied several *O*-demethylase systems from *T. acetatoxydans* in regard to their biochemical properties and phylogeny. Furthermore, we performed growth studies with *T. acetatoxydans* to investigate it"s still underexplored metabolic potential. We were able to show the growth of *T. acetatoxydans* on various MACs. Furthermore, we observed that one *O*-demethylase system from *T. acetatoxydans* converts next to the MACs syringate and vanillate also 2-MB and TMB, which is different to all yet characterized bacterial *O*-demethylase systems but similar to the archaeal one [1]. Different *O*-demethylases encoded by *T. acetatoxydans* seem to build a yet uncharacterized phylogenetic clade, which could indicate the use of different substrates. This work will lead to a better understanding of MAC conversion and the involved enzyme systems in *T. acetatoxydans* and resolve the acetogenic metabolism using MACs as carbon source.

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2. Westerholm M, Roos S, Schnürer A. (2011). *Syst Appl Microbiol*.34:260–6.

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P-AM-004

A BCR/HAD enzyme responsible for Plasmalogen production in *Clostridium perfringens*

*L. Peller[1], J. Fuchs[1], M. Boll[1]

[1]*University of Freiburg, Microbiology, Freiburg i.Br., Germany*

Plasmalogens are vinyl-ether containing glycerophospholipids and are produced by mammals and bacteria. In mammals, they play a vital role in cell membrane stability and physiological functions. However, their function in bacteria has not been fully clarified. While the oxygen-dependent biosynthetic steps for plasmalogen production in aerobic microorganisms are well-understood, the pathway for anaerobic bacteria has remained elusive. Recently, a plasmalogen synthase (plsAR) was identified in *Clostridium perfringens*. *PlsAR* is predicted to encode a multi-domain complex similar to benzoyl-CoA reductase (BCR)/2-hydroxyacyl-CoA dehydratase (HAD) enzymes. Although, the reactions may appear different at first glance, it is proposed that plsAR shares the same initial step as the BCR/HAD enzyme family. This involves an ATP-dependent one-electron reduction of a thioester to a ketyl radical anion. The prototypes of the BCR/HAD family include class I BCRs, which reduce benzoyl-CoA to 1,5-dienoyl-CoA, and HADs, which eliminate water from 2-hydroxyacyl-CoA. These oxygen-sensitive enzymes consist of four subunits, two of which form an electron-activation module, transferring the electrons from a reduced ferredoxin to a [4Fe-4S] cluster. Subsequently, these electrons are transferred to the active site Fe-S clusters located in the CoA-ester-binding module. Anabolic enzymes, such as plsAR, are also identified as part of the BCR/HAD enzyme family, expanding our understanding of this enzyme family. Plasmalogen production was confirmed through heterologous expression of *plsAR* in *Escherichia coli*. This discovery opened the door for further characterization of plsAR and we present initial insights into ATP-dependent plasmalogen synthesis.

P-AM-005

Thermophilic adaptations in the autotrophic pathways of *Ammonifex degensii*

*I. Krafeld[1], L. Schumann[1], A. Mall[1], S. König[1], I. A. Berg[1]

[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

Ammonifex degensii is an autotrophic, anaerobic and extremely thermophilic bacterium belonging to the class *Clostridia* (order *Thermoanaerobacterales*) (**1**). This organism is remarkable for its ability to use two different carbon dioxide fixation pathways for autotrophic growth. Depending on the H2 redox potential, *A. degensii* uses either the ferredoxin-dependent Wood-Ljungdahl pathway or the more ATP-intensive, ferredoxin-independent Calvin-Benson cycle (**2**). Notably, *A. degensii* is the most thermophilic

organism known to use the Calvin-Benson cycle, with an optimal growth temperature of 70°C, and the cycle shows specific adaptations to operate under thermophilic conditions. The formation of fructose 6-phosphate from triose phosphates and sedoheptulose 7-phosphate from erythrose 4-phosphate and dihydroxyacetone phosphate is catalyzed by an unprecedented promiscuous, bifunctional fructose 1,6-bisphosphate/ sedoheptulose 1,7-bisphosphate (FBP) aldolase/phosphatase encoded in the Calvin-Benson cycle operon. Furthermore, sedoheptulose 7-phosphate can be produced in the Calvin-Benson cycle from erythrose 4-phosphate and fructose 6-phosphate in a transaldolase reaction. Interestingly, *A. degensii* also encodes a promiscuous class II fructose 1,6-bisphosphate/ sedoheptulose 1,7-bisphosphate aldolase outside the Calvin-Benson cycle operon. As a stand-alone fructose 1,6-bisphosphatase or sedoheptulose 1,7-bisphosphatase is missing in the *A. degensii* genome, the functional role of sedoheptulose 1,7-bisphosphate aldolase reaction in this bacterium is unknown.

P-AM-006

A 4-hydroxybutyrate pathway of glutamate fermentation

*E. A. Cassens[1], M. Jaegers[1], C. Jansen[1], I. A. Berg[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

Glutamate is a common mammalian gut metabolite that can be fermented by several strictly anaerobic bacteria. Two pathways of glutamate fermentation are known, the methylaspartate and the 2-hydroxyglutarate pathway (Buckel and Barker 1973). Another form of glutamate fermentation occurs through the combined activities of two bacteria. In this case glutamate is decarboxylated to 4-aminobutyrate by one bacterium (e.g., *Escherichia coli*) and 4-aminobutyrate is then fermented via 4-hydroxybutyrate by the second bacterium (e.g., *Clostridium aminobutyricum*) (Gerhardt *et al*., 2000). Interestingly, glutamate fermentation via 4-hydroxybutyrate has never been shown in one organism. Here we show that strictly anaerobic *Anaerospora hongkongensis* possesses two pathways for glutamate fermentation. Besides the classical methylaspartate pathway, *A. hongkongensis* uses a novel 4-hydroxybutyrate pathway of glutamate fermentation similar to the 4-aminobutyrate fermentation pathway in *C. aminobutyricum* (Gerhardt *et al*., 2000). In this pathway, glutamate is deaminated by glutamate dehydrogenase to 2-oxoglutarate, which is further oxidized to succinyl-CoA by 2-oxoglutarate:ferredoxin oxidoreductase. Succinyl-CoA is reduced to succinic semialdehyde and finally to 4-hydroxybutyrate. Its activation leads to the formation of 4-hydroxybutyryl-CoA, which is dehydrated to crotonyl-CoA in a reaction catalyzed by 4-hydroxybutyryl-CoA dehydratase, which we consider to be the key enzyme of the pathway. Crotonyl-CoA is then converted to butyrate and acetate, the products of the pathway. Glutamate utilization may explain the widespread occurrence of genes for 4-hydroxybutyryl-CoA dehydratase in anaerobic heterotrophic bacteria. Previously, this enzyme was only known from autotrophic CO2 fixation in archaea and aminobutyrate fermentation in bacteria.

P-AM-007

Direct electron transfer between the respiratory cytochrome *bcc-aa$_3$* supercomplex and nitrate reductase 1 gives spores of *Streptomyces coelicolor* a selective energetic advantage

*S. Schnabel[1], D. Falke[1], M. Fischer[1], G. Sawers[1]
[1]*Martin-Luther-University Halle-Wittenberg, Microbiology, Halle (Saale), Germany*

Introduction: *Streptomyces coelicolor* A3(2) is a filamentous actinobacterium that requires oxygen for growth and uses a branched respiratory chain to respire with O_2. A menaquinol:cytochrome *bcc* oxidoreductase and copper-*aa$_3$*-type cytochrome *c* oxidase supercomplex forms one branch, while the other includes a menaquinol:cytochrome *bd* oxidase. The formation of an electron-transferring supercomplex is necessary because *S. coelicolor* has a membrane-bound *c*-type cytochrome. Although *S. coelicolor* can only grow with oxygen, it also encodes three related respiratory nitrate reductases (Nar); however, these do not support growth but are presumed to help maintain a membrane potential when O_2 becomes limiting and nitrate is available. The Nar enzymes are synthesized and active in different stages of the complex developmental life cycle of the bacterium. Nar1 is exclusively present and active in spores, Nar2 mainly in exponentially growing mycelium and Nar3 in stationary mycelium. Nar1 activity depends on a functional cytochrome *bcc-aa$_3$* supercomplex, but Nar2 activity in mycelium is not totally dependent on the respiratory supercomplex.

Objective: We wish to determine the mechanism underlying this differential dependence of Nar activity on the respiratory supercomplex at different developmental stages.

Methods: A combination of proteomic analyses, biochemical assays, use of defined *nar* mutants, physiological studies, including use of respiratory inhibitors, and determination of nitrate and oxygen consumption rates were used.

Results: Protein complex purification studies and proteomic analyses demonstrated that the respiratory supercomplexes differ between spores and mycelium. This finding was verified by determination of O_2 respiration rates combined with the use of respiratory inhibitors. The influence of *nar1* and *nar2* operon mutations on O_2 and nitrate reduction in spores and mycelium indicate direct electron transfer between Nar1 and the respiratory supercomplex in spores.

Conclusions: Our findings suggest that the cytochrome *bcc-aa$_3$* supercomplex is differently organized in spores compared with mycelium. Nar1 appears to receive electrons directly from the cytochrome *bcc-aa$_3$* supercomplex in spores, while Nar2 can functions as a menaquinol oxidase in mycelium. The ability of spores to switch rapidly between available electron acceptors (oxygen or nitrate) improves the chances of *S. coelicolor* spore survival.

P-AM-008

Isolation and characterization of novel carboxydotrophic *Moorella* strains for employment as potential thermophilic biocatalysts

*T. Böer[1], L. Engelhardt[2], A. Lüschen[1], L. Eysell[1], H. Yoshida[1,3], D. Schneider[1], L. T. Angenent[3], M. Basen[2], R. Daniel[1], A. Poehlein[1]
[1]*Georg-August University Göttingen, Institute of Microbiology and Genetics, Genomic and Applied Microbiology, Göttingen, Germany*
[2]*Institute of Biological Sciences, Microbiology, Rostock, Germany*
[3]*Environmental Biotechnology Group, Department of Geosciences, Tübingen, Germany*

Carboxydotrophic hydrogenogenic and acetogenic bacteria utilize carbon monoxide (CO) as their primary carbon and energy source. Hydrogenogenic bacteria employ the process of hydrogenogenesis to convert CO into H_2 gas, thereby

generating energy and H_2 as a renewable energy carrier. In contrast, acetogenic bacteria employ the acetyl-CoA pathway to convert CO into acetic acid or other organic acids, which can be utilized in the synthesis of bulk chemicals and biofuels. The use of these bacteria in biotechnological processes allows the conversion of waste gases or syngas containing CO into valuable end products such as H_2 and biofuels, thereby supporting sustainable energy solutions and a circular economy. Thermophilic carboxydotrophs, such as members of the genus *Moorella*, are of particular interest due to their capacity to grow at elevated temperatures. This trait reduces the costs associated with cooling of bioreactors, minimizes the risks of contamination, and facilitates the recovery of volatile products. The objective of this project is to isolate novel carboxydotrophic strains from the thermophilic *Moorella* genus, followed by a comprehensive genomic and physiological characterization of their CO-oxidizing metabolism.

Thermophilic enrichments resulted in the identification of a novel species, *Moorella carbonis* sp. nov., and six additional strains of the species *Moorella thermoacetica* and *Moorella humiferrea*. Growth experiments demonstrated that *M. carbonis* and one *M. humiferrea* strain exhibited carboxydotrophic hydrogenogenesis, resulting in the production of H_2 and CO_2 as end products. The remaining five strains performed acetogenesis, converting CO primarily to acetate. Genome analysis revealed that acetogenic *Moorella* strains encode a formate dehydrogenase upstream of the energy-conserving hydrogenase complex (Ech1), whereas hydrogenogenic strains encode a monofunctional carbon monoxide dehydrogenase (CooS) instead. Moreover, the CooS/Ech1 complex of hydrogenogenic *Moorella* strains differed from the highly conserved CooS/Ech-complex observed in all other described carboxydotrophic hydrogenogenic bacteria.

In conclusion, novel carboxydotrophic hydrogenogenic and acetogenic *Moorella* strains have been successfully isolated from environmental samples and primarily differed in the genes encoded in synteny to the Ech1 complex involved in energy conservation.

P-AM-009

Inoculation strategies for a synthetic co-cultures of a engineered *Acetobacterium woodii* and *Clostridium drakei* WT

*F. M. Wagenblast[1], K. S. Baur[1], F. R. Bengelsdorf[1]
[1]Ulm University, Molecular Biology and Biotechnology of Prokaryotes, Ulm, Germany

World climate change causes global higher temperatures and increasingly extreme weather phenomena to become more and more frequent. Thus, measures have to be taken to avoid reaching a point of no return. One possible step is to reduce emissions of greenhouse gases (e.g. CO_2) from the production of platform chemicals by switching from crude oil to microbial gas fermentation based production (Sanchez et al., 2023, https://www.iea.org/energy-system/industry/chemicals). The acetogenic bacterium *Acetobacterium woodii* has already been genetically engineered to produce lactate as a secondary product from H_2 and CO_2 next to acetate (Mook et al., 2022, doi: 10.1007/s00253-022-11770-z). Since *A. woodii* lacks the ability to produce higher value products through chain elongation, the addition of the bacterium *Clostridium drakei* in a synthetic co-culture allows the valorisation of lactate in combination with H_2 and CO_2 to more thought after chemicals such as butyrate or hexanoate. The inoculation strategy for this co-culture needs to be optimised and cell numbers from both species have to be quantifiable. Quantification of cells was done using fluorescence in situ hybridization (FISH) via distinguishable probes targeting both species individually. Two probes were constructed that hybridise to specific fragments of the V4 region of the 16S rDNA in *A. woodii* and *C. drakei* and carry differing fluorescent dyes (Pacific Blue for *C. drakei*, ATTO495 for *A. woodii*). These probes were then tested to find the optimal hybridisation conditions. Different inoculation strategies were tested for the co-culture by inoculating the strains both simultaneously and sequentially with different cell concentration ratios (*A. woodii*/*C. drakei*: 70/30, 50/50, 30/70). Additionally, carriers were added to test for possible improvements by allowing the cells to form stationary biofilms. Cell counts of both strains were quantified by hybridising the previously mentioned probes under the optimised conditions and observing them by both flow cytometry and fluorescent microscopy. The fermentation characteristics were determined by measuring gas consumption through loss in pressure, product spectra and production rates through gas chromatography and high pressure liquid chromatography, and capturing scanning and transmission electron microscopy images.

P-AM-010

Biochemical investigation of dichloromethane transformation reveals the role of MecE, MecB, and MecC methyltransferases in anaerobic fermenting bacteria

*J. M. Soder Walz[1], D. Deobald[1]
[1]Helmholtz Centre for Environmental Research - UFZ, Environmental Biotechnology, Leipzig, Germany

Dichloromethane (DCM) is a widely used chlorinated solvent and industrial chemical that is frequently detected as a contaminant in groundwater. As a regulated pollutant of high concern, anaerobic DCM bioremediation has been studied extensively in recent years. To date, four bacterial genera have been reported to transform DCM under anaerobic conditions: *Dehalobacter*, *Dehalobacterium*, *Ca*. Dichloromethanomonas, and *Ca*. Formimonas. Recently, a methyltransferase cassette has been implicated in the dechlorination of DCM. However, the key methyltransferases responsible for DCM transformation have remained elusive.

In this study, we investigated the DCM catabolism of the enriched *Dehalobacterium formicoaceticum* strain EZ94 as a model organism using a combination of biochemical approaches. Initially, blue native polyacrylamide gel electrophoresis was performed with cell-free protein extracts, and enzymatic assays were conducted. Protein mass spectrometry analysis revealed that the gel slices with the highest DCM-transforming activity showed the methyltransferase MecC as the most abundant protein. Heterologous expression of the candidate methyltransferases MecB, MecE, and MecC was performed in *Escherichia coli* BL21 (DE3). Subsequently, enzymatic assays were conducted with the recombinant proteins individually and in combination, with complete DCM transformation observed only when all three enzymes were present. The mass spectrometry analysis showed that the methyl group of DCM was transferred to acetate or coenzyme M, which functioned as methyl group acceptors *in vitro*. Computational analysis, including *in silico* protein structure predictions, multiple sequence alignments, and prediction of cofactor binding sites, together with inductively coupled plasma-triple quadrupole-mass spectrometry,

enabled us to predict the specific function of each protein in DCM transformation. Our data indicate that MecE and MecC function as zinc-dependent methyltransferases, facilitating DCM demethylation and subsequent re-methylation of a methyl group acceptor (*e.g.*, acetate or coenzyme M) *in vitro*, respectively. MecB acts as a cobalamin-dependent shuttle protein, transferring the methyl group between MecE and MecC.

This research presents the first biochemical evidence of enzymes that facilitate the anaerobic transformation of DCM, revealing a bacterial methylation system orchestrated by three methyltransferases – a system previously observed only in archaea.

P-AM-011
Investigation of the primary mechanism for entropy export of metal-reducing microorganisms
*B. Korth[1], T. Maskow[1], C. Dilßner[1], P. Haus[1], M. Schmidt[1], F. Harnisch[1]
[1]Helmholtz Centre for Environmental Research - UFZ, Microbial Biotechnology, Leipzig, Germany

Introduction
Two fundamental principles of life are coupling electron donor oxidation with electron acceptor reduction for energy harvest and exporting entropy into the environment by secretion of small molecules or heat dissipation. Chemotrophic microorganisms evolved to exploit numerous combinations of electron donor and acceptor, resulting in different mechanisms for entropy export ranging from purely entropy-driven to enthalpy-driven growth[1]. Metal-reducing microorganisms represent a unique variation as they couple their metabolism with insoluble electron acceptors by performing extracellular electron transfer (EET). This process allows the microbes to exchange metabolic electrons with metal ores in nature or electrodes in technical systems. Electrons undergo a phase shift from the liquid to the solid state during EET. Thus, the entropy export of metal-reducing microbes is supposed to differ from microbes utilizing soluble electron acceptors that has energetic consequences for the metabolism.

Goal
Analysis of the entropy export of metal-reducing microorganisms performing EET.

Materials&Methods
A bioelectrocalorimetric setup was employed to measure the heat flux of *Geobacter sulfurreducens* and *Geobacter* enrichment biofilms during EET with different electrode materials. Furthermore, the contribution of the electrolyte to the heat flux was assessed by applying the entropy of transport concept and fundamental equations[2]. The electrodes were microscopically and spectroscopically analyzed to elucidate the impact of EET on the elemental composition of the electrodes and to visualize biofilm formation.

Results
We show that the heat evolution depends on the electrode material amounting to 27.3±4.7, 22.1±4.5, and 13.2±5.0 kJ e-mol^{-1} for *Geobacter* enrichment biofilms at silver, copper, and gold electrodes. For *G. sulfurreducens* at graphite electrodes, 27.2±8.1 kJ e-mol^{-1} were measured, validating first experiments[3]. It is shown that *Geobacter*"s entropy export differs from microorganisms utilizing soluble electron acceptors as it mainly relies on EET and not the secretion of small molecules or dissipation of heat.

Summary
Bioelectrocalorimetric experiments show that electron transfer facilitates the main share of entropy export for metal-reducing microbes and depends on the electron acceptor material.

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P-AM-012
Unraveling the functional relevance of two *O*-demethylase paralogs encoded by *Methermicoccus shengliensis*
*J. Willemsen[1,2,3], J. Kurth[1,2,3]
[1]Philipps-University Marburg, Faculty of Chemistry & Microcosm Earth Center, Marburg, Germany
[2]Max Planck Institute, Microcosm Earth Center, Marburg, Germany
[3]Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany

Although methanogenic archaea were originally thought to exclusively feed on small metabolites released from microbial fermentation processed in anaerobic environments, it was demonstrated recently that the methanogen *Methermicoccus shengliensis* can produce methane from methoxylated aromatic compounds (MACs) [1]. Such compounds are readily released upon depolymerization of the abundant plant biopolymer lignin. It was discovered that the methyltransferase system involved in the demethylation of MACs (MtoABCD) is homologous to such systems of acetogenic bacteria [2]. In contrast to conventional methylotrophic methanogens, this enzyme system feeds the methyl moiety into the methanogenesis pathway using tetrahydromethanopterin (H4MPT) as a cofactor, rather than coenzyme M (CoM) [2]. The *mto* operon contains two copies of the *O*-demethylating methyltransferase (*mtoB1* and *mtoB2*), and it has been proposed that each of the paralogs has different substrate preferences [2]. To investigate the substrate usage, we have cultivated *M. shengliensis* with about 30 different MACs as substrates, but only a minority of these substrates can be used for growth. To understand if this finding is caused by a limitation of the substrate range of the two *O*-demethylases or by other effects such as toxicity or regulatory issues we will also perform *in vitro* activity assays using UV-Vis spectroscopy to compare reaction kinetics of MtoB1 and MtoB2 for different substrates. For the cultures that can be grown to feasible cell densities, an RT-qPCR based experiment was designed to compare transcription of *mtoB1* and *mtoB2* in response to the presence of different MACs. In conclusion, a combination of *in vivo* and *in vitro* work provides us with insights in the MAC preferences of *M. shengliensis* and potential differential regulation of the two encoded MtoB paralogs in response to MAC availability. Moreover, this project should shed light on the presumed specialization of the two MtoB homologs to demethylate different MACs based on kinetic insights.

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P-AM-013

Evaluation of *Eubacterium callanderi* for CO_2-neutral, recombinant bioplastics production

*F. Höfele[1], S. M. Schulz[1], F. R. Bengelsdorf[1]
[1]Ulm University, Molecular Biology and Biotechnology of Prokaryotes, Ulm, Germany

Rapid climate change and excessive plastics utilization lead to irreversible negative impact on our planet as well as on human society. The main accelerator of climate change is emission of greenhouse gases, which emerge primarily during fossil-based production processes. An alternative and in the best-case CO_2-negative process is microbial gas fermentation with acetogens. Acetogens harbor enzymes of the Wood-Ljungdahl pathway, which enables this group of anaerobic bacteria to use gaseous and liquid single carbon molecules. Some recently reclassified *Eubacterium callanderi* strains are to date not well characterized but indicate high potential for gas fermentation due to a wide substrate spectrum and genetic accessibility (Flaiz et al., Microb Cell Fact). As *E. callanderi* should serve as a bio catalysator for recombinant production of the bioplastic poly-3-hydroxybutyrate (PHB) the metabolic and transcriptomic characteristics of different *E. callanderi* strains should be analyzed to build the basis for selection of the best suitable strain for gas fermentation. The metabolic and transcriptomic characteristics of the strains *E. callanderi* "FD" (DSM 3662), "Marburg" (DSM 3468) and "2A" (DSM 2593) were examined by growth experiments using glucose and different methanol concentrations. For optimization of transformation of *E. callanderi* the protocol of Leang et al. (Appl Env Microbiol) was varied. Heterotrophic growth experiments of the *E. callanderi* strains "FD", "Marburg", and "2A" with glucose as carbon source revealed the ability of the acetogen to perform chain elongation via the reverse β-oxidation, which enables the bacterium to produce butyrate, hexanoate and octanoate. Acetogenic growth of all strains using methanol as carbon source resulted in production of butyrate and hexanoate. Furthermore, the product spectrum of *E. callanderi* includes acetate, ethanol, and isobutyrate as well as butanol. Antibiotic resistance tests elucidated sensitivity of *E. callanderi* "Marburg" to thiamphenicol and tetracycline, *E. callanderi* "FD" and "2A" showed natural resistances. Consequently, *E. callanderi* "Marburg" was selected for recombinant PHB production. In conclusion the acetogenic species *E. callanderi* shows great potential as bio catalysator for gas fermentation as it has both a wide substrate and product spectrum and is genetically accessible. Due to sensitivity of *E. callanderi* "Marburg" to antibiotics, it is the best suitable strain for recombinant PHB production.

P-AM-014

Engineering *Clostridium ljungdahlii* for sustainable acetone production

*L. Mühling[1], *F. Küchle[1], B. Molitor[1]
[1]Leipzig University, Leipzig, Germany

For a sustainable future, the reliance on fossil fuels must be further reduced. While significant progress has been made in replacing fossil fuels in electricity production, most industrial production of commodity chemicals still relies on fossil raw materials. Decarbonizing the chemical industry, therefore, requires implementing sustainable production routes. Acetone is a key chemical as a solvent and an essential resource in the production of various specialty chemicals, but it is currently primarily sourced from fossil raw materials.

Clostridia spp. have a long history in industrial bioprocesses. The solventogenic bacterium *Clostridium acetobutylicum* was already used in the early 20th century to produce acetone, butanol, and ethanol from starch. While this process is sustainable, its reliance on carbohydrates as substrate puts it in direct competition with food production. *Clostridium ljungdahlii,* on the other hand, can grow with hydrogen and carbon dioxide as sole electron and carbon sources, which makes it attractive for the sustainable bioproduction of chemical commodities. However, wild-type *C. ljungdahlii* only produces ethanol, acetate, and small amounts of 2,3-butanediol as metabolic end products.

In the project BETA (bioethanol to acetone), we aim to provide an efficient process for the sustainable production of the base chemical acetone from bioethanol. Here, we present the progress on the development of a microbial biocatalyst within the overall process. We illustrate ways to produce acetone with *C. ljungdahlii* and aim to optimize the production through pathway engineering and metabolic modeling.

P-AM-015

Exploring the Uncharted: Morphological changes, antibiotic resistance, and future perspectives on autotrophic growth of the strain M5a3

*B. Rühle[1], A. Poehlein[2], F. R. Bengelsdorf[1]
[1]Ulm University, Molecular Biology and Biotechnology of Prokaryotes, Ulm, Germany
[2]Georg-August University Göttingen, Institute of Microbiology and Genetics, Göttingen, Germany

The anaerobic bacterium designated as M5a3 was originally isolated in 1996 from human fecal samples (Bernalier et al., 1996, doi: 10.1007/s002849900081). However, since its initial isolation, M5a3 has received almost no attention in subsequent research despite its unusual property to produce lactate, along with acetate from H_2 + CO_2 by employing the Wood-Ljungdahl pathway. Lactate production is an uncommon metabolic feature within acetogens.

Heterotrophic reactivation of the lyophilized cells from the original 1996 stock was followed by amplifying and sequencing the 16S rDNA and the bacterium was identified as *Intestinibacter bartlettii*. *Intestinibacter* is often found to be a member of the human gut microbiota community. To further investigate the metabolic capabilities of *I. bartlettii*, whole-genome sequencing was performed using Illumina technology. Gram-staining was performed with cells obtained in its exponential and stationary growth phase. Gram-staining indicated that the bacterium showed a Gram-positive staining during the exponential phase, whereas in the stationary phase, a mixed staining pattern was observed. This staining variation may suggest Gram variability in this species. Also, a marked decrease in cell size was observed from the exponential phase (approx. 14 µm) to the stationary phase (approx. 3 µm). Furthermore, detailed morphological analysis using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were performed and respective micrographs recorded. The heterotrophic substrate spectrum was started to be investigated by growth experiments, which demonstrated its inability to utilize lactose and glycerol as carbon sources. In contrast, fructose served as a favorable substrate and enabled the production of 2.4 mM lactate, 17 mM ethanol and 27 mM acetate, which resulted in a pH decrease to 4.9 of the medium. Growth experiments utilizing H_2 + CO_2 as substrates were performed. The natural antibiotic resistance profile was analyzed using a selection of antibiotics, showing natural resistance against spectinomycin, kanamycin and

clarithromycin. In contrast, thiamphenicol and tetracycline consistently inhibited bacterial growth.

In summary, cells are still viable after being stored for 28 years and it could be shown that the heterotrophic metabolic profile fits the original description. Further analysis will be done to investigate the strains characteristics with elucidate its acetogenic properties.

P-AM-016
Metabolic engineering of *Clostridium kluyveri* for enhanced conversion of medium-chain carboxylates into medium-chain alcohols
*C. Schlaiss[1]
[1]*Max Planck Institute, Biology, Tübingen, Germany*

The microbial production of medium-chain alcohols from carboxylates is a promising approach for developing renewable biofuel and biochemical production. *Clostridium kluyveri* is well known for its native ability to produce short- and medium-chain carboxylates; however, its capacity to convert these carboxylates into alcohols is limited. To address this, we developed a genetic system tailored for *C. kluyveri*, to enable metabolic engineering of its endogenous pathways. Our strategy involved introducing different gene combinations and promoter sequences to increase the metabolic flux towards alcohol production. We incorporated different genes and promoters from solventogenic microbe *Clostridium acetobutylicum* to optimize the reduction of carboxylates to alcohols within *C. kluyveri*. We then evaluated both NADH and NADPH-dependent candidate genes associated with alcohol formation in combination and tested them with different promoters to determine the constructs with the highest alcohol conversion efficiencies. Across all constructs, we observed low concentrations of the desired alcohols. However, our results indicate that the tested promoter-gene combinations clearly increased alcohol yields compared to the strain carrying the empty vector. This study demonstrates the potential for metabolic engineering in *C. kluyveri* to achieve carboxylate-to-alcohol conversion, establishing a basis for optimizing microbial production of medium-chain alcohols. These findings highlight the role of gene and promoter selection in pathway regulation and lay the groundwork for further advancements in developing *C. kluyveri* as a robust platform for sustainable alcohol production.

P-AM-018
Characterization of promotor strenghts by thermostable reporter gene system to extend the promotor library in *Thermoanaerobacter kivui*
*V. Guttschuß[1], B. Zeldes[1], M. Basen[1]
[1]*University of Rostock, Microbiology, Rostock, Germany*

The anaerobic bacterium *Thermoanaerobacter kivui* belongs to the acetogenic bacteria, a group of microorganisms that recently have been proposed for the conversion of gases (hydrogen, carbon monoxide, carbon dioxide) to bioproducts (Leigh et al. 1981). Due to its thermophilicity and its capability of autotrophic and heterotrophic growth, non-requirement of vitamins and its genetic accessibility, *T. kivui* is a prime candidate for biotechnological applications (Basen et al. 2018; Weghoff et al. 2016).

A greater diversity of promoters, especially inducible promoters, is required in order to expand the application opportunities.

Since the genome of *T. kivui* does not encode a native β-galactosidase (β-gal), the thermostable β-gal enzyme from the related and extremly thermophilic strain *Caldicellulosiruptor bescii* (T_{OPT} 78 °C) was used to clone it into the genetically accessible Δ*pyrE* strain, as a reporter gene under the control of different promoters (glucose-inducible, phosphotransacetylase, S-layer) using an integrating plasmid (Zeldes et al. 2024).

Among the tested promoters, a constitutive S-Layer promoter (P_{SLP}) was found to be the strongest. A glucose-inducible promotor (Pgluc) was identified, and shown to lead to no β-gal activity when *T. kivui* was grown on fructose, and to a 10-fold reduction in activity when *T. kivui* was grown on mannitol, compared to glucose as a substrate. Overall, although the promoter was inducible the specific β-gal activity was very low compared to a reference strain using the β-gal controlled by P_{SLP}. As β-gal may be released by dying cells, the different strains were also to be tested for their ability to utilize lactose. According to the growth curve and metabolite analysis, one strain utilises lactose. This representing the first proof-of-concept for extending the substrate spectrum of *T. kivui*. Studies towards a targeted secretion of β-gal and exoenzymes for saccharide degradation are in progress.

These newly characterized promoters add to the promoter library available in *T. kivui*, providing the opportunity for greater control over recombinant gene expression in this industrially relevant acetogen.

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P-AM-019
Pathway and regulation of nitrate reduction in acetogenic *Sporomusa ovata*
*L. M. Waschinger[1], V. Müller[1]
[1]*Goethe University, Molecular Microbiology & Bioenergetics, Frankfurt a.M., Germany*

Introduction: Nitrate is known as an alternative electron acceptor in the acetogenic bacterium *Sporomusa ovata*[1]. Inspection of the genome sequence and biochemical analyses revealed the presence of a cytochrome *c*-containing periplasmic nitrate reductase. Moreover, transcriptome analyses revealed nitrite reductase and hydroxylamine reductase genes upregulated in nitrate-grown cells[2].

Goals: To get insights into the pathway of cytochrome *c*-dependent nitrate reduction of *S. ovata*.

Materials & Methods: Bioinformatics, growth experiments, metabolite analyses, resting cells experiments, determination of transcript levels, measurement of enzyme activities.

Results: Nitrate was reduced to ammonia as predicted from the transcriptome analyses. In the absence of ammonia, nitrate was also used as nitrogen source by *S. ovata*. Nitrate and nitrite reductase activities were measured in cell-free extract, cytoplasm and membrane fractions of *S. ovata*. These activities were induced only in cells grown in the presence of nitrate. Nitrate reductase (1.9 ± 0.4 U/mg) and nitrite reductase activity (6.1 ± 0.8 U/mg) were found almost exclusively in the membrane fraction. So far, only reduced methylviologen was used as artificial electron donor for nitrate and nitrite reductase. Both enzymes contain *c*-type cytochromes and the reduced cytochromes are speculated to be the physiological electron donors.

Summary: *S. ovata* performs an energy-conserving nitrate respiration. Nitrate-induced membrane-bound nitrate and nitrite reductase activities were identified in the membrane fraction of *S. ovata*. The pathway of nitrate ammonification will be described.

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P-AM-020

Formate as electron carrier in the gut acetogen *Blautia luti*

*R. Trischler[1], M. Sorbara[2], V. Müller[1]
[1]Goethe University, Molecular Microbiology & Bioenergetics, Frankfurt a.M., Germany
[2]University of Guelph, Department Molecular and Cellular Biology, Guelph, Canada

Introduction: The gut microbiome is a highly complex community, which is involved in the digestion of nutrients but also affects the development of diseases as well as the human well-being. Gut acetogens such as *Blautia* strains are often connected to the human well-being[1]. However, only little is known about the physiology of different *Blautia* species. Bacteria of this genus are often classified as acetogenic bacteria, but the use of a functional Wood-Ljungdahl pathway (WLP) has only been proven and characterized for a few strains. To shed light on the physiology of *Blautia* species, we characterized the WLP of the gut acetogen *Blautia luti* and its connection to its heterotrophic metabolism.

Goals: To unravel the role of the WLP in the physiology of human gut acetogens.

Materials & methods: Growth experiments, fermentation and metabolite analysis, enzyme assays, genome analysis.

Results: Bacteria of the genus *Blautia* can be divided into FDH-containing and FDH-lacking strains. While FDH-containing species such as *B. schinkii* can produce acetate from H_2 + CO_2 *via* the WLP, FDH-lacking strains such as *B. luti* cannot. However, analysis of the genome sequence revealed that all genes encoding the WLP with exception of the FDH encoding gene are present in the genome of these strains. Interestingly, these bacteria performed acetogenesis from CO + formate, showing an unusual but functional WLP. In addition, *B. luti* produced formate during heterotrophic fermentation by pyruvate formate lyase; in the presence of CO or H_2, formate is reduced to acetate by the WLP.

Summary: Many bacteria of the genus *Blautia* utilize an unusual WLP lacking a FDH. For these gut acetogens, formate plays an essential role as intraspecies electron carrier. Beyond that, formate might also be commonly used as interspecies electron carrier in the human gut.

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P-AM-021

Respiratory complex I in strictly anaerobic bacteria

*M. Odermatt[1], L. Zhang[2], D. Kosian[1], O. Einsle[2], M. Boll[1]
[1]University of Freiburg, Microbiology, Freiburg i.Br., Germany
[2]University of Freiburg, Institute of Biochemistry, Freiburg i.Br., Germany

Respiratory complex I is essential for cellular metabolism, serving as a primary entry point for catabolically derived electrons into respiratory chains. In canonical respiration, the NADH-dependent reduction of ubiquinone (E° = 100 mV) drives the translocation of four protons across the cytosolic membrane. This contributes to the establishment of a proton gradient that can be used for ATP synthesis. Membranes of strictly anaerobic microorganisms such as *Geobacter metallireducens* do not contain ubiquinone but menaquinone (MK) with a considerably lower midpoint redox potential (E° = -74 mV). The Gibbs free energy release of a MK-dependent NADH oxidation, however, is insufficient to support the translocation of four protons. This raises fundamental questions about the bioenergetics of these respiratory complex I enzymes.

Our *in silico* analysis of the NADH-binding module of complex I from the strictly anaerobic model organism *G. metallireducens* revealed homologies to electron-confurcating FeFe-hydrogenases. After enrichment of the hydrophilic, electron-transferring domain of the enzyme, we observed a higher iron content compared to canonical respiratory complex I, suggesting the presence of additional iron-sulfur clusters. Therefore, we propose that respiratory complex I from strictly anaerobic bacteria overcomes its thermodynamic limitation and enables the translocation of more than two protons per two electrons by simultaneous MK reduction using both NAD(P)H and reduced ferredoxin as electron donors by flavin-based electron confurcation.

P-AM-022

Isolation and characterization of *Sporomusa carbonis* sp. nov., a carboxydotrophic hydrogenogen in the genus of *Sporomusa* isolated from a charcoal burning pile

T. Böer[1], F. Rosenbaum[2], L. Eysell[1], V. Müller[2], R. Daniel[1], *A. Poehlein[1]
[1]Georg-August University Göttingen, Department of Genomic and Applied Microbiology, Göttingen, Germany
[2]Goethe University, Frankfurt a.M., Germany

With the description of *Sporomusa ovata* and *Sporomusa,* the genus *Sporomusa* was first mentioned in 1984 (1). Until now, seven other species were validly published under the ICNP. All members of the genus are able to grow on H_2 + CO_2, alcohols, N-methylated and methoxylated monoaromates. H_2 + CO_2 is converted via acetogenesis using the Wood-Ljungdahl pathway (WLP). Autotrophic

growth on CO has only been reported for *S. ovata* (1) and *Sporomusa termitida* (2). Energy conservation during acetogenesis in the mesophilic genus *Sporomusa* is achieved by building a chemiosmotic H^+-gradient using an Rnf complex, which is subsequently utilized by an ATP synthase for the phosphorylation of ADP to ATP. All *Sporomusa* strains contain both cytochromes b/c and ubiquinones (3). The presence of cytochromes and quinones is a rare trait of acetogens and besides *Sporomusa* members only present in acetogens of the thermophilic *Moorella* genus. Members of the genus *Sporomusa* are of interest for biotechnological applications.

A novel species, *Sporomusa carbonis* ACPt was isolated from the covering top soil of an active charcoal burning pile. In contrast to the other *Sporomusa* species this strain is not able to grow on H_2 + CO_2, but converts CO to H_2 + CO_2. This strain represents the first carboxydotrophic hydrogenogen within this genus. Phylogenomic analysis of showed that strain ACPt represented a novel species of the genus *Sporomusa*. The genome of ACPtT encoded cytochromes, ubiquinones, the Wood-Ljungdahl gene cluster and an Rnf complex, which were identified as common features all *Sporomusa* type strains.

Based on the genomic, morphological and physiological features presented in this study, strain ACPtT is proposed as a novel species in the genus *Sporomusa* with the name *Sporomusa carbonis* sp. nov. (DSM 116159^T and CCOS 2105^T).

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P-AM-023

Monitoring an anaerobic synthetic co-culture of a lactate producing *Acetobacterium woodii* mutant and *Clostridium drakei* FP for the production of butyrate and hexanoate

*K. S. Baur[1], F. M. Wagenblast[1], F. R. Bengelsdorf[1]
[1]*Ulm University, Molecular Biology and Biotechnology of Prokaryotes, Ulm, Germany*

Climate change is escalating into a global climate disaster. Rising greenhouse gas emissions (e.g. CO_2) are fuelling this trend. However, the use of fossil fuels such as crude oil is still increasing (Ripple et al., 2024) and to reverse this trend climate-friendly alternatives are needed. Microbial gas fermentation is investigated to produce platform chemicals such as hexanoate and butyrate. Our approach is using the acetogens *A. woodii* and *C. drakei* in a synthetic co-culture. *A. woodii* is capable of autotrophic growth utilising efficiently H_2 + CO_2, while producing natively acetate. By plasmid-borne expression of a D-lactate dehydrogenase (*ldhD*) from *Leuconostoc mesenteroides* and knocking out *A. woodii´*s native genes encoding the bifurcating lactate dehydrogenase complex, a strain was constructed, capable of producing lactate as a second product (Mook et al., 2022). *C. drakei* is capable of producing butyrate and hexanoate via reverse-β-oxidation using H_2 + CO_2 as well as lactate as a substrate. One challenge during co-cultivation is the monitoring of two different species over the cultivation time. Therefor quantitative PCR (qPCR) is an option to quantify the respective cells ampilification of unique genes. The idea is to define a correlation between respective c_q values and cell counts or OD_{600} values. Primers were designed to amplify the gene encoding the formate dehydrogenase from *A. woodii* and the gene encoding the phosphate butyryltransferase from *C. drakei*. To determine a strong correlation between cell counts and OD600 values several measurements were done over the course of heterotrophic growth experiments. Furthermore, dilution series of each specie obtained at the highest OD_{600} were also prepared and subsequently analysed. A correlation (R^2=0.96) was found between OD_{600} values and the logarhithm of the cell counts for *C. drakei* during growth. qPCR has been performed with this isolated genomic DNA from the dilution row and the results are currently evaluated. Another method to determine the number of cells in this co-culture is by labelling the cells with species specific fluorescent labelled DNA probes. Therefor probes binding in the V4 region of 16S rDNA of *A. woodii* and *C. drakei* have been designed. The probe binding in *A. woodii´*s DNA is labelled with the fluorophore ATTO495, while the probe binding in *C. drakei´*s DNA is labelled with Pacific Blue. The fixed and labelled cells will be analysed according to an established hybridization strategy.

Archaea

P-AR-024

Unraveling the distribution and diversity of methoxydotrophic archaea

*L. Hofmann[1,2,3], J. Kurth[1,2,3]
[1]*Philipps-University Marburg, Chemistry, Marburg, Germany*
[2]*Max Planck Institute, Marburg, Germany*
[3]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*

Methoxylated aromatic compounds (MACs) are a diverse group of aromatic ethers with significant relevance in biological systems. Lignin constitutes the primary source of MACs, comprising a substantial portion of organic matter in global ecosystems[1]. Utilization of MACs by bacteria and fungi has already been observed since over 40 years[2]. However only recently, it has been discovered that also archaea have this ability. These microbes harbor the so-called Mto system, a methyltransferase system for MAC conversion[3,4]. Given the abundance of MACs on Earth and the presence of *mto* homologs in a variety of archaeal taxa[3] methoxydotrophic archaea may contribute significantly to global carbon cycling by metabolizing MACs to CH_4 and/or CO_2.

Therefore, this project aims to elucidate the prevalence and diversity of methoxydotrophic archaea *via* amplicon sequencing with primers targeting *mto* methyltransferase genes. For instance, one of our primer pairs amplifies a variable region within *mtoB*-genes of *Halobacteriota* and selectively yields the amplicon of interest. Using such primer pairs designed for different archaeal clades, distinct environments are being screened for the presence of methoxydotrophic archaea.

In summary, our project investigates the environmental distribution of methoxydotrophic archaea. Based on potential findings in this project, future studies will further aim to illuminate the microbial ecology of these fascinating microbes in different ecosystems.

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P-AR-025

Investigating the gene regulation and physiology of methoxydotrophic archaea

*Z. Dogru[1,2,3], J. Kurth[1,2,3]
[1]*Philipps-University Marburg, Microbial Physiology, Marburg, Germany*
[2]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*
[3]*Philipps-University Marburg, Chemistry, Marburg, Germany*

Lignin, one of Earth's most abundant organic polymers, is a significant source of methoxylated aromatic compounds (MACs). While bacteria-mediated MAC conversion is well-documented, archaeal degradation of MACs has only recently been discovered. *Methermicoccus shengliensis*, a methanogenic archaeon, is the first archaeon identified to convert MACs, a process termed methoxydotrophy [1]. Recent studies reveal that *M. shengliensis* uses an *O*-demethylase system for MAC conversion, resembling methyltransferase systems of acetogenic bacteria [2]. Comparative transcriptomics identified an operon, the *mto operon*, within *M. shengliensis* that encodes essential MAC-converting proteins, with homologous genes found across diverse archaeal taxa. While the roles of most *mto* proteins in *M. shengliensis* have been elucidated, two hypothetical proteins with DNA-binding structural motifs encoded within this operon remain uncharacterized. We aim to elucidate *mto* gene regulation and characterize these putative DNA-binding proteins by confirming their DNA-binding abilities through EMSA (Electrophoretic Mobility Shift Assay) and performing a promoter pull-down assay with *M. shengliensis* cell extract, followed by MALDI-TOF analysis. Additionally, we seek to characterize the *O*-demethylase system of further archaea and understand their role within the metabolism of these organisms. *Methanolacinia petrolearia* is a hydrogenotrophic methanogen that primarily uses H_2 and CO_2 for CH_4 production and encodes the *Mto proteins, but no MAC transporters*. Growth experiments with *M. petrolearia* revealed tolerance to toxic methyl halides (e.g., chloromethane), suggesting these compounds might be converted/detoxified by the Mto system. We will determine the substrate spectrum of this Mto system by conducting enzyme activity assays while using methyl halides such as chloromethane and various MACs as substrates. Additionally, gene expression analysis (RT-qPCR) will be conducted to assess *mto* gene expression in response to chloromethane or MAC exposure. This research enhances our understanding of archaeal methoxydotrophy by studying the regulation of the *mto* genes and by characterizing Mto systems of further archaea alongside their role within the metabolism.

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P-AR-026

Approaching the function of archaeal ESCRT proteins from the lipid and protein side

*K. Grewe[1], H. Zivkovic[2], R. Reichelt[1], P. Schwille[2], D. Grohmann[1]
[1]*University of Regensburg, Microbiology & Archaea Centre, Regensburg, Germany*
[2]*Max Planck Institute, Munich, Germany*

Until recently, components of the ESCRT (endosomal sorting complex required for transport) machinery were considered to be eukaryotic signature proteins, but homologues of this complex have been identified in archaea as well, especially in the Asgard superphylum (Spang *et al*, 2015; Makarova *et al*, 2024). This discovery fuelled the hypothesis that membrane remodelling, and compartmentalization of the cell emerged already in archaeal cells. However, due to the unique lipid composition in Archaea, many questions about the function of archaeal ESCRT proteins and their interaction with native archaeal membranes arise. Here, we focus on an unusual ESCRT-associated protein identified within the Euryarchaeota, which appears to be a fusion protein composed of a Cas2-like and a TBP-like domain. Characterization of this protein might shed light on the evolution of both the ESCRT system and DNA-binding proteins.

The Cas2-like protein from *Pyrococcus furiosus* was successfully expressed and purified in recombinant form. AlphaFold predicts a dimerization of the protein via the Cas2-domain, which is supported by size exclusion chromatography data. To investigate the function of archaeal ESCRT proteins and ESCRT-associated proteins, giant unilamellar vesicles (GUVs) were prepared using native archaeal lipids and PVA swelling. While appearing perfectly round initially, the GUVs started to form tubular structures. This hints at unusual physical properties of archaeal membranes which provides the opportunity to further characterize the behaviour of archaeal lipid membranes and to study the ESCRT machinery and ESCRT-related proteins in native lipid environments.

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P-AR-027

Stable isotope labeling uncovers novel hexose degradation routes in *Saccharolobus solfataricus*

*K. Mucha[1], J. Wolf[1], A. Ebel[2], C. Bräsen[2], B. Siebers[2], M. Neumann-Schaal[3]
[1]*Leibniz Institute DSMZ, Braunschweig, Germany*
[2]*University of Duisburg-Essen, Essen, Germany*
[3]*Leibniz Institute DSMZ, Braunschweig, Germany*

Archaea are often found in extreme environments, tolerating for instance high temperatures, salinity and acidic or alkaline environments making them valuable models for understanding life under such conditions. Their metabolism is often characterized by unique and unusual metabolic pathways and enzymes that differ from bacteria and eukaryotes. Within the Thermoproteota, *Saccharolobus solfataricus* is a key model organism for studying archaeal metabolism. It thrives under thermoacidophilic conditions with an optimum temperature of 80 °C and a pH range of 2 to 4. Additionally, *S. solfataricus* is characterized by its metabolic versatility, allowing growth on various carbon sources. For some hexoses like D-glucose and D-galactose the central carbohydrate metabolism has been extensively investigated, revealing a modified branched Entner-Doudoroff (ED) pathway for degradation. This pathway notably avoids phosphorylated intermediates, which are common in other organisms. However, the degradation of other hexoses, such as D-mannose and D-fructose, remain unexplored. To address these metabolic gaps, a stable isotope labeling experiment was optimized to enable time-resolved tracing of D-mannose and D-fructose degradation. The labeling experiments suggest degradation analogous to the modified ED pathway, but with a surprisingly high flux through intermediates like mannose-6-phosphate, fructose-6-

phosphate, and trehalose, indicating a so far unknown degradation route within the *S. solfataricus* catabolism.

P-AR-028

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

*K. Premrajka[1], U. Johnsen[1], H. Preidel[1], G. Giacomelli[1], F. Meyer[1], M. Bramkamp[1]

[1]Christian-Albrechts-University of Kiel, Institute for General Microbiology, Kiel, Germany

Proper segregation of the genome is a fundamental process across all domains of life. Unlike bacteria and eukarya, genomic segregation in archaea has not been extensively studied. To maintain the stability of non-chromosomal genetic elements like plasmids in organisms with low plasmid copies, the cells require an active segregation system. The archeon *Haloferax volcanii* is a polyploid organism containing two large and two small plasmids as part of its genome. It is generally accepted that genomic segregation is carried out randomly due to its polyploidy.

However, we have identified multiple two-gene operons across its genome encoding a ParA homologue and an uncharacterized protein immediately downstream. We focus on one of these operons in the large plasmid pHV3, containing the loci *hpaB* and *hpaA* (haloarchaeal partitioning protein B/A). We show that HpaA has an altered localization upon mutation of key residues in the conserved walker motifs and that its presence is necessary for the proper spatial distribution of the HpaB. HpaB is a protein of archaeal origin capable of forming foci in the cytosol, and we think that it acts as a functional homologue of ParB. Comparative qPCR shows that HpaB is essential for maintaining the plasmid to chromosome ratio; deletion of *hpaB* leads to a decrease in the pHV3 plasmid copies in the *H. volcanii* population. Single particle tracking data suggests that HpaB is responsible for the *in vivo* dynamics of HpaA.

We thus propose the presence of an active plasmid segregation system in *H. volcanii*. We explore how the partitioning system could be necessary for two-dimensional segregation of its large plasmids, due to its pleiomorphism in different growth stages. *H. volcanii* shifts its cell shape from rod-shaped to flat, disk-like cells during logarithmic growth. Regular spatial distribution of all genome copies ensures proper genome segregation irrespective of the plane of division, which has been shown to not be influenced by the location of DNA in *H. volcanii*. We suggest that segregation of its large plasmids depends on a two-dimensional distribution of all its copies rather than a directional movement across the plane of division. Moreover, the presence and maintenance of a plasmid segregation system might be important in periods of stress when the cells have fewer genome copies, where random entropic distribution of DNA would lead to anucleate daughter cells.

P-AR-029

Insights in Small protein interactions with membrane proteins in *Methanosarcina mazei*

*T. Habenicht[1], E. Herdering[1], B. Hastedt[1], J. M. Schuller[2], R. A. Schmitz[1]

[1]Christian-Albrechts-University of Kiel, Institute for General Microbiology, Kiel, Germany

[2]Philipps-University Marburg, Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany

Small ORF encoded proteins, with a length less than 70 amino acids have been overlooked for long time in classical bioinformatics and biochemical approaches. By differential RNA sequencing, 1340 putative small open reading frames have been predicted in the model organism *Methanosarcina mazei*, of which 407 have been verified on translational level by ribosome profiling. 88 of those have been additionally confirmed by Mass Spectrometry (Tufail et al. 2024, Weidenbach et al. review). To this date, most of those novel small ORFs lack functional annotation, since often they do not incorporate known domains or have (functionally) characterized homologs.

Using a combination of biochemical and genetic approaches, we identified several of those novel small proteins that interact specifically with large membrane proteins in *M. mazei (e.g. Habenicht et al. 2023*). These interactions strongly suggest a regulatory or modulatory role of small ORF-encoded proteins in cellular processes involving membrane proteins, possibly influencing transport, signaling, or enzymatic functions. Our findings highlight the functional potential of these small proteins and underscore the importance of further characterizing sORFs in microbial systems.

P-AR-030

In silico* analyses of nucleotide tandem repeats in methanogens and differential expression of tandem repeats in *Methanosarcina mazei

*S. Haiss[1], M. Schölmerich[1]

[1]ETH Zurich, Department of Environmental Systems Science, Zurich, Switzerland

Methane emissions are largely caused by methanogenic archaea, which inhabit diverse anoxic ecosystems. Recently, pervasive nucleotide tandem repeats (TRs) have been uncovered in the genomes of methanogens, and these TRs are hypothesized to impact methanogen physiology and metabolism, possibly representing an overlooked feature explaining methanogens ecological adaptability and resilience. In this work, we computationally analyzed TRs in publicly available datasets of methanogens and carried out growth experiments and subsequent transcriptome analyses of the model methanogen *Methanosarcina mazei* Gö1. Among the 254 methanogen genomes analyzed, we detected a particularly high prevalence of TRs in the genomes of *Methanosarcina* (3.1 ± 0.7 TRs/100kb), and also in the animal gastrointestinal tract-associated methanogen *Methanobrevibacter* (2.8 ± 0.8 TRs/100kb) compared to other methanogens (0.37 ± 0.55 TRs/100kb). In *Methanosarcina*, TRs were enriched in adenine and thymine and were predominantly found in intergenic regions. On average, 20 % of TRs were located in open reading frames where they introduced intrinsicallydisordered regions (IDRs) in 62.7 % of TR-proteins (n = 2,296) compared to only 11.9 % across the entire proteomes (n = 369,548). We hypothesize that the IDRs mediate interactions with other proteins or nucleic acids, and that intergenic TRs serve as regulatory elements that enhance cellular flexibility. To corroborate a functional role of TRs, we analyzed their expression levels in *M. mazei* under different growth conditions. This revealed that 63 of the 168 TR regions were differentially expressed in the conditions tested, particularly in the stationary growth phase (34 % of TRs), supporting a potential role as regulatory RNAs. Notably, when cells were grown on trimethylamine (TMA), an intergenic TR downstream of a dimethylamine methyltransferase gene was highly upregulated, suggesting that this TR-RNA may be involved in regulating TMA metabolism. Moreover, two

differentially expressed TR-containing genes - one encoding an indole pyruvate ferredoxin oxidoreductase subunit and another a lactonase domain containing surface protein - were identified as targets for further biochemical and structural characterization of TR functions in proteins. This study is the first to survey and characterize TRs in methanogens and thus builds the basis for further research on the function of intergenic repeats and the role of tandem repeats in archaeal proteins.

P-AR-031
Insights into UV-induced cell aggregation and DNA exchange in Sulfolobales
*A. Banerjee[1], M. van Wolferen[1], A. Recalde[1], S. V. Albers[1]
[1]University of Freiburg, Faculty of Biology, Molecular biology of Archaea, Freiburg i.Br., Germany

Upon exposure to UV light, the hyperthermophilic archaeon *Sulfolobus acidocaldarius* forms aggregates in a species-specific manner, mediated by the UV-inducible type IV pili known as Ups pili. Within these aggregates, cells exchange DNA via the archaeal-specific Ced system, which enables them to repair DNA double-stranded breaks via homologous recombination.

Recently, we identified novel components of both the Ced and Ups systems (1): CedD, a VirD4-like ATPase essential for DNA import, and UpsC, a minor pilin protein essential for the function of the Ups pili. It is likely that other, currently unstudied proteins contribute to the Ced system. To identify these potential players, our current studies include pull down assays targeting both systems.

Here, we present our latest findings on the Ced and Ups systems, advancing our understanding of how these systems cooperate in *Sulfolobus acidocaldarius* to mitigate the effects of UV stress.

Recalde, Alejandra, et al. "New components of the community based DNA-repair mechanism in Sulfolobales." *bioRxiv*(2024): 2024-09.

P-AR-032
The global regulatory role of phosphatase PP2A in *S. acidocaldarius*
*L. Gayermann[1], M. van Wolferen[1], S. V. Albers[1]
[1]Albert-Ludwigs-University, Freiburg i.Br., Germany

In organisms across all domains of life, phosphatases are essential for adjusting to environmental changes. They regulate processes by modulating phosphorylation states of proteins, which can activate or inactivate enzymes and affect protein interactions and stability. This dynamic control is particularly important in stress responses, growth regulation, and adaptation mechanisms.

The hyperthermophillic archaeaon *Sulfolobus acidocaldarius* harbors only two phosphatases: a dual-specific tyrosine phosphatase (PTP) and a serine/threonine phosphatase (PP2A). Interestingly, the type IV pili like swimming organelle of *S. acidocaldarius,* the archaellum, is regulated by phosphorylation. A deletion of PP2A for instance results in hypermotility.

Previously, under starvation conditions, pull down experiments with PP2A revealed a stress regulation module involving archaellum repressors ArnA, and ArnB, as well as UspA (universal stress protein A) and a GTPase. In this study we investigated interactors of PP2A under normal growth conditions. For this, we pulled down a genomically HA-tagged PP2A mutant in early and late growth phases and analyzed its interactome by mass spectrometry.

The results revealed distinct core regulation modules that are specific to starvation and non-starvation conditions. Additionally, proteins involved in the regulation or biogenesis of other type IV pili were found to interact with PP2A. Using thermomicroscopy and electron microscopy, we observed that these surface structures were also impacted in deletion mutants of PP2A interactors. Our findings therefore suggest that phosphorylation via PP2A plays a key regulatory role in the function of all type IV pili appendages in *S. acidocaldarius*.

P-AR-033
Establishment of an efficient one-step enzymatic syntheses for cyclic-2,3-diphosphoglycerate
*C. Stracke[1], C. Bräsen[1], B. Siebers[1]
[1]University of Duisburg-Essen, Molecular Enzyme Technology and Biochemistry, Essen, Germany

Introduction: Extremolytes - unique compatible solutes synthesized exclusively by extremophiles - hold substantial promise for applications in pharmaceuticals, healthcare, and cosmetics. Cyclic-2,3-diphosphoglycerate (cDPG) has been identified as an extremolyte within hyperthermophilic methanogenic Archaea. However, the potential of cDPG for application as well as its ability to protect cellular components, such as membranes, proteins, and DNA, from damage under extreme environmental stress remains largely unexplored since feasible production procedures are missing.

Objective: Here, we present a one-step enzymatic *in vitro* approach for the synthesis of cDPG from 2,3-diphosphoglycerate (2,3-DPG) utilizing the cyclic 2,3-diphosphoglycerate synthetase (cDPGS) from *Methanothermus fervidus*.

Methods: Codon optimization enhanced the heterologous production of cDPGS in *Escherichia coli*. The purification process has been optimized by implementing a streamlined heat precipitation step, followed by optional size-exclusion chromatography for further refinement. Enzyme assays and 31P-NMR analysis were used to assess kinetic parameters and conversion efficiencies. Based on these data a kinetic model for the production of cDPG was implemented.

Results: From 1.7 g (wet weight) of *Escherichia coli* cells, we purified 3.5 mg of cDPGS. The recombinant protein showed a *V*max of 38.2 U mg-1, with *K*m values of 1.55 mM and 0.55 mM for 2,3 DPG and ATP, respectively. For enhanced activity and improved stability of the cDPGS, we added 400 mM KCl, 10 mM DTT, 5 mM Mg^{2+}, and 25% (v/v) glycerol (for storage at -70°C). This resulted in a retention of 95% enzyme activity after 1.5 months. The optimized *in vitro* reaction was scaled up (1-100 mM), achieving full conversion of 37.6 mg 2,3-DPG to cDPG at 55°C within 180 minutes.

Conclusion: These results represent an important step towards a simple one-step *in vitro* approach to produce cDPG. The complete substrate conversion simplifies

downstream purification, and the established kinetic model allows further up-scaling of the process. This approach paves the way for cost-effective cDPG production as a value-added product.

P-AR-034

Employing a novel plasmid system to decouple induction and selection for robust single-molecule sensitive imaging in *Haloferax volcanii*

*P. Schrage[1], U. Afonina[2], K. Martens[1], J. Sáenz[2], U. Endesfelder[1]
[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*
[2]*Technische Universität Dresden, Center for Molecular Bioengeneering, Dresden, Germany*

Fluorescence microscopy has become an indispensable tool in biological research, offering a powerful visual approach to study protein localization and dynamics and cellular biochemistry *in vivo*. Advances in fluorescence and super-resolution microscopy in the last decade have enabled the study of cellular functions and mechanisms in archaea [1], including *H. volcanii [*2, 3, 4], a model organism for haloarchaea, with an established collection of fluorescent proteins, plasmids, and promoter systems [2,5,6]. However, with the emergence of imaging routines, it has become evident that genetic modifications and overexpression of proteins can significantly alter native cell biology [7]. Therefore, careful consideration is required when choosing strains, expression systems, and growth conditions to study the native cell biology of *H. volcanii* using fluorescence imaging techniques. These issues become even more pronounced in sensitive techniques like single-molecule sensitive imaging, which we have successfully established in *H. volcanii* using an autofluorescence-free strain, WR806, and codon-optimized variants of photoswitchable FPs Dendra2 and PAmCherry1 [6]. Despite this important proof-of-concept work, challenges remain in this protocol due to the limitations of available plasmid systems. For example, we have observed pleomorphism, inclusion body formation, and insufficient control over protein expression levels.

To address these challenges, we developed and characterized a novel plasmid expression system for imaging cell biology in WR806. It combines a tryptophan-inducible promoter and an established selection marker system to explicitly decouple induction and selection in *H. volcanii* WR806. By doing so, our novel pUE001 expression plasmid enables robust fluorescence imaging of low-abundant, toxic, and growth-inhibiting proteins in *H. volcanii* WR806.

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[7] Patro et al., Front Microbiol. (2023) doi:10.3389/fmicb.2023.1270665.

P-AR-035

Cryo-EM structure of the ATP-dependent methyl-coenzyme M reductase activation complex

*A. Kumar[1], F. Ramírez-Amador[1], S. Paul[1], C. Lorent[2], S. Keller[3], S. Bohn[4], T. Nguyen[3], S. Lometto[5], J. Kahnt[5], D. Deobald[6], F. Abendroth[7], O. Vázquez[7], G. Hochberg[5], S. Scheller[3], S. Stripp[2], J. M. Schuller[1]
[1]*SYNMIKRO, Department of Chemistry, Philipps-Universität Marburg, Marburg, Germany*
[2]*TU Berlin, Berlin, Germany*
[3]*Aalto University, Espoo, Finland*
[4]*Helmholtz Centre Munich, Munich, Germany*
[5]*Max Planck Institute, Marburg, Germany*
[6]*Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany*
[7]*Philipps-University Marburg, Marburg, Germany*

Methyl-coenzyme M reductase (MCR) serves as the enzyme responsible for the production of nearly all biologically generated methane (CH_4). Its active site comprises coenzyme F_{430}, a porphyrin-based cofactor with a central nickel ion that is active exclusively in the Ni(I) state. How methanogenic archaea perform the reductive activation of F_{430} represents a major gap in understanding one of the most ancient bioenergetic systems in nature. Here, we purified and characterized the MCR activation complex from *Methanococcus maripaludis*. McrC, a small subunit encoded in the mcr operon, co-purifies with the methanogenic marker proteins Mmp7, Mmp17, Mmp3 and the A2 component. We demonstrate that this complex can activate MCR *in vitro* in a strictly ATP-dependent manner, enabling the production of CH_4. In addition, we determined the structure of the catalytic states of the MCR activation complex using cryo-EM single particle analysis, at a resolution ranging from 1.8 Å-2.1 Å when the sample is incubated with ATP. Strikingly, our data reveal three iron-sulfur (FeS) clusters that form an electron transfer pathway toward F_{430}. The topology and EPR spectra of these cofactors indicate that they are similar to [8Fe-9S-C] intermediates in the biosynthesis of the catalytic cofactor of nitrogenase. Altogether, our findings offer novel insights into the activation mechanism of MCR and prospects for the early evolution of nitrogenase.

P-AR-036

A closer look at the cell division in *Haloferax volcanii*

*J. C. Wähling[1], P. Nußbaum[1], D. Lefebvre[1], C. van der Does[1], S. V. Albers[1]
[1]*University of Freiburg, Molecular Biology of Archaea, Freiburg i.Br., Germany*

Cell division is one of the most essential processes across all domains of life, making its understanding crucial. In archaea, this process is still not fully understood due to the diverse mechanisms of cell division. Recent research on the FtsZ-based cell division mechanism in *Haloferax volcanii* has identified several proteins essential for this process. Studies have shown that cell division in *H. volcanii* requires homologs of bacterial proteins, including two FtsZ proteins that function as recruitment hubs for recruiting other proteins to build up the divisome including SepF, which acts as both an anchor for FtsZ2 and a recruiter for some cell division proteins [1]. Additionally, other important cell division proteins have been identified, including CdpB1 and CdpB2, which form heterodimer chains at the inner cell membrane [1]. These chains are connected with SepF via the CdpB1 protein, forming an anchoring complex for the FtsZ2 protein [2]. Recent research suggests that another protein, CdpA, is also part of this complex. CdpA interacts with CdpB1 and CdpB2 and has a transmembrane domain which potentially serves as the primary anchor to the cell membrane for the SepF-CdpB1-CdpB2 complex [3]. The recent discovery

regarding CdpA, an important component of cell division in *H. volcanii*, underscores the relevance of this research. Despite the seemingly foundational nature of cell division, this highlights that our understanding is still incomplete, indicating an active and ongoing need to uncover unknown factors within this critical process.

Our goal is to take a closer look at the cell division proteins to gain a deeper understanding of their formation into a mature divisome complex and their specific functions. In addition to examining currently known cell division proteins, we also aim to identify new factors involved in the FtsZ-based cell division process of archaea.

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[2] Nußbaum et al. (2021) Nature communications 12:3469
[3] Liao et al. (2024) bioRxiv 2024.07.17.603854

P-AR-037

***Methanosphaerula subterraneus* sp. nov., a hydrogenotrophic methanogen isolated from lacustrine subsurface sediment from the Eger Rift, exposed to natural high CO_2 concentrations**

*Z. Jia[1], D. Lipus[1,2], D. Wagner[1], L. Benning[3], V. Roddatis[3], C. Bahl[3], M. Syczewski[3,4], J. Kallmeyer[1]
[1]*GFZ German Research Centre for Geosciences, Geomicrobiology, Potsdam, Germany*
[2]*Thomas Jefferson University, Philadelphia, PA, United States*
[3]*GFZ German Research Centre for Geosciences, Interface Geochemistry, Potsdam, Germany*
[4]*University of Warsaw, Warsaw, Poland*

In natural environments, microbial communities react rapidly on changing physicochemical conditions. Such a reaction can be e.g. a change in community composition or overall metabolism, or both. Underground CO_2 storage represents such an example of physicochemical change. Stability over geologic time-scales is paramount for the success of underground CO_2 storage, but current knowledge of how the indigenous microbial community in a potential storage site would react to the introduction of CO2 and other perturbations is limited. In the Eger Rift area in the Czech Republic, constant mantle CO_2 degassing since roughly one million years has led to adaptation of subsurface microbial communities to high CO_2 conditions. It therefore allows a glimpse into the future of a subsurface environment perturbed by long-term high CO_2 conditions.

The Hartoušov Mofette Field (HMF) in the Eger rift is characterized by an extremely high flux of geogenic CO_2, reaching up to 50 kg/m^2 per day, thereby providing a natural analogue of long-term CO_2 storage. Previous geochemical monitoring at the surface already predicted an active methanogenic microbial community in the subsurface, which showed increasing methane production during the occasional swarm earthquakes in this area that may provide geogenic hydrogen that fuels methanogenesis. Efforts to identify these active members living in this analogue of long-term CO2 storage are still needed.

To assess how the subsurface microbial community adapts to high CO_2 conditions and unveil the active members, the ICDP project "Drilling the Eger Rift" recovered a drill core reaching 230 m below the surface from the HMF. Enrichment cultivation under H_2/CO_2 atmosphere in minimum mineral medium unveils a microbial community that is distinctly different to that of the original drill core samples. Methanogen and sulfate reducing bacteria exhibited high activity within the lacustrine sediment despite their undetectable abundance in the original drill core samples.

Our attempt to isolate an active methanogen resulted in a novel strain. Phylogenetic analysis of the 16S rRNA gene and conserved archaeal genes showed that the new strain fulfills the criteria of being a novel species of the genus *Methanosphaerula*. The name *Methanosphaerula subterraneus* sp. nov. is proposed. Strain EG is the type strain of *Methanosphaerula subterraneus*. Future study on the physiology of *Methanosphaerula subterraneus* could open a window to microbial adaptation to high CO_2.

P-AR-038

Utilizing *Methanothermobacter thermautotrophicus* ΔH as an expression host for recombinant enzymes

*S. Farmer[1], B. Molitor[1]
[1]*Leipzig University, Institute of Biochemistry, Leipzig, Germany*

Due to the increasing global temperature, research across various fields to counteract climate change remains an ever-present necessity. Reducing the amount of carbon dioxide in the atmosphere through carbon capture at industrial sites of emission and converting it into methane shows promise as one avenue to become independent of fossil fuels. Using *Methanothermobacter spp.* as biocatalysts in the power-to-gas platform to convert hydrogen produced from renewable electricity and captured carbon dioxide into methane piqued industrial interest. Another area of interest is taking the platform one step further and using *Methanothermobacter spp.* to produce more valuable chemicals from the same renewable sources. Recent research from our lab developed the fundamental tools for *Methanothermobacter thermautotrophicus* ΔH genetic research, such as functional conjugation and electroporation protocols, two selective markers in a shuttle vector system, and a thermostable Cas9 for genome editing. The immediate use of *M. thermautotrophicus* ΔH as a model microbe is to produce key homologous and heterologous recombinant enzymes for isolation and purification. We will present on the initial findings and methodology in the expression of recombinant enzymes, as well as further optimization of the electroporation protocol. Utilizing *M. thermautotrophicus* ΔH as a production host will provide key insight into the gene expression and role of these enzymes in methanogenesis and these developments would push M. thermautotrophicus ΔH forward as a model methanogen for fundamental research and industrial application.

P-AR-039

Function of KaiC homologs in cell shape regulation and motility in the archaeal model organism *Haloferax volcanii*

*K. Chou[1], J. Gassmann[1], A. Eulitz[1], S. Gfrerer[1], P. Nußbaum[1], S. V. Albers[1]
[1]*University of Freiburg, Freiburg i.Br., Germany*

The circadian clock is a fundamental time tracking system in organisms that regulate biological processes according to the light/dark cycle. KaiC is the core protein of the circadian clock in cyanobacteria, which autophosphorylates and interacts with KaiA and KaiB, regulating biological processes like photosynthesis and nitrogen fixation in *S. elongatus*. KaiA binds with de-phosphorylated KaiC and enhance its activity and KaiB binds to phosphorylated KaiC and catalyses its de-phosphorylation. Four KaiC homologs have

been identified in *Haloferax volcanii*, even though it lacks a light-dark cycle, and most of their function remains unclear. Nohomologs of KaiA and KaiB have been found in this modelorganism, but according to a recent publication by M. Pohlschroder et al, the deletion of a KaiC homolog (*cirA*) produced a hypermotile strain and an expression in trans decreased the motility.

The goal of our project is to obtain new insights about the function of the KaiC homologs in *H. volcanii*. To determine whether they play a regulatory play and if they are involved in other cell processes, deletion mutants of the four KaiC homologs were analysed for their cell shape, and motility. Moreover, all four proteins were expressed as GFP fusion proteins and their localisation was examined during different growth stages. The results provided hints that these proteins are indeed involved in the regulation of cell shape and motility

Biology of yeast and fungi

P-BYF-040

Draft genome sequence and description of a complete set of extracellular peptidases for an ascomycete fungus *Aspergillus ustus*

*A. Shestakova[1], A. Fatkulin[2], A. Osmolovskiy[1], E. Popova[1]
[1]*Lomonosov MSU, Microbiology, Moscow, Russian Federation*
[2]*HSE University, Department of Cell and Molecular Biotechnology, Moscow, Russian Federation*

Aspergillus fungi are ubiquitous, and found in environmental, indoor, patient, and other samples. For the organism, extracellular enzymes, particularly proteolytic enzymes, are crucial for interaction with their surroundings. In this research, we decided to characterize a full set of extracellular peptidases of *Aspergillus ustus*, a species frequently isolated from indoor samples and previously described as capable of producing proteolytic enzymes [1].

Therefore, we aimed to sequence the genome of the *A. ustus* MSU strain from the mycotheca of Lomonosov MSU and develop a tool for predicting peptidases encoded in the genome. Paired-end Illumina NovaSeq sequencing technology was chosen, and the genome was assembled using SPAdes v. 3.15.5 and annotated with AUGUSTUS. The peptidase prediction tool was written in Python, using MEROPS as a reference database and SignalP 6.0 as a localization filter. Additional analysis of predicted peptidases with higher E-values was done manually using InterProScan.

As a result, the whole genome of *A. ustus* was sequenced with a coverage of 92 and assembled (GenBank accession number JBCHKC000000000) with a total size of 42 Mb. A total of 16,459 genes were predicted for this assembly, and among them, 51 were predicted to be extracellular peptidase-encoding genes. The tool used for this prediction was named Protty and is available on GitHub [2]. Among the predicted peptidases, there are representatives of aspartic (10), serine (22), cysteine (1), glutamic (1), threonine (1), and metallopeptidases (16). Additionally, four internal inhibitors were also found in the genome.

Thus, a genome of A. ustus MSU was sequenced, assembled, and annotated for the first time with 16,459 predicted genes, including 51 genes encoding extracellular peptidases. Python-based peptidase prediction tool called Protty was created and released on GitHub.The identified peptidases of *A. ustus* included representatives from all major peptidase families, and four internal inhibitors were also discovered.

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[2] - Protty: Protty: A pretty simple tool for protease prediction. Github; Available from: https://github.com/ArtemF42/protty

P-BYF-041

The tRNA splicing endonuclease (SEN) complex localization in the model fungus *Ustilago maydis*

*M. Vázquez-Carrada[1], N. K. Stoffel[1], L. van Wijlick[1], M. Feldbrügge[1]
[1]*Heinrich Heine University, Microbiology, Düsseldorf, Germany*

Transfer RNAs (tRNAs) are synthesized by RNA-polymerase III in the nucleus and modified by multiple enzymes before entering the protein translation process. In recent years intron-containing tRNAs have been detected in all three kingdoms of life. Splicing of intron-containing tRNAs is accomplished by the well-conserved splicing endonuclease (SEN) complex. Although splicing of intron-containing tRNAs is an essential process, it has been discovered that the localization of the SEN complex differs significantly between species.

In eukaryotes, the SEN complex comprises four essential subunits, with Sen2 and Sen34 forming the catalytically active site and, Sen15 and Sen54 forming structural components of the complex. Surprisingly, in humans and vertebrates, it was discovered that the SEN complex is located in the nucleus whereas tRNA splicing in the budding and fission yeast ascomycetes takes place on the cytosolic surface of mitochondria. Despite its high grade of conservation, this finding indicates that species-specific mechanisms evolved to ensure subcellular trafficking of intron-containing tRNAs and the SEN complex.

The basidiomycete fungus *Ustilago maydis* has emerged as a new model system for cell biology. The genome-wide analysis demonstrated that *U. maydis* is more closely related to humans than to budding yeast and therefore offers a unique system to study mammalian processes. Interestingly, almost all tRNAs in *U. maydis* contain introns and sequence identities of SEN complex subunits with a human range between 28% to 50%, indicating a very high degree of conservation between the two organisms. Using state-of-the-art life-cell imaging coupled with genetic engineering we unravel the subcellular localization of the SEN complex in *U. maydis*, which might help to understand tRNA biology and its associated human diseases in the future.

P-BYF-043

Unraveling the complete toolbox *U. maydis* utilizes for sucrose metabolization

*T. Berwanger[1], J. C. Pohlentz[1], K. Schipper[1]
[1]*Heinrich Heine University, Düsseldorf, Germany*

Sucrose is a common carbon source in nature, which is utilized in plants as a transport sugar and metabolized by microbes. One microbe which thrives on sucrose during infection is the corn smut fungus *Ustilago maydis*. *U. maydis*

has a dimorphic lifestyle. It grows saprotrophically in a haploid yeast form, while together with a compatible mate it forms a dikaryotic hyphae, which infects the plant. It infects *Zea mays* in a biotrophic manner, resulting in the formation of tumors that spread spores. Till now, the sucrose metabolism of *U. maydis* during the different stages of the life cycle is not described in detail. So far it was assumed that *U. maydis* uses a high-affinity sucrose transporter (Srt1) for import, a dual localized invertase (Suc2), which can act both intra- and extracellularly and an additional intracellular invertase that we termed Suc1. The overall aim of this study was to understand the sucrose metabolism in the biotrophic plant pathogen *Ustilago maydis*.

We identified relevant enzymes bioinformatically and confirmed their activities by yeast complementation assays. Deletion mutants were generated to verify the observations. Axenic growth experiments on media supplemented with sucrose were conducted with *U. maydis* deletion mutants growing in the yeast form to determine the need of transporters and invertases. Maize infections with deletion mutants were conducted to study their relevance during infection.

A combination of bioinformatics and yeast complementation assays revealed that *U. maydis* employs additional two intracellular invertases. This results in a total of four invertases of which three are only intracellular and one can be secreted but also has an intracellular form. Additionally, to the known transporter Srt1, *U. maydis* utilizes Agt1 for sucrose import, which was described in *S. cerevisiae* as a maltose transporter. Growth experiments with deletion mutants uncovered, that Ima1 and Agt1 are the main responsible invertase and transporter, respectively, during axenic growth on sucrose.

We display that *U. maydis* employs a complex toolbox of invertases and transporters, which may result from its different life stages and reflect the competition between the plant immune system and the infection strategy of the fungus.

P-BYF-044
Itaconic acid: Logistics and cooperativity of mitochondrial metabolism
*A. Müsgens[1], L. M. Blank[1]
[1]RWTH Aachen University, Aachen, Germany

The smut fungus *Ustilago maydis* secrets itaconic acid under nitrogen limitation. Recently, there has been a significant scientific emphasis on increasing production parameters as itaconic acid is a valuable platform chemical for the envisaged bio-economy. However, the economic and logistical aspects of itaconic acid production have remained largely unexplored.

Since itaconate is directly synthesized from cis-aconitate, an intermediate of the TCA cycle, its production is highly dependent on mitochondrial activity. Transporters facilitating the exchange of carbon compounds between cytosol and mitochondrial matrix are essential for controlling this cross-compartment metabolic network between *Ustilago maydis* and its endosymbiont. Two mitochondrial transporters hold particular significance for itaconate production. Mtt1 mediates the export of cis-aconitate into the cytosol, while the pyruvate transporter enables carbon originating from glycolysis to enter the mitochondrial matrix. We aim to modify the mitochondrial activity by tuning the expression of mitochondrial transporters.

P-BYF-045
Morphological traits of ectomycorrhizal fungi in symbiosis
*H. Wege[1], H. Reichelt[1], E. Megalonidou[2], J. Muhr[2], I. Teichert[1]
[1]Georg-August University Göttingen, Forest Botany and Tree Physiology, Göttingen, Germany
[2]Georg-August University Göttingen, Laboratory for Radioisotopes, Göttingen, Germany

Mycorrhiza are a mutualistic interaction between numerous fungi and approximately 90 % of land plants, making them important for diverse ecosystems. Boreal and temperate forest trees interact with ectomycorrhizal fungi (EMF) that form three characteristic morphological traits: a hyphal mantle around the fine root tips, a network of hyphae between the root cortex cells (the Hartig net) and hyphae reaching into the soil for certain distances (extraradical mycelium). In ectomycorrhizal interactions, fungi gain carbohydrates from the trees, while the trees gain access to mineral nutrients and water. Additionally, current studies underline the role of EMF in protection of trees from biotic and abiotic stressors, such as drought and herbivore attack. Still, the role of mantle, Hartig net and extraradical mycelium in these different aspects of EMF function has not been elucidated.

Here, we study the symbiotic interaction between the basidiomycete EMF *Paxillus involutus* and the poplar tree *Paxillus x canescens* in a laboratory system. We employ different *P. involutus* strains that differ in their ability to form a Hartig net in poplar roots. Using Petri dish mycorrhization experiments, followed by microscopic analysis of root cross sections, we examine the extent to which different strains penetrate the root cortex. These experiments are carried out in different physiological conditions regarding the nutrition status of the fungus and / or the plant partner. We aim to test the impact of Hartig net formation on nutrient exchange and protection from drought stress in potted poplar plants. Further, we perform phosphorus uptake experiments with *P. involutus* to understand the dependency of nutrient transfer from the substrate to the EMF and further into the plant on Hartig net formation and on environmental conditions. Our results will further the understanding of EMF functions and point out means to conquer the effects of climate change on forest ecosystems.

P-BYF-046
High melanization of dark septate endophytes: An advantageous trait for plant colonization and stress tolerance (DARK&STRONG)
*D. Gaber[1,2], L. Lass[3], A. Maes[4], J. Zhoub[5], F. Hillmann[3], M. Chalot[5], P. Franken[1,6], D. Blaudez[4]
[1]Erfurt University of Applied Sciences, Research Centre for Horticultural Crops (FGK), Erfurt, Germany
[2]Assiut University, Faculty of Science, Department of Botany and Microbiology, Assiut, Egypt
[3]Hochschule Wismar University of Applied Sciences, Biochemistry/Biotechnology, Campus Insel Poel, Faculty of Engineering, Wismar, Germany
[4]Université de Lorraine, CNRS, LIEC, Nancy, France
[5]Université de Franche-Comté, CNRS, Chrono-environnement, Montbéliard, France
[6]Friedrich Schiller University, Institute of Microbiology, Jena, Germany

Dark septate endophytes (DSEs) are a sub-group of root ascomycetous endophytes that are highly melanized, commonly found worldwide, particularly under stress conditions. They have a high potential to form successful symbiotic association in plant production systems. The objective of the DARK&STRONG project is to better understand the melanization process in a model DSE species (*Rhexocercosporidium* sp.) and what role high melanization plays in stress tolerance and root colonization of host plants. The aim could be reached through, i) the production of new chemical and biological strains allowing further comparison of strains with contrasted levels of melanization, ii) production of new genetically engineered either melanin deficient strains or overexpressors of *Rhexocercosporidium* sp. and iii) study the impact of melanization on responses to abiotic and biotic stress. The genome of the model endophyte Me07 strain has been sequenced and the genome annotation is currently being analysed. Several albino strains were obtained by non-targeted agrotransformation and screening a library of KO mutants. The albino clones have been resequenced to identify the T-DNA insertion sites and will be tested phenotypically. Fungal melanins have also previously shown to protect from predation by fungivorous amoebae. Our preliminary results show that *Rhexocercosporidium* sp. strain Me07 is a potential food source of *Protostelium aurantium*, a fungivorous amoebae that feeds on conidia and hyphae of ascomycetous fungi. We are currently screening various melanin deficient mutants for their ability to serve as food source for *P. aurantium*. To study the melaninization impact on responses to abiotic stress, we successfully acclimatized the strain Me07 to salt, drought and heat stresses. In the ongoing experiments melanin content will be quantified in both acclimatized and non-acclimatized strains to identify biologically different melanin content strains which will be used in further experiments. The project results will facilitate the establishment of keystones for the development of highly melanized DSEs novel inocula to help plant crops in overcoming challenges caused by climate change, including drought, heat, salt, heavy metal contamination, and nutrient insufficiency.

P-BYF-047

Establishing transformation systems for ectomycorrhizal fungi

V. Kreszies[1], E. Friedrichs[1], B. Harders[1], T. Mosig[1], S. Tasnim[1], V. Woge[1], S. Herzog[1], *I. Teichert[1]

[1]Georg-August University Göttingen, Forest Botany and Tree Physiology, Göttingen, Germany

Mycorrhizal symbiosis between fungal microorganism and plants is an important feature in many ecosystems. Ectomycorrhizal fungi that remain outside of plants cells are important as partners for trees of temperate and boreal forests. The fungal partners are mostly ascomycetes or basidiomycetes that can be generalists or specialists. During symbiosis, the fungus transfers water and minerals to the plant, while the plant transfers carbohydrates to the fungus. Although many aspects of ectomycorrhizal fungi have been studied in detail, molecular genetic work and functional characterization of genes remains scarce.

Here, we aim to establish transformation methods for two ectomycorrhizal fungi, the ascomycete *Cenococcum geophilum* and the basidiomycete *Paxillus involutus*, which both undergo symbiosis with different tree species, including the genetically tractable poplar. We test selection markers for transformation, including different antibiotics and carboxin, and show strategies for cloning of the corresponding genes into suitable vectors. An important aspect in genetic transformation of basidiomycetes is the promoter; thus, we further show data on Golden Gate vectors for easy promoter exchange. Concerning transformation methods, we work on both, protoplast-mediated and *Agrobacterium*-mediated transformation. Since *P. involutus* is dikaryotic, we aim to generate monokaryons, which are easier to transform in some basidiomycete species due to their single nucleus and the formation of vegetative spores. With a robust transformation system for ectomycorrhizal fungi, we will be able to study gene functions and to learn more about the molecular mechanisms underlying ectomycorrhizal symbiosis, which is of major importance for temperate and boreal forests.

P-BYF-048

Proteome analysis of programmed cell death in *Aspergillus fumigatus* conidia as basis for a "dead or alive" reporter strain

*M. Händel[1], K. Gonzalez Rojas[1], M. Kawashima[1], T. Krüger[1], S. Schäuble[2], T. Heinekamp[1], A. Brakhage[1]

[1]Leibniz Institute for Natural Product Research and Infection Biology, Molecular and Applied Mikrobiology, Jena, Germany

[2]Leibniz Institute for Natural Product Research and Infection Biology, Microbiome Dynamics, Jena, Germany

Conidia produced by the human-pathogenic fungus *Aspergillus fumigatus* are the main cause of invasive aspergillosis in immunocompromised patients. In the lung, alveolar macrophages phagocytose the inhaled conidia and process them intracellularly, thereby clearing them from the airways. However, the fungus interferes with this mechanism to avoid being killed and antifungal compounds are needed to treat the infection and support the immune system. However, many currently available drugs exhibit adverse side effects, and the development of new therapeutic strategies is urgently needed. Now, it is challenging to distinguish between resting and dead conidia *in situ*, as they do not show easily detectable metabolic or morphological differences. The generation of a reporter strain that produces a cell death-associated protein fused with the green fluorescent protein would facilitate the differentiation between dead and living conidia by fluorescence microscopy. To identify a suitable reporter protein, we investigated the induction of programmed cell death in *A. fumigatus* conidia. An *in vitro* cell death assay was established by treating conidia with various cell death-inducing compounds, testing different concentrations, time points and cultivation media. The results demonstrated that H2O2 in a nutrient-rich medium kills resting conidia better than the antifungal drugs amphotericin B or voriconazole. On the basis of these data, the optimal conditions for proteome analysis were determined. By comparing the proteome of resting, swollen and dying conidia, we identified eleven proteins which are differentially abundant during regulated cell death. The further analysis of these proteins will provide mechanistic insights into programmed cell death in *A. fumigatus* conidia. Moreover, these proteins were used to generate GFP reporter strains and their potential use as an indicator of conidial cell death is to be tested in co-incubation studies of conidia with RAW 264.7 macrophages.

P-BT-049

Construction of a MoClo-compatible vector for efficient protein production and secretion in *Bacillus subtilis*

*L. N. Worster[1], M. Seidel[1], S. Gebhard[1]
[1]*Johannes Gutenberg-University Mainz, Biology, AG Gebhard, Mainz, Germany*

Heterologous protein production requires a suitable vector system to ensure adequate expression rates, correct protein folding and, where desired, efficient secretion. With a Modular Cloning (MoClo) approach, the construction of standardised parts allows the creation of complex genetic constructs using type II restriction endonucleases. These enzymes cut outside of their recognition sequence, allowing the design of individual four base pair overhangs to assemble multiple parts in a defined order within a one-pot reaction. MoClo can then be used to build genetic circuits for the production of complex proteins, such as spider silk proteins, spidroins. These proteins exhibit incredible chemo-mechanical properties, but researchers still face problems with low production rates due to their high molecular weight and repetitive nature. Here we show the construction of three MoClo-compatible *E. coli - B. subtilis* shuttle vectors, based on the parent vector pBSMuL1. This vector was constructed for the overproduction and secretion of heterologous proteins in *B. subtilis* [1]. Our MoClo-compatible vector system allows the exchange of the coding sequence or even the replacement of the entire transcription unit. This makes an individual change of the coding sequence and regulatory elements possible, allowing easy exchange of constitutive and inducible promoters, different ribosomal binding sites and transcription terminators. It is also possible to change signal sequences upstream of the coding sequence for protein secretion and add purification tags downstream for protein purification. In each construct, a constitutively expressed mRFP1 cassette, which leads to red colouration of *E. coli* colonies, provides a visible and economical selection for successful insertion during cloning. The functionality of the MoClo-compatible pBSMuL1 shuttle vector will be tested for the production of synthetic spider silk proteins in the Gram-positive host *B. subtilis*. Spidroins are known as high-performance materials because they combine strength, toughness and elasticity. This makes them an environmentally friendly component for building materials and, in the form of long fibres, ideal for use in the textile industry. [1] Brockmeier, U., Wendorff, M., & Eggert, T. (**2006**). Versatile expression and secretion vectors for *Bacillus subtilis*. *Current microbiology*, 52(2), 143–148. doi.org/10.1007/s00284-005-0231-7

P-BT-050

Comprehensive analysis of *Trametes sanguinea*-mediated wood degradation facilitated by mediators: Insights from SEM, FTIR and UV Spectroscopy

*A. Dulaj[1]
[1]*Institute of Biotechnology and Drug Research, Mainz, Germany*

Lignin and hemicellulose, key components of plant cell walls, are crucial for structural integrity but are difficult to be microbially degraded. Lignin, a complex and rigid polymer, strengthens the cell wall and resists microbial breakdown. Hemicellulose, a more flexible heteropolymer, links lignin and cellulose and is easier to hydrolyze. This study explores an eco-friendly method for degrading wood waste through fungi to enable its reuse in various applications.

White-rot fungi, particularly *Trametes sanguinea*, is are known for their degradation potential due to their many enzymatic systems. Genome sequencing of *T. sanguinea* revealed that there are seven distinct laccase enzymes in *T. sanguinea* that are likely involved in the degradation of wood.

The degradation efficacy of *T. sanguinea* was examined when supplemented with various mediators, including 1-hydroxybenzotriazole (HBT), gallic acid (GA), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), bipyridine (bpy), vanillic acid, and acetosyringone. A comprehensive analytical approach was employed, utilizing scanning electron microscopy (SEM), Fourier transform infrared spectroscopy, and ultraviolet spectroscopy to assess structural and chemical modifications in supplemented wood powder samples. SEM analysis revealed significant fragmentation and increased porosity in samples supplemented with HBT and GA. FTIR indicated the disruption of lignin and hemicellulose linkages, evidenced by changes in the C=O and C-O stretching regions. UV spectroscopy confirmed the formation of new functional groups, particularly with HBT and GA. Among the mediators tested, usage of HBT and GA lead to the highest enzymatic activity and degradation effect on the treated wood powder highlighting their potential to enhance fungal-mediated wood degradation for sustainable recycling processes.

P-BT-051

Evaluation of the white rot fungus *Phlebia radiata* for the application in the degradation and re-valorization of softwood Kraftlignin

*L. Schön[1]
[1]*IBWF, Mainz, Germany*

Fungi, particularly wood-degrading species, play a crucial role in ecosystems by breaking down complex organic materials such as lignin. White rot fungi, are of particular interest due to their lignin-modifying enzymes (LMEs), including laccases (EC 1.10.3.2), manganese peroxidases (EC 1.11.1.13), and lignin peroxidases (EC 1.11.1.14). These enzymes are highly efficient in degrading lignin, making them promising candidates for biotechnological applications such as managing industrial wastes like Kraftlignin (KL).

Tones of KL are produced annually as byproduct of the pulping process in the paper industry and often remain underutilized, i.e., being burned for energy rather than valorized into high-value products. In this study, we aimed to address this issue by screening nearly 100 fungal species from the collection of the IBWF for their ability to produce LMEs, using the redox-indicators ABTS, Poly-R, and Azure B as substrates. Among the top performers was *P. radiata*, a fungus known for its LME production.

In a liquid fermentation, supplemented with KL, the degradation capabilities of *P. radiata* were further investigated. Culture filtrate extracts were analyzed using high-performance liquid chromatography (HPLC) to assess the extent of KL degradation. After a two-week fermentation period a significant decrease in signals linked to KL could be seen in the chromatograms. Additionally, novel signals indicated the formation of so far uncharacterized degradation products.

P. radiata showed significant potential in the re-valorization of KL, offering a sustainable approach to converting industrial waste into valuable chemical products. This study

highlights the importance of fungal biotechnology in developing innovative solutions for waste management in the paper industry.

P-BT-052
Degradation of the hazardous chemical 2-Mercaptobenzothiazole by filamentous fungi
*K. Heeb[1], R. Morina[2], V. Hering[2]
[1]Institute of Biotechnology and Drug Research gGmbH (IBWF), Mainz, Germany
[2]Johannes Gutenberg-University Mainz, Microbiology and Biotechnology, Mainz, Germany

2-Mercaptobenzothiazole (2-MBT) is used as a vulcanisation accelerator in the production of rubber products. It is environmentally hazardous and potentially carcinogenic. We found that the filamentous fungi *Agrocybe pediades* and *Alternaria sp.* are capable of degrading 2-MBT in liquid culture. Therefore, *Agrocybe pediades* and *Alternaria sp.* are of significant interest for bioremediation of soil and industrial wastewater. *Agrocybe pediades* and *Alternaria sp.* were cultivated in various complete media with 30 mg/L 2-MBT. After a maximum of seven days, 2-MBT was completely degraded. Through protein purification using hydrophobic interaction chromatography, we isolated a protein fraction that achieved 50 % degradation of 2-MBT within 16 hours. Further analysis of this fraction offers potential for identifying enzymes that catalyse 2-MBT degradation.

We are further interested in elucidating the exact degradation pathway of 2-MBT, as little is known about this process in fungi. A potential metabolite with a mass of 151 Da was identified by HPLC mass spectrometry after cultivation of *Agrocybe pediades* in medium supplemented with 2-MBT. This mass might correspond to 2-hydroxybenzothiazole, marking the first indication of this metabolite in the fungal 2-MBT degradation pathway.

In the future, RNA sequencing will be conducted of fungi incubated with 2-MBT, compared to those incubated without 2-MBT. This approach aims to provide deeper insights into the gene regulatory processes essential for 2-MBT degradation. This may facilitate upscaling of the degradation process, for example through the manipulation of gene expression.

P-BT-053
Exploring a glutathione *S*-transferase from *Rhodococcus opacus* PD630 for aliphatic epoxide conversion
*M. Scholz[1], M. Haarmann[1], D. Tischler[1]
[1]Ruhr University Bochum, Faculty of Biology and Biotechnology, Bochum, Germany

Enantiopure epoxides are of great interest in industry as they are valuable intermediates in organic synthesis for the production of fine chemicals, perfumes, polymers or pharmaceuticals. Numerous enzymes have been identified for their ability to catalyze the production of enantiomerically pure epoxides. This includes styrene monooxygenases, cytochrome P450 monooxygenases or unspecific peroxygenases. Recently, investigations on a glutathione *S*-transferase (GST) from the glutathione-dependent styrene degradation pathway revealed its ability to be used for the production of enantiopure aromatic epoxides.

Interestingly, there are no known GSTs that demonstrate the ability to perform the enantioselective conversion of aliphatic epoxides. As a consequence, an actinobacterial GST capable of converting aliphatic epoxides enantioselectively was screened for. One promising GST, designated *Rho*IsoI, was found in the isoprene-degrading actinobacterium *Rhodococcus opacus* PD630 and was subsequently biochemically characterized.

In this study, the GST was initially analyzed for their ability to convert aliphatic epoxides with an enantiomeric preference *via* GC-FID. The results demonstrated that *Rho*IsoI exhibited significantly higher activity towards the aliphatic epoxide isoprene monoxide in contrast to the aromatic styrene oxide. An enantiomeric preference for both substrates was observed. Size exclusion chromatography revealed that this enzyme occurs as a homodimer. Spectrophotometric measurements using the model substrate CDNB showed optimal thermal stability at 25°C for this enzyme. Different additives had no significant positive or negative influence on the activity of *Rho*IsoI except for zinc and nickel sulphate. In this case, the relative activity decreased to 24% and 27%, respectively.

Overall, this study presents a promising GST capable of converting the aliphatic isoprene monoxide with high activity. However, detailed investigation on more aliphatic epoxides shall demonstrate the potential of using *Rho*IsoI to produce enantiopure epoxides.

P-BT-054
Design and assembly of a synthetic pyriform spider silk expression platform in *B. subtilis*
*C. Hammer[1], M. Seidel[1], S. Gebhard[1]
[1]Johannes Gutenberg-University Mainz, Institute for molecular physiology / Biology, Mainz, Germany

Spider silk possesses superior mechanical properties, like high tensile strength combined with low weight density, even if compared to Kevlar or steel fibres. As it is also biocompatible and biodegradable, it harbours a huge potential for many applications. So far, most focus has been on drag line silk, however there are many different types of spider silk with different properties. Pyriform silk, which is used by the spiders to anchor their web to the surroundings, forms a matrix-like structure that can be useful in a variety of applications in which co- and adhesion are needed. While the heterologous production of spider silk is possible, it still poses problems, due to the repetitive nature of the genes and the length of their ORFs, making DNA assembly and cloning a challenge. Synthetic biology is perfectly suited to solve these problems by creating standardized parts of the silk genes for an efficient construction process. Here, we present the design of a novel synthetic spider silk gene assembly and expression strategy with harmonised codon usage for protein production in *B. subtilis.* For this, we split the silk gene into three modular parts, the N-terminal element, a repeat element and the C-terminal element. In the wild-type pyriform silk gene, the repeat element occurs 17 times between the N- and C-terminal domains, rendering standard cloning protocols not viable for the assembly of expression constructs. To overcome this, we developed a new hybrid DNA assembly strategy, based on the Modular Cloning (MoClo) and the BioBrick RFC25 standards. This strategy enables an easy and flexible way of assembling expression constructs with increasing complexity and varying numbers of repeats from defined building blocks. With these constructs we aim to establish an expression system for spider silk in *B. subtilis*, which has great potential for

industrial applications. Especially the material sciences could benefit by this synthetic pyriform silk, which may provide an adhesive matrix for other compounds.

P-BT-055

Protein engineering of a Vanillyl alcohol oxidase for biocatalysis

*N. Weindorf[1], J. M. Sommer[1], D. Tischler[1]
[1]*Ruhr University Bochum, Faculty of Biology and Biotechnology, Bochum, Germany*

Vanillyl alcohol oxidases (VAOs) are flavin-dependent oxidases, belonging to the VAO/PCMH flavoprotein family and are found in ascomycetous fungi, where they are involved in the natural degradation of lignin. VAOs perform oxidation reactions on *p*-substituted phenols, including oxidations of alcohols, hydroxylation reactions, dehydrogenation reactions, deamination reactions and oxidative ether cleavages. To date, two VAOs have been identified, from *Penicillin simplicissimum* (*Ps*VAO), and from *Diplodia corticola* (*Dc*VAO). *Dc*VAO was identified by a phylogenetic comparison of the subgroup of the 4-phenol oxidases from the VAO/PCMH flavoprotein family. For this, focus was put on the comparison of the active sites, which has been elucidated for *Ps*VAO. VAOs harbour a relatively conserved active site however, there are differences, which are mainly found in regions, responsible for interactions with the substrate molecule. *Dc*VAO stood out as it harbours an exchange of two amino acids at positions 424 and 470 (*Ps*VAO numbering), where a Phe and a Cys are exchanged by an Ala and a Glu. We characterized *Dc*VAO and found that it has the same reaction pattern as *Ps*VAO however, *Dc*VAO can convert 2,6-dimethoxy substituted phenols, while *Ps*VAO cannot. In addition, *Dc*VAO also performed better in oxidative ether cleavages. We suspected that the gained space by the Phe Ala exchange allows for the conversion of 2,6-dimethoxy substituted phenols, while the Glu may enhance ether cleavages. It could be shown that the introduction of these two is allowing for the correct positioning of 2,6-dimethoxy substituted phenols, as an exchange of the Glu by Cys resulted in similar activities on ethers, while hydroxylation reactions were greatly reduced. *In silico* experiments showed that in *Dc*VAO, that substrates are differently oriented compared to *Ps*VAO, which seems to be needed to accommodate 2,6-dimethoxy substituted phenols. This would make *Dc*VAO a good candidate for a biocatalyst for the valorisation of lignin sourced from hardwood, as this contains almost exclusively 2,6-dimethoxylated phenolic building blocks. The initial characterization of *Dc*VAO however also indicated that in its wildtype form is not that applicable for biocatalysis.

To drive *Dc*VAO towards a more applicable biocatalyst, we set out to perform rational design to enhance its thermostability, influence its stereoselectivity and to optimize its reaction conditions.

P-BT-056

Exploring exopolysaccharide formation in *Vibrio natriegens* and its effect on anaerobic succinate production

*M. Hädrich[1], C. Scheuchenegger[1], J. Borger[1], B. Rühmann[2], M. Döring[2], F. Thoma[1], V. Sieber[2], B. Blombach[1]
[1]*Technical University Munich, Microbial Biotechnology, Straubing, Germany*
[2]*Technical University Munich, Chemistry of Biogenic Resources, Straubing, Germany*

In recent years, *Vibrio natriegens* emerged as new chassis for biotechnological applications due to its high growth and substrate consumption rates. The bacterium was engineered for various production processes, from amino acids over carboxylic acids and alcohols to pigments (reviewed in [1] and [2]). However, we noticed an increase of viscosity in larger lab-scale fermentations of *V. natriegens* due to the formation of exopolysaccharides (EPS), negatively affecting oxygen transfer to the culture broth. To overcome this issue, we deleted genes and gene clusters related to EPS formation, namely the *syp* and *cps* clusters, as well as the transcriptional regulator *cpsR* and the transporter *wbfF*. All strains continued to produce EPS in different quantities. However, deletion of *cpsR* and *cps* consistently led to low viscosity in the reactor broths throughout the 33-hour fermentation period.

The gained knowledge was then applied to a production process of succinic acid with *V. natriegens* Succ1 [3], which also showed an increase of viscosity over time during the process. Deletion strains of *cpsR* and *cps* in *V. natriegens* Succ1 had no observed viscosity increase during fermentation. The yield of the process was even increased by 27% to a $Y_{P/S}$ of 1.4 mol_{Suc} mol_{Glc}^{-1} [4]. In conclusion, we could successfully improve bioreactor fermentations with *V. natriegens* by addressing challenges imposed by EPS formation and resulting high viscosity to enhance succinate production.

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P-BT-057

Characterization of the dehydrogenase StyH and its role in a glutathione-dependent styrene degradation pathway

*T. Rapsch[1], M. Haarmann[1], D. Eggerichs[1], D. Tischler[1]
[1]*Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany*

Styrene is a precursor for thermoplastic polymers which are of industrial importance. A major issue is the anthropogenic release which burdens the environment. The toxic properties of styrene effectuated some soil-dwelling bacteria to develop degradation pathways detoxifying styrene or utilizing it as a carbon source. Consequently, these organisms became attractive targets for bioremediation. Recently, the unique degradation pathway of *Gordonia rubripertincta* CWB2 was described. A strain with further biotechnological relevance as CWB2 can degrade a styrene analog to produce ibuprofen in a co-metabolic process.

Herein, we present a newly characterized dehydrogenase (StyH) with the aim to elucidate its function and role in the pathway. Thus, improving the overall understanding of the CWB2 styrene degradation pathway and increasing its value as a potential candidate for bioremediation or biotechnological applications.

StyH was heterologously produced in *E. coli* BL21 (DE3), purified by IMAC, and characterized using bioinformatics as well as biochemical methods. The biochemical characterization of StyH was conducted spectrophotometrically to investigate optimal reaction conditions, kinetics and substrate range.

A phylogenetic analysis showed that StyH is a member of the SDR family. Protocols for enzymatic substrate synthesis and purification were established. The process yielded about 72 % (*S*)-GSH conjugate validated by NMR. Enzyme production was successful. StyH forms a homodimer (confirmed by bioinformatics and SEC). It displayed higher activity at basic pH values. Higher Vmax-values were observed with the (*S*)-GSH conjugate compared to the (*R*)-enantiomer. StyH acts specifically on glutathione-conjugated substrates and catalyzes two subsequent reaction steps. The product was identified *via* LC-MS.

Overall, this study provides new information on the function of StyH in the glutathione-dependent styrene degradation pathway. The enzyme is enantioselective, first catalyzing the (*S*)-enantiomer in a racemic mixture. StyH demonstrated a two-step catalysis by oxidation of (*S*)-GSH conjugate the corresponding phenylacetaldehyde, further to (*S*)-(1-phenyl-2-acetic acid) glutathione. Only short-chain alcohols were previously found to be catalyzed via two-step reactions.

P-BT-058

Selection of transcriptional parts as starting point for efficient expression of a synthetic spider silk protein construct in *Bacillus subtilis*

*M. M. Syre[1], M. Seidel[1], S. Gebhard[1]
[1]*Johannes Gutenberg-University Mainz, Mainz, Germany*

Spider silk exhibits outstanding mechanical properties often compared to steel and Kevlar. These properties make spider silk proteins, also known as spidroins, highly attractive for developing both medical and technical materials. The exceptional mechanical properties originate from their long length and the modular and repetitive protein structure. However, producing these proteins through heterologous expression yielded varying degrees of success. One of the challenges encountered is the construction of expression vectors, because of the size and repetitive nature of the coding sequence. To facilitate assembly of expression constructs with repetitive DNA sequences, a special cloning strategy is important. Here we show the adaptation of the Modular Cloning (MoClo) system and choice of suitable transcriptional parts to build an expression system for efficient production of a synthetic spidroin construct in *Bacillus subtilis*. Using a reportergene encoding superfolder GFP, we evaluated the effects of different promotors, and ribosomal binding sites on transcription and translation efficiency in *B. subtilis*. The results from testing individual and paired parts allows the selection of best-suited transcriptional elements as an effective starting point for optimizing recombinant spidroin production in *B. subtilis.* Future work will focus on assessing and comparing the heterologous production of the synthetic spidroin construct in *B. subtilis,* exploring both single- and multi-copy integration strategies. Notably, *B. subtilis* is an ideal host to produce synthetic spidroins on an industrial scale as it possesses an excellent protein secretion machinery, is highly amenable to genetic manipulation and can be easily cultivated on inexpensive substrates.

P-BT-059

Microbial metabolism of vulcanized rubber: Exploring sulfur utilization

*J. Graalmann[1], Y. Preim[1], M. Schlamkow[2], M. Fröse[1], M. Biel[2], P. Siegert[1], J. Pettrak[2], J. Bongaerts[1]
[1]*FH Aachen, Institute of Nano- and Biotechnologies, Jülich, Germany*
[2]*FH Aachen, Institute of Applied Polymer Chemistry, Jülich, Germany*

Due to the growing environmental impact of rubber waste, effective recycling methods are needed. Most rubber waste is downcycled into granulate or incinerated. A potential solution is recycling through devulcanization of waste rubber to allow revulcanization [1]. The goal of this project is to identify sulfur-cleaving bacteria. These bacteria are screened from environmental samples collected in areas exposed to rubber waste (e.g., road sink boxes, rubber conveyor belts) to understand the mechanisms of microbial devulcanization. Environmental samples were grown in minimal media with diallyl disulfide as the sole sulfur source to isolate organisms capable of cleaving sulfur-sulfur bonds. Various actinomycetes (*Streptomyces* sp. [2], *Gordonia* sp. [3]) and *Sphingomonas* sp. showed this ability [4]. The bacterial consortium from these samples will be further characterized for their sulfur bond-cleaving abilities in model substances. Future studies will focus on the analysis of identified bacteria for rubber devulcanization.

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P-BT-060

Utilization of a heterologous exocellular electron transfer pathway in *Escherichia coli*and *Vibrio natriegens*

*H. Fuchs[1], S. R. Ullrich[1], S. Hedrich[1]
[1]*Technical University Bergakademie Freiberg, Institute of Biosciences, Freiberg, Germany*

The Gram-negative acidophile *Acidithiobacillus ferrooxidans* is capable of iron reduction through exocellular electron transfer. Its electron transfer chain spans across both

membranes and the periplasm and consists of three c-type cytochromes and the copper redox protein rusticyanin. To circumvent the low biomass production of acidophilic microorganisms, the proteins were heterologously produced to accelerate protein characterization and interaction studies. The heterologous production of proteins from acidophilic microorganisms in neutrophilic expression hosts presents a challenge mainly due to the difference in extracellular and periplasmic pH which can affect protein folding and co-factor integration. Posttranslational modifications as well as the synthesis and incorporation of heme into the recombinant c-type cytochromes pose an additional challenge for the host. We reconstructed the iron reduction pathway from *At. ferrooxidans* in four different *E. coli* strains and *Vibrio natriegens,* an emerging biotechnology chassis. By screening the periplasmic, cytoplasmic and membrane fractions we showed that *V. natriegens* was able to produce more mature c-type cytochromes and rusticyanin than the *E. coli* hosts. The periplasmic cytochrome c Cyc1 and rusticyanin were correctly translocated into the periplasm of the neutrophilic hosts while the inner and outer membrane-bound c-type cytochromes were present in the membrane fraction exclusively. The recombinant c-type cytochromes heterologously produced by *V. natriegens* and *E. coli* were redox-active. We were able to demonstrate that *E. coli* C43(DE3) is able to utilize the recombinant proteins from *At. ferrooxidans* for ferric iron reduction. To our knowledge, this is the first demonstration of electron transfer functionality of recombinant redox proteins from an acidophile in neutrophiles.

P-BT-061

Designing Bioorthogonal Cu(II) catalyzed click chemistry for chemoenzymatic cascades

*J. Ochs[1], D. Tischler[2], C. E. Paul[3], N. Metzler-Nolte[1]
[1]*Ruhr University Bochum, Inorganic Chemistry I, Bochum, Germany*
[2]*Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany*
[3]*Delft Univerity of Technology, Biocatalysis, Delft, Netherlands*

Chemoenzymatic cascades impress with high overall yields, outstanding enantioselectivity, and favorable environmental impact.[1] A limiting factor for these cascades is the strict implementation of bioorthogonality. Especially, metal catalysts containing copper may cause deactivation of the enzyme by the undesired generation of reactive oxygen species or through non-specific binding to free amino acids.[2] Consequently, metal chelation has been identified as a crucial design feature to facilitate tandem reactions as it prevents non-specific binding to biological motifs and allows judicious modulation of the electronic properties of the catalyst.[3] In this poster, we present a novel chemoenzymatic "Click cascade" that is not affected by the presence of oxygen and does not require reductants. In the first step, a flavin-dependent styrene monooxygenase (SMO) enantioselectively generates *(S)*-styrene oxide as an intermediate, which is subsequently attacked by NaN3, and further functionalized via the bioorthogonal Cu(II) catalyzed Click reaction. For this purpose, we synthesized and characterized four distinct chelated Cu4O4 clusters[4] and one chelated Cu(II) monomeric complex with varying hydrophilicity to investigate the influence of water solubility on their respective performance in conducting the desired Click reactions while minimizing unwanted enzyme inhibition. Furthermore, different approaches to implement the enzyme into the chemoenzymatic cascade, i.e., free, immobilized, or whole cell, were evaluated. Product analysis by HPLC and GC was used to identify the best conditions to conduct the tandem reaction. The substrate scope will be extended to synthesize a variety of enantiopure 1,2,3-triazoles by this novel chemoenzymatic cascade reaction.

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P-BT-062

Artificially engineered P450 monooxygenase fusion constructs in oxyfunctionalization reactions

*M. Mirhadiyev[1], K. Yayci[1], M. Petrovic[1], C. Mügge[1]
[1]*Ruhr University Bochum, Bochum, Germany*

Cytochrome P450s (CYP) are heme-containing enzymes, present in a wide range of organisms, with manifold functions such as hydrocarbon degradation, fatty acid metabolism, hormone biosynthesis and detoxification. This enzymatic group contains CYP153A family(1), which has a large substrate scope with particularly high catalytic efficiency converting long carbon chain groups into alcohols. There are challenges associated with purified CYPs due to reliance on electron transfer partners (e.g., Ferredoxin Reductase and Ferredoxin), nicotinamide cosubstrates such as NAD(P)H and lowered activity outside of their native environment make it difficult for these enzymes to be scaled up for industrial use.

This work addresses the limitations of cytochrome P450 monooxygenases and proposes whole-cell biotransformation as a viable solution for scaling up their use, making CYPs a cost-effective and environmentally friendly option for the production of valuable chemicals. The experiments involved construction of fusion constructs of various class I bacterial cytochrome P450 enzymes (CYPs) artificially linked to different electron transfer partners using distinct linkers. The focus was set on medium-chain aliphatic compounds to evaluate whether these fusion constructs could effectively convert the substrates. Whole-cell biotransformation has the potential to address both the stability and cofactor dependency of P450 monooxygenase enzymes, while the fusion construct enhances the efficiency of the electron transport chain.

Reaction optimization experiments were conducted to increase the yield of the heme-incorporated fusion construct, enhancing the enzyme's catalytic efficiency. Resting cell assays were performed in phosphate buffer with various carbon sources to maintain cell activity without promoting growth. The focus of this research was on compounds where current literature reports low yields with existing biocatalytic methods. Specifically, improving the efficiency of hydroxylation reactions to produce industrially important chemicals has been challenging due to stability issues and the limited catalytic efficiency of known P450s.

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P-BT-063

Addressing the Difficulties in Transforming Non-Standard Microorganisms for Biotechnological Applications

*D. Kostner[1]
[1]*Hochschule München (HM), Research Group Industrial Biotechnology, Munich, Germany*

Question: Transforming non-standard bacterial microorganisms for biotechnological applications represents significant challenges. This study aims to develop a systematic approach to overcome these challenges by identifying unknown antibiotic resistances and sensitivities, selecting appropriate plasmids, and determining the most effective transformation methods.

Methods: The identification of unknown antibiotic resistances and sensitivities represents the first step using sterile culture methods in a multiwell plate reader. For plasmid selection, 2-3 plasmids with different *ori* types, previously validated across standard microorganisms, are chosen. In cases where organisms only replicate plasmids with still unknown origins, a suicide plasmid equipped with a homologous region of the recipient gDNA can be used. This plasmid, despite lacking replication capability, indicates successful transformation through an integrated plasmid with a selection marker. To avoid incompatibility with methylation patterns, the utilization of methylase-negative E. coli strains is recommended. Following plasmid selection, various transformation methods are evaluated (conjugation, electroporation, and chemically competent cells). Additionally, natural transformability is evaluated for its high practicality when successful.

Results: The systematic approach led to the successful development of a transformation method for the biopolymer-producing organisms *Zobellella denitrificans* and *Azohydromonas sp*. The time-saving identification of antibiotic resistances and sensitivities in a multiwell plate reader allowed for a quick selection of appropriate plasmids/resistance cassettes. The evaluation of different transformation methods highlighted conjugation as effective method for Azohydromonas sp. while for Zobellella denitrificans, transformation via natural competence was notably successful. Testing natural transformability, though rare, proved practical and should be considered.

Conclusion: This study presents a systematic approach to develop transformation protocols in non-standard organisms for biotechnological applications. By identifying antibiotic resistances/sensitivities, selecting suitable plasmids, and evaluating various transformation methods, the research successfully developed a transformation method for *Zobellella denitrificans* and *Azohydromonas sp*. This approach can be applied to other non-standard organisms, advancing biotechnological applications and research.

P-BT-064

A cyclic multiplex co-transformation approach to customize *V. natriegens* for protein production

*J. Hoff[1], B. Blombach[1]
[1]*Technical University Munich, Microbial Biotechnology, Straubing, Germany*

V. natriegens" fast growth and high carbon uptake rates render this bacterium a promising host for industrial applications. It is an emerging chassis with a versatile metabolism and a broad range of sophisticated genetic engineering tools being established in recent years [1, 2]. Several studies utilized this host for protein production [3], however, some with moderate success which is attributed to an incomplete understanding of the overall protein turnover in *V. natriegens*. Comparing different fluorophores and media compositions, we found that *V. natriegens* can efficiently degrade target proteins at distinct stages of the growth phase. This indicates that native proteases might play a crucial role in the life cycle of those proteins and shows our incomplete understanding of the overall protein turnover in *V. natriegens*. We mapped over 100 putative proteases and peptidases on the two chromosomes and designed a library of linear DNA to delete those proteases using natural transformation [4]. In a cyclic approach of co-transforming the library of linear DNA fragments alongside with two changeable selection markers we intend to enrich beneficial protease deletions. As naturally competent cells tend to take up and integrate multiple fragments at a time [4], we will receive strains with a random combination of deleted proteases. In every cycle, the antibiotic selection marker is swapped and thereby recovered, changing form chloramphenicol to carbenicillin and *vice versa*. An additional fluorescence selection marker is used to validate a positive impact on heterologous protein expression. Promising candidates are pooled by single cell screening and sorting for the upcoming cycle. Applying this approach, we aim to identify relevant proteases to eventually improve protein production and to gain a better understanding of the degradation machinery in *V. natriegens*.

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P-BT-065

Utilization of D-xylose by *Corynebacterium glutamicum* for the production of value-added products

*F. Gätgens[1], D. Höppner[1], J. Marienhagen[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

Corynebacterium glutamicum is a versatile microbial cell factory used for the industrial-scale production of a broad spectrum of valuable compounds, in particular for proteinogenic amino acids such as L-glutamate and L-lysine (Wolf et al., 2021). *C. glutamicum* can utilize a variety of different carbohydrates, but is not able to utilize the pentose D-xylose.

However, D-xylose is the second most abundant natural occurring sugar. Hence, its industrial utilization is of high interest. In particular since waste streams from different industries (e.g. agriculture, food) contain hemicellulose, which in turn contain pentoses, such as D-xylose or L-arabinose. Nature has developed a variety of different metabolic strategies, e.g. the Weimberg pathway or the isomerase pathway enabling the utilization of D-xylose. Both pathways have been already functionally introduced into

C. glutamicum to further broaden the spectrum of usable substrates (Kawaguchi et al., 2006; Radek et al., 2014).

This study focuses on the production of carbonic acids and alcohols from D-xylose in *Corynebacterium glutamicum*, utilizing heterologous metabolic strategies from different microorganisms with the aim to generate value-added products while keeping both, the production costs and the ecological footprint low.

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P-BT-066

Phosphate limited production of single cell oils with unconventional yeast

*K. E. Schulz[1], K. Ochsenreither[2], A. Neumann[1]
[1]Karlsruhe Institute of Technology (KIT), Institut für Bio- und Lebensmitteltechnik Teilinstitut Elektrobiotechnologie (EBT), Karlsruhe, Germany
[2]Technikum Laubholz GmbH, BIotechnological Conversion, Göppingen, Germany

Introduction

The continued rise of atmospherically CO2 concentrations through the release of millions of years old chemically captured carbon is one of the main reasons for climate change, needing to be replaced with a carbon neutral production of alternatives, mainly for fossil oils. By fixation of CO2, carbon neutral production of lipids is achievable by biotechnological production of single cell oils (SCO) providing a climate change friendly alternative not only for fossil resources but also for plant oils that are in strict concurrence with agricultural cultivation. Typically, SCO are produced by using oleaginous organisms in nitrogen limitation. Our study reports on the analysis of SCO production using phosphate limitation in comparison to nitrogen limitation and unlimited (that is not nitrogen or phosphate limited) conditions using the strains *Cutaneotrichosporon oleaginosum*, *Saitozyma podzolica*, *Apiotrichum porosum*, *Scheffersomyces segobiensis* in 2.5 L fermentation scale aiming to evaluate the feasibility of phosphate limitation as an SCO production inducer. Phosphate limitation would provide the advantage of enabling the usage of nitrogen rich side streams like molasses which are crucial for economic feasibility.

Goals

The analysis of phosphate limitation in comparison to nitrogen limitation investigates the production capabilities of unconventional yeast for generating desired single cell oils enabling a broader usage of side streams with nitrogen rich content. The direct comparison should also evaluate the changes in fatty acid profiles and production rates.

Materials & Methods

The strains were cultivated in 2.5 L stirred tank reactors in unlimited, nitrogen limited and phosphate limited cultivation for 144 h at pH 4. Ortho-phosphate and ammonium analytics were used for detection of limitation, measured cell dry weight, analyzed composition with indirect FAME analysis over GC, sulphur-anthrone assay and DC assay. The lipid production rate, yield and titer were determined.

Results

While phosphate limitation shows a higher specific production rate, the produced amount of lipids in 144 h was higher leading to interesting application possibilities.

Summary

Our results provide insides in different cultivation conditions and their influence on lipid production enabling the usage of high in nitrogen substrates like molasses for lipid production. We also show promising approaches for long- and short-term fermentations.

P-BT-067

Sustainable production of pharmaceutical drug precursors by engineered *Corynebacterium glutamicum*

*S. S. Poethe[1], N. Junker[1], F. Meyer[1], V. F. Wendisch[1]
[1]Bielefeld University, Genetics of Prokaryotes, Faculty of Biology and Center for Biotechnology (CeBiTec), Bielefeld, Germany

Tyramine has become a compound of great interest to the pharmaceutical industry, not only due to its utility as a starting material for hydrogel production, but also as a precursor for a multitude of pharmaceutically relevant compounds. An important example of one of these compounds is *N*-acetyltyramine, which has recently been found to be active against certain multi-drug resistant pathogens and is also a promising anticoagulant since it is inhibiting Factor Xa. However, the chemical synthesis of tyramine and its derivatives is associated with a lack of selectivity and the use of harsh conditions, which are environmentally unfavorable. Accordingly, we aimed to establish the biosynthesis of both tyramine and *N*-acetyltyramine as a sustainable alternative.

Corynebacterium glutamicum represents an optimal host for this objective, as it is a well-established workhorse in the industrial amino acid production, with an output of millions of tons per year. Moreover, it has been engineered for the utilization of a range of monomeric carbon substrates, allowing for a flexible feedstock strategy [1]. Testing the overexpression of various aromatic-l-amino acid decarboxylases in the tyrosine overproducing strain AROM3, the highest tyramine titer of 1.9 g L^{-1} was achieved upon the overexpression of the tyrosine decarboxylase gene of *Levilactobacillus brevis*. Further engineering for an extended substrate spectrum allowed for a tyramine production on the alternative carbon sources xylose, ribose and glycerol. Moreover, the upscaling of tyramine production to a 1.5 L

batch reactor was demonstrated to be stable [2]. In an effort to expand the tyramine synthesis pathway through the additional overexpression of acetyltransferase genes from diverse bacteria and insects, a *de novo* production of 2.5 g L^{-1} *N*-acetyltyramine was achieved by overexpressing the acetyltransferase gene from *Bombyx mori*. Concluding, an efficient production of tyramine and *N*-acetyltyramine from sustainable feedstocks was established, providing a scalable and eco-friendly alternative for chemical synthesis.

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P-BT-068

Substrate acceptance and activity of an alkane monooxygenase from *Marinobacter sp.*

*C. Kaiserer[1], A. Nigl[1], J. Spasic[1], R. Kourist[1]
[1]*TU Graz, Institute of Molecular Biotechnology, Graz, Austria*

For a sustainable production of polymers, the controlled functionalization of simple organic precursors is a key aspect, where highly precise enzymes are envisioned to replace intensive chemical reactions. The alkane monooxygenase AlkB from *P. putida* GPo1[1] is one promising candidate, which hydroxylates terminal methyl groups in a broad range of substrates with high regioselectivity[2]. It was reported to functionalize medium chain fatty acid methyl esters with even higher rates than its natural substrate octane[3], which makes AlkB especially interesting for the synthesis of bifunctional hydroxy fatty acids, as precursors of lactones and polymers[4,5]. This work focuses on AlkB from *Marinobacter sp.,* a related monooxygenase that was identified in a marine metagenomic sample. AlkB from *M. sp.* was heterologously expressed in *E. coli* and its acceptance of FAMEs and alcohol acetates was evaluated in whole-cell biotransformation experiments. To elucidate the role of selected amino acids affecting the substrate scope, single and double mutants of the monooxygenase were created in a rational enzyme engineering approach and compared regarding their relative activities.

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P-BT-069

Myxobacteria as natural benzoxazole producers

*L. Lernoud[1], R. Jakob[1], L. Warnick[1], M. L. Marx[1], L. Winand[1]
[1]*TU Dortmund, Technical Biology, Dortmund, Germany*

Benzoxazoles are heterocyclic ring structures, which occur in various commercially available drugs, such as the transthyretin amyloidosis medicine tafamidis or the non-steroidal antiinflammatory drug flunoxaprofen.[1,2] In recent years, several benzoxazole natural products with promising bioactivities have been discovered.[3] The identification of their biosynthetic gene clusters revealed the requirement of AMP ligases and condensing amidohydrolases for benzoxazole production. AMP ligases activate an aromatic carboxylic acid and ligate it to another carboxylic acid, while the amidohydrolase is in charge of condensing the ligation product into a benzoxazole.[4] Most of the reported benzoxazoles are produced by actinomycetes, such as the antibiotic caboxamycin from *Streptomyces* sp. NTK 937 and the antineoplastic agent nataxazole from *Streptomyces* sp. Tü 6176.[5,6] Upon a homology-based sequence search, potential benzoxazole biosynthetic gene clusters in various myxobacteria were identified. Among others, we found a biosynthetic gene cluster in *Pyxidicoccus fallax*, which we analysed further. For this, we heterologously expressed the detected biosynthesis genes in *E. coli* and performed in vitro assays with the gene products, which confirmed their ability to produce benzoxazoles. The results of these assays, along with the structures of the produced benzoxazoles, will be presented in this poster.

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P-BT-070

Integrated electro-biocatalysis for chemical production in flow system

*G. Lim[1], D. Calabrese[1], A. Wolder[2], P. Cordero[1], D. Rother[3], F. F. Mulks[4], C. E. Paul[2], L. Lauterbach[1]
[1]*RWTH Aachen University, iAMB, Aachen, Germany*
[2]*Delft Univerity of Technology, Delft, Netherlands*
[3]*Forschungszentrum Jülich GmbH, Jülich, Germany*
[4]*RWTH Aachen University, iOC, Aachen, Germany*

Introduction: Conventional batch reactions are commonly used in chemical synthesis but often fall short in terms of efficiency, scalability, and sustainability. Continuous flow systems offer a promising alternative by addressing these limitations, and incorporating electricity can further enhance process control and support sustainable chemical transformations1,2. Nonetheless, the potential of electro-biocatalytic flow systems remains underexplored.

Aim: Our aim was to evaluate H2-driven flavin-mediated reduction of cyclic enones in continuous flow setup for sustainable production of fine chemicals using electricity.

Method: This study utilized *Ts*OYE for ene reduction and soluble hydrogenase (SH) for H2-driven recycling of FMNH2. H2 was supplied from water electrolysis by a PEM electrolyzer and fed into the flow system through gas-permeable membrane tubing inside a gas addition module. SH was immobilized on Strep-Tactin resin and *Ts*OYE on EziG beads (SH-Tactin, *Ts*OYE-EziG), both packed in a column integrated into the flow system.

Result: We demonstrated electro-driven asymmetric reduction of ketoisophorone to levodione in the flow setup. Subsequently, the setup was used to evaluate the reusability of the immobilized enzymes, where it maintained product formation of >99 % even after seven cycles. Finally, we demonstrated scalability of the system by increasing the flow reaction volume, with TTN of SH-Tactin and *Ts*OYE-EziG reaching 3.2 x 105 and 2.6 x 104, respectively3.

Conclusion: In this study, we established a biocatalytic continuous-flow system using electrical energy to produce fine chemicals. Apart from integrating a PEM electrolyzer and highly gas-permeable tubing, this study also introduced Strep-Tactin resin and highlighted EziG beads as stable enzyme carriers suitable for applications in a scalable continuous-flow setup. Overall, we made advancements in continuous flow biocatalysis by incorporating H2 from water electrolysis into the flow system to fuel flavin-dependent reactions.

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P-BT-071

Spatio-Temporal dynamics of microbial co-cultures at the single-cell level in Picoliter-Scale microfluidic growth-chambers

*M. Pesch[1], M. Bund[1], B. Lückel[1], J. Zwoch[2], J. Wegmann[3], D. Kohlheyer[1]

[1]Forschungszentrum Jülich GmbH, Jülich, Germany

[2]Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

[3]Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

Microfluidic single-cell cultivation is a promising technology to study individual microbial cells and micro populations under precisely controlled and dynamic environments at the micrometre scale. Traditional bulk cell culture methods typically obscure interactions and the diversity within cell populations, making it difficult to examine individual cell performances in complex biological systems, such as mixed cultures. We have developed a microfluidic setup that enables microfluidic batch-mode cultivations of cultures growing in isolated and fixed-volume (pL – nL range) monolayer growth chambers. Our system has been successfully applied to analyse the behaviour of several co-cultures and we expect to present latest data from several collaborative research projects.

The first example is the CoNoS co-culture model, in which two amino acid auxotrophic *C. glutamicum* strains rely on each other for growth. This work is part of the Simbal 2.0 DFG Priority Program 2170 "InterZell". Another exemplary co-culture involves an *Escherichia coli* "producer" organism and a *Corynebacterium glutamicum* "cheater" organism that requires the producer"s iron chelators for the iron uptake. The third example inhibits microorganisms competing for a scarce iron resource. More specific, one of the interacting organisms is an iron storage protein deficient mutant, which will be outcompeted in low iron environments. These two co-cultures are being investigated within the SFB 1535 MibiNet "Microbial networking – from organelles to cross-kingdom communities".

All three investigated co-cultures exhibit distinct biological behaviours (cooperation, cheating, competition), demonstrating the versatility of our new microfluidic batch chip. Key analyses of these co-cultures include single-cell growth rates, colony composition, and insights into "spatial biology".

P-BT-072

Natural biofilm inhibitors from Microalgal microbiomes: A pathway to sustainable aquaculture disease management

*M. A. Sultani[1]

[1]Hamburg University, Institut für Mikrobiologie und Biotechnologie, Hamburg, Germany

Aquaculture represents one of the world"s most rapidly expanding food production sectors, with land-based systems garnering considerable attention. Bacterial pathogens such as *Pseudomonas* spp., *Aeromonas* spp., and *Edwardsiella* spp. pose significant challenges to aquaculture, often exacerbated by these bacteria's capacity to form biofilms. Despite rising concerns, antibiotics remain widely used in numerous regions, contributing to escalating antimicrobial resistance. This study investigates the potential of microalgae and their associated microbiomes as alternative agents for disease prevention and control in aquaculture.

Our research employed both functional and sequence-based screening techniques to assess microalgal and microbial consortia for anti-biofilm properties. Over 100 promising enzyme candidates were identified from various enzyme families—including ferrochelatases, dienelactone hydrolases, and endonucleases—demonstrating notable anti-biofilm activity. Comprehensive analyses of metagenomes, metaproteomes, and metatranscriptomes have yielded initial insights into the primary enzymes contributing to these anti-biofilm effects, highlighting candidates with potential industrial applications. However, substantial research opportunities remain to further explore and develop these promising biological resources.

P-BT-073

Establishing a deeper understanding of the terpene biosynthesis in the mushroom *Cyclocybe aegerita*

*N. Hoberg[1], K. Harms[2], F. Surup[2], M. Rühl[1,3]

[1]Justus Liebig University Giessen, Institute for Food Chemistry and Food Biotechnology, Gießen, Germany

[2]Helmholtz Centre for Infection Research – HZI, Department Microbial Drugs and German Center for Infection Research,

Braunschweig, Germany
[3]Fraunhofer-Gesellschaft, Business Area Bioressources, Gießen, Germany

With up to date more than 80,000 described compounds, terpenes and their derivatives are the largest group of known secondary metabolites with an undisputed diversity in structure and function. Although most of their natural purposes are still not fully understood, their potential for human applications is already intensely researched. For example, as odor-active compounds for food, cosmetics and perfumes, as future biofuels, as pesticides or as possible pharmaceuticals, terpenes and terpenoids exhibit promising properties.[1]

A biotechnological approach can help to avoid the disadvantages of other production methods, e.g. the need for relatively large quantities of raw materials for extraction from natural sources or the use of non-environmentally friendly chemicals in the (total) synthesis. However, a deep understanding of terpene biosynthesis is required for biotechnological production. In the current DFG funded project *MushTerp*, we shed light onto the four terpene biosynthetic gene clusters Agr2, Agr5, Agr6 and Agr7 of the mushroom *Cyclocybe aegerita* that is known for its versatile terpenome.[2,3] In contrast to previous studies, the heterologous production of the terpene synthase Agr2 in the agaric host *Coprinopsis cinerea* resulted in the production of two terpenes, one of which is the already known sesquiterpene viridiflorene.[3] The second is a previously unknown diterpene, which we isolated and purified by preparative RP-HPLC for structural elucidation. 1D and 2D NMR experiments showed that the compound was the new diterpene cyclocybene, indicating the bifunctionality of Agr2 in the formation of both a sesquiterpene and a diterpene.[4] Adjacent to *agr2* in the biosynthetic gene cluster, several genes coding for P450 monooxygenases are present that have been co-expressed with Agr2 in *C. cinerea* to identify intermediates in the biosynthesis of another, multi-functionalised terpenoid that has currently been detected in *C. aegerita*.

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P-BT-074
Deciphering the inhibition of isobutanol production in *Corynebacterium glutamicum* by acetate
*S. Müller[1], C. Gunkel[1], B. Blombach[1]
[1]Technical University Munich, Microbial Biotechnology, Straubing, Germany

Isobutanol is a promising precursor for biofuel production because isobutanol-derived fuels have advanced properties like high compatibility with existing engines. Various microorganisms have been engineered for the biotechnological production of isobutanol from renewable resources. However, these strains have mainly been optimized for the biotechnological isobutanol production from glucose in chemically defined medium. Complex substrates such as lignocellulosic hydrolysates contain, in addition to sugars, inhibitors such as phenolic acids, aldehydes and aliphatic acids that affect the production performance.

We aim to optimize the isobutanol production from wheat straw hydrolysate with *Corynebacterium glutamicum* Iso7 [1] through systems metabolic engineering and bioprocess development. To unravel the metabolic optimization targets, understanding the metabolic response of *C. glutamicum* Iso7 to wheat straw hydrolysate inhibitors is necessary. We focused on the inhibitor acetate, an uncoupling agent, and wanted to analyze its specific impact on isobutanol synthesis.

To investigate the uncoupling effect, an anaerobic production culture was treated with 10 µM of the protonophore CCCP (carbonyl cyanide m-chlorophenyl hydrazine) or 20 mM acetate. During the production phase, extracellular metabolites were analyzed by HPLC and intracellular metabolites by LC-MS (HILIC-QTOF). This allowed a direct comparison of the metabolic response of *C. glutamicum* Iso7 to CCCP- and acetate-containing medium.

The glucose uptake rate was reduced by approx. 60 % and 40 % in the acetate and the CCCP-treated cultures, respectively, compared to a control culture without an inhibitor. Furthermore, in both, the acetate and CCCP-containing cultures, the isobutanol yield was reduced, while the succinate yield was increased. It was assumed, that acetic acid uncouples the proton membrane gradient and thereby inhibits the NADH transhydrogenase, leading to an accumulation of NADH and the inhibition of glycolysis. This uncoupling effect, however, could not alone explain the strongly reduced glucose uptake rate of *C. glutamicum* Iso7 and an additional metabolic regulatory effect was suspected. Therefore, a deeper look into the metabolism was taken by intracellular metabolome analysis.

In summary, the inhibitory effect of acetate was characterized and provides new opportunities for further strain and process optimization.

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P-BT-075
***Dehalococcoides mccartyi* strain CBDB1 uses cobalt-containing metal complexes as electron acceptors in hydrogen-dependent respiration, enabling coupling to electrodes**
*M. Eberwein[1], D. Deobald[1], L. Adrian[1,2]
[1]Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany
[2]TU Berlin, Geobiotechnology, Berlin, Germany

Microorganisms capable of direct or mediated extracellular electron transfer (EET) are gaining significant attention for their potential in diverse biotechnological applications, such as bioremediation, metal recovery, wastewater treatment, microbial fuel cells, and microbial electrosynthesis. One particularly noteworthy microorganism in this context is the organohalide-respiring bacterium *Dehalococcoides mccartyi* strain CBDB1, which stands out due to its ability to reductively dehalogenate many toxic and persistent halogenated organic compounds *via* organohalide respiration (OHR). In this process, CBDB1 cells use organohalides as terminal electron acceptors, with electron transfer facilitated by a membrane-bound OHR protein complex. This complex conduct electron transfer linked to proton extrusion,

generating a proton motive force that sustains both metabolic functions and growth.

To explore alternative electron acceptors for the OHR, we employed a photometric high-throughput assay based on methyl viologen to screen for electron mediators capable of replacing halogenated compounds. This screening identified cobalt-containing metal complexes (cobalt chelates) as promising mediators. In further experiments with hydrogen as the electron donor, we demonstrated that CBDB1 reduces cobalt chelates in a process coupled to hydrogen oxidation, suggesting the involvement of the full OHR complex. Subsequent biochemical analysis indicated that the cobalt chelates are reduced by CBDB1 cells, likely through the reductive dehalogenase subunit (RdhA) of the OHR complex. Molecular docking studies using the AlphaFold 2-predicted RdhA structure showed that one of the identified cobalt chelates binds favorably to RdhA with a binding energy of approximately -28 kJ mol^{-1}. To further validate the potential for electron transfer, we conducted cyclic voltammetry experiments that confirmed cobalt chelates can indeed exchange electrons with a gold electrode, making them promising for bioelectrochemical cultivation.

Our findings collectively indicate that *D. mccartyi* strain CBDB1 can utilize cobalt chelates as electron acceptors, establishing EET between the OHR complex and an electrode. This approach allows cobalt chelates to serve as anode mediators in place of halogenated compounds, opening new possibilities for bioelectrochemical cultivation and broadening the potential applications of strain CBDB1 in biotechnology fields such as bioremediation and microbial electrosynthesis.

P-BT-076

Anoxic microbial transformation of antibiotics by *Nitratidesulfovibrio vulgaris*

*J. Köpke[1,2], C. Akay[1], I. Vergara-Luis[3], C. Ding[1], A. S. Ruhl[2,4], L. Adrian[1,5]

[1]*Helmholtz Centre for Environmental Research - UFZ, Molecular Environmental Biotechnology, Leipzig, Germany*

[2]*German Environment Agency (UBA), II 3.3 Water Treatment, Berlin, Germany*

[3]*University of the Basque Country, Department of Analytical Chemistry (UPV/EHU), Leioa, Spain*

[4]*TU Berlin, Chair of Water Quality Engineering, Berlin, Germany*

[5]*TU Berlin, Chair of Biogeochemistry, Berlin, Germany*

Introduction and objectives

Wastewater treatment plants do not fully remove antibiotic residuals such as sulfonamides and their human metabolites and hence discharge considerable amounts into surface water leading to detection even in groundwater. Bank filtration represents a well-established process to eliminate antibiotic residuals. Despite the essential role of redox zone sequences during bank filtration to naturally attenuate antibiotic residuals, the responsible microbial community governing their fate and fate of their transformation products (TPs) are not fully revealed. Particularly under sulfate reducing conditions sulfonamide antibiotics can be transformed, however knowledge about the molecular process, the microbial communities and the extent of antibiotics transformation is scarce.

The objective of the presented study was to investigate the microbial transformation of several classes of antibiotics and their human metabolites by the strain *Nitratidesulfovibrio vulgaris*. Additionally, we aimed to elucidate transformation products and pathways and link them to the anaerobic metabolism of *N. vulgaris* using different electron donors and acceptors.

Methods

We conducted batch experiments cultivating *N. vulgaris* coupling the oxidation of various electron donors to the dissimilatory reduction of different electron acceptors with given antibiotics at concentrations between 10 and 650 µM. Tandem mass spectrometry was employed to investigate the formation and persistence of TPs and perform shotgun proteomics. Additionally, spectral photometry and ion chromatography were used to monitor growth and catabolic activity of *N. vulgaris*.

Results

We observed the cometabolic transformation of the antibiotic sulfamethoxazole (SMX) and it´s human metabolite N-Acetyl-SMX at 10 to 650 µM under sulfate-reducing conditions using lactate or hydrogen as electron donors. We identified several TPs with shorter half-lives then SMX also suggesting different transformation mechanism, e.g. isoxazole ring cleavage. Comparative proteomics suggested house-keeping enzymes to transform both compounds. The antibiotics sulfamethazine, sulfadiazine and trimethoprim were not transformed.

Conclusion

The conducted batch experiments showed the capability of *N. vulgaris* to cometabolically transform several antibiotics and their human metabolites under anoxic conditions highlighting the important role of anoxic conditions for their natural attenuation.

P-BT-077

Insights into the substrate profile of *Mycolicibacterium smegmatis* aminoacylase for the synthesis of *N*-acyl amino acids

*J. Wirges[1], P. Schmidt[1], L. Sumaila[1], M. Wieczorek[2], G. Haeger[3], J. Bongaerts[1], P. Siegert[1]

[1]*FH Aachen, Nano- and Biotechnologies, Jülich, Germany*

[2]*FH Aachen, Applied Polymer Chemistry, Jülich, Germany*

[3]*Novo Nordisk, Måløv, Denmark*

Acyl amino acid biosurfactants are a versatile class of lipopeptide surfactants with excellent properties in terms of biodegradability and mildness [1]. Due to the requirement for harmful chemicals and the stoichiometric generation of sodium, the chemical synthesis *via* the Schotten-Baumann method poses significant environmental challenges [2]. However, these challenges could be overcome by developing a sustainable biocatalytic synthesis route. In preliminary studies, an aminoacylase termed *Mycolicibacterium smegmatis* aminoacylase (MsAA) was successfully expressed in *Escherichia coli* BL21 (DE3) and presented as a promising catalyst for the synthesis of acyl amino acids [3]. MsAA showed a strong preference for small and hydrophobic amino acids and C8-12 fatty acids during synthesis. To gain an understanding of the catalytic reaction mechanism, *in silico* molecular docking studies with lauric acid and methionine were performed. Further comparison with other aminoacylases and related enzymes allowed the identification of residues that could mediate substrate binding. To validate these results and shift the substrate

scope of MsAA towards larger and hydrophilic amino acids, a mutagenesis study is in progress [4].

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P-BT-078

Benchmarking an in-gel zymography assay for enzyme discovery in microbial communities

*M. Prisacar[1], L. Spannenkrebs[1], D. Sander[1], F. Dicks[1], L. I. O. Leichert[1]
[1]*Ruhr University Bochum, Microbial Biochemistry, Bochum, Germany*

Functional metaproteomics is a powerful approach to discovering new enzymes from microbial communities that can be used for medical or biotechnological applications. This method simultaneously analyses the functional metaproteome and the metagenome, overcoming the need for bacterial cultivability, while still directly measuring the desired enzyme activity. In a functional metaproteomics approach proteins from environmental samples are screened in in-gel-based assays for the desired activity and then selectively identified by mass spectrometry, using microbial metagenome information as template for a computational protein identification.

Here, we optimize and benchmark an in-gel zymography-based activity assay to identify microbial enzymes that catalyze the hydrolytic cleavage of short chain fatty acid (SCFA) esters. For this, a commercially available lipase from *Aspergillus oryzae* (AOL) and the fluorogenic substrates 4-methylumbelliferyl butyrate (4-MUB) and 4-methylumbelliferyl propionate (4-MUP) were selected. The evaluation of the sensitivity, reproducibility and efficiency of our approach shows that an amount as low as 62.5 ng of AOL can be detected. Overall, we could detect and quantify activity over three orders of magnitude.

Using our method to analyze metaproteomes from a variety of sources, we could determine the presence of SCFA ester-degrading enzymes. Since there is increasing evidence that reduced levels of SCFAs can be associated with pathogenesis of autoimmune and neurodegenerative diseases like multiple sclerosis, identifying compositional alterations of microbiota-derived SCFA-degrading enzymes could contribute to the understanding of the role of the gut microbiome in these pathologies.

P-BT-079

***P. taiwanensis* cell surface engineering for the improved production tolerance to organic solvents**

*T. Redeker[1], B. Wynands[1], N. Wierckx[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

To support the transition toward a sustainable bioeconomy, our research focuses on *Pseudomonas taiwanensis* VLB120, engineered for efficient biosynthesis of hydrophobic aromatic compounds within a second-phase system. Through the PROSPER project, we aim to develop a scalable, sustainable, bio-based production platform, providing a greener alternative to conventional chemical manufacturing. Due to toxic effects of hydrophobic compounds, we focus on enhancing solvent tolerance in the bacterium applying different strategies. One of the approaches is the reduction of the cell surface hydrophobicity (CSH), thereby mitigating negative cell-solvent interactions. Additionally, we analyze extracellular matrix components and their function as a diffusion barrier to optimize product transport.

In our work, adaptive laboratory evolution (ALE) was used to harness the adaptive capacity of *Pseudomonas taiwanensis* under selective conditions, while genome editing enabled precise, targeted modifications to the bacterial genome. Phenotypic analyses included the Bacterial Adhesion to Hydrocarbons assay (BATH) to assess changes in cell surface hydrophobicity (CSH), biofilm formation, and emulsification assays. By integrating these approaches, we generated strains with significantly reduced CSH and identified the genetic basis for these adaptations. Reverse engineering further confirmed the mutations' role in decreasing CSH, with strains exhibiting reduced biofilm formation and emulsification to hydrophobic phases.

Our results validate ALE as a robust tool for adjusting cell surface properties, linking reduced hydrophobicity to lower emulsification and biofilm formation. As these are beneficial traits for industrial bioprocesses, these advancements mark progress toward a more efficient, biotechnology-driven production platform.

P-BT-080

Effect of lignocellulose-derived inhibitors on isobutanol production with *Corynebacterium glutamicum*

*C. Gunkel[1], S. Müller[1], B. Blombach[1]
[1]*Technical University Munich, Campus Straubing for Biotechnology and Sustainability, Straubing, Germany*

Isobutanol is a precursor of the chemical building block isobutylene and a sustainable alternative to fossil fuels when derived from renewable resources. Various microorganisms have been engineered to synthesize isobutanol from defined substrates, but therefore often perform poorly when complex non-food carbon sources such as lignocellulosic hydrolysates are used as substrate. Lignocellulose-derived carbon fractions are intricate mixtures, containing carbohydrates but also potentially toxic compounds. A broader understanding of how lignocellulosic inhibitors impact anaerobic isobutanol biosynthesis is lacking.

We aimed to characterize how 20 mM of exemplary inhibitors affect *Corynebacterium glutamicum* Iso7's anoxic isobutanol production in synthetic glucose medium and compared these effects to those observed in wheat straw hydrolysate (WSH) medium. We also explored potential inhibition patterns and cellular detoxification strategies using LC/MS and HPLC analysis to deduce metabolic optimization targets for isobutanol synthesis from WSH.

The presence of acetate reduced the glucose uptake by more than 60 % to 0.10 ± 0.00 g_{Glc} g_{CDW} h^{-1} compared to the culture with glucose only. Strikingly, the fermentation course in synthetic glucose-acetate medium was very similar to the inhibition in early WSH-based fermentations. In WSH medium, however, the isobutanol precursor 2,3-dihydroxyisovalerate started to accumulate after about 24 h, indicating that the dihydroxy-acid dehydratase IlvD limited sustained isobutanol synthesis. Furfural, formate, ferulate and vanillate hardly affected isobutanol production, demonstrating that *C. glutamicum* Iso7 is robust against high concentrations of diverse lignocellulosic inhibitors. Vanillin even increased the yield of isobutanol by about 9 % to 0.50 ± 0.01 C-mol_{iBuOH} C-mol_{Glc}^{-1} and diminished isobutyrate formation. As vanillin was predominantly oxidized into vanillate, we propose that the cells employed vanillin for redox balancing.

Our results contribute to the understanding of complex-substrate fermentations with *C. glutamicum*. Considering the intricacy of the observed inhibition aspects, we propose that granting some degree of metabolic flexibility is beneficial for a robust valorization of complex renewable substrates. In this regard, we emphasize that the various components of lignocellulosic hydrolysates are not necessarily toxic for fermentations, but clever integration might offer potential for process enhancement.

P-BT-081

Engineering tyrosine ammonia lyase for uninhibited production of phenyl propanoic acids

*P. Schrank[1,2], B. Moritz[1], P. Harati Sheshagiri[1], A. R. Thadani Acosta[1], M. D. Davari[2], M. Pietzsch[1]
[1]Martin-Luther-University Halle-Wittenberg, Institute of Pharmacy, Downstream Processing, Halle (Saale), Germany
[2]Leibniz Institute for Plant Biochemistry, Bioorganic Chemistry, Halle (Saale), Germany

Tyrosine ammonia lyases (TALs) are part of the aromatic amino acid lyase family, which catalyze the non-oxidative deamination of L-tyrosine to p-coumaric acid. Besides their potential as therapeutic enzymes for the treatment of hereditary tyrosinemia, they are also utilized for *in vivo* biocatalysis production of several high value plant metabolites f. e. phenyl propanoic acids. Till now biotechnological productions utilizing TALs are still limited by their pronounced product inhibition, leading to carbon flux restrictions in engineered biosynthetic pathways. To understand the mechanism of product inhibition, we analyzed the TAL from *Rhodotorula glutinis* (*Rg*TAL) kinetically and computationally. Continuous kinetic measurements revealed, that strong competitive inhibition is not only limited to the reaction product coumaric acid (Ki = 20 µM) but also extends to other phenyl propanoic acids like ferulic acid (Ki = 12 µM). Further we developed and applied engineering strategies for the *Rg*TAL to screen for variants with increased resistance to product like inhibitors. For the selection of sensitive amino acid (aa) positions, we came up with different hypothesis regarding substrate binding and substrate access. We first modeled and analyzed the 3D structure of *Rg*TAL to detect binding pockets and molecular access tunnels. For assigning the binding pocket design, we generated binding conformations of the substrate and the phenol propanoic acids by molecular docking. Dynamic protein ligand interaction patterns (PLIPs) were then elucidated using MD simulations of the complexed structures. Analysis of these PLIPs revealed stable binding conformations for both the tyrosine and inhibitors like ferulic acid with similar modes of non-covalent interactions. On the other hand, inhibitors like sinapic acid couldn"t be bound in substrate-like manner, presumably due to steric clashes with the active site residues. For redesign of the access tunnel, steered MD simulations of substrate/inhibitor translocations through the prior identified tunnel regions were carried out. Two bottleneck regions in the tunnel were elucidated, showing increased energy barriers for bulkier ligands like sinapic acids. Based on these results we selected nine aa positions with increased probability to alter inhibitory properties of *Rg*TAL upon mutagenesis. Finally, we established an *in vivo* screening procedure to screen focused libraries of 14 substitutions per position in deep well plates.

P-BT-082

Biotechnological production of hydroxy cinnamic acid conjugates in *E. coli* BL21 catalyzed by recombinant plant enzymes

*I. Mohamed[1], B. Moritz[1], R. Milde[1], M. Pietzsch[1]
[1]Martin-Luther-University Halle-Wittenberg, Institute of Pharmacy, Downstream Processing, Halle (Saale), Germany

Feruloyltyramine (FerT), also known as Moupinamide, Alfrutamide or N-trans-Feruloyltyramine, is one of the main representatives of hydroxycinnamic acid amides (HCAAs), a diverse group of plant secondary metabolites which has functions in plant growth, development and defense. It was isolated from several medicinal plants including *Allium sativum*, *Smilax aristolochiifolia*, *Arcangelisia gusanlung* and *Lycium barbarum*, and is proposed to have antioxidant, anti-inflammatory, hypoglycemic, hypotensive, anti-melanogenesis and anti-cancer activities. Therefore, FerT is a promising pharmaceutical agent for the treatment or prevention of a wide variety of diseases.

Barriers limiting its availability for pharmaceutical research and agricultural applications include the low yield from plants and the costly and multi-step processes of chemical synthesis. Biotechnological approaches offered a suitable alternative meeting the criteria of green chemistry. FerT can be synthesized biotechnologically by a two-step enzymatic conversion of ferulic acid (FerA) and tyramine (TyN). We cloned two plant enzymes, 4-coumarate-CoA ligase (4CL) and tyramine hydroxycinnamoyltransferase (THT), on a bicistronic expression plasmid and investigated the temperature dependence of recombinant protein expression.

Initial results showed whole cell catalysis with product accumulation in the supernatant. However, the activity of the recombinant cells is differentially sensitive to the temperature at which proteins are expressed, showing higher activity at lower expression temperatures. Furthermore, we tested the dependence of 4CL expression in regard to time and expression temperature and analyzed the recombinant protein amount compared to total protein by SDS-PAGE. We observed a substantial lack of stability of the recombinant plant enzyme at elevated temperatures. Between the three tested temperatures (16 °C, 22 °C and 28 °C), 16 °C resulted in the highest stability. These results suggest that the temperature is a critical parameter to consider in process

development for the biotechnological synthesis of FerT. Exemplary production processes for the one-liter bioreactor scale and the production of other HCAAs are to be discussed.

P-BT-083

Expanding the molecular toolbox to explore the enigmatic cell biology in the phylum *Planctomycetota*

*T. Haufschild[1], *V. Plut[1], N. Rabold[1], J. Hammer[1], N. Kallscheuer[1], C. Jogler[1]
[1]*Friedrich Schiller University, Microbial Interactions, Jena, Germany*

Strains belonging to the bacterial phylum *Planctomycetota* show several enigmatic features: their cell plan differs from that of canonical Gram-negative model bacteria and phylum members propagate by an uncommon form of asymmetric budding cell division. A detailed analysis of these conspicuous traits is hindered by the lack of sophisticated molecular biological tools for the genetic manipulation of these non-model microorganisms. Previous studies have reported the transposon-driven introduction of heterologous genes into random loci in the genome and more recently the targeted construction of single gene deletion mutants [1,2]. Still, the investigation of cell biological features of *Planctomycetota* remains largely untapped without more advanced genetic engineering tools.

We here expand the molecular toolbox applicable for limnic and marine model planctomycetes. Extensive testing of selection marker genes yielded the first strain carrying deletions at multiple genomic loci. While testing different chromosomal locations for the introduction of heterologous genes, the stable expression of a set of reporter genes encoding (monomeric) fluorescent proteins and the multi-purpose HaloTag was achieved. The investigation of native promoters of the model strain *Planctopirus limnophila* yielded multiple promoters with varying strengths. A naturally-occurring sugar-dependent regulator/regulated promoter pair from this strain was turned into an inducible gene expression system.

Applying this enhanced molecular toolbox to members of the phylum *Planctomycetota* will support deciphering the molecular mechanisms of their enigmatic cell biology while more challenging tools such as replicative plasmids are yet to be developed.

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P-BT-84

Metabolic engineering of *Aspergillus violaceofuscus* enables discovery of cryptic NRPS-like product violafuranone A

*C. Wieder[1,2], C. Laubenstein[1,2], R. Wiechert[3], K. Seipp[3], A. Schüffler[2], T. Opatz[3], E. Thines[1,2]
[1]*Johannes Gutenberg-University Mainz, Microbiology and Biotechnology, Mainz, Germany*
[2]*Institute of Biotechnology and Drug Research gGmbH (IBWF), Mainz, Germany*
[3]*Johannes Gutenberg-University Mainz, Chemistry, Mainz, Germany*

Fungal secondary metabolites (SMs) are a rich source for bioactive compounds with various applications in agriculture, biotechnology and medicine. In laboratory cultivation, however, fungi produce only few of the hypothetically encoded metabolites with fewer yet at high titers. Various different strategies have been employed in order to access and elucidate cryptic biosynthetic gene clusters (BGCs), potentially encoding novel scaffolds and lead structures. In this study, we aimed to explore the metabolic potential of *Aspergillus violaceofuscus*, a biosynthetically talented fungus, harboring more than 90 predicted BGCs. Establishing an efficient Cas9-mediated microhomology-directed deletion strategy allowed for performing genetic dereplication, linking the most abundantly produced metabolites to their respective BGCs. In order to activate silent biosynthetic gene clusters, we performed homologous overexpression of select biosynthetic core genes and transcription factors. Overexpression of a cryptic NRPS-like coding gene lead to the production of a new metabolite we termed violafuranone A. We demonstrate metabolic manipulation of *A. violaceofuscus* and the potential of this species as a source for novel SMs. These findings pave the way for further investigations into the secondary metabolism of *A. violaceofuscus*.

P-BT-085

Converting phototrophic biomass into value-added products

*P. Ernst[1,2], M. Denter[1], M. Feldbrügge[2], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*
[2]*Heinrich Heine University, Microbiology, Düsseldorf, Germany*

Introduction: Nowadays, a large proportion of chemicals and fuels are derived from fossil resources such as oil, gas and coal. The heavy reliance on these fossil resources results in excessive emissions of greenhouse gases, which are a major contributor to global warming (Khoo et al., 2019). Thus, the development of technologies for a more sustainable energy and chemical production attracts concerns worldwide. Much of the attention has been drawn to the utilization of microalgae, which convert sunlight, CO2 and water into organic compounds, and possess a number of advantages over traditional plant-based biomass (Singh & Olsen, 2011).

Goal: The aim of this study is to cultivate the phototrophic microalgae *Galdieria sulphuraria* and *Cyanidioschyzon merolae* in shake flasks, Multi-Cultivator photobioreactors as well as in custom-made flat panel photobioreactor and to utilize the resulting biomass as 3rd generation feedstock for the production of value-added compounds using heterotrophic platform organisms.

Results: Initial characterizations of the microalgae strains regarding temperature, illumination intensity and CO2 concentration were performed in Multi-Cultivator photobioreactors, with the highest growth rate of 0.05 h-1 reached at 42 °C, 400 µE and 5 % CO2. The scale-up to custom-made flat panel photobioreactors reduced the growth rate to 0.02 h-1, but enabled much higher optical density values of 35.7 ± 0.1. Heat-stable phycocyanin was identified as a valuable product extracted from algal biomass. The remaining algal biomass was successfully utilized by the biotechnological platform species *Ustilago maydis* for both growth and itaconic acid production.

Summary: This work provides blueprints for converting photosynthetic biomass into bioproducts essential for the

pharmaceutical and polymer industries, supporting both carbon utilization and sequestration.

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P-BT-086

Towards the production of mushroom-derived sesquiterpenoids in a light-driven microbial consortium

*J. C. Pohlentz[1], V. Paderi[1], D. Hasenklever[2], P. Westhoff[3], V. Wewer[4], S. Metzger[4], M. Feldbrügge[1], K. Schipper[1]
[1]Heinrich Heine University, Düsseldorf, Germany
[2]Heinrich Heine University, Düsseldorf, Germany
[3]Heinrich Heine University, Düsseldorf, Germany
[4]University of Cologne, Cologne, Germany

Synthetic microbial consortia exhibit extended metabolic capacities compared to monocultures and therefore have a great potential for biotechnological applications. Here, we envision to combine the photoautotrophic features of cyanobacteria with the versatile production potential of the fungal chassis *Ustilago maydis* to produce mushroom-derived sesquiterpenoids from CO_2 and light. Mushroom-forming basidiomycetes are a rich source of sesquiterpenoids, but their slow growth and low isolation yields limit the access to those valuable compounds. Moreover, successful expression of the underlying biosynthetic pathways in traditional heterologous expression platforms can be challenging. *U. maydis,* a basidiomycete itself, constitutes a promising alternative.

While modified cyanobacteria will serve as the "breadwinner" of our consortium, supplying *U. maydis* with sucrose, we aim to engineer *U. maydis* to efficiently produce fungal sesquiterpenoids, such as α-cuprenene and its oxygenated antimicrobial derivative lagopodin B. Our approach involves the transplantation of heterologous sesquiterpenoid biosynthesis pathways, as well as metabolic engineering to increase sesquiterpenoid yields. To facilitate this, we are currently implementing a modular cloning (MoClo) system for *U. maydis* while also expanding our genetic engineering toolbox with e.g. additional strong and/or inducible promoters.

Initial metabolic engineering efforts to enhance the yield of the sesquiterpene α-cuprenene indicate that the expression strength of the heterologous α-cuprenene synthase gene, *cop6*, represents a bottleneck. To address this issue, we are now focusing on single- or multi-copy integration of *cop6* using different promoters. Additionally, through the heterologous expression of two cytochrome P450 monooxygenase genes, we have successfully produced oxygenated α-cuprenene derivatives, bringing us closer to the valuable sesquiterpenoid lagopodin B. Furthermore, initial proof-of-concept co-cultivation experiments with sucrose-producing cyanobacteria are promising. Thus, optimized production strains will next be tested for their performance in co-culture.

P-BT-087

Establishing an open-source, custom-built turbidostat for enhanced microbial growth in a stabilized environment

*L. Rehm[1], M. Hecker[1], K. Martens[2], U. Endesfelder[1]
[1]Rheinische Friedrich-Wilhelms-Universität Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany
[2]Delft Univerity of Technology, Department of Imaging Physics, Delft, Netherlands

The reproducibility of experiments and thus consistency of observations is a significant challenge in biological studies. In microbial research, bioreactors and turbidostats offer great potential for eliminating external factors that can influence the growth and physiology of microbes. They enable a reproducible and efficient production of high-quality proteins through precise regulation of e.g. temperature, nutrient supply, pH or oxygen levels. Moreover, the inter-batch variability is remarkably diminished [1,2]. Using bioreactors has been demonstrated to accelerate not only protein production, but also the cultivation of microbes [3]. In comparison to bioreactor operation in batch or fed-batch mode, turbidostats adapt the culture dilution rate according to the culture"s optical density (OD). They continuously add fresh medium and discard an equal volume of effluent culture, thereby maintaining a pseudo-static environment for microbes. Thus, turbidostats offer precise control of growth conditions and continuous monitoring of environmental parameters like pH and nutrient availability, while preventing the buildup of toxins and by-products [4]. This makes them ideal for maintaining stable, controlled environments for long-term microbial culture in cell biological research [1,2].

This work presents an open-source turbidostat which we named "Turbino". Next to cost-effectiveness, Turbino is characterized by its reliable and robust functionality which we showcase for multiple use-scenarios. Our device has been designed to be lightweight and compact, allowing for placement in any laboratory setting. It is operated in a very versatile, general multi-purpose scheme, so the experimental possibilities that can be facilitated by automation provided by Turbino are extensive. As an open-source and fully documented instrument, it can be extended and further developed for specific experimental requirements. We demonstrate the potential of Turbino by investigating the dynamics of NreA, a recently discovered protein involved in DNA repair in the haloarchaeon *Haloferax volcanii* [5]. As this model archaeon can be challenging to work with in conventional lab settings due to its slow and pleomorphic growth, which highly depends on culturing conditions, we showcase the benefits of cultivating *H. volcanii* using Turbino.

[1]10.1371/journal.pone.0181923
[2]10.1371/journal.pbio.3000794
[3]10.3389/fbioe.2019.00420
[4]10.1016/b978-0-444-63663-8.00016-1
[5] 10.1111/mmi.13210

P-BT-088

Investigating the impacts of process conditions on biological phosphorous removal and biofilm formation in a developed microfluidic cultivation platform

*B. Akgün[1], J. Lapp[1], A. Elreedy[1], J. Gescher[1]
[1]Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany

Biological phosphorus removal (BPR) is an advanced process utilized in wastewater treatment to eliminate phosphorus effectively. This process is primarily performed by specific microorganisms known as polyphosphate-accumulating organisms (PAOs). PAOs are enriched to achieve efficient BPR by adapting activated sludge with cyclic anoxic feeding and oxic starvation conditions. This study aims to use a developed microfluidic biofilm cultivation platform (MBCP) to enrich PAOs. Using MBCP was found advantageous for mimicking the BPR process and enriching PAOs under varying operational conditions. To achieve this goal, a series of experiments were conducted, including the use of different carbon sources, COD/P ratios of 10, 40 and 80, pH values of 6.5, 7.5 and 8.0, and temperatures of 12 and 22 °C.

Activated sludge was used as inoculum, collected from the Langenhagen wastewater treatment plant in Germany. During cultivation, the development of the biofilm structure was monitored by optical coherence tomography (OCT) analysis. In addition, samples were taken in the oxic and anoxic phases to detect phosphate release and uptake amounts by ion chromatography (IC). The results show that using glycerol, COD/P ratio of 10, pH 8.0 and 12 °C resulted in the best biofilm growth. However, P-removal capacity increased with the use of glycose, COD/P ratio of 40, pH 7.5 and 12 °C.

After cultivation, the metagenomic analysis of the enriched biofilm was compared to the initial inoculum composition to determine the abundance of PAOs. To further corroborate the bioinformatic results, fluorescence in situ hybridization (FISH) analysis of the cultured cells was performed as an endpoint analysis and with a sample from the inoculum. Lastly, principal component analysis (PCA) was conducted among the abundance of potential PAOs and corresponding parameters.

This implementation shed light on how the process conditions affect the biofilm formation and microbial community composition. This enables us to understand the influence of key operating conditions on process stability and efficiency to optimize the relevant design parameters used in full-scale wastewater treatment plants.

P-BT-089

Development of a high throughput screening platform for electroactive microorganisms

*A. Kuchenbuch[1], R. Frank[2], C. Wagner[3], N. Wilfred[3], M. Bulst[3], H. G. Jahnke[2], F. Harnisch[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Microbial Biotechnology, Leipzig, Germany*
[2]*Leipzig University, Centre for Biotechnology and Biomedicine, Biochemical Cell Technology, Leipzig, Germany*
[3]*Sciospec Scientific Instruments GmbH, Bennewitz, Germany*

The oxidation of organic matter coupled with the reduction of metal ores, like Fe (III) or Mn (IV), is the most important biogeochemical process in soils, aquatic sediments and groundwater. These processes are based on metal-reducing microorganisms that perform anaerobic respiration and utilize metals as terminal electron acceptor [1]. This is achieved by microbial extracellular electron transfer, which these so-called electroactive microorganisms (EAM) are capable of. EAM include both obligate, such as *Geobacteraceae*, as well as facultative anaerobes, such as *Shewanella* spp. In bioelectrochemical systems, EAM transfer electrons to a solid electrode instead of metals [2]. A distinct ecological niche cannot be defined and due to the enormous microbial diversity in different habitats, systematic screening for EAM is one of the greatest challenges that is currently methodically hampered due to unavailable electrochemical screening tools [3].

Our aim is to establish a high throughput screening platform that allows independent electrochemical systems to be investigated in parallel and real-time. The requirements are to perform basic electrochemical measurement methods such as chronoamperometry and cyclic voltammetry in a 3-electrode-setup to screen for EAM in long-term operation under anaerobic conditions.

To tackle this challenge, we developed a 96 electrochemical deepwell plate and a 96-channel multipotentiostat to investigate 96 independent controllable samples. Electrochemical cultivation of the model organisms *Geobacter sulfurreducens* and *Shewanella oneidensis* achieved maximum current densities and coulombic efficiencies that were well in-line with literature [4].

This easy-to-use system will allow to gain deeper insights into the physiology and diversity of EAM and allow their targeted development for use in a biobased and electrified economy.

Funding: This research is funded by the German Federal Ministry of Education and Research (BMBF) under the grant number 031B1378C.

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P-BT-090

Metabolic engineering of *Clostridium kluyveri* DSM555^T for increased CO tolerance

*A. Abstreiter[1], L. Glanz[1], C. Raab[1], V. Burgmaier[2], D. Weuster-Botz[2], W. Liebl[1], A. Ehrenreich[1]
[1]*Technical University Munich, Microbiology, Freising, Germany*
[2]*Technical University Munich, Chair of Biochemical Engineering, Garching, Germany*

In biotechnology, microbial co-cultures offer the potential to combine metabolic properties from different organisms. As an example, the gas-fermenting *Clostridium carboxidivorans* supplies the carbon chain elongating *Clostridium kluyveri* with its substrates ethanol and acetate for the production of medium chain fatty acids, which in turn are finally reduced to the corresponding alcohols like butanol, hexanol and even octanol. The use of syngas as the initial substrate, which is derived from gasification of municipal or food waste or as a by-product from the steel industry, has the advantage of producing these bulk chemicals solely from waste materials as a sustainable alternative to petrochemical synthesis. Unfortunately, analysis of this co-culture revealed that at CO concentrations that are most favorable for good ethanol and acetate production by *C. carboxidivorans*, reversible growth-inhibition of *C. kluyveri* was observed, presumably by inhibition of the [FeFe]-hydrogenases.

In an effort to adapt *C. kluyveri* genetically to high CO partial pressures, a plasmid-encoded heterologous alcohol aldehyde dehydrogenase (AdhE2) was introduced in the organism as a potential alternative route for redox factor regeneration together with the organism"s Rnf complex. To this end, a protocol for triparental conjugation enabling reliable transfer of plasmids into the *C. kluyveri* DSM555^T wildtype strain was established. Protection against a dominant type II restriction-modification (RM) system was achieved by *in vivo* methylation of the plasmid in the *E. coli* donor strain. Furthermore, chromosomal insertion of the AdhE2 is attempted with a *codAB*-counterselection system and a highly efficient CRISPR-Cas9 system controlled by a theophylline riboswitch. For these engineering efforts the expression strength of different inducible and constitutive promoters from various *Clostridium* species, *Bacillus subtilis* as well as some synthetic promoters were characterized in *C. kluyveri* using FAST fusions as reporters. The repertoire of promoters is the basis for adjustments of the expression levels of the components of the gene editing systems and the introduced enzymes like AdhE2.

P-BT-091

Heterotrophic and phototrophic multi-step biocatalysis for the production of nylon building blocks from renewables

*L. K. Möhrlin[1], A. Tüllinghoff[1], J. Toepel[1], B. Bühler[1]
[1]Helmholtz Centre for Environmental Research - UFZ, MIBITECH, Leipzig, Germany

Multi-step whole-cell biocatalysis is a versatile approach to facilitate the sustainable synthesis of value-added compounds, such as polyamides (PA) building blocks, from renewables. Currently, PAs are produced via multiple process steps, energy-, and waste- intensive reaction processes, heavily based on fossil resources. [1] Whereas whole-cell biocatalysis benefits from highly specific enzymatic reactions under mild conditions, resulting in less by-product formation. It also enables multiple reactions in a single process step via the design of orthologous pathways (*in-vivo* cascades) thereby avoiding intermediary purification steps. [2; 3] Nylon 6 (PA6), an industrially widely used PA, can be synthesised from 6-aminohexanoic acid (6-AHA), which not only can serve as key monomer for PA6, but also constitutes a crucial pharmaceutical agent for the treatment of aneurysmal subarachnoid haemorrhages.

Our research aims to engineer biocatalysts capable of efficiently producing 6-AHA through an enzymatic cascade assembled from genes of diverse microbial origin. The goal is to maximize, scale up, and compare production efficiencies of heterotrophic strains, with a focus on *Escherichia coli* JM101, *Pseudomonas taiwanensis* VLB120, as well as the phototrophic cyanobacterium *Synechocystis* sp. PCC 6803 as host strains, the latter with the goal to enable light-driven 6-AHA synthesis. Substrates for this process are plant oil-derived hexanoic acid methyl esters or hexanoic acid, which can be produced from lignocellulosic biomass via an anaerobic digestion process.

To quantitatively analyse, detect bottle necks in, and to optimize the transgenic cascade, the enzymes are tested individually and in combinations. Promising *in-vivo* enzyme activities for the terminal functionalization of hexanoic acid methyl ester *inter alia* by the alkane monooxygenase AlkBGT will be reported.

We aim at the implementation of the resulting reaction cascade in a suitable bio-process concept mitigating substrate toxicity. The toxic effect of the apolar substrate HAME is likely caused by accumulation in the cell membrane. [3] Thus, various feeding strategies and a two-phase reactor approaches will be presented.

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P-BT-092

A new split GFP-based linker and biosensor system for *in vivo* protein immobilization

*J. Jetten[1], O. Klaus[1], T. Gensch[2], T. Drepper[1]
[1]Heinrich Heine University, Institute of Molecular Enzyme Technology, Jülich, Germany
[2]Forschungszentrum Jülich GmbH, Institute of Biological Information Processing IBI-1: Molecular and Cellular Physiology, Jülich, Germany

In vivo immobilization of proteins on biogenic carriers, such as polyhydroxybutyrate (PHB) granules, is a commonly used technique to enhance their stability and reusability in industrial applications. Although many strategies for protein immobilization have been developed, e.g. the use of linker systems instead of direct fusion of the protein of interest (POI) to a matrix, *in vivo* immobilization still represents a major challenge. Moreover, online-analysis of the immobilization process is yet not feasible. Here, we present a new approach for PHB-based *in vivo* immobilization of POIs in *Escherichia coli* where split fluorescent proteins such as split GFP are used as a linker and biosensor. The split GFP system consists of the superfolder GFP that is split into two non-fluorescent fragments GFP1-10 and GFP11 that have the ability to specifically self-assemble resulting in a green fluorescent signal. In our approach, GFP11 is fused as an anchor protein to the PHB synthase PhaC that is covalently bound to the surface of the PHB granule, while the POI is linked to GFP1-10, resulting in a green fluorescence signal after successful immobilization. To test the system, the red fluorescent protein mCherry, the squalene synthase (SQS) from *Methylococcus capsulatus and* the ester hydrolase LipD from *Alcanivorax borkumensis* were used as target proteins. The immobilization process was verified using fluorescence spectrometry, microscopy, and fluorescence lifetime imaging. Online-monitoring of the corresponding immobilization processes in *E. coli* was performed by determining fluorescence signals during the cultivation and production process. The activity of immobilized enzymes could successfully be demonstrated via HPLC analyses (analysis of squalene formation by the SQS) as well as by the conversion of 4-nitrophenyl butyrate (analysis of LipD activity).

In summary, we successfully showed that the split GFP-mediated system for *in vivo* immobilization of POIs on PHB granules represents a promising alternative to existing concepts for protein immobilization. Furthermore we could demonstrate that successful immobilization can be online monitored and quantified via the biosensor function of split GFP even when using candidate enzymes that have not yet been used as target proteins for split GFP-mediated immobilization. The new system therefore enables a detectable decoration of the PHB surface and thus represent

a promising approach for future biotechnological applications.

P-BT-093

A dual-fluorescent biosensor for *in vivo* analysis of protein secretion in *Escherichia coli* and *Corynebacterium glutamicum*

*T. Horbach[1], S. H. Paik[1], A. Woop[1], T. Drepper[1]
[1]*Heinrich Heine University, Institute of Molecular Enzyme Technology, Jülich, Germany*

A flavin-binding fluorescent protein (FbFP)-based biosensor was developed to enable real-time monitoring of protein expression and secretion in Gram-negative and Gram-positive bacteria. The applicability of this biosensor was first demonstrated in *Escherichia coli* and *Corynebacterium glutamicum* as a proof of concept. Both species are frequently used in industrial biotechnology, with *C. glutamicum* valued for its ability to secrete proteins directly into the culture supernatant, simplifying downstream processing.

This biosensor enables non-invasive, continuous tracking of both intracellular and extracellular protein levels during bacterial cell growth, providing detailed insights into secretion dynamics. When FbFPs remain in the cytoplasm after expression, they incorporate the endogenous chromophore FMN (flavin mononucleotide), resulting in a specific cyan-green fluorescence signal. In contrast, secretion of the biosensor protein into the periplasm or supernatant via the Sec secretion pathway requires an externally supplied FMN derivative. This addition results in a red-shifted fluorescence signal, enabling fluorescence-based differentiation between cytoplasmic and secreted proteins and providing real-time insights into protein localization and secretion pathways.

After demonstrating its functionality, the biosensor was used to comparatively analyze the expression and secretion of recombinant proteins consisting (i) different signal peptides from *Bacillus subtilis*, (ii) the cutinase from *Fusarium solani pisi*, and (iii) the FbFP domain. For this purpose, corresponding *E. coli* and *C. glutamicum* expression strains were cultivated, and green and red-shifted fluorescence signals as well as cutinase activities were subsequently measured. The results demonstrated that a direct correlation between fluorescence signals and cutinase activity could be achieved, validating the biosensor's ability to reliably report on protein localization and secretion.

Overall, this biosensor system expands the applicability of FbFPs by enabling *in vivo* investigation of Sec-dependent secretion processes, thereby providing valuable information for the optimization of biotechnological production processes in bacteria.

P-BT-094

Optogenetic multicolor light control with photocaged inducers for microbial bioprocess engeneering

*L. Müller[1,2], S. H. Paik[1], S. Helgers[2], F. Knapp[1], F. Hogenkamp[2], T. Polen[3], J. Pietruszka[2,3], T. Drepper[1]
[1]*Heinrich Heine University, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Heinrich Heine University, Institute of Bioorganic Chemistry, Jülich, Germany*
[3]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

Photocaged inducers are molecules whose biological activity is masked by photolabile protecting groups that are released upon exposure to light of a specific wavelength. This innovative approach to molecular regulation permits precise spatial and temporal control over biological processes, a significant advantage in fields like synthetic biology and biotechnology. Photocaged inducers are particularly appealing due to their ability to control gene expression and other cellular processes with high specificity and non-invasive triggers.

These compounds find applications across various biotechnological and medical fields, enabling targeted regulation of gene expression to optimize processes such as protein production. For instance, the synthesis of valuable biochemicals can be modulated at specific growth stages of microorganisms, facilitating enhanced yields and product quality. Research has shown successful application of photocaged inducers, including IPTG derivatives, in microorganisms like *Escherichia coli* and *Pseudomonas putida*, highlights their versatile use.

Advancements in this field involve exploring new protecting groups that respond to different light spectra, extending from UV to visible light. This expansion is critical for developing bioprocesses that leverage multichromatic control, allowing for multifactorial process engineering where different pathways can be activated or inhibited using distinct wavelengths. The adaptation of such tools for use in alternative hosts, such as the phototrophic bacterium *Rhodobacter capsulatus*, has demonstrated potential for complex, layered bioprocess control.

The ongoing development focuses on synthesizing green light-sensitive protecting groups, enabling less invasive control compared to UV-based systems. These enhancements promise more efficient and reliable control over cellular processes, contributing to novel strategies in optogenetics for applications ranging from bio-manufacturing to medical therapeutics.

The ultimate goal of this expanding field is to create robust, multifactorial bioprocessing systems. The ability to selectively regulate different pathways using light as a precise trigger heralds a future where metabolic pathways can be dynamically modulated, enhancing efficiency and productivity in microbial production systems. These optogenetic innovations promise to bolster the production of fine chemicals and pharmaceuticals, optimizing outputs and reducing process-related challenges.

P-BT-095

Modifiying *Comamonas thiooxydans* to upcycle PET in biofilms

*M. Jaegers[1], R. Dierkes[1], P. Perez-Garcia[1], C. Vollstedt[1], W. Streit[1]
[1]*Hamburg University, IPM, Hamburg, Germany*

Introduction

The accumulation of plastics, among others PET, poses a serious threat to the environment due to its extensive and short-term use and insufficient recycling processes. Microbial degradation of PET can contribute to combat this problem. One promising organism is *Comamonas thiooxydans* S23, since it is capable to import and metabolise TPA, a degradation product of PET. Also, a reporter strain of *C. thiooxydans S23* namely *C. thiooxydans* ReporTPA_UHH04

has already been constructed by Dierkes et al. (2022), which exhibits TPA induced fluorescence of sfGFP. [1]

Goal

We aim to equip *C. thiooxydans* ReporTPA_UHH04 with suitable PETases to enable it to degrade PET to TPA. To optimize expression and secretion, different promotors and signal peptides will be compared. The strains will be grown as biofilms on PET-film as well as with PET-powder and should be able to directly report PET degradation through fluorescence. This should serve as a first proof of concept of *C. thiooxydans* being able to degrade PET to TPA, to further modify the strain to finally being able to convert TPA into a useful platform chemical in later work.

Methods

The formation of biofilms was visualised via confocal laser scanning microscopy and scanning electron microscopy. The performance of the PET degrading reporter strains for degrading PET as well as the TPA induced fluorescence itself will be analysed through confocal laser scanning microscopy and microtiter plate assays. PET degradation products will furthermore be verified through HPLC and MS.

Results

We were already able to identify conditions under which the *C. thiooxydans* could form biofilms on PET film. Further, potential PETases were already cloned into ReporTPA_UHH04 and right now their general activity is getting evaluated. The degradation ability of the promising strains to degrade PET as a biofilm on PET film as well as for PET powder will be examined in upcoming experiments.

Conclusion

C. thiooxydans can form biofilms on PET and if it can successfully be employed to degrade PET in different forms to TPA, the strain is well suited to be further manipulated to convert the imported through PET degradation released TPA into useful platform chemicals.

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P-BT-096

Harnessing the potential of methyltransferases utilizing C1 compounds as primary substrates for methylation reactions

*W. Mehak[1], J. M. Soder Walz[1], D. Deobald[1]
[1]Helmholtz Centre for Environmental Research - UFZ, Environmental Biotechnology, Leipzig, Germany

Methylation reactions play a crucial role in the chemical and pharmaceutical industries, where they are widely used for product diversification. Unlike conventional chemical methylation methods that rely on strong bases, metal catalysts, and toxic methyl donors, enzymatic methylation processes occur under mild conditions, offering both chemo- and regioselectivity. Methyltransferases (MTases) present a biological alternative for methylation reactions, with various low-molecular-weight methyl donors such as methanol and acetyl-CoA, or cofactors like methyl-tetrahydrofolate, *S*-adenosylmethionine (SAM), and methyl cobalamin available for use. While SAM-dependent methyltransferases have been extensively studied, cobalamin (B12)-dependent methylation systems remain largely underexplored. B12-dependent methylation systems consist of three main components: *i)* an MTase I that demethylates a methyl group donor, *ii)* an MTase II that transfers the methyl group to an acceptor molecule, and *iii)* a B_{12}-shuttle protein that carries the cofactor as a prosthetic group, enabling the reversible transfer of a methyl group between MTase I and MTase II.

My project will focus on the investigation and characterization of MTase I enzymes that use C1 compounds, such as methanol, as methyl group donors, which can be sustainably produced through electrochemical CO_2 reduction. To enable biotechnological applications of methanol-demethylating MTase I enzymes (MTase I), expression vectors will be constructed, followed by screening of various conditions for heterologous expression, protein production and purification, as well as enzyme activity assays. Recombinant MTase I enzymes will be identified, and metal cofactor characterization will be conducted using mass spectrometry and inductively coupled plasma-triple quadrupole mass spectrometry. Additionally, structural modeling with AlphaFold2, coupled with multiple sequence alignments, will help identify key amino acids for site-directed or saturation mutagenesis. Enzyme variants will be developed to enhance specific activity, affinity, and stability of MTase I.

Overall, this research aims to develop a sustainable and circular biological methylation process,using C1 compounds as a methyl group donor, that overcomes the limitations of conventional methods and supports a shift toward CO_2-neutral chemical processes. The outcomes hold promise for applications in sectors such as pharmaceuticals and biofuels.

P-BT-097

Engineering the amoeba *Dictyostelium discoideum* for biosynthesis of a cannabinoid precursor and other polyketides

*C. Reimer[1], L. Regestein[2], F. Hillmann[3], J. E. Kufs[1]
[1]Bielefeld University, Genome Engineering and Editing, Bielefeld, Germany
[2]Leibniz Institute for Natural Product Research and Infection Biology, Bio Pilot Plant, Jena, Germany
[3]Wismar University of Applied Sciences Technology, Business and Design, Faculty of Engineering, Biochemistry/Biotechnology, Wismar, Germany

Polyketides are structurally and functionally diverse natural products with a broad spectrum of biological activities and relevance as therapeutics. Advances in genome sequencing technologies are continuously increasing the number of new polyketide synthase (PKS) genes, presenting an untapped source for the identification of novel polyketides among all major taxonomic groups. Especially the genomes of social amoebae like *Dictyostelium discoideum* comprise a wide, but largely unexplored, repertoire of mostly silent PKS genes. Among those genes, there are two exceptions that encode for unique hybrid enzymes, having a type I fatty acid synthase (FAS) fused to a plant-like type III PKS. Due to its biosynthetic potential, we aimed to exploit *D. discoideum* as novel chassis for the production of native polyketides but also pharmaceutically relevant compounds of plant origin.

By expressing its native and cognate plant PKS genes, we show production of phlorocaprophenone, methyl-olivetol, resveratrol, and olivetolic acid (OA) as the central

intermediate in the cannabinoid biosynthesis. Cannabinoids are known for their psychoactive, sedative and analgesic properties, and are extensively studied for their use as alternative painkillers. The production of such phytochemicals in model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* has only been achieved by extensive metabolic engineering to ensure a proper supply of the required precursors. As *D. discoideum* is a native producer of polyketides and terpenoids, we aimed to realize OA biosynthesis in amoeba without additional accessory pathways. To address this, we engineered an amoeba/plant inter-kingdom hybrid enzyme, where a type III PKS from *Cannabis sativa* occurs as a C-terminal fusion to the amoeba FAS domain. This hybrid enzyme enabled OA synthesis from primary metabolites in two enzymatic steps and provides a shortcut in a synthetic cannabinoid pathway. To complete the cannabinoid biosynthesis in amoeba, we finally expressed genes encoding the prenyltransferase and tetrahydrocannabinolic acid synthase from *C. sativa* and demonstrated *in vitro* functionality of those enzymes. As alternative to chemical syntheses and laborious extractions from native plant material, an amoeba-based bioprocess may offer an innovative and environmentally friendly approach for cannabinoid production from renewable resources.

P-BT-098

Turning straw to gold: How *Ustilago maydis* converts corn stover to high quality oil

K. Müntjes[1,2], M. Philipp[1,2], *L. Plücker[1,2], S. Robertz[3,2], P. Richter[4,2], M. Pauly[3,2], M. Mann[4,2], M. Feldbrügge[1,2], K. Schipper[1,2]
[1]Heinrich Heine University, Microbiology, Düsseldorf, Germany
[2]Forschungszentrum Jülich GmbH, Bioeconomy Science Center (BioSC), Jülich, Germany
[3]Heinrich Heine University, Institute for Plant Cell Biology and Biotechnology, Düsseldorf, Germany
[4]RWTH Aachen University, Aachener Verfahrenstechnik - Biochemical Engineering, Aachen, Germany

The development of a bioeconomy is an important contribution to the UN"s Sustainable Development Goals Agenda 2030, that has been extensively promoted in recent years. In the framework of the BioSC, the NextVegOil project focuses on the production of a microbial palm oil substitute from the agricultural waste stream corn stover. Although plant oils like palm oil are a renewable material, the cultivation requires large deforested areas. In the case of palm oil, the CO2 emissions caused through deforestation of the rain forest as well as its worldwide transport are critical. Here we use the fungal chassis *Ustilago maydis* to produce a microbial oil that closely resembles the composition of palm oil. Currently, genetic engineering to tailor the fatty acid profile to further adapt the oil composition and to further increase the oil yields is conducted. In addition, a fluorescent reporter for online monitoring of oil accumulation was established. As a maize plant pathogen, *U. maydis* naturally possesses a set of carbohydrate-active enzymes that theoretically enables it to utilize complex substrates. By deregulating intrinsic enzymes and complementing the repertoire with heterologous hydrolytic enzymes we aim to further increase the utilization of the waste stream corn stover as sole carbon source for oil production.

In general, the biovalorization of corn stover on the one hand and the immense possible applications for microbial oil due to the huge marked volume of palm oil -containing products in the food industry on the other hand, underline the enormous potential of this product.

P-BT-099

Exploring oil-degrading microbes for environmental remediation

*L. Medina[1], D. Calderón[1], M. del Carmen Sánchez Olmos[1], D. Schindler[1], P. Del Portillo[1], M. M. Zambrano[1]
[1]Max Planck Institute for Terrestrial Microbiology, RG Schindler, Marburg, Germany

Introduction

The use of petroleum-based products can result in environmental pollution, mainly due to extraction, transport of oil, damage to infrastructure, and weather conditions. To provide solutions to the environmental impact of oil spills, this study analyzed microorganisms for their capacity to grow on crude oil and be used to clean up contaminated ecosystems. As the organisms are to be used for environmental remediation, they cannot be genetically modified and must comply with Government guidelines.

Methods

Previously isolated yeast strains capable of growing on hydrocarbons (Delgadillo-Ordóñez, 2017) were analyzed by growth on solid (YPD) and liquid medium (Czapek or M9) supplemented with crude oil. The growth and capacity of cultures to utilize crude oil was assessed visually. Synthetic communities of these strains were assessed using the same strategy. Individual strains were profiled using ITS sequence analysis and genomes of strains showing the most promising results were sequenced using long-read Nanopore sequencing.

Results

Of the twelve analyzed yeast strains, a subset of five showed the best growth in the presence of crude oil after 21 days of incubation, apparently reducing crude oil and resulting in the formation of granules indicative of degradation. ITS profiling identified the isolates as belonging to the genera *Candida, Cystobasidium* and *Rhodotorula.* Antagonistic activity (growth inhibition) was evident in some communities, while in others, communities seemed to potentiate the use of crude oil.

Conclusions and Perspectives

We identified yeast isolates capable of utilizing hydrocarbons from crude oil in the lab, individually or as a community. Future work involves quantification of degradation via HPLC and assessing their performance when adhered to a biodegradable mesh and applied in the environment. Furthermore, we aim to elucidate the metabolic pathways allowing for crude oil utilization based on genome mining of our sequenced isolates.

Reference:

Delgadillo-Ordóñez et al. 2017. Rev. Colomb. Biotecnol. XIX: 123-133.

Computational microbiology

P-CM-100
BACAI: A novel multi-modal approach to bacterial image analysis and classification
*A. Mostafa[1], E. Grohmann[1], E. Rodner[2], M. Koddenbrock[2]
[1]*Berlin University of Applied Sciences and Technology, Microbiology, Berlin, Germany*
[2]*Hochschule für Technik und Wirtschaft Berlin, Berlin, Germany*

The rapid and accurate identification of bacteria remains a critical challenge in microbiology, with far-reaching implications for healthcare, environmental monitoring, and industrial applications. We present BACAI (Bacterial Analysis and Classification through Artificial Intelligence), an innovative multi-modal platform designed to revolutionize bacterial image analysis and classification.

BACAI employs a multi-modal approach, integrating various data types to enhance bacterial analysis. As a foundational step, we are comparing state-of-the-art segmentation models, including convolutional neural networks and U-Net architectures, to establish a performance baseline for fluorescence microscopy image analysis. This comparison will inform the development of our multi-modal framework, which aims to synthesize image data with other relevant bacterial characteristics.

The platform's multi-modal capabilities extend beyond simple classification, aiming to extract meaningful biological insights from image data. By incorporating additional imaging modalities and metadata, BACAI has the potential to elucidate microbial cell biology characteristics, including morphological variations, growth patterns, and potentially, antibiotic resistance profiles.

Our initial database focuses on three clinically relevant bacterial species: Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. These species serve as a starting point for our model development, with plans to expand to a broader range of bacterial species in subsequent phases. This approach allows us to refine our methodologies on well-characterized organisms before tackling more diverse microbial communities.

Our ongoing research focuses on optimizing the neural network architecture, expanding the training dataset, and developing robust data augmentation techniques to enhance the model's generalization capabilities. Future directions include exploring the platform's potential for analyzing microbial interactions within mixed populations and integrating genomic data to provide a more comprehensive understanding of bacterial physiology and behavior.

BACAI represents a significant step towards automated, high-throughput bacterial analysis, with potential applications ranging from clinical diagnostics to environmental monitoring. This presentation will outline the theoretical framework of BACAI discuss the challenges, and present our roadmap for advancing the field of AI-assisted microbiology.

P-CM-102
BakRep DB release 2.0
*L. Fenske[1], L. Jelonek[1], A. Goesmann[1], O. Schwengers[1]
[1]*Justus Liebig University Giessen, Bioinformatics and Systems Biology, Gießen, Germany*

Bacteria serve as intriguing subjects of study across various disciplines for numerous reasons, and whole-genome sequencing has emerged as the primary method for enhancing our understanding of microbiology. The advent of cost-effective sequencing technologies has significantly accelerated bacterial whole-genome sequencing, presenting new challenges related to data accessibility, computational requirements, the diversity of analysis workflows, and ultimately, its scientific applicability. To address these challenges, *Blackwell et al.* released a consistently processed dataset of 661,405 bacterial genome assemblies sourced from the European Nucleotide Archive (ENA) as of November 2018. Building on this foundation, we utilized this dataset to compute assembly metrics, perform robust taxonomic classifications and MLST subtypings, and annotate all genomes. Previously, we introduced BakRep, a searchable large-scale web repository featuring this initial set of genomes, with plans to continuously expand the database by adding more genomes over time.

In March 2024, as a follow-up to Blackwell"s initial dataset, the AllTheBacteria project released an update with a total of 1,932,812 assemblies. We processed all these additional genome assemblies using our previous workflow and made them accessible through our web repository. BakRep is an innovative web repository designed to improve the findability and accessibility of sequenced bacterial genomes housed in public data repositories. By offering a uniformly processed and thoroughly annotated dataset, BakRep tackles key challenges in the field of microbiology, such as data integration, standardization, and usability. We believe that this large-scale yet accessible genome repository will empower microbiology researchers across various disciplines to leverage this extensive collection of genomes as a foundation for their targeted analyses. With its user-friendly interface and comprehensive dataset, BakRep serves as an invaluable resource for the scientific community, facilitating a wide array of comparative and clinical studies and fostering new breakthroughs in microbial genomics.

P-CM-103
Bac*Dive*: The core database for prokaryotic strain data
*I. Schober[1], J. Koblitz[1], J. Sardà Carbasse[1], E. Christian[1], J. Overmann[2], L. C. Reimer[1]
[1]*Leibniz Institute DSMZ, Bioinformatics, IT and Databases, Braunschweig, Germany*
[2]*Leibniz Institute DSMZ, Microbial Ecology and Diversity, Braunschweig, Germany*

Since its initial release more than a decade ago, the bacterial diversity database Bac*Dive* (https://bacdive.dsmz.de) has grown tremendously in terms of content and functionality. Today, Bac*Dive* is a comprehensive resource covering the phenotypic diversity of prokaryotes with data on taxonomy, morphology, physiology, cultivation, and more. As the leading database for strain-level information on bacteria and archaea, it has been designated as an *ELIXIR Core Data Resource* and a *Global Core Biodata Resource*. These distinctions underscore its importance to the wider microbiological research community. As part of the newly established DSMZ Digital Diversity infrastructure, Bac*Dive* is now closely linked to several other databases of fundamental

importance to the life sciences, including BRENDA, LPSN and SILVA. These databases will be further developed in a coordinated manner and benefit from frequent data exchange.

Here we present the current status and recent developments in Bac*Dive*. The database currently contains 2.6 million data points for 97,334 strains. This includes the world's largest collection of Analytical Profile Index (API) test results, which have now been fully translated into easily understandable and searchable data fields. Increasingly, the standardized high-quality data provided by Bac*Dive* is being used to train artificial intelligence models. The high-confidence genome-based predictions produced by our own models are now also being used to fill content gaps in the database. We further present a novel Bac*Dive* knowledge graph that provides powerful new search capabilities via a SPARQL endpoint to directly search and analyze the knowledge provided through Bac*Dive* in a standardized way, supported by a new descriptive ontology.

P-CM-104

NFDI4Microbiota supporting microbiome research providing data access, services, training and workflows

*M. Müller[1], C. Hege[1], B. Götz[2], K. Förstner[2], A. C. McHardy[2]
[1]Helmholtz Centre for Infection Research – HZI, Bioinformatics, IT and Databases, Braunschweig, Germany
[2]ZB MED, Information Centre for Life Sciences, Cologne, Germany

Introduction: NFDI4Microbiota aims to support the microbiome research community by providing access to data, analysis services, data/metadata standards, and training. It belongs to the National Research data Infrastructure (NFDI), which aims to develop a comprehensive research data management. Different consortia ensure a broad coverage from cultural sciences, engineering to life sciences and natural science. NFDI4Microbiota intends to facilitate the digital transformation in the microbiological community (bacteriology, virology, mycology, and parasitology).

Goals: NFDI4Microbiota aims to support the German microbiome research network through training and community building activities, and by creating a cloud-based system that will make the storage, integration, and analysis of microbial data and (microbial) omics data, consistent, reproducible, and accessible. Thereby, NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable and Re-usable) principles and Open Science.

Results: To enable FAIR data management, the NFDI4Microbiota consortium develops and provides computational infrastructure and analytical workflows to store, access, process, and interpret various microbiome-related data types. NFDI4Microbiota works on developing and implementing software and standardized workflows for users to analyze their own data. Further, NFDI4Microbiota offers trainings, spanning from metagenomics, over courses about programming in R, to research data management and ELN (electronic lab notebooks). To interact with young scientists, the consortium launched an ambassador program, thereby helping to identify the needs of their local community. All relevant information and specific services are available via the web portal.

Summary: NFDI4Microbiota has established community services providing access to data, analysis services, data/metadata standards, and training thereby promoting FAIR principles and Open Science in the microbiology community.

P-CM-105

EDGAR 3.5: Advancing comparative genomics with new tools for microbial genome analysis

*M. Pfister[1], L. Fenske[1], A. Jain[2], M. Dieckmann[1], F. Knopp[1], J. Blom[1], A. Goesmann[1]
[1]Justus Liebig University Giessen, Bioinformatics and Systems Biology, Gießen, Germany
[2]Justus Liebig University Giessen, Bioinformatics and Systems Biology, Gießen, Germany

Introduction

The significant increase of whole genome sequence data availability, made possible by next generation sequencing methods, has caused the emergence of a variety of tools for the comparative analysis of related genomes. The web-based, EDGAR platform, is one of the most established tools, to highlight similarities and differences, conserved and divergent regions within microbial sequence data. EDGAR is being constantly updated for the last 15 years to serve the emerging needs of the scientific community. This work focuses on the latest developments on the EDGAR platform and expands on upcoming changes.

Objective

Since its inception, the average number of genomes analyzed per EDGAR project has steadily increased, as has the variety of methods employed by its community. As a consequence, the development of EDGAR pursues two main goals: First, to create the necessary infrastructure to accommodate the mounting data volumes. Second, to constantly improve the EDGAR website with new ways for scientists to examine their genomes.

Methods

We iterated further on our recently released functional category features and will soon offer the interactive exploration of KEGG subcategories organized in a sunburst plot. The GO term categorization has received a major upgrade, with it now being possible to calculate an enrichment analysis between a chosen subset of genomes. This is being additionally expanded upon in the form of a GO-Net visualization, which will make it possible to browse through the web of correlated genes and hierarchical GO-terms. Furthermore, we are working on a feature that enables our users to find, save, visualize and compare conserved syntenic gene clusters. The core ortholog calculation has been rewritten, making it faster by a factor of 50x, enabling EDGAR to scale into the ultra-high-throughput era. This will allow us to use binned subsets of the BakRep project to provide a significantly scaled-up version of EDGAR's public database in the near future.

Results

EDGAR continues to grow its capabilities, offering an increasingly diverse array of analysis methods for comparative genomics and phylogenomics in a single software framework. Our infrastructure and web platform offer researchers a time-effective way to gain various perspectives on their microbial genomes. For unpublished data, EDGAR offers private, password protected projects.

Our web server is accessible at: https://edgar3.computational.bio

P-CM-106
Thermodynamic limits of microbial growth
*O. Ebenhöh[1]
[1]*Heinrich Heine University, Düsseldorf, Germany*

Microbial growth is often described as an energy converter, in which the catabolic breakdown of nutrients yields free energy which is partly used to drive the anabolic processes that assemble nutrients into new biomass. The combined effect of anabolism and catabolism can be described by a so-called "macrochemical" equation, which summarizes the chemical conversion of nutrients into catabolic waste products, biomass, and possibly other side products. Because such a description of microbial growth completely ignores the details of intracelleular metabolism, it is often termed a "black box" model of microbial metabolism. A diametrically different approach to describe metabolism is by genome-scale metabolic network models. Such models are derived from annotated genome sequences and ideally comprise all known biochemical reactions that can occur inside an organism. We here investigate how these two concepts can be combined to study the thermodynamics of microbial metabolism. We illustrate how experimentally determined macrochemical equations can be systematically separated into a catabolic and an anabolic part. We then characterize these separate chemical equations by their thermodynamic properties and derive the generalized thermodynamic forces and flows that correspond to the affinities of catabolism and anabolism, and the corresponding catabolic and anabolic rates. With these data we challenge the common view that microbial energy conversion can be described by a linear energy converter model. Moreover, we employ genome-scale models to study how catabolism and anabolism are coupled through the production and consumption of ATP, and determine thermodynamic efficiencies. Finally, we observe a linear relation between the growth rate and the catabolic power, which appears to be species independent and may hint at a general principle in microbial growth.

P-CM-107
NFDI4Microbiota – Enhancing microbiota research through data integration and digital transformation
*M. Bole[1], K. Förstner[2], A. C. McHardy[3], N. Consortium[2], *U. Nunes da Rocha[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Applied Microbial Ecology - AME, Leipzig, Germany*
[2]*ZB MED, Data Science and services, Cologne, Germany*
[3]*Helmholtz Centre for Infection Research – HZI, Computational Biology for Infection Research, Braunschweig, Germany*

Over recent years, advances in omics data generation have opened up significant opportunities for researchers working with "big data." As a result, the challenges in the field have evolved, shifting from data collection to the reuse, interpretation, and analysis of vast, heterogeneous datasets. The NFDI4Microbiota consortium was formed to meet these new demands by offering data access, analytical services, training, and infrastructure to support the broader microbiological community.

NFDI4Microbiota aims to serve as a central hub established to assist both the German and international microbiological research communities. It comprises ten partner institutions backed by five professional societies and over 50 participating organizations. The consortium aims to accelerate the digital transformation within the microbiological community by providing solutions for data management through cutting-edge computational methods. It also seeks to streamline the entire research workflow, from initial data generation to the final stages of publication, including data submission. The consortium offers specialized training programs, infrastructure, and advanced computational tools to achieve this streamlining process.

The wide array of tools, methodologies, and resources offered by NFDI4Microbiota is designed to facilitate the transformation of new and existing data into meaningful scientific knowledge. Training opportunities are available in open science, research data management, databases, programming, data science, and data analysis. Furthermore, implementing a cloud-based infrastructure provides access to analytical tools, a knowledge base, and systems for analyzing, integrating, and storing microbiological data.

By creating this centralized resource, NFDI4Microbiota aims to become the essential connection for microbiota research in Germany and beyond. It offers researchers the infrastructure, expertise, and easy access to tools and training that enhance their work while supporting Open Science principles and ensuring that data remains Findable, Accessible, Interoperable, and Reusable (FAIR) in the future.

P-CM-108
Pan-BGC Atlas: An in-depth visual analysis of biosynthetic gene cluster families
*D. Paccagnella[1], B. Pourmohsenin[1], A. Gavriilidou[1], H. Link[1], C. Loureiro[2], M. Medema[2], N. Ziemert[1]
[1]*Eberhard Karls University Tübingen, Ziemert Lab, Tübingen, Germany*
[2]*Wageningen University, Wageningen, Netherlands*

Due to environmental pressures such as microbial competition and adaptation to diverse ecological niches, biosynthetic gene clusters (BGCs) in microorganisms have evolved a remarkable natural diversity. This diversity enables microbes to produce a wide array of structurally and functionally similar yet distinct secondary metabolites, many of which play essential roles in survival and interaction within their environments.

Using bioinformatics tools, we can group BGCs into gene cluster families (GCFs) based on genetic similarity. GCFs can vary widely in size, ranging from single clusters per family to hundreds. Compounds produced by BGCs within a family typically share a core chemical structure with only slight variations. This raises important questions: What are the underlying mechanisms and building blocks that lead to this diversity? Can we harness this potential, and gather more insights into selection mechanisms?

To better understand and systematically explore the natural diversity in GCFs, we developed a comprehensive visual GCF database, the Pan-BGC Atlas, by adapting the pangenome concept to BGCs. Our approach involved grouping bacterial BGCs from the antiSMASH database into similarity-based families, followed by a series of bioinformatics analyses. In the Pan-BGC Atlas, each GCF is visually represented and can be easily adjusted, allowing for rapid and intuitive analysis.

The Atlas includes both an "open" BGC, encompassing all possible genes within a GCF, and a "closed" BGC,

representing the minimal set of genes needed to produce the core chemical structure shared across the family. Comparing the open and closed BGCs, along with other information, reveals enzymatic diversity and could offer new insights for synthetic biology.

Beyond exploration, users can compare their own BGCs against the database to identify the closest matching families. Additionally, a downloadable pipeline enables users to create similar visual analyses for their own gene cluster families, fostering reproducibility and broader application of these tools. This platform is a valuable resource for microbial genomics and natural product research, enhancing navigation and interpretation within GCFs.

P-CM-109

Computational modelling of metabolic interactions in a virtual colon

*G. Marinos[1,2], J. Zimmermann[1,3,4], J. Taubenheim[1], C. Kaleta[1]
[1]*Christian-Albrechts-University of Kiel, Research Group Medical Systems Biology, Kiel, Germany*
[2]*Christian-Albrechts-University of Kiel, CAU Innovation GmbH, Kiel, Germany*
[3]*Max Planck Institute, Antibiotic Resistance Group, Plön, Germany*
[4]*Friedrich Schiller University, Junior Research Group Mechanisms of Microbial Metabolic Interactions, Jena, Germany*

Question

Metabolic interactions are important for the function, health, and disease of host-microbial systems. Reconstructing metabolic models of organisms from host and microbial genomes allows us to study these interactions. However, these interactions are often considered static, neglecting spatial and temporal factors.

Methods

The software BacArena enables the co-simulation of those models in a computational space over time and has been widely used to decipher such phenomena. Here, we present Virtual Colon, an application of this software which is specialised to replicate the structure of the mammalian colon. The host models are separated from the bacterial ones, which reside in a simulated lumen including mucus layers.

Results

Our proof-of-concept experiments include transcriptomics-based, context-specific colonic models combined with individual bacterial species and a bacterial collection, such as the extended simplified human intestinal microbiota (SIHUMIx). The Virtual Colon notably visualises those interactions either predicting known ones (e.g., short-chain fatty acids exchange) or novel ones (e.g., amino acids exchange).

Conclusions

Virtual colon enhances genome-scale metabolic modelling, providing new insights into spatiotemporal host-microbiome systems. Like BacArena, it is freely available and can be adapted for other spatially structured environments (www.github.com/maringos/VirtualColon, www.doi.org/10.1101/2024.06.11.598488).

P-CM-110

Importance of data Provenance in genome sequencing studies. A guideline to understand the gaps in sequencing projects

*S. Kamath[1], U. Nunes da Rocha[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany*

There is a growing need to make data from life sciences findable, accessible, interoperable, and reusable(FAIR), which is incredibly challenging in metagenomics. This field deals with the study of microorganisms' functional potential in natural, host-associated, and constructed environments. It uses high-throughput sequencing data from total DNA isolated from microbial communities. There is often a disconnection between wet labs and computational analysis in this field, leading to provenance issues among scientists.

This study aims to improve FAIR principle usage in metagenomics by creating guidelines to connect the wet and dry lab parts of projects in the context of genomic sequencing. To this end, we explored provenance in over 50 projects involving more than 1000 samples from multiple sources, i.e., bioreactors, agricultural and forest soils, freshwater, wastewater, and the gut microbiome. We organized the guidelines into five parts: sample preparation, sequencing logistics, data downloading, integrity and quality checking of sequences, and preprocessing the sequenced data until assembly.

First, we analyzed provenance from sample preparation (sample storage and DNA extraction) for metagenomics. Our data indicated that it is necessary to pay attention to the DNA yield and quality (particularly if interested in long-read sequencing) during sample preparation for sequencing. We observed that both low and high yields in the samples can lead to failed library preparation. DNA quality check must be performed in every sample in BioAnalyzer-like machines. We also highlight that memory configurations and resources required for sequencing projects to facilitate reproducibility must be considered, particularly for sequencing data preprocessing, as it demands a high usage of resources. Reviewing and commenting must be implemented on automatic reports in existing data processing pipelines to improve the interoperability and reuse of metagenomics data.

In Conclusion, small research groups and data stewards responsible for organizing data in local or large sequencing facilities may use our guidelines to bridge the gap between wet and dry lab researchers. Our policies may help to improve FAIR usage of metagenomics as we concentrate on data interoperability and reuse aspects in genome sequencing.

P-CM-111

Comprehensive hybrid genome assembly reveals mobile genetic elements, antimicrobial-resistance genes and antimicrobial peptides in *Bacteroidota* spp. using machine learning approaches

*M. A. Tufail[1], H. Bethge[1], L. Ladewig[1], R. A. Schmitz[1]
[1]*Christian-Albrechts-University of Kiel, Institute for General Microbiology, Kiel, Germany*

Bacteroidota species, dominant members of the human gut microbiome, are emerging as promising candidates for Next

Generation Probiotics (NGPs) due to their extensive metabolic capabilities, antimicrobial properties, and beneficial interactions with the host immune system. The emergence of *Bacteroidota* spp. as NGPs necessitates detailed genomic characterization to understand their therapeutic potential. This study presents a comprehensive genomic and pangenome analysis of 18 Bacteroidota isolates from diverse sources including mouse cecum, animal feces, and human stool samples, utilizing an innovative hybrid sequencing approach combining Illumina short reads and Oxford Nanopore long reads. Our optimized hybrid assembly pipeline, employing Unicycler with Canu-corrected long reads, achieved exceptional genome completeness (≥99.68%) with minimal contamination. Three isolates achieved single-contig circular genomes, while seven others demonstrated minimal complexity in their assembly graphs. The assembled genomes showed consistent coding density (90.5-98.5%) with gene counts ranging from 3,968 to 5,150, as annotated by Bakta. Taxonomic classification using GTDB-TK identified the isolates as members of the *Bacteroides* and *Phocaeicola* genera, with Fast-ANI scores above 97.07%. Comprehensive screening using multiple databases (NCBI, CARD, and Resfinder) through ABRicate revealed the presence of diverse antimicrobial resistance genes among isolates. Novel potential antimicrobial peptides were predicted using an enhanced prediction approach combining hyperparameter tuning of machine learning models, optimized amPEPpy v1.1.0, trained on ADAPTABLE and AMPSphere databases, complemented by Macrel v1.4.0 for high-quality AMP candidate identification and small open reading frame prediction. Characterizing the isolates experimentally demonstrated significant probiotic potential, with all strains showing capacity to inhibit biofilm formation against clinically significant pathogens. Notably, *Bacteroides caecimuris* and *Bacteroides muris* exhibited exceptional biofilm inhibition and quorum quenching activity. This comprehensive genomic, pangenomic and functional characterization establishes these *Bacteroidota* strains as promising candidates for therapeutic applications, advancing our understanding of their potential as next-generation probiotics.

P-CM-112

Extending the enzyme-constrained genome scale model of *Corynebacterium glutamicum* with multi-omics datasets

*L. Wormer[1], D. Thiele[2], M. Nusser[3], T. Alter[4], A. K. Kaster[2], K. Bleher[3], N. Henke[1]
[1]*Karlsruhe Institute of Technology (KIT), Institute of Process Engineering in Life Sciences - Microsystems in Bioprocess Engineering, Karlsruhe, Germany*
[2]*Karlsruhe Institute of Technology (KIT), Institut of Biological Interfaces – IBG5, Karlsruhe, Germany*
[3]*Karlsruhe Institute of Technology (KIT), Institute of Functional Interfaces (IFG), Karlsruhe, Germany*
[4]*RWTH Aachen University, Microbiology, Aachen, Germany*

Introduction

Advances in computational methods continue to shift the landscape of what is feasible when modeling complex biological systems *in-silico,* while modern omics analysis tools can deliver the broad *in-vivo* quantification of biomolecules required to calibrate these models. Genome-scale metabolic models (GEMs) represent the entirety of known biochemical reactions encoded in a genome as a network of constrained and balanced fluxes. Several GEM extensions have been developed, integrating details from systems biology (e.g. transcriptional regulation [1]) and biochemistry (e.g. turnover numbers [2]).

Goals

This work focuses on a novel enzyme-constrained GEM (ecGEM) for *C. glutamicum* integrating data both from systems biology and biochemistry.

Materials & Methods

The ecGEM formulation presented here combines (i) enzyme abundances derived from proteomics with (ii) catalytic turnover numbers estimated via data mining and machine learning methods based on the public databases BRENDA and SABIO-RK as well as the (iii) incorporation of transcriptomics derived from RNA-seq.

Results

Via data mining, 457 turnover numbers could be retrieved for the catalytic enzymes of *C. glutamicum* (30% of 1539). The remaining 70% were predicted via recently published deep learning models. Protein and RNA abundances were determined across a broad set of culture conditions. In general, more than 1000 proteins were quantified via LC-MS-MS with data-independent acquisition, while the total RNA profile was investigated with RNA-seq. Those datasets served for the calibration of the newly developed ecGEM. Computational methods were applied for the prediction of protein abundances from mRNA, enabling the direct use of transcriptome profiles as condition-specific constraints for the analysis of the ecGEM.

Summary

The resulting ecGEM can be used to investigate *C. glutamicum* on the transcriptomic, proteomic and metabolic level, providing new impetus to metabolic engineering as well as a proof of concept for the extension of ecGEMs beyond the proteome.

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Environmental microbiology & ecology

P-EME-113

Promoting autotrophic denitrification in shallow porous aquifers for drinking water production

*F. Pfaff[1], A. Seeholzer[2], A. Wunderlich[2], V. Falchi-Bernardo[3], J. Fleckenstein[3], F. Einsiedl[2], T. Lueders[1]
[1]*University of Bayreuth, Ecological Microbiology, Bayreuth, Germany*
[2]*Technical University Munich, Hydrogeology, Munich, Germany*
[3]*Helmholtz Centre for Environmental Research - UFZ, Hydrology, Leipzig, Germany*

Nitrate pollution in groundwater is a global challenge since groundwater serves as a vital drinking water source across Europe. Avoiding a nitrate overload requires sustainable management schemes for shallow aquifers with high groundwater recharge. However, their attenuation potential against pollutants like nitrate is often limited. High nitrate levels persist in many aquifers even long after reducing nitrogen inputs. Especially for otherwise oligotrophic shallow groundwater systems, innovative and cost-effective technologies to eliminate nitrate loading and to ensure a sustainable supply of drinking and utility water are urgently needed to meet rising water demands.

In this project we will implement a novel approach for nitrate removal from shallow groundwater. Via the injection of reduced gaseous electron donors (H_2 & CH_4), we aim to stimulate autotrophic denitrification directly upstream of burdened drinking water wells. This has the advantage of avoiding an enrichment of heterotrophic, potentially pathogenic microorganisms.

In lab scale microcosms, we have simulated a range of in-situ conditions and aquifer materials. We identified temperature as a key driver for denitrification rates. Enrichment cultures of autotrophic denitrifying microorganisms for a potential augmentation and stimulation of denitrification inside natural aquifers were successfully introduced to a sediment matrix in a bioreactor. We inoculated non-denitrifying aquifer material with enriched stable denitrifying communities and successfully reestablished denitrification in the presence of hydrogen and methane. In a currently running mesoscale pilot plant experiment we are testing the principles of gas injection into porous media and monitor the stimulation effects over time. In a final stage, we hope to successfully implement our approach at the field scale to eliminate nitrate in an actual drinking water production system.

We aim to offer proof that injecting H_2/CH_4 can effectively eliminate nitrate from groundwater aquifers without adversely impacting water quality or hydraulic characteristics through secondary reactions, like mineral dissolution and precipitation. Harnessing the intrinsic potential of autotrophic microorganisms native to groundwater systems and using gasses that can be produced with green biotechnology offers a new and sustainable solution to a notorious problem.

P-EME-114

Impact of land use on sediment and mayfly larval prokaryotic communities in temperate freshwater ecosystems

*R. Martínez-Cuesta[1,2], R. Hoess[3], J. Geist[3], M. Schloter[1,2], S. Schulz[2]
[1]*Technical University Munich, Chair of Environmental Microbiology, Freising, Germany*
[2]*Helmholtz Centre Munich, COMI, Munich, Germany*
[3]*Technical University Munich, Chair of Aquatic Systems Biology, Freising, Germany*

Host-microbe interactions are key for host health, behavior and function. Besides vertical transmission, hosts acquire part of their microbiome from the surrounding environment. Thus, environmental microbiome alterations can disrupt host microbial communities and may induce dysbiosis. We hypothesized that intensive land use resulting in increased nutrient runoff and suspended organic matter (SOM) would impact the microbiome of sediments (< 5 cm in depth) and mayfly larvae hosts (*Ephemera danica*) feeding on SOM within the sediment. The prokaryotic communities of sediment and mayfly larvae guts were analyzed in relation to land use gradients including forest, extensive grassland and intensive agriculture of two different stream catchments (Otterbach and Perlenbach) in the Forstmühler Forest, Upper Palatinate, Germany.

Five sediment replicates and larvae samples were collected from each site at two time points in 2023 (August and November). Three dissected larvae guts were pooled for each replicate, resulting in five biological replicates per site. Prokaryotic community composition was assessed through 16S rRNA gene amplicon sequencing. Sequencing results were processed with DADA2 v1.28.0 until the obtention of amplicon sequencing variants (ASVs), which were taxonomically classified using the Silva v138.1 database.

Nutrient gradients played a significant role in shaping beta-diversity, particularly in sediment samples. PERMANOVA tests indicated that sampling time and land use significantly influenced clustering within sample types. The analysis of compositions of microbiomes with bias correction 2 method was used to detect differentially abundant ASVs comparing forest and intensive site samples for each sample type. This approach identified potentially pathogenic, fertilization and sewage-associated taxa in the larvae gut and sediment samples from the site under intensive management, such as *Aeromonas*, *Gaiella*, *Illumatobacter* or *Terrimonas*, which can harbor type VI secretion systems, antimicrobial resistance or denitrification, while taxa with beneficial properties, such as *Anaerovorax*, *Lachnospiraceae* or *Christensenellaceae*, typically known as short-chain fatty acids producers and plant material degraders, were detected in the forest site larvae guts.

Our findings demonstrate downstream effects of terrestrial land use on freshwater ecosystems, with potentially negative effects on host-microbe interactions, entailing loss of function or behavioral changes.

P-EME-115

Resistance properties among different *Clostridioides difficile* isolates

*F. Poth[1,2,3], D. Jahn[2,3], M. Neumann-Schaal[1,3]
[1]*Leibniz Institute DSMZ, Braunschweig, Germany*
[2]*Technical University of Braunschweig, Microbiology, Braunschweig, Germany*
[3]*Technical University of Braunschweig, Braunschweig, Germany*

The rod-shaped, motile and obligate anaerobic bacterium *Clostridioides difficile* occurs ubiquitously in the environment and colonizes the gastrointestinal tract of mammals. As a leading causative agent of antibiotic-associated diarrhea worldwide, it is particularly relevant in clinical settings. The pathogenicity of *C. difficile* is based on the formation and secretion of toxins and the associated damage to the intestinal epithelial tissue. If the *C. difficile* infection (CDI) progresses severely, the consequences for the patient can be fatal. Treating CDI is challenging due to *C. difficile's* ability to form metabolic dormant endospores, which leads to the risk of reinfection.

Since the first description in the 1930s, numerous isolates have been characterized. Common protocols recommend heat or ethanol shock during sample preparation, using the endospore's resistance for isolating purposes. It became apparent that both, the vegetative cells and the corresponding endospores of several isolates react differently to certain treatments. As a result, the isolation

conditions used to monitor *C. difficile* populations might not reflect the actual situation. To address this issue, a panel of *C. difficile* isolates were tested regarding their resistance properties over different exposure times applying a plating procedure. Here, defined concentrations of vegetative cells as well as endospores were used to determine classical characterization parameters based on the inactivation kinetics. This knowledge provides information to improve isolation and enrichment strategies to obtain a better understanding of the environmental distribution of *C. difficile* populations.

P-EME-116
Quantification of the influence of a Rhine flood on the microbiology of groundwater
*I. Erdmann[1], V. Brauer[1], R. U. Meckenstock[1]
[1]University of Duisburg-Essen, Essen, Germany

Bank filtration is a widely used method for the pre-treatment of drinking water. During a flood situation, surface water can penetrate the groundwater to a greater extent due to flooding or erosion of the colmation layer. This can affect the microbial quality of the groundwater and is relevant for drinking water protection, as the introduction of microorganisms must be prevented and monitored. This study determined whether and how microorganisms are introduced from river Rhine into the groundwater during floods. The selected groundwater wells represented a gradient in distance from the Rhine (17 to 78 m) and were sampled at three different depths (18 to 27 m). In January 2024, surface and groundwater samples were taken five days after a flood situation from the Rhine in Düsseldorf and from neighboring groundwater wells. In April, the procedure was repeated at normal water level. Community composition was analyzed using 16S rRNA gene sequencing, cell density was measured using flow cytometry and internal ATP was determined using BacTiter-Glo Microbial Cell Viability Assay. A principal component analysis (PCA) revealed that the microbial composition of the groundwater in the samples near the Rhine was more similar to that of the Rhine water in January than in April. During floods, up to 65.5 % of the identified amplicon sequence variants (ASVs) were transported from the Rhine into the groundwater. In April this value dropped to 10.8 %. In general, the more distant the wells were from the Rhine, the fewer ASVs from the Rhine are found in the groundwater. The cell count in the wells closest to the Rhine was higher in January than in April. This also applies to the internal ATP. We conclude that the Rhine water transports microorganisms into the groundwater to a considerable extent and changes its composition. The effect is increased during floods. The effect of bank filtration can be measured based on the number of cells and the microbial composition in the groundwater. The groundwater community appears to be resilient to this influence and can return to its original state.

P-EME-117
Influence of oxygen supply on bacterial degradation of various azo dyes by *Rhodoccocus opacus* 1CP
*P. Hanisch[1,2], A. C. Ngo[2], D. Tischler[2], R. Huber[1]
[1]Technical University Munich, Department of Engineering and Management, Munich, Germany
[2]Ruhr University Bochum, Faculty of Biology and Biotechnology, Bochum, Germany

Azo dyes are a primary group of synthetic dyes widely used for textiles, food technology, and paper printing. These compounds contain one or more azo bonds linking aromatic rings. Some dyes and their aromatic biotransformation products are toxic or carcinogenic, which makes them significant environmental pollutants. Azo dyes can be degraded by microorganisms under anaerobic, facultative aerobic, or aerobic conditions, depending on the type of organism used. Anaerobic degradation involves nonspecific enzymatic processes, whereby resulting amine compounds cannot be further broken down under these conditions and may be toxic. Under aerobic conditions, these amine compounds can be further degraded. It has also been shown that oxygen can inhibit dye decolorization, as evidenced by comparing static and shaking culture conditions. Additionally, the primary organic carbon source present can influence the degradation process under both anaerobic and aerobic conditions.

However, the effects of varying oxygen supply levels remain not fully understood, particularly for the organism *Rhodococcus opacus* 1CP. The study will focus on investigating the influence of oxygen supply on biodegradation efficiency. Oxygen transfer rate (OTR) measurements in 96-well plates allow for varying oxygen availability for bacterial cultures depending on the filling volume of each well. This high-throughput screening method enables simultaneous investigation of numerous different settings, combining azo dye decolorization measurements with mass spectrometric analysis of degradation products to examine the impact of dissolved oxygen on the complete biomineralization of various azo dyes.

Experiments confirmed the theory that rapid decolorization of the azo dyes methyl red and brilliant black occurs at lower OTRs due to reduced oxygen availability by the organism *R. opacus* 1CP. Additionally, a significant difference in the OTR profile during the cultivation of the organism was observed.

The data provided deeper insight into these processes, enabling targeted adaptations for cultivation settings that could lead to more efficient azo dye decolorization and by-product degradation through optimized oxygen supply.

P-EME-118
Towards deciphering interactions within the living Skin of the Earth
*S. Herrera-Chairez[1], C. Jogler[1], *V. S. Hotter[1]
[1]Friedrich Schiller University, Institute of Microbiology, Jena, Germany

Biocrusts (BC) are intriguing communities composed of heterotrophic bacteria and fungi as well as photoautotrophic cyanobacteria, algae, mosses and lichens. Often referred to as the *Skin of the Earth*, latest estimates state that they cover about 12% of all terrestrial surface. BC are found globally where light is available but otherwise harsh conditions make the prevailing growth of higher plants impossible. They dominate arid regions such as the poles, deserts, and high alpine environments, where they are pivotal ecosystem engineers that offer a wide array of ecosystem services stabilising the soil surface, preventing erosion, fixing carbon and nitrogen, and building up organic soil biomass that enables later plant settlement. Moreover, BC immobilise heavy metals in contaminated soils, making them promising communities for bioremediation applications.

Despite their long-known ecological importance, many aspects of BC are still poorly understood. While their biogeography and ecology is well-researched, detailed

studies are confined to few geographical regions, resulting in limited knowledge about both photo- and heterotrophic species composition. Biochemical and molecular processes underlying their ecological functions, interactions between the many different microorganisms that build a BC, and the role of secondary metabolites in these interactions are, with very few exceptions, still unknown. To investigate these intricate and important communities on a molecular level, model systems consisting of at least two organisms are needed. Such a system, however, so far only exists for cyanobacteria-dominated BC from temperate North American deserts, which is unsuited to study BC from other ecoregions like Europe, where BC are also found in cold climate zones like the Alps or the Arctic.

To develop a model system to investigate molecular mechanisms in BC from Europe, we isolated bacteria from an Arctic BC for further studies. Here, we present the first results of our work towards deciphering interactions within the living Skin of the Earth.

P-EME-119

Forest tree holes as models to study the island biogeography of microorganisms

*F. Bajerski[1], J. Sikorski[1], S. Vieira[1], C. Baschien[2], A. Methner[1], A. Roberts[3], M. Gossner[4,5], J. Petermann[3], J. Overmann[1]
[1]Leibniz Institute DSMZ, Microbiology, Braunschweig, Germany
[2]Leibniz Institute DSMZ, Bioresources for Bioeconomy and Health Research, Braunschweig, Germany
[3]University of Salzburg, Department of Environment and Biodiversity, Salzburg, Austria
[4]Swiss Federal Research Institute WSL, Forest Entomology, Birmensdorf, Switzerland
[5]ETH Zurich, Department of Environmental Systems Science, Zurich, Switzerland

Water-filled tree holes are naturally formed semi-enclosed cavities in which environmental conditions such as water availability strongly fluctuate. These small aquatic ecosystems feature island-like characteristics that render them suitable models to test island biogeography theory and to study the mechanisms of microbial community assembly.

Our study was conducted within the German Biodiversity Exploratories framework and is one of the first targeting both bacterial and fungal communities present in tree holes. Microbial biomass collected from 55 water-filled tree holes *(predominately Fagus sylvatica)* in three different geographic regions were analyzed by Illumina high-throughput amplicon sequencing (bacteria: V3 16S rRNA gene, fungi: ITS2 rRNA gene region). Community composition was used to elucidate the factors shaping biogeographic patterns and to examine the extent to which tree holes represent microbial islands.

Tree holes were found to harbour complex microbial communities. We observed bacterial species known to be associated with freshwater and sediment (*Limnohabitans*, *Flavobacterium*, *Polynucleobacter*), soil (*Pseudomonas*) and plant-associated (degrading) (*Duganella*, *Paludibacter*) environments, as well as phytopathogenic (*Fusarium*), insect-associated (*Zoophthora*), and aquatic fungi (*Alatospora*, *Flagellospora*, *Gorgomyces*) including yeasts (*Camptobasidium*). Overall, we observed a limited overlap of bacterial or fungal taxa between individual tree holes. In contrast to previous reports, species richness of the bacterial communities decreased with increasing tree hole volume. However, we found evidence that deterministic processes had little overall influence on microbial community assembly, whereas stochastic processes were predominant in these systems. Especially dispersal limitation was important, which had a greater impact on bacteria than on fungi. Since community assembly was largely mediated by stochastic processes, our results support the conclusion that tree holes act as islands for microbial communities.

P-EME-120

Exploring microbial communities and methane cycling in Northern Peatlands

*G. Fiorito[1], M. H. Schroth[1], M. Sander[1], M. Schölmerich[1]
[1]ETH Zurich, Zurich, Switzerland

Peatlands are significant organic carbon pools, naturally acting as carbon dioxide (CO_2) sinks but emitting methane (CH_4), potent greenhouse gas, as a product of the slow decomposition of organic matter under anoxic conditions. This study illuminates the unexplored microbial communities of pristine Northern peatlands in Värmland, central Sweden, with a particular focus on microorganisms involved in CH_4 cycling. Peat samples were collected up to 60 cm depth from four peatlands traced back to the Holocene (Björsmossen [BM], Norra Romyren [NR], Lungsmossen [LM] and Havsjömossen [HM]) and subjected to 16S rRNA amplicon sequencing and biogeochemical measurements to compare the biogeochemistry and microbial diversity across the sites. Additional samples retrieved up to 700 cm depth were subjected to amplicon sequencing to shed light on the microbial communities across the entire peat layer. This revealed that deep samples generally showed a higher microbial diversity and were dominated by archaea, mainly *Crenarchaeota*, encompassing many uncultivated lineages. Eight microbial taxa were identified across all samples as potential contributors to CH_4 cycling, comprising aerobic methanotrophic bacteria and methanogenic archaea, but no anaerobic methanotrophic archaea. Methanotrophic bacteria represented 20% of the community in the top peat layers, but significantly decreased in abundance below 30 cm sampling depth. At the same time, elevated CO_2 to CH_4 ratios measured at two of the sites at these depths suggest that there is a hitherto unidentified microbial process causing the low CH_4 concentrations relative to CO_2 concentrations in the lower layers. These findings provide unprecedented insight into the microbial communities inhabiting pristine Northern peatlands and their correlation with peat biogeochemistry. Upcoming metagenomic and metatranscriptomic data will help clarify their metabolic roles and potential involvement in CH_4 cycling.

P-EME-121

Targeted ecological effects of phage cocktails against a pathogen of foals in the soil of horse breeding farms

*G. O'Reilly[1], K. Muscat[1], C. Giles[2], M. Barton[3], G. Muscatello[1], C. Venturini[4,5]
[1]The University of Sydney, School of Life and Environmental Sciences, Sydney, Australia
[2]Centre for Aquatic Animal Health, Department of Primary Industries, Parks, Water and Environment, Hobart, Australia
[3]University of South Australia, UniSA Clinical and Health Sciences, South Australia, Australia
[4]The University of Sydney, Sydney School of Veterinary Science, Sydney, Australia
[5]Westmead Institute for Medical Research, Centre for Infectious Diseases and Microbiology, Westmead, Australia

Virulent *Rhodococcus equi* is a globally recognised pathogen of young foals, best known for causing aggressive pyogranulomatous bronchopneumonia. The lack of a vaccine, emergence of multi-drug resistance and the soil-

animal lifestyle of *R. equi* necessitates novel approaches to disease control. The specificity of bacteriophages (phages) offers a promising opportunity to manage *R. equi* burdens in soil. The aim of this study was to formulate phage cocktails (combinations of phage) and assess their capacity for *R. equi* burden control in soil. This study involved *in vitro* laboratory and soil intervention experimental design. Five phage candidates isolated from fecal and soil samples from Thoroughbred farms were screened against a collection of virulent *R. equi* isolates. Collectively, the phages showed lytic activity against 20/22 historical (1991-2004) and 17/21 contemporary (2021-2023) environmental and clinical *R. equi* isolates. Two cocktails were trialled against mixed *R. equi* populations and were able to limit bacterial growth to within 1.5 log CFU/g of the starting concentration in sterile soil and actively reduce total *R. equi* populations by 1 log CFU/g in natural soil. Additional parameters of successful application include phage persistence and analysis of the effect of phage on the surrounding soil microbiota. This work demonstrates the targeted and robust nature of phages as bactericidal agents in the soil environment.

P-EME-122

How to co-habit with algae: Adaptive traits of *Planctomycetota* to thrive on algal surfaces

*M. Staack[1], J. Hammer[1], N. Kallscheuer[2], C. Jogler[1]
[1]*Friedrich Schiller University, Department for Microbial Interactions, Jena, Germany*
[2]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*

Bacteria of the phylum *Planctomycetota* are common colonizers of phototrophs in marine and limnic habitats. They frequently co-occur with a variety of microalgal taxa [1] and have been shown to be abundant in the particle-attached fraction of cyanobacterial blooms [2]. On the surfaces of macroalgae and on the leaves of seagrass they can even dominate the microbial community. To study the interactions of *Planctomycetota* bacteria with phototrophs, we performed co-cultivation experiments with the marine microalga *Emiliania huxleyi*, the limnic cyanobacterium *Microcystis aeruginosa* and the marine macroalga *Ulva* with a selection of *Planctomycetota* bacteria. Furthermore, we introduced a second heterotrophic bacterium, *Phaeobacter inhibens* (*Rhodobacterales*) to our co-cultivations.

Here, we present new insights in the cross-feeding of nutrients, such as carbon and vitamins, in dual and tripartite algal-bacterial interactions. We further investigated how chemical mediators influence the morphology of bacteria as well as phototrophs. We show that algal exudates trigger the elongation of *P. inhibens* cells, thereby influencing the buoyancy. We hypothesize that this allows *P. inhibens* to stay in a nutrient-rich zone and to prevent a fast sinking to deeper ocean layers where nutrients are scarce. Furthermore, we show that the cyanobacterium *M. aeruginosa* underwent morphological changes upon co-cultivation with a planctomycetal model strain. This suggest a stress response triggered by the bacterial partner, possibly involving chemical mediators produced by *Planctomycetota* in response to the toxin microcystin.

Our results highlight the complex interactions between *Planctomycetota* bacteria and phototrophs, including both symbiotic and competitive aspects. The discovery of cross-feeding, chemical mediators and morphological adaptations contributes significantly to the understanding of microbial dynamics in aquatic ecosystems and opens new perspectives for the study of bacterial factors influencing algal growth and ecology.

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P-EME-123

Deciphering the cabanillasin biosynthesis pathway using limited proteolysis coupled with mass spectrometry (LiP-MS)

*C. Pavesi[1], G. Angelidou[1], T. Glatter[1], H. B. Bode[1]
[1]*Max Planck Institute, Marburg, Germany*

Cabanillasins are rare polyguanidine natural products from entomopathogenic *Xenorhabdus* bacteria with good antifungal bioactivity.

Although we were able to identify building blocks and intermediates in the biosynthesis of these unusual compounds, we were not able to identify the actual biosynthetic gene cluster (BGC) nor the genes or enzymes involved in their biosynthesis. To address this knowledge gap, we wanted to apply limited proteolysis coupled with mass spectrometry (LiP-MS), a proteomics method developed by the group of Paola Picotti in 2017. We adjusted it to study the *Xenorhabdus doucetiae* proteome, and to find the proteins involved in the cabanillasin biosynthesis pathway. Briefly, the proteome of *X. doucetiae* was studied in the absence or presence of building blocks and precursor already identified in the cabanillasin biosynthesis by digestion using proteinase K (PK), followed by trypsin digestion.

The difference in the overall protein structures binding to the building blocks and intermediates led to different cleavage sites for PK and trypsin, resulting in different peptides (in the absence or presence of the building blocks), and enabling the identification of candidate proteins probably involved in the cabanillasin biosynthesis pathway.

We also tested LiP-MS to identify candidate targets of bioactive natural products showing antibiotic activity since the advantage of this method is that it does not require the chemical modification of the original natural product and is therefore also suitable for very low molecular weight natural products.

P-EME-124

Phycobilin biosynthesis for light harvesting in the cryptophyte alga *Hemiselmis cryptochromatica*

*T. Schmidt[1], N. Frankenberg-Dinkel[1], F. Frascogna[1], M. Greenwold[2], T. Richardson[3]
[1]*Rheinland-Pfälzische Technische Universität, Biology, Kaiserslautern, Germany*
[2]*University of Texas at Tyler, Biology, Tyler, TX, United States*
[3]*University of South Carolina, Biological Sciences, Columbia, SC, United States*

In cyanobacteria, chromatic acclimation (CA) is a well-studied process in which the organism adapts its light-harvesting machinery to varying light qualities. Therefore, the phycobilisomes (PBS) get restructured, depending on the type of CA used. A similar process was recently described for the cryptophyte alga *Hemiselmis cryptochromatica.* Cryptophytes are microalgae, originating from a secondary endosymbiosis event, where a red alga cell was ingested by an unknown eukaryote and are capable of living in a variety of aquatic habitats. For light harvesting, phycobiliproteins (PBPs) are used, which in contrast to cyanobacteria are present as soluble proteins in the thylakoid lumen. Color differences amongst cryptophytes depend on the type of PBP pigment. In total, nine major differently absorbing PBPs have been identified so far. The PBPs can either be phycocyanin (PC) or phycoerythrin (PE), designated by the maximum absorption wavelength. Cryptophyte PBPs are soluble α-/β-hetero dimers, with the β-subunit highly homologous to the ancestral red algal one. The α-subunit, in contrast, evolved separately. Overall, multiple α-subunits are encoded on the nucleus genome, while one β-subunit is encoded on the plastid genome. α-subunits covalently bind one light absorbing linear tetrapyrrole (phycobilin) and β-subunits have three phycobilins covalently attached. In contrast to other organisms, cryptophytes possess rather unusual phycobilins incorporated in their PBPs. These are 18^1,18^2-dihydrobiliverdin (MBV) and 15,16-dihydrobiliverdin (DHBV) and the unique phycobilins bilin 584 and bilin 618. They are acryoyl-derivatives of PEB and DHBV and their biosynthesis is still unknown. The project aims at providing deeper understanding for the molecular mechanisms of the observed CA. In particular, we will isolate and identify the PBP chromophores under different light qualities, as well as look at their biosynthetic pathway. We bioinformatically identified five FDBRs in the *H. cryptochromatica* transcriptome which are now investigated in a recombinant protein approach. Results indicate the production of PCB by two of the encoded FDBRs showing at least one possible function while the functions of the other three remains to be elucidated. Overall, this study will help in understanding the specialized phycobilin biosynthesis and CA in cryptophytes.

P-EME-125

Investigations on bile acid catabolism in *Sphingobium* sp. strain Chol11 suggests a Rieske monooxygenase-catalyzed hydroxylation that occurs prior to the degradation of the carboxylic side chain

*A. Fuentes[1], J. Holert[1], B. Philipp[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

Bile acids are steroids produced by vertebrates in their digestive system, and after excretion, they can be used as carbon and energy sources by environmental bacteria. Bile acids have a hydroxylated steroid skeleton with a C_5 carboxylic side chain attached to C17. Two pathway variants, named $\Delta^{1,4}$ and $\Delta^{4,6}$, are known for bile acid catabolism under aerobic conditions. While the $\Delta^{1,4}$-variant is common in pseudomonads and *Actinobacteria*, the $\Delta^{4,6}$-variant seems unique to the *Sphingomonadaceae* family.

Proteomic analysis of *Sphingobium* sp. strain Chol11 grown with bile acids revealed a gene cluster specifically upregulated during side-chain degradation, which is conserved in this family. It contains genes for a CoA ligase (*sclA*), an acyl-CoA-dehydrogenase (*scd4AB*), as well as genes for an amidase and a Rieske monooxygenase (RMO). Notably, this cluster lacks genes for the stepwise side-chain degradation described in bacteria using the $\Delta^{1,4}$ variant. Additionally, Chol11 Δ*scd4A* cannot grow with bile acids and accumulates hydroxylated dead-end metabolites with a C_5 side-chain. These instances suggest that side-chain degradation in strain Chol11 involves a hydroxylation catalyzed by the RMO.

The potential role of the RMO was investigated through gene deletion and complementation experiments. Gene deletion attempts led to the removal of a 64 kb genomic region, encompassing a substantial fraction of the side-chain degradation cluster containing the genes coding for the RMO, the amidase, and the acyl-CoA dehydrogenase Scd4AB, plus a downstream genomic region. Complementation of this mutant with the genes coding for RMO only, as well as with the amidase and RMO genes together, resulted in the production of hydroxylated metabolites containing an intact side chain. These metabolites resembled the dead-end metabolite production by strain Chol11 *Δscd4A*. These results indicate that RMO catalyzes the hydroxylation of intermediates in the *Δscd4A* mutant and suggest that these hydroxylated compounds might be the substrate for Scd4AB before the side-chain degradation.

This study provides further evidence that bile acid side-chain degradation in *Sphingomonadaceae* proceeds via an uncharacterized pathway involving an RMO-catalyzed hydroxylation. Current efforts are focused on complementing strain Chol11 m25 with different gene combinations comprising *scd4AB*, RMO, and amidase genes in order to elucidate the putative reaction sequence catalyzed by the respective enzymes.

P-EME-126

Candidate Phyla Radiation bacteria in a groundwater enrichment showing genomic signatures of a free-living lifestyle

R. S. Andräs[1], V. Reilly-Schott[2], S. Riedel[1], J. Bach[1], M. Sprenger[3], K. Papenfort[3], E. Gabashvili[1], *M. Taubert[1]
[1]*Friedrich Schiller University, Aquatic Geomicrobiology, Jena, Germany*
[2]*Helmholtz Centre for Environmental Research - UFZ, Solar Materials Biotechnology, Leipzig, Germany*
[3]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*

Bacteria of the widespread Candidate Phyla Radiation (CPR) often lack biosynthetic genes for essential building blocks like amino acids and nucleotides, causing dependence on other organisms. Available cultures of some CPR taxa, like *Cand.* Saccharimonadia and *Cand.* Absconditabacteriales, showed physical attachment of CPR to bacterial host cells. However, CPR taxa that typically dominate the planktonic community in groundwater habitats, like the *Cand.* Paceibacteria that can reach abundances of up to 45%, have not been obtained in culture so far. Recent studies have indicated that some members of these taxa might favor a free-living lifestyle.

Here, we present results of a metagenomics analysis on an aerobic groundwater-derived enrichment containing *Cand.* Paceibacteria that has been continuously maintained for more than two years. CPR constitute up to 25% of the enrichment community, based on coverage of metagenomic reads. Metagenome-assembled genomes (MAGs) affiliated with CPR in the enrichment were compared to CPR-MAGs previously obtained from a groundwater metagenomics campaign, with the aim to determine differences that might indicate a free-living lifestyle. In total, 14 MAGs affiliated with

Cand. Paceibacteria, belonging to the order *Cand.* Kaiserbacteria, as well as 4 MAGs of CPR class ABY1, which is also common in groundwater, were recovered. The enriched CPR showed a significantly higher GC content and genome size than those found in groundwater CPR. Genes involved in nitrogen and sulfur cycling, like nitrite reductase *nirK* and sulfur dioxygenase *sdo*, as well as F-type ATPase genes, were more frequently present in enriched compared to groundwater MAGs, suggesting mechanisms for host-independent energy generation. Pathways for nucleotide biosynthesis were also more often present in enrichment versus groundwater MAGs, potentially enabling de-novo synthesis of these building blocks. Furthermore, CAZymes and peptidases that may provide access to organic carbon sources in the enrichment were abundant in the CPR-MAGs. Interestingly, the ATP-citrate lyase genes *aclAB*, key genes in the reverse TCA cycle for CO_2 fixation, were present in most *Cand.* Paceibacteria from the enrichment. The lack of a complete reverse TCA cycle, however, suggests a different function of these genes in CPR. Taken together, this shows that the specific, distinct functions in the enriched CPR-MAGs could benefit a host-independent lifestyle.

P-EME-127

Identification of microbial keystones in complex soil ecosystems and determination of their activity and niche preferences

*S. Vieira[1], J. Sikorski[1], K. Goldmann[2], F. Buscot[2], L. Prada-Salcedo[2], M. Schloter[3], P. Espíndola-Hernández[3], M. Schrumpf[4], D. Portik[5], B. Bunk[6], J. Overmann[1]
[1]*Leibniz Institute DSMZ, Microbial Ecology and Diversity, Braunschweig, Germany*
[2]*Helmholtz Centre for Environmental Research - UFZ, Soil Ecology, Halle (Saale), Germany*
[3]*Helmholtz Centre Munich, Comparative Microbiome Analysis, Munich, Germany*
[4]*Max Planck Institute, Biogeochemical Processes, Jena, Germany*
[5]*PacBio, Menlo Park, CA, United States*
[6]*Leibniz Institute DSMZ, Bioinformatics, IT and Databases, Braunschweig, Germany*

Determining the functional role of individual types of microorganisms and their specific relevance in the environment remains very challenging, especially in highly complex environments such as soils. One promising approach is to try to detect and characterize so-called keystone taxa, which are critical to the stability and functioning of the entire communities. We used co-occurrence networks from high-throughput sequencing data to elucidate significant associations, and to thereby infer microbial sequence types that might be of particular importance to the overall microbial communities, hence representing keystones. This approach was applied to bacterial and fungal communities in 300 different soils of the German Biodiversity Exploratories. The sites encompass grassland and forest ecosystems under different land use regimes, which were sampled over a period of 11 years. 16S rRNA and the internal transcribed spacer (ITS2) were used as markers for bacteria and fungi, respectively. Keystones were identified by a ranking procedure based on the susceptible-infected-recovered model after perturbation of the original networks. Keystone taxa were specific to distinct soil environments within different geographic regions and remained rather stable in their habitat over time. We hypothesized that keystone taxa must be more active than co-occurring microorganisms to exert their ecological influence over longer time periods. Accordingly, active bacterial taxa were identified using both 16S rRNA/ 16S rDNA ratios and also by determining bacterial growth rate from the peak-to-trough ratio of genomic sequence coverage. Notably, we found a significant overlap between keystone and active species, corroborating the network analysis approach. Using the molecular signatures of keystone taxa, their distinct ecological niche preferences were analysed by modelling abundances against environmental parameters, and metagenome assembled genomes (MAGs) from bacterial keystones provided further insights into their functional potential. Our results thus pave the way towards dissecting the ecological roles of individual microbial taxa in highly complex environments.

P-EME-128

Wastewater-borne pollutants affected the soil resistome, mobilome and rhizosphere microbial community composition in monolithic soil columns planted with cilantro

*S. Gallego[1], L. Soufi[2], I. Kampouris[1], K. C. Lüneberg[3], B. J. Heyde[2,4], C. Siebe[3], J. Siemens[4], E. Grohmann[2], K. Smalla[1]
[1]*Julius Kühn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany*
[2]*Berlin University of Applied Sciences and Technology, Microbiology, Berlin, Germany*
[3]*Universidad Nacional Autónoma de México, Instituto de Geología, Mexico City, Mexico*
[4]*Justus Liebig University Giessen, Institute of Soil Science and Soil Conservation, iFZ Research Center for BioSystems, Land Use and Nutrition, Gießen, Germany*

Over decades, untreated wastewater from Mexico City has been used to irrigate crops in the Mezquital Valley. After the construction of the Atotonilco wastewater treatment plant, the irrigation wastewater has been progressively shifted from untreated to treated. We hypothesized that with the change of irrigation water quality, pollutants accumulated over decades may be released favoring the environmental spread and dissemination of antibiotic resistance genes (ARGs) through mobile genetic elements (MGEs) in a soil type specific manner. Experiments ranging from incubation experiments with homogenized soils under well-defined conditions to experiments with intact soils with plants were performed to investigate the effects of changing irrigation water quality on the resistome, mobilome and microbial community composition in different soil types by means of DNA-based methods (qPCR, 16S amplicon sequencing).

The incubation experiment with homogenized soils showed that wastewater spiked with a mixture of antibiotics and disinfectants affected the soil resistome and mobilome with increases in the relative abundance of specific ARGs and MGEs, but not the soil microbial community composition that was mainly shaped by the soil type. Subsequent experiments with monolithic soil columns with two soil types from the Mezquital Valley planted with cilantro and irrigated with spiked or unspiked untreated or treated wastewater confirmed the results observed in the incubation experiment. Soil collected from preferential flow paths (stained with a non-toxic dye) harbored a higher relative abundance and diversity of ARGs and MGEs compared to unstained soil, which was less exposed to the infiltrating wastewater. In contrast to the bulk soil microbial community, the rhizosphere microbial community was strongly influenced by the quality and level of antibiotics and disinfectants in the irrigation wastewater. Exogenous plasmid capturing experiments will contribute to understand the role of wastewater pollutants and plasmids on the spread of environmental antimicrobial resistance.

P-EME-129

Bacterial bile acid degradation as a parameter to evaluate effects of a fungicide on selected bacterial taxa both in soil microcosms and in laboratory cultures

*J. C. Coutiño-Montes[1], A. Bruder[1,2], K. Schlich[2], C. A. Diaz Navarrete[2], Y. Abdullaeva[1], B. Philipp[1,2,3]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*
[2]*Fraunhofer-Gesellschaft, Environmental Microbiology, Schmallenberg, Germany*
[3]*University of Münster, Centre for Interdisciplinary Sustainability Research (ZIN), Münster, Germany*

Plant protection products (PPPs) have effects on non-target organisms including prokaryotes. In soil this can impair the transformation of organic and inorganic matter. For regulatory purposes, the influence of PPPs on nitrification and respiration is measured but this does not provide information about the affected taxa. For this, a substrate that can only be degraded by selected bacteria, which do occur ubiquitously in soil, would be desirable. Bile acids could be a suitable substrate because they are excreted by all vertebrates and can be used as a carbon and energy source by only a few defined bacterial taxa frequently found in soil. These taxa mainly comprise the genera *Pseudomonas*, *Comamonas* and *Rhodococcus* all of which are also known as degraders of synthetic compounds.

It was investigated whether the microbial community in a reference soil (01A) without addition of other nutrients can degrade different bile acids. Chromatographic analyses coupled to mass spectrometry showed that degradation of bile acids instantly started in soil microcosms. Determination of colony forming units revealed an enrichment of bile-acid degrading bacteria. Amplicon sequencing of 16S rRNA genes showed that bacteria of the genera *Pseudomonas* and *Comamonas* accumulated in the soil microcosms with cholate and ursodeoxycholate, respectively, which was confirmed by quantitative PCR and isolation of abundant bacteria. Such soil microcosms were then used to analyze the effect of the fungicides Propamocarb and Thiram on bile acid degradation. While Propamocarb had no effect, Thiram delayed degradation of both bile acids. Molecular analysis showed that Thiram reduced the proportion of *Pseudomonas* in cholate-containing microcosms while the proportion of *Comomonas* increased. Laboratory cultures with two different isolated strains of each genus showed that Thiram inhibited the degradation of cholate and also of succinate much more in the *Pseudomonas* than in the *Comamonas* strains.

These results showed that bile acids are a suitable substrate for addressing the metabolic activity of defined bacterial taxa in soils because the bile acids, which only slightly differ in the number and configuration of hydroxyl groups, were utilized by different taxa. This selective enrichment could be used to analyze the effect of a PPP because it consistently showed stronger inhibition of cholate degradation for *Pseudomonas* than for *Comamonas* both in soil communities and in laboratory cultures.

P-EME-130

Effects of lowland rainforest conversion to managed land use systems on bacterial community composition and diversity

*S. Meliah[1], J. Hollensteiner[1], T. Klein[2], A. Polle[2], R. Daniel[1]
[1]*Georg-August University Göttingen, Department of Genomic and Applied Microbiology, Göttingen, Germany*
[2]*Georg-August University Göttingen, Forest Botany and Tree Physiology, Göttingen, Germany*

Tropical rainforest is a biodiversity-rich habitat. The conversion of rainforests to managed land use system shifts the soil prokaryotic community. In this study, we investigated the bacterial community in litter, soil and root of four different land use systems, consisting of lowland rainforest (RF), oil palm plantation (OP), rubber plantation (RP) and shrubland (SL) in Jambi, Indonesia. The samples were collected from the topsoil and the bacterial community was assessed based on 16S rRNA gene amplicons. The total amplicon sequence variants (ASVs) generated from 370 selected samples was 113,296. The analysis showed that Proteobacteria and Actinobacteria were dominant across the samples, especially in litter samples. Soil samples were dominated by Actinobacteria, Cloroflexi, and Firmicutes. Some well-known predatory bacteria phyla, such as Myxococcota and Bdellovibrionota were also present in soil. Proteobacteria exhibited the highest relative abundance in root samples. The composition of bacterial communities in litter and root harbor members of Armatimonadota, Bacteroidota, and Cyanobacteria phyla. Analysis of bacterial diversity in different land use systems based on Shannon diversity index revealed that the conversion of rainforest negatively affected the diversity in litter of rubber plantation and positively affected the diversity in roots of oil palm ($P<0.05$). The conversion of rainforest into managed land use system also significantly affected the phylogenetic diversity in litter of rubber plantation, soil of rubber and oil palm plantation, and root of rubber and oil palm plantation. The bacterial diversity in shrubland, which is an intermediate of rainforest and managed land use systems, was not significantly different from that of rainforest. Redundancy analysis suggested that the total carbon was the main driver in bacterial community composition across the samples.

P-EME-131

Diversity and distribution of genomic potential for sulfoquinovose degradation across gut and soil microbiomes

*T. S. Tanabe[1], J. Krasenbrink[1], S. Chen[1], A. Loy[1]
[1]*Centre for Microbiology and Environmental Systems Science, Vienna, Austria*

Introduction

Sulfoquinovose is a sulfonated derivate of glucose and one of the most abundant organosulfur compounds in the biosphere. As headgroup of the sulfonolipid sulfoquinovosyl diacylglycerol (SQDG) it is an integral component of thylakoids of photosynthetic organism. So far at least five bacterial degradation mechanisms have been described, which are all initialized by the cleavage of sulfoquinovose from SQDG. Sulfoquinovose is then degraded via transaldolase or transketolase-driven sulfoglycolytic variants of the pentosephosphate, the Embden-Meyerhof-Parnas or the Entner-Doudorff pathway, or an oxygen dependent mono- or dioxygenase driven pathway. Although these pathways differ in their degradation products and contribution to sulfur and carbon cycles, their distribution across environmental and host-associated microbiomes is not fully explored.

Goals

Here, we developed a bioinformatic pipeline for the automated generation of hidden Markov models (HMM). We applied this tool for genome-based analysis of microbial SQDG degradation to study the diversity and distribution of SQDG catabolism across soil habitats and in the mouse gut.

Material & Methods

The selection of functionally equivalent reference sequences for the creation of HMMs is a time-consuming task, which was accelerated by the development of an automatized HMM generation pipeline. HMMs are automatically generated based on the combined sequence similarity, genomic vicinity and phylogenetic information from the sequences of a single gene cluster. This tool enabled the automated generation of HMMs that differentiate between the enzymes of glucose and sulfoquinovose catabolism.

Results

The reliability of the HMMs was verified for several sulfur metabolism related gene clusters, including those of the sulfoquinovose catabolism, in a cross-validation procedure. When applied to large collections of metagenome-assembled genomes from soil and gut microbiomes, mono- and dioxygenase driven aerobic SQDG degradation pathways and the sulfo-Entner-Doudoroff pathway were primarily detected by the generated HMMs. In contrast, the sulfo-Embden-Meyerhof-Parnas and transaldolase driven pathways were more abundant in gut microbiomes, revealing an ecosystem dependent pattern in SQDG catabolism diversity.

Summary

Depending on the ecosystem, the genetically predominant sulfoquinovose catabolism differs, which probably affects the availability of sulfur and organosulfur compounds.

P-EME-132
Resilience of Arctic vs. Temperate phytoplankton to temperature and light conditions in the Arctic Ecosystem

*A. von Hoyningen-Huene[1,2], L. Kopplin[2,3], A. M. Eren[1,2,4,5], U. John[1,2]
[1]Helmholtz Centre for Infection Research – HZI, Ecosystem Data Science & Eco-Evolutionary Genomics, Oldenburg, Germany
[2]Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
[3]Technical University Munich, Munich, Germany
[4]Carl von Ossietzky University, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany
[5]Max Planck Institute, Bremen, Germany

Phytoplankton is estimated to account for 50 % of oxygen production on the planet. It drives the marine food web and major marine biochemical cycles, acting as an important carbon sink. The Arctic is affected by global warming four times faster than the average. This includes changes in sea ice cover and the influx of warm, nutrient rich water from the Atlantic Ocean, a process called Atlantification.

In light of this, we aim to determine which mechanisms allow arctic algae, adapted to cold temperatures and long dark winter periods, to persevere in their environment in comparison to incoming temperate ecotypes. By understanding whether they use similar adaptations to persist, we will be able to better understand their ability to contribute to ecosystem reassembly in the warming Arctic.

The experimental setup included six arctic and five temperate microalgae belonging to four phyla. To understand whether temperate ecotypes can replace their arctic counterparts, we tested for differences in their biotic interactions with one another. The algae were grown in single batch culture or in co-culture with the other species at 1°C and a 16:8 h light:dark cycle. To test for an effect of polar daylengths, we recorded growth rates of the single strains at 1°C with a 2:22 h, 16:8 h or 24:0 h light:dark cycle. To survey adaptive responses to changing light and temperature conditions, we used long-read isoform sequencing to obtain extensive transcriptomic profiles for temperate and arctic phytoplankton species.

First results show, that not all tested temperate strains are able to survive at 1°C and a 2:22 h light:dark cycle expected for winter and early spring in the arctic. This indicates that less robust algae need to be re-introduced to the arctic by the currents. Temperate algae that are able to persist at low light and temperature conditions grow at reduced rates, which are comparable to the growth rates of their arctic counterparts. This applies to both single and co-cultures, thus, showing their ability to compete in the arctic system.

This dataset serves as a foundation for further transcriptomic exploration of an arctic winter/summer scenario. Leveraging the data, we will search for common patterns of adaptation and metabolic plasticity among arctic survivors versus less robust taxa. The resulting patterns will then be compared to field-transcriptomes, to determine whether they can be detected consistently in the environment.

P-EME-133
Buried Secrets – A journey into subsurface habitats

*M. Kündgen[1], *S. Siebert[1], J. S. Niegisch[1], C. E. Wurzbacher[1], V. S. Hotter[1], A. Soares[2,3], A. Probst[2,3], C. Jogler[1]
[1]Friedrich Schiller University, Department of Microbial Interactions, Jena, Germany
[2]University of Duisburg-Essen, Environmental Metagenomics, Essen, Germany
[3]University of Duisburg-Essen, Centre of Water and Environmental Research (ZWU), Essen, Germany

Beneath our feet lies a world full of mystery—a realm where secrets of life await discovery. In our investigation of subsurface habitats, including groundwater and seepage water from the Hainich CZE (Critical Zone Exploratory) and a cave in Baden-Württemberg, we initiated a quest to cultivate the hidden diversity of subsurface habitats and the untapped microbial life they harbor. The main aim of the investigation: planctomycetes, with their complex cell structure, multiple genes of unknown function and partly mysterious lifestyles. Equipped with cultivation techniques that employed both oxic and anoxic approaches, we proceeded to investigate these enigmatic environments. Each habitat with varying nutrient-, oxygen-, and pH- conditions but with the one common factor of being completely shrouded in darkness. As the cultivation of these samples proceeded, a sense of anticipation arose from the darkness. Who are these microbial players, and what secrets might they hold? And then on a random Friday the breakthrough was achieved – The first groundwater Planctomycete was isolated from an enormous number of plates. And there was more to come, more to investigate and more to be fascinated by. Several isolates from the five phyla of Actinomycetota, Bacilli, Bacteroidota, Planctomycetota, and Pseudomonadota were obtained, including novel species that had never seen the light of the day. An overview of these isolates and novel species for all investigated subsurface habitats and a characterisation of all isolated planctomycetal species will be presented to uncover the buried secrets of subsurface habitats.

P-EME-134

Strategies for cultivating elusive CPR Bacteria: Integrating antibody engineering and epicPCR

*S. Sayyadi[1,2,3], O. M. P. Carrascal[3,4], S. Hengoju[1], M. Taubert[3,4], K. Küsel[3,4], M. A. Rosenbaum[3,4]

[1]Leibniz Institute for Natural Product Research and Infection Biology, Biopilot Plant, Jena, Germany

[2]Friedrich Schiller University, Biology, Jena, Germany

[3]Friedrich Schiller University, Cluster of Excellence Balance of the Microverse, Jena, Germany

[4]Friedrich Schiller University, Aquatic Geomicrobiology, Institute of Biodiversity, Jena, Germany

Candidate Phyla Radiation (CPR) bacteria, known for minimal genomes, slow growth, and symbiotic tendencies, represent a challenging yet vital part of bacterial diversity. Their unique physiological traits often defy conventional cultivation techniques, necessitating new strategies. This study presents two ongoing approaches for cultivating CPR bacteria from groundwater samples in the Hainich Critical Zone Exploratory, Germany, a site where CPR phyla are notably dominant. The first approach uses antibodies, carefully engineered from metagenomic data, to target a surface membrane protein from *Candidatus Kerfeldbacteria* (ABY1 class). This protein shares high sequence similarity (70%) with homologous proteins in *Candidatus Parcubacteria*, suggesting conservation across CPR classes. Imaging confirmed successful antibody labeling, and FACS was then employed to isolate cells exhibiting fluorescence from this labeling, enriching for a subset likely to contain CPR bacteria. By narrowing down to this specific subset, FACS enhances the chances of cultivating CPR bacteria. The sorted cells are now being cultivated, with initial colony formation observed. Sequencing of both the emerging colonies and DNA from the entire sorted population is underway to verify CPR presence, validating this targeted approach as a means to overcome traditional cultivation barriers. The second approach utilizes epicPCR (emulsion, paired isolation, and concatenation PCR) within a droplet microfluidic system. This method, adapted to maintain droplet integrity post-PCR, enables mapping of CPR-host associations, which is crucial for understanding the symbiotic relationships. epicPCR provides the foundation for targeted co-cultivation, where identified hosts can be paired with CPR bacteria under controlled conditions to promote growth. Initial gel electrophoresis confirmed the expected PCR bands, and subsequent sequencing identified three potential host species that could facilitate CPR growth. Currently, these findings are being validated through repeated experiments to ensure robustness. Following validation, these identified hosts will be used for targeted co-cultivation with CPR bacteria, aiming to overcome cultivation barriers by mimicking their natural associations. These complementary approaches—targeted antibody enrichment and epicPCR for host identification—are paving the way for more effective CPR cultivation, enhancing our understanding of their ecological roles.

P-EME-136

Microbial and environmental controls of methane emissions across Arctic permafrost landscapes

*N. Eves[1,2,3], K. Ivanova[1,4], J. Vogt[1], M. Mairl[5], A. Bieniada[1], M. Marz[3,6], M. Goeckede[1], J. Rahlff[2,3,7]

[1]Max Planck Institute, Department for Biogeochemical Signals, Jena, Germany

[2]Friedrich Schiller University, Aero-Aquatic Virus Research Group, Faculty of Mathematics and Computer Science, Jena, Germany

[3]Fritz Lipmann Institute (FLI), Jena, Germany

[4]Friedrich Schiller University, Institute of Geoscience, Jena, Germany

[5]University of Innsbruck, Department of Ecology, Innsbruck, Austria

[6]Friedrich Schiller University, RNA Bioinformatics Research Group, Faculty of Mathematics and Computer Science, Jena, Germany

[7]Linnaeus University, Ecology and Evolution in Microbial Model Systems (EEMiS), Department of Biology and Environmental Science, Kalmar, Sweden

Permafrost thaw releases previously frozen organic carbon making it vulnerable to microbial transformation into carbon dioxide (CO_2) and methane (CH_4). While CO_2 emissions dominate in terms of total carbon emissions, CH4 has 35-times the warming potential of CO_2 over a 100-year period, so has a pronounced effect on future warming and permafrost thaw feedback. Understanding the microbial drivers behind CH_4 emissions is complicated by the high spatial heterogeneity of the permafrost landscape. Features such as polygonal mires and thermokarst lakes change the physical and chemical conditions of soils and freshwaters even across small spatial scales. The related impacts on microbial community structure have important implications on microbially mediated transformation of organic carbon.

Here, we propose ideas to investigate 1) how physical and chemical changes across diverse features of permafrost environments impact microbial community 2) how this impacts microbial CH_4 metabolism, and 3) whether considering microbial community improves the interpretation of controls on CH_4 emissions, through the integration of *in situ* CH_4 flux measurements with metagenomic analyses. We completed sampling across two polygonal mires and ten permafrost lakes in a region of continuous permafrost around the Trail Valley Creek site in the Northwest Territories, Canada. Active layer soil was collected for metagenomic analysis and air-soil CH_4 fluxes were measured across polygonal mires using a static chamber method. At each lake, water, sediment, and surface microlayer samples were collected for metagenomic analysis and air-water CH_4 fluxes were measured around the shoreline using a floating chamber.

Preliminary results suggest that diffusive CH_4 fluxes did not significantly differ between lakes but found several occasions of CH_4 ebullition. We also found that CH_4 fluxes vary widely across different structural parts of polygonal mires, ranging from -0.28 to 7.54 mg CH_4 m^2 h^{-1}, and can be largely explained by physical soil properties, namely soil moisture at 12cm depth and soil temperature at 20cm depth. We will analyse metagenomes to determine microbial taxonomic composition, identify relevant methane metabolism genes and study how these correlate with soil and freshwater properties across the permafrost environment. This will provide insight to the microbial processes driving CH_4 emissions and how these may change as permafrost environments change under continued warming.

P-EME-137

Intercropping wheat and aromatic crops: Impacts on the root associated microbiome

*E. Ollo[1], W. Burzik[2], A. Gattinger[1], M. Athmann[2], S. P. Glaeser[1]

[1]Justus Liebig University Giessen, Gießen, Germany

[2]University of Kassel, Faculty of Organic Agricultural Sciences, Kassel, Germany

The root microbiome plays a crucial role in plant health, growth, and yield promotion. Under draught stress, the important roles played by root associated bacteria and arbuscular mycorrhiza (AMF) are more pronounce. Mixed cropping which involves the cultivation of different crops enables the efficient utilization of nutrient by combining deep rooters and shallow rooting plants during a production time

period. This enables increased plant diversity and hence diversity of root exudates**.** The composition of root associated microbiome is controlled by the production of root exudates. The extent of the effects of mixed cropping/intercropping involving wheat and medicinal aromatic plants on the root microbiome is not well studied.

In the TRIO project intercropping of wheat-aromatic crops (wheat-coriander, wheat-caraway) and monocrops (wheat, caraway, and coriander) will be investigated to determine the effects of intercropping systems on the root associated microbiome. The project involves two experimental fields under ecological and two experimental fields under conventional agricultural management system in Hesse. The field trials in the four experimental fields will be carried out under randomized complete block experimental design. Each field has 20 plots with 12 plots under monocrops of wheat, caraway, and coriander. For mixed crops eight plots are studied, four plots with wheat-coriander and four plots with wheat-caraway combinations. Annual and perennial crop production will be studied. The microbial diversity and community composition in bulk soil (at sowing and flowering of herbs) and rhizosphere and roots samples at flowering of wheat and herbs will be compared.

Our first hypothesis is that mixed cultures have a positive effect on the bacterial diversity and affect nutrient uptake. To answer this hypothesis, the bacterial communities will be analysed by 16S rRNA gene amplicon next generation sequencing. We also hypothesize that crop diversity in mixed crops positively affect AMF colonization benefitting perennial as compared to annual crops. To answer this, fluorescence microscopy of plant in monocrops and mixed will be carried out to determine the colonization abundances of AMF. Quantification of root colonizing AMF using qPCR and diversity analysis using amplicon sequencing will be carried out in parallel.

P-EME-138

Control of *Blumeria graminis* f. sp. *hordei* on Barley Leaves by Treatment with Fungi-Consuming Protist Isolates

*J. Sacharow[1], E. Salehi-Mobarakeh[1], S. Ratering[1], J. Imani[2], A. Österreicher Cunha-Dupont[1], S. Schnell[1]
[1]Justus Liebig University Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany
[2]Justus Liebig University Giessen, Department of Phytopathology, Gießen, Germany

The obligate biotrophic fungal pathogen *Blumeria graminis* causes the powdery mildew disease of cereals, which results in large crop losses. Control of *B. graminis* in barley is mainly achieved by fungicide treatment and by breeding resistant varieties. Vampyrellid amoebae, just like mycophagous protists, are able to consume a variety of fungi. To reveal the impact of some selected fungus-consuming protists on *Blumeria graminis* f. sp. *hordei* (Bgh), and to evaluate the possibility of using these protists as biological agents in the future, their feeding behaviour on *B. graminis* spores on barley leaves was investigated. An experiment was carried out with five different protist isolates (*Leptophrys vorax*, *Platyreta germanica*, *Theratromyxa weberi* U 11, *Theratromyxa weberi* G7.2 and *Acanthamoeba castellanii*) and four matched controls, including the food sources of the cultures and the medium. Ten-day-old leaves of barley (*Hordeum vulgare* cv. Golden Promise) were first inoculated with *Blumeria graminis* (f. sp. *hordei* race A6) spores, then treated with protists and fungal colonies on the leaf surfaces were counted under the microscope after 5 days. The isolates *L. vorax*, *P. germanica*, and *T. weberi* U11 did not show a significant reduction in the number of powdery mildew colonies whereas the isolates *T. weberi* G7.2 and *A. castellanii* significantly reduced the number of powdery mildew colonies on the leaf surfaces compared to their respective controls. This indicates that these two isolates are capable of reducing *B. graminis* colonies on barley leaves and are suitable candidates for further investigation for possible use as biological agents. Nevertheless, the susceptibility to dryness and the cell division rate should be considered during the optimisation of the next steps like application procedure and whole plant treatment.

Sacharow, J., Salehi-Mobarakeh, E., Ratering, S., Imani, J., Österreicher Cunha-Dupont, A., & Schnell, S. (2023). Control of *Blumeria graminis* f. sp. *hordei* on Barley Leaves by Treatment with Fungi-Consuming Protist Isolates. Current Microbiology, 80(12), 384. https://doi.org/10.1007/s00284-023-03497-5.

P-EME-139

Microbial abundance and community composition in topsoil profiles of differently cropped and fertilized arable soils

*A. Pausch[1], M. Speth[2], A. Gattinger[3], S. P. Glaeser[1]
[1]Justus Liebig University Giessen, Mikrobiologie der Recyclingprozesse (Kämpfer), Gießen, Germany
[2]Department of Soil Science and Soil Conservation, Professur für Bodenressourcen und Bodenschutz, Gießen, Germany
[3]Justus Liebig University Giessen, Professur für Ökologischen Landbau m.d.S. Bodennutzung, Gießen, Germany

Soil microbial communities are crucial for ecological processes such as organic matter turnover and nitrogen cycling in agricultural soils. In the LOEWE project Green Dairy, we investigated the effects of two different cow manures from high- and low productivity feeding and crop rotatio on the soil microbiome in organic farming systems. Bulk soil samples of 0-10 and 10-30 cm depth collected in spring 2024 from eight arable plots were comparatively analyzed. Alfalfa (one and two year old), spelt, maize, potatoes, oats, rye, wheat and field beans were grown on the plots. Using quantitative PCR (qPCR), we determined the total abundance of bacteria, archaea and fungi as well as ammonium oxidizing bacteria and archaea (detected by *amoA* genes). In addition, we analyze the diversity and phylogenetic composition of bacterial and archaeal communities by 16S rRNA gene amplicon Illumina sequencing. Current results showed that the abundance of bacteria, archaea and *amoA* genes in bulk soil was effected by crops. Different manure types only affected the abundance of bacterial 16S rRNA and archaeal *amoA* genes. In general no significant differences were obtained among the two studied top soil layers. These findings underline the influence of crop rotation on the soil microbiome and thus on soil fertility and plant performance and provide important information for the sustainable management of agricultural land.

P-EME-140

Dynamics of soil microbial communities: The role of dormancy and dispersal in recovery from temperature shifts

*A. Ulmer[1], A. Shade[2], R. Allen[1]
[1]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*
[2]*Universite Claude Bernard Lyon, Institute of Ecology and the Environment, Lyon, France*

In natural environments, microbial communities regularly experience perturbations that can disrupt their stability. Such disturbances may temporarily alter community composition or, in more extreme cases, lead to cell death and species extinction, resulting in a new composition. An important example is the disturbance of soil communities by temperature shifts.

In soil, a majority fraction of the microbial community is dormant. Dormant microbes do not grow but are expected to resist unfavourable perturbations. This 'microbial seed bank' is thought to enhance community resilience (the ability to recover after perturbation), as the resuscitation of dormant cells contributes to community reassembly. Previous experiments with soil communities have demonstrated that resuscitation and microbial dispersal contribute to microbiome recovery following temperature shifts. However, the relative contribution of resuscitation and dispersal to resilience remains an open question. Quantifying the factors driving community reassembly after perturbation is an essential step in helping to restore microbial-contributed ecosystem functions following disturbance.

Mathematical modelling is important in distinguishing the contributions of different factors to microbial community dynamics. Therefore, we have developed an individual-based mathematical model that accounts for individual cells from various species, each with a distinct temperature-dependent response and the ability to switch between active (growth and death) and inactive (dormant) states and to proliferate or die. The model simulates the impact of temperature perturbations on a microbial community. Our model is linked to existing dynamical data on the response of soil microbiomes to temperature shifts and provides insights into how dormancy, growth, mortality, and dispersal impact community structure.

Using this model, we aim to quantify the potential for microbiome restoration via the reactivation of dormant cells and the dispersal of cells following perturbations.

P-EME-141

Insights into amino acid utilization and regulation in Galdieria sulphuraria

*P. Westhoff[1]
[1]*Heinrich Heine University, Plant Metabolism and Metabolomics Labor, Düsseldorf, Germany*

The extremophilic microalga *Galdieria sulphuraria* thrives in nutrient-limited, fluctuating environments, displaying remarkable metabolic adaptability. We investigated this adaptability through intra- and extracellular metabolomics using LC-MS, complemented by heavy isotope tracers to track amino acid and carbon fate. Our study examined how *G. sulphuraria* modulates amino acid metabolism in response to environmental amino acid and carbon availability, assessing uptake rates, inhibitory interactions, and metabolic pathway shifts. By following labeled amino acids and carbon, we mapped key metabolic adjustments that support resilience in challenging conditions. These findings provide new insights into *G. sulphuraria*"s regulatory mechanisms and nutrient utilization strategies, underscoring its potential in biotechnological applications for environments with limited resources.

P-EME-142

Simulated brood cell matrix for stingless bee larvae and symbiotic fungus rearing

*R. Montagnolli[1], G. do Amaral Reis Pinto[1], R. Cornélio Ferreira Nocelli[1]
[1]*Federal University of São Carlos - Araras, São Carlos, Brazil*

1. INTRODUCTION

Stingless bees are essential pollinators closely associated with microorganisms. Notably, *Scaptotrigona* sp. larvae acquire ergosterol—a hormone essential for pupation—by consuming *Zygosaccharomyces* sp. (Paludo *et al*., 2019). The mycelium grows within the brood cells and its consumption has been observed during *in vitro* rearing. Standardizing laboratory procedures involving this system is necessary for conservation studies.

2. GOALS

Develop a simulated brood cell matrix for *Scaptotrigona postica* larval rearing and establish parameters for symbiotic fungus cultivation.

3. MATERIAL AND METHODS

The matrix was developed using acrylic and microtitration plates with 92 and 300µL wells, respectively. The cerumen component was sterilized by autoclaving, melting at 60°C, or fragmented and transferred directly. Larval food samples were autoclaved by 120°C for 20 min., frozen at -80°C for 20 min. and thawed at 60°C for 3 min. or microfiltrated through a 0.45µm membrane; supplemented with 10% $MgSO_4$ or $(NH_4)_2SO_4$; and diluted in distilled water at 100%, 75%, or 50%.

During incubation, humidity was kept at 100% using distilled water, followed by 85% by using a KCl solution, and 75% with NaCl. Fungal biomass was quantified by centrifuging the samples at 2000g and weighing. Larval development (n=200) was assessed under the optimized conditions.

4. RESULTS

Highest fungal biomass values were obtained using the cerumen fragmentation approach and under the freeze-thawing condition (0.047g), without supplementation (0.039g) or with $(NH_4)_2SO_4$ (0.047g), and at 100% larval food concentration (0.035g). *Zygosaccharomyces* sp. growth was restricted to the regions of the wells coated with cerumen.

Larval rearing assays showed average pupation rates of 72% in microtitration plates and 8% in acrylic plates. The former proved to be more suitable for larval development when incorporating cerumen to the matrix, while the latter had higher mortality and malformation rates.

5. **SUMMARY**

The study confirmed the viability of the simulated matrix for fungal growth and larval development, emphasizing the importance of replicating brood cell dimensions and components.

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Microbial diversity and evolution

P-MDE-143
The gender gap in names of prokaryotes honouring persons
*H. M. Freese[1], L. Giner-Pérez[2], A. Oren[3], M. Göker[1], D. R. Arahal[2]
[1]Leibniz Institute DSMZ, Bioinformatics, IT and Databases, Braunschweig, Germany
[2]Universitat de València, Valencia, Spain
[3]The Hebrew University of Jerusalem, Jerusalem, Israel

Gender inequalities in microbiology can manifest themselves in many different ways. While there are well-known unresolved issues, gender equality should be reflected in public recognition. In biology, the formation of scientific names derived from personal names (eponyms) is one way of expressing this. However, prokaryotic names which honour persons have never been considered from a gender perspective. To investigate this, we retrieved data from the nomenclatural database LPSN (List of Prokaryotic names with Standing in Nomenclature). The etymologies of 23,315 unique names at the rank of genus, species and subspecies were analysed excluding new combinations. A total of 2018 names (8.7%) honour persons. For these names, the proportion of females honoured and, in particular, the change in this proportion over time, was further investigated. Women started to be honoured relatively recently (1947) compared to men (1823). Overall, only 14.8% of all prokaryotic eponyms refer to females. In contrast, about 50% of prokaryotic names derived from mythological characters refer to females. Furthermore, the ratio of honoured women has hardly improved since 1947, although the number of women whose contributions to microbiology could have been recognized has increased over time. To reduce this gender gap, we encourage authors proposing new taxon names to honour female scientists who can serve as role models for new generations.

P-MDE-144
Natural competence of *Shewanella*
*F. Eck[1]
[1]Justus Liebig University Giessen, Mikro- & Molekularbiologie, Gießen, Germany

The ability of many organisms to take up free DNA from their environment and use it either to modify their own genome or as a source of nutrients is an ability to adapt to environmental changes and is a driving force in bacterial evolution. This ability is known as natural competence. The gammaproteobacterium *Shewanella sp*. plays a role in various processes such as biocorrosion, spoilage or even as a pathogen of fish and humans. *Shewanella sp.* are widely distributed in nature. While extracellular DNA can be used as a nutrient, particularly for phosphorus, no natural competence has been demonstrated for species of the genus. Here, we have identified the complete genetic setup required for competence in the model species *S. oneidensis*. We show that the corresponding proteins are produced in a nutrient-dependent manner during growth on surfaces. Accordingly, we have shown that surface-cultivated *S. oneidensis* is competent for natural transformation, as the cells take up and incorporate linear and circular DNA from various sources. The rate of transformation is affected by the presence of a type IV pilus as well as several extracellular nucleases produced by *Shewanella sp.* and required for the use of DNA as a nutrient. Orthologous competence/transformation systems are widespread in *Shewanella sp*., suggesting that competence may have helped this species to acquire genetic traits that allow it to adapt to and proliferate in the wide range of different environments in which this genus thrives.

P-MDE-145
The Mobilome drives the differentiation in *Clostridioides difficile*
*P. Halama[1], T. Riedel[2], I. Schober[1], C. Rodriguez[3], D. Paredes-Sabja[4], B. Bunk[1], U. Groß[5], R. Daniel[5], J. Overmann[6]
[1]DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Bioinformatics, IT and Databases, Braunschweig, Germany
[2]DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, MÖD/DZIF, Braunschweig, Germany
[3]Universidad de Costa Rica, Facultad de Microbiologia, San José, Costa Rica
[4]Texas A&M University, Biology, College Station, TX, United States
[5]Georg-August University Göttingen, Medical Microbiology, Göttingen, Germany
[6]DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Scientific Director, Braunschweig, Germany

Clostridioides difficile causes antibiotic-associated diarrhea which can progress to pseudomembranous colitis. Most genomic information on this pathogen is based on incomplete draft genome sequences from mainly clinical strains. However, recent findings indicate that the untapped mobilome of non-clinical and environmental isolates is large and highly variable.

To further elucidate the genomic diversity and evolutionary dynamics of *C. difficile*, we sequenced 163 isolates *de novo* and compared their closed, high-quality genomes to delimitate their pangenome, lineage-specific genes and mobile genetic elements (MGE). Clade-specific differences in the mobilome and the arsenal of defense mechanisms were observed, suggesting that the five existing clades exploit different evolutionary strategies. While Clades 1 and 4 contain diverse genomes and MGE, Clade 5 remains highly clonal and seems to be undergoing accelerated speciation. Numerous small chromosomal MGE, such as insertion sequence or transposons co-located with lineage-specific resistance genes, defense systems or virulence factors, indicating different mechanisms for gene acquisition. Several prophages and putative phage-plasmids included defense systems and virulence factors, but not resistance genes. Anti-defense systems to bypass the restriction of horizontal gene transfer (HGT) were often observed within the genomes. Remarkably, 94.9% of the CRISPR arrays observed matched *C. difficile* phages, suggesting they are functional and advantageous. Nearly all detected phages were *Caudoviricetes* and were consistently targeted by

CRISPR-Cas spacer sequences. Genomes from Clade 5 showed an increased number of spacer regions and spacer-phage matches per genome despite having the least number of prophages. Furthermore, type I and II restriction-modification systems have been detected frequently among the genomes, where at least five methylation motifs were successfully matched to their putative methyltransferases.

These findings contribute to our understanding of *C. difficile* genome evolution and the role of MGE and defense systems on the diversification and, ultimately, speciation of *C. difficile*.

P-MDE-146
Miniaturizing Evolution: Adaptation of whole-cells using a microfluidic chip
*C. Stolle[1], C. M. Niemeyer[1], K. S. Rabe[1]
[1]*Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces 1 (IBG 1), Eggenstein-Leopoldshafen, Germany*

Introduction

Adaptive laboratory evolution (ALE) is a methodology used for the generation of novel optimized traits for bioproduction. Conventional ALE techniques involve axenic cultivation and do not consider that microorganisms preferably life in communities known as biofilms. Microfluidics have fundamentally changed the field of microbial ALE as it can mimic naturally occurring microenvironments promoting the growth of biofilms alongside planktonic cells.

Goals

The aim of the presented work was to develop a user-friendly and cost-effective microfluidic chip device to perform miniaturized, automated ALE experiments.

Materials & Methods

This poster introduces the microfluidic evo.S (*evo*lution under *s*tress) chip, which was designed to create tunable, spatially stable stressor concentration gradients across consecutive growth wells to adapt microbial cells to defined profiles of stressors such as antibiotics.

Results

The cultivation of microbial cells in the interconnected growth wells results in microenvironments within the chip creating mixed populations, ranging from planktonic cells over to stiff biofilms. The generation of controlled gradient profiles leads to the adaptation of *Escherichia coli* to antibiotics and revealed the chip"s ability to differentiate between persistence and resistance. Importantly, this approach was effectively employed for the discovery of previously unknown mutations conferring resistance to nalidixic acid in *E. coli*. Recently, a customized chip setup was used to successfully adapt the thermophilic organism *Thermus thermophilus* to the antibiotic kanamycin, and also to the utilization of an alternative carbon source.

Summary

The microfluidic evo.S chip enables the cultivation of microbial cells under quasi-static conditions with tunable and spatially stable stressor profiles, enhancing the occurrence of mutations conferring resistance to antibiotics. This kind of chip-based ALE can be applied to adapt virtually any microorganism to desired environmental conditions and offers crucial insights into the mechanism of antibiotic resistance and biofilm formation.

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P-MDE-147
Computational analyses of prophages in acetogenic bacteria and experimental evidence of prophage induction in *Clostridium ljungdahlii*
*D. Crippa[1], M. Schölmerich[1]
[1]*ETH Zurich, Environmental System Sciences, Zurich, Switzerland*

Acetogenic bacteria (acetogens) use the ancient Wood-Ljungdahl pathway (WLP) to grow at the thermodynamic limit of life, often coupling it to other metabolic modules to utilize a wide range of organic compounds as energy and carbon sources for acetogenesis. This metabolic flexibility enables acetogens to coexist and compete in anoxic ecosystems despite the low energy yield of acetogenesis. These metabolic modules and the fact that acetogenesis does not follow an exclusive phylogenetic pattern, suggests that the spread and success/persistence of acetogenesis is attributed to horizontal gene transfer (HGT) via still elusive extrachromosomal DNA elements (ecDNA).
In this study, we use computational tools to shed first light on ecDNA that is present in isolated acetogens and investigate their potential functions. We found that 71 of 95 acetogen isolates were predicted to harbor 1-8 prophages. The prophages are 8-172 kilobases in length and encode many hypothetical proteins, structural phage proteins, lytic genes, toxin-antitoxin systems, but also metabolic proteins such as the bifunctional aldehyde/alcohol dehydrogenase (adhE2), which could be impacting central metabolism.To assess the prophages function, the two model acetogens Clostridium autoethanogenum and Clostridium ljungdahlii which carry two and five prophages, respectively, were selected for further experimental analyses. Whole genome sequencing revealed that two prophage regions in C. ljungdahlii showed a 3 and 40-fold higher read recruitment than the overall genome in stationary phase, indicating that these two prophages become induced and excised. Furthermore, these regions were also highly expressed in transcriptomic datasets from the same growth phase. Taken together, these findings suggest that two of five prophages in C. ljungdahlii are excised to form high-copy ecDNA which is actively expressed in the stationary phase under standard laboratory conditions. In conclusion, our research suggests that the potential to make ecDNAs from prophages encoded in their own genomes is widely distributed among isolated acetogens. Future investigations are aimed at illuminating molecular and environmental triggers of prophage induction and test what impact these elements have on the physiology and metabolism of acetogens.

P-MDE-148

Insights into the microbiota of commercial poultry farms in Pakistan: A 16S rRNA gene based metagenomics study

*M. M. Khan[1], M. A. Mushtaq[1], M. Suleman[2], U. Ahmed[3], R. Aslam[3], M. Mohsin[3], S. Rödiger[1], Y. Sarwar[4], P. Schierack[1], A. Ali[4]
[1]BTU Cottbus-Senftenberg, Multiparameteric Diagnostics, Senftenberg, Germany
[2]Clemson Universi, Clemson, SC, United States
[3]University of Agriculture, Institute of Microbiology, Faisalabad, Pakistan
[4]National Institute for Biotechnology and Genetic Engineering College, Pakistan Institute of Engineering and Applied Sciences (NIBGE-C, PIEAS), Faisalabad, Pakistan

Question: This observational and descriptive study—the first of its kind in Pakistan—examines the fecal microbiota of two commercial bird types i.e., broiler and layer farms to understand how genetic breed, age, and farm type influence microbial communities in commercial settings. Additionally, functional analysis was conducted to identify key metabolic pathways and investigate the abundance of potentially pathogenic bacteria.

Methods: Fecal samples from 18 commercial poultry farms (12 layer and 6 broiler farms) in Faisalabad, Pakistan, were analyzed using 16S rRNA gene sequencing of the V3-V4 region to assess bacterial composition across different breeds, ages, and farm types. Co-occurrence network analysis was performed to explore interactions among bacterial taxa, while functional profiling using PICRUSt2 was employed to identify metabolic pathways within the microbial community. The total abundance of potentially pathogenic genera was also analyzed across all farms.

Results: The dominant phylum, *Firmicutes*, accounted for 58.72% of the microbial population, with *Lactobacillus* being the most abundant genus in both broilers and layers. Layers exhibited greater microbial richness and diversity compared to broilers, and the Karachi cage system (KCS) farm type showed higher microbial richness than the Floor system (FS). Breed significantly influenced microbial diversity, while age did not. Co-occurrence analyses revealed close interactions among the phyla (*Actinobacteriota*, *Proteobacteria*, *Firmicutes*, *Fusobacteriota*, and *Bacteroidota*) and genera (*Lactobacillus*, *Brevibacterium*, *Enterococcus*), suggesting their key roles in the microbial community. Additionally, layers showed higher abundances in amino acid, carbohydrate, cell wall/membrane, cofactor/vitamin, energy, lipid, and nucleotide metabolism pathways. Ten potentially pathogenic genera were detected at varying abundance levels across the farms, with *Enterococcus* and *Corynebacterium* being the most common across all farms, regardless of bird type.

Conclusions: Despite limitations, such as the need for broader geographic sampling, more detailed dietary and medicinal data, and additional farm types, this study establishes a foundation for microbiome research in Pakistan's poultry sector. It highlights consistent taxa, important metabolic pathways, and pathogen traces, providing new insights that could enhance poultry health and productivity.

P-MDE-150

Uncovering Viral Diversity of the freshwater sponge species *Metania* sp using Metagenomics

*T. C. Yeo[1], P. Istvan[2], C. P. Pereira Alves[3], R. H. Krüger[3], J. Rahlff[1,4,5]
[1]Friedrich Schiller University, Aero-Aquatic Virus Research Group, Jena, Germany
[2]University of Oslo, Centre for Bioinformatics,Department of Informatics, Oslo, Norway
[3]Universidade de Brasília (UnB), Enzymology Laboratory, Cell Biology Department, Brasilia, Brazil
[4]Fritz Lipmann Institute (FLI), Leibniz Institute on Aging, Jena, Germany
[5]Linnaeus University, Ecology and Evolution in Microbial Model Systems (EEMiS), Department of Biology and Environmental Science, Kalmar, Sweden

Sponge-associated ecosystems represent a significant reservoir of microbial diversity, contributing especially to nutrient cycling and habitat complexity. However, the role of viruses within sponge microbiomes remains underexplored. Using viral metagenomics and bioinformatic predictions, this study compares the taxonomic composition and functional potential of viral communities across Brazilian freshwater sponges to those in the surrounding water.

The primary objectives of this research were to study the viral operational taxonomic units (vOTUs) from sponge samples and their surrounding water, and to compare the efficiency of different bioinformatics pipelines in vOTU identification.

Eight samples were collected from the Veredas Waterfall Complex in Brazil, half representing sponge tissue samples and the other half corresponding to water samples collected from around the sponge. Illumina sequencing data from these samples were processed using three bioinformatics pipelines: a custom manual pipeline, VirMake (https://github.com/Rounge-lab/VirMake) and MVP [1](Coclet *et al*. 2024).

All pipelines identified several vOTUs unique to the sponge samples, ranging from 15% (VirMake), 18.6% (custom), to 21.9% (MVP) of all vOTUs. In total, 51 out of 113 of the VirMake vOTUs were predicted phages. Among the identified families, tailed phages of the *Peduoviridae* were the most prevalent. Principal Component Analysis showed that the vOTUs of water samples clustered closely together, while sponge samples exhibited greater diversity and were distinctly separated from the water sample cluster. By building a proteomic tree, we found vOTUs associated with lake virophages, and vOTUs showing protein identities to known marine phages. Two vOTUs carried genes functionally annotated as Neurochondrin (Circoviridae) and Sortilin (Caudoviricetes), which are neuro-modulatory genes, that could benefit signalling between bacteria symbionts and sponges [2](Xiang *et al.* 2023).

Our findings indicate distinct sponge viromes and the potential role of sponge viruses in sponge-bacteria interactions. The comparative analysis of different vOTU identification pipelines underscores the importance of methodological approaches in capturing the breadth of viral diversity.

1. https://doi.org/10.1128/msystems.00888-24
2. https://doi.org/10.3389/fncir.2023.1250694

P-MDE-151
Evolving two Thermophiles to thrive at suboptimal temperatures
*C. Prohaska[1], A. Poehlein[2], R. Daniel[2], M. Basen[1]
[1]University of Rostock, Microbiology, Rostock, Germany
[2]Georg-August University Göttingen, Department of Genomic and Applied Microbiology, Göttingen, Germany

Phylogenetic studies suggest that LUCA, the last universal common ancestor of Bacteria and Archaea, was a thermophile (Weiss M., et al. 2016 NatMicrob). This implies that at some point in the history of Early Earth, mesophilic organisms may have evolved from thermophilic organisms.

In this study we explore the thermal adaptability of two thermophilic bacteria, *Thermoanaerobacter kivui* and *Thermotoga maritima*, through Adaptive Laboratory Evolution (ALE). *T. kivui*, an acetogenic bacterium with an optimal growth temperature of 66 °C, was selected due to its ancient acetogenic physiology. In contrast, *T. maritima*, a hyperthermophile from a basal lineage of bacteria, was chosen for its evolutionary connection to mesophilic relatives such as *Mesotoga prima*, allowing insights into temperature adaptation across phylogenetic extremes. Initial experiments determined a minimum growth temperature of 39 °C for *T. kivui* and 45 °C for *T. maritima.* Electron microscopy was employed to visualize structural changes in both organisms and revealed alterations in cell morphology.

ALE was performed through serial transfers at 45 °C for *T. kivui* and 50 °C for *T. maritima* to promote low-temperature adaptation. Surprisingly, the evolved *T. kivui* strain displayed no improvement at 45 °C but a reduced growth rate of 0.31 h^{-1} at 66 °C (compared to typestrain 0.4 h^{-1}) and an increased growth rate of 0.41 h^{-1} at 60 °C (compared to 0.32 h^{-1}). This lead to a new optimal growth temperature of 60 °C.

Similarly the evolved *T. maritima* strain shown shifts in growth rates with lower growth rates at higher temperatures compared to the typestrain (80 °C: 0.29 h^{-1}/ 0.37 h^{-1}) and higher growth rates at lower temperatures (60 °C: 0.11 h^{-1}/ 0.08 h^{-1}, 50 °C: 0.03 h^{-1}/ 0.025 h^{-1}).

In contrast to *T. kivui* the adapted *T. maritima* demonstrated altered fermentation products with an increase in lactate fermentation at temperatures of 50 °C providing insights into the thermal plasticity and metabolic flexibility of hyperthermophiles.

Notably, genomic analyses revealed single nucleotide polymorphisms (SNPs) in both organisms that correlated with their adaptive responses. These genetic changes suggest that specific mutations may drive phenotypic adaptations to temperature fluctuations.

These ongoing studies reveal adaptive responses to temperature shifts in thermophilic bacteria, emphasizing the evolutionary dynamics of thermal preference and metabolic adaptation under laboratory conditions.

P-MDE-152
The role of transposases in accumulation of adaptive mutations in the industrially relevant acetogen *Thermoanaerobacter kivui*
*B. Zeldes[1], M. Basen[1]
[1]University of Rostock, Microbiology, Rostock, Germany

The thermophilic acetogen *Thermoanaerobacter kivui* (Topt 66°C) is a promising candidate for the industrial fermentation of syngas - a mixture of H2, carbon monoxide (CO) and CO2, due to its ability to rapidly acclimate to growth on high concentrations of CO. Genomic analysis of CO adapted strains to identify specific CO adaptive mutations revealed a highly reproducible mutation leading to truncation of a hydrogenase subunit. In addition, whole genome sequencing revealed an ISLre2 family transposase to be highly mobile. This, combined with other evidence that *T. kivui* has a "dynamic" (alternatively, "unstable") genome led the hypothesis that the transposase may play an important role in evolutionary adaptation in *T. kivui*.

To investigate further, *T. kivui* cells were subjected to targeted selective pressure with 5-fluoroorotic acid (5-FOA) in an effort to induce transposition in the purine biosynthesis operon. Primers were used to screen for possible transposon insertion. Unexpectedly large PCR products were indicative of transposition into the site of selection, which was confirmed by sanger sequencing. A similar approach was used to determine if the transposase also plays a role in the CO-adaptive truncation mutation. These targeted approaches were supplemented with whole genome resequencing of strains from multiple lineages generated during a long-term adaptive laboratory evolution (ALE) experiment.

Transposon insertions leading to disruption of counter-selected genes were identified following application of both forms of selective pressure (5-FOA and CO), confirming that the transposase can mediate adaptive mutations in *T. kivui*. In addition, genome resequencing identified frequent miss-matches between ALE strains and the reference genome of *T. kivui* in genome regions adjacent to the five ISLre2 genes. These results suggest the transposase is important for rapid adaptation to extreme stresses, but also remains active and may play a role in generating genomic diversity even in un-stressed cells. Interestingly, the ISLre2 transposase is found in almost all published *Thermoanaerobacter* genomes, except the strain X514. Therefore, future work will examine the *T. sp.* strain X514 to see if its genome is more stable, and adaptive mutations less frequent, without the transposase.

P-MDE-153
Phenotypic divergence and genetic adaptations of *Escherichia coli* in the presence of silver nanoparticles
*M. Wernecki[1], A. Kędziora[1], G. Bugla-Płoskońska[1]
[1]University of Wrocław, Faculty of Biological Sciences, Department of Microbiology, Wrocław, Poland

Question: How does the model organism *Escherichia coli* BW25113 adapt phenotypically, genotypically and epigenetically in response to silver nanoparticles (AgNPs) and what are the potential mechanisms of resistance?

Methods: In this continuation of our group's research revealing diverse bacterial adaptations to silver nanoparticles, we investigated the adaptations of *E. coli* BW25113, a derivative of the K-12 strain, by adaptive laboratory evolution (ALE). Cultures were exposed to 20 nm spherical AgNPs in 26 serial transfers in three replicates. Phenotypic changes were assessed by observing colony growth characteristics using the ScanLag method, while genotypic adaptations were analysed using whole genome nanopore sequencing. Gene enrichment analysis focused on pathways potentially altered by the selective pressure of

AgNP exposure, and differential methylation was assessed to identify the epigenetic state.

Results: Phenotypic diversity emerged, with one subpopulation retaining wild-type growth patterns, while another exhibited prolonged lag phase, reduced growth rates and cell filamentation. Genomic analysis identified single nucleotide polymorphisms (SNPs) distributed across coding and non-coding regions, with no uniform pattern of mutation, suggesting individualised adaptation pathways. Pathway enrichment highlighted shifts in sulphur metabolism, ribosomal function, pentose phosphate pathway, aminoacyl-tRNA biosynthesis and flagellar assembly. In addition, differential methylation was observed in sequences of regulators including OmpR, OxyR, NsrR, NtrC and FlhDC, indicating potential epigenetic regulation in response to AgNPs.

Conclusions: The results reveal a complex adaptive response of E. coli to AgNPs, involving distinct phenotypic subpopulations and diverse genetic and epigenetic modifications. The variability in adaptive pathways highlights the need for integrated phenotypic, genotypic and epigenetic approaches to understand bacterial resistance mechanisms to AgNPs.

P-MDE-154

The collection of *Myxococcota* at the Leibniz Institute DSMZ

*N. Reimann[1], *B. Trümper[1], *R. L. Hahnke[1]
[1]Leibniz Institute DSMZ, Mikroorganisms, Braunschweig, Germany

The collection of *Myxobacteria* at the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures comprise 8,800 strains covering 50 species, 24 genera, 5 families and all four classes of the phylum *Myxococcota* Waite *et al.* 2021.

With the opening of the HZI/DSMZ Reichenbach collection in 2022 to the wider scientific community additional 5,800 strains of the unexplored part will become available for the scientific community. These strains are accompanied by a fantastic documentation and a collection of 2,800 specimens from all over the world sampled before 2014. Reichenbach, Dworkin, White and Rosenberg developed novel isolation and cultivation methods and paved the way for the isolation of novel *Myxobacteria* of broader taxonomic range.

Investigations by Mohr *et al.* and Octaviana *et al.* showed a huge diversity of novel species and genera among *Sorangium* and "*Pseudowusel*" strains from this collection (Mohr *et al.* 2018; Octaviana *et al.* 2022). We further investigated the genera "*Pseudowusel*" and "*Wusel*" by reactivating and classifying more than 200 strains. Surprisingly, we did observe strains of the described genera *Dawidia* and *Pseudochryseolinea* but also strains representing four novel genera and seven novel species. Another aspect is the successful isolation of novel taxa from the specimen collection as shown by Garcia *et al.* 2024. In a practical course, we confirmed successful isolation of *Myxobacteria* from soil samples from 1983.

To gather experts in the taxonomy of *Myxobacteria* (*Myxococcota*), the ICSP subcommittee on the taxonomy of *Myxobacteria* (*Myxococcota*) was established in 2022 (Hahnke *et al.* 2023). The subcommittee invites interested people to participate, provides expert knowledge on the current nomenclature, and define the minimal standards to describe new taxa within the phylum *Myxococcota*.

We present along with the diversity of *Myxococcota* at DSMZ, methods for long term preservation, give advice on depositing *Myxobacteria* at DSMZ (preservation, authentication), and show a glimpse on Reichenbach's documentation on specimens and strains.

References:

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P-MDE-155

Chloracidobacterium validum, sp. nov., a thermophilic chlorophotohetrotrophic bacterium of the phylum _Acidobacteriota_ from an alkaline hot spring mat, emendation of _Chloracidobacterium and C. thermophilum_, with descriptions of _Chloracidobacteriaceae_ fam. nov. and _Chloracidobacteriales_ ord. nov.

M. Kumar Saini[1,2], S. B. Kuzyk[3], C. Villena-Alemany[2], S. Haruta[1], S. Kirstein[3], J. Wolf[3], S. Hanada[1], M. Koblížek[2], *V. Thiel[1,3,4], M. Tank[1,3,4], D. A. Bryant†[4]
[1]Tokyo Metropolitan University, Department of Biological Sciences, Tokyo, Japan
[2]Institute of Microbiology CAS, Laboratory of Anoxygenic Phototrophs, Třeboň, Czech Republic
[3]DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany
[4]Pennsylvania State University, Department of Biochemistry and Molecular Biology, University Park, PA, United States

†Posthumous authorship; Donald A. Bryant sadly passed away on August 28th, 2024

A novel chlorophototrophic bacterium, strain BV2-C^T, was isolated from a microbial mat (~40 °C) at Rupite hot springs, Bulgaria. While rod-shaped, Gram-negative, and producing numerous fimbriae/pili, it was non-motile. Optimal growth occurred under microoxic conditions in the light (20–50 µmol photons m^{-2} s^{-1}) at 45 °C (range, 40–50 °C) and pH 7.2 (range, pH 5.5–9). Bacteriochlorophyll (BChl) *c* and BChl a_P were the most abundant chlorophylls (Chls), with echinenone and γ-carotene as the predominant carotenoids, in cells capable of producing chlorosomes and type-1 homodimeric reaction centers. Dense liquid cultures appear greenish-brown, forming small clumps and aggregates. Strain BV2-C^T grows photoheterotrophically using organic compounds as carbon source, thioglycolate as reduced sulfur source, obligately requiring branched-chain amino acids (isoleucine, leucine, and valine) and lysine. Strain BV2-C^T was closely related to *Chloracidobacterium thermophilum* and *Candidatus* Chloracidobacterium aggregatum with 16S rRNA gene sequence identities of 96.97 % and 97.78 %, respectively. The genome of strain BV2-C^T comprises two circular chromosomes of 2,659,040 and 1,000,103 bp (total genome, 3.65 Mb) and a mol % G+C content of 59.9 %. Phenotypic, phylogenetic and genomic analyses demonstrated strain BV2-C^T as a novel species of the genus *Chloracidobacterium,* which we hereby name *Chloracidobacterium validum* sp. nov. Furthermore, based on phylogenomic analyses we propose *Chloracidobacterium* to be the type genus of a new family *Chloracidobacteriaceae* fam. nov., among a new order *Chloracidobacteriales* ord. nov., within the class *Blastocatellia.* Thus,

Chloracidobacterium validum strain BV2-C^T = DSM 113832 = JCM 39534 represents the type species of all associated taxa.

Keywords: Chlorophotoheterotroph, anoxygenic photosynthesis, thermophile, microaerophile, *Chloracidobacteriaceae*; *Chloracidobacteriales*

Infection biology

P-IB-156

Mechanistic insights into the role of extracellular vesicles at the bacterial-host interface

*M. Battista[1], M. Buchholz[2], Y. Bachelot[3], M. T. Figge[3,4], C. Vilhena[1,2]
[1]*Friedrich-Alexander University, Pharmaceutical Biology - Bacterial Interface Dynamics lab, Erlangen, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology, Infection Biology, Jena, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology, Applied Systems Biology, Jena, Germany*
[4]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*

Introduction*:* Bacterial and human cells produce extracellular vesicles (EVs) in response to diverse stimuli, e.g. toxins, oxidative stress, nutrient depletion, or high cell density. EVs function as transport systems for proteins, enzymes, DNA and RNA, thereby contributing to interkingdom communication. Several studies have characterized isolated EVs from both cell types under distinct conditions.

Objectives: There is currently no platform that enables the simultaneous production of bacterial and host EVs within the same environment. We present a method for bacteria-host co-cultivation using the *Transwell*® insert system, which allows the exchange of solutes between the two cell types whilst preventing their direct contact.

Methods: We investigated both Gram-positive and Gram-negative bacteria, harboring distinct pathogenicity traits: *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Neisseria meningitidis*. Bacteria were co-incubated with peripheral blood mononuclear cells (PBMCs) isolated from healthy human donors. The co-cultivation allowed recovery of the shared supernatant and further isolation and characterization of the mixed pool of bacterial and host extracellular vesicles (mix-EVs).

Results: Dynamic light scattering microscopy revealed a negative correlation between the bacterial concentration and the formation of mix-EVs. Exposing healthy human PBMCs to mix-EVs led to an increased expression of pro-inflammatory cytokines (e.g. IL-6, TNF-α) and several chemokines (e.g. MIP-1a, CCL2, CCL5). Additionally, the transcriptome profile of the PBMCs after exposure to mix-EVs revealed an enrichment of IL-1β transcripts, which was further confirmed at the protein level. By utilizing toll-like receptor pathway specific inhibitors, we could show that this increased expression of IL-1β is mediated by IRAK and TRAF6 related pathways.

Conclusions: This work provides a new platform for the study of EVs at the pathogen-host interface and presents mechanistic insights into the effect of EVs on an infected host.

P-IB-157

Staphylococcus aureus as a proficient intracellular pathogen inducing cell death in different human host cell lines

*A. J. Walter[1], S. Mühlen[2], G. K. Mannala[3], W. Schneider-Brachert[1], J. Fritsch[1]
[1]*University Hospital Regensburg, Department of Infection Prevention and Infectious Diseases, Regensburg, Germany*
[2]*Ruhr University Bochum, Molecular Immunology, Bochum, Germany*
[3]*University Hospital Regensburg, Trauma Surgery, Regensburg, Germany*

The opportunistic pathogen *Staphylococcus aureus* is a well-known cause of life-threatening infections in humans. Many isolated strains are multidrug resistant, making the treatment of infections challenging. Formerly designated a merely extracellular pathogen, *S. aureus* is now recognized to invade and survive within both phagocytic and non-phagocytic host cells, providing a protective hiding niche from antibiotic and immune control. Once inside the cell, the pathogen employs an arsenal of regulatory mechanisms and virulence factors to adjust to the hostile environment, to orchestrate survival in and escape out of intracellular compartments, and to eventually disseminate within the host. However, many studies focus partially or exclusively on non-human models and observations obtained from human cell lines still vary extensively, considering the variety of staphylococcal strains and the host cell types analyzed.

Our aim is to unravel and compare post-invasion events and strategies of host cell remodeling to promote intracellular survival of four different *S. aureus* isolates, of distinct infection backgrounds and virulence patterns. Intracellular fates are analyzed in three different human cell lines, which reflect host tissues affected upon clinical manifestations. Accordingly, strains were assessed microscopically for intracellular uptake, their co-localization with markers of lysosomal activity, their ability to escape from autophago-/endolysosomal compartments into the cytosol, and cytotoxicity.

A key strategy for *S. aureus* to escape the intracellular compartment and disseminate within the host is the manipulation of both timing and mode of host cell death. The extent, pathway, and kinetics of host cell death induction were investigated using several techniques, including flow cytometry, western blot, and xCELLigence RTCA. We present data derived from *S. aureus*-infected human U937 monocytes, A549 lung epithelial and SaOS-2 osteoblast-like cells, showing that *S. aureus* isolates are taken up and reside intracellularly both membrane-enclosed and/or cytosolically. Intracellular *S. aureus* induces host cell death of different kinetics and magnitude in a strain- and cell type-dependent manner, and includes (but is likely not limited to) apoptotic signaling, as shown by the activation of apoptosis key effectors.

Altogether, we show distinct host cell responses upon intracellular infection of different *S. aureus* isolates in selected human host cell lines.

P-IB-158

Streptococcus agalactiae in elephants – a comparative genomic analysis

*L. Fenske[1], T. Eisenberg[2], A. Goesmann[1]
[1]*Justus Liebig University Giessen, Bioinformatics and Systems Biology, Gießen, Germany*
[2]*Hessian State Laboratory, Department of Veterinary Medicine, Gießen, Germany*

Streptococcus agalactiae (GBS) is a bacterium well-known for causing neonatal infections in humans and is also a major pathogen in the dairy industry, responsible bovine mastitis. While GBS research has largely focused on human and bovine infections, this pathogen has a broader host range, infecting various animals, including dolphins, camelids, rats, fish, seals, and elephants. However, studies of isolates from elephants have rarely been conducted. In recent years, our group hast occasionally isolated GBS from African and Asian elephants kept in various zoos in Germany and characterized with molecular methods. This study presents the first whole-genome sequencing analysis of GBS isolates from elephants, aiming to expand understanding of GBS infection mechanisms in this unique host.

Next-generation-sequencing was conducted with 24 GBS isolates followed by comparative genomic analyses to determine the pan- and core genome and identify singleton gene. We searched for antimicrobial resistance genes and performed an in-depth analysis of the capsular polysaccharide (cps) locus. Additionally, we screened for mobile genetic elements and performed a genome-wide association study to identify gene enrichment specific to the elephant host group.

Twelve isolates were assigned to multilocus sequence type 2019, while the remaining isolates represented a previously unidentified sequence type. All isolates carried the mprF gene, linked to host cell uptake and disease progression. Serotyping efforts revealed the only four isolates could be assigned to a serotype. Primers flanking the cps-locus were used to screen for this region. Only one isolate contained the full region, nine isolates lacked both primer sequences, while ten had a deletion within the region. No plasmids were identified in the isolates. However, several regions indicative of potential phage presence were detected. The marR gene was significantly associated with the elephant host, which plays a role in controlling the oxidative stress response. Two additional proteins (Maf family protein; acyl-CoA thioester hydrolase/BAAT C-terminal domain-containing protein) were uniquely associated with the elephant isolates, while one histidine kinase was found in all but one of the elephant isolates.

Future studies should include additional isolates from elephants and other wildlife animals to better understand the pathogenic properties and potential zoonotic impact of this pathogen.

P-IB-159
The fate of intracellular EPEC
*A. Kaiping[1], M. Müsken[2], S. Mühlen[1]
[1]*Ruhr University Bochum, Molecular Immunology, Bochum, Germany*
[2]*Helmholtz Centre for Infection Research – HZI, Zentrale Einheit für Mikroskopie, Braunschweig, Germany*

Infections with enteropathogenic *E. coli* (EPEC) are a major cause of diarrheal disease in children under the age of three in developing countries. While EPEC are generally considered non-invasive pathogens that bind to the surface of enterocytes of the small intestine and inject effector proteins into host cells via a Type III Secretion System, several early studies provided evidence that EPEC is taken up into the host cell during infection. The uptake process appears to be dependent on the presence of the EPEC adherence factor (EAF) plasmid encoding a type IV pilus that mediates bacterial attachment, and the bacterial outer membrane protein intimin, which is involved in the intimate adhesion of EPEC to the host cell. Studies on the fate of intracellular EPEC and their role during infection are still missing. However, a more thorough understanding of the process is essential for the development of future treatment strategies as an intracellular lifestyle enables bacteria to evade immune responses as well as antibiotic treatment. In preliminary studies, we were able to show that EPEC was taken up into both, HeLa as well as HT-29 cells during infection. Using transmission electron microscopy, we found intracellular bacteria in both, membrane-bound vesicles as well as in the cytosol, suggesting, that the bacteria are able to survive in and escape the pathogen-containing vacuole and thereby lysosomal degradation. In the future, we will assess the ability of EPEC to be taken up into both phagocytic and non-phagocytic cells, determine the fate of EPEC inside host cells, and assess whether intracellular bacteria can survive inside and escape from the cells to cause reinfection.

P-IB-160
***In Vitro* Synergy of Colistin in combination with Farnesyltransferase inhibitors against ESKAPE Bacteria**
*M. Klose[1], L. Weber[1], H. S. Bachmann[1]
[1]*Witten/Herdecke University, Pharmacology and Toxicology, Witten, Germany*

Introduction

The emergence of antibiotic resistance is steadily increasing. One way to partially overcome these problems is to test already approved drugs (drug repurposing). Farnesyltransferase inhibitors (FTIs), primarily used in cancer therapy, have shown antimicrobial effects against some gram-positive bacteria [1]. To explore their impact on other gram-positive as well as gram-negative bacteria and assess potential synergy, FTIs are tested with colistin, which is known to disrupt the outer membrane. This study includes not only the key ESKAPE pathogen panel but also additional bacterial strains to gain a broad understanding of differential bacterial responses to FTIs. This approach aims to better understand and categorize FTIs" properties and identify dose-dependent synergistic effects relevant for the treatment of resistant bacteria.

Materials & Methods

Ten bacterial strains were tested to evaluate potential synergy between colistin and various drugs, focusing on FTIs and the ESKAPE panel. Antimicrobial susceptibilities were determined by broth microdilution. Synergy was assessed using a checkerboard assay to calculate ∑FIC values.

Results

When combined with sub-MIC colistin, FTIs inhibited the growth of gram-negative bacteria. Tipifarnib, an imidazole-containing heterocyclic FTI, demonstrated stronger effects on gram-negative bacteria than lonafarnib, a non-peptidic tricyclic FTI. Additionally, the peptidomimetic FTIs B581 and FTI-277 inhibited the growth of gram-negative bacteria in combination with colistin but showed no effect on the gram-positive strains tested. In contrast, bempedoic acid and αHFP, which both target the mevalonate pathway, exhibited no inhibitory activity.

Discussion

FTIs inhibited both gram-positive and gram-negative bacteria when combined with sub-MIC colistin, potentially reducing the required colistin dosage and thus minimizing side effects. This combination may also target colistin-resistant bacteria carrying the *mcr-1* gene, the most prevalent and well-characterised colistin resistance gene. The mechanism of action likely differs from the eukaryotic pathway. The species-dependent effects suggest FTIs disrupt multiple biosynthetic pathways. Further studies will be conducted to clarify the mechanisms.

1. Weber et al. 2019 Front. Microbiol. 12, 628283

P-IB-161
Activation and biological role of the T6SS/T1F secretion/adhesion system in the human pathogen *Yersinia enterocolitica*

*K. Götz[1], A. Diepold[1]
[1]*Karlsruhe Institute of Technology (KIT), Applied biology, Karlsruhe, Germany*

Bacterial secretion systems are crucial for the virulence and competition of many bacterial pathogens. Among these, the type VI secretion system (T6SS) functions as a contractile molecular nanomachine, delivering toxic effector proteins into eukaryotic or prokaryotic target cells. The T6SS consists of 13 highly conserved core components, typically organized in gene clusters. The human pathogen *Yersinia enterocolitica* harbors a special type of T6SS cluster that also incorporates genes for type I fimbriae (T1F), structures typically involved in surface adhesion. We found that these genes are co-regulated, and their activation leads to a distinctive switch in bacterial cell biology and attachment. To determine the biological role and function of the T6SS/T1F cluster we aimed to characterize the expression and activation conditions of the T6SS/T1F cluster and identify T6SS effector proteins. Live cell microscopy of bacteria expressing functional fluorescent labeled TssB, a core component of the T6SS sheath allowed to visualize the expression and assembly of the T6SS under various conditions. We identified regulatory elements that strongly increased the expression levels of T6SS/T1F components. Proteomic analysis in these strains led to the identification of the first T6SS effector in *Y. enterocolitica* and its putative immunity protein. Examining the role of this effector yielded insights into bioactivity and target specificity of the T6SS/T1F system and its function in *Y. enterocolitica* virulence.

P-IB-162
Microorganisms represent a dormant phenotype during long-term starvation in tap water

M. Hummert[1], J. Diehr[1], A. Mellmann[1], *T. Kuczius[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*

The expression of the dormancy state is a widespread mechanism of microorganisms to swerve environmental stress situations. Dormant cells assumed by low metabolism activity indicated by a lack of multiplication under these conditions.

We investigated the transfer of faecal, skin and infectious pathogens as well as environmental microorganisms to this dormant state by starvation and long-term persistence in tap water, and we analysed long-term survival and re-cultivation. Bacteria were adapted to tap water by pre-cultivation in Luria both producing high bacterial masses followed by addition of the nine-fold sterile tap water volume and ongoing incubation for further 72h at room temperature. After harvesting and suspension in tap water, cells rotated at low speed to minimize biofilm formation for up to 12 weeks. Re-growth verified long-term viability, and the cell number was determined weekly by counting the colony forming units (CFU/mL) in dilution series.

During a 6-week incubation period, the survival rate decreased less than 2 $\log_{10}$ levels for *Pseudomonas aeruginosa*, *Bacillus subtilis*, *E. coli* and the enterococcal species *E. faecium* and *E. faecali*s. A stronger drop in the cell counts was observed up to week 12. The survival curve of the human colonizers *Staphylococcus aureus* and *S. epidermidis* decreased more rapidly over time. No colonies came up after 4 weeks. *S. lentus* and *S. vitulinus,* isolated from the environment, were more robust within a 6-week period showing only a slight decrease by few $\log_{10}$ levels in the cell counts. Overall, no increase in cell numbers was determined.

Many microorganisms are able to express the dormancy state and to survive in tap water over weeks. Despite the stress conditions caused by lack of food in tap water, the bacteria showed a dormant state with a good re-cultivation rate. The ability of bacterial long-term survival displays a major infection risk, because water reservoirs can play a role as source for contamination and subsequent infections. The long-term existence of dormant bacteria cells in tap water should be viewed critically in order to maintain health and hygiene.

P-IB-163
Exploring the spectrum of human encounters with bacterial pathogens by profiling pathogen-specific antibody repertoires in a population-based setting using the SHIP-TREND cohort

*M. S. Illenseher[1], D. Raafat[2,3], D. Dittmar[1], T. C. Meyer[1], L. Scheunemann[1], S. Weiss[1], N. Friedrich[4], H. Völzke[5], T. Kocher[6], C. Kohler[7], K. Surmann[1], C. Hentschker[1], S. Holtfreter[3], L. Kaderali[8], S. Simm[8], B. M. Bröker[3], S. Michalik[1], U. Völker[1]
[1]*University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany*
[2]*Faculty of Pharmacy, Alexandria University, Department of Microbiology and Immunology, Alexandria, Egypt*
[3]*University Medicine Greifswald, Greifswald, Germany*
[4]*University Medicine Greifswald, Greifswald, Germany*
[5]*University Medicine Greifswald, Greifswald, Germany*
[6]*University Medicine Greifswald, Greifswald, Germany*
[7]*University Medicine Greifswald, Greifswald, Germany*
[8]*University Medicine Greifswald, Greifswald, Germany*

The Study of Health in Pomerania (SHIP) is a comprehensive population-based project that investigates the health dynamics of the population of Northeast Germany, a region historically associated with lower life expectancy. SHIP's extensive data collection provides a longitudinal perspective on health trends, enabling the analysis of the complex interplay of biological and environmental factors. For instance, *Staphylococcus aureus* (*S. aureus*)-specific IgG and IgA responses were measured using a dilution-based multiplexed bead array to monitor the immune response across the SHIP-TREND-0 cohort. The findings revealed a heterogeneous antibody response to different *S. aureus* antigens influenced by factors such as colonization status, sex, and age.

We now expanded the study to track the antibody response to 35 pathogens associated with human infections, such as pneumonia, sepsis, and joint infections. We recorded the profile of the antibody response to these bacteria in the SHIP-TREND cohort using the Infection Array, and then examined its correlation with the composition of the microbiome.

The Infection Array comprises antigens of the secretomes of these pathogens, which are conjugated to individual bead species. Incubation of plasma samples in seven-step dilution series with the multiplexed beads allow the binding of the plasma antibodies to their respective presented antigens over a high dynamic range. The binding is detected *via* the addition of a secondary R-phycoerythrin-conjugated antibody, which induces a fluorescence signal that is measured with the Luminex® Flexmap 3D®. In total, 2041 plasma samples of the SHIP-TREND (0 and 1) and SHIP-START (2 and 3) cohorts (0/1 as baseline and 2/3 as follow-up) were included in the analysis to gain a longitudinal perspective on pathogen-specific antibody titers. The studies offered valuable insights into the dynamics of health in the general population as male sex is associated with higher *S. aureus* carriage and female sex correlates with higher antibody responses against uropathogenic bacteria. With increasing age there is a slightly negative correlation with pathogens that cause vaccine-preventable diseases such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, thereby indicating a decrease in the efficiency of the immune system.

These findings highlight how age, sex, or colonization status shape pathogen-specific antibody responses, revealing important insights into immune dynamics within the population.

P-IB-164
Identification of novel molecular targets for inhibition of *E. faecalis* biofilms

*F. Martin[1], E. Grohmann[1]
[1]*Berlin University of Applied Sciences and Technology, Fachbereich V - Biotechnologie, Berlin, Germany*

Enterococcus faecalis, a common gut commensal and opportunistic pathogen, is a major cause of hospital-acquired infections. Its ability to form biofilms enhances antibiotic tolerance and persistence, making infections difficult to treat (de la Fuente-Núñez *et al.*, 2013). Given that 80% of infections are associated with biofilms (Park and Sauer, 2022), there is a critical need for new therapeutic approaches.

The primary objective of our research is to identify new biofilm inhibitors for *E. faecalis* through two complementary approaches. First, an automated high-throughput (HTP) screening process is being developed, integrating machine learning to accelerate drug discovery. Data from biofilm screening assays will be leveraged to train machine learning models, streamlining the identification of promising compounds by predicting their efficacy more efficiently.

The second approach focuses on discovering novel drug targets through comparative transcriptomics paired with proteomics, comparing biofilm and planktonic states of the bug. Identified targets will be analyzed using protein chemistry techniques, followed by rational drug design to develop therapeutic compounds aimed at disrupting biofilm formation.

Preliminary results from transcriptomic and proteomic analyses, which reveal differential expression patterns of the planktonic and biofilm state, that may serve as potential drug targets, will be presented. Early-stage evaluations of the automated biofilm assay will also be shown.

By connecting HTP screening with machine learning, the drug discovery process becomes iterative, where each method refines the other, potentially improving the quality of outcomes.

Since no biofilm treatments have advanced beyond clinical trials, novel drug targets are essential. An integrated omics approach, overcoming the limitations of single-omics, can reveal new therapeutic pathways.

De la Fuente-Núñez, C. *et al.* (2013) "Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies", *Current Opinion in Microbiology*, 16(5), pp. 580–589. https://doi.org/10.1016/j.mib.2013.06.013.

Park, S. and Sauer, K. (2022) "Controlling Biofilm Development Through Cyclic di-GMP Signaling", in A. Filloux and J.-L. Ramos (eds) *Pseudomonas aeruginosa*. Cham: Springer International Publishing (Advances in Experimental Medicine and Biology), pp. 69–94. https://doi.org/10.1007/978-3-031-08491-1_3.

P-IB-165
MraY and WbpL from *Pseudomonas aeruginosa are* inhibited by uridyl-peptide antibiotics

*J. M. Daniel[1], M. Arts[1], H. Groß[2], B. Gust[2], P. H. Koutsandrea[2], T. Schneider[3], R. Sonnengrün[2]
[1]*University Hospital Bonn, Institute for pharmaceutical microbiology, Bonn, Germany*
[2]*Eberhard Karls University Tübingen, Institute for pharmaceutical biology, Tübingen, Germany*
[3]*University Hospital Bonn, Institute for pharmaceutical biology, Bonn, Germany*

Antibiotic resistance is one of the most serious health threats and the therapeutic options to treat infections caused by multidrug-resistant strains are seriously compromised. Especially for infections caused by *Pseudomonas aeruginosa* isolates which have emerged resistance towards all carbapenems, aminoglycosides, and fluoroquinolones, novel antibiotics with new targets or unprecedented mechanisms of action are urgently needed. Integral component of the drug development process is the analysis of the mechanism of action of an antibiotic, as well as identification of the molecular target. Without this detailed knowledge, rational drug design is strongly hampered.

Uridyl-peptide antibiotics (UPA) are a promising group of nucleoside natural products, which show potent activity against *P. aeruginosa*. An important molecular target of this compound class is the phospho-N-acetylmuramoyl-pentapeptide-transferase (MraY), which catalyses the transfer of phospho-MurNAc pentapeptide to the membrane-standing lipid carrier undecaprenyl phosphate (C_{55}-P) resulting in the formation of Lipid I. Using a fluorometric assay with the heterologously expressed MraY from *P. aeruginosa*, eight UPA"s were characterized, and all showed IC_{50} values in the nanomolar range. Further, a second periplasmatic binding site was identified and confirmed by using site-directed mutagenesis resulting in significant elevation of the IC_{50} compared to the wildtype MraY, making

UPA"s the first antibiotics with a dual binding motifs against the MraY of *P. aeruginosa*. To corroborate the data, a bioinformatical approach was used, by calculating the ΔG of the wildtype MraY and the mutants with AutoDock Vina (Trott & Olson, 2010). The theoretical binding showed very similar results to the biological data. Additionally, we could identify LPS synthesis of *Pseudomonas* as a new target of the UPA"s, by developing an in vitro assay for the first membrane standing protein of LPS biosynthesis (WbpL). Furthermore we investigated the effect of UPA's on the functional inhibition of LPS biosynthesis and could notice a decrease in LPS with an increase of an inhibitor.

P-IB-166

Understanding how biofilms develop on catheters in liver transplant patients

*A. Busch[1], O. Rohland[2], A. Barborski[1], G. Doman[1], L. Rathnow[1], C. Schaudinn[3], F. Dondorf[2], F. Rauchfuss[2], M. Bauer[4], R. Allen[1]
[1]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*
[2]*Friedrich Schiller University, Department of General, Visceral and Vascular Surgery, Jena, Germany*
[3]*Robert Koch Institute, Advanced Light and Electron Microscopy, Berlin, Germany*
[4]*Friedrich Schiller University, Department of Anesthesiology and Intensive Care Medicine, Jena, Germany*

In liver transplant patients, catheters facilitate bile drainage but also provide surfaces for bacterial biofilm formation, elevating infection risks, particularly under immunosuppressant therapy. This pilot study investigated surgical and microbiological factors influencing biofilm formation and infection risk in liver transplantation. Results showed that in surgery duct-to-duct reconstruction lowers infection risk relative to biliodigestive anastomosis. This can be correlated to biofilm formation on biliary catheters associated with duct-to-duct reconstructions correlated with higher bacterial loads and adverse outcome as cholangitis and hospital stay. Microbiome analysis of the catheter biofilms revealed a bile duct microbiome composition similar to the gut, suggesting an ascending infection pathway. Notably, Enterobacteriaceae constituted 90–95%, whereas Enterococcaceae represented only 5–10%, contrasting with the usual 50/50 distribution observed in standard cases of cholangitis.

To assess the pathogenic potential of bile duct microbiome, live bacteria were isolated from catheters, identified through genetic analyses and antibiotic resistance analysis. Although many isolates harbored antibiotic resistance genes, most did not show phenotypic resistance, suggesting that biofilm formation, rather than antibiotic resistance, may be a primary pathogenic factor. Using these isolates, we developed an *in vitro* model for biofilm formation on catheters. Single-species, dual-species, and complex microbiome biofilms were profiled using 16S rRNA sequencing, and live-dead cell ratios evaluated through LIVE/DEAD staining in confocal laser scanning microscopy (CLSM) and CFU enumeration. Enterobacteriaceae consistently formed dense biofilms with high viable cell content, while Enterococcaceae biofilms were sparse to nonexistent. Dual-species biofilms and microbiome biofilms were thicker and exhibited higher survival ratios. As biofilm-forming bile duct microbiome bacteria can disperse into the abdominal cavity due to bile leakage post-operation, polymicrobial biofilms potentially spreading across various catheters and can include nosocomial bacteria, posing considerable challenges in clinical settings. These findings provide valuable insights into biofilm dynamics and may inform strategies for treatment or prevention of infection.

P-IB-167

Establishing a quantitative chemoproteomic labeling method for protein *N*-chloramines

*L. Kühn[1], L. R. Knoke[1], F. M. L. Peeters[2], F. Schulz[2], L. I. O. Leichert[1]
[1]*Ruhr University Bochum, Biochemistry, Bochum, Germany*
[2]*Ruhr University Bochum, Chemistry and Biochemistry of Natural Products, Bochum, Germany*

In humans, neutrophils represent the initial line of defense against bacterial pathogens. Invading bacteria are killed by neutrophils by a process called phagocytosis, during which bacteria are exposed to a cocktail of oxidative substances. One of these substances is the highly toxic hypochlorous acid (HOCl), which is generated by the Myeloperoxidase (MPO) in the phagolysosome. In proteins HOCl chiefly oxidizes thiols but also reacts with free amines to form *N*-chloramines, often resulting in a loss of protein function. However, there are also proteins that are turned into effective holdase-like chaperones by *N*-chlorination. Despite the clear effect of N-chlorination on protein function, there are currently no strategies to study the extent of *N*-chloramines *in vivo*.

We have recently shown that protein *N*-chlorination can be detected *in vitro* using the DNASyl derivative DANSyl sulfinic acid ($DANSO_2H$). Additionally free amines can be labeled by DANSyl chloride (DANSCl). Both reactions yield the same DANSylated fluorescent protein product. To advance insights into the role of *N*-chlorination in host-pathogen interactions, this study aims to harness this chemistry in combination with isotopic labeling to establish a robust, quantifiable labeling protocol to detect *N*-chloramines *in vitro* and *in vivo*.

Labeling of *N*-chloramines and free amines was initially optimized on peptides and proteins *in vitro* and followed up by the establishment of labeling procedures *in vivo*. The addition of detergents during the labeling process was found to significantly increase labeling efficiency. This increase in labeling suggests that not all amines/*N*-chloramines are accessible for DANSyl labeling without denaturation. The labeling degree was quantified through in-gel fluorescence measurements of the DANSyl products and confirmed by mass spectrometry analysis.

The DANSyl labeling established here lays a foundation for the quantification of the occurrence of *N*-chloramines *in vivo* and to study their pathophysiological role.

P-IB-168

A widespread SCC*mec*-located gene cluster protects MRSA against toxic polysulfide

*V. Wiemann[1], J. S. Puls[1,2], T. S. Tanabe[3,4], T. Schneider[1], C. Dahl[3], S. Heilbronner[2,5], F. Grein[1], T. Fließwasser[1]
[1]*University Hospital Bonn, Institute for Pharmaceutical Microbiology, Bonn, Germany*
[2]*Ludwig Maximilians University, Microbiology, Munich, Germany*
[3]*Rheinische Friedrich-Wilhelms-Universität Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*
[4]*University of Vienna, Division of Microbial Ecology, Centre for Microbiology and Environmental Systems Science, Vienna, Austria*
[5]*Eberhard Karls University Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Department of Infection Biology, Tübingen, Germany*

Colonization of the human body presents challenges for opportunistic pathogens, which must overcome host defense

mechanisms, antimicrobial compounds, and competition with the commensal microbiota. One of these opportunistic pathogens is *Staphylococcus aureus* (*S. aureus*), with methicillin-resistant *S. aureus* (MRSA) strains being one of the most common antibiotic-resistant nosocomial pathogens, responsible for millions of life-threatening infections worldwide8. *S. aureus* often colonizes mucosal surfaces of the human body and is therefore exposed to environmental stress factors, including hydrogen sulfide (H2S). H2S is ubiquitous in the human body, and while low concentrations act as a signaling molecule, high concentrations are toxic. The elevated H2S levels are primarily a consequence of bacterial sulfur compound metabolism. On mucosal surfaces, such as the nose and gut, H_2S concentrations can reach up to 0.4 mM9 and between 0.3–3.4 mM10,11, respectively, due to the degradation of sulfated sugar residues and l-cysteine from mucin12. Under oxic conditions, which prevail on mucosal surfaces13, H_2S oxidizes into highly reactive polysulfides, creating another toxic stressor for the bacteria. While it is known that *S. aureus* protects itself from H_2S via its *cst* gene cluster (*tauE, cstR, cstA, cstB, sqr*)14, the detoxification of polysulfides is virtually unknown. We showed that *S. aureus* uses an SQR-independent variant of the *cst* pathway to detoxify polysulfides. Furthermore, our research revealed that the *cst* gene cluster is heterogeneously distributed in staphylococcal genomes, with multiple clinically relevant SCC*mec* types introducing an additional *cst* (*cst2*, without *sqr*) into the genomes of MRSA strains. Phenotypic analyses of a wide range of clinical and laboratory-derived strains demonstrated that the additional *cst2* confers high polysulfide tolerance to MRSA, providing a significant fitness advantage in polysulfide-rich environments that results in the displacement of MSSA strains in direct intraspecies competition.

8 **Murray *et al.* (2022):** *in: The Lancet 399, 629–655*
9 **Ikeda *et al.* (2019):** *in: Molecules (Basel, Switzerland) 24*
10 **Braccia *et al.* (2021):** *in: Frontiers in microbiology* 12: 705583
11 **Dordević et al. (2021):** *in: Journal of advanced research* 27:55–69
12 **Stümmer *et al.* (2023):** *in: Antioxidants (Basel, Switzerland)12*
13 **Espey (2013):** *in: Free radical biology & medicine* 55: 130–140
14 **Shen, *et al*. (2015):** *in: Biochemistry* 54 29, 4542–4554

P-IB-169

Identification of anti-TB therapy induced ADRs genetic markers using In-Silico approaches

*K. Kishor[1]
[1]IIPS, Population Sciences, New Delhi, India

Introduction Adverse drug reactions (ADRs) are associated with clinical morbidity and, in severe cases, even mortality. Globally billions of dollars are spent on managing these ADRs for common and uncommon diseases. Due to these reasons drug resistant strains have emerged and are now a serious challenge to TB eradication. To effectively deliver the available treatment regimen and ensure patient compliance it is important to manage ADRs more efficiently. Recent studies have demonstrated that drug outcomes are patient-specific and can, therefore be predicted. A few of these drugs, including a few administered for TB, have shown excellent correlation with response rates and development of ADR.

Method: ADRs selected based on frequency of occurrence (≥1%). Anti-TB drugs were reviewed to identify the candidate genes (DMETs, HLA). Genes analysed with different web tools and databases to extract their SNPs. MAF >0.01 shortlisted using NCBI Gene and dbSNP databases (built 141). SNPs which lay in a functional domain of the gene were prioritized using SNPinfo web server (www.snpinfo.niehs.nih.gov/). Additionally, same analysis was done for Indian population.

Result: We identified 10 genes which maybe directly linked to ADRs to various anti-TB drugs, 4 of these have been documented earlier. Nearly 47 genes were identified for indirect association with ADRs–by virtue of them being off-targets of the drugs. Lastly, 5 genes were reported for their allelic association with anti-TB DIH. To our knowledge, this is the first review reporting a list of possible genetic markers in context to TB ADRs to such a vast extent.

Conclusions: New gens are identified that may be associated potentially with anti-TB drug ADRs. This would translate into not just patient welfare but would also save billions of dollars spent annually on managing drug induced ADRs.

P-IB-170

Active downregulation of the type III secretion system at higher local cell densities promotes *Yersinia* replication and dissemination

F. Ermoli[1], G. Malengo[1], C. Spahn[1], T. Glatter[1], *A. Diepold[2,1]
[1]Max Planck Institute, Marburg, Germany
[2]Karlsruhe Institute of Technology (KIT), Institute of Applied Biosciences, Karlsruhe, Germany

The Type III secretion system (T3SS) is used by Gram-negative bacteria, including important pathogens, to manipulate eukaryotic target cells by injecting effector proteins. T3SS secretion is tightly regulated and accompanied by a phenomenon called secretion-associated growth inhibition (SAGI). Since actively secreting bacteria cannot grow and divide, a non-uniform distribution of T3SS expression is conceivably beneficial during infection. *Yersinia enterocolitica*, a main T3SS model organism that uses the system to evade the host immune response, had so far been found to uniformly express and assemble T3SSs, which are then activated by target cell contact. However, in this study, we found that *Yersinia* actively suppress T3SS expression, assembly and activity at higher cell densities, such as inside microcolonies. This effect is highly specific to the T3SS, reversible, and distinct from stationary phase adaptation. It is conferred by the T3SS transcriptional activator VirF, which is downregulated at the higher cell densities and whose *in trans* expression restores T3SS assembly and activity. Transcript analysis showed that this effect is mediated by increased levels of the regulatory RNAs CsrBC, which sequester the regulatory protein CsrA and destabilize the *virF* transcript. Cell adhesion, an essential trait for *Yersinia* virulence, linked to T3SS secretion and regulated by VirF also showed to be affected by local higher cell densities. The concomitant downregulation of the VirF-dependent adhesin YadA led to a drastic reduction in bacterial cell adhesion. We propose that this active suppression of T3SS secretion and cell attachment at higher local bacterial densities promotes a switch during *Yersinia* infection from a T3SS-active colonization stage to a bacterial replication and dissemination phase.

P-IB-171

Chlamydia-like bacterium *Simkania negevensis* exploits host sphingolipids for infection and progeny formation

*A. Mohanty[1], J. D. Weinrich[1], F. Schumacher[2], M. Rühling[1], B. Kleuser[2], V. Kozjak-Pavlovic[1]
[1]*University of Würzburg, Microbiology, Würzburg, Germany*
[2]*Free University of Berlin, Berlin, Germany*

The obligate intracellular pathogen *Simkania negevensis* (Sne) resides within a *Simkania*-Containing Vacuole (SnCV), establishing close interactions with the endoplasmic reticulum (ER) and mitochondria. Due to limited metabolic capacity, Sne relies on host sphingolipids for survival. Previous findings showed that Sne does not depend on the *de novo* ceramide synthesis or ceramide transport by CERT. To investigate the role of salvage pathway in sphingolipid acquisition, we studied the importance of acid ceramidase (AC) and sphingomyelin synthases (SMS1 and SMS2) in modulating *Simkania* infection.

We first inhibited AC with a fluoxetine derivative inhibitor AKS466, which both reduced Sne infection rates and altered SnCV morphology. In contrast, AC knockdown or knockout in HeLa229 cells showed no significant effect on infection. Lipidomic analysis showed accumulation of ceramide and dihydroceramide, indicating an off-target inhibition of dihydroceramide desaturase (DEGS) by AKS466. This was further confirmed through DEGS activity assay, suggesting that a combined inhibition of AC and DEGS might be necessary to reduce infection.

The Sne infection of SMS1/2 double knockout (DKO) HeLa229 cells was significantly reduced. SMS1 is localized to Golgi and SMS2 is a plasma membrane/Golgi distributed enzyme. Using cell lines that inducibly expressed either the mutated, miss localized SMS2-M64A-HA or the wild type SMS2-HA, we observed that only the expression of the wildtype enzyme restored the infection rates of Sne, pointing to the importance of SMS2 localization for Sne infection. Currently, we are analyzing in which way SMS1 contributes to infection.

Finally, plasma membrane sphingomyelin depletion using *Staphylococcus aureus* β-toxin did not affect infection rates, indicating that sphingomyelin itself may not be the sole cause of infection reduction, but that the function and cellular localization of SMS1 and SMS2 may be critical. Together, our findings highlight that Sne utilizes both lysosomal- and Golgi-associated sphingolipid pathways for infection. We are currently examining whether SnCV surface similar to *Chlamydia*, serves as a platform for sphingomyelin or ceramide synthesis within the host. Additionally, we aim to explore the role of cholesterol trafficking and modifications in Sne infection.

P-IB-172

Overcoming resistance via reactivation – Mode of action of vancomycin conjugates

*A. Abdelrahman[1]
[1]*University Hospital Bonn, Pharmazeutische Mikrobiologie, Bonn, Germany*

Glycopeptide antibiotics (GPAs) are drugs of last resort to treat infections caused by (multi-) resistant Gram-positive pathogens. They inhibit peptidoglycan biosynthesis mainly by binding to the D-alanyl-D-alanine terminus of lipid II peptide stem, thereby blocking transglycosylation and transpeptidation reactions in a sterical manner. Vancomycin (VAN) and teicoplanin (TEIC) represent the prototype GPAs in clinical use today. However, widespread GPA resistance has become a serious threat to global health. Resistance resulting from alteration of the PGN precursor, e. g. by exchange of the terminal D-alanine to D-lactate or D-serine, is conferred by the acquisition of a van-resistance operon. Enterococci, which harbour a vanA or vanB cluster, are most significant in the clinical setting. Research is focused on structural modification of glycopeptide antibiotics to overcome resistance. Lead candidate FU002, a vancomycin-hexa-arginine derivative, with high potency against vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) was generated by conjugation of polycationic peptides to VAN. Cell-based and in vitro assay are used to elucidate the precise mechanism of action to build a rational basis for targeted structure optimization. Differences in potency are not based on interaction with a secondary binding site on lipid II and remain still elusive.

P-IB-173

Role of alpha-hemolysin and its variants in Hemolytic *E. coli pathogenicity*

*M. A. Mushtaq[1], R. Kolenda[2], P. Schierack[1], M. Mohsin[3], S. Rödiger[1], M. M. Khan[1]
[1]*BTU Cottbus-Senftenberg, Multiparametric Diagnostics, Senftenberg, Germany*
[2]*Quadram Institute Bioscience, Norwich Research Park, Norwich, United Kingdom*
[3]*University of Agriculture, Microbiology, Faisalabad, Pakistan*

Question

What roles do different alpha hemolysin (*hlyA*) variants and associated adhesins play in the cytotoxicity and host cell specificity of hemolytic *Escherichia coli* strains, and how do plasmid-encoded versus chromosomal *hlyA* variants contribute to this effect?

Methods

78 alpha hemolysin encoding hemolytic *E. coli* isolates from various human and animal sources were previously sequenced and genome placement (plasmid or chromosome encoded) was determined. Sequence based analysis to identify the alpha hemolysin variants was carried out. Cytotoxicity of all 78 strains was assessed on four human cell lines i.e., LoVo (intestinal), Caco-2 (colon), HEp-2 (epithelial), and 5637 (urinary bladder) using advanced imaging to detect and quantify monolayer disruption. Gene deletions of selected *hlyA* variants were performed to evaluate variant-specific cytotoxicity. Complementation with plasmids (pBAD33) encoding various *hlyA* variants against the same genetic background confirmed variant specific phenotypic impacts. Plasmid-encoded *hlyA* variants underwent long-read sequencing to map genetic architecture. Plasmids were transferred into non-hemolytic *E. coli* strains to examine acquired cytotoxicity. Proteomic analysis as well as characterized hemolysin and adhesins (*fimH*, *ecp*, *csgA*); deletions assessed their influence on hemolysin expression and function.

Results

We identified 19 *hlyA* variants among the 78 strains and cytotoxicity assays revealed strongest activity on 5637 and Caco-2 cells for most variants, except plasmid-encoded *hlyA* variants, which depicted mild effects on 5637 but strong effects on Caco-2 and LoVo cells. Variant 7, the most

prevalent across multiple sources, showed significant cytotoxicity on Caco-2, 5637, and LoVo cells. Variant 11 exhibited the highest cytotoxicity across all cell lines tested. HEp-2 cells remained unaffected by any strains. Deletion of *hlyA* in variant 7 strains reduced cytotoxicity, confirming *hlyA*'s role. Plasmid transfer experiments demonstrated that plasmid-encoded *hlyA* confers cytotoxicity to non-hemolytic strains.

Conclusion

Alpha hemolysin variants significantly contribute to differential exfoliative cytotoxicity and host cell specificity. Gene deletion studies confirm *hlyA*'s role in cytotoxicity. The observed effects on specific human cell lines highlight the importance of *hlyA* variants and adhesins in *E. coli* pathogenicity, advancing insights into molecular mechanisms of cytotoxicity and hemolytic activity.

P-IB-174

Assessing the direct antibacterial activities of synthetic host defense peptides

*C. L. Knopp[1], I. Wiegand[1], A. Müller[1], T. Schneider[1]
[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Pharmaceutical Microbiology, Bonn, Germany*

Antimicrobial peptides (AMPs), also known as host defense peptides (HDPs) are effectors of innate immunity present in all forms of life and have co-evolved with bacteria for millions of years. AMPs bear great potential for future antibiotic therapy. Although bacteria are frequently exposed to these AMPs, emergence of resistance is rarely reported. Furthermore, they combine direct antimicrobial activities together with immunomodulatory properties boosting their effectiveness in infection control. Many HDPs are known to disturb bacterial membrane functions, but the specific mechanisms of action (MoA) are not fully understood. Artificially designed AMPs based on the Anti-Lipopolysaccharide factor (ALF) of *Limulus polyphemus* were supposed to bind free lipopolysaccharides from Gram-negative pathogens and prevent sepsis. Here, we show that synthetic anti-lipopolysaccharide peptides (SALPs) Pep19-2.5 and Pep19-4LF demonstrate promising antibacterial activity and that their MoA is more direct, likely involving the interaction with specific bacterial target molecules.

P-IB-175

The mechanism of action of semisynthetic guanidino lipoglycopeptides with potent antibacterial activity

*M. Arts[1], E. van Groesen[2], E. Mons[2], I. Kotsogianni[2], K. H. M. Tehrani[2], N. Wade[2], V. Lysenko[2], F. M. Stel[2], J. T. Zwerus[2], S. De Benedetti[1], A. Bakker[3], P. Chakraborty[4], M. van der Stelt[3], D. J. Scheffers[4], J. Gooskens[5], W. K. Smits[6], K. Holden[7], P. S. Gilmour[8], J. Willemse[9], C. A. Hitchcock[10], J. G. C. van Hasselt[11], T. Schneider[1], N. I. Martin[2]
[1]*University Hospital Bonn, Institute for Pharmaceutical Microbiology, Bonn, Germany*
[2]*Leiden University, Biological Chemistry Group, Institute of Biology Leiden, Leiden, Netherlands*
[3]*Leiden University, Department of Molecular Physiology, Leiden Institute of Chemistry, Leiden, Netherlands*
[4]*University of Groningen, Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, Groningen, Netherlands*
[5]*Leiden University, Department of Medical Microbiology, Leiden University Center for Infectious Diseases (LUCID), Leiden, Netherlands*
[6]*Leiden University, Experimental Bacteriology, Leiden University Center for Infectious Diseases (LUCID), Leiden, Netherlands*
[7]*Evotec (U.K.) Ltd., Cheshire, United Kingdom*
[8]*The Drug Development Team, Leiden, Netherlands*
[9]*Leiden University, Institute of Biology, Leiden, Netherlands*
[10]*6 Carnoustie Close, Chestfield, Whitstable, Kent, United Kingdom*
[11]*Leiden University, Division of Systems Biomedicine and Pharmacology, Leiden Academic Centre for Drug Research, Leiden, Netherlands*

Increasing numbers of infections with antibiotic-resistant pathogens, like vancomycin-resistant enterococci or staphylococci, are a major global health problem. To overcome resistance, the semisynthetic glycopeptide derivatives, like the clinically used telavancin, have been developed. These lipoglycopeptides exhibit increased antibacterial potency but additionally raised toxicity concerns. The group of Nathaniel I. Martin, Leiden University, The Netherlands, recently developed semisynthetic lipoglycopeptide antibiotics, which contain a positively charged guanidino moiety and a variable lipid group. These guanidino lipoglycopeptides exhibit a highly increased activity against a variety of Gram-positive bacteria, including vancomycin-resistant strains. Moreover, they exhibited minimal to mild toxicity towards eukaryotic cells suggesting an improved therapeutic safety profile compared to vancomycin. In-depth analysis of the modes of action of the guanidino lipoglycopeptides revealed that they bind the peptidoglycan precursor lipid II-D-Ala-D-Ala with a higher affinity than vancomycin. Additionally, in contrast to vancomycin, they showed a high affinity interaction with lipid II-D-Ala-D-Lac found in resistant strains, providing a rational for the enhanced activity against vancomycin-resistant isolates. According to these findings, guanidino lipoglycopeptides represent promising candidates for further development of antibiotics against clinically relevant multidrug-resistant Gram-positive infections.

van Groesen, E., Mons, E., Kotsogianni, I., Arts, M., Tehrani, Kamaleddin H. M. E., Wade, N., *et al.* (2024) Semisynthetic guanidino lipoglycopeptides with potent *in vitro* and *in vivo* antibacterial activity. *Sci Transl Med* **16** (759).

P-IB-176

Unraveling bacterial-archaeal interactions within the human oral microbiome – insights into the etiology of periodontal disease

*S. D. Akinci[1], A. Gante[1], A. Hechler[2], M. Munz[2], C. von Ohle[2], B. Molitor[1]
[1]*Eberhard Karls University Tübingen, Dept. of Geosciences, Environmental Biotechnology Group, Tübingen, Germany*
[2]*University Hospital of Tübingen, Poliklinik für Zahnerhaltung, Tübingen, Germany*

Methanogenic archaea produce methane as the metabolic end product for energy conservation. Among other methanogenic pathways, the ability to produce methane from CO_2 and H_2 in hydrogenotrophic methanogenesis is the most widespread. The hydrogenotrophic species *Methanobrevibacter oralis* is the predominant methanogen residing in human subgingival plaque. Its prevalence has recently been linked to periodontitis, indicating a pivotal role in oral health and disease. Previous studies yielded evidence of close interactions between *M. oralis* and various bacterial species, as they synergistically participate in the anaerobic degradation of organic compounds. Importantly, this syntrophic cross-feeding between methanogens and hydrogen-producing bacteria, referred to as interspecies hydrogen transfer, is suggested to indirectly promote pathological conditions because it facilitates the growth and colonization of secondary fermenting pathogens. However,

much is still unknown about the complex microbial interplay between *M. oralis* and oral community members that contribute to dysbiosis and, ultimately, to the pathogenesis of periodontitis. A more profound knowledge of the role of *M. oralis* within the oral microbial community is required to gain deeper insight into the etiology and manifestation of this polymicrobial disease.

In this context, we aim to gain information from three different perspectives. First, we will investigate the relationships (mutualism and competitiveness) between *M. oralis* and potential bacterial interaction partners through co-culturing and quantitative PCR (qPCR) techniques. Second, we will employ qPCR to quantify *M. oralis* in patient samples. Third, an additional information layer will be added by quantifying and visualizing *M. oralis* in an established biofilm model using qPCR and FISH. Ultimately, these findings will contribute to a greater understanding of the dynamic interplay between *M. oralis* and other oral microbes, offering valuable perspectives for developing potential therapeutic strategies against periodontal infections.

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P-IB-177

Metabolic adaptation of *Acinetobacter baumannii* to the human host: The kynurenine degradation pathway and its role in virulence

*J. D. Enzmann[1], B. Averhoff[1]

[1]Goethe University, Molecular Microbiology & Bioenergetics, Frankfurt a.M., Germany

Introduction: *Acinetobacter baumannii* is an opportunistic human pathogen responsible for nosocomial infections. The emerging antibiotic resistances but also its viable but not culturable (VBNC) state have contributed to its success[1]. Another factor contributing to the adaptation of the human host is its broad metabolic versatility. *A. baumannii* uses different amino acids, fatty acids, polycyclic compounds and aromatic compounds, that are abundant in the human host, as carbon and energy source[2]. The later include compounds derived from amino acid metabolism of the host, such as kynurenine. Previous studies led to the identification of a gene cluster encoding the kynurenine degradation pathway[3].

Goals: Elucidation of the function of the genes encoding the kynurenine degradation pathway and its role in virulence.

Material & Methods: Mutant studies, growth studies, antibiotic and reactive oxygen species resistance, serum resistance, *Galleria mellonella* and mouse infection studies.

Results: Mutant studies revealed that kynurenine is converted by a kynureninase to anthranilic acid which is oxidized by an anthranilate dioxygenase to catechol which is converted to acetyl-CoA and succinyl-CoA *via* the ß-ketoadipate pathway. Deletion of a potential regulatory gene of the *kyn* gene cluster, *kynR*, abolished growth on kynurenine, indicating that *kynR* encodes a transcriptional activator. A Δ*kyn* mutant was significantly impaired in mice infection, indicating a role of kynurenine degradation in host adaption. This is currently verified by *Galleria mellonella* infection studies and serum resistance analyses.

Summary: Here we report an analyses of the kynurenine degradation pathway in *A. baumannii* and its role in virulence.

P-IB-178

Infection of cornea models with different *Neisseria gonorrhoeae* derivatives to study the importance of the Type IV pilus for bacterial adherence and infection outcome

*L. M. Kafuri Cifuentes[1], N. Knetzger[2], V. Kozjak-Pavlovic[1], C. Lotz[2], T. Rudel[1]

[1]University of Würzburg, Microbiology, Würzburg, Germany

[2]Fraunhofer-Gesellschaft, Translational Center Regenerative Therapies, Würzburg, Germany

Neisseria gonorrhoeae (Ngo) is a human obligate pathogen and is the main cause of *ophthalmia neonatorum*, a type of conjunctivitis that affects newborns, which untreated can lead to blindness (1). One of the most important virulence factors of Ngo is the Type IV pilus, filamentous protein structures that facilitate the adhesion of the bacteria to their target tissue.

In this project, we studied the importance of the Type IV pilus in Ngo infection of cornea tissue models, derived either from cell lines or from primary cells (2). We infected the models with MS11 Ngo derivatives: MS11 F3 (Pili+, RecA+), MS11 N159 (Pili+, RecA-, Adh+cornea) and MS11 N191 (Pili+, RecA-, Adh-cornea). We observed that the bacterial adherence was highest for N159, which could be confirmed using fluorescence microscopy and SEM. However, no significant difference in the tissue integrity between the non-infected and infected models was detected. Cytotoxicity assessment by measuring LDH in the supernatant showed that the highest cytotoxicity was present after 72 h of infection with N159 Ngo. In the primary-cell-derived models the cytotoxicity was comparable between the different derivatives. Measuring of the cytokines showed that IL-8 was the most secreted cytokine; its concentration was the highest in N159-infected cell line-derived models after 72 h, suggesting a specific response that could be possibly pilus-dependent. However, there was no significant difference in the IL-8 secretion between primary-cell derived models infected with F3 and N159 after 72 h. To challenge the pilus relevance, the cell-line derived models were treated with trifluoperazine, a drug that has shown an effect on pilus retraction. We observed a reduction of more than 50% of the bacterial adherence when treated in an early stage of the infection. There was also a reduction of the secretion of IL-8, suggesting a possible decrease also on the severity of the infection outcome. These results show that cornea models can be used for studying Ngo infection, as well as the importance of the functional Type IV pilus and its role not only in bacterial adherence but also in the outcome of the infection.

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P-IB-179

Development of a novel airway model for Aspergillus fumigatus infection research

*T. N. M. Hoang[1], K. Graf[1], M. Raasch[1], K. Rennert[1]
[1]*University Hospital Jena, Jena, Germany*

Invasive pulmonary aspergillosis (IPA), primarily caused by *Aspergillus fumigatus*, is a life-threatening condition in immunocompromised individuals. Patients with respiratory diseases, such as chronic obstructive pulmonary disease (COPD), cystic fibrosis, and severe asthma, are particularly vulnerable due to impaired mucus clearance, which exacerbates IPA susceptibility. However, existing models often fail to accurately mimic human-specific lung responses, limiting our understanding of IPA pathogenesis. Organ-on-chip (OOC) technology offers a promising solution to overcome these limitations.

Building on a previously established proof-of-concept invasive aspergillosis chip model (IAC) [1], we present a human bronchus-on-chip model that faithfully replicates the architecture and function of human bronchial tissue under air-liquid interface conditions, providing a more physiologically relevant platform for studying IPA in obstructed airways.

This model consists of two compartments: a differentiated bronchial epithelial layer and a perfused vascular compartment, separated by a porous membrane. The bronchial epithelium exhibits key cell types, including basal cells, ciliated cells, secretory club cells, and mucus-producing goblet cells, as confirmed by expression of cell-specific markers (MUC5AC, acetylated-α tubulin, and CC10). Quantitative image analysis revealed that the ciliary coverage of the differentiated bronchial epithelium is 60-70%, closely resembling the in vivo condition.

To model *A. fumigatus* conidia transportation in the airway, conidia were introduced to the differentiated epithelium, and their movement was tracked. This bronchus-on-chip model offers a powerful tool to investigate how *A. fumigatus* conidia being transported in the large airway, the pathogenesis of IPA in compromised airways and has potential applications in therapeutic development, including antifungal drug testing and personalized medicine approaches.

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P-IB-180

Overcoming resistance via reactivation – Mode of action of vancomycin conjugates

*A. Abdelrahman[1], E. Mühlberg[2], F. Umstätter[2], K. Ohlsen[3], W. Mier[2], T. Schneider[1], A. Müller[1], P. Uhl[2]
[1]*University Hospital Bonn, Pharmaceutical Microbiology, Bonn, Germany*
[2]*University of Heidelberg, Heidelberg, Germany*
[3]*University of Würzburg, Würzburg, Germany*

Glycopeptide antibiotics (GPAs) are drugs of last resort to treat infections caused by (multi-) resistant Gram-positive pathogens. They inhibit peptidoglycan biosynthesis mainly by binding to the D-alanyl-D-alanine terminus of lipid II peptide stem, thereby blocking transglycosylation and transpeptidation reactions in a sterical manner. Vancomycin (VAN) and teicoplanin (TEIC) represent the prototype GPAs in clinical use today. However, widespread GPA resistance has become a serious threat to global health. Resistance resulting from alteration of the PGN precursor, e. g. by exchange of the terminal D-alanine to D-lactate or D-serine, is conferred by the acquisition of a van-resistance operon. Enterococci, which harbour a vanA or vanB cluster, are most significant in the clinical setting. Research is focused on structural modification of glycopeptide antibiotics to overcome resistance. Lead candidate FU002, a vancomycin-hexa-arginine derivative, with high potency against vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) was generated by conjugation of polycationic peptides to VAN. Cell-based and in vitro assays are used to elucidate the precise mechanism of action to build a rational basis for targeted structure optimization.

P-IB-181

Elucidating structure and function of CE-clan protease-related bacterial pathogenicity factors with dual ubiquitin(-like) protease and acetyltransferase activity

*O. Schmöker[1], B. Girbardt[1], L. M. Mayer[2], K. Schoknecht[3], L. Steil[3], R. Al-Abdulla[4], K. Garz[4], G. J. Palm[1], S. Schulze[1], J. Hoppen[1], V. Boll[5], K. Hofmann[5], V. Kozjak-Pavlovic[2], U. Völker[3], E. Krüger[4], M. Lammers[1]
[1]*University Medicine Greifswald, Synthetic and Structural Biochemistry, Greifswald, Germany*
[2]*University of Würzburg, Microbiology, Würzburg, Germany*
[3]*University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany*
[4]*University Medicine Greifswald, Department for Microbial Physiology and Molecular Biology, Greifswald, Germany*
[5]*University of Cologne, Institute for Genetics, Cologne, Germany*

Regulation of protein function by ubiquitination and acetylation has been investigated extensively and is known to be exploited by pathogens to hijack their host. Especially gram-negative bacteria (e.g. *Yersinia* sp., *Vibrio* sp., *Chlamydia* sp.) encode for effector proteins that are injected into host cells in order to promote infection and ensure bacterial survival. According to the MEROPS database, many of these bacterial virulence factors belong to the CE-clan, an enzyme family mainly comprised of cysteine proteases with deubiquitinase (DUB) or ubiquitin-like protease (ULP) activity, but also acetyltransferases (AcT). Some effectors even exhibit a dual activity as DUB/ULP and AcT. However, the underlying mechanisms regulating their enzymatic activity on a molecular level are often not completely understood and their exact role within the infection process remains elusive.

By using tools from structural biology, biochemistry and cell biology we elucidate the structure-function relationship of a bacterial CE-clan effector in context of host cell infection. The *Chlamydia*-like pathogen *Simkania negevensis* encodes for a CE-clan member called SnCE1. Besides its protease activity as a deubiquitinase and deSUMOylase, we report strong autoacetylation for SnCE1, suggesting a dual specificity similar to *Chlamydia* effector ChlaDUB1. ChlaDUB1 has been reported to carry out both, AcT and DUB activity, by the same active site. Despite structural homology between these two bacterial effectors, X-ray crystallographic analysis reveals differences in variable regions, therefore hinting at alternative mechanistics in SnCE1. Modelling of SnCE1 in complex with acetyl group donor Coenzyme A (CoA) identifies key residues for CoA-binding, supported by mutational studies and mass-spectrometric data. To further elucidate the physiological role

of SnCE1 we set up infection studies in cell culture. Upon infection of HEK293T cells with *Simkania*, SnCE1 localizes to the ER and also mitochondria. The exact pathways targeted by SnCE1 still have to be identified. If there is a potential cross-talk between acetylation and ubiquitination/SUMOylation, as well as an impact of the acetylation status on infection, remains question of our research.

Our studies contribute to a comprehensive understanding of these type of bacterial virulence factors and their role in infection, as it is crucial for our understanding of host-pathogen interactions.

P-IB-199
Bacterial cell wall biosynthesis as target for novel antibiotic compounds
*M. Bingel[1], M. Arts[1], S. De Benedetti[1], T. Schneider[1], A. Müller[1]
[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany*

Antimicrobial resistance represents a major global health threat as the treatment options for bacterial infections caused by (multi-) drug-resistant pathogens are hardly limited. The majority of antibiotics in clinical use today are derivatives of molecules discovered in the early to mid-20th century and novel antibiotics with new modes of action or targets are urgently needed (1). The cell wall biosynthesis pathway was the first one to be utilized for antibiotic intervention and is still the most important target despite the development of resistance (2).

First whole-cell experiments using *B. subtilis*- and *S. aureus*-based bioreporters confirmed interference with cell wall biosynthesis as evidenced by induction of the specific stress response. In depth biochemical analyses led to identification of the molecular target and provided further information on the detailed mode of action.

Phage biology

P-PB-183
Understanding the autonomously replicating plasmid pDolos acting as phage parasite of the Shewanella phage Dolos
*D. Fischer[1]
[1]*Justus Liebig University Giessen, Gießen, Germany*

We recently isolated a novel phage, *Shewanella* phage Dolos, which belongs to the family *Inoviridae.* Phages of this family are characterized by a rather small genome, a filamentous morphology and a chronic-productive life cycle. Accordingly, Dolos genome has a size of about 9 kbp, and its production has little effect on host cell proliferation. We found that phage Dolos uses the type IV MSHA pili of its *Shewanella* host as primary recognition structure to attach to and enter the cell. Of note, together with phage Dolos we isolated a small (3 kbp) rolling circle-replicating (RCR) plasmid called pDolos. In the presence of phage Dolos, pDolos hijacks phage functions to be packaged into phage virions instead of the viral genome. This drastically reduces the virion output of its helper phage. Thus, pDolos acts as a parasitic phage ("phage satellite"). We show that Dolos together with pDolos not only reverses the reduction of the host cell biofilm caused by Dolos alone, but also leads to an increased relative biofilm biomass. Furthermore, pDolos can disseminate genetic material, which, for instance, encode resistance or fluorophores for host cells that are susceptible to Dolos infection. pDolos can be regarded as the first member of a new family of phage satellites, characterized by autonomously replicating plasmids without phage genes that hijack inoviruses. The mechanism is akin to that of phagemids, which have long been used to produce single-stranded DNA with helper phages such as M13.

P-PB-184
The role of phage Thanatos ADP-ribosyl-transferases Alt1 and Alt2 in disarming and preparing the host
*A. Kemmler[1], N. Schmid[1], Z. C. Heinek[1], K. Thormann[1]
[1]*Justus Liebig University Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany*

ADP-ribosyl-transferases (ARTs) are enzymes with various functions that are widespread in prokaryotes, eukaryotes, and viruses. Recent genome analyses reveal that many phages possess genes encoding ARTs. However, to date, only a few functions of ARTs (ModA, ModB, and Alt) from a single phage, the well-known *Escherichia* phage T4, are known. These enzymes NADylate or RNAylate host proteins involved in transcription and translation, thus participating in the reprogramming of the host cell. Here, we explore the role of the two ARTs, Alt1 and Alt2, of *Shewanella* phage Thanatos. Both proteins are present in the fully assembled phage and actively modify target proteins by NADylation immediately after phage infection. Purified proteins also show pronounced self-modification. In the absence of functional ARTs there is an influence on the phage fitness as the number of produced phage Thanatos progeny drops by a level of 10. We have identified some potential target proteins, which include proteins of the central metabolism and also several potential phage defense systems, such as toxin-antitoxin systems or a dNTPase. First results suggest that Thanatos ART activity may, in fact, interfere with the host defense. Moreover, Alt1 can partly restore the infectivity of the *E. coli* T5 phage when it is co-expressed with a dGTPase, an anti-phage defense strategy of bacteria. Therefore, we postulate that Alt1 and Alt2 represent the phage's first line of attack to disarm and prepare the host.

P-PB-185
Bacteriophage infection and matrix processing in a glycan-based biofilm of plant pathogens
*A. Nowicki[1], N. K. Bröker[2], S. Chiantia[3], S. Barbirz[1]
[1]*MSB Medical School Berlin, Berlin, Germany*
[2]*HMU Health and Medical University, Potsdam, Germany*
[3]*University of Potsdam, Potsdam, Germany*

Phages and biofilms are ubiquitous in natural environments, and their interaction has contributed to accelerate the evolution of phages and their bacterial hosts in these particular niches. Biofilms have represented a protective mode of microbial life against harsh environments for millions of years, and it appears that biofilms are also a beneficial trait in pathogenesis, as the majority of chronic infections are caused by cells organized into biofilms. Most bacterial species have the ability to colonize inorganic or biological surfaces and embed themselves in a gel formed by polysaccharides, DNA and proteins they excrete, the extracellular polymeric substance (EPS). EPS regulates the selective transport of molecules and is a diffusion barrier for bacteriophages.

We chose Salmonella P22 podovirus as a model to analyze hydrodynamic behavior of bacteriophages inside biofilms,

using polysaccharide matrices produced by *Pantoea stewartii* and *Erwinia amylovora* as a model for a glycan-based biofilm. To this end, the hindrance factor of the phage as well as polystyrene beads of different sizes were determined using fluorescence microscopy with single particle tracking (SPT) in the polysaccharide biofilm. We describe bacteriophage diffusion in relation to physicochemical properties of the stewartan/amylovoran matrix like polysaccharide concentration, the solvent pH and the tracer properties. In addition, we show that the degradation of stewartan by glycan-depolymerizing enzymes is an important regulatory mechanism that can rapidly modulate particle dynamics within the matrix [1]. In the following, we will link bacteriophage diffusion properties to their host infection dynamics to understand how bacteriophages address their associated pathogens depending on the hydrodynamic properties of viscous biofilms.

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P-PB-186

Expression dynamics of the *Cfr*BI restriction-modification system and its impact on the restriction of phage restriction

*J. Gibhardt[1], A. Kirillov[2], N. Morozova[2], M. Khodorkovskii[2]

[1]University of Hohenheim, FG 190h Molecular Microbiology, Stuttgart, Germany

[2]Peter the Great St. Petersburg Polytechnic University, Laboratory of Molecular Microbiology, Research Center "Nanobiotechnology", Saint Petersburg, Russian Federation

Restriction modification (R-M) systems are one of the most widespread and due their often plasmid-based nature easily transmittable systems bacteria can utilize to protect themselves against infections by bacteriophages (1). The here studied *Cfr*BI R-M system is a type II R-M system and consists of a methyltransferase (MT) and a restriction endonuclease (RE) that are divergently expressed and share a promoter region (2,3). The MT and RE recognize the same DNA sequence, with the MT conferring protection against the RE that can only act on unmethylated DNA. Interestingly, in the promoter region of the *Cfr*BI R-M system a single *Cfr*BI recognition site is present that has been shown to regulate the expression of the system (4,5). Here, we show that the expression dynamics of the *Cfr*b R-M system, as well as its protective properties, depend on the copy number of the plasmid harboring it. The higher the plasmid copy number, the higher the overall expression, but the expression dynamics and MT to RE expression ratios in the background of a medium copy number plasmid interestingly conferred the highest phage resistance. After transformation in naïve cells however, expression of the RE that only occurs after methylation of the *Cfr*BI site in the promoter was fastest in the high copy plasmid background. To conclude, the results indicate that while for protection against phages the overall expression strength might, in case of the *Cfr*BI R-M system, not be the predominant factor, while for the swiftness of establishment of the system the initial expression rate of the MT seems to be the determining factor.

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P-PB-188

The hidden arsenal: A plasmid-encoded defence system confers resistance against the *Bacillus subtilis* phage SPβ

*K. Kohm[1], R. Warneke[2], M. Uhde[1], J. Hoffmann[1], L. Flachsbart[1], B. Schmidt[1], R. Hertel[3], F. M. Commichau[1]

[1]University of Hohenheim, FG 190h Molecular Microbiology, Stuttgart, Germany

[2]TU Berlin, FG Bioanalytic, Institute for Biology, Berlin, Germany

[3]Georg-August University Göttingen, Department of Genomic and Applied Microbiology, Göttingen, Germany

As a natural component of the environment, bacteriophages are omnipresent and outperform their hosts in both diversity and abundance. The constant evolutionary arms race leads to the development of many different antiphage defence systems in bacteria and archaea (1). Currently, over 150 different defence systems have been discovered, with ongoing studies likely to uncover new mechanisms or better characterize known ones (2).

In this study, we present a novel plasmid-derived antiphage defence system from *Bacillus thuringiensis*, which, upon introduction into the prophage-free *Bacillus subtilis* strain TS01 (3), confers resistance against the temperate phage SPβ c2 (4). The mediated resistance is observed by a decrease in phage titre and an alteration in plaque morphology. The genomic region responsible for conferring resistance, designated as the *spbB* locus, ensures the stable segregation of the plasmid in *B. thuringiensis* and *Bacillus subtilis* (5). The resistance is primarily attributed to *spbB* and its adjacent region, encompassing a small noncoding RNA of the skipping-rope type (6) and a subsequent intrinsic terminator (5). In the presence of the *spbB* locus phage propagation or replication is impaired due to cellular death or growth inhibition. This applies not only to phages of the SPβ family but also to *B. subtilis* phages from other genera.

The defence system outlined in this study represents a previously unrecognized category of antiphage defence that shows no homology to any systems previously described. Studying antiphage defence systems provide further knowledge about phages and phage-host interactions. Furthermore, understanding the diverse defence strategies employed by bacteria against phages can aid in the development of novel antimicrobial therapies (7).

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P-PB-189

Catch them if you can: Multicellular strategies of *Streptomyces* for the inactivation of free phage particles

*M. Kabuu[1], L. Kever[1], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*

Viral predation exerted by bacteriophages (or phages) has driven the continual evolution of diverse antiphage defense mechanisms. The majority of the antiphage defense systems described so far act at the cellular level targeting the process of phage infection at various levels[1]. Considering that *Streptomyces spp.* are characterized by a complex developmental lifestyle, which is intricately linked to the production of specialized metabolites and other molecules like proteins, we hypothesize that these multicellular bacteria also evolved strategies aiming at the inactivation of free phages after bursts of infections. This would ultimately protect the younger, more susceptible regions of the mycelium from phage attacks, thereby conferring protection on a multicellular level.

To test this, we screened different *Streptomyces* spent media and observed that some lead to the inactivation and degradation of extracellular phage particles. We further observed that a burst of infection of a *Streptomyces* culture is frequently followed by regrowth of phage-resistant mycelium coinciding with a significant drop in the titer of infectious phage particles. Efforts aiming at dissecting the observed phenomenon underline the importance of mycelial development and the transient secretion of antiphage molecules[2].

Taken together, these results on extracellular phage degradation and the development of phage-resistant mycelium highlight the diverse multicellular strategies[3,4] employed by *Streptomyces* in the defense against their most abundant predator.

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P-PB-190

Outer membrane vesicles as a transport system after bacteriophage contact

*H. M. Monks[1], M. Stephan[2], S. Chiantia[3], N. K. Bröker[4], S. Barbirz[1]
[1]*MSB Medical School Berlin, Medicine, Berlin, Germany*
[2]*Fraunhofer-Gesellschaft, Department of Immunology and Allergology, Berlin, Germany*
[3]*University of Potsdam, Institute of Biochemistry, Potsdam, Germany*
[4]*HMU Health and Medical University, Medicine, Potsdam, Germany*

Bacteria use a variety of defence systems to control bacteriophage infection, amongst others extracelluar vesicles (EVs) [1, 2, 3]. In particular, bacteriophage infection or antibiotic treatment trigger vesicle production [3]. Such EVs demonstrate a proficient capacity to bind and reduce the number of infectious bacteriophage particles within a bacterial population [2]. However, the precise mechanism underlying the interaction between vesicles and bacteriophages is still not fully understood. Outer membrane vesicles (OMVs) of Gram-negative bacteria like *Salmonella* (*S.*) contain a variety of surface receptors used in bacteriophage infection. The tailed *S.* Typhimurium-specific phages P22 (podovirus), 9NA (siphovirus), and Det7 (myovirus) use lipopolysaccharide as a receptor. We have recently shown that phage P22 binds to *S.* Typhimurium OMVs and injects its DNA into the vesicle lumen [2]. Aim of this study is to characterise these "DNA loaded" OMVs and to analyse their properties. We study the change in the surface composition of the OMVs upon phage challenge with emphasis on their LPS composition. We also investigate the fate of bacteriophage particles bound to the OMV surface. For this, we compare different OMVs obtained either from explosive cell lysis or when budded into culture supernatants. We characterise them in complex with phages using dynamic light scattering, fluorescence spectroscopy and electron microscopy.

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P-PB-191

Loss of SPFH-domain protein YdjI renders *B. subtilis* WT168 insensitive to Φ29 phage infection

*A. Mondry[1], S. Shafquat[1], S. Baur[1], U. Repnik[2], M. Bramkamp[2,1]
[1]*Christian-Albrechts-University of Kiel, Institute for General Microbiology, Kiel, Germany*
[2]*Christian-Albrechts-University of Kiel, Central Microscopy, Kiel, Germany*

In their natural habitat bacteria are exposed to a variety of external stresses such as changes in temperature and pH levels, osmotic shock, antibiotics, and phage infection. To maintain viability and fitness, including cell shape and mobility, it is important for bacteria to detect these stress and limit potential damages. The *pspA-ydjGHI* operon in the model organism *Bacillus subtilis* is upregulated under various cell envelope stresses, however, its precise role in the stress response has not yet been fully uncovered [1, 2]. Our previous research showed that the SPFH-domain protein YdjI, encoded in the *pspA-ydjGHI* operon, is necessary for foci formation of the phage-shock protein A (PspA) in *B. subtilis* [2]. This prompted us to further investigate the influence of the *pspA-ydjGHI* operon on phage infection. Surprisingly, deleting *pspA* did not affect phage infection with Φ29, however, we found that deletion of *ydjI* led to significantly reduced phage sensitivity. Furthermore, we observed changes in the localization and oligomerization of YdjI upon phage infection, suggesting an additional regulatory role for YdjI. This hypothesis was supported by differences in RNA levels between the wild type and the *ydjI* deletion strain during phage infection, indicating changes in key regulatory pathways. Further analysis revealed that deletion of motility-related genes belonging to the *degS-degU* regulon caused resistance to Φ29, suggesting the importance of functional motility regulation for phage infection. Overall, our results indicate a connection between the SPFH-domain protein YdjI, motility regulation, and successful phage infection in *B. subtilis* WT168.

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P-PB-192

How holins form holes – Insights based on phage T4 holin characterization

J. M. F. Schwarzkopf[1], *T. Brüser[1]
[1]*Leibniz University Hannover, Microbiology, Hannover, Germany*

Lytic phages hydrolyze the host cell wall at the end of their reproductive cycle to induce lysis and release of their progeny. To do this, they produce endolysins, which hydrolyze the cell wall, and holins, which allow the endolysins access to the cell wall. The mechanism by which the holins permeabilize the cytoplasmic membrane is not clear. However, it is currently assumed, primarily based on studies on phage T4 and lambda systems, that holins form two-dimensional aggregates (rafts) that dissolve large areas of the cytoplasmic membrane at the required time point. Using the phage T4 system as model, we now show that smaller assemblies are sufficient to enable the release of endolysin into the periplasm and that positions of the cytoplasmic domain of the holin and therein especially in the N-terminal amphipathic helix are essential for this. AlphaFold 3 predicts a reasonable ring structure for T4 holin, which is consistent with our data, as the important amphipathic helix generates a hydrophilic hole that could enable a passage of the endolysin. Ring formation and a thereby induced flipping of the amphipathic helix might be a general mechanism for hole formation by holins, which would clarify an old key question of phage cycle biology.

P-PB-193

Exploring the role of the auxiliary metabolic gene cpeT from the cyanophage Syn9 during infection

*M. Fernández Contreras[1]
[1]*Rheinland-Pfälzische Technische Universität, Kaiserslautern, Germany*

Infection of a bacterium by a phage transforms the host cell into a so-called virocell which shifts its normal cellular activities towards producing viral components. During phage infection, cyanobacteria undergo significant changes in host metabolic pathways such as photosynthesis, carbon-, and fatty acid- metabolism. These changes are supported by so called auxiliary metabolic genes (AMGs) encoded in the phage genome. Those host-like genes are thought to improve the host metabolism during the infection cycle. AMGs are frequently found in cyanophage genomes(1,2). We are investigating the role of a particular AMG, the gene cpeT encoding a putative phycobiliprotein lyase from the cyanophage Syn9. Lyases in the host are involved in the assembly of the light harvesting phycobilisome and similar functions are to be expected from the phage copy. A recombinant Syn9 phage lacking the cpeT gene was generated by homologous recombination in the infected host Synechococcus sp. WH8109. Recombinant phage particles were first enriched and subsequently purified. Recombinant phage lysates are assessed for their efficiency in infection, viral particle production and host phycobilisome assembly. Furthermore, complementation of the recombinant phages during infection, protein-protein interactions, and biochemical characterization of CpeT will be performed to demonstrate the specific role and effect of CpeT from Syn 9. All strategies aim to gain insight into the role of phage CpeT during infection and how it shapes the virocell metabolism.

P-PB-194

Isolation of novel bacteriophages against rare actinomycetes

*C. Rolland[1], I. Nouioui[1], S. Peter[1], M. Döppner[1], R. N. Ekici[1], M. Müsken[2], Y. Mast[1], J. Wittmann[1]
[1]*Leibniz Institute DSMZ, Braunschweig, Germany*
[2]*Helmholtz Centre for Infection Research – HZI, Braunschweig, Germany*

Actinomycetes are a heterogenous group of Gram-positive bacteria belonging to the phylum *Actinomycetota*. They constitute one of the largest bacterial phyla and are present in terrestrial and aquatic ecosystems, mainly in soil. Some genera such as *Streptomyces* are well known as a rich source for novel antibiotics, insecticides and other secondary metabolites that could be useful in medicine, biotechnology, and agriculture, which makes them valuable bacteria for research and applications. However, the phylum also includes pathogenic species, the most well-known is *Mycobacterium tuberculosis*, the causative agent of tuberculosis. Other species can induce diseases in humans and animals such as *Nocardia brasiliensis* or *Actinomadura madurae*, which causes Nocardiosis and mycetoma (e.g. madura foot). One approach to control these pathogenic agents might be the use of bacteriophages. For rare actinomycetes, bacteriophages have been used as a selective tool to isolate novel natural product producer rather than as potential therapeutic agents. Our aim was to isolate new bacteriophages against rare actinomycetes from the DSMZ collection as an alternative control agent against infections with pathogenic actinomycetes and/or for selective isolation of specific actinomycetes. A set of 22 phages were isolated against seven different strains of actinomycetes notably three against *Lentzea rhizosphaerae* and one against *Actinomadura meyerae*. The next steps to fully characterize all the newly-obtained phages are to acquire TEM images for each of them, establish their host range and test their endurance, mainly their stability at different pH and temperatures. Another essential step is to sequence their genome and to analyze their characteristics in relation to their host. This work is currently in progress. Morphological characterisation identified the *Lentzea* phages as a siphovirus. They present all a narrow host range and preliminary results about their stability at different pH and temperature conditions point to a high stability. Another interesting phage is the *Actinomadura meyerae* phage vB_AmeS_Stercus. Host range analysis revealed a narrow host range, genomic analysis identified genes of a lysogeny cluster, thus identifying it as a temperate phage. Further experiments are in progress to express the endolysin of vB_AmeS_Stercus as an alternative to fight against *Actinomadura* pathogenic strains (e.g. *A. madurae, A. latina*).

P-PB-195

Siphovirus interactions with the Gram-negative bacterial surface: Perceptions from supported lipid bilayers enriched with lipopolysaccharides

*A. Borges[1,2], H. Pace[3], D. Conca[3], M. Bally[3], N. K. Bröker[1], S. Barbirz[2]
[1]*HMU Health and Medical University, Potsdam, Germany*
[2]*MSB Medical School Berlin, Berlin, Germany*
[3]*Umeå University, Clinical Microbiology, Umeå, Sweden*

The bacteriophage 9NA is a siphovirus infecting *Salmonella enterica* Typhimurium. The 9NA tail baseplate contains tailspike proteins that bind the lipopolysaccharide (LPS) surface using the LPS O-antigen as a receptor. However, the O-antigen alone does not trigger DNA release. In fact, the presence of a complete LPS structure (i.e., lipid A, core, and O-antigen) is essential for both *Salmonella* infection and *in vitro* DNA release. Such observations resulted from fluorescence analyses using either LPS aggregates in solution or outer membrane vesicles to set off DNA ejection. These *in vitro* experiments were performed in bulk and allowed us to hypothesize that the presence of membrane-like structures is sufficient to set in motion conformational rearrangements in the baseplate and to start bacteriophage infection. To test this hypothesis, we employed supported lipid bilayers (SLBs) enriched with different LPS types as defined model membrane platforms to study bacteriophage behavior in the presence of their receptor. Using total internal reflection fluorescence (TIRF) microscopy, we are able to track 9NA single particles as they make contact with the distinct SLBs and investigate the dynamics leading to DNA ejection.

P-PB-196

Viral take-over of host photosynthetic electron transport: Investigating the role of Cyanophage-encoded Plastocyanin

*N. Sekularac[1], M. Fernández Contreras[1], R. Tahan[2], D. Lindell[2], J. C. Soares[3], A. J. Pierik[3], E. P. Patallo[1], S. Zehner[1], N. Frankenberg-Dinkel[1]
[1]*Rheinland-Pfälzische Technische Universität, Microbiology, Kaiserslautern, Germany*
[2]*Technion-Israel Institute of Technology, Biology, Haifa, Israel*
[3]*Rheinland-Pfälzische Technische Universität, Institute of Biochemistry, Kaiserslautern, Germany*

Cyanobacteria perform oxygenic photosynthesis and can be found in almost every habitat on earth. They are infected by specific viruses, cyanophages. During phage infection, key metabolic pathways of the cyanobacterial host (such as photosynthesis, carbon-, and fatty acid- metabolism) are significantly altered. So-called auxiliary metabolic genes (AMGs) encoded in the genome of the infecting phage are believed to contribute to the takeover of the cyanobacterial cell by maintaining the host metabolism during the infection cycle. AMGs are frequently found in cyanophage genomes.[1,2] We are investigating the role of a particular AMG, the plastocyanin-like gene *petE* from the cyanophage Syn9. Plastocyanins are small blue copper proteins that transfer electrons from the cytochrome b_6f complex to photosystem I and are commonly found in most cyanobacteria, algae, and plants.[3]

To verify the functionality of the phage gene, *Syn9petE* was heterologously expressed in *E. coli*. The purified recombinant protein displayed the blue colour typical for plastocyanins and an absorption maximum at ~600 nm. The protein can shift between different redox states. Furthermore, EPR spectroscopy confirmed its identity as a type I copper-binding protein and a midpoint potential of 286 mV was determined by redox titration. In parallel, a recombinant Syn9 phage lacking a functional *petE* was generated. In parallel, a recombinant Syn9 cyanophage lacking a functional copy of *petE* was generated. Recombinant phage particles were first enriched, subsequently purified and assessed for their efficiency in infection and viral particle production.

In summary, our objective is to understand the role of cyanophage-encoded plastocyanin, its importance for phage progeny production and to elucidate how cyanophage proteins interact with host metabolism.

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P-PB-197

RNA stability in T4 phage infected *E. coli*: The dual role of NAD-capping and NudC Decapping

*H. Keuthen[1], K. Höfer[1]
[1]*Max Planck Institute, Marburg, Germany*

Bacteriophages, viruses that infect bacteria, are promising alternatives to antibiotics in fighting multidrug-resistant infections. However, the advancement of phage therapy is hindered by a lack of understanding regarding the molecular mechanisms underlying bacteriophage-mediated hijacking of host gene expression machinery.

Recent discoveries have underscored the importance of RNA modifications, such as NAD caps, which stabilize RNA transcripts and protect them from degradation by nucleases like RNase E. Our lab has established NAD capture sequencing (NAD captureSeq), a novel technology for identifying and quantifying NAD-capped RNAs, to explore their regulatory functions during phage infection [1].

This study examines the role of Nudix hydrolases in modulating RNA stability in *Escherichia coli* during T4 phage infection. We hypothesize that while NAD caps may stabilize phage RNA, Nudix hydrolase-mediated decapping could act as a bacterial defense by destabilizing viral RNA and thereby limiting phage replication [2]. By comparing NAD-capped RNA profiles in *E. coli* strains during infection, we aim to clarify the impact on RNA stability using an integrated approach of NAD captureSeq, transcriptomics, and proteomics.

This project''s outcomes will clarify the role of Nudix-hydrolases in selective RNA decapping during phage infection, providing new insights into how RNA modifications and decapping enzymes contribute to phage-host interactions. Insights from the T4 phage infection model in *E. coli* will also serve as a valuable framework for exploring phage-host interactions more broadly, informing the design of effective phage therapies against resistant bacterial infections and contributing to efforts addressing global health challenges posed by antibiotic resistance.

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P-PB-198
CRISPR-Cas9 shaped viral metagenomes associated with *Brevundimonas goettingensis*
*I. Friedrich[1], V. Beck[1], R. Hertel[1], R. Daniel[1]
[1]Georg-August University Göttingen, Department of Genomic and Applied Microbiology, Göttingen, Germany

The predominant use of dsDNA as the genomic material of bacterial viruses (phages) is supported by many studies. *Brevundimonas goettingensis* was characterized and employed as a viral host system for isolation- and metagenomic-based phage investigation to validate this observation. The host strain was utilized for phage enrichment with water samples from the primary treatment step of a local sewage plant (Göttingen, Germany). We specifically targeted phages containing dsDNA, ssDNA, dsRNA, or ssRNA as genetic material. In total, five *Brevundimonas*-associated dsDNA phages ranging from 62 to 265 kb were isolated. All of them utilized dsDNA as genomic material, isolates with a different genome type were not obtained. Metagenomic data, however, were retrieved from dsDNA- and ssRNA-based viruses. By combining genome data from the obtained isolates with the dsDNA metagenomic data, we demonstrated that even stringent isolation covers, at best, only 70% of potential phages infecting a host present in a sample. However, a CRISPR-Cas9 system with an artificial CRISPR array was established and used to discriminate abundant and well-known *B. goettingensis* phages from a host-based metagenome enrichment. Here, we were able to isolate further five dsDNA phages and showed that the isolation of more phages resulted in a coverage of 96% of potential metagenome-based detected phages infecting a host. In conclusion, we showed that dsDNA phages are the easiest to obtain. We highlighted the need to expand our experimental procedures to obtain isolates with a different genome type by using the CRISPR-Cas9 system.

P-PB-200
Prophage diversity and metabolic signatures of viral induction in groundwater bacterial isolates
*N. Ghanem[1], S. Schroeter[2], F. Borim Corrêa[1], S. Jurburg[1], L. Wick[1], G. Gleixner[2], A. Chatzinotas[1,3,4]
[1]Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany
[2]Max Planck Institute, Molecular Biogeochemistry Group, Jena, Germany
[3]Leipzig University, Biology, Leipzig, Germany
[4]German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

Viral lysis of bacterial hosts results in the release of metabolites and cell lysates that promote energy and material fluxes (viral shunt). Viruses can lyse their host immediately after infection (lytic cycle), or replicate their genome alongside the host genome (lysogenic cycle). The integrated viral genome (i.e. prophage) can be induced to enter the lytic cycle under certain conditions (e.g. environmental stress). While much is known about these processes in marine systems, the role of prophage diversity and virus-driven cell lysis in terrestrial food webs, particularly in groundwater environments, remains less understood.

Our study examines the effects of prophage-host interactions in groundwater, focusing on the release of dissolved organic matter (DOM) following prophage induction and identifying factors that influence prophage distribution and diversity. We used mitomycin C to induce prophages in 146 bacterial isolates from groundwater. Of these, 37 strains had inducible prophages and underwent further analysis. The genomes of inducible strains were sequenced using nanopore technology, while induced phages were quantified by fluorescence microscopy, and the DOM released during induction was analyzed by direct injection mass spectrometry.

We identified 120 prophages across the 37 inducible strains representing 15 bacterial clusters and 58 unique viral clusters. Notably, isolates within the same bacterial cluster did not consistently share phage clusters, suggesting a weak correlation between bacterial relatedness and prophage composition. Phage diversity varied widely, with a positive correlation between the total phage count and phage diversity per isolate. Mass spectrometric analysis of DOM showed consistent metabolic responses across many isolates after induction, revealing 993 distinct molecular species with significant changes. Approximately 39% of these changes were linked to species-specific factors, while 3% were attributable directly to phage induction, highlighting the combined influence of species identity and viral induction on the metabolic landscape. This pool of molecules provides a basis for identifying potential biomarkers of viral lysis and for advancing our understanding of the role of viruses in nutrient cycling and ecosystem dynamics in groundwater environments.

Systems & synthetic microbiology

P-SSM-201
A marker-free plasmid selection system in *E. coli*
*A. K. Berndt[1], C. Schiklenk[2], D. Degreif[2], T. Waldminghaus[1]
[1]TU Darmstadt, Molecular Microbiology, Darmstadt, Germany
[2]Sanofi-Aventis Deutschland GmbH, Frankfurt a.M., Germany

For decades, genes encoding antibiotic resistance have served as selection markers for maintaining plasmids in bacterial cells. However, in an industrial context, challenges such as antibiotic resistance gene transfer, high cost for antibiotics, and metabolic burden imposed by marker gene expression necessitate alternative strategies. In our study, we propose a marker-free plasmid selection system based only on the presence of non-coding RNAs transcribed from the origin of replication. In ColE1 plasmids, two partially complementary RNAs, RNAI and RNAII, regulate plasmid replication. RNAII initiates replication, while the interaction of RNAI with RNAII forming a stable RNA duplex inhibits replication.

We designed a novel system by fusing an antisense RNAI (asRNAI) to an mRNA encoding a repressor that regulates the transcription of an essential gene, both integrated into the *E. coli* chromosome. In absence of a plasmid, the repressor inhibits the essential gene, killing the cell. In presence of a ColE1 plasmid, the ori-encoded RNAI binds to

asRNAI, forming a stable complex that blocks the repressor"s translation, thereby allowing cell survival.

One of the primary challenges in designing asRNAI is ensuring efficient translational repression of the repressor by RNAI without disrupting its translation. To tackle this, we developed a test system with a reporter gene instead of the essential gene. We evaluate 14 different designs, yielding a range of repression efficiencies. Further analysis demonstrates that the system is compatible with various ColE1 plasmids, offering significant flexibility.

P-SSM-202

Advanced workflows for the systematic identification of metabolic optimization targets in DBTL-cycles: A demonstrator for producing aromatic compounds in *C. glutamicum*

*N. Hollmann[1,2], D. Koesters[1,2], J. Gaetgens[1], S. Noack[1], J. Marienhagen[1,2]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*
[2]*RWTH Aachen University, Biotechnology, Aachen, Germany*

The establishment of rational Design - Build - Test – Learn (DBTL) cycles based on modularization, standardization and automation enables a reduction in development times and an increase in reproducibility and effectiveness of microbial strain construction for competitive biomanufacturing.

Here, we present an advanced strain design and analysis workflow to systematically improve the understanding of microbial production pathways and to enable faster identification of metabolic optimization targets. We focus on aromatic compound production in *Corynebacterium glutamicum* with the specific goal to enhance the carbon flux towards the three aromatic amino acids as platform chemicals through the native shikimate pathway.

The rational approach harnesses the power of *in silico* metabolic modelling; modular, high-throughput assembly of synthetic transcription units and parallelized phenotyping of producer strains. The production pathway is screened systematically by episomal balancing of shikimate pathway enzyme levels utilizing *MoClo*-based workflows on our *AutoBioTech* platform [1]. Quantitative and fast characterization of growth and production phenotypes is realized by employing robotic-assisted micro-cultivation experiments with (un)targeted metabolomics and proteomics via LC/ GC-ToF-MS. Quantitative comparison of producer strains is therein enabled utilizing bioprocess modelling (estimation of specific uptake & production rates) with recently developed python tools [2].

Ultimately, a standardized and modular strain construction and analysis toolbox enables the design and analysis of a broad range of production strains and represents an essential component for the operation of biofoundries.

P-SSM-203

Reconstitution of the unusual PE-III phycobiliprotein of *Prochlorococcus marinus* SS120 in *Escherichia coli*

*J. Hackh[1], B. Gu[1], N. Frankenberg-Dinkel[1]
[1]*Rheinland-Pfälzische Technische Universität, Biology, Kaiserslautern, Germany*

The marine cyanobacterium *Prochlorococcus* sp. is known to be the smallest photosynthetic organism on this planet, nonetheless, its sheer abundance makes it ecological one of the most significant cyanobacteria in the ocean. Unlike other cyanobacteria, *Prochlorococcus* has abandoned the effective light harvesting complexes, called phycobilisomes (PBS). Instead, it relies on divinyl-chlorophyll-antenna, harvesting blue light very efficiently. Interestingly, *Prochlorococcus* kept small amounts of a remnant of PBSs in form of a single phycobiliprotein (PBP), phycoerythrin III (PE-III). This PE-III of low-light adapted *P. marinus* SS120 is composed of an α- and β-subunit (SU) and likely carries covalently attached phycoerythrobilin (PEB) and phycourobilin (PUB) chromophores in a 1:3 ratio. Genes required for the assembly of PE-III are encoded in a ~10 kb gene cluster, encoding SUs and five putative PBP lyases for the proper stereochemical attachment of chromophores to apo-PE-III. In addition to the cluster, genes are found encoding biosynthetic enzymes for PEB and phycocyanobilin (PCB). The function of lyases and their role in the assembly of apo-PE-III were investigated by a heterologous *E. coli* expression system. To date, only the function of lyase CpeS was confirmed by this approach, ligating (3Z)-PEB to Cys82 on the β-SU CpeB. The other lyases remained uncharacterized mainly due to protein folding issues. A new approach with genes that replicate the codon usage pattern (harmonization) of the original organism in the heterologous expression host was able to bypass folding problems. With the help of this method, we identified MpeX as the first isomerase-lyase in *Prochlorococcus*, attaching PUB at Cys51/60 on CpeB. Interestingly, MpeX shows only activity in the presence of chaperon-like "helper-lyase" CpeZ and a prior attachment of PEB to Cys82 by CpeS. The remaining lyase CpeT will be used to presumably attach PEB to cysteine residue Cys163 on the β-SU, while CpeY might attach PEB to Cys73 on the α-SU CpeA. Isolated recombinant PBPs are examined by UV-Vis- and fluorescence spectroscopy, Zinc-blot, HPLC and MS to evaluate their composition. The ultimate goal is to reconstitute the whole PE-III in *E. coli* and on the long run, to transplant PE-III into the model cyanobacterium *Synechocystis* sp. PCC 6803 to investigate its light harvesting capabilities in a non-native host.

P-SSM-204

Engineering *E. coli* for H_2 -driven hydroxylation reactions through the heterologous production of hydrogenases and methane monooxygenases

*K. Brunsbach[1], A. C. Ngo[2], R. Rad[3], D. Tischler[2], U. P. Apfel[3], L. Lauterbach[1]
[1]*RWTH Aachen University, Microbiology, Aachen, Germany*
[2]*Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany*
[3]*Ruhr University Bochum, Inorganic Chemistry I, Bochum, Germany*

Introduction: The conversion of the potent greenhouse gas methane to methanol presents a promising method for reducing emissions while producing valuable chemicals. Unlike standard industrial catalysts, soluble methane monooxygenases (sMMO) offer an environmentally friendly alternative by converting methane to methanol under mild conditions[1,2]. *In vivo* systems maintain enzyme stability and activity in a controlled cellular environment, thereby increasing the overall efficiency of the reaction. *E. coli* offers faster growth rates and easier genetic manipulation than methanotrophic organisms, making it a more practical host for bioengineering applications targeting methanol production.

Goals: This study explores using an engineered *E. coli* system for methane-to-methanol conversion by introducing synthetic metabolic pathways alongside a soluble NAD^+-reducing hydrogenase (SH) for cofactor recycling to increase process sustainability[1,3]. A connected zero-gap electrolyzer system with selective permeability decouples mass and electron transfer, allowing precise redox control and facilitate proton exchange without direct contact between the cathode and anode[4].

Materials & Methods: Our engineered *E. coli* features two plasmid integrations encoding 1) SH for H_2-driven NAD^+-reduction and 2) sMMO for methane oxidation. In addition, the adaptable plasmid design allows rapid sMMO mutagenesis to expand the substrate range.

Results: We successfully achieved robust heterologous expression of active SH in *E. coli* under challenging aerobic conditions. We also demonstrated sMMO production in *E. coli*, opening new ways for methane-to-methanol conversion in a highly versatile host system[1].

Summary: We develop a sustainable method to convert methane to methanol in engineered *E. coli*, reducing emissions and creating valuable chemicals. Enhancing sMMO activity and NADH recycling via SH with a zero-gap cell offers a low-energy alternative to traditional methanol production and enables precise redox control for broader electrobiochemical applications.

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P-SSM-205

Utilization of methane through biocatalysts and developed production strains

*C. Tahiraj[1], K. Brunsbach[1], L. Hille[2], M. Macgregor-Fairlie[2], S. Lindner-Mehlich[2], K. Vincent[3], R. Banerjee[4], L. Lauterbach[1]
[1]RWTH Aachen University, Microbiology, Aachen, Germany
[2]Charité Berlin, Institute of Biochemistry, Berlin, Germany
[3]Oxford University, Chemistry, Oxford, United Kingdom
[4]Auburn University, Department of Chemistry and Biochemistry, Auburn, AL

Introduction

Methane (CH_4), the most reduced one-carbon (C1) molecule, is both a potent greenhouse gas and a promising carbon source for biotechnological applications. Methanotrophic bacteria utilize CH_4 as their sole carbon and energy source, however, complex genetics, restricted genomic accessibility, and specific growth requirements have hindered their broader application in CH_4 bioprocesses[1].

Goals

This work aims to advance CH_4 bioconversions by targeting key enzymes in microbial C1 metabolism, particularly soluble methane monooxygenase (sMMO)[2]. A deeper understanding of sMMO-mediated catalysis and interactions with auxiliary proteins (MmoD, MmoE, MmoG) is pursued[3–5]. Finally, the engineering of a synthetically methanotrophic *Escherichia coli* seeks to enhance CH_4-driven biosynthesis of valuable compounds.

Materials & Methods

The sMMO operon from *Methylomonas methanica* MC09 was optimized and rearranged to enable heterologous production of sMMO in *E. coli*. Co-production will be performed, to assess the effect of each auxiliary protein on the production of the hydroxylase module MMOH.

Results

Optimization of the *M. methanica* MC09 sMMO operon enabled successful production of MMOH in *E. coli*, confirmed by Western blot of strep-tagged subunit MmoX in the soluble fraction. Ongoing optimization efforts focus on temperature, chaperone-mediated folding[5], and inducer strength to enhance MMOH production. Additionally, the heterologous production of strep-tagged MmoE in *E. coli* was confirmed by Western Blot.

Summary

Sufficient production and proper folding of sMMO in *E. coli* is essential for bioactivity in CH_4 conversion. Overall, this work contributes to engineering a synthetically methanotrophic *E. coli* that supports CH_4-driven bioprocesses, advancing a sustainable C1 bioeconomy.

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P-SSM-206

Investigation of the autotrophic metabolism in the carboxydotrophic "Knallgas" bacterium *Hydrogenophaga pseudoflava*

*S. Grieshaber[1], A. Ebert[2], P. Kehr[1], B. Ebert[2], E. Marcellin[2], B. Blombach[1]
[1]Technical University Munich, Microbial Biotechnology, Straubing, Germany
[2]University of Queensland, Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia

The sustainable production of chemicals and fuels is becoming increasingly important in reducing greenhouse gas emissions and global pollution. One interesting approach is aerobic gas fermentation, converting C1 building blocks to valuable compounds using carboxydotrophic "Knallgas" bacteria like *Hydrogenophaga pseudoflava*. This Gram-negative β-proteobacterium shows comparable high growth rates on CO, H_2 and CO_2 and mixtures thereof under aerobic conditions. Moreover, a basic genetic engineering toolbox is available, rendering this bacterium an interesting host for biotechnological applications. [1, 2]

To obtain a holistic understanding of the autotrophic metabolism and its regulation, proteomic analyses were conducted under different conditions (fructose, H_2/CO_2, and CO). Additionally, key enzymes involved in gas utilization were deleted, and the resulting effect on growth as well as the overall activities of hydrogenase and CO dehydrogenase were determined.

Cells were harvested in the exponential phase for protein extraction (five biological replicates). Proteins were digested with 1 µg Trypsin and peptides were subjected to LC-MS/MS analysis. DIA data processing was performed with Spectronaut™ in default settings and collected mass spectra searched against the corresponding database (*H. pseudoflava* DSM 1084). Thresholds for comparisons: unique number of peptides ≥ 2, adjusted p-value ≤ 0.05, q-value ≤ 0.01, $|\log_2 FC| \geq 0.585$ (FC ≤ 0.667 and ≥ 1.5).

Applying these parameters, a total of 415 or 1011 proteins showed increased abundance, while 322 or 883 proteins showed decreased abundance when comparing autotrophic growth on H_2/CO_2 or CO gas mixtures to heterotrophic growth with fructose. These findings reflect a significant shift in the cellular proteome, indicating an adaptation towards autotrophic pathways. Additionally, the proteomic data revealed an increased abundance of soluble hydrogenase subunits under CO gas conditions compared to "Knallgas". This finding supports growth and enzyme assay results, suggesting that the soluble hydrogenase in *H. pseudoflava* has a different physiological role than expected.

The data provided new insights into the regulatory mechanisms and adaptive responses of *H. pseudoflava* under varying conditions, highlighting key enzymes involved in its autotrophic metabolism.

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P-SSM-207

Using synthetic yeast chromosomes to map and probe large-scale deletions in high-throughput

*T. Lindeboom[1], M. d. C. Sanchez Olmos[1], D. Schindler[1]

[1]Max Planck Institute, Marburg, Germany

Introduction: The Synthetic Yeast Genome Project (Sc2.0) aims to produce the first synthetic eukaryotic genome.[1,2] All synthetic chromosomes have been synthesized and characterised in individual strains. The sixteen synthetic chromosomes and the tRNA neochromosome are being consolidated in a single cell[DS1] . All chromosomes have been redesigned according to strict principles.[3] One notable feature is the insertion of symmetrical *loxP* sites downstream of almost every non-essential gene. Upon activation of Cre recombinase, this leads to highly complex structural variations, turning a single genotype into a population. Here we present our strategy and technology for using synthetic yeast to systematically study the effects of large-scale deletions, complementing our knowledge of single gene deletion libraries.

Approach: The *loxP* sites are 34 nucleotides in length. We use this as a landing pad to randomly integrate a modified marker by homologous recombination (HR). The resulting population is transformed with a plasmid overexpressing a system to introduce a double-strand break in the marker. The DNA is degraded *in vivo* until HR occurs at *loxP* sites. Counter selection ensures that only candidates that have lost the marker are obtained.

Materials & Methods: We use laboratory automation to select and characterise large numbers of candidates. Based on our high-throughput, low-cost end-point genotyping, we can rapidly identify the deletion size for each strain.[4,5] This is followed by phenotyping of the relevant strains and identification of consistently deleted loci.

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

Summary: Knowledge-based development of synthetic genomes relies on the combinatorial deletions of non-essential genes. Here, a pipeline is presented using synthetic *S. cerevisiae* chromosomes as a platform for investigating large-scale deletions.

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P-SSM-208

Optimization of different membrane-bound dehydrogenases and their expression in *Gluconobacter oxydans*

*N. Kucher[1], L. Pütthoff[1], E. Bieringer[2], A. Zimmermann[2], D. Weuster-Botz[2], W. Liebl[1], A. Ehrenreich[1]

[1]Technical University Munich, Molecular Life Sciences, Freising, Germany

[2]Technical University Munich, Energy and Process Engineering, Garching, Germany

Gluconobacter oxydans has great biotechnological potential due to its ability to incompletely oxidize many sugars, polyols, and related compounds with the preservation of their carbon skeletons. It can be used in fermentations with highly concentrated substrates and tolerates low pH. These oxidations are catalyzed by membrane-bound dehydrogenases (mDHs), with the active site facing toward the periplasm, circumventing the necessity to transport substrates and products. We developed a platform for the functional expression of heterologous mDHs in *G. oxydans* BP9.1, devoid of its native mDHs, thereby increasing the specific activity of the heterologous mDHs and avoiding unwanted side reactions.

This platform was used to produce various chemicals of industrial importance. The membrane-bound glucose dehydrogenase (mGDH) and the membrane-bound polyol dehydrogenase (mSldAB) from *G. oxydans* are responsible for the production of sugar acids (cellobionic acid and galactaric acid) and ketoses (L-xylulose and D-tagatose) respectively. L-xylulose and D-tagatose are valuable in the food industry as low-calorie sweeteners and potential prebiotics, supporting health-conscious consumer trends. Cellobionic- and galactaric acid are important intermediates

for biodegradable materials and pharmaceuticals, promoting sustainable practices in various manufacturing sectors.

To construct a strain that produces these sugar acids and ketoses with high space-time yields, a collection of BP9.1 strains expressing various homologous mGDH and mSldAB enzymes based on sequence and phylogeny was constructed. Additionally, new isolates from environmental samples were utilized as sources of novel mGDH and mSldAB enzymes, expanding our repertoire of biocatalysts. The expression was carried out using both plasmid-based systems and genes integrated into the chromosome of BP9.1.

For further optimization of enzyme activity, random mutagenesis is used to generate diverse enzyme variants, enabling the identification of mutants that enhance activity, specificity, and stability. This approach should increase the likelihood of obtaining improved enzymes for industrial needs. To facilitate the transfer of large libraries of mutated enzyme genes into BP9.1, we optimized and modified the triparental conjugation method to establish a high throughput protocol, significantly accelerating the screening process and enabling efficient selection of the most promising enzyme variants for industrial applications.

P-SSM-209

Engineering of synthetic small RNAs for multi-targeting in *Escherichia coli*

*S. Dittmar[1], R. Melo Palhares[1,2], D. Schindler[2,3], B. Berghoff[4]
[1]Justus Liebig University Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany
[2]Max Planck Institute, Marburg, Germany
[3]Philipps-University Marburg, Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany
[4]Ulm University, Molecular Biology and Biotechnology of Prokaryotes, Ulm, Germany

Synthetic biology is a rapidly evolving field focused on designing and constructing novel biological systems with specific functions. A key emerging area within synthetic biology is the development of synthetic small RNAs (sRNAs). In bacteria, sRNAs typically repress translation of their target mRNAs. Typical sRNAs consist of a seed region and a scaffold region, which are modular elements that can be engineered and combined to create synthetic sRNAs. The seed region is essential for binding and repression of mRNAs, while the scaffold provides structural features, enhancing sRNA stability and facilitating interactions with proteins, such as the RNA chaperone Hfq. Synthetic sRNAs hold significant potential for various applications. To efficiently repress translation, different synthetic seed regions and scaffolds should be evaluated. Furthermore, this study aims at constructing an sRNA array for simultaneous modulation of several phenotypes in *Escherichia coli*. Golden Gate cloning and Modular Cloning (MoClo) were used to assemble seed regions and scaffolds to construct synthetic sRNA expression plasmids. The functionality of synthetic sRNAs was evaluated using phenotypic screens. It was found that the regulatory activity of synthetic sRNAs varies depending on the chosen seed and scaffold regions. The seed region is critical for translational repression, but we also observed that certain scaffolds are more effective for regulation of specific mRNAs. After the initial screening, sRNAs were selected to construct an sRNA array for simultaneous repression of antibiotic resistance, flagella-based motility, and biofilm formation. We predict that the presented multi-targeting approach has numerous potential applications.

P-SSM-210

Expansion of the genetic toolbox for *Corynebacterium glutamicum*: Golden gate cloning and pathway assembly for 1,4-butanediol production

*M. Quack[1], B. Blombach[1,2]
[1]Technical University Munich, Microbial Biotechnology, Straubing, Germany
[2]Technical University Munich, SynBiofoundry@TUM, Straubing, Germany

To facilitate genetic engineering in *Corynebacterium glutamicum*, we expanded the toolbox for the expression and integration of genes and gene clusters. This includes adapting the pEKEx2 and pK19mobsacB vectors for Golden Gate-based cloning, building on the previously established Marburg collection (Stukenberg et al. 2021). The modifications of these two vectors now allow flexible assembly of genes or gene clusters into the pEKEx2 and pK19mobsacB backbones. The toolbox facilitates the assembly of functional transcription units (level 1) by providing a basic selection of promoters, ribosome binding sites (RBSs), and terminators with varying strengths, as well as an inducible promoter (Ptac). Additionally, the expansion provides specific connector modules for the pK19mobsacB vector that enable targeted genomic integration via homologous recombination at designated landing pads. These connectors facilitate seamless integration of single or multiple genes into the genome, with 500-bp homologous regions flanking the inserted DNA on the pK19mobsacB vector. This adaptable system allows for the efficient transfer of genes and gene clusters between vectors. As a demonstration of its utility, the toolbox was used to assemble optimized coding sequences under the control of a set of different promoters, RBSs, and terminators to generate a library of transcription units for a CoA-dependent reduction pathway derived from succinate. These transcription units were then combined into gene clusters (level 2) to form a library of 1,4-butanediol (1,4-BDO) synthetic pathways. The pathways incorporated different enzyme variants that vary in their cofactor requirements, enabling the identification of the most suitable pathway configuration. The episomal expression of these pathways was assessed based on 1,4-BDO production from acetate. The expanded toolbox enables construction and integration of genes and gene clusters in *Corynebacterium glutamicum*, simplifying the genetic manipulation of this organism and further expanding metabolic engineering possibilities.

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P-SSM-211

Gas fermentation with recombinant *Cupriavidus necator* for lactic acid production and PLA production

*H. Fan[1,2], P. Cordero[1,2], L. Lauterbach[1,2]
[1]RWTH Aachen University, Microbiology, Aachen, Germany
[2]RWTH Aachen University, Aachen, Germany

Introduction:

Polylactide (PLA) is recognized for its high mechanical strength, rigidity, effective barrier properties, and moderate thermostability, rendering it a well-suited biopolymer for

applications demanding structural robustness and stability. A critical precursor for PLA synthesis is lactic acid (*L*- or *D*-isomer) and a high optical purity (≥ 99%) is essential to achieving the desired polymer quality[1]. The use of chemolithoautotrophic bacterium *Cupriavidus necator* presents a sustainable approach for lactic acid production through gas fermentation, using CO_2 as the sole carbon source.

Goals:

This study aims to produce optically pure *D*- and *L*-lactate in *C. necator*, and to eventually produce a biopolymer co-polymerized from *L*-lactate with 3-hydroxybutyrate(3HB). Throughout the study, we will assess the potential of gas fermentation as a strategy for biopolymer synthesis, emphasizing its potential to reduce reliance on conventional feedstocks while supporting a circular bioeconomy.

Materials & Methods:

To enable reduction of pyruvate to *D*- or *L*-lactate, a *D*-lactate dehydrogenase (*D*-LDH) from *Leuconostoc mesenteroides* and three *L*-LDHs from *Bos taurus*, *Lactobacillus helveticus*, and *Lactiplantibacillus plantarum* were selected to be produced in *C. necator* H16 and PHB-synthesis deletion strains. The genes responsible for converting *D*-lactate or *L*-lactate into pyruvate were targeted for knockout using a pLO3 plasmid-based double-crossover homologous recombination to prevent reverse conversion and enhance lactate production.

Results:

To date, *D*-LDH from *L. mesenteroides* was heterologously produced in *C. necator* H16 wildtype and PHB-synthesis deletion mutant showing by immunoblot.

Summary:

With the successful heterologously production of *D*-LDH from *L. mesenteroides*, this study presents the potential of *C. necator* for sustainable lactic acid production via gas fermentation. This lays the groundwork for developing *C. necator* as an autotrophic biotechnological platform for PLA and its copolymer synthesis. Further strain engineering is required to maximize the yield while minimizing metabolic burden on the organism.

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P-SSM-212

Engineered living materials for biotechnology: proof-of-principle for metabolic activity in bacterial coacervates

*K. Meyer zu Riemsloh[1], D. Di Iorio[2], G. Otte[2], S. Wegner[2], B. Philipp[1]
[1]University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany
[2]University of Münster, Institute of Physiological Chemistry and Pathobiochemistry, Münster, Germany

Engineered Living Materials (ELM) represent innovative approaches in the design of novel adaptive materials, in which living cells are used to impart biologically active functionalities to manmade synthetic materials. Here, we present Engineered Bacterial Coacervates (EBCs) as a novel hybrid living material for the development of microbial biotechnology platforms. On one hand, polymer-based coacervates are used as membrane-less cages for localizing bacteria in a confined space, allowing a controlled uptake and release of metabolites. On the other hand, the encapsulated engineered living bacteria provide the coacervates with unique ´on demand´ properties that can be finely tuned by genetic engineering. Towards this aim, we report at the encapsulation of several biotechnologically relevant bacteria.

In particular, the polymer composition of coacervates was optimized in order to achieve the formation of stable polymeric compartments together with the successful encapsulation of *Escherichia coli* and *Pseudomonas putida* inside the polymeric matrix. The viability as well as the metabolic activity of the embedded bacteria was also assessed. Results showed that *E. coli* cells preserved their metabolic activity for several hours after the encapsulation, with EBCs being able to express fluorescent proteins. More importantly, we showed the possibility to externally induce the protein expression in time in EBCs with external stimuli such as chemicals or visible light. Particularly, we observed the increasing expression of fluorescent GFP proteins induced by the external addition of IPTG or arabinose, as well as the blue-light induced expression of mCherry in bacteria engineered with the pAurora system. Cells of *P. putida* could also be encapsulated successfully and are currently being investigated regarding their ability to degrade benzoate within the coacervates.

In order to investigate the interaction of bacterial surface structures with the polymeric matrix, we are currently deleting and overexpressing, respectively, the gene for curlin protein CsgA. Such studies will aim to explore the ability of bacteria to produce inside the EBCs extracellular polymeric substances (EPS) which are crucial for biofilm formation/cell aggregation.

P-SSM-213

Investigation of the polyether biosynthetic machinery and its products in *Streptomyces chartreusis*

*M. Ashraf[1], J. Santos-Aberturas[2], A. W. Truman[2], J. E. Bandow[1]
[1]Ruhr University Bochum, Microbiology, Bochum, Germany
[2]John Innes Centre, Molecular Microbiology, Norwich, United Kingdom

S. chartreusis is a gram-positive bacterium that produces a divalent cation ionophore named calcimycin that inhibits the growth of gram-positive bacteria and is a widely used biochemical tool to study calcium signalling. However, of the well-characterized calcimycin biosynthesis gene cluster a few genes, such as *calU1* and *calU2*, remain catalytically uncharacterized [1]. Moreover, in the light of calcimycin transporting manganese and iron and 4-Br-calcimycin transporting copper, we hypothesize that naturally occurring calcimycin derivatives offer altered divalent cation transport properties [2].

We aim to elucidate the catalytic roles of the unknown genes and to generate calcimycin derivatives through mutasynthesis to explore divalent cation transport properties. To this end, knockout mutants of the corresponding gene were generated by Redirect technology [3].

In the *calU1* deletion mutant an intermediate accumulated, suggesting CalU1 to be a putative novel enzyme to close the heterocycle ring to produce the benzoxazole ring of calcimycin. Bioinformatics analysis and literature review indicate that CalU2 is a putative spirocyclase [4]. In a feeding experiment with a 3-hydroxy anthranilic acid producing deletion mutant, a new derivative (m/z: 470.2638 [M+H]+) of calcimycin was identified by LC-MS.

The characterization of the unknown genes will provide a more thorough understanding of the biosynthesis of this class of polyether ionophores that consists of three ring systems, only one of which is synthesized by a PKS. The generation of calcimycin derivatives by modifications at the benzoxazole moiety might expand the capability of this ionophore class as biochemical tools.

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P-SSM-214

SporoBeads: Using the inner and outer coat of *Bacillus subtilis* endospores as a protein displaying platform

*L. Scheufler[1], E. Öztel[1], T. Mascher[1]

[1]Technische Universität Dresden, Dresden, Germany

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 contains 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 using 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 among other candidates for protein auto-immobilization inside the endospore coat.

Fluorescence microscopy was chosen to be the first detection method to determine putative anchor proteins on the isolated spores. All constructs were put under the transcriptional control of the crust gene promoter P*cotYZ* , which is active during sporulation, and ensures a proper timing of fusion protein expression. The reporter gene *sfgfp* was genetically fused to a selection of anchor proteins at both the N- and C-terminus, with a variable set of linker sequences, and subsequently transformed into *B. subtilis*. In addition to the crust protein CotY, which was demonstrated to be an appropriate anchor in previous experiments, new results indicate that CotB, OxdD, CotD and CotP are also suitable for sfGFP display. In line with previous studies, N-terminal fusions generally resulted in a higher fluorescence intensity, which would render this the most promising fusion side for the putative anchor proteins.

The newly acquired knowledge will allow for further development of SporoBeads as a protein displaying platform. The successful display of a laccase fused to the same set of anchor proteins demonstrated the potential for biotechnological applications.

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P-SSM-215

Isolation of pre-magnetic magnetosome membrane vesicles by synthetic affinity tags

*J. Beierl[1], S. Markert[1], F. Mickoleit[1], D. Schüler[1]

[1]University of Bayreuth, Ecological Microbiology, Bayreuth, Germany

Magnetosomes are sensory organelles which are formed by magnetotactic bacteria and consist of magnetite crystals (~ 40 nm) enveloped by a proteinaceous lipid-bilayer membrane. In the alphaprotebacterium *Magnetospirillum gryphiswaldense* magnetosomes are formed by a genetically encoded pathway, which starts with the invagination of empty magnetosome membrane vesicles (eMMs) from the cytoplasmic membrane, probably simultaneous with the targeting of ca. 30 MM-specific proteins, and followed by iron uptake and biomineralization of the magnetite core [1, 2]. While purification and analysis of mature magnetite-containing magnetosomes (magMM) by sedimentation and magnetic separation is relatively straightforward, isolation of pre-magnetic eMMs has proven to be challenging due to their low abundance and the lack of magnetic and density tags. Thus, little is known about the early stages of magnetosome biosynthesis represented by eMMs.

In this study, we assessed if eMMs can be purified using genetically encoded affinity tags. To this end, a multi-adaptor construct was assembled, which comprises multiple tags, molecular adaptors and fluorescence reporters. A MBP/His6-Tag hybrid protein was fused to the abundant magnetosome protein MamG as an eMM anchor, while an eGFP-SpyTag-Twin-StrepTag protein [3] was fused to the MamC protein. Upon expression of the construct in mutant strains of *M. gryphiswaldense* producing eMMs, chromatography of cell extracts using complementary affinity columns yielded conspicuous spherical membranous structures, which were absent in similar preparations from mutant strains expressing the same construct but incapable of eMM formation. Analysis of these vesicles by various imaging and biochemical methods revealed a similar size and appearance, but somewhat distinct protein composition as compared to magMMs.

In summary, our findings seem to indicate that pre-magnetic eMMs vesicles can be isolated. In future approaches, this may set the stage for their use as novel engineerable bacterial membrane vesicles with potential for various applications.

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P-SSM-216

CRISPR/Cas-mediated activation in the social amoeba *D. discoideum* for natural product discovery

*A. Krieger[1], C. Reimer[1], J. E. Kufs[1]
[1]Bielefeld University, Bielefeld, Germany

The discovery of novel natural products is essential for the modern pharmaceutical industry and the development of potent new drugs. The social amoeba *Dictyostelium discoideum* holds significant potential for uncovering bioactive molecules with therapeutic relevance. *D. discoideum* lives in soil and leaf litter, and has a unique life cycle, transforming from a unicellular organism to a multicellular fruiting body with specialized cell types. This life cycle makes *D. discoideum* a valuable model organism for studying biological processes and discovering elusive secondary metabolites. Its haploid genome encodes a diverse array of biosynthetic genes, including up to 45 potential type I polyketide synthase and 11 terpene synthase genes. However, most of these genes are not expressed in the vegetative cell state, limiting natural product discovery in wild-type strains. Thus, innovative approaches to activate these transcriptionally silent genes are crucial for revealing the corresponding secondary metabolites.

In this study, we aim to establish a CRISPR/Cas-based activation (CRISPRa) system for *D. discoideum* to transcriptionally activate biosynthetic genes. Typically, CRISPR/Cas systems include a Cas nuclease, and programmable RNAs that guide the Cas protein to specific genomic loci. Here, we employ a nuclease-deficient Cas9 mutant fused to the potent transactivation domain VP16, previously used for the inducible TetON expression system in amoeba. This construct, together with target-specific guide RNAs, is encoded on a single vector, creating a user-friendly, all-in-one system. As proof of concept, the CRISPRa system will be used to activate the artificial TetON promoter to induce the expression of *gfp* as an easy and fast readout. The promoter possesses three Cas9 sgRNA sites, which occur in seven repeats, allowing the Cas9-VP16 protein to bind at multiple sites simultaneously. The reporter cassette will be expressed extrachromosomally to verify the functionality of the CRISPRa system without the influence of genomic content or chromatin condensation. The system will be further applied to activate native genes that are silent in the vegetative cell state, such as the terpene synthases *tps3* and *tps5*. This CRISPRa approach would enable a rapid, convenient and simultaneous activation of multiple silent biosynthetic genes in *D. discoideum*, enhancing the exploration of their biological functions and biotechnological potential.

P-SSM-217

Race for Iron: Controlling siderophore production in *P. putida via* RGB & UV-light

*S. H. Paik[1], L. Müller[1,2], A. Krüger[3], M. Bund[3], J. Wiechert[3], J. Pietruszka[2,3], D. Kohlheyer[3], J. Frunzke[3], T. Drepper[1]
[1]Heinrich Heine University, Institute of Molecular Enzyme Technology, Jülich, Germany
[2]Heinrich Heine University, Institute of Bioorganic Chemistry, Jülich, Germany
[3]Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany

Microbial communities are an essential component of various global ecosystems and participate in crucial biological processes. The complex social interactions, characterized by intra- and interspecies communication, significantly influence community behavior and composition. In this context, the exchange of various secondary metabolites plays a central role. Notably, siderophores are an important class among these metabolites, due to their central role in microbial iron acquisition. Pyoverdine (PVD), a fluorescent siderophore produced by various *Pseudomonas* species, exemplifies this by mediating vital social interactions and serving as a virulence factor in the infection process of *Pseudomonas aeruginosa* and other pathogenic Pseudomonads. Furthermore, PVD is becoming increasingly important for different biotechnological applications such as plant growth promotion and soil bioremediation.

Despite the depth of research on PVD-related interactions, the spatial and temporal dynamics governing these processes are still poorly understood. To this end, we developed and applied different optogenetic strategies to precisely control PVD synthesis in *Pseudomonas putida* non-invasively and with high spatiotemporal resolution. To achieve this goal, we implemented light-responsive two-component systems and caged-compounds as optogenetic switches for the control of PVD biosynthesis in *P. putida*. This approach allowed a dynamic transition from PVD production to non-production states, and even to overproduction, thereby facilitating detailed analysis of how siderophore-mediated interactions influence community composition over time and space. The reprogrammability of bacterial social behavior was demonstrated by modulating light exposure/intensity conditions of RGB and UV-light to fine-tune PVD synthesis.

The optogenetic switches will thus enable precise control over cellular behavior, offering insights into intra- and inter-species microbial cooperation, competition, and resilience. This approach can thus deepen our understanding of the ecological and pathogenic roles of siderophores and will provide new insights into community dynamics and pathogen-host interactions.

P-SSM-218

Rubisco's interaction with its small subunit: Understanding and engineering allosteric effects

T. J. Erb[1], *F. Otto[1]
[1]Max Planck Institute, Biochemistry and synthetic metabolism, Marburg, Germany

Rubisco, a crucial enzyme in photosynthesis, catalyzes the carboxylation of ribulose 1,5-bisphosphate, incorporating CO_2 into sugars. However, it also reacts with oxygen, producing toxic phosphoglycolate, necessitating energy-intensive photorespiration.[1] Improving Rubisco's specificity without compromising activity is a key research goal. Our approach focuses on enhancing catalytic activity through

protein-protein interactions. We've demonstrated that the small subunit acts as an allosteric modulator, activating silent mutations in the large subunit. We generated AncL, an evolutionarily regressed Rubisco independent of the small subunit but unable to interact with it.[2] By introducing seven substitutions at the large-small subunit interface (AncL+7) and an additional e170N substitution, we observed increased specificity, but only in the presence of the small subunit. This substitution enabled hydrogen bond formation between subunits, doubling specificity compared to AncL without structural changes visible in CryoEM studies.

Based on these results, we aim to develop artificial small subunits that have a similar allosteric effect on the catalytic activity. In addition, we will investigate whether a similar or even improved effect can be achieved by introducing further binders. The goal is to investigate whether not only interactions at the interface of the small and large subunit have an influence on catalysis, but also whether the introduction of further (possibly also staggered) interactions on the large subunit has a positive effect on catalytic activity by inducing allosteric modulation. To this end, the large subunit of Rubisco has already been studied for possible interaction residues based on proteins that are already known to interact with Rubisco.[3],[4]

Novel artificial binders will probe diverse regions of Rubisco's large subunit, enabling targeted allosteric modulation. This approach aims to enhance Rubisco's activity without altering its active site, potentially revolutionizing photosynthetic efficiency and carbon fixation.

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Membranes and transport

P-MT-219
Comprehensive elucidation of glutathione import in *Escherichia coli*

*M. Muskietorz[1], L. R. Knoke[1], L. Kühn[1], L. I. O. Leichert[1]
[1]*Ruhr University Bochum, Microbial Biochemistry, Bochum, Germany*

Glutathione is the major thiol-based antioxidant in a wide variety of biological systems, ranging from bacteria to eukaryotes. As a redox couple, consisting of reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG), it is crucial for the maintenance of the cellular redox balance. Glutathione transport out of and into cellular compartments and the extracellular space is a determinant of the thiol-disulfide redox state of the organelles and bodily fluids in question, but is currently not well understood.

Here we use the genetically-encoded, glutathione-measuring redox probe Grx1-roGFP2 to comprehensively elucidate the import of extracellular glutathione into the cytoplasm of the model organism *Escherichia coli*. The elimination of only two ATP-Binding Cassette (ABC) transporter systems, Gsi and Opp, completely abrogates glutathione import into *E. coli*"s cytoplasm, both in its reduced and oxidized form. The lack of only one of them, Gsi, completely prevents import of oxidized glutathione (GSSG), while the lack of the other, Opp, substantially retards the uptake of reduced glutathione (GSH). Thus, this study provides evidence that Opp is the main transporter for the import of reduced glutathione while Gsi is the only transporter for oxidized glutathione in *E. coli*.

P-MT-220
Influence of the putative lipid bridges YhdP, TamB and YdbH on lipid composition of the cell envelope and outer membrane vesicles of Gram-negative bacteria

*L. Stadler[1], G. Angelidou[2], J. Baldus[1], T. Rath[1], C. Glaubitz[1], K. M. Pos[1]
[1]*Goethe University, Institute of Biochemistry, Frankfurt a.M., Germany*
[2]*Max Planck Institute, Marburg, Germany*

In Gram-negative bacteria, the first line of defence against most hydrophobic antibiotics is the asymmetric outer membrane (OM), comprising of phospholipids in its inner leaflet and lipopolysaccharide (LPS) in the outer leaflet. While the transport of lipopolysaccharides to the outer leaflet of the OM via the MsbA/WzmWzt/Lpt machinery is well-researched, the transport pathways and systems putatively facilitating the movement of phospholipids from the IM to the inner leaflet of the OM were only recently discovered. Three proteins—YhdP, TamB, and YdbH—, while redundant in function, appear to play a crucial role in membrane phospholipid homeostasis. These proteins are postulated to bridge the periplasm, thereby facilitating anterograde lipid transport in an analogous manner to the LPS transport via the Lpt bridge.

Our aim is to study the distribution of lipid species, including alkyl chain lengths and modification for the IM, OM, and OMVs and their dependence of the YhdP, TamB, and YdbH lipid bridges. The different membrane preparations of *E. coli tamB*, *yhdP* and *ydbH* knock-out strains will be subjected to a combination of LC/MS analysis (lipidomics) and ssNMR to verify their role in Phospholipid transport. Initial results indicate a great variety of lipid species with different levels of alkyl chain lengths and modifications in the IM, OM and OMVs.

P-MT-221
Structure of a surface-layer protein covering Anammox cells

*D. Dallemer[1], G. Nuijten[2], L. Van Niftrik[2], K. Parey[1]
[1]*Osnabrück University, Structural biology division, Osnabrück, Germany*
[2]*Radboud University Medical Center, Nijmegen, Netherlands*

The discovery of anammox bacteria in the 1990s changed our understanding of the global nitrogen cycle (1). These extraordinary microorganisms with unusual morphology derive their energy from the oxidation of ammonium coupled with nitrite reduction, which relies on highly toxic intermediates such as hydrazine and nitric oxide (2). Approximately 50% of the dinitrogen gas released is produced by anammox bacteria. In biotechnology, the Anammox process is being used as a sustainable alternative to current wastewater treatment systems for the removal of nitrogen compounds.

We have made significant contributions to elucidating the nature of the catabolic pathway and characterizing the key

soluble enzymes. Central to the harvesting of energy from hydrazine is the hydrazine dehydrogenase complex, which converts hydrazine to dinitrogen gas, releasing four extremely low-potential electrons (-750 mV) (3). In addition, anammox bacteria obtain additional reducing equivalents from the oxidation of nitrite to nitrate, catalyzed by a nitrite oxidoreductase (NXR) (4). Currently, using single-particle cryo-electron microscopy and cryo-electron tomography, we are working on uncovering the complete atomic structure of the Surface-layer protein (SLPs), which form a para-crystalline layer covering the entire anammox cell. SLPs are the most abundant macromolecules in anammox bacteria and play several roles such as membrane scaffolding and external cell protection. In the context of anammox bacteria, we believe that SLPs also play a role in nutrient uptake by sequestering ammonium and nitrite ions inside the cells, which are essential for the anaerobic ammonium oxidation process.

P-MT-222

Contribution of FocA to pH Homeostasis and ATP generation during *E. coli* fermentation

*C. Erdmann[1], M. Kammel[1], G. Sawers[1]
[1]*Martin-Luther-University Halle-Wittenberg, Microbiology, Halle (Saale), Germany*

Introduction: Formic acid is a major product of enterobacterial mixed-acid fermentation. During exponential-phase growth, formic acid passes from the cytoplasm to the periplasm via the pentameric channel, FocA. Re-uptake of formate into the cytoplasm during the late exponential and stationary phases of growth, or upon a decrease in external pH below 6.5, allows its disproportionation into the gaseous products CO2 and H2 by the membrane-associated formate hydrogenlyase (FHL) complex. Uptake of formate by FocA is dependent on an active FHL complex, suggesting coupling between both systems and this depends on conserved histidine (H209) and threonine (T91) residues. A H209N variant of FocA has a serious fermentative growth deficiency caused by to rapid efflux of formic acid from the cell and the inability to take up formate from the growth medium.

Objectives: Our aim was to determine mechanistically the reason for the growth defect of a cell excreting formic acid. This could be due to a deficiency in balancing pH, a loss of cellular carbon or a deficiency in ATP generation.

Methods: We use a formate-responsive reporter system, together with defined *E. coli* mutants to assess changes in various physiological parameters during fermentation, including changes in H2 production, intracellular pH, CO2, and ATP levels.

Results: A cell producing the FocAH209N variant fails to produce H2, is not restricted for heterotrophic CO2 utilization, but generates less ATP than wild type. The intracellular pH also remains at a near-neutral value during growth. Mutants that lack an FHL complex do not exhibit the same phenotype.

Conclusion: Together, our findings suggest that formate production coupled with H2 production are together important in balancing intracellular pH, controlling carbon flow through glycolysis, and thus contribute to optimize energy conservation during mixed-acid fermentation. Restricting carbon flux to formate appears to be important in optimizing ATP generation during fermentation.

P-MT-223

Anaerobic measurement of NADH:ubiquinone oxidoreduction by the NADH sodium pump (Na^+-NQR) from *Vibrio cholerae*

*S. Herdan[1], S. Strobel[1], C. Lindauer[1], J. L. Hau[1], G. Fritz[1], J. Fritz-Steuber[1]
[1]*University of Hohenheim, Cellular Microbiology, Stuttgart, Germany*

The Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR) is a central enzyme in the respiratory chain of many Gram-negative bacteria including pathogens such as *Vibrio cholerae*. Oxidation of NADH and reduction of ubiquinone by Na^+-NQR is usually followed in the presence of oxygen, but this results in the formation of harmful reactive oxygen species and sub-stoichiometric reduction of ubiquinone. Therefore, activity measurements were performed under anaerobic conditions revealing high affinities of Na^+ and ubiquinone towards the Na^+-NQR from *Vibrio cholerae*.

P-MT-224

Elucidating the role of the *Campylobacter jejuni* Mla pathway and the putative lipid transporter CltA for outer membrane homeostasis

*L. Fernandes da Costa[1], T. Rath[2], M. Lazarova[1], W. E. Foong[1], N. Morgner[2], K. M. Pos[1]
[1]*Goethe University, Institute of Biochemistry, Frankfurt a.M., Germany*
[2]*Goethe University, Institute of Physical and Theoretical Chemistry, Frankfurt a.M., Germany*

Gram-negative bacteria can tolerate harsh environments and prevent the entry of toxic compounds in part due to their unique outer membrane bilayer, which contains phospholipids in the inner leaflet and lipopolysaccharide molecules in the outer leaflet. To maintain this penetration barrier function, Gram-negative bacteria have evolved the highly conserved maintenance of lipid asymmetry (Mla) system. In the model organism *Escherichia coli*, this system comprises the outer membrane lipoprotein MlaA, a periplasmic shuttle protein MlaC and the ATP-binding cassette transporter complex MlaFEDB in the inner membrane, which mediate the retrograde transport of mislocalized phospholipids from the outer membrane to the inner membrane. In the Gram-negative bacterium *Campylobacter jejuni*, which is responsible for most foodborne illnesses, MlaA and MlaC are encoded on an operon together with a Resistance-nodulation-cell division (RND) transporter, which was named *Campylobacter* lipid transporter A (CltA). Since the pathways driving anterograde lipid transport are not well understood to date, there is a particular focus on investigating whether CltA could function together with same operon-derived MlaA and MlaC in the anterograde transport of lipids. Our results suggest that *C. jejuni* MlaA complements an *E. coli* mlaA-deficient strain, whereas *C. jejuni* MlaC appears to function independently of the *E. coli* Mla pathway. In addition, *C. jejuni* MlaC exhibits a broad lipid preference as observed by thin-layer chromatography and electrospray ionization mass spectrometry and adopts a dimeric conformation, what is different from monomeric *E. coli* MlaC. With respect to CltA, it has been shown that it confers better growth to a wild-type *E. coli* strain when exposed to antibiotics, which is no longer observed when one of the *E. coli* Mla components is chromosomally deleted.

P-MT-225

Understanding the catalytic mechanism of bacterial phospholipid *N*-methyltransferases

*I. Shevyreva[1], L. S. Fritsch[1], L. M. Koch[1], A. Effing[1], M. Aktas[1], F. Narberhaus[1]

[1]Ruhr University Bochum, Bochum, Germany

Phosphatidylcholine (PC), a typical eukaryotic phospholipid, is an important lipid in some bacteria as well. The amount of PC in bacteria ranges from a few percent of total membrane lipids in *Xanthomonas campestris* to nearly 70% in *Acetobacter aceti*. Notably, in certain pathogenic or symbiotic bacteria, PC plays a crucial role in facilitating interactions with their eukaryotic hosts.

One of the most common PC biosynthesis pathways in bacteria is a three-step *S*-adenosylmethionine (SAM)-dependent methylation of phosphatidylethanolamine (PE) to PC mediated by a single or a combination of multiple phospholipid *N*-methyltransferases (Pmts). Pmts are classified based on their sequence similarities into the *Sinorhizobium* (*S*) and *Rhodobacter* (*R*) types. These enzymes display different substrate preferences regardless of their type. For instance, the *S*-type *Agrobacterium tumefaciens* PmtA (*At*PmtA) catalyzes all three methylations of PE to produce PC, while another *S*-type enzyme, *Rhizobium leguminosarum* PmtS1, only catalyzes the first methylation step. Although *At*PmtA has been extensively studied biochemically, and recent structural insights have been gained about an *R*-type representative from *Rubellimicrobium thermophilum* (*Rt*PmtA), we still lack information about the catalytic mechanism and structural details of *S*-type Pmts.

The main goal of this project is the elucidation and comparative analysis of catalytic mechanisms of *S*-type and *R*-type Pmts by a combination of computational and biochemical research. Despite the low amino acid sequence identity, the two Pmt classes exhibit similar reaction mechanisms based on "proximity and desolvation". However, the number and localization of the key tyrosine residues, essential for activity, vary between the two Pmt types. *Rt*PmtA features two highly conserved tyrosines in the binding pocket, located in the N-terminal αA-helix. In contrast, in the *S*-type *At*PmtA, only one tyrosine, positioned in the protein core, has an impact on the enzyme´s function. This data suggests that while the basic catalytic mechanism is conserved, the specific structural features in the active site of these Pmt types reflect their evolutionary divergence.

P-MT-226

Analysis of MHYT domain proteins and the regulation of enzymatic functions

*N. Bäuerle[1], M. T. Agbadaola[1], J. Rehmel[1], M. Schösser[1], E. Pérez Patallo[1], S. Zehner[1]

[1]Rheinland-Pfälzische Technische Universität, Microbiology, Kaiserslautern, Germany

The MHYT domain is named after its conserved amino acid motif. It has seven transmembrane domains, three of which have the conserved amino acid motif. The transmembrane domains are connected by arginine-rich cytoplasmic and histidine- and tyrosine-rich periplasmic loops. The residues of the conserved methionine, histidine and tyrosine amino acids are thought to bind copper, allowing them to sense diatomic gases. Although the first description of this domain is more than 20 years old, it was only this year that it could be shown experimentally that the MHYT domain is involved in gas perception [1, 2].

This study aims to investigate the heme binding to the MHYT domain and the influence of divalent cations on the modulation of activity of connected enzymatic domains. For this purpose, the genes coding for the MHYT domain proteins CdgB and NbdA were cloned for truncated and full-length variants and produced heterologously in presence of heme and the first precursor of heme, 5-aminolevulinic acid (ALA), in *E. coli*. In addition to the MHYT domain, both proteins have a diguanylate cyclase (DGC) and phosphodiesterase (PDE) domain. The solubilization of the full-length protein from *E. coli* membrane fractions using polymer nanodiscs enabled spectroscopic characterization of heme binding to the protein and activity assays. Absorption spectra of oxidized and reduced NbdA and CdgB revealed characteristic Soret bands for hemoproteins. The cytosolic NbdA variant showed PDE activity while interestingly the activity of the full-length protein was only shown when produced in *E. coli* Nissle 1917 in the presence of heme and iron ions. The cytosolic CdgB variant showed a possible DGC and PDE activity.

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P-MT-227

Enzymatic key players involved in syntrophic conversion of fatty acids into methane

*D. Kosian[1], L. Zhang[2], L. Heidinger[2], F. Krier[1], M. Agne[1], O. Einsle[2], M. Boll[1]

[1]University of Freiburg, Microbiology, Freiburg i.Br., Germany

[2]University of Freiburg, Institute of Biochemistry, Freiburg i.Br., Germany

Microbial methane formation from organic matter plays a major role in the global carbon cycle, contributing to more than half of the annual methane production worldwide[1]. In nature, the conversion of organic substrates into CH_4 and CO_2 is catalyzed by strictly anaerobic, methanogenic archaea in syntrophic association with fermenting bacteria. The latter thereby produce the methanogen"s substrates formate, H_2, CO_2 and acetate from short-chain fatty acids or alcohols. For many years, the endergonic electron transfer from β-oxidation-derived electrons via an electron-transferring flavoprotein (ETF, $E^{0'} \approx -10$ mV) to CO_2 ($E' \approx -290$ mV at formate concentrations < 10 µM) remained hypothetical. In a recent study, we identified a key enzyme in the model organism *Syntrophus aciditrophicus*, a membrane-bound FeS cluster- and heme b-containing ETF:(methyl)menaquinone oxidoreductase (EMO). This enzyme enables the proton motive force-driven reduction of CO_2 via a reverse redox loop between EMO and membrane-bound formate dehydrogenase with methylmenaquinone (MMK, $E^{0'} = -156$ mV) serving as membrane-bound electron carrier[2].

Until recently, structural data of EMO and its interaction partners was lacking. Based on experiments evaluated by blue native PAGE, we observed the formation of a complex between enriched ETF and EMO *in vitro*. Using cryo electron microscopy, we obtained several 3D structures of the EMO-ETF complex and EMO alone, with atomic resolutions down to 2.7 Å. The data suggests the presence of two non-cubane [4Fe−4S] clusters, to date exclusively known from B subunits

of heterodisulfide reductases (Hdr) of methanogenic archaea[3], in the cytoplasm-facing soluble domain of EMO. In addition, we observed several conformational states of the flavin-containing region of ETF while bound to the HdrB-like domain of EMO, providing first insights into the interaction between flavin cofactor and non-cubane [4Fe−4S] clusters. Given the high abundance of *emo* genes in a variety of (M)MK-containing organisms, including the pathogen *Mycobacterium tuberculosis*[2], knowledge of this interaction is essential for understanding the endergonic electron transfer during β-oxidation in these prokaryotes.

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P-MT-228

A back-up multidrug resistance system of *E. coli*: AcrEF-TolC

*P. Y. Yu[1], L. Prinz[1], K. M. Pos[1]

[1]Goethe University, Department of Membrane Transport, Frankfurt a.M., Germany

Multidrug resistance (MDR) poses a severe public health problem worldwide. Bacterial efflux pumps, particularly those in the Resistance Nodulation and Cell Division (RND) superfamily, often confer resistance to multiple classes of antibiotics and play a significant role in clinically relevant antibiotic resistance. RND antibiotic efflux transporters are secondary H+/drug antiporters and mainly function as homotrimers to execute drug extrusion powered by the proton motive force. Of the RND superfamily, AcrB, which forms a tripartite complex with a periplasmic adaptor AcrA and an outer membrane channel TolC, is the major drug efflux system in gram-negative bacteria. Due to its broad ability to transport various classes of antibiotics, several specific inhibitors of AcrB have been developed to tackle bacterial antibiotic resistance.

AcrEF-TolC has recently been identified as a backup system when AcrAB-TolC is dysfunctional in *E. coli*. This tripartite efflux system consists of an RND transporter AcrF, a periplasmic AcrE, and an outer membrane channel, TolC. Genetic studies have revealed that AcrF has 67% identity with AcrB, while AcrE shares 75% identity with AcrA. To date, the detailed structural and functional information of AcrF is still elusive. Here, we employed a combination of in vivo and in vitro assays, including the two-dimensional minimum inhibitory concentration (2D-MIC) assay, nano differential scanning fluorimetry (nanoDSF), and a tryptophan fluorescence quenching assay to investigate the binding affinity of AcrF to different AcrB-specific inhibitors. Furthermore, we reported two cryogenic electron microscopy (cryo-EM) maps of AcrF in detergent and nanodisc at resolutions of 2.9 and 3.4 Å, respectively. By comparing with the structure of AcrB, we further discussed the structural and functional differences between AcrB and AcrF at the molecular level.

Microbial communities

P-MC-229

Tracing active members in microbial communities by BONCAT and click chemistry-based enrichment of newly synthesized proteins

*P. Hellwig[1], D. Kautzner[2], R. Heyer[2,3], A. Dittrich[4], D. Wibberg[2,5], T. Busche[2,6], A. Winkler[2,6], U. Reichl[1,7], D. Benndorf[1,7,8]

[1]Otto von Guericke University Magdeburg, Bioprocess engineering, Magdeburg, Germany

[2]Bielefeld University, Bielefeld, Germany

[3]Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany

[4]Otto von Guericke University Magdeburg, Systems Biology, Magdeburg, Germany

[5]Forschungszentrum Jülich GmbH, Jülich, Germany

[6]Bielefeld University, Genetics of Prokaryotes, Faculty of Biology and Center for Biotechnology (CeBiTec), Bielefeld, Germany

[7]Max Planck Institute, Bioprocess engineering, Magdeburg, Germany

[8]Anhalt University of Applied Sciences, Koethen, Germany

Motivation and aims

Understanding microbial community (MC) dynamics is key to advancing environmental microbiology, human health, and biotechnology. Metaproteomics analyzes all proteins in an MC, providing taxonomic and functional insights. Microbial activity and adaptation involve the synthesis of new proteins (nP), but differentiating nP from bulk proteins is challenging. Applying bioorthogonal non-canonical amino acid tagging (BONCAT) with click chemistry has demonstrated efficacy in enriching nP in pure cultures. However, transferring this technique to MC has been proven challenging due to inefficient BONCAT processes, resulting in low nP yield and high contamination from non-labeled bulk proteins. To address this issue, a new workflow to enrich nP from complex MC was developed. Initially tested with *E. coli* (labeled) and yeast (unlabeled control), the workflow was applied to an anaerobic MC from a laboratory biogas reactor.

Methods

After shifting from glucose to lactose, *E. coli* proteins were labeled with 4-Azido-L-homoalanine (AHA) and mixed with unlabeled yeast. AHA-labeled proteins were biotinylated via click chemistry, enriched using magnetic streptavidin beads, digested, and analyzed using LC-MS/MS. Protein identification was performed with MASCOT and MetaProteomAnalyzer. The workflow was then applied to an anaerobic MC shifted from glucose to ethanol, labeling the nP with AHA.

Results and discussion

Using breakable biotin linkers, amino acid bead blocking, and optimized washing resulted in specific enrichment of nP from *E. coli* (555 *E. coli*, 21 yeast in enriched sample versus 885 *E. coli*, 590 yeast in non-enriched sample). In the unlabeled control, only 7 proteins were detected. Applying the optimized workflow to the anaerobic MC identified 441 metaproteins, compared to 12 in the non-labeled control. Identifying a bifunctional alcohol dehydrogenase and syntrophic interactions between an ethanol-utilizing bacterium and two methanogens (hydrogenotrophic and acetoclastic) demonstrates the potential of metaproteomics targeting nP to trace microbial activity in complex MC.

P-MC-230

Functional responses of *Segatella bryantii* in the rumen environment

*J. Fritz-Steuber[1], J. Seifert[1]
[1]*University of Hohenheim, Biology, Stuttgart, Germany*

Prevotellacea is a predominant family in the rumen microbiome, there they are important for non-cellulose polysaccharide and protein degradation. Because of their metabolic activities, Prevotellaceae maintain the redox potential in the rumen and balance the rumen microbial activities. *Segatella bryantii* (formerly named *Prevotella bryantii*) can be used as a typical model organism and we showed that growth on glucose in a synthetic, defined medium resulted in the formation of succinate as major product [1, 2]. The energy conservation is driven by an electron transport chain including Na^+-translocating NADH:quinone oxidoreductase (NQR) and the quinol:fumarate oxidoreductase (QFR) which are assembled into a membrane-bound supercomplex participating in the build-up of a sodium-motive force [1]. In a healthy animal, short chain fatty acid (SCFA) levels in the rumen are counterbalanced by the uptake of these metabolites by the cow. Under high energy demand (e.g. lactation), SCFA production by the rumen microbiota is limited, eventually leading to ketosis in dairy cows. The ionophore monensin is used to treat such imbalances and disease patterns. Monensin has a strong impact on the microbial population in the rumen, leading to a shift in carbon balance towards increased succinate and propionate formation and decreased methane production [3]. The resilience of *S. bryantii* against monensin was approved by an enhanced NQR activity as well as an active detachment of the extracellular polysaccharide layer and an increased abundance of iron uptake related proteins. During monensin challenge, we also observed a change of abundance of metalloproteases [4]. This opens new questions about the importance of metal requirements and availabilities for an effective bacterial metabolism in the rumen such as how proteolysis and energy conservation dependent on metal availability.

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P-MC-231

RNA-Based characterization of metabolically active microbial communities during beef slaughtering

*M. S. Chaughtai[1], F. F. Roch[1], S. Thalguter[1], B. Conrady[2], M. Dzieciol[1], M. Wagner[1], E. Selberherr[1]
[1]*University of Vienna, Clinical Department for Farm Animals and Food System Science, Centre for Food Science and Veterinary Public Health, Vienna, Austria*
[2]*University of Copenhagen, Department of Veterinary and Animal Sciences, Animal Welfare and Disease Control, København, Denmark*

Microbiological contamination on beef carcasses not only shortens shelf-life but also poses significant risks to food safety. RNA-based analysis offers an accurate detection of metabolically active microbes. In this study, we investigated the metabolically active portion of the microbiota at key processing stages (skinning, splitting, chilling) in an Austrian slaughterhouse. A total of nine surface samples were collected from the carcasses during the skinning and chilling stages, while ten samples were collected during the splitting stage, all preserved using RNAlater. Total RNA was extracted, converted to cDNA, and subjected to 16S rRNA gene sequencing (Illumina MiSeq). Analyses were performed with QIIME 2, R and DESeq2. While alpha diversity did not change significantly during slaughter, PERMANOVA analysis identified the processing step as a significant factor influencing sample variance. The genera *Pseudomonas*, *Serratia* and *Escherichia-Shigella* were highly abundant during the skinning and chilling stages but showed comparatively lower abundance during carcass splitting. Among the Firmicutes, *Staphylococcus* was more prevalent during skinning while *Mycoplasma* exhibited higher abundance during both the splitting and chilling stages. The relative abundance of *Corynebacterium* was higher during both the skinning and splitting stages, while *Bifidobacterium* was more prevalent during skinning compared to the other stages. Notably, there were substantial differences in ASVs representing *Gulbenkiania* and *Exiguobacterium* between the skinning and carcass splitting stages, as well as between skinning and the chilling stages. In contrast, a higher expression of ASVs associated with *Halomonas* and *Bacillus* was observed at the chilling station compared to the skinning station. The high activity of *Pseudomonas*, a known psychrotrophic spoilage organism, during skinning and chilling highlights its role in cold storage spoilage, while *Serratia* and *Escherichia-Shigella* are indicators of faecal contamination and hygiene lapses. The increased abundance of active *Mycoplasma* suggests its resilience in low-nutrient and stressed environments, potentially contributing to biofilm formation. Variations in ASVs for *Gulbenkiania* and *Halomonas* suggest their involvement in oxidative stress response and biofilm development, which could influence meat quality and contribute to microbial persistence during the chilling process.

P-MC-233

Engineering cyanobacteria as the basis for a synthetic microbial community

*D. Hasenklever[1], T. Berwanger[2], J. C. Pohlentz[2], T. Hassan[3], K. Schipper[2], A. Matuszy'nska[3], I. M. Axmann[1]
[1]*Heinrich Heine University, Institute for Synthetic Microbiology, Düsseldorf, Germany*
[2]*Heinrich Heine University, Microbiology, Düsseldorf, Germany*
[3]*RWTH Aachen University, Computational Life Science, Aachen, Germany*

In nature, different microorganisms form highly complex communities in which every species has a distinct role. To increase our understanding of the complex structures and interactions within microbial communities and the role of each microbial partner, the design and analysis of simplified microbial model communities is necessary.

In this collaborative project, our goal is the *de novo* design of a synthetic microbial cross-kingdom community based on the well characterized and genetically amenable model organisms representing cyanobacteria (*Synechocystis* sp. PCC 6803, or *Synechococcus elongatus* PCC 7942), ascomycete (*Saccharomyces cerevisiae*) and basidiomycete fungi (*Ustilago maydis*).

Co-cultivation is based on the carbon source sucrose, which is produced by the phototrophic cyanobacterium using light and carbon dioxide. Sucrose secretion into the culture medium is achieved by inducible, heterologous expression of a sucrose permease gene in the cyanobacteria. This

approach is often combined with salt stress or metabolic pathway engineering to increase sucrose production and secretion.

An important aspect for creating a synthetic community is to design and establish tools for the formation of stable co-cultures and the analysis/quantification of the microbial partners of the community. Thus, in this part of the project, we test different cultivation devices and photobioreactor setups for online monitoring of co-cultures as well as single-cell flow cytometry analysis for quantification of individual populations within the co-culture. Another important aspect for the formation of a synthetic microbial community is the characterization of the optimal cultivation conditions for cyanobacterial sucrose production and simultaneous growth of all co-culture partners. In order to track the carbon source sucrose and other important metabolites within the co-culture, we also intend to establish biosensors in cyanobacteria.

Exploiting these established tools, we will create a stable synthetic microbial community which will then be further used to characterize the nutrient exchange in microbial consortia with a special focus on carbon economics and logistics.

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – SFB1535 - Project ID 458090666.

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Major Research Instrumentation INST 208/808-1.

P-MC-234

Microgels – A game changer in microfluidic single-cell analysis

*M. Bund[1], A. Krüger[1], S. H. Paik[2], J. Frunzke[1], T. Drepper[2], D. Kohlheyer[1,2]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*
[2]*HHU Düsseldorf at Forschungszentrum Jülich GmbH, IMET, Jülich, Germany*

Spatial bursts in nutrient availability, such as carbon or iron sources, are key drivers of spatiotemporal dynamics in microbial communities. Consequently, the position of individual cells and the resulting neighborhood effects ultimately have a significant impact on cellular growth outcomes. To date, these effects can be studied in a static environment using microfluidic single cell analysis. However, the natural environment of cells is dynamic. Therefore, complex microfluidic environments are required to mimic natural cell behavior. Dynamic control of environmental conditions, such as nutrient concentrations, allows more accurate replication and observation of physiological conditions. Structured environments within a microfluidic chip are therefore urgently needed to understand cellular behavior and interactions, such as iron homeostasis. Therefore, we developed a new approach allowing the integration of microgels inside of the microfluidic growth chambers, whose tunable structure can be used to incorporate and release nutrients and minerals such as iron and glucose in respond to different stimuli (e.g. microgel degrading enzymes), providing a versatile platform for structured microfluidic environments.

P-MC-235

Exploring microbial dynamics of Polymicrobial biofilms on Bilioenteric catheters

*A. Baborski[1], L. Rathnow[1], C. Schaudinn[2], O. Rohland[3], R. Allen[1], A. Busch[1]
[1]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*
[2]*Robert Koch Institute, Advanced Light and Electron Microscopy ZBS4, Berlin, Germany*
[3]*University Hospital Jena, Department of General, Visceral and Vascular Surgery, Jena, Germany*

During liver transplantations gut microbiota may infiltrate the biliary system. This can lead to postoperative polymicrobial infections and thus cholangitis, suspected to result from bacterial colonization of bilioenteric catheters as well as by immunosuppressive treatment. Cholangitis can lead to systemic, life-threatening infections such as sepsis or organ failure. Therefore, understanding the dynamics of polymicrobial biofilms on bilioenteric catheter is of high importance.

The most prevalent families of microorganisms found in biofilms on biliary catheters are gram-positive *Enterococcaceae* and gram-negative *Enterobacteriaceae. Enterococcaceae* are less abundant than *Enterobacteriaceae* in the biofilms but have an equivalent tendency to be isolated from cholangitis patients – suggesting that potentially pathogenic *Enterococcaceae* may be sustained within mixed bilioenteric catheter biofilms by interactions with *Enterobacteriaceae.*

This study therefore aimed to elucidate the inter- and intraspecies communication between representative isolates of *Enterococcaceae* and *Enterobacteriaceae* isolated from bilioenteric catheters, in a standardized *in vitro* system. We investigate spatial and temporal changes in biofilm structure and composition using microscopy and viability staining, colony forming units (CFU) counting and 16S rRNA gene sequencing for microbiome analysis. We determined how the **microbial dynamics of polymicrobial interaction changed** between *Enterococcaceae* and *Enterobacteriaceae* comparing planktonic to biofilm growth on various catheter materials. Additionally, we investigated the influence of intrinsic factors such as antibiotic resistance genes or external factors such as bile acids and antibiotics on these dynamics. Furthermore, we use bioinformatical tools to explore **the microbial dynamics of polymicrobial interaction** *Enterobacteriaceae* and *Enterococcaceae* at a genetic level in clinical samples and reconstituted lab microbiomes.

This multifaceted approach aims to provide insight into the mechanisms of polymicrobial biofilm formation in the biliary system and identify potential interventions to mitigate postoperative complications in patients.

P-MC-236

The close-knit association between endosymbiont and host glycosomes in *Angomonas deanei*

*N. Kuenzel[1], E. Nowack[1]
[1]*Heinrich Heine University, Institute of Microbial Cell Biology, Düsseldorf, Germany*

Critical for the origin and diversification of eukaryotic life has been the evolution of endosymbiosis-derived organelles (mitochondria and plastids), which originated from endosymbiotic events occurring more than one billion years ago. To understand which early steps led free-living bacteria

to be fully integrated into the cellular networks of their host cells - a process called organellogenesis - we use the model trypanosomatid *Angomonas deanei* which acquired its single β-proteobacterial endosymbiont (ES) more recently. *A. deanei* harbors a single ES per host cell which is achieved by synchronized cell cycles of host and ES. This synchronization is likely host-controlled and mediated by so-called ETPs (ES-targeted proteins) which are nucleus-encoded but localize to specific ES structures including the division site. The host-ES integration is further marked by a metabolic exchange between the eukaryotic host and its bacterial ES. Genomic analyses revealed highly complementary sets of enzymes encoded by host and ES which suggest that the host is supplied with metabolites like heme and riboflavin by the ES which in turn depends on its host to synthesize certain amino acids. Interestingly, the OCD (ornithine cyclodeaminase), which is the only known nuclear-encoded protein in *A. deanei* resulting from endosymbiotic gene transfer (EGT), localizes to host glycosomes. These specialized peroxisomes, which contain the enzymes for the first few steps of glycolysis, are tightly associated with the ES. Localized in host glycosomes, the OCD protein converts ornithine to proline, which is likely consumed by the physically close, proline-auxotrophic ES. Therefore, apparently, host glycosomes became specialized to support ES integration. To further assess how the metabolic capacity of the glycosome has been recalibrated to aid the metabolic integration of the ES we analyse the glycosomal proteome to identify complementary metabolic pathways and potentially exchanged metabolites. Selected pathways will be further described and analyzed regarding their role in host-ES integration. To understand if/how the exchange of metabolites is facilitated by the close spatial positioning of ES and glycosomes we aim to investigate the ES-glycosome interface in greater detail and experimentally break the ES-glycosome association. With these analyses, we will gain greater insights into the first steps taken in the evolution from endosymbiont to organelle.

P-MC-237

Establishment of a conditional knockout system in the endosymbiont-harboring trypanosomatid *Angomonas deanei*

*L. Kröninger[1], Z. Kim[1], E. Nowack[1]
[1]Heinrich Heine University, Institute of Microbial Cell Biology, Düsseldorf, Germany

Introduction: Mitochondria and plastids originate from endosymbiotic events more than one billion years ago in which free-living bacteria were taken up by a host cell and subsequently evolved into organelles that are now fully integrated into the cellular networks of the host. Since the acquisition and evolution of these organelles date far back in time, we use the trypanosomatid *Angomonas deanei,* which acquired a bacterial endosymbiont (ES) more recently, to study early steps of endosymbiosis. *A. deanei* harbors a single β-proteobacterial ES whose cell cycle is synchronized with and likely controlled by the host by so-called endosymbiont-targeted proteins (ETPs) that are encoded by the host but transported to the ES.

Goal: Preliminary work revealed that homozygous deletions of several ETP-encoding genes cannot be obtained, suggesting that these genes are essential for survival of the symbiosis. Thus, to study how the ETPs effect the host-ES interaction, we aimed to develop genetic tools to generate inducible homozygous knockout (or knockdown) mutants.

Methods & results: To this end, we generated an *A. deanei* strain that produces the T7 RNA polymerase (T7RNAP) from a highly expressed locus. To avoid interference with the endogenous expression machinery we identified at least seven potentially silent loci that appeared suitable for transgene insertion and were expected to be transcriptionally inactive under native conditions. Using a blasticidin resistance cassette with T7 promoter as a reporter gene in the T7RNAP background, we demonstrated T7RNAP-dependent gene expression from one of these silent loci. Finally, implementation of the transcriptional regulator TetR enabled us to induce expression in a ligand-depended manner. Furthermore, we are exploring the integration of a blue light-inducible T7RNAP in *A. deanei*.

Summary: *A. deanei* is an emerging model system to study early steps of organellogenesis. The conditional knockout system, that enables now inducible perturbations of the system, will be key for the in-depth exploration of the host-ES interaction, and, in particular, to observe effects of ETP losses.

P-MC-238

The role of ferritin-like proteins in the microbial iron metabolic network

*B. Lückel[1], M. Baumgart[1], M. Bott[1]
[1]Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany

Introduction: Iron (Fe) is an essential element for bacterial metabolism involved in numerous processes like DNA synthesis, gene regulation, or respiration [1]. Many enzymes require iron for their catalytic activity. It is bound, for example, in the form of heme groups or iron-sulfur clusters. Since Fe2+ causes oxidative stress through the Fenton reaction, bacteria must keep the concentration of free Fe2+ in the cell low. Therefore, many bacteria employ iron storage proteins (ISPs) such as ferritin (Ftn), bacterioferritin (Bfr), or Dps (DNA-binding proteins from starved cells), to oxidize and store iron in the non-toxic Fe3+ state [1].

Objectives: Since ISPs play a role in protecting cells from oxidative damage through the Fenton mechanism, the response of *Corynebacterium glutamicum* wild type (WT) and mutants lacking ISPs [2] to oxidative stress should be assessed using a disc diffusion assay. Furthermore, we are interested in elucidating the significance of ISPs in the race for iron in microbial communities.

Materials & Methods: The sensitivity of *C. glutamicum* WT, *ftn*, *dps* and *ftn-dps* deletion strains to H2O2 was tested by disc diffusion assays. For competitive growth experiments *C. glutamicum* WT, Δ*ftn*, Δ*dps and* Δ*ftn*Δ*dps* mutants were equipped either with mVenus or with E2-Crimson. Cells of WT and mutant strains were co-cultivated in glucose minimal medium and serially transferred to fresh medium. After each transfer, cells were analyzed by flow cytometry to determine the ratio of mVenus- and E2-Crimson-labelled cells.

Results and Summary: The loss of ISPs increased the sensitivity to oxidative stress with the Δ*ftn*Δ*dps* mutant being most susceptible to H_2O_2-triggered growth inhibition. Competitive co-cultivations under iron limitation revealed a significant disadvantage for the ferritin deletion mutant. After three transfers, the Δ*ftn* mutant was outcompeted by the wild type. The same behavior was observed for the Δ*ftn*Δ*dps* mutant, but not for the Δ*dps* mutant, which was not outcompeted by the wild type, suggesting that Dps is not

essential under iron scarcity as long as ferritin is present. These results demonstrate the critical role of Ftn for coping with oxidative stress and for survival under iron limitation in natural habitats with numerous competitors.

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P-MC-239

Polyamine cross-feed interactions in microbial gut communities

*S. Beck[1], K. Jung[1]

[1]*Ludwig Maximilians University, Microbiology, Martinsried, Germany*

In the complex milieu of the human gut microbiome, understanding the intricate interactions among microbial communities is essential for unraveling the dynamics that govern health and disease. In our study, we analyze the cross-feeding of polyamines by utilizing gut community models including the Oligo-Mouse-Microbiota (Oligo-MM^{12}) and the commensal *Escherichia coli* Mt1B1 (leading to Oligo-MM^{13})[1,2]. Thereby, we investigate how *E. coli* contributes to the acid resistance capability of the community. For their cultivation, under single-species and community cultivation conditions, we use high-performance liquid chromatography to analyze polyamine secretion to identify the key player in the polyamine cross-feeding. Thereby we found *E. coli* Mt1B1 to be the main producer of cadaverine and putrescine. Since the metabolic pathways leading to the production of these polyamines also facilitate acid resistance systems[3,4], we hypothesize that *E. coli"s* addition to the community contribute to its acid resistance capability. Additionally, we reveal a binary genotypic system in the putrescine synthesis pathway across different *E. coli* strains, where one variant exhibits higher ornithine decarboxylase (SpeF) activity, resulting in enhanced putrescine production. These insights underscore the crucial role of polyamine cross-feeding in modulating microbial resilience and interactions, offering new perspectives on gut microbiome stability and its potential therapeutic applications.

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P-MC-240

Towards a circular economy through microbial stability and in situ cultivation in a photobioreactor - chicken coop system

*M. Velaz Martín[1], L. Meisch[1], A. K. Kaster[2], K. S. Rabe[1], C. M. Niemeyer[1]

[1]*Karlsruhe Institute of Technology (KIT), Institut of Biological Interfaces - IBG1, Karlsruhe, Germany*

[2]*Karlsruhe Institute of Technology (KIT), Institut of Biological Interfaces – IBG5, Karlsruhe, Germany*

Finding sustainable systems that minimize waste and maximize resource efficiency especially in agriculture is a major challenge for circular economy.[1] In this context, biological systems such as photobioreactors offer an attractive way to convert under-utilized nutrient streams such as CO_2 into useful biomass products, such as photosynthetic microorganisms (PMOs) like *Spirulina* (Cyanobacteriota). In this context, we investigated microbial interactions and community stability in an integrated photobioreactor-chicken coop system, where waste and nutrients were retained, to enable closed loop operations.[2]

First analysis demonstrated the impact of chicken house exhaust on the cyanobacterial culture via CO_2 fixation and provided insights into NH_3 metabolism. Taxonomic classification of the microbial community suspended in the photobioreactor revealed a predominance of bacterial organisms before aeration, with a notable shift to a more diverse community, including eukaryotic species, after exposure to chicken coop exhaust.

In perspective we aim to integrate a novel macroporous material into the system to allow direct monitoring of microbial dynamics.[3] This could provide further valuable insights into microbial interactions in this particular context and allow us to directly assess community stability. Understanding community stability is essential to run integrated, closed-loop biological production systems. It illustrates how microbiome research can contribute to circular economy and serves as a precursor for possible integration into biological systems for resource efficient and sustainable solutions.

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P-MC-241

Does the vitamin B_2 analogue roseoflavin have the potential to influence the composition of the oral microbiome?

*N. Schwendenmann[1], B. Fritz[1], L. Steiner[1], S. Burger[1], L. Seufert[2], M. Mack[2], M. Egert[1]

[1]*Furtwangen University of Applied Sciences, Institute of Precision Medicine, Villingen-Schwenningen, Germany*

[2]*Mannheim University of Applied Sciences, Institute of Technical Microbiology, Mannheim, Germany*

Streptococcus mutans is auxotrophic for riboflavin (RF, vitamin B_2) and thus depends on its uptake from the environment [1]. In its natural soil habitat, *Streptomyces davaonensis* produces the antivitamin roseoflavin (RoF), an antimicrobial RF analogue, to reduce growth of competing microorganisms [2]. We hypothesized that RoF might be useful as a microbiome modulator against potential oral pathogens, e.g. involved in caries formation. However, little is known about the antimicrobial efficacy of RoF against members of the human oral microbiota.

In the cultivation-based arm of our study, more than 300 microbial isolates were obtained from saliva samples of 41 healthy human volunteers and characterized by MALDI-TOF-MS. Securely identified species were analysed with regard to the presence of RF biosynthesis and uptake genes and tested for susceptibility to RoF in RF-free media supplemented with RF and/or RoF, respectively. For cultivation-independent studies, we obtained saliva samples from 15 healthy volunteers, which were pooled and incubated with or without RoF for 24 hours, respectively. After DNA and RNA extraction, 16S rRNA (cDNA) and 16S rRNA gene sequencing was used to investigate shifts in the community composition of the present (DNA-based) or active (cDNA-based) saliva microbiota.

None of the isolated gram-negative species were found to be RF auxotrophic, while 7 out of 19 gram-positive species grew significantly better when supplemented with RF (100 µM). Similarly, growth of 11 out of 12 tested gram-negative species was not negatively affected by RoF (100 µM), whereas 11 out of 19 gram-positives showed growth reduction, including *S. mutans*. Our data tentatively suggested that RoF-treatment shifts the human oral microbiota towards the gram-negative fraction [3]. However, our 16S-sequencing-based incubation experiment did not confirm a relative increase of the gram-negative fraction, neither on DNA- nor on RNA-basis. We believe that under the growth conditions used, too few cell divisions took place to reduce the cell number of the riboflavin-auxotrophic species. Consequently, future experiments will address monitoring of RF-concentrations and different bacterial growth conditions.

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P-MC-242

Material based approach to study microbial communities

*L. Meisch[1], M. Velaz Martín[1], S. Moench[1], A. K. Kaster[2], K. S. Rabe[1], C. M. Niemeyer[1]

[1]Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces 1 (IBG 1), Eggenstein-Leopoldshafen, Germany

[2]Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces 5 (IBG 5), Eggenstein-Leopoldshafen, Germany

Introduction

Microorganisms exist in immense variety on Earth, thriving in a wide range of habitats and often forming biofilm communities. However, studying these microbial communities in laboratory settings is challenging, since the environmental conditions are hard to mimic. Using a macroporous elastomeric silicone foam (MESIF) as a material to cultivate complex environmental microbial communities aids the transition from the environment to the laboratory.

Goals

Modifying the surface characteristics of the MESIF matrix to evaluate the influence on biofilm formation and the microbial composition of the communities being cultivated.

Methods

MESIF materials were chemically modified or functionalized with biomolecules, characterized and integrated in a chip system. The influence of the different surface properties on microbial growth and biofilm formation were evaluated with model organisms and environmental microbiomes.

Results

To establish reproducible surface modifications methods, the silanization and functionalization were analyzed using fluorophobic, fluorescent dyes as well as labeled oligonucleotides and proteins. These were then used to modify MESIF materials for growth studies of model organisms equipped with plasmids expressing fluorescent proteins. We found that the surface modification methods required for the functionalization of biomolecules led to different biofilm formation patterns. This was also reflected by the bioinformatic analysis of environmental microbial communities colonizing different MESIF materials. While chemical modifications did not alter microbial composition, biomolecule functionalization enriched different microbial consortia compositions.

Summary

Different surface modification techniques for MESIF materials were established with reproducible surface characteristics. The surface modifications enable the enrichment of different consortia of microorganisms, facilitating the systematic exploration of Microbial Dark Matter.

Literature:

Zoheir AE, Meisch L, Velaz Martín M, Bickmann C, Kiselev A, Lenk F, Kaster A-K, Rabe KS and Niemeyer CM.: Macroporous Silicone Chips for Decoding Microbial Dark Matter in Environmental Microbiomes. *ACS Appl. Mater. Interfaces* (2022) *14* (44):49592

P-MC-243

Tripartite interaction between wood-decay Basidiomycetes andhabitat sharing microbes

*R. Jesse[1]

[1]Friedrich Schiller University, Jena, Germany

Wood-decay basidiomycetes, such as the ubiquitous white-rot fungus *Schizophyllum commune*, fulfill an important ecological role by contributing to global carbon cycling through the degradation of lignocellulose and woody material. However, the brown-rot fungus *Serpula lacrymans* also represents an economic burden, by invading buildings and destroying wood construction material. Natural environments of both fungi are characterized by the interaction with habitat sharing microorganisms. Besides competition with other fungal decomposers, both fungi interact with bacteria. Such bacterial-fungal interactions can be classified as mutualistic or antagonistic, with levels in between, and often involve the production of natural products. While *Bacillus subtilis* attaches to the hyphae of *S. commune* (fungal highway), it induces the production of natural products including terpenoids in *S. lacrymans*.

The aim of our study is to understand how consortia stimuli are recognized and transduced inside basidiomycete cells.

Special attention is given to elucidate the production of terpenoids in *S. commune* and how they mediate microbial interactions. We use knockout and overexpression of genes in signaling cascades of *S. commune* to identify pathways involved in the interaction between both fungi. Growth and combative ability are evaluated on defined media with different carbon sources associated with wood and stressors of signaling pathways. Changes in gene expression due to bacterial cues are anaylzed by RT-qPCR. Furthermore, we characterize the inter- and intra-kingdom interactions by micromorphology and fluorescence microscopy. Putative terpene synthases of *S. commune* are characterized by heterologous expression.

Overall, our results help to elucidate the dynamic interactions between fungi and bacteria and highlight the importance of terpenoids as predominant natural product of basidiomycetes.

P-MC-244
Understanding the role of antimicrobial effector proteins secreted by the lichen-forming fungus *Peltigera rufescens*
*C. Kelly[1], V. Joisten-Rosenthal[2], H. Rövenich[1], B. Usadel[2], B. Thomma[1]
[1]*University of Cologne, Biology, Cologne, Germany*
[2]*Heinrich Heine University, Institute of Biological Data Science, Düsseldorf, Germany*

Symbiotic associations are found across the fungal kingdom, among which lichens are one of the most successful mutualistic fungal symbioses. By classical dual definition, lichens are comprised of two primary partners. While one is a lichen-forming fungus, termed the mycobiont, the other is a photosynthetic partner, called the photobiont. We believe effector mediated microbiome manipulation previously discovered for pathogenic fungi is also fundamental to fungi with different lifestyles and therefore aim to understand the role of antimicrobial proteins for lichen-forming fungi. Initial analyses of a high quality *Peltigera rufescens* genome identified potential effectors with antimicrobial activity that might play a role in shaping the community structure of the lichen microbiota. A candidate, for which RNA-sequencing counts support the expression of the protein coding gene in the natural lichen, displays antimicrobial activity *in vitro* on bacteria and fungi from the lichen"s natural environment. Beyond we recorded a reduction in photosynthetic activity when the protein is applied to phototrophic organisms. The selected ~18 kDa protein contains a ricin B lectin domain which in studies on the ricin protein, a plant toxin, has been shown to bind simple sugars. In the future we will gather evidence for the secretion of our protein in the natural *Peltigera* lichen and uncover its mode of action.

P-MC-245
Modelling tripartite microbial population dynamics
*T. Hassan[1], A. Matuszy'nska[1]
[1]*RWTH Aachen University, Institute of Biotechnology, Aachen, Germany*

Microorganisms are omnipresent in nature, where they form diverse communities consisting of genetically different strains or even a variety of species that interact and co-exist. Mathematical models can be used to systematically unravel the intricacies of coexistence and competition dynamics among the members of microbial colonies over time. This study extensively explores the metabolic interactions occurring over time within the heterotrophic members of microbial communities by emphasizing the co-cultivation of photoautotrophic and heterotrophic partners within the community structure. The main objective is to employ a population dynamics model along with an evolutionary game theoretical framework to investigate how three distinct metabolic strategies—Public metabolizer, Private metabolizer, and Cheater (also referred to as defector or free rider) affect the dynamics, composition, and stability of a consortium. Our suggested population model is based on the Nonlinear Lotka-Volterra framework, where the growth of other strains is influenced by the glucose extracted by the Public metabolizer and the available sucrose. This analytical approach enables us to understand the stability and instability conditions of cooperation and competition which indicate the general rules for the survival and extinction of the members of our designed synthetic cross-kingdom community linked vai nutrient exchange. This work provides valuable insights into the ecological dynamics of microbial systems and offers a foundation for further investigations into the impact of cheater and privatizer on stability and resilience. The anticipated outcomes may influence various domains, including environmental management, ecosystem engineering, and applications in biotechnology, by shaping creative approaches based on the study's discoveries.

P-MC-246
Designing a consumer-resource model for understanding interactions in complex microbial communities
*J. F. Pohlkotte[1]
[1]*Heinrich Heine University, Institute of Quantitative and Theoretical Biology, Düsseldorf, Germany*

Mathematical modelling of microbial communities is essential for understanding interactions between their members, the process of community assembly and what determines the dynamics, stability and composition of a microbiome. MacArthur consumer-resource models describe simple communities driven by competition for resources. Expanding this framework to include the exchange and consumption of metabolites enables modelling microbial communities and explaining community assembly, taxonomic composition and the structure of the metabolic network of a system. A commonly used approach assumes a base metabolism where the only differences between species are their resource uptake preferences. Here, we expand upon these microbial consumer-resource models with the goal of creating a more mechanistic and biologically interpretable framework. Metabolic processes are designed to incorporate enzyme kinetics while ensuring the validity of mass balances. This approach allows microbial communities to be modelled on a flexible scale between two extremes: Firstly, treating each species as a black box where the entire metabolism is summarized as one metabolic macro-reaction, which requires only a small amount of knowledge of the species, and secondly, modelling each part of a species" metabolism as a separate process which allows a more accurate and in-depth look at a microbial community and the exact interactions, but requires large amounts of knowledge and data. We show that this model can exhibit a wide range of behavioural patterns and reproduce findings of previous works. We demonstrate that this model can be applied to simple systems consisting of two bacterial species, algal-bacterial communities, and more complex communities containing the alga *Chlamydomonas reinhardtii*, the fungus *Verticilium dahilae* and a number of different bacteria.

P-MC-247

Effect of soil and microorganisms in truffle growth

*A. Gopa Kumar Nair[1], K. Krause[1], E. Kothe[1]
[1]*Friedrich Schiller University, Microbial Interactions, Jena, Germany*

Truffles are hypogeous ascomycetous fungi belonging to *Tuber* genus, which form ectomycorrhizal association with oak, hazel and beech. The expensive edible mushrooms are known for its unique flavour and smell. The role of microbial communities associated with truffles are rather unknown. The aim of the project is to understand the effect of soil and microorganisms on cultivation of *Tuber* on truffle field sites.

There were two different type of sampling sites in Thüringia- two test field sites and a natural site. Both the test field sites were treated with truffle growth supplements- Truf up and Rhizovital. Soil community analyses and element analyses of test field and natural sites were done along with isolation of microorganisms from soil nearby of fruiting bodies and the gleba of fruiting bodies of the natural site. Interaction studies of *T. borchii* with isolated bacteria was carried out in Petri dishes under different conditions. Based on the results of interaction with *T. borchii*, a consortia was prepared and applied it on the test field sites.

Soil community analysis of natural site revealed dominance of *Proteobacteria, Actinomycetia, Mortierellomycetes, Sordariomycetes* and *Dothideomycetes*. Whereas in test field sites, lower diversity was found. After three years, the microbial community had a significant rise of *Tremellomycetes* and a drop of *Mortierellomycetes* in the treatment plots.

A strain collection with 355 microorganisms contained predominantly of *Streptomyces* sp., *Pseudomonas* sp. and *Bacillus* sp were created from the soil and fruiting bodies of natural sites. First analyses of bacterial interaction with truffles showed that species of *Proteobacteria* and *Actinobacteria* induced faster growth of truffle mycelium with visible mycelium after 4 days interaction. Moreover, the absence of light showed a positive growth of truffle mycelium, when compared with light conditions.

Over the span of three years, a change in the microbial communities in the test field sites were observed which were similar with natural sites of truffles. Along with that, the interaction of truffle helper bacteria with truffles were effective, which were then used as consortia for the test field sites.

P-MC-248

The effect of fungal-derived antimicrobial proteins is context- and condition-dependent

*G. Bauerecker[1], E. Chavarro-Carrero[1], H. Rövenich[1], B. Thomma[1]
[1]*University of Cologne, Biology, Cologne, Germany*

Plants actively recruit mutualists and beneficial endophytes through root exudates into their microbiota, allowing to alleviate abiotic stress and suppress pathogen invasion. Most soil-borne pathogens infect the plant through the root, encountering the rhizosphere microbiota as a first layer of plant defense. The soil-borne, xylem-invading fungus *Verticillium dahliae* was shown to secrete antimicrobial effector proteins (AMPs) to manipulate the host microbiota, facilitating its invasion into the plant. Those AMPs show selective, yet distinct, activity spectra and can be antibacterial or antifungal or both. Individual expression patterns differ, and collectively they are not only expressed during host-colonization stages but also during soil-dwelling life stages of the fungus. Hence, AMPs potentially play a crucial role in facilitating fungal competition with various soil microbes. Considering that fungal evolution predates the emergence of land plants, and the likely intense competition with soil bacteria in early terrestrial systems in the quest for carbon, AMPs may initially have evolved as tools for microbial competition and might be conserved throughout the entire fungal kingdom. Here, we set out to investigate the effect of fungal-derived AMPs on communities of soil-derived microbes. The sensitivity of the individual bacteria to the AMPs was tested in different growth media. Subsequently, we assessed if the observed sensitivities change when other bacteria are present. Remarkably, we observe that certain bacteria, which are sensitive to an AMP when tested individually, become insensitive in the presence of certain other bacteria and *vice versa*. Collectively, our data suggest that antimicrobial activities of fungal-derived AMPs depend on environmental contexts.

P-MC-249

A versatile microfluidic platform for the cultivation of cyanobacteria at single-cell resolution

*L. Witting[1], J. M. Hofer[2], T. Schulze[3], A. Weber[2], M. Eisenhut[3], D. Kohlheyer[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany*
[2]*Heinrich Heine University, Plant Biochemistry, Düsseldorf, Germany*
[3]*Bielefeld University, Faculty of Biology / Computational Biology, Bielefeld, Germany*

1. Introduction

The primary endosymbiosis theory states, that about one billion years ago a heterotrophic host organism incorporated a cyanobacterium, which subsequently evolved into the chloroplast we find in algae and plants today. However, the early steps in the establishment of endosymbiosis and the transition towards an organelle have not been experimentally tested yet. Furthermore, cyanobacteria are expected to play a key role a future bio-economy.

2. Goals

Establish microfluidic single-cell cultivation for high-throughput characterization of cyanobacteria. The technology will later be used to trap and observe single cells in picoliters of medium, mimicking the inclusion vacuole during early endosymbiosis.

3. Materials & Methods

Cyanobacteria are cultivated in a strict monolayer, therefore self-shading of cells is avoided. The use of the highly gas-permeable polydimethylsiloxane (PDMS) to fabricate cultivation chips allows cultivation without carbon limitation.

A method was developed to generate a linear light-intensity gradient along the microfluidic chip, allowing growth data to be generated at high-throughput. Cells were segmented using a deep learning based algorithm. Python scripts were used for cell segmentation and subsequent data analysis.

Dynamic CO2 control was enabled by stacking an additional PDMS layer on top of the thin film cultivation chip. CO2-depleted air was then perfused through the gas layer in countercurrent to the medium flow.

4. **Results**

The cyanobacterial strains *Synechococcus elongatus* UTEX2973 and PCC7942 as well as *Synechocystis* sp. PCC6803 were used as model organisms. Using the image analysis pipeline, TB of image data containing millions of cells were analysed. It was shown that UTEX2973 grows without carbon limitation in the microfluidic cultivation system when operating it at ambient air. Using dynamic CO2 control, it was shown that carbon limitation first occurs at a CO2 concentration of 15 ppm. In comparison to UTEX2973, PCC7942 showed higher CO2-dependent growth rates. PCC6803 was the slowest cyanobacterial strain tested. No divergence of growth rates in the direction orthogonal to the light intensity-gradient was observed.

5. **Summary**

A versatile platform for the characterization of cyanobacteria at single cell resolution was developed and published1. In the near future, the platform will be used to test hypotheses related to endosymbiosis.

1. Witting et al., Lab Chip, p., 2024, doi: 10.1039/D4LC00567H.

P-MC-250

Advancing Microbial Research Infrastructure: MibiNet's Data Management Strategies and the LichenMetaImage Collaboration

*S. Zander[1], V. Joisten-Rosenthal[1], K. Krooß[2], D. Wang[1], X. Zhou[3], A. Kranz[3], S. Weidtkamp-Peters[2], B. Usadel[1,3]
[1]Heinrich Heine University, Institute of Biological Data Science, Düsseldorf, Germany
[2]Heinrich Heine University, Center for Advanced Imaging, Düsseldorf, Germany
[3]Forschungszentrum Jülich GmbH, IBG-4: Bioinformatics, Jülich, Germany

The CRC 1535 MibiNet consortium aims to enhance our understanding of microbial networks, providing insights into organelle evolution and microbiome functionality [1]. We implement effective data management strategies for consortium members by applying the FAIR principles (Findable, Accessible, Interoperable, Reusable) throughout data storage and processing workflows. A key component of our data management strategy is the Annotated Research Context (ARC) structure, developed by DataPLANT. This structure enables researchers to consolidate data, metadata, and computational workflow in a single location [2]. Enhanced by ontology-driven metadata templates and the DataHUB platform, this ARC framework ensures streamlined data sharing and interoperability within MibiNet. Integrating the eLabFTW electronic lab notebook and the eLab2ARC tool supports comprehensive experimental documentation [3,4].

The *LichenMetaImage* use case [5], a joint project between CRC 1535 MibiNet and the NFDIs NFDI4Microbiota, DataPLANT and NFDI4BIOIMAGE, focuses on developing standardized workflows and metadata annotations for bioimage data related to lichens and their associated microbiome. Addressing challenges of large file sizes and proprietary formats, we are creating tailored metadata templates based on REMBI [6] to meet the unique requirements of lichen bioimage. These templates, implemented in OMERO [7] and ARC structures, support comprehensive documentation of imaging data. Future plans include integrating complementary omics data, such as sequence information, to map microbial compositions and spatial distributions within lichen thalli. By enhancing metadata accessibility and usability, these initiatives improve reproducibility in microbial research, foster interdisciplinary collaboration, and advance our understanding of complex microbial ecosystems.

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P-MC-251

From cyanobacteria to cell organelle – Engineering and studying minimal endosymbiotic metabolism

*J. M. Hofer[1], *T. Schulze[2], L. Witting[3], J. Volke[1], D. Kohlheyer[3], M. Eisenhut[2], A. Weber[1]
[1]Heinrich Heine University, Institute for Plant Biochemistry, Düsseldorf, Germany
[2]Bielefeld University, Faculty of Biology / Computational Biology, Bielefeld, Germany
[3]Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany

In eukaryotes, photosynthesis evolved through endosymbiotic incorporation of a photosynthetic cyanobacterium, which became the ancestor of plastids. The transformation entailed gene transfer from the endosymbiont to the host nucleus, evolution of protein targeting systems, as well as synchronization of host and endosymbiont cell cycles. The *ménage-à-trois* (MAT) hypothesis proposes that the linkage of carbon and energy metabolism, established by the integration of transporters gained by horizontal gene transfer from *Chlamydia*-like endoparasites, was the primary driver of the endosymbiosis (Karkar *et al.*, 2015).

In order to investigate whether the linkage of host and endosymbiont carbon and energy metabolism could be sufficient for the establishment of endosymbiosis, we aim to enforce a synthetic endosymbiont-like metabolism in the model cyanobacterium *Synechocystis* sp. PCC 6803. By deletion of the *glgC* gene, the precursor for the energy storage compound glycogen will no longer be produced. Implementation of the *Chlamydia*-like transporter UhpC will alleviate carbon overflow reactions (Gründel *et al.*, 2012) via export of excess carbon out of the cell during periods of light. Implementation of the transporter NTT will allow uptake of external ATP for energy during periods of darkness. Markerless deletion will be used to sequentially erode the genome, while diurnal growth, transcriptomes and metabolomes will be characterized. Our microfluidic platform (Witting *et al.*, 2024) will be used to mimic the host cytoplasm during early stages of endosymbiosis, enabling analysis of cyanobacterial physiology in response to metabolomic rerouting and special constraints at single cell resolution. Additionally, the introduction of metabolite sensors will enable real-time readouts of metabolic changes within the cell.

The successful knockout of *glgC* has been achieved and three independent *Synechocystis* Δ*glgC* knockout lines have

been characterized under diurnal growth conditions. The transcriptomes and metabolomes of the mutant lines reveal stressed phenotypes and increased energy charge as well as metabolic re-routing. Strains expressing *Chlamydia*-like transporters are tested for their efficiency to complement the metabolic overflow in light and energy shortage in darkness.

P-MC-252
Spatial structure and public goods drive microbial community ecology
*M. Bäcker[1,2], T. Schubert[1,2], B. E. Dutilh[1,2]
[1]*Friedrich Schiller University, Viral Ecology and Omics, Jena, Germany*
[2]*Friedrich Schiller University, Cluster of Excellence Balance of the Microverse, Jena, Germany*

Microbes self-organize into spatially structured communities, profoundly impacting their interactions with each other and their environment. Metagenomic sampling inherently destroys spatial patterns, complicating our ability to predict ecological dynamics from bulk metagenomic data. To bridge this gap, we generated a multi-dimensional dataset on soil communities stabilized under various conditions, examining their functional and compositional characteristics.

Using soil sampled from the Jena Experiment, we cultivated a bacterial microbiome in minimal media with either the complex polymer chitin or its monomer as the sole carbon source under structure-permissive or -destructive conditions. Through metagenomic analysis of longitudinal samples, we tracked community composition and assembled high-quality metagenome-assembled genomes (MAGs) of key microbes. Chitin degradation necessitates the costly production of extracellular chitinases, which are shared with the community depending on the self-assembled spatial structure. Hence, we will use biochemical assays to quantify public goods production, including chitinases, which we will further analyze using computational modeling to understand their impact on community function, stability, and productivity.

Ultimately, we aim to understand how spatial structure and complex carbohydrate degradation via public goods affect microbial community ecology. This will allow us to better understand the microbial interactions in soil and other structured biomes.

P-MC-253
The microbiome of a paper mill
*F. Langguth[1], D. Skalski[2], D. Beck[2], J. Roedig[2], D. Benndorf[1], P. Vrizas[3], R. Schubert[3]
[1]*Anhalt University of Applied Sciences, FB 7, Mikrobiologie, Koethen, Germany*
[2]*Anhalt University of Applied Sciences, FB7, Angewandte Biotechnologie, Koethen, Germany*
[3]*Progroup Paper PM3 GmbH, Sandersdorf-Brehna, Germany*

1. **Introduction**

Paper production consumes large amounts of water and energy. This can be reduced by using a closed water cycle and producing primary energy by anaerobic water treatment. This water treatment cycle consists of UASB reactors for anaerobic water treatment, a subsequent stripping of carbon dioxide and waste gases, a lime trap and the final treatment of waste air using a trickle bed reactor and a biofilter. Continuous operation revealed the formation of biofilms and odor as some challenges related to the microbial communities established in the systems.

2. **Goals**

The study aims at a better understanding of microbial functions and to identify key targets for process optimization.

3. **Materials & Methods**

Combined metagenomics and metaprotomics were applied to analyze the microbial communities. Extracted microbial proteins from water cycle were tryptically digested, transferred to nanoHPLC-MS/MS (TimsTOF Pro) and identified by search against a protein sequence database based on corresponding metagenome data.

4. **Results**

The combined metagenomics and metaproteomics approach showed a complex microbial community which clearly differs between the process stages: (i) samples from paper production and contaminated water showed higher abundance of *Bacillalles* and *Lactobacillales*, (ii) anaerobic treatment in UASB reactors contained more *Methanomicrobiales*, *Enterobacterales* and *Thiotrichales*, (iii) aerobic treatment and cleaned water and finally (iv) exhausted air treatment showed higher abundance of *Burkholderiales.* These differences correlated well with the different process conditions.

Functional analysis showed hydrogenotrophic methanogenesis in the UASB reactors and acidogenesis from organics as well as methanogenesis from methylated amines in the contaminated water from paper production. Detecting methanogenesis from methylated amines was surprising but correlated with anaerobic conditions in these samples. The detection of methanogenic functions in the final aerobic cleaned water could be explained by a partial loss of methanogenic biomass from the UASB reactor.

5. **Summary**

The metaproteome analysis of microbial communities from closed water cycle of a paper mill provided insight into community structure and functions. Data can be used to further optimize the system.

P-MC-254
Effects of head-down tilt bed rest on the human gut microbiome
*K. Siems[1], A. Rehm[2], A. Warkentin[1], D. Engel[1], S. Janssen[2], S. Leuko[1]
[1]*German Aerospace Center (DLR), Applied Biology, Cologne, Germany*
[2]*Justus Liebig University Giessen, Algorithmic Bioinformatics, Gießen, Germany*

A healthy gut microbiome, particularly including probiotic microorganisms that metabolize complex carbohydrates and produce short-chain fatty acids (SCFAs), plays a critical role in maintaining intestinal homeostasis and is generally associated with improved health outcomes. Stressors, both physical and psychological, result in the release of stress-related neurochemicals to which gastrointestinal bacteria are particularly sensitive. This can lead to gastrointestinal distress and inflammation, and a dysbiotic gut microbiome

can lead to anxiety and depression. During spaceflight, astronauts are exposed to numerous stressors such as microgravity, radiation, sleep disruption, and isolation, which can lead to challenges such as muscle wasting, immune dysfunction, and neuro-ocular syndrome. However, the impact of space conditions on the human microbiome is not well understood. Since the availability of samples from astronauts during spaceflight is limited, bed rest studies on Earth offer a great opportunity to study the simulated effects of spaceflight-induced changes in the human body in more detail and with a larger number of subjects. In particular, head-down tilt (HDT) bed rest studies provide valuable insights into the effects of microgravity on the human body. The DLR Institute of Aerospace Medicine has conducted several HDT studies, including "AGBRESA", "SANS-CM" and "SMC", to evaluate the influence of different countermeasures on physiological changes. Subjects underwent 30 to 60 days of HDT with countermeasures such as exercise and centrifugation. To assess microbiome changes, stool samples were collected from subjects in these bedrest studies at baseline, during HDT, and after recovery and analyzed using next-generation sequencing of the 16S rRNA gene. Results show high stability of gut microbial communities but interindividual variability in gut microbiome composition. Improving this understanding of the interactions between the gut microbiome and individual host responses to prolonged bed rest, might provide valuable insights to help mitigate the risks associated with long-duration space missions.

P-MC-256

Antimicrobial effectiveness across copper content gradients on surfaces against a reference microbial community

*Y. T. Ly[1,2], R. Anton[2], L. Kopruch[3], C. L. Krämer[2], S. Leuko[2]
[1]*Justus Liebig University Giessen, Gießen, Germany*
[2]*German Aerospace Center (DLR), Cologne, Germany*
[3]*RWTH Aachen University, Aachen, Germany*

Microbial spread is occurring especially in confined environments, such as passenger cabins. Pathogens can be easily spread through contact with surfaces. The ongoing silent pandemic is driven by antimicrobial resistance, that are already affecting millions of patients. Copper is known to harbor antimicrobial properties, and in consideration of sustainability and cost-effectiveness, we conducted an experiment to assess the effectivity of varying copper concentrations on surfaces against a reference microbiome, which represents the most prevalent bacterial genera in public transportation. Further, the effect of the used materials is additionally tested against two selected species of the reference microbiome. The test materials are glass slides that are coated with varying amounts of copper, specifically 100 at.%, 79 at.%, 53 at.% and 24 at.%. The results demonstrate that a copper content of 100 at.% is the most effective in reducing the survival of the tested organisms. In contrast, the 79 at.% and 49 at.% copper content surfaces only exhibit an antimicrobial effect on single species, which is not visible in the survival rate but is evident in a reduced metabolic activity of the cells. In general, the reference microbial community is more resilient than the tested single species. Consequently, community testing may be a more realistic approach compared to single model organisms, and higher doses of copper (at least 80 at.%) or increased coating thickness may be necessary to achieve the desired antimicrobial effect.

P-MC-257

Insights into establishment and maintenance of lichen communities through structural bioinformatics and synthetic microbial consortia

*M. Heinen[1]
[1]*Institute of Microbiology, AG Altegoer, Düsseldorf, Germany*

Lichens are remarkable examples of complex microbial cross-kingdom communities, thriving in some of the harshest environments on Earth. These symbioses, comprising fungi (mycobionts) and photosynthetic partners like algae or cyanobacteria (photobionts) but also additional bacterial members, have been traditionally difficult to study due to their complexity. A detailed molecular understanding of interactions between the community members is still missing.

The main focus of our research is therefore to elucidate the mechanisms that control the formation, stabilization, and maintenance of lichens. From other fungal interactions, it is already known that secreted proteins play a vital role in recognition, facilitation of cell-cell contact, regulation of growth, and production of metabolites. Known as effector proteins, these molecules have key roles in both symbiotic but also pathogenic interactions.

For this reason, we are focusing on the identification and classification of secreted proteins through bioinformatic mining of sequenced genomes of *Peltigera* lichens. By leveraging structural prediction and clustering algorithms, our bioinformatic pipeline is able to identify effector proteins that traditional sequence-based methods often miss. This approach already allowed us to identify structurally conserved proteins also found in pathogenic fungi but revealed a large number of currently uncharacterized protein families. However, a detailed functional understanding of these proteins is currently lacking.

As lichens are not efficiently amenable to genetic manipulation, we will use synthetic communities composed of well-characterized model microbes. These communities comprise *Synechocystis*, *Saccharomyces cerevisiae*, and the dimorphic fungus *Ustilago maydis*, and allow us to investigate the effects of candidate effector proteins. As the members in a lichen community are spatially closely associated, we plan to incorporate surface-presented proteins that allow us to connect the individual organisms in a controllable manner. To this end, we develop a *U. maydis*-based surface display system, making use of the carbohydrate-binding properties of lectins – a class of secreted proteins that have been shown to facilitate cell-to-cell contacts and are also present in lichens.

By unraveling the molecular secrets of lichen effector proteins, we hope to shed light on the fundamental principles of microbial cooperation using these ancient symbionts as exemplars.

P-MC-258
Development of a novel model for the analysis of the human gut microbiotica
*N. Hager[1], U. Deppenmeier[1]
[1]*Institut für Mikrobiologie und Biotechnologie, Bonn, Germany*

The human gut microbiota is a complex system that not only plays a crucial role in the metabolism of endogenous and dietary compounds, but also significantly influences the modification of exogenous chemical substances (xenobiotics). Xenobiotics include various additives, pharmaceuticals and industrial chemicals that can be converted into metabolites with altered toxicity, bioavailability and activity. While host-specific xenobiotic metabolism has been extensively described, suitable models for studying the gut microbial influence are lacking. Existing Systems often rely on complex fermentation approaches or synthetic gut communities, which are not only costly and time consuming, but can also lead to reduced overall microbial diversity. However, many crucial modifications, such as the reduction of digoxin or the decarboxylation of levodopa, are mediated through less abundant members of the microbiota. In this work, an innovative in vitro model of the human colon was developed, based on the isolation of the entire bacterial population from fecal samples, which allows high-throughput screening of xenobiotic modifications. Our system represents a pure bacterial population reflecting the entire microbiota. Modifications of drugs and xenobiotics can be analyzed without eukaryotic influences and without absorption events that could reduce availability of the xenobiotics. To confirm physiological functionality, extensive activity profiles of the most important key enzymes of bacterial metabolism as well as the production of short-chain fatty acids were investigated in comparison to original stool samples. In addition, the microbial composition during the incubation period was recorded using 16S amplicon sequencing. Finally, the applicability of the model for toxicokinetic studies was confirmed for various xenobiotics with known microbial conversion. Our model offers a simple method to investigate the microbial influence on the metabolism of xenobiotics. The approach represents an important advance for the evaluation of drug safety and toxicological risk assessment.

P-MC-259
Siderophore typing, a powerful tool to study their role in mediating microbial networks
*S. Krueger[1], P. Westhoff[1]
[1]*Heinrich Heine University, Plant Metabolism and Metabolomics Labor, Düsseldorf, Germany*

Siderophores are iron chelating molecules that are produced by a variety of microorganisms and have essential functions in building microbial communities. An important group of siderophores are the pyoverdines, a structurally diverse group of chromopeptides with species-specific differences in the peptide sequence and side group structure. Here we describe the development of an LC-MS/MS-based approach to investigate the role of pyoverdines in the interaction of microbial communities. This method utilizes the characteristic fragmentation pattern of all pyoverdines that occurs in the all-ion fragmentation mode and takes advantage of the fact that all pyoverdines are ionized as M2+ ions. In this way, we are able to explore the diversity of pyoverdines and their possible modification.

Prokaryotic cell biology

P-PCB-260
Molecular basis for the targeting process of injectisome substrate proteins in *Salmonella enterica*
*H. Grabietz[1], M. Erhardt[1,2]
[1]*Humboldt University, Institut für Biologie - Molecular Microbiology, Berlin, Germany*
[2]*Max Planck Institute, Berlin, Germany*

A major virulence factor of many pathogenic gram-negative bacteria, including *Salmonella enterica* Typhimurium, is the injectisome, a needle-like structure that injects toxins into host cells during infection. The injectisome employs a type-III secretion system (T3SS) for the export of toxin proteins. Before initiating toxin secretion, it secretes its own structural components through a highly controlled self-assembly process. However, because T3SS substrates do not contain a typical cleavable secretion signal in the aminoterminal region of the peptide sequence [1], it remains unknown how these substrates are selected from thousands of cytoplasmic proteins and specifically targeted to the T3SS. This project explores the hypothesis that substrate gene transcription and translation occur in close proximity to the T3SS, enabling efficient recognition and secretion. This concept is inspired a process known as transertion in which transcription, translation, and membrane insertion of proteins are spatially coordinated. Recent findings suggest that *Vibrio parahaemolyticus* utilizes transertion for T3SS substrate secretion [2], but its role in *Salmonella* remains unclear. To test this hypothesis, co-localization analyses of chromosomal substrate gene loci and the T3SS, as well as spatial studies on membrane proximity will be conducted. In summary, this project will provide critical insights into the type III secretion process, shedding light on the assembly mechanism of the injectisome and the secretion of toxins during infection. Ultimately, it will elucidate the potential role of local transcription and translation of substrate genes in these processes.

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P-PCB-261
The role of FimV in cellular processes in *Pseudomonas putida*
*L. M. Schmidt[1], K. Thormann[1], T. S. Bentele[1], L. Zehner[1]
[1]*Justus Liebig University Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany*

It is important for bacteria to be able to precisely coordinate intracellular processes such as the placement of motility structures, e.g. the flagellum or the pilus machinery. Therefore, polar landmark proteins such as HubP or FimV are widely distributed in different bacterial species and help them to control the spatiotemporal regulation of polar regions within the bacterial cell. While the HubP proteins of *Vibrio* or *Shewanella* are comparably well characterized and their involvement in various essential processes such as chromosome segregation or the correct placement of the chemotaxis machinery has been shown, the role of FimV in *Pseudomonas putida* remained unclear. To characterize the

role of FimV in *P. putida*, we examined FimV with respect to cellular processes known to involve FimV or HubP in other species. Motility assays showed that deletion of FimV significantly reduces the spreading behavior of the bacteria, while it does not alter the flagellation pattern of the bacteria. We could also show that FimV is not essential for the correct placement of the chemotaxis system, whereas deletion of the polar marker FlhF delocalizes the chemotaxis protein CheA from the cell pole. Replacement of the entire HubP/FimV protein from *Shewanella putrefaciens* and *Pseudomonas putida*, respectively, showed that the spreading behavior is phenotypically similar to a deletion mutant. In contrast, the introduction of the C-terminal FimV domain of *Pseudomonas* into *Shewanella* HubP led to a restriction of the spreading behavior of *Shewanella*, whereas the introduction of the FimV domain of *Shewanella* into *Pseudomonas* slightly improved the spreading of the *Pseudomonas* bacteria. Taken together, the results show that the polar landmark proteins HubP and FimV are not necessarily functional orthologs but that their function is highly species-specific.

P-PCB-262

The bacterial predator *Myxococcus xanthus* uses two LcrH-like chaperones with unprecedented substrates to lyse prey cells

*A. L. Hampe[1], S. Thiery[1], C. Kaimer[1]
[1]*Ruhr University Bochum, Microbiology, Bochum, Germany*

In soil environments, microorganisms constantly compete for limited nutrients. To secure their resources, selected bacterial species act as predators, i.e. they kill other bacteria to consume their biomass. An important model to study bacterial predation is *Myxococcus xanthus*, which uses a broad prey spectrum and possesses a distinct predation machinery. *M. xanthus* kills its prey in direct cell-cell contact using two protein secretion systems [1]. A Tad-like "Kil" system seems to mediate prey killing, and an atypical type 3 secretion system (T3SS*) is responsible for prey lysis [2]. Compared to other T3SS, the T3SS* in *M. xanthus* lacks a needle and other outer membrane components, yet it is fully functional.

To address the question of whether and how the T3SS* might secrete effector proteins across the *M. xanthus* cell envelope to the prey cell, we initially investigate the role of putative chaperones. These chaperones presumably bind specific substrates to recruit them for secretion. Phenotypic analysis showed that two out of four LcrH-like chaperones within the T3SS* gene cluster are required for T3SS*-dependent prey lysis. However, homologues to known T3SS effectors were not found in the *M. xanthus* genome. We are currently looking into candidate effectors for each chaperone by screening deletion mutants. First interactions between chaperone-effector pairs were identified using *in vitro* pulldown assays. To localize these T3SS* components upon prey contact, we perform fluorescence microscopy and aim to unravel their role within the predation machinery.

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This work is funded by Deutsche Forschungsgemeinschaft DFG.

P-PCB-263

Chromosome maintenance in *Escherichia coli* – the role of SeqA in mismatch repair

*J. Felder[1], D. Schindler[2], T. Probanowski[1,3], M. Eskander[2], T. Waldminghaus[1]
[1]*TU Darmstadt, Molecular Microbiology, Darmstadt, Germany*
[2]*Philipps-University Marburg, Zentrum für Synthetische Mikrobiologie, Marburg, Germany*
[3]*Forschungszentrum Jülich GmbH, Biotechnologie, Jülich, Germany*

To achieve faithful transfer of the genetic information from one generation to the next, bacteria have evolved different chromosome maintenance systems. In *Escherichia coli* two such chromosome maintenance systems are based on the DNA sequence motif GATC, which is methylated at the N6 position of the adenines by the DNA adenine methyltransferase (Dam). During DNA replication, unmodified nucleotides are incorporated into the newly synthesized DNA strand, which results in a hemi-methylation of the GATC motifs for a short period of time after the passing of the replication fork. These hemi-methylated GATC motifs are targeted by MutH, a component of the mismatch repair system in *E. coli*, in order to enable efficient mismatch repair. Hemi-methylated GATCs are further targeted by SeqA, which sequesters the replication origin and is thought to act in chromosome organization and segregation. These chromosome maintenance systems have been studied individually for many years, but little is known about their interplay. As SeqA and MutH target the same DNA motif, the question arises how these factors functionally interact with one another.

We designed several experimental approaches to analyze the functional interaction between MutH and SeqA in *E. coli,* by investigating the efficiency of mismatch repair in presence and absence of SeqA. In addition to deletion mutants we designed and constructed synthetic secondary chromosomes. Based on the arrangement of GATC sites on these replicons, the binding of only MutH or both MutH and SeqA or none of these proteins is expected. The respective mutation rate should allow to assess the role of SeqA in mismatch repair. First mutation accumulation assays and next generation sequencing experiments showed that the mutation rates in a *seqA* deletion mutant are slightly increased compared to the wildtype. To support these results, further mutation accumulation assays are performed analyzing the synthetic secondary chromosomes.

P-PCB-264

YejL is involved in Gram-negative outer membrane biogenesis

*L. J. Tilg[1], S. Weber[1], R. Voigt[1], A. M. Möller[1], B. Kutscher[1], F. Narberhaus[1]
[1]*Ruhr University Bochum, Biology of microorganisms, Bochum, Germany*

The outer membrane of Gram-negative bacteria consists of two leaflets that differ in their composition: the inner leaflet contains mostly phospholipids (PL) whereas the outer leaflet is mostly composed of lipopolysaccharides (LPS). LPS

provide a physical barrier against hydrophobic molecules like antibiotics and bile salts, therefore, the balance of the two major components is essential for viability. To maintain the optimal ratio of LPS and PL, their biosynthesis pathways are connected at multiple sites, resulting in a complex protein network (Möller *et al*., 2023). Since an overproduction of LPS leads to cell death, the first committed enzyme in the LPS pathway, LpxC, is posttranslationally degraded by the protease FtsH, which is facilitated by the adaptor protein LapB. The adaptor function of LapB is inhibited by the inner membrane protein YejM (LapC) upon lack of LPS, resulting in stabilisation of LpxC. Interestingly, the *yejM* gene is located downstream of *yejL*, which encodes for a so far uncharacterised protein. Unlike other proteins involved in the regulatory interplay, YejL is not essential. However, YejL is conserved among gammaproteobacteria, contains 75 amino acids and forms a dimer with a tight conformation.

To investigate the function of YejL in the complex regulatory network, we performed protein-protein interaction studies using bacterial two-hybrid, pull down, microscale thermophoresis (MST) and far western dot blot analysis. We identified various proteins involved in outer membrane biosynthesis that might interact with YejL. The bacterial two-hybrid assay and MST showed that the first protein of the PL pathway, FabZ, as well as the first protein of the LPS pathway, LpxA, bind to YejL. In addition, MST and far western dot blot revealed an interaction between YejL and MreB which coordinates cell shape.

Overall, our studies indicate that YejL is likely involved in the regulatory interplay of outer membrane biogenesis.

P-PCB-265
Protein-protein interactions at the LapB interface, a central player in cell envelope synthesis
*H. Bille[1], A. M. Möller[1], B. Kutscher[1], F. Narberhaus[1]
[1]*Ruhr University Bochum, Microbiology, Bochum, Germany*

The asymmetric outer membrane (OM) of gram-negative bacteria consists of phospholipids (PL) as well as lipopolysaccharides (LPS), with LPS exclusively located in the outer leaflet. LPS levels in *Escherichia coli* are strictly controlled by post-translational regulation of LpxC, the key enzyme of LPS synthesis. LpxC is degraded by the membrane-bound protease FtsH. This process is regulated by the adaptor protein LapB (YciM), which binds LpxC and directs it towards FtsH. Further, based on periplasmic LPS levels, the activity of LapB itself is controlled by the anti-adaptor protein YejM. Our previous studies demonstrated that LapB interacts with other proteins in the lipopolysaccharide synthesis pathway, namely LpxA and LpxD (Möller *et al.*, 2023). In addition, we found that LapB binds FabZ, the first enzyme in the phospholipid synthesis (PL) pathway. Since the proper balance between LPS and PL is mandatory for bacterial survival, LapB is believed to be a central player in cell envelope synthesis by serving as an anchor for the involved cytosolic enzymes. We now investigate, how the interaction between those proteins is navigated.

We study the formation of protein complexes with purified LapB and its interaction partners to understand if the protein-protein interactions of LapB can occur simultaneously. Therefore, we use different *in vitro* approaches, such as size exclusion chromatography, blue native PAGE and pull downs. In addition, we employ targeted mutagenesis to investigate the interaction sites where LapB binds to its interaction partners, since it harbors nine tetratricopeptide repeat motifs (TPR) in its cytosolic domain. The results will provide insights into how LapB coordinates the assembly of a dynamic protein complex to orchestrate cell envelope biogenesis.

P-PCB-266
Functional analysis of CRISPR-Cas systems in antibiotic-producing actinomycetes
*L. Mitousis[1], E. Musiol-Kroll[1], W. Wohlleben[1]
[1]*Eberhard Karls University Tübingen, IMIT-Organismic Interactions/Microbial Glycobiology, Tübingen, Germany*

Many Actinomycetes are important producers of valuable secondary metabolites with potent properties like antimicrobial, anti-tumor or immunosuppressive activity. However, their genetic engineering is often prohibited by mechanisms that prevent the stable establishment of foreign DNA. CRISPR-Cas systems are known as adaptive prokaryotic immunity systems. They play an important role in the defence against foreign mobile genetic elements like plasmids or phages. Recently, also the involvement of CRISPR-Cas in other cellular processes including DNA-repair or cell-differentiation was described. But so far the knowledge about the function of CRISPR-Cas in Actinomycetes is still very limited.

The goal of this project is the functional analysis of CRISPR-Cas systems in antibiotic-producing actinomycetes. This includes adaptive immunity as well as functions beyond immunity, such as a potential role of CRISPR-Cas in regulation of antibiotic production. For this, we use the industrial aminoglycoside antibiotic producer *Streptoalloteichus tenebrarius* as model strain. It is particularly interesting, as its genome encodes 33 predicted biosynthetic gene clusters and three CRISPR-Cas systems (Region 1-3).

Bioinformatic sequence analysis on the origin of the spacers did not result in matches to known sequences from databases. Gene expression studies revealed the transcription of all *cas*-genes under standard laboratory conditions. The immunity function can be investigated with DNA-uptake assays based on transformation, conjugation and/or phage infection. To identify a phage that infects *S. tenebrarius*, an extensive screening of newly isolated phages as well as known actinomycetes phages was conducted. It was shown, that *S. tenebrarius* is not included in the host range of all tested phages.

Since the genetic manipulation of *S. tenebrarius* is challenging, the CRISPR-Cas systems of *S. tenebrarius* were heterologously expressed in *Escherichia coli* and *Streptomyces coelicolor*. The DNA-Uptake assays performed with the heterologous hosts expressing CRISPR-Cas Region 1 did not reveal activity as adaptive immunity system so far.

Furthermore, CRISPR-Cas deletion mutants in *S. tenebrarius* were generated. The deletion of CRISPR-Cas Region 1 led to a sporulation-deficient phenotype. This suggests an involvement of CRISPR-Cas in cell-differentiation processes in *S. tenebrarius*.

P-PCB-267

Influence of an SPFH protein on developmental programs in *Bacillus subtilis*

*S. Baur[1], U. Repnik[2], A. Mondry[1], L. Rau[1], M. Bramkamp[1]
[1]*Christian-Albrechts-University of Kiel, Institute for General Microbiology, Kiel, Germany*
[2]*Christian-Albrechts-University of Kiel, Central microscopy, Kiel, Germany*

In its natural environment *Bacillus subtilis* is often exposed to life-threatening and fluctuating conditions. To respond to these environmental changes *B. subtilis* use alternative σ-factors which activate gene clusters to help to adapt to the given conditions. Perceiving these environmental changes is often facilitated by membrane-associated proteins. One notable protein complex is the PspA-YdjGHI complex, which encodes the SPFH (Stomatin/Prohibitin/Flotillin/HflK-C) protein, YdjI (1). Members of the widely conserved SPFH family are associated with regulation of membrane fluidity (2,3) and are known for their impact on a variety of membrane bound processes, including developmental processes in *B. subtilis* (4,5). Here, we show that YdjI is required for efficient motility and development of social communities. Absence of *ydjI* in a biofilm leads to changes in metabolism associated genes clusters, indicating that cells within the biofilm are starving. This results in a decreased secretion of BslA, one of the major matrix genes, preventing the formation of the hydrophobic coat and rendering the biofilm surface hydrophilic. Interestingly, phenotypic complementation is only achieved in a background strain that renders YdjI cytosolic suggesting that YdjI has dual functions depending on its subcellular localisation.

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P-PCB-268

Effects of cell shape changes on *Listeria monocytogenes in vitro* virulence

*S. Wamp[1], J. Rismondo[1,2], J. Döhling[1], G. Holland[3], S. Halbedel[1,4]
[1]*Robert Koch Institute, FG11 - Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany*
[2]*Georg-August University Göttingen, Department of General Microbiology, Institute for Microbiology & Genetics, GZMB, Göttingen, Germany*
[3]*Robert Koch Institute, ZBS4 - Advanced Light and Electron Microscopy, Berlin, Germany*
[4]*Otto von Guericke University Magdeburg, Institute for Medical Microbiology and Hospital Hygiene, Magdeburg, Germany*

Introduction: *Listeria monocytogenes* is a foodborne pathogen characterized by its capability to invade and replicate in multiple human cell types and to spread from cell to cell. In addition to known virulence factors, also the absence of the late cell division protein DivIVA leads to severe virulence defects (1, 2). Based on the characteristic cell-chaining phenotype of the *L. monocytogenes divIVA* mutant, the question arose how the cell shape affects *in vitro* virulence. Therefore, we here compare the virulence properties of coccoid and filamentous *mreB* (involved in lateral cell growth) and *ezrA* (involved in Z-ring formation) mutants to the cell-chaining phenotype of the *divIVA* mutant.

Methods: IPTG-dependent *mreB* and *ezrA* depletion strains were constructed and the presence of the expected coccoid and filamentous phenotypes was verified during growth under standard laboratory conditions and in *in vitro* infection experiments. All cell morphotypes were tested for replication, invasion and cell-to-cell spread in various eukaryotic cell lines. Additionally, the expression of actin tails, required for movement and spreading during infection, was analysed by phalloidin staining.

Results: The coccoid and filamentous growth of the *mreB* and *ezrA* depletion strains did neither impair the multiplication in infected macrophages nor did it influence the invasion in human hepatocytes, as opposed to the replication and invasion deficient *divIVA* mutant. On the other hand, filamentous growth of the *ezrA* depletion strain led to reduced plaque sizes compared to the wild type. No cell-to-cell spread could be observed for the coccoid growing *mreB* depletion strain as well as the *divIVA* mutant, which coincided with a reduced abundance or absence of actin filaments.

Conclusions: Our findings indicate that the cell shape plays a minor role for efficient *in vitro* infection. The severe virulence defect of the *divIVA* mutant therefore seems to be associated with a DivIVA-dependent influence on other virulence factors or their release via the SecA2 secretion route (1).

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P-PCB-269

The virulence regulator VirB from *Shigella flexneri* uses a CTP-dependent switch mechanism to activate gene expression

*S. Jakob[1], W. Steinchen[2,3], J. Hanßmann[1], J. Rosum[1], K. Langenfeld[4], M. Osorio-Valeriano[1,5], T. Glatter[4], G. Bange[2,4,3], A. Diepold[4], M. Thanbichler[1,4,3]
[1]*Philipps-University Marburg, Biology, Marburg, Germany*
[2]*Philipps-University Marburg, Chemistry, Marburg, Germany*
[3]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*
[4]*Max Planck Institute, Terrestrial Microbiology, Marburg, Germany*
[5]*Blavatnik Institute, Harvard Medical School, Cell Biology, Boston, MA, United States*

Previous studies on the transcriptional antisilencer VirB from *S. flexneri* have shown that it activates virulence gene expression by counteracting the nucleoid-organizing protein H-NS in a *virS*-dependent manner. The loading and spreading on the *virS* site lead to a local decrease in negative supercoiling, thereby destabilizing the H-NS complexes to activate gene expression. However, the underlying process remains unclear. In contrast to conventional transcriptional activators, VirB does not directly interact with the RNA polymerase (RNAP). VirB belongs to a novel class of molecular switches, which act as DNA sliding clamps that use cytidine triphosphate (CTP) binding and hydrolysis to control their opening and closing state. The newly discovered CTPase activity of ParB that allows its binding and sliding along the DNA raises the question of whether VirB uses a similar mode of action. Here, we show that VirB binds CTP, embraces DNA in a clamp-like fashion upon its CTP-dependent loading at *virS* sites and slides laterally on DNA after clamp closure. CTP-binding mutations inhibit VirB loading in vitro, and abolish the formation of VirB nucleoprotein complexes as well as virulence gene expression in vivo. Therefore, VirB functions as a CTP-dependent molecular switch that regulates transcription through a loading-and-sliding mechanism during bacterial pathogenesis.

P-PCB-270

Identification of EcpK, a BY-pseudokinase important for exopolysaccharide biosynthesis in *Myxococcus xanthus*

*L. Blöcher[1], J. Schwabe[1], T. Glatter[2], L. Søgaard-Andersen[1]
[1]*Max Planck Institute, Ecophysiology, RG Søgaard-Andersen, Marburg, Germany*
[2]*Max Planck Institute, Core Facility for Mass Spectrometry and Proteomics, Marburg, Germany*

Bacteria secrete a diverse array of polysaccharides that have important functions in many physiological processes and industrial applications. In the ubiquitous Wzx/Wzy-dependent pathway, polysaccharide biosynthesis is initiated by a phosphoglycosyltransferase transferring a sugar-1 phosphate to undecaprenyl phosphate. Next, glycosyltransferases add sugar units to synthesize the repeat unit, which is then "flipped" to the periplasm by a Wzx flippase. Subsequently, a Wzy polymerase in conjunction with a polysaccharide-co-polymerase (PCP) polymerizes repeat units. Finally, in Gram-negative bacteria, an OPX protein in conjunction with the PCP translocate the polysaccharide across the outer membrane. Thus, PCPs in Wzx/Wzy-dependent pathways in Gram-negative bacteria are at the nexus between polymerization and translocation of the polysaccharide. These PCPs belong to the 2a family and interact directly with the Wzy polymerase and the OPX protein. Generally, PCP-2a proteins are inner membrane proteins with an extended periplasmic domain, a C-terminal cytoplasmic bacterial tyrosine kinase (BYK) domain and form octameric assemblies. Interestingly, PCP-2a proteins lacking either the BYK domain entirely or motifs important for Tyr phosphorylation have been described. Here, we focus on one such protein, EpsV for exopolysaccharide (EPS) biosynthesis in *Myxococcus xanthus*. Computational structural biology confirmed that EpsV lacks the BYK domain entirely. Using phylogenomics and computational structural approaches, we identify EcpK as a standalone cytoplasmic BY-pseudokinase, lacking key residues required for tyrosine phosphorylation and show experimentally that EcpK is essential for EPS biosynthesis. Using mass-spectrometry based proteomics, bacterial-two-hybrid assays and computational analyses, we show that EcpK directly interacts with EpsV. Based on these findings, we suggest that EcpK functions as a scaffold protein and by direct protein-protein interactions, rather than by Tyr phosphorylation, facilitates EpsV activity. Notably, EcpK and EpsV orthologs are present in other bacteria, suggesting a broad conservation of this novel, non-canonical PCP-2a mechanism.

P-PCB-271

Unraveling the role of a new input receiver in a complex cyanobacterial circadian clock

*F. P. Stirba[1], A. Wiegard[1], N. Küppers[1], F. Lansen[1], A. Pawlowski[1], P. Kolkhof[1], A. Wilde[2], I. M. Axmann[1]
[1]*Heinrich Heine University, Institute for Synthetic Microbiology, Düsseldorf, Germany*
[2]*University of Freiburg, Biology, Freiburg i.Br., Germany*

Circadian rhythms are biological processes that synchronize an organism"s physiology and behavior with the 24-hour day-night cycle. The primary model organism for procaryotic circadian research is *Synechococcus elongatus* PCC 7942 (*Synechococcus*). At the core of this unicellular freshwater cyanobacterium"s timekeeping mechanism is a simple posttranslational oscillator consisting of the proteins KaiA, KaiB and KaiC. The clock of *Synechocystis* sp. PCC 6803 (*Synechocystis*), another unicellular freshwater cyanobacterium, is more complex (Köbler et al. 2024). Due to its genetic tractability, phototrophic lifestyle, and ability to grow hetero-/mixotrophically, *Synechocystis* is a model organism for synthetic biology, biotechnology and photosynthetic research. However, its circadian clock is less characterized than that of *Synechococcus*. While *Synechocystis* exhibits the core clock proteins KaiA1, KaiB1 and KaiC1, our recent discovery of the chimeric KaiA3 revealed a second fully functional oscillator consisting of KaiA3, KaiB3 and KaiC3. In both oscillators, KaiA stimulates the phosphorylation of KaiC while KaiB sequesters KaiA and leads to the dephosphorylation of KaiC. Additionally, these two oscillators are interconnected as KaiC1 interacts with KaiB3 and KaiC3 with KaiB1 (Wiegard et al. 2020). Both oscillators are required for the maintenance of the circadian rhythm and for the switch between phototrophic and heterotrophic growth. However, little is known about the input and output factors that integrate environmental signals and relay the status of the clock to downstream processes such as gene expression. Based on pull-down assays and bioinformatic analysis, we found two candidate kinases that could interact with the response receiver domain of KaiA3 and act as putative input factors for the clock. We are further investigating the interaction of these kinases with KaiA3 using yeast two-hybrid assays. Additionally, we measure the circadian backscatter oscillations of knockout mutants of these kinases to observe their effect on the circadian rhythm of *Synechocystis*. Our aim is to shed light on the complex circadian clock of *Synechocystis*, including its input factors, that leads to the metabolic flexibility of this model organism.

P-PCB-272

Characterization of luciferase-fluorophore reporters for tracking of single molecules

*S. Peschkes[1], M. Eilers[1], U. Endesfelder[1]
[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Mikrobiologie und Biotechnologie, Bonn, Germany*

Single-Molecule Microscopy is a powerful tool for studying molecular dynamics in living cells. Monitoring the kinetics of intracellular processes, single-molecule tracking (smt) grants a deeper understanding of behavioral states of biomolecules as these often correlate with diffusive behavior [1].

Crucial for smt is a constant signal and specific labelling. While the latter poses a major problem with conventional dye labeling systems, the use of fluorescent proteins prevents non-specific binding and still achieves sufficient quantum yields [2]. Even more advantageous is the use of luciferases as a marker due to their long signal half-life. However, their quantum yield is typically too low for smt, as smt requires a high temporal resolution in the millisecond range [3].

The combination of luciferases and FPs, often referred to as Nano-lanterns, unifies their advantages by exploiting Fluorescence Resonance Energy Transfer (FRET) to enhance the light output of the luciferase [4]. FRET describes the non-radiative energy transfer via resonance from a donor- to acceptor-fluorophore and depends upon the overlap of the emission and excitation spectra of the donor and acceptor molecules, as well as close proximity of the donor and acceptor and their respective orientation.

In this work, we present our efforts to characterize different luciferase-fluorophore reporters for single-molecule tracking in living cells. Therefore, we established a stable read-out system employing the monomeric inner membrane protein LacY from *E. coli* as the biological test system. We chose LacY due to its slow diffusion properties [5], its monomeric

structure, and the localization of its two termini on the cytoplasmic side of the membrane [6]. Regulating gene expression by a L-Rhamnose inducible promoter, this low expression system enables laser free high-resolution tracking of low abundant biomolecules. We tested and characterized different constructs in which fluorophores and luciferases were fused to the distinct termini of LacY.

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P-PCB-273

Morphological characterisation of star-shaped bacteria from the genus *Stella*

*J. Eschenhagen[1], *C. E. Wurzbacher[1], M. C. F. van Teeseling[1], C. Jogler[1]

[1]Friedrich Schiller University, Department for Microbial Interactions, Jena, Germany

The physiological and morphological diversity of bacteria is enormous, ranging from simple rods or cocci to extraordinary asymmetrical cell shapes. 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 strains, which are potential members of the genus *Stella* within the Alphaproteobacteria. Members of this genus are described to form unusually shaped cells, which resemble flat, six-pointed stars (Vasilyeva, 1970). In order to understand their peculiar cell structure and morphology better, we examined the cells employing multiple light microscopy techniques. Furthermore, the phylogenetic relationships of *Stella spp*. were analysed to complement the microscopic examination. In addition to their exceptional morphology, strains belonging to the genus *Stella* have been reported earlier to change their shape in response to the nutrient compositions of the surrounding media and other environmental factors (Vasilyeva et al., 1974). Therefore, we investigated the influence of different factors on their cell shape and growth.

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P-PCB-274

Cytosolic factors controlling *in vivo* phosphorylation of ReoM, an important player in control of *Listeria monocytogenes* peptidoglycan biosynthesis

P. Rothe[1], S. Wamp[1], L. Rosemeyer[1], J. Rismondo[2], A. Gründling[3], J. Doellinger[4], *S. Halbedel[5,6]

[1]Robert Koch Institute, FG11 - Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany

[2]Georg-August University Göttingen, Department of General Microbiology, Institute of Microbiology and Genetics, Göttingen, Germany

[3]Imperial College London, Section of Molecular Microbiology and Medical Research Council Centre for Molecular Bacteriology and Infection, London, United Kingdom

[4]Robert Koch Institute, ZBS6 - Proteomics and spectroscopy, Berlin, Germany

[5]Robert Koch Institute, FG 11 Division of Enteropathogenic Bacteria and Legionella, Consultant Laboratory for Listeria, Wernigerode, Germany

[6]Otto von Guericke University Magdeburg, Institute for Medical Microbiology and Hospital Hygiene, Magdeburg, Germany

Listeria monocytogenes is characterised by a thick cell wall consisting mainly of peptidoglycan (PG). The cell wall counteracts the high internal turgor pressure and ensures viability. To fully understand PG biosynthesis, we are studying a regulatory pathway that controls PG production in *L. monocytogenes* and related Gr+ bacteria1,2,3. This pathway controls the proteolytic degradation of MurA, the enzyme that catalyses the first committed step of PG biosynthesis. MurA is degraded by the protease ClpCP in the presence of unphosphorylated ReoM. However, when ReoM is phosphorylated by the PASTA-domain containing protein serine/threonine kinase PrkA, the interaction of ReoM with MurA is inhibited and MurA degradation is prevented. The phosphorylation can be reversed by the phosphatase PrpC. In this way, PrkA and PrpC are involved in the decision whether PG biosynthesis is switched on or off. We here have studied the phosphorylation profile of ReoM in living *L. monocytogenes* cells to clarify under which growth conditions ReoM is phosphorylated and thus PG biosynthesis activated, respectively. Our results show that ReoM is fully phosphorylated under most tested laboratory growth conditions. However, we also found factors that prevented the phosphorylation of ReoM as, for example, ReoM phosphorylation was reduced in a mutant lacking the divisome gene *gpsB*. Additionally, ReoM phosphorylation decreased in the late stationary growth phase and this decrease was dependent on the presence of the phosphatase PrpC. Furthermore, overexpression of MurA exerted a negative feedback on ReoM phosphorylation and this required a direct contact between ReoM and MurA, as well as MurA enzymatic activity and substrate binding. These findings indicate that MurA and PrkA compete for ReoM as their shared interaction partner and suggest that the involved proteins constitute a safety valve mechanism ensuring controlled degradation of excess MurA to align the MurA amounts and the rate of PG biosynthesis with nutrient supply and growth.

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P-PCB-275

Investigation of minor pilin functions in the cyanobacterium *Synechocystis* sp. PCC 6803

*L. Wittig[1], N. Schürgers[1], F. Drepper[2], A. Wilde[1]

[1]University of Freiburg, Freiburg i.Br., Germany

[2]University of Freiburg, Freiburg i.Br., Germany

Type IV pili (TFP) are flexible and versatile fibers on the surface of many gram-negative bacteria, primarily composed of multiple copies of protein subunits called major pilins. In addition to the cyanobacterial major pilin PilA1, *Synechocystis* sp. PCC 6803 encodes less abundant pilin-

like proteins, known as minor pilins, which are crucial for pilus assembly and facilitate various functions, including natural competence, aggregation, adhesion, and motility.

Recent studies have highlighted the importance of different transcriptional units of minor pilins in distinct pilus functions. TU2300, which encodes PilA5 and PilA6, is essential for natural transformation but is dispensable for motility and flocculation. Similarly, we demonstrated that TU2301, which encodes PilA7 and PilA8, is crucial for natural transformation, but not for motility and flocculation. TU763, encoding PilA9-PilA12, is essential for motility and flocculation but dispensable for natural transformation. Investigations of the minor pilins PilX1, PilX2, and PilX3 indicate their importance for flocculation.

The absence of extracellular PilA1 in mutant strains lacking specific sets of minor pilins suggests that TFP assembly depends on the presence of either PilA5-8 or PilA9-12. We hypothesized that two different priming complexes are assembled, forming the tip of a pilus fiber with distinct functions. Here, we provide mass spectrometry (MS) data for sheared pili fractions and co-immunoprecipitation of specific minor pilins to elucidate the composition of these distinct pilus subtypes.

Furthermore, we visualized spatiotemporal dynamics of pilus subtypes by confocal microscopy employing a PilA1(T77C) cysteine mutant that can be stained with Alexa Fluor 488. The combination of this PilA1-variant with the deletion of minor pilin genes allowed us to detect phenotypic differences in cell surface piliation. Preliminary data showed long, less dynamic pilus fibers in *pilA9-12* mutants, whereas *pilA5-6* mutants exhibited short, dynamic fibers. The machine learning algorithm ILASTIK was used to automatically detect pili and analyze the pilus subtypes of knock-out mutants. We present a rapid and standardized method for analyzing the microscopic images of cells decorated with TFP.

P-PCB-276

Novel Cag outer protein interactions of the Helicobacter pylori Cag Type 4 SecretionSystem and direct physical network of outer membrane proteins HopQ and HopZ withT4SS components

*C. Josenhans[1], F. Metz[1], J. Beilmann[1], S. Bats[1], B. Sedlmaier[1], A. Latoscha[1], G. Witte[2], R. Megens[3], K. P. Hopfner[2], K. Thorell[4], W. Fischer[1], L. Terradot[5], S. Suerbaum[1,6,7]

[1]Ludwig Maximilians University, Microbiology, Munich, Germany
[2]Ludwig Maximilians University, Gene Centre, Department for Biochemistry, and Protein Facility, Munich, Germany
[3]Ludwig Maximilians University, IPEK, Munich, Germany
[4]University of Gothenburg, Department of Chemistry and Molecular Biology, Gothenburg, Sweden
[5]Université de Lyon, Institut de Biologie et Chimie des Protéines, Lyon, France
[6]German Center for Infection Research (DZIF), Munich, Germany
[7]Ludwig Maximilians University, National Reference Center for Helicobacter pylori, Munich, Germany

Background. Type IV secretion systems, for instance the CagT4SS of Helicobacter pylori, encode membrane-spanning protein complexes in Gram-negative bacteria which can transport various molecules. To elucidate mechanisms and new functionalities, we use the CagT4SS as a model, which is an important virulence factor in the human H. pylori infection [1,2,3]. Recently, structural information on the CagT4SS has been substantially improved by cryo-EM [4]. However, important structural and functional information is lacking, in particular on protein interactions between T4SS outer proteins [5], and of T4SS outer proteins with other proteins,

Methods and Results. In the present study, we followed the hypothesis that H. pylori T4SS outer proteins may form an outer protein complex, together with other, non-CagT4SS proteins, which may also be essential for T4SS function under different conditions. Using interaction screens of H. pylori CagT4SS outer proteins, followed by biochemical and functional characterization, we have enhanced here our knowledge on functional protein-protein interactions of the CagT4SS outer proteins. This extends to newly identified physical interactions of outer membrane proteins HopQ and HopZ with CagT4SS proteins. We have further identified and quantitated direct physical interactions of T4SS outer proteins with HopZ and HopQ, which play an important role in T4SS function, and of both HopZ and HopQ with themselves and with host cell factors CEACAM and integrin. Furthermore, we determined an influence of pH on Hop-Cag interactions. Utilizing chromosomal tag insertions in H. pylori, we confirmed surface-exposed co-localization of HopQ with the T4SS in the absence or presence of human gastric epithelial cells using high-resolution microscopy. Functionally opposing roles of HopQ and HopZ for the T4SS were uncovered in pro-inflammatory human cell activation.

Conclusions. In summary, we identified novel interactions between H. pylori outer membrane proteins and CagT4SS outer proteins and characterized them as functionally important for membrane transport processes. This will help to refine structural and functional details of the dynamic surface-exposed complex of the CagT4SS.

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Prokaryotic physiology and metabolism

P-PPM-278

Physiological interplay of iron and biotin metabolism in *Corynebacterium glutamicum*

*K. Dietz[1], P. Peters-Wendisch[2], M. Meyyappan[1], V. F. Wendisch[2], B. Blombach[1]

[1]Technical University Munich, Microbial Biotechnology, Straubing, Germany
[2]Bielefeld University, Genetics of Prokaryotes, Faculty of Biology and Center for Biotechnology (CeBiTec), Bielefeld, Germany

Growth of *Corynebacterium glutamicum* in minimal medium relies on the supplementation of essential biotin and the siderophore protocatechuate (PCA) to compensate for transient iron limitation [1,2]. Previously, biotin-prototroph *C. glutamicum* was engineered [1] and iron-responsive, enforced PCA synthesis was implemented in strain *C. glutamicum* IRON+ to improve intracellular iron availability [2].

Here, a prototrophic *C. glutamicum* strain was engineered that is independent of both, biotin and PCA. Therefore, *C. glutamicum* IRON+ was genomically equipped with heterologous *bioF* and *bioI* genes catalyzing the missing

steps of biotin synthesis [1] which yielded the designated strain *C. glutamicum* IRON+BioFI. Growth properties were characterized in shake flask cultivations in biotin- and PCA-free CgXII minimal medium ($CgXII_{MM}$) with 20 g glucose L^{-1}.

In the shake flask, *C. glutamicum* IRON+BioFI grew to a final biomass of 11.3 ± 0.8 g CDW L^{-1} in $CgXII_{MM}$, however, showed biphasic growth with reduced growth rates of 0.27 ± 0.00 h^{-1} and 0.34 ± 0.02 h^{-1} in each phase, respectively. In contrast, the wild type (WT) exhibited continuous exponential growth with a µ of 0.40 ± 0.01 h^{-1} reaching 10.2 ± 0.5 g CDW L^{-1} in fully supplemented CgXII medium. The biphasic growth was observed before for the WT grown in PCA-depleted CgXII medium which was traced back to a temporary iron limitation [2,3]. Thus, we assume that intracellular iron availability of *C. glutamicum* IRON+BioFI is impaired again.

Upon *bioFI* integration, the regulator of the biotin metabolism *bioQ* was deleted which previously led to decreased transcription levels of genes involved in the iron metabolism, such as *cg0924* [4] which encodes a component of a putative siderophore ABC transporter [5]. To investigate the underlying physiological effects of *bioQ* deletion on the iron metabolism, we deleted *cg0924*, integrated the *bioFI* genes into the neutral landing pad CgLP5 [6] and characterized growth of the engineered strains on different carbon sources.

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P-PPM-279

Investigation of a non-canonical fumarate-based glutamate biosynthetic pathway in *Bacillus subtilis* and *Escherichia coli*

*C. S. Götz[1], J. M. Ebel[1], M. S. Y. Mardoukhi[2], F. M. Commichau[2], J. Gibhardt[1]

[1]University of Hohenheim, FG 190h Molecular Microbiology, Stuttgart, Germany

[2]University of Hohenheim, FG 190h Molecular Microbiology, Stuttgart, Germany

The amino acid glutamate plays important roles in cellular processes in the Gram-positive and -negative model bacteria *Bacillus subtilis* and *Escherichia coli,* respectively. It is the major amino group donor in anabolic reactions and a key molecule connecting carbon with nitrogen metabolism (1,2). *B. subtilis* synthesises glutamate via the glutamate synthase (GOGAT)/glutamine synthetase (GS) pathway (1), while *E. coli* can utilize the GOGAT/GS pathway, as well as the glutamate dehydrogenase (GDH) for glutamate biosynthesis (1,2). The GDHs of *B. subtilis* are strictly catabolically active. Recently, a non-canonical, fumarate-based pathway – involving the *ansB*-encoded L-aspartase and the *aspB*-encoded aspartate transaminase – was found that restored glutamate-prototrophy of a glutamate-auxotrophic *B. subtilis* strain lacking the GOGAT-encoding genes *gltAB* (3). The aim of this project is to better understand the mechanism and regulations of the different glutamate synthesizing pathways. To further analyse the fumarate-based pathway for glutamate biosynthesis, we constructed plasmids allowing for *ansB* and *aspB* expression in *B. subtilis* and *E. coli* glutamate-auxotrophic strains and further we characterized the novel pathway by growth experiments under defined growth conditions with different carbon and nitrogen sources. Results show that the novel pathway is able to restore glutamate-prototrophy not only in *B. subtilis* but also in *E. coli* and highlight different needs of the novel-pathway to the canonical glutamate biosynthetic pathway.

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P-PPM-280

In-depth characterization of *Bacillus subtilis* mutants lacking the essential target of glyphosate

*A. Rütten[1], I. Schwedt[2], M. Eckert[1,2], F. Kemmler[1], F. M. Commichau[1]

[1]University of Hohenheim, FG 190h Molecular Microbiology, Stuttgart, Germany

[2]Philipps-University Marburg, RNA Biochemistry, Marburg, Germany

The best known and most widely used herbicide worldwide is glyphosate [1]. The non-selective, systemic broad-spectrum herbicide glyphosate (N-(phosphonomethyl)-glycine) is used against all types of weeds that compete with agricultural crops [1]. Glyphosate acts specifically on the 5-enolpyruvyl shikimate-3-phosphate (EPSP) synthase, which makes the EPSP synthase of bacteria an attractive target for antibiotics and herbicides. The EPSP synthase is involved in the synthesis of chorismate from shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP), which is an essential precursor for the *de novo* synthesis of aromatic amino acids, folates (essential for purine and pyrimidine synthesis) and quinones (essential electron carrier in the respiratory chain) [2]. It has already been shown that the enzyme function of EPSP synthase in *Bacillus subtilis* is essential for growth in minimal medium and complex medium such as LB [3]. A *B. subtilis* deletion mutant in which the *aroE* gene coding for EPSP synthase has been deleted can still grow in complex BHI medium [3]. This is possible because *B. subtilis* apparently has unknown uptake systems for all chorismate derivatives and aromatic amino. Nevertheless, it has not yet been possible to cultivate an *aroE* deletion mutant stably in a minimal medium. For this purpose, the exact, minimal nutrient composition is to be determined. This can be achieved by characterizing suppressors of the *aroE* deletion mutant, which frequently contain mutations in genes encoding the ResED 2-component system, which is responsible for switching from aerobic to anaerobic metabolism [4]. This indicates that there is an imbalance in the menaquinone pool and the NADH+H+/NAD+ ratio during growth of the *aroE* mutant in minimal medium, which can be restored by suppressor mutations. Comprehensive suppressor analysis to understand the effect of *aroE* deletion on the physiology of the cell will be done. Also, stably growing *aroE* mutants will be established to identify additional glyphosate targets in *B. subtilis*. Subsequently, identification of aromatic amino acid transporters in *B. subtilis* will be performed.

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P-PPM-281

Redirecting carbon flux towards products in cyanobacteria by engineering the regulatory mechanism of the 2,3-bisphosphoglycerate-independent phosphoglycerate mutase reaction

*F. Hufnagel[1], K. Otec[1], N. S. Becker[2], K. Forchhammer[2], S. Klähn[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Solar Materials Biotechnology, Leipzig, Germany*
[2]*Eberhard Karls University Tübingen, IMIT - Organismic Interactions/Microbial Glycobiology, Tübingen, Germany*

Introduction

Among prokaryotes oxygenic photosynthesis is a unique feature in cyanobacteria. In the context of establishing a sustainable bioeconomy, they receive growing interest as biocatalysts in photo-biotechnological applications. To rationally engineer cyanobacteria and to direct metabolic fluxes towards chemical products, it is important to consider native molecular processes that control metabolism. Recently, the 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (PGAM) has been shown to serve as a central control point determining the carbon flux from the Calvin-Benson-Bassham cycle to lower glycolysis. In particular, PGAM activity is controlled by the small protein PirC that also binds to the central regulatory protein P_{II}. Upon PirC-binding, PGAM is inactivated leading to glycogen accumulation within the cell.[1]

Here we aim to target the PirC-PGAM switch in the model strain *Synechocystis* sp. PCC 6803. In particular, flux through PGAM shall be blocked to increase product yields that derive from gluconeogenic routes.

Material and Methods

Expression of the genes encoding PGAM and PirC was manipulated by using controllable promoters that respond to copper ions. To reduce PGAM activity we aimed for an inducible downregulation of *pgam* and simultaneous upregulation of *pirC*. Similar cloning strategies were used to rationally design sucrose production strains, achieved by overexpression of the sps gene and by installing an invertase gene knockout (similar to Du et al.[2]). Glycogen and sucrose formation were analyzed in the obtained strains.

Results

Recombinant *Synechocystis* strains showing Cu2+-inducible downregulation of *pgam* and overexpression of *pirC* were cloned and glycogen accumulation was analyzed in the corresponding cultures. In both mutant strains an accumulation of glycogen after addition of copper ions was determinable. This glycogen accumulation did not occur in the wild type control or in the mutant strains before changing expression through addition of copper ions. These data confirm that PGAM activity can be controlled through downregulation of *pgam* expression and overexpression of *pirC* routing higher amounts of the fixed carbon into glycogen.

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P-PPM-282

The role of small proteins in *Escherichia coli* under acid stress

*T. Starikova[1], K. Jung[1]
[1]*Ludwig Maximilians University, Microbiology, Munich, Germany*

In the human gastrointestinal tract, bacteria have survival strategies in response to acidic environments. *Escherichia coli*, known for its high level of acid resistance, employs multiple acid defense mechanisms to ensure its survival under these harsh conditions. The main acid resistance mechanism involves three proton-consuming protection strategies, each consisting of an amino acid decarboxylase and an antiporter to take up the amino acid and release the more alkaline polyamine into the surrounding medium (Brameyer et al. 2022).

In recent years, due to the advances in detection techniques, a significant number of previously uncharacterized small proteins have been identified in bacteria (Fuchs and Engelmann 2023). These small proteins encoded by small open reading frames (sORFs) are often overlooked during annotation and are difficult to characterize using conventional biochemical techniques.

In 2023, ribosome profiling revealed 18 novel stress-induced sORFs in *E. coli*, found particularly under severe (pH 4.4) acid stress conditions (Schumacher et al. 2023). The current project aims to gain further insights into the subcellular location, properties, potential interactions, and the role in the acid resistance mechanism of the newly identified sORF candidates. sORF15 is located between *gadW*, encoding one of the major transcriptional regulators of the Gad acid resistance system, and *mdtF*, encoding a multidrug resistance protein. Interestingly, the last 64 bp of sORF15 overlaps with 3′ of *mdtF*. We found that *E. coli* MG1655 was more tolerant to stress imposed by organic acids and antibiotics than *E. coli* MG1655 Δ*sORF15.*

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P-PPM-283

CRISPR Cas9-based genome editing of *Hyphomicrobium denitrificans*

*M. G. Mohr[1], C. Kümpel[1], P. Schilling[1], J. Thöne[1], H. Hartenfels[1], W. Flegler[1], M. Keul[1], K. Grewe[1], C. Dahl[1]
[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*

Hyphomicrobium denitrificans X^T is a chemoorganoheterotroph that grows on C_1 and C_2 compounds. It serves as an important model organism for the analysis of dissimilatory sulfur oxidation because it can oxidize thiosulfate as a supplementary electron donor [1]. Establishing gene deletions and insertions in its chromosome has been instrumental in elucidating the sulfur oxidation pathway. Currently, genomic mutagenesis in *H. denitrificans* is based on suicide plasmid-driven homologous recombination (HR) systems. These systems work through an initial single-crossover (SCO) event, which integrates a non-replicating vector like pK18*mobsacB* into the genome, followed by a second SCO event, which either restores the wild-type sequence or induces the intended modification [1, 2]. A major challenge is the high rate of wild-type reversion, especially when targeting important genes. This leads to time-intensive mutant screening with low success rates. In fact, the lack of efficient markerless genome editing tools for *H. denitrificans* is a bottleneck for fundamental studies and also for possible future biotechnological exploitation.

The Cas9 RNA-guided DNA-endonuclease from the type II CRISPR-Cas system of *Streptococcus pyogenes* (SpCas9) has been extensively used to develop genome engineering tools for both prokaryotes and eukaryotes, but not for *H. denitrificans*, an Alphaproteobacterium of the order Hyphomicrobiales. Here, we present a SpCas9-based genome editing system for this organism, integrating plasmid-borne HR templates. We began with plasmid pBBR_Cas9_NT, which contains a *cas9* gene codon-optimized for the Alphaproteobacterium *Cereibacter sphaeroides* (formerly *Rhodobacter sphaeroides*, order Rhodobacterales) [3]. We further optimized the guide RNA for *H. denitrificans* and constructed plasmids designed to delete and replace the *shdrI* gene within the *H. denitrificans* genome. The protein sHdrI, which plays an important role in sulfur oxidation, has a distinctive dynamite bundle structure with an unknown function, making it a particularly interesting target. Finally, we compared the performance of our new CRISPR-Cas system with the traditional suicide plasmid-driven HR method.

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P-PPM-284

Observation and control of sporulation failures in *Bacillus subtilis*

M. Högner[1,2], C. Kaspar[1,2], B. Mucha[1,2], D. Wies[1,2], M. C. Elizondo-Cantú[1,2], S. E. Santos[3], R. Öberg[4], D. Malyshev[4], *M. Kilian[1,2], I. B. Bischofs[1,2]
[1]*Max Planck Institute, Marburg, Germany*
[2]*University of Heidelberg, Heidelberg, Germany*
[3]*Novonesis A/S, Microbial Physiology R&D, Hørsholm, Denmark*
[4]*Umeå University, Department of Physics, Umeå, Sweden*

Spore formation in *Bacilli* is a complex developmental process in which vegetative cells form dormant endospores. However, not all produced spores are necessarily viable, yet high purity of spore preparations is crucial for many spore-based applications.

Here, we characterized how such sporulation failures arise by tuning sporulation medium conditions in liquid batch culture in small-scale bioreactors. Supplementation of a chemically defined sporulation medium with L-alanine improved the sporulation outcome of *Bacillus subtilis*. We attribute this to alanine catabolism by alanine dehydrogenase, which prevents premature germination triggered by the major germinant receptor GerA. Pyruvate biosynthesis from alanine catabolism in turn supports spore formation via central metabolism.

By using automated time-lapse microscopy, we further investigated spore morphogenesis during successful and failed sporulation events. We found that sporulation failures were associated with premature mother cell lysis and linked to an additional, understudied late step in the sporulation program: a centering motion of the forespore body within the mother cell.

Our observations suggest a tight link between central metabolism and the outcome of the late stages of spore development. Furthermore, our new methods and insights offer new opportunities for improving spore production of *Bacilli* in applied contexts.

P-PPM-285

Elucidating the role of a cryptic small protein in the assimilation of inorganic carbon in cyanobacteria

*C. Poppitz[1,2], V. Reimann[3], W. Hess[3], P. Walke[2], M. Hagemann[2], S. Klähn[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Biotechnologie Solarer Materialien (SOMA), Leipzig, Germany*
[2]*University of Rostock, Rostock, Germany*
[3]*Albert-Ludwigs-University, Freiburg i.Br., Germany*

Cyanobacteria have evolved a sophisticated carbon concentrating mechanism (CCM) to significantly enhance the CO_2 concentration in close proximity to the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase and thereby diminish the frequency of its detrimental oxygenation reaction[1]. While previous research has extensively studied the sodium dependent bicarbonate transporter SbtA and its regulator SbtB[2], both crucial for the CCM, a so far non-annotated gene for a small protein of 80 amino acids has been identified upstream of the *sbtAB* operon in many cyanobacterial genomes. We hypothezise a function linked to the CCM and SbtA or B in particular and hence termed the protein SbtC. The 3D structure of SbtC, conserved regions as well as potential binding sites for interactions with DNA or other proteins were identified *in silico*. Structural analysis of SbtC identified a central transmembrane helix and a downstream region displaying a protein-binding motif. Moreover, Δ*sbtC* mutants have been generated using the cyanobacterial model *Synechocystis* sp. PCC 6803 and comparative growth analyses have been conducted compared to wild type and further mutants deficient in other inorganic carbon transporters such as NDH-1_3, NDH-1_4, and CmpA. We also performed transcriptomics with the Δ*sbtC* strain to uncover the impact on the gene regulatory network potentially associated with SbtC. Microarrays revealed a significant decrease in transcript abundance of ten protein encoding genes, including *sigA*, *rbcS*, and *cpcG2*. In combination with the reduced growth exhibited under diurnal illumination this may point towards an involvement of SbtC in the mediation of cyanobacterial photoacclimation, potentially through interaction with a transcription factor.

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P-PPM-286

Biosynthesis and Bioorthogonal labelling of Cyclopeptides harboring reactive Furyl side chains

*F. J. Ehinger[1], S. P. Niehs[1], B. Dose[1], K. Scherlach[1], F. Trottmann[1], C. Hertweck[1]
[1]Leibniz-HKI Jena, Biomolecular Chemistry, Jena, Germany

3-Furylalanine (Fua) is a reactive amino acid that occurs naturally in a limited number of cyclopeptides. For example, hepatotoxic rhizonins are produced by bacterial endosymbionts of *Rhizopus microsporus*,[1] and their toxicity critically depends on the Fua residues.[2] Fua moieties are also found in endolides, where the presence of Fua moieties confers receptor binding specificity.[3] Beyond, furyl-substituted peptides are amenable to bioorthogonal Diels-Alder-type derivatization.

Despite its relevance, Fua biosynthesis and incorporation by nonribosomal peptide (NRP) synthetases has remained elusive. By genome sequencing and gene inactivation, we discovered the gene cluster responsible for rhizonin biosynthesis. Mutational analysis and heterologous reconstitution identified the dioxygenase RhzB as a Fua synthase, and isotope labeling pinpointed tyrosine and L-DOPA as Fua precursors.[4] Interestingly, RhzB-like Fua synthases form a novel clade of heme-dependent aromatic oxygenases and are encoded in the genomes of phylogenetically distant bacterial phyla.

The prevalence of cryptic Fua-containing NRPs motivated us to devise a strategy to rapidly access novel clickable Fua-harboring bioactives. First, we screened various RhzB orthologs for Fua formation in a recombinant strain. After treating the native hosts" culture extracts with a dienophilic maleimide, LC-MS/MS analysis identified Diels-Alder-derivatized Fua-carrying compounds. This way, we discovered two groups of strongly antiproliferative Fua-containing cyclopeptides from Actinobacteria. We translated this approach to a tailor-made maleimide resin that allows the solid-support-based purification of Fua-harboring peptides.

The presented work provides insight into the biosynthesis and incorporation of the Fua pharmacophore, which permits bioorthogonal labelling, and shows the power of combining genome mining with reactivity-based screening to discover novel antiproliferative cyclopeptides.

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P-PPM-287

Investigation of temperature-regulated mechanisms in *Yersinia pseudotuberculosis*

*D. Meggers[1], F. Mrosek[1], D. Scheller[2], S. Pienkoß[1], F. Narberhaus[1]
[1]Ruhr University Bochum, Bochum, Germany
[2]Umeå University, Umeå, Sweden

Pathogenic bacteria are confronted with frequently changing conditions during infection of warm-blooded hosts and after excretion into the environment. They therefore must be able to respond to these changes rapidly and adapt their metabolism accordingly. Among various environmental factors, temperature plays an important role. Bacteria can react to changes in temperature at different levels of regulation. At the DNA level, transcription factors can up- or downregulate gene expression. At the RNA level, non-coding RNAs, such as small regulatory RNAs (sRNAs) and RNA thermometers (RNATs) can have a post-transcriptional effect on the translation efficiency of various mRNAs through temperature-induced conformational changes.

Yersinia pseudotuberculosis is a food-borne human pathogen that can lead to diseases of the gastrointestinal tract and the intestinal lymphatic system. Its optimum growth temperature is ~28 °C, but the cells can quickly react to an increase in host temperature to 37 °C and adapt their metabolism and virulence. In addition, *Y. pseudotuberculosis* can survive cold temperatures, allowing growth on food in the refrigerator.

While some regulatory mechanisms of *Y. pseudotuberculosis* are well described once the cells are taken up from the environment by a host, we aim to determine how the bacterium reacts when it is released from the host to low temperatures in the environment. RNA-Seq revealed numerous genes that might play an important role for *Yersinia* within 30 min after the temperature is reduced from 37 °C to 25 °C, 17 °C or 10 °C.

We are also interested in the function of the outer membrane protein OmpX of *Y. pseudotuberculosis*. It is primarily produced at 25 °C due to the temperature-mediated regulation by the sRNA CyaR (1). Interestingly, *Yersinia* produces a structurally similar, virulence-related protein, Ail, at 37 °C, which is upregulated by an RNAT. To examine the reciprocal production of OmpX and Ail at 25 °C or 37 °C, respectively, we carry out phenotypic characterizations of mutant strains to understand a potential connection between these outer membrane proteins. We found that single or combined deletions of both genes lead to an increased sensitivity at elevated salt concentrations.

1. Guanzon et al. Two temperature-responsive RNAs act in concert: The small RNA CyaR and the mRNA *ompX* bioRxiv 2024

P-PPM-288

Ensuring anaerobic survival – the critical role of UspK in metabolic adaptation during pyruvate fermentation

*S. Holland[1,2,3], A. Mustafayeva[4], A. Arce-Rodriguez[4,5], G. Molinari[6], J. M. Borrero de Acuña[4,5], D. Jahn[3,4], M. Neumann-Schaal[1,3]
[1]Leibniz Institute DSMZ, Metabolomics & Services, Braunschweig, Germany
[2]Technical University of Braunschweig, Institute of Biochemistry, Biotechnology and Bioinformatics, Braunschweig, Germany
[3]Technical University of Braunschweig, Braunschweig, Germany
[4]Technical University of Braunschweig, Microbiology, Braunschweig, Germany
[5]Universidad de Sevilla, Microbiology, Sevilla, Spain
[6]Helmholtz Centre for Infection Research – HZI, Central Facility for Microscopy, Braunschweig, Germany

Pseudomonas aeruginosa is a versatile opportunistic human pathogen associated with acute and chronic infections, particularly affecting immunocompromised individuals, including those with cystic fibrosis (CF). The bacterium's ability to thrive under diverse environmental conditions, such as oxygen limitation in the mucus of CF patients, is central to its pathogenicity and a key contributor to persistence in hostile settings. Under anaerobic conditions and the absence of other energy-generating systems, pyruvate fermentation serves as an alternative strategy ensuring long-term survival and basal metabolism. In these growth-limiting, nutrient-deficient conditions, the universal stress protein UspK was found to be highly upregulated. This study investigates the role of UspK in facilitating adaptation to anaerobic stress, revealing a significantly decreased anaerobic survival rate of the UspK mutant. Upon switching to pyruvate fermentation, we observed alterations in LPS levels and a significant increase in AMP levels. Further analysis showed that UspK displays a nucleotide-binding motif capable of binding AMP, suggesting high AMP levels may act as a signal for starvation beyond stringent response. Supported by phenotypic analysis, our results highlight the critical role of UspK in anaerobic stress adaptation via pyruvate fermentation and survival in such famine conditions. This study provides new insights into stress response and niche adaptation of *P. aeruginosa*, which are crucial for targeting chronic infections.

P-PPM-289

Aerobic gas fermentation with carboxydotrophic 'Knallgas' bacteria

*Á. Salazar-Quirós[1], D. Siebert[1], S. Grieshaber[1], B. Blombach[1]
[1]Technical University Munich, Microbial Biotechnology, Straubing, Germany

Industrial waste gases emit large amounts of pollutants into the atmosphere and may contain significant amounts of CO_2, CO, H_2, and O_2 depending on the source. Such gas streams represent an abundant feedstock for gas fermentation to produce chemicals with microbial catalysts. Current gas fermentation technologies enable the transformation of gaseous feedstocks into products such as ethanol, utilizing anaerobic acetogenic bacteria. However, the energy-limited anaerobic metabolism makes the synthesis of ATP-intensive products challenging.

To overcome this limitation, we propose the utilization of carboxydotrophic 'Knallgas' bacteria as cell factories to convert gaseous streams into valuable chemicals. These organisms possess the ability to utilize CO as a sole energy source with O_2 to produce CO_2, which is subsequently used for biomass formation via the CBB cycle. Moreover, these organisms can also oxidize H_2 with hydrogenases to drive the reduction of CO_2 in the absence of CO. One representative is *Hydrogenophaga pseudoflava* DSM 1084, which shows comparably high growth rates under autotrophic conditions [1, 2].

Several knowledge gaps remain regarding the essential genes for CO utilization and electron transport of carboxydotrophic 'Knallgas' bacteria. A comparative genomic analysis between the carboxydotrophic strain *H. pseudoflava* DSM 1084 and the H_2-oxidizing, non-carboxydotrophic *H. pseudoflava* DSM 1034 elucidated genetic elements that might be the key for growth on CO. Therefore, we conducted a combined approach involving genetic engineering and adaptive laboratory evolution on *H. pseudoflava* DSM 1034, to produce the first-reported synthetic carboxydotrophic organism, able to grow with CO as its sole carbon and energy source. Genome sequencing and re-engineering of the key mutations found on the evolved strain, together with the knowledge gained from proteome-level analysis might allow the transferability of carboxydotrophy to other microbial hosts, thus broadening the scope of biocatalysts that can be utilized for converting gaseous feedstocks into value-added chemicals and biofuels.

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P-PPM-290

Fosfomycin perception by CreBC: Novel insights into *Escherichia coli*'s response to MurA blockage

*M. Bianchi[1], M. Borisova-Mayer[1], T. Harbig[2], K. Nieselt[2], H. Brötz-Oesterhelt[3], C. Mayer[1]
[1]Eberhard Karls University Tübingen, IMIT-Organismic Interactions/Microbial Glycobiology, Tübingen, Germany
[2]Eberhard Karls University Tübingen, IBMI, Tübingen, Germany
[3]Eberhard Karls University Tübingen, IMIT - Organismic Interactions/Microbial Glycobiology, Tübingen, Germany

Introduction:

Antimicrobial resistance will be a leading cause of mortality within a few decades, due to the current antibiotic discovery pipeline crisis. Thus, it remains essential to optimize the use of existing antibiotics by better understanding their broad cellular impact on bacteria. Fosfomycin (FOS), first discovered in 1969, remains active against multi-drug resistant (MDR) bacteria and its excellent pharmacological properties and unique mode of action make it ideal for synergistic therapies. Within bacteria, it covalently inhibits the enzyme MurA, blocking the first committed step of peptidoglycan synthesis. However, its clinical use against *Escherichia coli* often leads to high rates of resistance development and infection relapse.

Goal:

A better understanding of FOS cellular effects and off-target actions in *E. coli* could enable improved treatments, reducing the risk of regrowth of resistant or adapted bacteria.

Materials and methods:

Susceptibility to FOS was tested with gradient strip tests and broth dilutions in LB medium, monitoring the growth with a microplate reader. Transcriptional analyses were conducted

with Illumina RNA sequencing. *E. coli* BW25113 and KO-mutants of the genes of interest were tested. The induction conditions of these genes of interest were further assessed with a *lacZ*-reporter plasmid.

Results:

E. coli alters its transcriptional state in response to FOS, shortly before drug-induced lysis. Transcriptional analyses of exponentially growing *E. coli* treated with FOS showed the rapid up-regulation of genes of the CreBC core regulon (*cbrA*, *cbrB, cbrC, creD*). The CreBC two-component system (TCS) is known to be responsive to carbon source changes in *E. coli*, but its real purpose remains unclear. Knockout mutants of these genes showed heightened sensitivity to FOS, and a similar effect was observed in strains completely lacking the CreBC TCS. The link between FOS and the upregulation of the CreBC regulon core genes was further investigated with a *lacZ*-reporter of *creD* expression.

Summary:

FOS-treated *E. coli* upregulated CreBC-related genes, and their role in response to FOS bactericidal action was investigated. This work advances efforts to better exploit the potential of FOS in MDR therapy, while preventing resistance development.

P-PPM-291

In vitro characterisation of central glycogen synthesis enzymes in _Synechocystis_ sp. PCC 6803 reveals complex regulatory mechanisms

*K. Lee[1]
[1]*Eberhard Karls University Tübingen, Organismic Interactions, Tübingen, Germany*

In cyanobacteria such as the model strain *Synechocystis* sp. PCC 6803, ADP-Glucose (ADP-Glc) is produced from Glucose-1-phosphate (Glc-1P) by Glucose-1-phosphate adenylyltransferase (GlgC). ADP-Glc is subsequently added to a growing glycogen molecule via a 1,4-alpha bond by glycogen synthases, present as two isoenzymes in *Synechocystis* (GlgA1 and GlgA2). This reaction is assisted by the branching enzyme (GlgB) which introduces branches that increases the number of potential extension sites for glycogen synthases. In this work, we reveal the fine-tuned regulatory mechanisms behind these enzymes using a NADH coupled-enzyme colorimetric assay, expanding upon the previously documented regulation of GlgC by 3-phosphoglycerate and inorganic phosphate [1,2]. In addition to that, we reveal the primer specificity and redox regulation of glycogen synthases, as well as modulatory effects of GlgC and other regulatory proteins, shedding light on the interconnected nature of glycogen synthesis regulation.

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P-PPM-292

Dormancy of the probiotic strain _Escherichia coli_ Nissle 1917 after long-term storage: A physiological and molecular characterization

*M. Thamm[1], F. Braas[2], M. Wegmann[2], B. Klinkert[3], R. von Bünau[3], B. Berghoff[1]
[1]*Ulm University, Molecular Biology and Biotechnology of Prokaryotes, Ulm, Germany*
[2]*Justus Liebig University Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany*
[3]*Pharma-Zentrale GmbH, Herdecke, Germany*

Mutaflor Suspension is a medical drug containing the probiotic strain *Escherichia coli* Nissle 1917 (EcN) as active ingredient. It is used to restore the human intestinal microbiota after e.g. diarrheal infection. After long-term storage (LTS) of Mutaflor Suspension at 4°C, EcN cells are impeded in their ability to form colonies on agar plates, while short-term storage (STS) does not affect culturability. The observed plating defect after LTS can be overcome by regeneration of suspensions at 37°C. Here, we use Mutaflor Suspension as a model system for bacterial dormancy and investigate the physiological and molecular differences. STS and LTS suspensions were either tested directly or after regeneration at 37°C using cultivation-based approaches in solid and liquid media. Furthermore, experiments with antibiotics, microscopic analysis, and ATP assays were performed and compared to exponential and stationary phase laboratory cultures. To elucidate the molecular response, total RNA was isolated and analyzed by RNA-sequencing. We found that Mutaflor suspensions showed an increased antibiotic tolerance as compared to exponential EcN cultures, clearly indicating the expected stress tolerance of dormant cells. The cultivation-based approaches further demonstrated that EcN in LTS suspensions regenerate in dependence of their cell density. Finally, the RNA-sequencing analysis revealed several candidates that might be either involved in establishing dormancy upon LTS or the regeneration process after LTS. Our study provides new insights into the dormancy and regeneration of bacteria, and represents an important basis for future research.

P-PPM-293

The path of the iron-sulfur clusters into the proteins: Unveiling the Sulfur Mobilization Machinery

*L. Schmück[1], J. M. Schuller[1], S. Bohn[2]
[1]*Philipps-University Marburg, AG Schuller, Marburg, Germany*
[2]*Helmholtz Centre Munich, Munich, Germany*

Introduction: Iron-sulfur cluster containing proteins are ubiquitous in nature and conserved across all kingdoms of life; however, iron-sulfur clusters are not assembled within their target proteins. Instead, iron-sulfur clusters are assembled on scaffold proteins assisted by specialized assembly machinery. Currently, four iron-sulfur cluster machineries are known: the NIF (nitrogen-fixation system), ISC (iron-sulfur cluster assembly machinery), CIA (cytosolic iron-sulfur cluster assembly machinery), and SUF (sulfur mobilization machinery) [1]. The SUF machinery is the most ancient of the identified iron-sulfur cluster biogenesis systems. The SUF machinery contains the proteins SufS, SufE or SufU, SufBCD or SufBC, and SufA. The SufBCD / SufBC protein complex"s postulated function is the assembly of iron-sulfur clusters on its SufBD subunits [2]. SufC belongs to the highly diverse ABC ATPase-family, whose function within the iron-sulfur cluster biogenesis pathway remains unresolved [3]. To overcome this, structures in the enzyme"s conformational cycles are required to understand how the ATPase movement is related to the formation of a nascent iron-sulfur cluster.

Objective: We determined a high-resolution structure of the SufBC protein complex, concentrating on the proposed iron-sulfur cluster forming residues and the proposed ATPase activity-induced, conformational changes.

Materials and methods: We utilised single-particle cryo-electron microscopy (cryo-EM) to create structural models of the purified SufBC protein complex.

Results: The SUF machinery components were overexpressed, purified and cryo-EM sample preparation was carried out under redox controlled conditions. UV/Vis spectroscopy confirmed the presence of an iron-sulfur cluster in the anaerobically purified SufBC complex. Preliminary data processing revealed SufBC undergoes conformational changes, possibly in relation to the presence of an iron-sulfur cluster and bound substrate.

Conclusion: Overall, preliminary structural information acquired by single particle cryo-EM shows that the SufBC protein complex adopts different structural conformations during iron-sulfur cluster assembly.

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P-PPM-294

Structural and mechanistic investigation of the key enzyme orchestrating backbone rearrangement in rishirilide biosynthesis

*H. Weddeling[1], S. Sowa[1], E. Bialas[2], A. Bechthold[2], R. Teufel[1]
[1]University of Basel, Department of Pharmaceutical Sciences, Basel, Switzerland
[2]Albert-Ludwigs-University, Institute of Pharmaceutical Sciences, Freiburg i.Br., Germany

Rishirilide A and B, first isolated in 1984 during a α2-macroglobulin inhibitor screen, belong to the class of tricyclic aromatic polyketides and are produced by multiple *Streptomyces* strains (1). Labeling studies validated the polyketide synthase-dependent biosynthesis and already suggested an unusual backbone rearrangement of the isopentyl substituent (2). Knockout studies in the rishirilide producer *Streptomyces bottropensis* in combination with *in vitro* enzyme reactions revealed RslO9, a FAD-dependent Monooxygenase (FPMO), as the key player in the last steps of rishirilide biosynthesis (3). However, the catalytic mechanism of this biosynthesis leading to rishirilide A/B and lupinacidin A remains unknown. Unraveling the intriguing activity could give insights into the structural diversification of aromatic polyketides and FPMO chemistry.

For in depth analysis, RslO9 and N-terminal deletion variants were cloned and heterologously expressed, followed by purification and structure elucidation by X-ray crystallography. The molecular structure of RslO9 was obtained at a resolution of 2.4 Å. Cocrystallization attempts with the native substrate prevented formation of crystals, while soaking experiments revealed a conformational change in the active site, although no clear electron density for the substrate could be observed. Activity assays with numerous substrate-like compounds showed conversion of multiple naphthoquinones and implied a facilitating effect of alkyl substituents. On the other hand, anthraquinone derivatives were not converted, suggesting a terminal paraquinone moiety as an important feature for catalysis. Furthermore, docking and molecular dynamics simulations as well as site-directed mutagenesis studies are ongoing to pin down essential residues for substrate binding and catalysis.

In conclusion our studies give insights into the backbone rearrangement performed by RslO9, further deepening our understanding of flavoprotein chemistry and polyketide tailoring reactions.

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P-PPM-295

Analysis of the antibacterial mechanism of Farnesyltransferase inhibitors

*L. Mokhlessi[1], L. Weber[1], S. Schäkermann[2], J. E. Bandow[2], H. S. Bachmann[1]
[1]Witten/Herdecke University, Pharmacology and Toxicology, Witten, Germany
[2]Ruhr University Bochum, Microbiology, Bochum, Germany

Introduction

Farnesylation is a posttranslational modification, involving the attachment of a C15 isoprene unit (farnesyl) to proteins with a specific CAAX motif. Farnesylation enables over 200 proteins to bind to the membrane and fulfil their function in processes like cell growth, differentiation or apoptosis. The inhibition by farnesyltransferase inhibitors (FTI), such as lonafarnib or tipifarnib, came in focus for the treatment of various diseases, e.g. cancer. Furthermore, we demonstrated antibacterial effects of lonafarnib and tipifarnib in gram-positive bacteria such as *S. aureus* or *S. epidermidis* and even in the multi-resistant strain MRSA [1]. However, the underlying molecular mechanism remains elusive.

Material & Methods

We determined the minimal inhibitory concentration (MIC) as well as the physiological effective concentration (PEK) of lonafarnib and tipifarnib on *B. subtilis*. To analyse the effect of FTIs on the proteome, we performed high-resolution liquid chromatography followed by mass spectrometry analysis.

Results

In total, 1060 proteins were detected via mass spectrometric analysis. Treatment with tipifarnib resulted in the upregulation of 38 proteins in *B. subtilis*, whereas treatment with lonafarnib upregulated 89 proteins. 33 proteins were regulated by both FTI. Among the identified marker proteins, those involved in the biosynthesis and modification of cell wall components, such as DitE, YocH or PbpE, were particularly important. Additionally, marker proteins related to protein biosynthesis, general stress response, oxidative stress and various transport processes were detected.

Discussion

It is known that both lonafarnib as well as tipifarnib show antibacterial effects in different bacterial strains, however, the underlying mechanism remains elusive. Here, we could show that both lonafarnib and tipifarnib treatment resulted in a significant change in several proteins, whereas the treatment with lonafarnib led to a change in twice as many proteins as tipifarnib. Strikingly, many marker proteins were proteins involved in processes affecting the cell envelope. Therefore, we assume that the cell wall and cell membrane are the main targets for the FTI in *B. subtilis*.

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P-PPM-296

Kinetic and mechanistic characterization of the carboxyvinyl transferase isozymes (MurAA & MurAB) of *B. subtilis*

*Y. Stahl[1], S. Friz[1], P. Stincone[2], D. Petras[2], C. Mayer[1]
[1]*Eberhard Karls University Tübingen, IMIT - Organismic Interactions/Microbial Glycobiology, Tübingen, Germany*
[2]*Eberhard Karls University Tübingen, IMIT - Infectionbiology, Tübingen, Germany*

Introduction:

The carboxyvinyl-transferase MurA catalyses the first committed step of the peptidoglycan biosynthesis allowing bacteria to sustain a functional cell wall, thus, it is generally essential. MurA is of medical importance, since it is the molecular target for the broad-spectrum antibiotic fosfomycin (FOS). FOS binds to an active-site cysteine of MurA, forming a highly stable thioether bond, which covalently blocks the enzyme, preventing substrate binding and keeping MurA in an inactivated state. While Gram-negative have one isoform, Gram-positive usually have two or more isoforms of the enzyme. *Bacillus subtilis* has two MurA isoforms: while MurAA is essential, MurAB is expandable. The reason for *B. subtilis* having two isoforms of MurA is unknown and their physiological roles, and kinetic and mechanistic differences have not been investigated, so far.

Goal:

Our aim is to gain a better understanding of the MurA isoforms of *B. subtilis*, in terms of catalytic properties, susceptibility to FOS, and physiological functions.

Materials and methods:

MurAA and MurAB of *B. subtilis* were cloned, heterologously expressed, purified and biochemically characterized. A malachite-green based phosphate assay was used to determine their kinetic parameters and the IC50 for FOS. With native mass spectrometry (nMS) we investigated the occupation of the active site cysteine, after incubation with substrate or antibiotic at varying concentrations.

Results:

MurAA appears to have a higher affinity towards phosphoenolpyruvate (PEP) and FOS, while MurAB has a higher maximal velocity. Most interestingly, purified MurAA and MurAB both carried a PEP molecule covalently bound to the active-site cysteine residue, shown with nMS, and we were able to demonstrate that, in this form, both isozymes are no molecular targets for FOS. Addition of second substrate UDP-N-acetylglucosamine triggers the enzymatic reaction, relieves the active site and, in this form, enabled binding of FOS. Further, the two isozymes have differences in their active-site occupation, where multiple substrates/products are bound to MurAB, but not to MurAA.

Summary:

Using nMS, we were able to directly show, for the first time, that MurA isozymes are isolated from cells in a FOS-insensitive form with a PEP covalently blocking the active site cysteine. Although the MurA isozymes of *B. subtilis* have rather similar kinetic properties, differences occur in reaction rate and the affinity for PEP and FOS.

P-PPM-297

Impact of flavor enhancers on the structure and function of the intestinal microbiome

*N. Nematzadeh Somehsaraei[1,2], P. Schmitt-Kopplin[1,3], J. Lemuel Hadi[4,5], M. Khan Mirzaei[4,5], L. Deng[4,5], C. Dawid[1,6], M. Schloter[1,2], S. Gschwendtner[2]
[1]*Technical University Munich, Institute for Food & Health (ZIEL), Freising, Germany*
[2]*Helmholtz Centre Munich, Research Unit for Comparative Microbiome Analysis, Munich, Germany*
[3]*Helmholtz Centre Munich, Research Unit Analytical Biogeochemistry, Munich, Germany*
[4]*Helmholtz Centre Munich, Institute of Virology, Munich, Germany*
[5]*Technical University Munich, Chair of Prevention of Microbial Diseases; Central Institute of Infection Prevention (ZIP), Freising, Germany*
[6]*Technical University Munich, Chair of Food Chemistry and Molecular Sensory Science, Freising, Germany*

Introduction: The human intestinal microbiome plays a vital role in human health and is influenced by both dietary and non-dietary factors. Food additives like monosodium glutamate (MSG) are commonly used in the food industry as taste enhancers; however, their impact on the gut microbiome is still not well understood. Glutamate acts as a signaling molecule in the enteric nervous system, affecting neuroendocrine responses, suggesting that the gut microbiome could also be responsive to MSG.

Goals: Using an in vitro simulator of the human intestinal microbial ecosystem (SHIME®) this study investigated the effects of MSG on the colonic microbiome in a case-control design focusing on transcriptional and metabolic responses.

Materials and Methods: SHIME® is a chemostat system that can simulate the luminal colon region via different bioreactors representing the stomach/small intestine, as well as proximal (PC) and distal colon (DC). The study was conducted using a defined community of eight bacterial strains (SIHUMIx) representing the important gut phyla Firmicutes (*Blautia producta*, *Clostridium butyricum*, *Clostridium ramosum*, *Lactobacillus plantarum*, *Anaerostipes caccae*), Bacteroidetes (*Bacteroides thetaiotaomicron*), Actinobacteria (*Bifidobacterium longum*), and Proteobacteria (*Escherichia coli*). We applied MSG in a mixture with a defined medium containing 25% starch at a concentration of 0.5 mg/mL, which was adjusted to pH 2 and then heated by autoclaving.

Results: Over a three-week period (stabilization, treatment, and post-treatment phases), shifts in microbial community composition and gene expression patterns were analyzed. In addition, short-chain fatty acid (SCFA) profiling was performed. As expected, the relative abundance of members of the synthetic communities varied in the different simulated

gut compartments. In the proximal colon, *B. thetaiotaomicron*, *L. plantarum*, *E. coli and C. butyricum* were predominant, while the distal colon was dominated by *B. thetaiotaomicron*, *B. producta*, *E. coli and A. caccae*. The application of MSG led to a reduction in the abundance of *B. thetaiotaomicron*, *L. plantarum*, and *C. butyricum* in the proximal colon, as well as a decrease in *B. thetaiotaomicron* and *E. coli*, and an increase in *B. producta* in the distal colon. A reduction in SCFA concentrations was detected across both colon regions. Further findings, including transcriptomic data and detailed results, will be presented in the frame of the poster.

P-PPM-298

Regulation of the carbon flux in *Synechocystis* using the PGAM-PirC Switch

*K. Forchhammer[1], N. S. Becker[1], T. Orthwein[1]
[1]Eberhard Karls University Tübingen, IMIT - Organismic Interactions/Microbial Glycobiology, Tübingen, Germany

Cyanobacteria are autotrophic organisms, which perform oxidative photosynthesis. Their ability to oxidize water and use the electrons for reducing carbon dioxide (CO2) to form organic matter by using solar energy makes them an interesting organism for biotechnological applications. Therefore, *Synechocystis* sp. PCC 6803 is studied as a model organism for the construction of green cell factories.

An important control point of the carbon flux is the 2,3-bisphosphoglycerate-independent phosphoglycerate-mutase (PGAM), which converts the first CO2 fixation product 3-phosphoglycerate to 2-phosphoglycerate. This reaction directs carbon flow towards lower glycolysis for the production of amino acids, fatty acids, biopolymers, such as polyhydroxybutyrate (PHB) and more. PGAM activity is inhibited through binding of the small protein PirC, which itself is repressed by the PII protein, the sensor protein for the carbon/nitrogen status [1].

The aim is to use this PGAM-PirC key hub to direct the metabolic flux towards lower glycolysis and the production of PHB and other feedstock chemicals. Therefore, a strain constitutively overexpressing *pgam* was constructed and the level of PGAM and glycogen, PHB production and metabolite excretion were analyzed. In addition, several deletion mutants were tested, *e.g.,* a Δ*phaEC* mutant, which cannot produce PHB. Furthermore, we analyzed the PGAM enzyme biochemically and identified structural elements required for regulation by PirC [2]. Strains with the corresponding PGAM variants were also constructed.

Our results revealed that PGAM overproduction led to lower glycogen levels, whereas PHB amounts were higher under nitrogen depletion compared to the WT. Deletion of *pirC* or *phaEC* resulted in higher concentrations of the tested metabolites pyruvate, succinate and 2-oxoglutarate extracellularly. The double mutant lacking *pirC* and *phaEC* showed the highest excretion of the tested metabolites.

Our work shows the important role the PGAM-PirC switch is playing in the metabolism. By tuning this regulation further, a platform will be established to redirect carbon flow for enhanced valuable chemical production.

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P-PPM-299

The heterodisulfide reductase-like (sHdr) complex for dissimilatory sulfur oxidation

*M. Grosser[1], T. Wagner[2], C. Dahl[1]
[1]Rheinische Friedrich-Wilhelms-Universität Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany
[2]Max Planck Institute, Bremen, Germany

Many dissimilatory sulfur-oxidizing bacteria and archaea pursue a pathway for sulfur oxidation that involves heterodisulfide reductase-like (sHdr) enzymes. Its core proteins are encoded in a strictly conserved *shdrC1B1AHC2B2* cluster in Pseudomonadota and Aquificae and are similar to the subunits of the heterodisulfide reductase from methanogenic archaea. In the Alphaproteobacterium *Hyphomicrobium denitrificans* the sHdr proteins are essential for the use of thiosulfate as an accessory electron donor [2].

We aim at the structural and biochemical characterization of the sHdr complex, including the elucidation of its cofactors and the identification of possible interaction partners. The sHdrAB1B2C1 and -C2 proteins were co-purified from membrane fractions of the hyperthermophile *Aquifex aeolicus* [2]. In contrast, hyphomicrobial sHdrA is soluble [1,3]. Here, we show that all sHdr proteins in the obligately chemolithoautotrophic Gammaproteobacterium *Thioalkalivibrio thiocyanoxidans* are soluble, as evidenced by immunodetection. sHdrAB1B2C1C2 is co-purified from *T. thiocyanoxidans* as a heterocomplex, containing FAD and multiple iron-sulfur clusters after two anion exchange chromatography steps. SDS-PAGE, Blue-Native-PAGE followed by 2D-SDS-PAGE and mass spectrometry revealed that it not only interacts with several other proteins encoded in the vicinity of the core *shdr* genes, such as the sulfur transferase DsrE3B, but that the complex composition may be dynamic with regard to the number and identity of the core subunits present. The sHdrH protein, which is inevitably encoded within the *shdr* genes and has no counterpart in methanogens, does not appear to be a component of the sulfur-oxidizing sHdr complex although it is not only co-transcribed and transcriptionally co-regulated with the core *shdr* genes in *H. denitrificans*, but also indispensable for thiosulfate oxidation. Together, our results provide first glimpses into the reaction mechanism of the sHdr complex.

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P-PPM-300

Investigating the regulation of the carbon switch in central metabolism of *Synechocystis* sp. PCC 6803

*R. S. Ojha[1], L. Shen[1], C. Peraglie[1], C. Bräsen[1], B. Siebers[1]
[1]University of Duisburg-Essen, Molecular Enzyme Technology and Biochemistry, Essen, Germany

Dependent on the light/nutrient conditions *Synechocystis* is able to grow auto-, hetero-, and mixotrophically for which the reversibility of sugar metabolism particularly the Embden-Meyerhof-Parnas pathway (EMP) is essential. To switch between anabolic and catabolic direction sophisticated regulation is required and also the interplay of e.g. EMP and Calvin-Benson-Bassham (CBB) cycle for CO_2 fixation must be tightly balanced. The antagonistic PFK and FBPase operate exclusively in the catabolic or anabolic direction of

the EMP, respectively, and PFK is the classical control point of glycolysis. Two paralogous copies of both enzymes are present in *Synechocystis*. Furthermore, under mixotrophic conditions the reversible phosphoglucose isomerase (PGI) shunt has been shown to direct fluxes via the non-oxidative pentose phosphate pathway (NOPP) to the CBB cycle.[1] However, the control points and the function and regulatory capacities of the enzymes are not established in *Synechocystis*.

Detailed enzymatic characterization revealed that both PFKs are ADP dependent, thereby defining a new class of ADP-dependent PFKs within the PFK-A superfamily. PFK-A1 is inhibited by 3-phosphoglycerate (3PG), and PFK-A2 by ATP.[2] FBPase is not regulated by various effectors whereas F/SBPase is bifunctional and inhibited by AMP and activated by DTT.[3] The PGI displays a preference for the gluconeogenic direction and is inhibited by erythrose 4-phosphate (E4P) and 6-phosphogluconate (6PG).

Our investigations reveal that in line with the ADP-dependence both PFKs are active under conditions of low energy-charge, facilitating the breakdown of glycogen/D-glucose. The inhibition of PFK-A1 and PFK-A2 by 3PG and ATP, respectively, helps in rapid generation of CO_2 acceptor molecule. The sustained FBPase activity in presence of various effectors suggests its housekeeping function to enable reversibility of the EMP pathway in response to substrate availability/growth conditions. E4P and 6PG regulation enables PGI function as a valve to balance the flux via the PGI shunt into NOPP or the oxidative pentose phosphate pathway, respectively.

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P-PPM-301

Wadjet System Inactivation Boosts Plasmid Acquisition in Phylogenetically Distant Gram-positive Bacteria *Clostridium cellulovorans* and *Micrococcus luteus*

A. Schöllkopf[1], *M. Baudrexl[1], A. Ehrenreich[1], W. Liebl[1]
[1]*Technical University Munich, Microbiology, Freising, Germany*

Innate defense mechanisms heavily influence the ability of some bacteria to take up and maintain plasmid DNA. The Wadjet system is a recently characterized defense system that targets and cleaves circular plasmid DNA. In this study, we report the consequences of the knockout of Wadjet system-encoding genes in two Gram-positive model bacteria, *Clostridium cellulovorans* (phylum *Bacillota*) and *Micrococcus luteus* (phylum *Actinomycetota*), for the uptake of plasmid DNA via transconjugation and natural transformation, respectively.

Genome sequencing of a spontaneous *C. cellulovorans* mutant with a dramatically enhanced frequency of plasmid DNA acquisition through conjugation revealed three chromosomal deletions, one of them including a putative Wadjet system-encoding *jetABCD* gene cluster. After deletion of only this gene cluster in the wildtype, the efficiency of conjugative plasmid transfer from *E. coli* to *C. cellulovorans* Δ*jetABCD* increased by about five orders of magnitude, confirming that the Wadjet system represents an efficient barrier to plasmid transfer in *C. cellulovorans*. Additional *in vivo* methylation of the plasmid in the *E. coli* donor to protect it from restriction by a type II RM endonuclease present in *C. cellulovorans* further improved uptake by conjugation.

In *M. luteus* strain TrpE16, a model organism for the study of DNA uptake by natural transformation in high-GC Gram-positive bacteria, deletion of the chromosomal *jetABCD* gene cluster likewise substantially increased the frequency of plasmid uptake, in this case by natural transformation. Via introduction of the Δ*jetABCD* deletion it was possible to overcome a known limitation of *M. luteus* TrpE16 to be transformed with circular plasmid DNA. Notably, reisolation and analysis of plasmids from the Wadjet-deficient and the parental *M. luteus* strains seemed to yield different topologies or concatemeric states of the plasmid.

These findings collectively underscore the restrictive role of Wadjet systems in two phylogenetically distant Gram-positive bacteria from the *Actinomycetota* (high-GC) and *Bacillota* (low-GC) on plasmid stability and consequently on the efficiency of gene transfer via natural transformation and conjugation. This demonstrates the importance of considering Wadjet systems in addition to RM and CRISPR-Cas systems as defense systems that can seriously hinder the introduction of recombinant plasmids for genetic engineering purposes.

P-PPM-302

A naturally isolated co-culture of *Streptomyces* species displays antifungal synergy

*G. Sohl[1], D. Iliasov[1], T. Mascher[1]
[1]*Technische Universität Dresden, Institute of Microbiology, General Microbiology, Dresden, Germany*

Fungal pathogens pose unique threats to modern healthcare: current treatment is limited to four antifungal drug classes, and many produce adverse side effects in patients[1]. Exploration of new environments could lead to the isolation of novel antifungal-producing microorganisms.

We report the isolationed and characterization ofed a mixed isolate of two *Streptomyces* species from dried moth larvae (*Cirina forda*) that exhibited antifungal synergy against filamentous fungi. Antimicrobial profiles were obtained through overlay assays with mature colonies. The observed antibacterial and antifungal activity was further characterized in regards to cellular targets of produced compounds using a panel of whole-cell biosensors containing the luciferase cassette under the control of an antibiotic-specific inducible promotor. Variation in colony morphology led to the subculturing of two morphologically distinct isolates, both of which displayed reduced activity against filamentous fungi. Whole genome sequencing identified these sub-strains as *Streptomyces albidoflavus*. Nucleotide variations in regulatory, metabolic genes, and biosynthetic gene clusters (BGCs) were found that may explain the observed differences in strain morphology, physiology, and antimicrobial activity. Biosensor tests indicated the production of antifungal polyene macrolide-like compounds in the strains and mixed isolate, and genome mining confirmed the presence of the BGC for the polyene macrolide candicidin. Further analysis revealed discrepancies between the antimicrobial profiles of candicidin and the mixed isolate. Determination of produced antimicrobial compounds via HPLC-MS is currently ongoing.

This work demonstrates the co-isolation of antifungal compound-producing organisms from a niche habitat.

Differences in antimicrobial activity between the sub-strains and the observed synergistic relationship in the mixed isolate indicate cross-regulation and activation of antifungal BGC expression in one or both strains. Further research towards these microbial interactions could aid the discovery and development of new antifungals as well as further our understanding of antifungal BGC regulation and the effects and mechanisms of strain interaction on secondary metabolite production in *Streptomyces* species.

1. WHO fungal priority pathogens list to guide research, development and public health action. Geneva: World Health Organization 2022; Licence: CC BY-NC-SA 3.0 IGO.

P-PPM-303

Characterization of the key enzyme of Isoprenoid Biosynthesis DXS from *Corynebacterium glutamicum*

*M. Rogowski[1], P. Peters-Wendisch[1], V. F. Wendisch[1]

[1]Bielefeld University, Genetics of Prokaryotes, Faculty of Biology and Center for Biotechnology (CeBiTec), Bielefeld, Germany

In isoprenoid biosynthesis, 1-Deoxy-D-xylulose 5-phosphate synthase (DXS) catalyzes the first step of the methyl erythritol phosphate (MEP) pathway by converting pyruvate and glyceraldehyde-3-phosphate to 1-deoxy-D-xylulose 5-phosphate (DXP). DXP serves as a primary intermediate in the biosynthesis of thiamine and terpenoids, such as carotenoids. Previously, overexpression of DXS in *Corynebacterium glutamicum* resulted in a twofold increase in carotenoid production [1], corroborating that DXS catalyzes a rate-limiting step in terpenoid biosynthesis. However, the available knowledge on the regulation of DXS in *C. glutamicum* is limited. Previously, the lysin residue 352 of DXS from *C. glutamicum* was shown to be pupylated by a prokaryotic ubiquitin-like regulatory mechanism [2], but the physiological consequences are unknown.

In order to gain insight into the regulation of DXS in *C. glutamicum*, K352 was changed to either glutamic acid or alanine to obtain DXS variants that cannot be pupylated. These variants were expressed in *E. coli*, purified using a HisC tag and enzymatically characterized *in vitro*. Interestingly, the pupylation deficient variants DXS^{K352E} and DXS^{K352A} showed almost twice the enzymatic activity than the wild-type DXS (0.68 U/mg and 0.72 U/mg vs 0.37 U/mg, respectively). The overexpression of wild-type DXS in the *C. glutamicum* WT strain led to a shorter lag-phase, but reduced the decaprenoxanthin titer compared to the overexpression of the pupylation deficient mutants. By contrast, in the pupylation deficient strain *C. glutamicum* Δ*pup* no such differences occurred. The mechanism of regulation of DXS by pupylation in *C. glutamicum* remains to be disclosed.

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P-PPM-304

Scavenging nitrogen in the gut: the next step of *Nε*-carboxymethyllysine metabolism in *Escherichia coli*

*E. F. Aveta[1], J. Mehler[1], K. I. Behringer[2], N. Gericke[1], M. Walczak[3], P. Vougioukas[3], M. Hellwig[3], J. Lassak[1]

[1]Ludwig Maximilians University, Microbiology, Munich, Germany

[2]Technical University of Braunschweig, Institute of Food Chemistry, Braunschweig, Germany

[3]Technische Universität Dresden, Special Food Chemistry, Dresden, Germany

Amino acids are modified through a plethora of enzymatic and non-enzymatic reactions. Glycation, or Maillard reaction, takes place at high temperatures between reducing sugars and free amino groups of proteins, producing compounds commonly found in processed foods (Maillard *et al*. 1912). One of the best-known glycated amino acids, *Nε*-carboxymethyllysine(CML), is consumed widely by humans reaching a daily intake of 24.6 mg(Mark *et al*. 2014). Most of it remains undigested, and the limited absorption in the gastrointestinal tract suggests a major role in its degradation by colonic bacteria (Lassak *et al*. 2023). *E. coli* produces novel metabolites when exposed to CML, mainly carboxymethylcadaverine, however no enzyme responsible for this degradation has been identified (Hellwig *et al*. 2019). We have unveiled an underground metabolism proceeding through promiscuous enzymes that accept CML and its downstream metabolites as substrates. Ornithine decarboxylase SpeC is the first enzyme that generates CM-Cad. Furthermore, *E. coli* can utilize CML as a sole nitrogen source, and based on the identified metabolites, we discovered that GABA transaminase GabT accepts CM-Cad and CML as substrates, with the formation of glutamate. Our findings suggest that the degradation route proceeds similarly to other biogenic amines like cadaverine or putrescine, likely involving other enzymes with broad substrate specificity. This study reveals a new metabolic route within *E. coli*, with the generation of unique compounds that may influence both gut microbiota dynamics and host physiology. The unknown effects of these metabolites on other gut bacteria and the human host warrant further exploration.

P-PPM-305

Structure function analysis of ADP-dependent cyanobacterial phosphofructokinase reveals new phylogenetic grouping in the PFK-A family

*C. Peraglie[1], L. Shen[1], D. Podlesainski[2], C. Stracke[1], R. S. Ojha[1], F. Caliebe[3], M. Kaiser[2], K. Forchhammer[4], M. Hagemann[5], K. Gutekunst[3], J. L. Snoep[6], C. Bräsen[1], B. Siebers[1]

[1]University of Duisburg-Essen, Molecular Enzyme Technology and Biochemistry, Essen, Germany

[2]University of Duisburg-Essen, Chemical Biology, Centre of Medical Biotechnology (ZMB), Essen, Germany

[3]University of Kassel, Molecular Plant Physiology, Kassel, Germany

[4]Eberhard Karls University Tübingen, IMIT - Organismic Interactions/Microbial Glycobiology, Tübingen, Germany

[5]University of Rostock, Plant Physiology, Rostock, Germany

[6]University of Stellenbosch, Institute of Biochemistry, Stellenbosch, Germany

In photosynthetic organisms, efficient carbohydrate metabolism is crucial for adapting to varying environmental conditions, particularly during transitions between light and dark cycles. Phosphofructokinase (PFK) plays a pivotal role in glycolysis, acting as one of two key regulatory points that govern the flow of metabolites via the Embden-Meyerhof-Parnas (EMP) pathway. Three different types of PFKs have been described that vary in their phosphate donor, i.e. ATP, ADP or PPi and regulatory properties [1]. *Synechocystis* PFK activity has been analysed in cell extracts [2], and it was

classified as member of the PFK-B family, even though sequence comparisons indicated them to be members of the PFK-A family [3].

We aimed to characterize the enzymatic properties, regulatory mechanisms, and evolutionary adaptations of the two PFK isoenzymes in *Synechocystis*, particularly their roles in adjusting to changing growth conditions and metabolic states.

The genes encoding PFK-A1 and PFK-A2 from *Synechocystis* were cloned, expressed, and purified. Kinetic parameters were determined under varying substrate and effector concentrations, and the phylogenetic relationships were examined through sequence comparisons and structural modeling.

Both PFK-A1 and PFK-A2 showed exclusive dependence on ADP for catalytic activity, with distinct regulatory profiles: PFK-A1 is inhibited by 3-phosphoglycerate, while PFK-A2 is inhibited by ATP. Both of these inhibitors accumulate during photosynthesis, facilitating enzyme inactivation during light. Phylogenetic analysis identified these ADP-dependent PFK-A isoenzymes as monophyletic group within the PFK-A family, comprising cyanobacterial evolutionary lineage and structural adaptation among cyanobacteria and homologs [4].

The study provides new insights into the regulatory properties and evolution of PFK-A isoenzymes in cyanobacteria, emphasizing an adaptive mechanism for balancing glycolysis and gluconeogenesis in response to light and energy charge of the cell. In addition, their ADP dependence offers interesting potential for optimization of carbohydrate metabolism in metabolic engineering and synthetic biology approaches.

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P-PPM-306

Elucidating the mechanisms of symbiosis in bacterial consortia formed by cyanobacteria with proteobacterial epibionts

*S. B. Kuzyk[1], F. Burkart[1], A. Methner[1], M. Mohammadi Livari[2], M. Müsken[3], M. Neumann-Schaal[4], S. Pradella[5], A. Röthke[2], O. Rienitz[2], J. Overmann[1]

[1]DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Mikrobielle Ökologie und Diversitätsforschung, Braunschweig, Germany

[2]PTB-Physikalisch-Technische Bundesanstalt, Inorganic Chemistry, Braunschweig, Germany

[3]Helmholtz Centre for Infection Research – HZI, Central Unit for Microscopy, Braunschweig, Germany

[4]DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Services Department, Braunschweig, Germany

[5]DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Cyanobacteria and Protists Collection, Braunschweig, Germany

Cyanobacteria are key primary producers in almost every illuminated environment and often represent a significant proportion of natural microbial communities. Consequently, they serve as sources of organic carbon for many other surrounding microorganisms. While the resulting bacterial interactions are often antagonistic, commensal and mutualistic adaptations are also known. A cyanobacterium of the genus *Anabaena* has been shown to closely interact with the heterotrophic proteobacterium *Hoeflea* sp., constituting a conspicuous form of bacterial heterologous multicellularity. Within this multicellular consortium the *Hoeflea* epibiont adheres in high numbers specifically to the heterocysts of the filamentous cyanobacterial partner. This model system offers unique opportunities for the study of symbiosis as (1) it involves two bacteria with medium-sized genomes, (2) each partner can be grown axenically in the presence of oxygen, (3) combining pure cultures of both restores the consortium phenotype.

We applied genomics, transcriptomics, metabolomics, metallomics, fluorescent and electron microscopic techniques to study the biotic interactions underlying this bacterial heterologous multicellularity. Following each partner in axenic culture, or as consortium, we demonstrated that specific metabolites and metals are sequestered by either species prior to being shared, which in turn enhances their combined growth. Cell signalling pathways were further identified via gene expression data that suggest this consortium to be related (and perhaps a predecessor) to the symbiosis of plants with nodulating rhizobia. Since the *Anabaena* and *Hoeflea* can be cultivated independently, and then mixed to form consortia, diversity assays were conducted to check the host and epibiont range by exchanging either partner for alternative species. In this way, we discovered that several members of *Nostocales* could form consortia, whereas each required the specific species of *Hoeflea*, providing greater context to the possible symbiosis detected in nature.

P-PPM-307

Triggering natural product synthesis in *Streptomyces sp.*

*F. M. L. Peeters[1], C. Anders[1], M. Ashraf[1], L. Sagurna[1], J. E. Bandow[1]

[1]Ruhr University Bochum, Microbiology, Bochum, Germany

Streptomyces sp. are the most prolific source of antibiotics in nature. In face of the rise in antibiotic resistance of pathogens, the numerous biosynthetic gene clusters (BGCs) encoded in *Streptomyces'* genomes give rise to undiscovered natural products (NPs) and may be a treasure trove for antibiotic discovery. Nevertheless, searching for new NPs through variation of cultivation conditions (1. Bode *et al.*) suffers from high rediscovery rates and non-annotated BGCs often remain silent. Commonly, this approach involves the following steps: cultivation, detection of bioactive extracts, dereplication and compound isolation. Activity-based screening has the disadvantage that low-abundant, bioactive metabolites may not be discovered because their activity is masked by that of other more abundant compounds. Subsequent fractionation often fails to yield active fractions because low abundant substances are lost. Here, eight new *Streptomyces* isolates with up to 39 non-annotated silent BGCs are cultivated using small molecular elicitors to trigger production of bioactive compounds (2. Alwali *et al.*). Using liquid-chromatography mass-spectrometry and dose-dependent response experiments, rapid dereplication of antimicrobials in bioactive extracts is achieved, identifying previously discovered compounds and annotating the corresponding BGCs. With CRISPR-Cas9, knock-out mutants are then generated that are unable to produce these compounds. Hereby the masking effect of the dereplicated compound is eliminated in the mutant strains

and low abundant bioactive molecules may in principle be detected using an identical workflow. Since in the mutant the functionality of the natural product is missing and no resources are spent on the production of the dereplicated molecule, it is also investigated whether resources are redirected to an increased production of other antimicrobials. The goal of this "knock-out and rescreen"-approach is to identify and isolate new and rare antimicrobial compounds.

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P-PPM-308

A cryptic acetyltransferase-like protein drives spiroketal ring contraction in rubromycin biosynthesis

*S. Sowa[1], H. Weddeling[1], R. Teufel[1]
[1]*University of Basel, Basel, Switzerland*

Rubromycins are a class of aromatic polyketides with potent antibiotic properties and are produced by a wide variety of actinobacteria. A distinctive feature of rubromycins is the presence of a [5,6]-spiroketal moiety at the core of the molecule. Previous work in our group elucidated the mechanism behind the initial formation of a [6,6]-spiroketal unit during rubromycin maturation through the concerted action of GrhO1 and GrhO5.[1] The resulting intermediate is subsequently transformed by the flavoprotein monooxygenase GrhO6, leading to the contraction of ring C to form the [5,6]-spiroketal structure found in mature rubromycins. Interestingly, efficient ring contraction only takes place in presence of GrhJ, which belongs to the family of GCN5-related N-acetyltransferases (GNAT).[2,3] However, the precise catalytic mechanism of GrhO6 and the role of GrhJ in this process remain to be elucidated. Here, we aimed to investigate the catalytic mechanism of GrhO6 and the role of GrhJ in the [5,6]-spiroketal formation by recombinantly producing these proteins and employing a combination of biochemical assays, biophysical methods, and X-ray crystallography. The crystal structure of GrhO6, together with biochemical and mutagenesis studies, indicates that a potential acetylation of GrhO6 by GrhJ is unlikely to be responsible for the spiroketal ring contraction. Furthermore, differential scanning fluorimetry data indicate that GrhJ binds to rubromycin-type molecules, despite the absence of N-acetylation sites in rubromycins or their biosynthetic intermediates. We propose that GrhJ may not act as an acetyltransferase. Instead, it might facilitate the release of the product from GrhO6 or stabilize reaction intermediates to minimize side reactions during spiroketal ring contraction. These insights advance our understanding of rubromycin biosynthesis and suggest new functional roles for members of the GNAT family. Further investigation is required to elucidate the detailed mechanism by which GrhJ promotes this reaction.

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P-PPM-310

Acetone and acetophenone metabolism in *Rhodococcus aetherivorans*

*K. Müller[1], J. Kahnt[2], J. Heider[1]
[1]*Philipps-University Marburg, Marburg, Germany*
[2]*Max Planck Institute, Marburg, Germany*

Acetone and acetophenone are common intermediates of natural secondary metabolism. As these substrates are chemically inert, they must be activated prior to microbial degradation. In the known metabolic pathways, ketone activation proceeds either by oxygenation or by ATP-dependent carboxylation [1,2].

Acetone carboxylase (Acx) and acetophenone carboxylase (Apc) are homologous enzymes, but have different subunit compositions. While Acx forms a hexameric complex out of three different types of subunits ($[\alpha\beta\gamma]_2$), the octameric Apc core complex comprises four distinct subunits ($[\alpha\alpha'\beta\gamma]_2$). Furthermore, Apc activity requires a fifth subunit (Apcε), which is not present in Acx [2,3].

The strictly aerobic actinobacterium *Rhodococcus aetherivorans* encodes an apparent *apc* operon. Surprisingly, acetone rather than acetophenone induces expression of this gene cluster. In contrast, a phenol monooxygenase homolog is up-regulated during growth on acetophenone. Under both conditions, we observe strong expression of isocitrate lyase, which is involved in acetyl-CoA assimilation.

Our current data suggest that acetone and acetophenone degradation in *R. aetherivorans* is accomplished by different principles. We propose that acetone catabolism is initiated by a carboxylase with Apc-like subunit structure and then proceeds in the canonical pathway via acetoacetate and acetyl-CoA. In contrast, acetophenone may be activated by oxygenation. The responsible enzyme and the subsequent reaction steps are not known yet, but we hypothesize that phenol and acetate are intermediates of the acetophenone degradation pathway.

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P-PPM-311

From mineral to microbe: lanthanide mobilization, uptake, and gene expression dynamics in a lanthanide-utilizing methylotroph

L. Gorniak[1], S. M. Gutenthaler-Tietze[2,3], A. Lobe[3], L. J. Daumann[2,3], F. Steiniger[4], M. Westermann[4], *C. E. Wegner[1,2]
[1]*Friedrich Schiller University, Aquatic Geomicrobiology, Jena, Germany*
[2]*Heinrich Heine University, Bioinorganic Chemistry, Düsseldorf, Germany*
[3]*Ludwig Maximilians University, Chemistry, Munich, Germany*
[4]*Jena University Hospital, Electron Microscopy Centre, Jena, Germany*

The lanthanides (elements 57–71, [La–Lu], Ln), along with the chemically similar elements scandium (Sc) and yttrium (Y), are known as "rare-earth elements" (REE). Ln are critical resources for the ongoing green energy transition, but mining and recycling them is expensive and environmentally harmful. They are omnipresent in our everyday lives and represent the most recently described biometals. Ln-utilizing microbes are mostly studied using soluble Ln chloride salts, which are a poor mimic for natural Ln bioavailability. We performed an in-depth characterization of selected Ln minerals and alloys (e.g. bastnaesite, gadolinite, monazite, xenotime, lighter flint) that differ by type (carbonate, oxide,

silicate, phosphate) and Ln content through energy-dispersive X-ray spectroscopy (EDX), inductively coupled plasma mass spectrometry, and complementary methods. Using our model strain Beijerinckiaceae bacterium RH AL1, which naturally depends on light Ln (La - Nd) for methanol oxidation, we applied transcriptomics and transmission electron microscopy (TEM) to understand how this strain mobilizes, takes up, and discriminates Ln during methylotrophic growth. We could show Ln mobilization for methanol oxidation from all tested Ln sources. TEM combined with EDX revealed that Beijerinckiaceae bacterium RH AL1 takes up certain Ln and selectively accumulates them in the periplasm as Ln phosphates, confirming our previous hypothesis that periplasmic Ln accumulation contributes to intracellular Ln homeostasis. Transcriptomics indicated that gene expression dynamics—up to 53% of the encoded genes were differentially expressed—were strongly governed by the type of Ln source. These aspects were not restricted to genes known to be involved in Ln utilization but reached into many aspects of cellular metabolism. Ln mobilization is presumably enabled by Ln-based chemotaxis, bacteria attaching to the Ln source, and the concerted action of extracellular pH modulation, and the release of potential Ln-binding metallophores. We noted significant crosstalk with Fe metabolism and could pinpoint candidate genes encoding proteins involved in intracellular Ln discrimination. Adaptations to low Ln bioavailability and types of Ln sources make Ln-utilizing microbes a treasure chest for developing strategies for the sustainable use of Ln as key elements for our modern way of living in the 21st century.

P-PPM-312

Trace metal availability drives microbial metabolic shifts and shapes community functional diversity

*A. Ricciardelli[1], B. de Pins[1], J. Brusca[2], M. Correggia[1], L. Di Iorio[1], M. Cascone[1], M. Giardina[1], S. Castaldi[1], N. Nappi[1], R. Iacono[1], R. Isticato[1], M. Moracci[1], A. Pollio[1], V. Costantino[3], S. Leone[4], A. Cordone[1], D. Giovannelli[1]

[1]University of Naples "Federico II", Biology, Napoli, Italy

[2]Ca' Foscari University of Venice, Venice, Italy

[3]Rutgers University, Department of Marine and Coastal Science, New Brunswick, NJ, United States

[4]Stazione Zoologica Anton Dohrn, Department of Biology and Evolution of Marine Organisms (BEOM), Napoli, Italy

Microbial life relies on redox reactions to power essential cellular and metabolic processes, in which oxidoreductases are the main players. These enzymes use trace elements, and especially transition metals, as their cofactors, crucial in controlling the electron flow from electron donors to final electron acceptors, and contribute to maintaining the redox balance and thermodynamic disequilibria of natural systems. Despite their low abundance in the environment, trace metals distribution and availability might play a key role in influencing microbial functional diversity, shaping microbial community composition, and modulating biogeochemical cycling processes.

Here, we report findings from both field and laboratory experiments elucidating the impact of trace elements availability on microbial functional diversity. Metagenomic data obtained from environmental samples suggest that trace elements availability shapes the diversity of the oxidoreductases in microbial communities, driving functional diversity in natural ecosystems. Furthermore, through physiological, proteomic, and functional analyses on microbial model organisms, we show that trace elements deficiency imposes energetic costs on microbial growth, delays microbial respiration transitions, alters electron acceptor utilization order, and enhances metal scavenging mechanisms. Our findings provide valuable insights into how trace metals regulate microbial functions, offering new perspectives for optimizing microbial processes in both environmental and industrial applications.

RNA biology

P-RNA-313

YloC, a hexameric enzyme

*M. Meiser[1]

[1]Philipps-University Marburg, Chemistry, Marburg, Germany

YloC, a novel ribonuclease in *Bacillus subtilis,* functions as an endoribonuclease, specifically cleaving single-stranded RNA (ssRNA) and preferentially targeting uridine residues [1]. It was classified into the highly conserved YicC family of endoribonucleases, which is present in many bacteria like *Escherichia coli* (*E. coli*). Understudied proteins of this group are assumed to play a role in small RNA regulation [1,2]. It was suggested that YicC from *E. coli* and YloC have similar enzymatic activities and substrate preferences and might also share a common catalytic mechanism, but the clear function is still unknown [3].

It was also shown that downstream of *yloC* in all firmicutes *remA* (the regulator of biofilm) and *gmk* (an essential guanylate kinase) are located in an operon [4]. However, at the genetic level, the transcript of *yloC* is expressed in different ways and is therefore subject to various levels of regulation [1]. An involvement of YloC in biofilm formation due to gene proximity to *remA* might be present.

Our preliminary *in vitro* analyses show that YloC exists as a hexamer, a characteristic also observed in homologous proteins like YicC from *E. coli*. In our *in vivo* analyses we demonstrated that the deletion of *yloC* leads to a modified biofilm phenotype, similar to that of SinR mutants, indicating that YloC may influence biofilm formation. Additionally, YloC could be linked to the genetic competence of *B. subtilis*, as higher competence correlates with an increased number of YloC-mNeonGreen foci.

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P-RNA-314

RNA structures as targets for antimicrobial compounds

*D. Scheller[1], R. N. Das[2,3], K. Islam[4], E. Chorell[2,3], N. Arnberg[4], J. Johansson[1]
[1]*Umeå University, Molecular Biosciences, Umeå, Sweden*
[2]*Umeå University, Chemistry, Umeå, Sweden*
[3]*Chemical Biology Consortium Sweden, Umeå, Sweden*
[4]*Umeå University, Clinical Microbiology, Umeå, Sweden*

Regulatory RNA structures play an important role for pathogens. Many virulence-related genes encode a temperature-sensitive mRNA element, a so-called RNA thermometer (RNAT) in their transcript. Upon entry into a warm-blooded host, this RNAT structure melts open, allowing translation of the downstream gene. The virulence regulator PrfA of *Listeria monocytogenes* is controlled by such an RNAT. Further important RNA structures can be found in RNA viruses, such as the viral frameshift element. The viral frameshift element leads to a frameshift during translation, allowing different expression ratios of viral polyproteins located in the orf1a and orf1ab open reading frames. Disturbing this ratio of frameshifting, severely cripples coronavirus replication. Due to the importance of RNA structures for virulence, targeting by antimicrobial compounds could be a suitable alternative to classical protein-targeting antibiotics. By different Förster Resonance Energy Transfer (FRET) approaches we will screen a compound library for RNA structure altering compounds. As a proof-of-concept approach, we have used aminoglycosides and validated their inhibitory effect on viral translation by a dual-luciferase reporter system. Furthermore, we could observe a reduction of viral infection by determining the viral titer after infection. Therefore, targeting RNA structures seems to be a promising way to treat clinically relevant infections.

P-RNA-315

Cuckoo sRNAs in *Agrobacterium tumefaciens*

*J. S. Tischler[1], Y. Pfänder[1], S. Schäkermann[2], J. E. Bandow[2], F. Narberhaus[1]
[1]*Ruhr University Bochum, Microbiology, Bochum, Germany*
[2]*Ruhr University Bochum, Microbiology, Bochum, Germany*

Small regulatory RNAs (sRNAs) are essential for bacterial adaptation to environmental changes. Since they play a significant role in regulating both mRNA translation and stability, sRNAs are involved in controlling various cell processes, including stress responses. sRNAs of the αr14 family appear to play a role in stress responses and are characterized by a CCUCCUCC anti-Shine Dalgarno sequence in each loop, which led Reinkensmeier and Giegerich to refer to them as "cuckoo" sRNAs (1). The soil bacterium and plant pathogen *Agrobacterium tumefaciens* contains three cuckoo sRNAs, which are dependent on the RNA chaperone Hfq and are positively regulated by the LysR-type transcriptional regulator LsrB. Deletion of one cuckoo sRNA resulted in minor effects, raising the question of whether the paralogs complement each other. Therefore, we constructed a mutant lacking all three cuckoo sRNAs and analyzed the transcriptome and proteome of the triple deletion mutant to better understand the function of these sRNAs.

The transcriptome and proteome analysis of the triple deletion mutant showed an altered expression of numerous ABC transporter substrate-binding proteins. The most upregulated target was *afuA*, coding for an iron(III) ABC transporter substrate-binding protein. The increased *afuA* mRNA may explain the growth defect observed in the triple deletion mutant under elevated iron concentrations. Despite the increased iron uptake, the oxidative stress response remained unaffected. Additionally, several ABC transporter substrate-binding proteins, that bind sugars, were downregulated in the transcriptome of the cuckoo sRNAs deletion strain. Oxidation of sucrose in the periplasm leads to ketosucrose, which can be transported into the cytoplasm or released into the cultivation medium. We found an increased ketosucrose concentration in the medium of the cuckoo sRNAs deletion mutant compared to the wild type. This suggests that one of the downregulated ABC transporter substrate-binding proteins is involved in ketosucrose uptake. Both phenotypes can be compensated by a single cuckoo sRNA suggesting that the three cuckoo sRNAs have redundant functions and are able to complement each other. In summary, the cuckoo sRNAs of *A. tumefaciens* seem to be involved in the regulation of the uptake of iron and ketosucrose.

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P-RNA-316

Small proteins and dual RNA regulators in *Vibrio cholerae*

*L. M. Luke[1], D. Vega[1], M. A. Rahman[1], S. L. Svensson[2], M. Lakemeyer[3], C. M. Sharma[2], K. Papenfort[1,4]
[1]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*
[2]*University of Würzburg, Department of Molecular Infection Biology II, Würzburg, Germany*
[3]*Friedrich Schiller University, Institute for Organic and Macromolecular Chemistry (IOMC), Jena, Germany*
[4]*Microverse Cluster, Friedrich Schiller University Jena, Jena, Germany*

Dual RNA regulators are a unique group of small regulatory RNAs (sRNAs) that merges base-pairing sRNA function with small protein (SP) encoding function of an mRNA. In the previous decades, the importance of sRNAs has been extensively studied ranging from stress responses, antibiotic tolerance, virulence, carbon metabolism, and intercellular communication. In contrast, only little is known about dual RNA regulators and SPs. The problem is mainly due to the small size of SPs (≤ 50 amino acids), poor annotation, and the lack of genetic screens aiming to characterize SPs in bacteria.

To address this problem in the major human pathogen *Vibrio cholerae*, we performed ribosome profiling to identify potential small open reading frames at the genome-wide level. These experiments revealed 75 potential sORFs in *V. cholerae*, of which we confirmed 44 for *in vivo* translation by GFP reporter and validated 21 by Western Blotting. Characterization of SPs led us to discover the *V. cholerae* dual RNA and protein (VcdRP) harboring the VcdP SP and the VcdR sRNA, which together synchronize carbon uptake and central metabolism in *V. cholerae* (1). Following the search for additional dual regulators pivotal during carbon utilization, we identified an additional dual RNA regulator in *V. cholerae* named VcsRP (*V. cholerae* carbon starvation dual RNA and Protein). The *vcsRP* gene encodes the VcsP SP and the VcsR sRNA, which is processed from 3" UTR by ribonuclease E. Expression of *vcsRP* is activated under carbon limitation and is repressed under glucose-rich conditions. Further, we obtained evidence showing that VcsR acts post-transcriptionally and in concert with Hfq to inhibit the expression of enzymes involved in galactose metabolism and oxidative phosphorylation.

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P-RNA-317
A novel bisulfite-PCR method for nucleotide-specific detection of stress-induced changes in m^5C RNA modification in *Escherichia coli*
*L. Vasquez Camus[1], S. Riquelme-Barrios[1], K. Jung[1]
[1]*Ludwig Maximilians University, Microbiology, Planegg, Germany*

Gene expression is regulated by numerous DNA and RNA modifications, with over 150 RNA modifications identified. Among these, methylations occur across mRNA, rRNA, and tRNA. One such modification, 5-methylcytosine (m^5C), is reversible and impacts RNA stability, protein synthesis, and transcription. Increasing evidence also links RNA m^5C to tumorigenesis [1].

Traditional methods for measuring RNA methylation, such as LC-MS and bisulfite sequencing, are costly, time-consuming, and lack the sensitivity needed for nucleotide-specific detection/quantification. A more detailed understanding of how RNA modifications vary by condition, tissue, and species is essential for insights into transcriptional regulation, antibiotic resistance, and disease prognosis.

We have developed a novel approach that first employs bisulfite treatment to distinguish m^5C from unmodified cytosine—converting unmodified cytosines to uracil while preserving m^5C as cytosine. In a subsequent step, we apply a reported combination of rolling circle PCR and loop-mediated isothermal amplification (LAMP) [2]—both isothermal amplification techniques—to detect the specific modified and unmodified bases. By using different mixes containing dGTP or dATP, we were able to map all m^5C sites in *E. coli* rRNA.

This method was used to test stress-dependent changes of the m^5C level at three positions of rRNA after exposure of *E. coli* to oxidative or heat stress. We observed that the level of m^5C1407 modification in 16S rRNA increased in response to both stresses, while the level at the other positions remained constant. Previously, quantitative mass spectrometry had revealed an overall increase in m^5C in heat stressed *E. coli* RNA, but failed to identify the specific sites [3]. However, our bisulfite-PCR approach enables precise, site-specific detection of modifications.

This approach provides new insights into the analysis of RNA modification changes and highlights the importance of m^5C dynamics in bacteria. It opens up avenues for exploring RNA methylation as a regulatory mechanism in bacterial stress response.

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P-RNA-318
A bacterial ADP-ribosyltransferase RNAylates plant proteins
*M. Weber[1,2], D. Staiger[3], K. Höfer[1,2]
[1]*Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*
[2]*Max Planck Institute, Marburg, Germany*
[3]*Bielefeld University, Bielefeld, Germany*

NAD-capped RNAs (NAD-RNAs) are RNA molecules with a nicotinamide adenine dinucleotide (NAD) attached at their 5' end, a modification that influences RNA stability and degradation. Although found across various organisms, including plants, the functions of NAD-RNA remain poorly understood. One proposed function of this modification is the RNAylation, a posttranslational protein modification in which ADP-ribosyltransferases (ARTs) covalently attach NAD-RNA to proteins. To date, only the T4 bacteriophage ART ModB has been shown to RNAylate proteins in *E. coli*.

Since NAD-RNA and ARTs are present in all domains of life, we hypothesized that RNAylation could be widespread in nature. This study aims to identify ARTs with RNAylation ability and investigate the occurrence of RNAylation in biological systems beyond the described interaction of *E. coli* and bacteriophage T4.

Using structure predictions of NAD-bound ARTs, we identified an ART that binds NAD similar to ModB. This ART originates from a plant pathogenic bacterium, ADP-ribosylates arginine residues of RNA-binding proteins from *Arabidopsis thaliana to suppress the plant´s innate immune system*.

Using recombinantly expressed proteins and fluorophor-labeled NAD-RNA, we demonstrate that this ART can catalyze RNAylations of a target protein *in vitro*. Our proteomic analyses showed that the ART has the same site-selectivity for RNAylations and ADP-ribosylations. We further observed that linear NAD-RNA is preferred as an RNAylation substrate over structured NAD-RNA. Additionally, the ART catalyzed RNAylation of another target protein in plant lysates.

These findings indicate that RNAylation is catalyzed by various ARTs and suggest potential roles for RNAylation in plant-pathogen interactions, which will be subject of further study.

P-RNA-319
RNAylation of Ribosomal Proteins Regulates RNA-Protein interaction During T4 Phage Infection of *E. coli*
*L. Garrido García-Dorado[1], K. Höfer[1]
[1]*Max Planck Institute, Bacterial epitranscriptomics, Marburg, Germany*

RNAylation is a recently identified post-translational modification where NAD-capped RNAs are covalently attached to host proteins, catalyzed by the ADP-ribosyltransferase ModB of T4 bacteriophage. Traditionally, ADP-ribosylation was the only known modification of this kind, involving the transfer of an ADP-ribose group from NAD to a protein acceptor. In contrast, RNAylation involves the attachment of NAD-RNA molecule to ribosomal proteins, such as rL2 and rS1, which are key players in translation initiation and elongation (1). However, the biological role of RNAylation in translation remains elusive. Interestingly, T4-

infected *Escherichia coli* cells with a catalytically inactive ModB show phenotypic changes, including delayed lysis, smaller burst size, and reduced phage adsorption (2), suggesting ModB"s critical role in modulating the translational machinery during infection. This study aims to investigate the impact of RNAylation on ribosomal proteins and translation.

Understanding these mechanisms will enhance our knowledge of how RNAylation modulates translation during viral infections, new insights into translation regulation and valuable comprehension in manipulating viral replication and host responses.

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P-RNA-320

Identification of RNA-binding proteins involved in cell differentiation in cyanobacteria

*M. Brenes-Álvarez[1], H. Ropp[1], D. Papagiannidis[2], C. Potel[2], F. Stein[2], I. Scholz[1], C. Steglich[1], M. Savitski[2], A. Vioque[3], A. Muro-Pastor[3], W. Hess[1]

[1]University of Freiburg, Biology, Freiburg i.Br., Germany

[2]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

[3]University of Sevilla, Plant Biochemistry and Photosynthesis, Sevilla, Spain

RNA-binding proteins (RBPs) are central components of gene regulatory networks. The differentiation of heterocysts in some filamentous cyanobacteria and their cooperation with vegetative cells in the same filament is one of the evolutionarily oldest cases of true multicellularity. Although multiple non-coding transcripts are involved in this process, no RBPs have been implicated thus far. We used quantitative mass spectrometry to analyze the differential fractionation of RNA-protein complexes after RNase treatment in density gradients yielding 333 RNA-associated proteins in *Nostoc* sp. PCC 7120. We validated *in vivo* the RNA-binding capacity of 6 RBP candidates. Some participate in essential physiological aspects, such as photosynthesis, thylakoid biogenesis or heterocyst differentiation, but their association with RNA was unknown. Validated RBPs Asl3888 and Alr1700 were not previously characterized. Alr1700 is an RBP with two OB-fold-like domains that is differentially expressed in heterocysts and interacts with non-coding regulatory RNAs. Deletion of *alr1700* led to complete deregulation of the cell differentiation process, a striking increase in the number of heterocyst-like cells, and was ultimately lethal in the absence of combined nitrogen. These observations characterize this RBP as a master regulator of the heterocyst patterning and differentiation process, highlighting the role of RNA-binding proteins in developmental processes of multicellular prokaryotes.

Brenes-Álvarez, M., Ropp, H. R., Papagiannidis, D., Potel, C. Stein, F., Scholz, I., Steglich, C. Savistski, M. M., Vioque, A., Muro-Pastor, A. M., Hess, W. R. (2025) R-DeeP/TripepSVM identifies the RNA-binding OB-fold-like protein PatR as regulator of heterocyst patterning. *Nucleic Acids Research* (*in press*).

Signal transduction & gene regulation

P-STGR-321

Exploring the relationship between the two-component system CroRS and the second messenger c-di-AMP in regulating β-lactam resistance in *Enterococcus faecalis*

*L. Wiens[1], S. Morris[2], S. Gebhard[1]

[1]Johannes Gutenberg-University Mainz, Mainz, Germany

[2]University of Bath, Bath, United Kingdom

The opportunistic pathogen *Enterococcus faecalis*, which is responsible for serious hospital-acquired infections, has a high level of intrinsic antibiotic resistance, especially to β-lactam antibiotics. *E. faecalis* uses the CroRS two-component system to control antibiotic resistance against cell-wall targeting antibiotics, such as the β-lactam ampicillin. A distinct phenotype was observed upon deletion of *croRS*, including a high susceptibility towards β-lactams, low overall growth rate, lager cell size and a thinned cell wall. However, previous genome profiling studies exploring the CroRS regulon did not show the exact genes responsible for the CroRS dependent β-lactam resistance. To gain further insights into the mechanistic link between CroRS regulation and β-lactam resistance, we here used experimental evolution to restore ampicillin resistance in a *croRS* deletion strain. The four resulting strains exhibited a 10-fold increase in ampicillin resistance, which is equivalent to that of the wild type. Genome sequencing showed that the only mutations common to all evolved lines were in genes encoding enzymes responsible for the synthesis and degradation of the second messenger c-di-AMP. Consistent with this, deletion of *croRS* led to a marked increase in c-di-AMP levels, while the evolved strains exhibited a decrease in c-di-AMP levels similar to the wild type. This observation may begin to explain the sensitivity of the *croRS*-deleted strain, because in other Gram-positive bacteria excessive c-di-AMP production has been shown to coincide with β-lactam sensitivity. Curiously, in the Δ*croRS* background single gene deletion of tow of the phosphodiesterases fully restores β-lactam resistance. These deletions also improved the overall growth rate of the *croRS*-deleted strain. While we do not yet know the mechanistic basis for this effect, our findings clearly show that a tight correlation between c-di-AMP concentration and β-lactam resistance exists in *E. faecalis*. These findings will help to improve our understanding of how CroRS regulation controls β-lactam tolerance in *E. faecalis*.

P-STGR-322

Redox-sensing of quinones by the MhqR repressor in *Staphylococcus aureus*

*T. P. T. Nguyen[1], V. L. Vu[1], P. Weiland[2], V. N. Fritsch[1], V. Z. Leon[2], G. Bange[2], H. Antelmann[1]

[1]Free University of Berlin, Institut für Biologie-Mikrobiologie, Berlin, Germany

[2]Philipps-University Marburg, 2Center for Synthetic Microbiology (SYNMIKRO) and Department of Chemistry, Marburg, Germany

Introduction: During infection, *Staphylococcus aureus* is exposed to reactive electrophilic species, such as quinones and aldehydes. The MhqR regulon was previously shown to confer resistance towards methylhydroquinone (MHQ) and quinone-like antimicrobials, such as pyocyanin and ciprofloxacin [1]. However, the quinone-sensing mechanism of the MhqR repressor is unknown thus far. **Question:** We hypothesized that quinones bind to a ligand-binding pocket of MhqR, leading to its inactivation and derepression of transcription of quinone detoxification genes. **Materials & Methods:** To investigate the quinone-sensing mechanism of MhqR, we resolved the crystal structure of MhqR in the

presence of MHQ and conducted ITC analyses, DNA binding assays and phenotype analyses. **Results:** The crystal structure of MhqR in complex with MHQ identified potential quinone interaction sites, which supported the predictions of the potential quinone binding pocket. Transcriptional analysis identified F11, R16, F39, and H111 of MhqR as essential for MHQ sensing *in vivo*, while L115 is important for DNA binding *in vivo*. In phenotype analyses of *mhqR* variants, the *mhqR-F11A*, *mhqR-R16A*, *mhqR-F39A*, and *mhqR-H111A* mutants were impaired in growth under MHQ stress in *S. aureus*. However, MHQ binding was not affected in the MhqR variants *in vitro*, as shown by ITC experiments and EMSAs *in vitro*. **Summary:** Structural and mutational analyses identified a quinone-binding pocket of MhqR, which is essential for MHQ sensing *in vivo*. Our results provide novel insights into the redox-mechanism of MhqR, that contributes to antimicrobial resistance in *S. aureus*.

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P-STGR-323

First insights into the putative role of AidA for the bacteria-host interactions of *Photorhabdus luminescens*

*M. Kara[1], L. Willy[1], L. Wiens[1], L. Kirsch[1], G. Schiemenz[1], F. Platz[1], N. Dominelli[1], R. Heermann[1,2]

[1]Johannes Gutenberg-University Mainz, Mainz, Germany

[2]Institute of Biotechnology and Drug Research gGmbH (IBWF), Mainz, Germany

Bacteria engage in a complex interplay with their host organisms. *Photorhabdus luminescens* is a Gram-negative insect pathogenic bacterium, that undergoes pathogenic as well as symbiotic interactions with several eukaryotes, including nematodes, insects and plants. The bacteria live in close symbiosis with soil nematodes that infect insect larvae and upon entering they inject their bacterial symbiosis partner into the insect's hemolymph, where the bacteria kill the insects. In the insect, the bacteria undergo a phenotypic switch that splits the bacterial population into two parts. Up to 50% of the cells switch from the so-called primary (1°) to the secondary (2°) cell form. Whereas 1° cells reassociate with the nematodes, this trait is absent from 2° cells. They remain in the soil where they interact with plant roots, protecting them from infection with fungi. Little is known how *P. luminescens* mediates the biotic interactions to these three different hosts. We recently identified a protein of yet unknown function, AidA, that is assumed to be involved in bacteria-host interactions. AidA is a crucial virulence factor in the plant pathogen *Ralstonia solanacearum* playing a significant role in pathogenicity. In *P. luminescens*, *aidA* was differentially regulated in 1° and 2° cells. Furthermore, SdiA, a LuxR solo in *P. luminescens*, which is involved in plant recognition and colonization is known regulating the expression of *aidA*. Here we demonstrate that the addition of several plant root exudates to *P. luminescens* 2° cells increases the *PaidA* promotoractivity, indicating a regulatory role of AidA in response to various plants species. Furthermore, the deletion of *aidA* in 1° cells results in increased swimming motility and reduced biofilm formation, especially under the influence of plant root exudates. Moreover, the 1° *ΔaidA* mutant exhibited an impaired ability to associate with nematodes, suggesting that AidA is also crucial for nematode symbiosis. Finally, the deletion of *aidA* in both 1° and 2° cells resulted in the loss of the ability to kill insect larvae since neither the 1° nor the 2° *ΔaidA* mutant showed any pathogenic effects. In summary, we could demonstrate that AidA plays a crucial role for the interaction of *P. luminescens* with its three eukaryotic hosts, however, the molecular mechanism of AidA action remains yet unclear.

P-STGR-324

Characterization of the cobalt ECF-importer CbiMNQO and its cobalt/nickel dependent regulation by the ArsR/SmtB-like regulator CbiR in *Mycobacterium smegmatis*

*E. Goethe[1], M. Ackermann[1], L. Ende[2], M. Jarek[3], R. Goethe[1]

[1]Hannover Medical School, Department of Infectious Diseases, Hannover, Germany

[2]Leibniz University Hannover, Hannover, Germany

[3]Helmholtz Centre for Infection Research – HZI, Genome Analytics, Braunschweig, Germany

Cobalt is an essential trace metal, incorporated in important metalloproteins, e.g. corronoid proteins. Best-known representatives of this group are cobalamins, such as vitamin B12. Despite the presence of vitamin B12 dependent enzymes involved in central metabolic processes and host-pathogen interaction in most bacteria, only 25-30% of bacterial species are able to produce vitamin B12 *de novo*, including many mycobacteria species. For the production of vitamin B12, cobalt uptake is essential. An important system for cobalt import is the energy-dependent ECF transporter CbiMNQO (1,2). Associated regulators of this system are not yet known. No cobalt importers, including CbiMNQO, have been identified in mycobacteria.

By analyzing the genome of the non-pathogenic model organism *Mycobacterium smegmatis* (MSwt), we identified an operon that encodes for a putative *cbi*MNQO transporter (*msmeg*_2607-2610). BLAST and TMHMM analyses of *msmeg*_2607-2610 encoded proteins showed typical elements of *cbi*MNQO transporters. The *cbi*MNQO operon is associated with a regulator of the ArsR/SmtB family (*msmeg*_2606, here *cbi*R) and genes involved in vitamin B12 biosynthesis (*msmeg*_2616-2618). We hypothesized that CbiR is responsible for *cbi*MNQO expression in a metal dependent manner. The regulon of CbiR was determined by transcriptome analyses of MSwt and an MS∆*cbi*R deletion mutant. Amongst 29 DEG"s (>5 fold differentially expressed), expression of *cbi*MNQO was increased in MS∆*cbi*R compared to MSwt. To analyze the impact of different metals on *cbi*MNQO expression, MSwt was grown in metal depleted Sauton"s medium (SM, control) or SM supplemented with $CoCl_2$, $NiCl_2$, $ZnSO_4$ or $FeNH_3$ and performed qRT-PCR analyses. Compared to the control *cbi*M expression was reduced only upon addition of $CoCl_2$ and $NiCl_2$, indicating a Co/Ni dependent binding of the putative regulator CbiR. Reporter studies with MSwt and MS∆*cbi*R carrying a plasmid with the *cbi*M promoter fused to *lac*Z and subsequent ß-galactosidase assays confirmed the impact of Co/Ni in CbiR binding. Growth experiments with a MS∆*cbi*MN mutant in SM with different metals showed the influence of *cbi*MNQO on Co/Ni uptake. This is the first report of a transporter involved in mycobacterial Co/Ni uptake. These findings might help understanding Co/Ni homeostasis in other mycobacteria and identifying potential treatment strategies.

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P-STGR-325

A novel regulatory module important for cell cycle regulation in the stalked budding bacterium *Hyphomonas neptunium*

*J. Harberding[1,2], O. Leicht[1], M. C. F. van Teeseling[3], C. Reif[1,3], U. S. Mies[4], W. Steinchen[1,2], T. Glatter[4], A. Brune[4], G. Bange[1,2], M. Thanbichler[1,2,4]
[1]*Philipps-University Marburg, Biology, Marburg, Germany*
[2]*LOEWE Center for Synthetic Microbiology, Marburg, Germany*
[3]*Friedrich Schiller University, Jena, Germany*
[4]*Max Planck Institute, Marburg, Germany*

Unlike many bacterial model organisms, the alphaproteobacterium *Hyphomonas neptunium* does not undergo binary fission but generates offspring through the formation of buds at the end of a stalk-like cellular extension. The control of this intricate biphasic life cycle requires a precise regulation of cell cycle progression, morphogenesis, and cell division. Here, we report the identification of a two-component signaling protein that is critical for normal growth and development in *H. neptunium*. A deletion mutant shows pleiotropic defects, including severely swollen mother cell bodies and growth defects. This new regulator is part of a regulatory module whose components mediate essential cellular functions and show cell cycle-dependent localization patterns. Together, our results reveal the existence of a novel and conserved, regulatory pathway that contributes to the regulation of cellular development in *H. neptunium* and, potentially, also other members of the alphaproteobacterial lineage.

P-STGR-326

Beyond ParB: Evolution of the chromosome partitioning protein ParB and discovery of novel CTP-dependent proteins

*J. Hanßmann[1,2], S. Lometto[3], W. Steinchen[1,3], L. Schnabel[2], M. Lechner[1], G. Bange[1,4], G. Hochberg[1,3,4], M. Thanbichler[1,2,4]
[1]*Zentrum für Synthetische Mikrobiologie, AG Thanbichler, Marburg, Germany*
[2]*Philipps-University Marburg, Biology, Marburg, Germany*
[3]*Philipps-University Marburg, Chemistry, Marburg, Germany*
[4]*Max Planck Institute, Marburg, Germany*

The precise coordination of cellular processes such as cell-cycle regulation is critical for every organism. Therefore, a vast repertoire of regulatory proteins including proteins regulated by small molecules such as nucleotides has evolved. Recent studies on ParB, a protein involved in bacterial DNA segregation, led to the discovery of a novel class of CTP-dependent molecular switches. Importantly, database searches suggested that the ParB/Srx domain of ParB, which mediates its ability to bind and hydrolyze CTP, may be widely conserved among bacteria and archaea. In this study, we combined bioinformatic and biochemical approaches to identify novel CTP-regulated proteins. Apart from that, we used ancestral sequence reconstruction and biochemical studies to unravel the evolution of ParB-type DNA segregation proteins. We show that ParB is an ancient protein whose core functions were preserved over billions of years of evolution. Collectively, this work highlights the prevalence and importance of ParB/Srx-type CTPases in bacteria, thereby shedding light on a widespread new mode of regulation in bacteria.

P-STGR-327

First insights into host-dependent regulation of biofilm formation and virulence by cNMP second messengers in *Photorhabdus luminescens*

*F. Platz[1], H. Bähre[2], R. Seifert[2], R. Heermann[1,3]
[1]*Johannes Gutenberg-University Mainz, Institut für Molekulare Physiologie, Mikrobiologie und Weinforschung, Mainz, Germany*
[2]*Hannover Medical School, ZFA Metabolomics, Hannover, Germany*
[3]*Institute of Biotechnology and Drug Research gGmbH (IBWF), Mainz, Germany*

Photorhabdus luminescens interacts with different eukaryotic hosts - insects, nematodes and plants. While the bacteria form a mutualistic symbiosis with nematodes and plants, they are highly pathogenic towards insects. The bacteria exist in two phenotypic different cell forms designated as primary (1°) and secondary (2°) cells, whereas only the 1° cells interact with the nematodes and 2° cells with plants. Both cell forms are pathogenic against insects. *P. luminescens* needs to specifically adapt to the different hosts, especially by regulation of sessility and pathogenicity. In other bacteria, second messengers play a central role for those biotic interactions. In *P. luminescens* we found presence of 2',3'- and 3',5'-cyclic nucleotide monophosphates (cNMPs), while no cyclic di-GMP could be detected. In many bacteria, c-di-GMP is often involved in the regulation of motility and sessility. To get first insight into the role of second messengers in the regulation of *P. luminescens*-host interactions, we incubated the bacteria in the presence of the three different host signals and analysed the cells for presence of different cNMPs. Thereby, increased levels of 3',5'-cAMP were detected when host signals were present. For that reason, a bioinformatics analysis was conducted, proposing 3',5'-cAMP to regulate virulence in *P. luminescens*. While adenylate and guanylate cyclases involved in 3',5'-cNMP production are well characterized and could be bioinformatically identified in *P. luminescens*, the synthesis and regulatory role of the non-canonical 2',3'-cNMPs was vastly unknown. As the prominent second messenger cyclic di-GMP is absent in *P. luminescens*, we investigated the role of 2',3'-cNMPs for biofilm formation. We identified a RNase T2 (RnsA) that is a homologue to RNase1 of *E. coli* where it is known to be involved in 2',3'-cNMP production and regulation of sessility. The deletion of *rnsA* let to a 10-fold decrease of the 2',3'-cNMP levels compared to the wildtype in both *P. luminescens* 1° and 2° cells. Moreover, we observed an increased biofilm formation in both cell types but especially in 1° cells, suggesting a central role of these second messengers for regulation of sessility and therefore probably also for host colonization. Overall, here we identified RnsA as a central enzyme for 2',3'-cNMP synthesis and raised first insights into the regulation of sessility by 2',3'-cNMPs and virulence by 3',5'-cAMP.

P-STGR-328

Two ArsR-type transcription factors, sHdrR and SoxR, are global regulators of sulfur oxidation in *Hyphomicrobium denitrificans*

*J. Li[1], N. Schmitte[1], K. Törkel[1], *C. Dahl[1]
[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*

Organisms use different strategies to sense and respond to reduced sulfur compounds such as sulfide or thiosulfate. In the methylotrophic Alphaproteobacterium *Hyphomicrobium denitrificans*, which uses thiosulfate as an accessory electron donor, this involves two distinct but related ArsR-type transcriptional repressors, sHdrR and SoxR. Here, we

focused on identifying target genes regulated by these repressors. This was achieved by generating individual deletions of each regulator gene, performing comparative RNA-seq analysis, and mapping sHdrR and SoxR binding sites using electrophoretic mobility shift assays (EMSA). In our mRNA-seq experiments, the number of identified mRNAs was between 95 and 96% of the 3529 predicted genes in all cases. The absence of the repressors sHdrR and SoxR affected the abundance of a total of 165 (4.8%) and 170 (5.0%) of the detected mRNAs, respectively, with a high overlap of 138 genes, of which 48 were upregulated and 90 were downregulated. In the reference strain, the presence of thiosulfate significantly altered the transcription of 136 (4.1%) genes. Of these, 85 (65%) overlap with genes affected by the absence of one of the two repressors. We can therefore state with confidence that SoxR and sHdrR are important factors in the cellular response to the availability of oxidizable sulfur. SoxR affects the *sox* genes for periplasmic thiosulfate oxidation and sulfane sulfur import into the cytoplasm, as well as the *lip-shdr-lbpA* genes, which encode the cytoplasmic enzymes essential for sulfite formation. sHdrR affects only a subset of these genes. The transcription of *sox* genes remains unchanged in its absence. The absence of SoxR and sHdrR had a strong negative effect on the transcription of set of genes for enzymes of fatty acid biosynthesis. How this effect is exerted and how sulfur oxidation and fatty acid biosynthesis are linked remains to be elucidated. The combined regulatory role of both repressors was confirmed in vitro by EMSA experiments. EMSA was used to map common binding regions. These overlap the putative -35 and -10 RNA polymerase binding sites upstream of the divergently transcribed *soxY* and *soxA*, and *soxT1A* and *shdrR* gene sets. Whether the interaction between the two repressors is direct or indirect in vivo is an important question for future research.

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P-STGR-329

Regulation of azalomycin F production in *Streptomyces iranensis* required for activation of fungal silent gene clusters

*L. Zehner[1,2], S. Edenhart[1], M. Rosin[1,2], P. Hortschansky[1], T. Netzker[3], M. C. Stroe[1], T. Krüger[1], M. K. C. Krespach[1,2], V. Schroeckh[1], A. Brakhage[1,2]

[1]*Leibniz-HKI Jena, Molecular and Applied Microbiology, Jena, Germany*

[2]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*

[3]*Fritz Lipmann Institute (FLI), Jena, Germany*

Streptomycetes are renowned producers of secondary metabolites, which are compounds that are not directly required for the survival of the producing organism but rather provide an advantage under certain ecological conditions. Thus, the biosynthetic gene clusters (BGCs) encoding the machinery to produce these compounds are often only active when these specific conditions are met. As we have previously shown, *Streptomyces iranensis* HM35 (DSM 41954) has the ability to activate the production of orsellinic acid and various derivatives thereof in the fungus *Aspergillus nidulans* 1,2. Furthermore, we were able to show that *S. iranensis* can activate silent BGCs in various fungi through the secondary metabolite azalomycin F33. To gain a better understanding of this microbial interaction, we further investigated the genetic regulation of azalomycin F production. By conducting deletion experiments, we identified a group of major regulatory genes located in a regulatory island. It encodes four LuxR-type transcription factors and is required for the interaction between *S. iranensis* and *A. nidulans*. Furthermore, we were able to verify that the encoded regulators are involved in the production of azalomycin F3, which mediates the interaction of *S. iranensis* and *A. nidulans*. Through transcriptomic analysis and protein-DNA interaction studies we aim to identify the target genes and exact binding motifs of the regulators as well as their potential involvement in mechanisms of export or transport of azalomycin F3 to the recipient fungus. Our data provide insight into the molecular regulation of bacterial-fungal interactions and the complex network of transcription factors involved.

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P-STGR-330

Deciphering regulatory networks and functional roles of the two-component system MxtR/ErdR in *Pseudomonas putida* KT2440

*F. Burr[1], *I. Heinzelmann[1], M. Eder[1], T. Henriquez[2], H. Jung[1]

[1]*Ludwig Maximilians University, Microbiology, Planegg, Germany*

[2]*University of Siena, Medical Biotechnology, Siena, Italy*

Two-component systems (TCS) allow bacteria to sense and adapt to various extracellular stimuli by triggering cellular responses in the form of altered gene expression, inducing metabolic changes. The TCS MxtR/ErdR of *Pseudomonas* and *Vibrio* species has been shown to control the expression of genes essential to acetate utilisation [1,2]. MxtR contains a solute/sodium symporter (SSS) domain and transporters of the SSS family are known to be involved in the acquisition of solutes as carbon sources [3,4]. MxtR's SSS domain senses an unknown signal, transports the solute into the cell, affecting signal transduction and expression of target genes. We aim to (1) unravel the regulatory network of MxtR/ErdR and (2) identify the functional roles and significance of individual MxtR domains.

Pursuing Aim 1, the expression of anticipated target genes of the TCS was analysed in *mxtR/erdR* mutants. Thus, target genes were deleted and growth was investigated on different carbon sources. Moreover, binding between the response regulator ErdR and promoters of target genes was examined. Furthermore, we investigated the possibility of MxtR being a part of a sensor kinase network. To address Aim 2, domains of MxtR were deleted and growth on different carbon sources was analysed. Single amino acid substitutions in the SSS and receiver (REC) domain of MxtR, as well as the REC domain of ErdR were performed to evaluate their importance in signal transduction and carbon source utilisation.

Our results show MxtR/ErdR dependent expression of target genes encoding enzymes involved in the methylcitrate cycle i.e. the degradation of propionate. Deletion of the target genes reveal a role in propionate utilisation. Moreover,

binding assays underline ErdR-target gene interaction and binding. In addition, we observed, that the SSS and STAC domains of MxtR are essential to its function and growth on various carbon sources, while the REC and PAS domains appear to be dispensable. We also identified amino acids in the SSS domain that are crucial to MxtR function and are hypothesized to play a key role in signal transduction.

The abovementioned results emphasize the role of MxtR/ErdR, its domains and target genes in short-chain fatty acid metabolism.

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P-STGR-331

The role of CtsR and ClpX in the regulation of the heat stress response in *Bacillus subtilis*

*C. Scheidhauer[1], M. Harms[1], M. Schaffer[1], U. Gerth[2], U. Völker[1], A. Reder[1]

[1]Interfaculty Institute for Genetics and Functional Genomics, Department of Functional Genomics, Greifswald, Germany

[2]University of Greifswald, Department for Microbial Physiology and Molecular Biology, Greifswald, Germany

B. subtilis possesses several mechanisms to adapt to and withstand different kinds of stress. If the bacteria are exposed to heat stress, among others, stress specific heat shock proteins of the CtsR regulon are synthesized at increased rate. The members of this regulon, ATPases, proteolytic subunits and their adaptors, inherit a crucial role in the cellular proteostasis under stress conditions [Miethke M. et al., J Bacteriol. 2006.]. During vegetative growth CtsR (**c**lass **t**hree **s**tress **r**epressor) represses the transcription of the *clpC* operon (*ctsR-mcsA-mcsB-clpC*), *clpE* and *clpP* gene [Krüger E. et al., J Bacteriol. 1998.]. Upon heat shock CtsR detaches from its operator caused by a conformational change in a thermosensing glycine rich loop leading to the transcription of the regulon [Elsholz A.K. et al. EMBO J. 2010.]. Novel data of *clpX* deficient strains revealed a positive effect of the ATPase ClpX on the induction of *clpE* upon heat stress. Hence, we constructed several strains of deletional and complemental mutants of *clpX* and *ctsR* to uncouple the induction of these genes from their natural stimuli. The results demonstrated that full induction of the CtsR regulon and especially *clpE*, so far believed to be solely regulated by CtsR and Spx, is dependent on the presence of heat and ClpX. This observation was made on transcriptional as well as protein level indicating a key role of ClpX in the transcriptional activation. Additionally, the data suggests that ClpX might act as a chaperone on CtsR to achieve full induction of *clpE* under heat shock conditions. Taken together we are able to show a so far unknown influence of ClpX on the induction of the CtsR regulon, including an interaction of ClpX and CtsR and thereby adding a new layer of complexity to the understanding how *B. subtilis* responds to heat stress.

P-STGR-332

The human microbiome-derived antibiotic lugdunin self-regulates its biosynthesis by a feed-forward mechanism

*L. Schulze[1], L. Reetz[1], T. Kronenberger[2], K. Selim[3], T. Scheafle[4], T. Dema[4], A. Zipperer[5], J. Mößner[5], A. Poso[2], S. Grond[4], A. Peschel[5], B. Krismer[5]

[1]University of Tübingen, IMIT - Infectionbiology, Tübingen, Germany

[2]University of Eastern Finland, School of Pharmacy, Kuopio, Finland

[3]University of Tübingen, IMIT - Organismic Interactions/Microbial Glycobiology, Tübingen, Germany

[4]University of Tübingen, Organic Chemistry, Tübingen, Germany

[5]University of Tübingen, IMIT - Infectionbiology, Tübingen, Germany

Many human microbiome members inhibit bacterial competitors by production of antimicrobial compounds whose expression needs to be tightly controlled to balance costs and benefits of compound biosynthesis. The nasal commensal *Staphylococcus lugdunensis* outcompetes *Staphylococcus aureus* using the antimicrobial lugdunin. The lugdunin biosynthetic gene cluster (BGC) encodes two potential regulators whose role has remained unknown. Deletion of the regulator genes *lugR* or *lugJ* led to increased lugdunin production and/or immunity. Whereas LugR was found to repress transcription of the biosynthetic *lugRABCTDZ* operon, LugJ repressed the *lugIEFGH* export and immunity genes. Both regulators bound to different inverted repeats in the controlled promoter regions. Notably, both repressors were released from cognate promoters to allow transcription upon addition of exogenous lugdunin. Even minor structural changes disabled lugdunin derivatives to induce expression of its BGC, which is consistent with inferior binding to the predicted LugR and LugJ binding pockets. Thus, lugdunin controls its own biosynthesis through a feed-forward mechanism, probably to avoid futile production.

P-STGR-333

Structural and functional insights into dual-signal recognition by a LysR-type transcriptional regulator in *Agrobacterium tumefaciens*

*R. Fiedler[1], J. J. Schmidt[1], M. Peters[1], H. Elders[2], E. Hofmann[2], S. Shahzad[3], F. Narberhaus[1]

[1]Ruhr University Bochum, Microbiology, Bochum, Germany

[2]Ruhr University Bochum, X-Ray Structure Analysis of Proteins, Bochum, Germany

[3]Forschungszentrum Jülich GmbH, Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons (ER-C), Jülich, Germany

The soil-borne plant pathogen *Agrobacterium tumefaciens* is the causal agent of the crown gall disease in plants. Its transition between host and soil environments depends on tightly regulated response systems. A crucial factor in environmental adaptation of *A. tumefaciens* is the LysR-type transcriptional regulator (LTTR) LsrB. Deletion of *lsrB* leads to misregulation of over 1,200 genes, which results in pleiotropic phenotypes such as reduced virulence and motility and increased sensitivity to antibiotics and reactive oxygen species (ROS) (1–3). Recent results from our group indicate that LsrB senses two distinct signals: ROS and the plant-derived signal molecule acetosyringone. In the present study we focus on refining the structural and functional mechanisms underlying LsrB-dependent regulation.

Size exclusion chromatography and *in vivo* crosslinking revealed that LsrB assembles as a homotetramer, consistent with other known LTTRs(4). AlphaFold3 modelling of the LsrB structure suggests that this regulator harbors I) redox-responsive cysteines and II) a conserved ligand-binding site, putatively involved in the recognition of the two distinct signals. Therefore, we performed site-directed mutagenesis of amino acids likely involved in signal recognition and assessed their contribution to LsrB function *in vivo* by phenotypic assays. Further, *in vitro* thiol trapping coupled to mass spectrometry support our model of cysteine-based redox sensing. Currently we are carrying out cryo-EM to

resolve the LsrB structure and elucidate the precise signal recognition mechanism. Taken together, we present evidence for LsrB"s dual-signal detection mechanism and contribute to the knowledge of signal-driven regulation by LTTRs.

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P-STGR-334

Exploring the regulatory role of TipA in *Streptomyces*: implications for cobalamin synthesis under environmental stressors

*M. Höller[1], T. Schubert[1], K. Krause[1], E. Kothe[1]

[1]Friedrich Schiller University, Institute of Microbiology, Jena, Germany

The Gram-positive filamentous soil bacteria belonging to the genus *Streptomyces* possess a relatively large genome. Their extensive regulatory elements and vast repertoire of secondary metabolites allow them to thrive in the extremely heterogeneous and complex soil environment. One such regulator is TipA, a MerR family type transcription factor, a putative regulator of cellular processes responding to thiopeptide antibiotics and metal stress.

The responses of *S. coelicolor* A3 (2) and *S. mirabilis* P16B-1 to thiostrepton and metal were investigated. RNA-Seq analysis comparing wild type and a *tipA* knockout in both species revealed significant changes in the expression of genes involved in cobalamin biosynthesis under either nickel or thiostrepton stress, depending on the presence of TipA. The cobalt containing cobalamin, or vitamin B12, acts as a coenzyme for multiple proteins but also is known to regulate gene translation *via* a riboswitch mechanism. Differences in selected cobalamin synthesis gene expression between wild type and knockout strain were confirmed in *S. coelicolor* A3(2) *via* qPCR. Less cobalamin was extracted from *S. coelicolor* A3(2) cultures treated with either nickel or copper, although this response, unlike the observations on RNA level, did not depend on the presence of TipA. Analysis of cellular metal content revealed that the presence of TipA significantly impacts the concentration of cobalt, iron and nickel in cells of *S. coelicolor* A3(2) but not *S. mirabilis* P16B-1 under thiostrepton stress.

The auxotrophic soil community members depend on the vitamins produced by other organisms. Additionally, metals and antibiotics play a role in structuring the soil microbiome. In this context, cobalamin may serve as a common good produced by *Streptomyces*. Understanding the regulatory response of *Streptomyces* to various environmental stressors, particularly in the context of TipA presence, could provide insights into the possibility of thiopeptides being involved in microbial communication and the ecological role of *Streptomyces* within soil habitats.

P-STGR-335

Resistant Bacteria – RUNOr better – WalKThe influence of established and novel antibiotics on the activity of the WalRK and the LiaRS two-component systems of *Bacillus subtilis*

*M. Karcher[1], G. Bierbaum[1], V. Franz[1], M. Gajdiss[1], A. Siskowski[1], S. Kirsch-Dahmen[2], R. Müller[2]

[1]University Hospital Bonn, AG Bierbaum, Bonn, Germany

[2]The Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany

Introduction: Bacterial two-component systems (TCS) are not present in mammalian cells and, therefore, represent attractive targets for new antimicrobials (Gotoh *et al.*, 2010,*J Antibiot (Tokyo)* **63** (3): 127–134). WalRK (YycG) is the only essential TCS in Gram-positive bacteria with a low G+C content, e.g. *Bacillus subtilis* and *Staphylococcus aureus* (Dubrac *et al.*, 2008). WalRK influences the maturation and turnover of the peptidoglycan as well as the separation of daughter cells. Therefore, the WalRK regulon comprises mainly genes that are involved in cell wall lysis *(Dubrac et al.,* 2008,*Mol Microbiol **70** (6): 1307–1322)*. Phosphorylated WalR activates transcription of several autolysins, e.g. SsaA in *S. aureus* (Dubrac *et al.*, 2008,*Mol Microbiol* **70** (6): 1307–1322), and inhibits transcription of the autolysin inhibitor IseA in *B. subtilis* (Dobihal *et al.*, 2022,*J Bacteriol* **204** (2): e0053321). However, the activity of WalRK is also influenced by the Ser/Thr kinase PrkC, which phosphorylates the amino acid Thr101 of WalR (Libby *et al.*, 2015,*PLoS Genet* **11** (6): e1005275).**Method:** Promoter sequences of *iseA* and *ssaA* were fused to the luminescence gene cluster of *Photorhabdus luminescens* and integrated into the *B. subtilis* genome. A *liaI* reporter (Kobras *et al.*, 2017,*Methods Mol Biol* **1520:** 121–131) was included in the experiments to detect inhibition of cell wall biosynthesis. These reporter strains were validated with known antibiotics and natural products. Additionally, Δ*prkC* knockout mutants of the reporter strains were constructed. *In vitro* assays were performed with the purified WalRK of *S. aureus*.**Results:** The activity of the reporter strains showed typical expression patterns for all three strains. Lipid II binders enhanced the expression of *liaI* and *iseA* and β-lactam antibiotics led to an upregulation of *iseA*, indicating that the activity of WalRK is inhibited in the presence of cell wall biosynthesis inhibitors. The expression of all three promoters was downregulated in the presence of protein, RNA and DNA biosynthesis inhibitors, indicating that the signal is specific for cell wall biosynthesis or WalK inhibitors. An additional knockout of *prkC* in the reporter strains resulted in differences in the expression of the promoters in presence of glycopeptide antibiotics.**Summary:** In this study, reporter strains for screening of WalRK inhibitors were created. Validation with established antibiotics showed expression patterns dependent on the antibiotic group and the presence of PrkC.

P-STGR-336

Ribosome heterogeneity in alphaproteobacteria

*T. Dietz[1], J. Cabrera[1,2], S. Mesa[2], E. Evguenieva-Hackenberg[1]

[1]Justus Liebig University Giessen, Institute of Microbiology and Molecular Biology, Gießen, Germany

[2]Consejo Superior de Investigaciones Científicas, Department of Soil Microbiology and Symbiotic Systems, Granada, Spain

Ribosome heterogeneity in bacteria refers to variations in ribosome composition and function, but little is known about its physiological role. Many bacteria harbor multiple *rpsU* genes encoding bS21, one of the smallest ribosomal proteins. Due to their small size, some *rpsU* genes were

missed in early annotations. *Bradyrhizobium diazoefficiens* and *Sinorhizobium meliloti* harbor *rpsU1* and *rpsU2* with unknown specific roles. In *S. meliloti*, *rpsU1* is a part of a cold shock operon (1), and *rpsU2* was annotated only in 2017. In a ribosome profiling (Ribo-seq) of *S. meliloti*, *rpsU2* was identified as a translated small ORF, and bS21-2 but not bS21-1 was detected by mass spectrometry (2). Here, we aimed to study the ribosome heterogeneity based on *rpsU1* and *rpsU2* homologs in *S. meliloti* and *B. diazoefficiens.* We applied RNA-seq, qRT-PCR, immunoblot, and phenotypic assays to analyze wild type and mutant bacterial strains. We found that in exponentially growing *S. meliloti* 2011, the levels of *rpsU1* mRNA are at least 30-fold lower than that of *rpsU2*. However, since according to Ribo-seq the translation efficiency (TE) of *rpsU1* is approximately 10-fold higher than the TE of *rpsU2*, *rpsU1* seems to be translated in exponential cultures, a suggestion supported by the failure to delete *rpsU1* unless it was expressed ectopically. Depletion and overexpression of *rpsU1* caused growth changes at 15°C. Upon cold shock, the level of *rpsU1* mRNA level was strongly increased and that of *rpsU2* mRNA was decreased till the differences were evened. Furthermore, we obtained a Δ *rpsU1* mutant of *B. diazoefficiens*, which is currently compared to the parental strain. To address the specific roles of the *rpsU* homologs in both species, we are analyzing changes in ribosome-associated mRNA pools upon deletion or manipulated expression of *rpsU1* and *rpsU2*. Altogether, the results suggest that *rpsU1* plays a role in the adaptation of *S. meliloti* to cold stress, but is also essential under standard conditions, when *rpsU2* is predominantly used. By contrast, *rpsU1* is not essential in *B. diazoefficiens*. The specific roles of the *rpsU* homologs in *S. meliloti* and *B. diazoefficiens* are still under investigation and most recent results will be shown.

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P-STGR-337

Consequences of Hyper- and Hypo-Cannibalism on biofilm formation in *Bacillus subtilis*

*M. Frank[1], L. Friebel[1], T. Mascher[1]

[1]Technische Universität Dresden, Institute of Microbiology, General Microbiology, Dresden, Germany

Bacillus subtilis is a gram-positive, soil-dwelling model organism that has evolved various strategies to handle nutrient limitations. Differentiation is a survival mechanism that challenges the traditional view of bacteria as simple single cells. Structures such as biofilms, pattern formation, and sporulation underscore the complex structure and tight regulatory networks within bacterial communities. To cope with nutrient shortages, *B. subtilis* engages in cannibalism, sacrificing a biofilm subpopulation to prolong energy-intensive sporulation and ensure population survival. This study investigates the effects of the epipeptide EPE on biofilm architecture and differentiation, using the undomesticated strain NCIB 3A38, which is known to form complex biofilms. Strains lacking or overexpressing EPE were created to examine its influence on processes like sporulation, competence, and matrix production. Results showed that the absence or overexpression of EPE significantly impacts biofilm morphology, emphasizing a link between cannibalism and matrix production. EPE gene expression was associated with the Lia stress response, sporulation, and SDP/SKF expression, displaying an oscillating pattern in biofilm formation, possibly tied to nitrogen stress. Overproducing EPE delayed sporulation while deleting the biosynthetic genes increased sporulation in liquid cultures. Deleting the regulatory region between *epeX* and *epeE* led to marked changes in biofilm structure and gene expression. Understanding the interaction between differentiated subpopulations and cannibalism toxins like EPE may offer insights into biofilm formation and the multicellularity of bacterial communities.

P-STGR-338

Specific interaction of the light-dependent antirepressor LdaP with the PpsR repressor mediates the activation of the photosynthetic gene cluster of *Dinoroseobacter shibae*

*S. Pucelik[1], S. K. Hotop[2], M. Brönstrup[2], D. Jahn[3], E. Härtig[1]

[1]Technical University of Braunschweig, Institute of Biochemistry, Biotechnology and Bioinformatics, Braunschweig, Germany

[2]Helmholtz Centre for Infection Research – HZI, Department of Chemical Biology, Braunschweig, Germany

[3]Technical University of Braunschweig, Braunschweig Integrated Centre of Systems Biology (BRICS), Braunschweig, Germany

Introduction: In the marine bacterium *Dinoroseobacter shibae* the photosynthetic gene cluster (PGC), encoding all components for aerobic anoxygenic photosynthesis, is specifically activated under dark growth conditions. The photosynthetic repressor PpsR inhibits PGC transcription under white & blue light conditions by binding to promoter sequences and preventing RNA polymerase binding. In our current regulatory model, the light-dependent, FMN containing LOV protein LdaP interacts with PpsR only in its dark state, while light state LdaP is inactive. Thereby LdaP acts as a PpsR antirepressor only under dark growth conditions. (Pucelik et al., 2024, *Front. Microbiol.* 15:1351297. doi: 10.3389/fmicb.2024.1351297).

Goals: Characterising the light-dependent interaction of the antirepressor LdaP with the PpsR repressor and its influence on DNA binding properties of PpsR.

Methods: Homologous produced & purified LdaP protein was incubated under blue light or dark conditions with SPOT peptide arrays representing the PpsR protein sequence and vice versa. Specific interactions were detected using LdaP or PpsR specific antisera. The DNA binding properties of PpsR were analysed by EMSA using a 100 bp *bchF* promoter DNA fragment and recombinantly produced PpsR. In addition, *in vivo* DNA binding studies of PpsR were performed using chromatin immunoprecipitation followed by qPCR. The oligomeric state of LdaP & PpsR was investigated using *in vitro* crosslinking.

Results: The SPOT peptide arrays showed specific interaction of LdaP with PpsR at the DNA binding domain of PpsR only under dark conditions. Single amino acid exchanges involved in the FMN coordination of LdaP abolished this interaction. EMSA analyses revealed specific PpsR binding to the conserved binding motif 5´-TGT-N12-ACA-3". In EMSA assays, LdaP did not abolish PpsR binding, but instead altered complex formation only under dark conditions. *In vitro* crosslinking experiments indicated the formation of PpsR tetramers and LdaP dimers as well as potential tetramers. This led to the suggestion that, under dark conditions, PpsR, LdaP and DNA form an altered complex that enables the transcription of the PGC.

Summary: LdaP mediates the activation of PGC expression, presumably by acting as an antirepressor of PpsR. Specific

binding regions within the LdaP and PpsR proteins were identified. Light-dependent complex formation of PpsR, LdaP and DNA was demonstrated. An octameric protein complex of PpsR and LdaP is proposed.

P-STGR-339

The *Helicobacter pylori* orphan ATTAAT-specific methyltransferase M.Hpy99XIX plays a central role in the coordinated regulation of genes involved in iron metabolism and is highly conserved in *Ubiquitous H. pylori* vs. absent from the *Hardy* ecospecies

W. Gottschall[1], F. Ailloud[1,2], C. Josenhans[1,2], *S. Suerbaum[1,2,3]
[1]*Ludwig Maximilians University, Medizinische Mikrobiologie und Krankenhaushygiene, Munich, Germany*
[2]*DZIF partner site, Munich, Germany*
[3]*National Reference Center for Helicobacter pylori, Munich, Germany*

H. pylori genomes contain a large and variable portfolio of methyltransferases (MTases), creating a highly diverse methylome. Here, we characterize a highly conserved ATTAAT-specific MTase, M.Hpy99XIX, the only H. pylori MTase never associated with an endonuclease ("orphan" MTase). Inactivation of M.Hpy99XIX resulted in a significant change in the transcription of >100 genes, despite the fact that only a small subset of their promoter regions contained an ATTAAT target motif. Patterns of transcriptional change showed significantly correlations with changes reported for H. pylori mutants in the ArsRS regulators involved in iron regulation. The MTase inactivation also caused a higher susceptibility to diverse metal ions as well as iron chelation and oxidative stress. These phenotypes could be traced back to the methylation of single motifs in the promoter regions of iron transporters frpB1 and fecA1. Altogether, methylation of individual motifs in promoters can have a large downstream effect causing major changes to metabolic pathways. These findings suggest that the methylome represents a universal and dynamic interface connecting genome diversity and transcriptional regulation. Very recently, a new ecospecies Hardy of H. pylori has been reported. M.Hpy99XIX is present in the majority of "normal" (Ubiquitous) H. pylori strains, whereas no single Hardy strain contained this gene, consistent with other reported differences between Hardy and Ubiquitous strains related to iron/metal homeostasis. ATTAAT methylation is intricately connected with the bacterial transcriptional network, highlighting the important role of bacterial epigenetic modifications in bacterial physiology and pathogenesis.

Open topics

P-OT-340

COG0523-family proteins CobW2 and CobW3 and the interplay between zinc and cobalt homeostasis in *Cupriavidus metallidurans*

*D. Galea[1], D. H. Nies[1]
[1]*Martin-Luther-University Halle-Wittenberg, Microbiology, Halle (Saale), Germany*

Background: Cupriavidus metallidurans CH34 can handle both zinc limitation and zinc toxicity. At the core of the zinc starvation response lies the Zur regulon. This regulon contains Zur as transcriptional regulator, ZupT as the main zinc importer, and three G3E-type P-loop GTPases from the COG0523 family, named CobW1, CobW2 and CobW3. The cell preferably accommodates a quota of 70.000 zinc atoms when cultivated in zinc replete conditions, but it can also survive with lower amounts. Recently, Co (II) has been proposed to substitute for Zn (II) in case of zinc starvation. This leads to the question of zinc limitation responses in this betaproteobacterium and whether Co (II) can have a similar effect.

Objective: We aim to understand the interplay between zinc and cobalt homeostasis and the role of the metal-binding GTPases CobW2 and CobW3.

Methods: The plasmid free strain AE104 and knock-out mutants were physiologically characterized in combination with cellular metal determination by ICP-MS.

Results: When faced with zinc limitation, parent strain AE104 imported higher amounts of cobalt ions. Loss of the ZupT protein led to a reduced cellular zinc and cobalt content. DmeF, a member of the CDF family of efflux proteins, was needed to export surplus cobalt ions. Mutants devoid of CobW2 and CobW3 exhibited different cobalt quotas in comparison with the parent strain. Loss of CobW3 reduced the cobalt content in both the parental and Δ*dmeF* background, but not in Δ*zupT* background.

Conclusions: An interplay between zinc and cobalt homeostasis emerges from the action of the zinc importer ZupT, the cobalt efflux system DmeF and the two GTPases CobW2 and CobW3. In combination with a cobalt efflux system, CobW2 and CobW3 reveal a new physiological role in cobalt homeostasis in *Cupriavidus metallidurans*. This opens up the possibility that members of the bacterial COG0523 family of P-loop GTPases might also transfer cobalt, not only zinc, to client proteins, as a response to zinc starvation. Metal cambialistic enzymes, such as the GTP cyclohydrolase FolE_IB2, could be metalated with cobalt ions when the cell is facing zinc starvation conditions.

P-OT-341

Heme-degrading bacteria from blood-sucking organisms

*M. Hahn[1], S. Barthels[1], M. Neumann-Schaal[1,2], M. Jahn[1], D. Jahn[1]
[1]*Technical University of Braunschweig, Microbiology, Braunschweig, Germany*
[2]*Leibniz Institute DSMZ, Braunschweig, Germany*

Many ectoparasites that frequently change hosts, especially hematophagous parasites, often serve as vectors for pathogenic microorganisms and thus contribute to their transport and spread. The microbiome of these parasites can also contain pathogenic bacteria that can be transmitted on contact with the host. Especially in times of progressive climate change, which favors the spread of such parasitic vectors for pathogens, the precise research and associated prevention of such vector-borne infectious diseases and their bacteria is essential.

Since heme is a major component of the blood meal of these hematophagous parasites, the aim of this work is to investigate the different microbiomes of such parasites for heme-degrading bacteria, since these bacteria have to survive in an environment with a lot of, partly also toxic, free heme. The degradation and thus the potential utilization of heme also plays an important role for these pathogens in the case of infectious transmission into the bloodstream of a host.

The microbiome was extracted from leeches, ticks, mosquitoes and other bloodsuckers using various methods

and individual bacterial strains were isolated and analyzed using Maldi Tof. Subsequently, these bacterial strains were examined for their ability to grow in heme-containing M9 medium with heme as the only carbon source.

Some bacteria showed no growth at all in this medium, others showed slightly increased growth with a slight but significant difference to the control without heme, which could indicate the utilization of heme. Genomic and proteomic studies of these strains will follow in the future to better elucidate the degradation and utilization pathway.

P-OT-342
Tools for the analysis of SplB under infection-mimicking conditions
*G. Wockenfuß[1], H. Wolfgramm[1], K. Surmann[1], L. Steil[1], C. Hentschker[1], M. Gesell Salazar[1], L. M. Busch[1], S. Michalik[1], P. Hildebrand[1], U. Völker[1], A. Reder[1]
[1]University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany

Staphylococcus aureus, a prevalent nosocomial pathogen, exhibits genomic heterogeneity across strains. Extracellular proteases, including serine-like proteases (Spls), play a crucial role in the pathogen's survival within the host (Singh V. et al. Med Microbiol Immunol. 2019). Interestingly, distribution of these proteases among individual *S. aureus* strains differs significantly.

Our research objective is to investigate the genomic and proteomic differences of Spls among *S. aureus* strains. To this end, we initially examined levels of SplB and other proteases by mass spectrometry and Western blots in different media in the stationary growth phase across different strains.

In order to explore the temporal expression of *spls* in cell culture infection experiments and the different regulators involved we designed a specific reporter system. We adapted the vector pTricolor to integrate different measurements during an infection experiment. In brief, the *cfp* reporter, expressed from a SigA promoter, facilitates normalization and copy number control. The *spl* promoter region, driving *gfp* reporter expression, enables time resolved recording of *spl* expression at single cell level. Finally, *dtomato* expressed from promoters controlled by specific regulators of Spls, reveals the drivers of *spl* operon expression in an infection experiment.

Our findings reveal variations in the amount of the secreted protease among different strains, and even within a specific *S. aureus* lineage (NCTC8325). The molecular background of regulation of *spl* expression and its impact during infection are currently being analyzed.

The results obtained so far suggest that Spl regulation is more intricate than previously assumed.

P-OT-343
Proteomics analysis of biofilm matrix of *Stenotrophomonas maltophilia* in single and mixed species biofiolms
*C. Tu[1], I. Alio[1], R. Moll[1], T. Mair[2], P. Nissen[2], H. Sondermann[3], W. Streit[1]
[1]Hamburg University, IPM, Hamburg, Germany
[2]UKE, Proteomics Facility, Hamburg, Germany
[3]Christian-Albrechts-University of Kiel, CSSB, Hamburg, Germany

Introduction :

Stenotrophomonas maltophilia is a gram-negative bacterium constitutively occurring in man but has drawn more concern due to its notable adaptability and opportunistic nosocomial infections especially in immunocompromised patients. As an opportunistic pathogen, this organism has the propensity to form biofilms rendering it highly resilient to antibacterial measures and host immune system. Biofilms are three-dimensional structures of multicellular aggregates encased in a self-made matrix of biofilm associated EPS (extracellular polymeric substances) wherein understanding the protein content is imperative in the study of biofilm"s pathobiology.

Objectives:

So far little is known what peptides are secreted by the pathogen *S. maltophilia* in single and mixed species biofilms and what they paly role in biofilm formation and pathogenicity.

Materials and methods:

This study focuses on the identification of proteins secreted into the biofilm matrix by *S. maltophilia* in single or mixed species biofilms. Biofilms were grown under static conditions for 48 hours. Biofilm matrix was isolated using the NaCl method as proposed in ref: Pan, Xiangliang, et al. (2010). After a purification step using the C18 spin Columns, a liquid chromatography (LC) mass spectrometry (MS) tandem system was employed for protein identification[IA1] .

Results:

Our results showed that the majority of proteins identified were exported hypothetical protein, secreted proteases, ton B receptors and adhesins. Most of the ton b receptors have been associated with iron acquisition and the some of the identified proteases have been inked to **virulence.**

Conclusion:

We have successfully established a method for the isolation and identification of peptides from the biofilm matrix of *S. maltophilia*. Our Proteomics data showed that the majority of identified proteins are extracellular proteases, receptors involved in iron acquisition , adhesins for cell adhesion and exported hypotheitical proteins.

We are currently analyzing the proteomics profile of *S. maltophilia* planctonic cultures and *S. maltophilia* in a mixed species biofilm with *P. aeruginosa.*

P-OT-344
Spoilage of UHT milk by psychrotrophic bacteria
L. Au[1], L. Robles[1], J. Vahle[1], T. Büthe[1], M. Plötz[1], *N. Jeßberger[1]
[1]Hannover Medical School, Hannover, Germany

A number of chemical and physical changes can occur during the storage of UHT milk. These include ageing gelation, creaming, excessive sedimentation, off-flavours or undesirable browning. Product spoilage due to proteolysis is

also feared. This can be triggered by the milk's natural proteases, such as plasmin and cathepsin D, or by heat-resistant proteases produced by psychrotrophic bacteria. These include Gram-negative (*Pseudomonas, Aeromonas, Serratia, Acinetobacter, Alcaligenes, Achromobacter, Enterobacter, Flavobacterium*, etc.) and Gram-positive (*Bacillus, Aneurinibacillus, Brevibacillus, Geobacillus, Clostridium, Corynebacterium, Microbacterium, Micrococcus, Streptococcus, Arthbacter, Carnobacterium, Staphylococcus*, etc.) germs.

Of approx. 200 isolates of the genera *Bacillus, Pseudomonas, Aeromonas* and *Serratia* tested in this project, 95 % showed distinct degradation on skim milk agar. Heating the culture supernatants and adding protease inhibitors indicated that the casein degradation was caused by various heat-labile and heat-stable exoproteases. These are currently being identified using zymography and MALDI-ToF-MS. At the meantime, UHT milk was inoculated with selected isolates. Clear differences in product spoilage (colouring, sedimentation, flocculation, odour, etc.) were identified with regard to the different genera, the initial bacterial count (101 or 105 cfu/ml), the fat content of the milk and the storage temperature (4, 22, 40 °C).

Overall, it was shown that even a minimal bacterial contamination (10 germs per ml) is sufficient to spoil UHT milk, even when stored under refrigerated conditions. As spore formers in particular can survive exposure to high heating temperatures, stringent hygiene standards are of utmost importance.

P-OT-345

Modulations of the microbiome from Atlantic salmon epidermal mucus after changes in diet and stressing conditions

*J. Clols-Fuentes[1], P. Unger[1], H. W. Palm[1]
[1]*University of Rostock, Aquaculture and sea ranching, Rostock, Germany*

The development of sustainable aquaculture is a prerequisite to minimize environmental impacts associated to aquaculture industry [1]. The replacement of fishmeal for plant-based diets is a key element to improve sustainability and fish welfare [2]. A balanced diet is important to counteract environmental changing circumstances that cause stress to fish [3]. The interpretation of the bacterial communities residing in the mucosal surfaces has been very poorly studied, and could act as a promising tool to use as welfare indicator during the fish production cycle [4, 5], since the epithelial mucus acts as a barrier against external stressors.The first aim of the study is to determine whereas environmental stressors cause changes in the microbiome from the mucosal surfaces of the fish. The second aim investigates whereas a microalgae-based diet modulates the microbiome from the epidermal mucus, and promotes a positive health condition during stressing conditions.

Each tank from the Recirculating Aquaculture System contained 20 Atlantic salmon. Water parameters were constant over the trial period. Swabs were collected before the start of the trial (T0) and after eight weeks of feeding the experimental diets (T1). Three different diets were formulated: Group A was fed with commercial feed; Group B feed contained cortisol (100 mg kg-1) as a stressor factor; Group C feed contained *Schizochytrium* sp. and cortisol. Eight fish were sampled per tank to collect one swabs from the epithelial mucus. Sample DNA was extracted and sent for the amplification of the V3-V4 16S rRNA gene region. Illumina MiSeq sequencing produced a total of 19,339,640 reads. DADA2 workflow for Amplicon Sequence Variants (ASV) prediction was used for data processing and analysis [6]. Relative abundances, diversity index and PERMANOVA were calculated.

The microbiome from the mucosal surface of the fish from all treatments was composed mainly by Proteobacteria, Bacteroidetes, Actinobacteria and Verrucomicrobia. Shannon diversity index was significantly different between T0 and T1 from the control group A. At T1, diversity was significantly different between group A and C, and between group B and C. Microbial diversity was significantly higher when fish was fed with microalgae, even under stressful conditions. The microbial diversity decreased after feeding the fish for 8 weeks with a commercial feed.

We observed a positive response from the fish and its external mucosal microbiome to the diet with microalgae during stress conditions

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P-OT-346

The future of the SILVA database as part of the DSMZ Digital Diversity

*M. Frentrup[1], J. Gerken[1], M. Chuvochina[1], Y. Sandikci[1], R. Goldmann[1], L. C. Reimer[1]
[1]*Leibniz Institute DSMZ, Braunschweig, Germany*

Motivated by the need to catalog rapidly growing sequence-based diversity, the SILVA database was developed to provide a comprehensive online resource of quality-controlled and aligned ribosomal RNA (rRNA) gene sequences from the *Bacteria*, *Archaea* and *Eukaryota* domains. The database quickly emerged as a central resource for microbial community studies worldwide, allowing users to align, classify, and design primers and probes based on gene sequences from both small and large rRNA subunits.

Over 30 years of development and community service provided by SILVA, starting with its main underlying software ARB, have been recognised by designating SILVA as a Core Data Resource in 2018 and Global Core Biodata Resource in 2023 by European ELIXIR and Global Biodata Coalition, respectively. As a member of these international organizations, SILVA is dedicated to maintaining high standards as a data platform for life science resources. This underlines SILVA"s efforts to provide users with easy access to a comprehensive collection of high-quality microbial rRNA sequences and associated metadata.

With the integration into the biodata infrastructure DSMZ Digital Diversity (https://hub.dsmz.de/#/), SILVA now has stable support for ongoing developments and improvements. The newly funded platform will facilitate the integration and interoperability of taxonomic, sequence, phenotypic data, and metadata among its principal databases such as Bac*Dive*, LPSN, Media*Dive*, and SILVA. Furthermore, the permanent hosting of the DSMZ will allow for continuous updates of the SILVA database and its taxonomy in the future. This will help to strengthen SILVA"s role as a leading rRNA database for microbial taxonomy and diversity studies.

P-OT-348

A novel metal-based antibiotic containing an N-heterocyclic carbene ligand with multiple modes of actions

*S. Schäkermann[1,2], D. Siegmund[3], P. Dietze[1,2], L. Janzen[3], L. Alejo Esquembre[4], J. Schubert[4], J. Straetener[4], T. Wannenwetsch[4], M. Engelhardt[3], C. Dunham[5], H. Brötz-Oesterhelt[4,6], N. Metzler-Nolte[3], J. E. Bandow[1,2]

[1]Ruhr University Bochum, Angewandte Mikrobiologie, Bochum, Germany

[2]Ruhr University Bochum, Center for System-based Antibiotic Research (CESAR), Bochum, Germany

[3]Ruhr University Bochum, Bioinorganic Chemistry, Bochum, Germany

[4]Eberhard Karls University Tübingen, IMIT - Infection Medicine, Microbial Bioactive Compounds, Tübingen, Germany

[5]Emory University, Chemistry, Atlanta, GA, United States

[6]Eberhard Karls University Tübingen, Cluster of Excellence 2124 Controlling Microbes to fight infections (CMFI), Tübingen, Germany

Metal complexes with N-heterocyclic carbene (NHC) ligands are a novel class of organometallic antibiotics with promising antibacterial activity. In this work we aimed to unravel the mode of action of a novel metal-based antibiotic containing an N-heterocyclic carbene ligand. Target fishing and chromatographic co-elution experiments revealed potential protein targets in *Bacillus subtilis*. The substance interferes with translation but also transcription in precursor incorporation assays. The proteomic response of *Bacillus subtilis* to treatment with sublethal concentrations of the compound was compared to responses to well-characterized antibiotics hitting the same target [2]. The comparison of proteome responses suggests that the mode of interference with the target protein is unprecedented. It further reveals similarities to the response to membrane-active antibiotics that are lytic and depolarize the membrane like gramicidin S and MP196 [1,3], which is congruent with a dose-dependent bacteriolytic activity and membrane depolarization caused by the metal-based antibiotic. We conclude that the compound is a novel promising lead structure for antibiotic development with multiple modes of action.

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P-OT-349

Extraintestinal *Escherichia coli* employ the membrane protein RcrB to protect against reactive chlorine species

S. Sultana[1], P. O. Tawiah[1], C. Jackson[1], M. Bennis[1], *J. U. Dahl[1]

[1]Illinois State University, School of Biological Sciences, Normal, IL, United States

Members of the *Escherichia coli* species are remarkably diverse and comprise commensal, probiotic and pathogenic strains. While some pathogenic *E. coli* cause intestinal diseases, extraintestinal *E. coli* (ExPEC) can colonize and infect environments outside the gut, including the urinary tract. Upon entry of the urinary tract, ExPEC must counter a multitude of bactericidal host defense strategies, which require specialized genetic adaption for survival from this *E. coli* pathotype. For instance, ExPEC must defend highly antimicrobial oxidnts such as hypochlorous acid (HOCl), a potent reactive chlorine species (RCS) generated during neutrophil-mediated phagocytosis and by enzymes in uroepithelial cells to control bacterial colonization. The increasing rate of ExPEC infections in humans due to changing infection dynamics demonstrate the critical need for a better understanding of ExPEC pathogenesis, which is desperately needed to improve approaches for infection prevention and treatment given the rise in antibiotic resistance spreading among *E. coli*. Our lab has reported that members of the ExPEC pathotype are more resistant to RCS *in vitro* and to neutrophil-mediated phagocytosis when compared to non-pathogenic and enteropathogenic *E. coli*. We identified the defense system responsible for these phenotypes and characterized its regulation during RCS stress: the RcrR regulon consisting of the *rcrARB* genes is controlled by the RCS-sensing transcriptional repressor RcrR, which reversibly loses its repressor activity upon oxidation by RCS, resulting in de-repression of its downstream targets. Induced expression of RcrB, which we identified as a membrane protein, contributes significantly to ExPEC"s increased RCS resistance. We show that ExPEC cells that lack RcrB are characterized by elevated protein and DNA damage, indicating increased RCS influx into the cell. Moreover, RcrB quenches RCS in the media, which it does through select redox-active amino acids located on its periplasmic side. Overall, our data indicate that RcrB works as an RCS reductase, which is novel in the field. Thus, RcrB plays a crucial role for ExPEC in maintaining bacterial redox homeostasis in RCS-rich environments.

Late poster

LP-01

From taxonomy to metabolism: Exploring the Methane Production Potential of Anaerobic Microbiomes

M. F. Villegas Torres[1], *V. H. Rúa López[2], A. F. González Barrios[2]

[1]Centro de Investigaciones Microbiológicas (CIMIC), Biology, Bogotá, Colombia

[2]Grupo de Diseño de Productos y Procesos (GDPP), Departamento de Ingeniería Química y de Alimentos, Bogotá, Colombia

Anaerobic digestion is a sustainable strategy for the treatment of organic waste, notable for its ability to produce biogas and reduce environmental impact. This study addresses the challenges of understanding the complex dynamics of microbial communities in this process using an approach that integrates metagenomic analyses and genome-scale metabolic modelling (GEM). Combinations of inoculants (wastewater treatment plant sludge and cattle manure) with substrates (pig manure, poultry manure and food waste) were evaluated under different conditions and their impact on microbial composition, metabolic dynamics and methane production was analysed.

Reconstruction of microbial communities from MAGs (genomes assembled from metagenomes) was a major challenge due to the complexity and diversity of the environmental samples. Nevertheless, high quality genomes were obtained, allowing the characterisation of key methanogenic archaea and fermentative bacteria. These results highlight the importance of syntrophic interactions in methane production and substrate degradation.

Metabolic modelling with GEMs facilitated the simulation of processes such as methanogenesis and the production of intermediate metabolites (acetate, lactate and butyrate). The

models predicted clear differences in metabolic performance according to inoculum and substrate combinations, showing an increase in methane production under certain co-digestion conditions. Furthermore, temporal analysis revealed how communities evolve to maximise metabolic efficiency throughout the process.

This study highlights the importance of reconstructing microbial communities from MAGs as a tool for understanding and optimising anaerobic digestion. The results highlight the critical role of microbial interactions in process efficiency, providing a basis for improving biogas production and designing more sustainable and efficient waste treatment systems.

Keywords: Anaerobic digestion, microbial communities, metagenome-assembled genomes (MAGs), genome-scale metabolic models, methane production, Co-digestion, Syntrophic interactions, waste treatment optimization

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LP-02

Integrating Bioinformatics for Microbial Discovery: Unlocking Polyhydroxyalkanoates (PHA) Potential in Hypersaline Environments

*J. Elgazel[1,2], F. Meyer[2], S. M. Diaz Valerio[2], A. Bathy[1], N. Mhiri[1], D. Schneider[2], S. Tounsi[3], H. Liesegang[2], F. Karray[1]
[1]University of Sfax, Center of Biotechnology of Sfax, Sfax, Tunisia
[2]Göttingen Genomics Laboratory (G2L), Georg-August-Universität, Göttingen, Germany
[3]University of Sfax, Biopesticides Laboratory, Center of Biotechnology of Sfax, Sfax, Tunisia

Background and Aim: Petroleum-based plastics contribute to persistent environmental pollution and health risks. Polyhydroxyalkanoates (PHAs) offer a promising biodegradable and biocompatible alternative. This study investigates metagenomic data from hypersaline environments to identify novel metabolic pathways involved in PHA production. Methods: Water and sediment samples were collected from hypersaline ecosystems in southern Tunisia. Oxford Nanopore and Illumina sequencing technologies were used to generate metagenomes. Hidden Markov Model (HMM)-based bioinformatics pipelines were developed to identify PHA biosynthetic gene clusters in halophilic microorganisms. Results: Analysis revealed key genes involved in PHA biosynthesis, including PHA synthases, depolymerases, and regulatory proteins. These genes are central to the polymerization and degradation of PHAs. The identification of shared operon structures indicates that PHA-synthesis is encoded in conserved operon structures. Functional validation through genetic engineering is planned to confirm their roles in PHA production. Conclusion: This study highlights the biosynthetic potential of halophilic microorganisms in hypersaline environments, emphasizing their promise for sustainable bioplastic production. Keywords: Bioplastics, Polyhydroxyalkanoates, Hypersaline Microorganisms, Metagenomics, Hidden Markov Models, Sustainable Alternatives

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LP-03

Utilizing photosystem I to drive CYP153A monooxygenase activity by light

*L. Pospisil[1], A. Frank[2], A. Volkmann[2], F. Schultes[3], C. Mügge[3], D. Tischler[3], M. M. Nowaczyk[1,2]
[1]Ruhr University Bochum, Mikrobielle Biotechnologie, Bochum, Germany
[2]University Rostock, Molecular Machines of Photosynthetis, Department of Biochemistry, Rostock, Germany
[3]Ruhr University Bochum, Microbial Biotechnology, Faculty of Biology & Biotechnology, Bochum, Germany

Nowadays, the chemical industry has a great interest in the sustainable production of chemicals such as ω-hydroxylated hydrocarbons or epoxides as chemical feedstocks. As an alternative to the chemical synthesis, the enzymatic approach is considered promising. CYP153A oxidoreductases, a family of the P450 monooxygenase superfamily, are known to catalyze diverse oxidation reactions, e.g. the terminal hydroxylation of various carbon hydrates or the epoxidation of carbon double bounds in a highly regio- and stereoselective manner. In situ, CYP153A enzymes are part of a NAD(P)H dependent three component system consisting of a reductase and a ferredoxin as an electron carrier.

The accessibility of NAD(P)H as the electron donor is one of the most relevant limitations for the enzymatic biocatalysis. Various NAD(P)H regeneration systems are available but are also harboring unfavorable downsides. A strategy to overcome the dependence of NAD(P)H is the functional coupling of the CYP153A enzymes to the photosynthetic electron transport chain to enable a light dependent biocatalysis. As a proof of concept, a photosystem I (PSI) driven cascade was established, utilizing combinations of two ferredoxins from Thermosynechococcus vestitus and Acinetobacter sp. OC4 as an electron mediator to enable the monooxygenase activity of two CYP153A candidates from Polaromonas sp. JS666 or Gordonia rubripertincta CWB2.

Active PSI trimers were isolated from the thermophilic cyanobacterium T. vestitus via hydrophobic interaction chromatography, whereas the ferredoxins and monooxygenases were expressed as recombinant proteins in E. coli and purified via Ni-NTA affinity chromatography. For light driven biocatalysis, the conversion of different alkanes to their respective alkanols, the formation of perillyl alcohol from limonene and the epoxidation of aromatic and aliphatic substrates were tested, analyzed and quantified via GC-FID. In addition, P700+ reduction kinetics were established as a screening tool for electron acceptors for PSI.

In conclusion, the formation of 1-octanol, 1- hexanol, perillyl alcohol and 1,2-epoxyhexane using the in vitro cascade serves as a proof of concept for PSI driven monooxygenase activity and paves the way for the implementation of CYP153A enzymes in cyanobacteria for in vivo models. Additionally, P700+ reduction kinetics could be utilized to determine the electron transport efficiency between PSI and various potential electron acceptors.

LP-04

The effect of flavonoids on cyanobacterial motilitiy and their role in symbiotic relationship with land plants

*D. Büyüktaş[1], W. D. M. Mesquita[1], A. Dadras[1], C. G. Köhne[1], A. Pöhlein[2], C. Herfurth[3,4], I. Feussner[3,4], R. Daniel[2], T. Darienko[1], M. Lorenz[5], S. de Vries[1]
[1]University of Goettingen, Department of Applied Bioinformatics, Institute for Microbiology and Genetics, Göttingen, Germany
[2]University of Goettingen, Department of Genomic and Applied Microbiology, Göttingen, Germany
[3]Goettingen Center for Molecular Biosciences (GZMB), Service Unit for Metabolomics and Lipidomics, Göttingen, Germany
[4]University of Goettingen, Department of Plant Biochemistry, Göttingen, Germany

[5]University of Goettingen, Department of Experimental Phycology and SAG Culture Collection of Algae, Göttingen, Germany

Cyanobacteria have symbiotic relationships with diverse land plants, from bryophytes to ferns, gymnosperms, and angiosperms. One specific example is the symbiosis between Azolla filiculoides and its cyanobiont Nostoc azollae: the cyanobiont is vertically inherited, and both partners co-evolve, which results in full co-dependency. Essential for all cyanobacterial symbioses is the ability to differentiate into motile filaments, hormogonia, upon plant cues. In Azolla, hormogonia is required to colonize new tissue and inheritance; however, the molecular cues underlying the symbiotic transfer are only poorly understood. Salicylic acid (SA) is produced by A. filiculoides and in land plants as a defense response to different pathogenic microbes in the environment. When SA is exogenously applied to A. filiculoides, its cyanobiont's abundance and gene expression are altered. Our preliminary transcriptomic data suggest that SA may control flavonoids, metabolites that in the past have been suggested to influence cyanobacterial motility. We want to test whether SA and flavonoids play a role in cyanobacterial symbiotic relationships. To do that, we investigated the effect of SA and flavonoids produced by A. filiculoides on cyanobacterial symbionts. We induced motility in cyanobacteria using far-red light and tested the ability of the phenolic compounds to modulate the transition into hormogonia stages. The results of the treatments were documented using light microscopy and RNA was extracted for further analyses and then sequenced. We set up a machine-learning-based image analysis pipeline and analysed light microscopy images to quantify differentiation into hormogonia and the amount of vegetative cells. We found that SA and the majority of flavonoids tested enhanced hormogonia transition in a symbiotic cyanobacterium. Now, we are analysing the RNA-Seq data obtained from this experiment to connect differential gene expression to the motility phenotype, hereby focusing on genes known to modulate motility, nitrogen-fixation, and those suggested to be related to the communication between cyanobacteria and their hosts.

LP-05

A microfluidic droplet-based system for the phototrophic cultivation of cyanobacteria to optimize growth for physiological and analytical data acquisition from bulk to single cell level

*M. Herzberg[1], M. Schirmer[1], P. Böhme[1], C. Dusny[1], A. Schmid[1]
[1]Helmholtz-Zentrum für Umweltforschung GmbH - UFZ, Department of Solar Materials Biotechnology (SOMA), Leipzig, Germany

Introduction: Microfluidic systems offer the possibility of combining and integrating many process steps in a very small space, enabling highly miniaturized continuous workflows. In combination with miniaturization in the field of quantitative bioanalytic and their integration into microfluidic systems, they are the key to a better understanding of cellular functioning and will also be crucial for further advances in the field of miniaturized and microfluidic from bulk to single cell analysis. Cyanobacteria such as *Synechocystis* sp. PCC 6803 are photosynthetic microorganisms with considerable biotechnological potential in the bio recovery of commodities, the production of various bioactive compounds and renewable biofuels. And could thus support the transition from the traditional linear economy to a circular economy in the near future. By means of phototrophic growth through oxygenic photosynthesis and the associated ability to produce photobiological hydrogen, the potential of an eco-friendly and renewable future-oriented energy source is also given.

Objectives: The microfluidic systems and droplet generation on the pL to nL scale bring bulk cultivation to the level of single cell cultivation for analyzing the heterogeneity of cell populations. Cell proliferation should be trackable online to determine optimal growth conditions and parameters of hydrogen production driven by oxygenic photosynthesis. The microfluidic LOC (Lab-on-Chip) device acts as a photobioreactor in these studies.

Materials & methods: Microfluidic chips for droplet generation, incubation and analysis. Light-coupled microfluidic Photobioreactor. Optical online data acquisition and flow cell endpoint determination.

Results: Non-invasive optical cell mass determination with sub-pg sensitivity, microfluidic perfusion cultivations for establishing physiological steady-states, and nano- to picoliter batch reactors, enabled the quantification of all physiological parameters. The heterogeneity of the cellular reactivity of single biocatalysts per droplet could be demonstrated by parallelizing the droplet incubation. These results enable the resolution of biocatalysis beyond averages of populations.

Conclusion: Microfluidic droplet-based system for the phototrophic cultivation as nano size batch photobioreactors enables the quantification of physiological growth parameter and allows the analysis of the heterogeneity of cell population on from bulk to the single cell level.

LP-06

Actinospongicola halichondria* gen. nov. sp. nov., the first sponge-derived cultivated representative of a new genus within the class *Acidimicrobiia

J. Huang[1,2], J. Wiese[1], L. X. Steiner[1], T. Rahn[1], E. Borchert[1], Y. Ying[2], *U. Hentschel[1]
[1]GEOMAR Helmholtz Centre for Ocean Research Kiel, RU Marine Ecology, RD3 Marine Symbioses, Kiel, Germany
[2]Chinese Academy of Sciences (IMCAS), Institute of Microbiology, Beijing, China

The current, valid taxonomic class *Acidimicrobiia* includes two orders (*Acidimicrobiales, Iamiales*), three families (*Acidimicrobiaceae*, *Iamiaceae* and *Ilumatobacteraceae*) and 16 species. Bacteria from the family *Acidimicrobiaceae* are often described from acid mine drainage or geothermal vents while members of the *Iamiaceae* and *Ilumatobacteraceae* are found in neutral or mildly alkaline pH environments, such as marine sediments and invertebrates or the human skin. Recent studies using culture-independent methods have shown a wide distribution of this clade ranging from coastal to deep sea environments as well as various marine invertebrate hosts. *Acidimicrobiia* isolation has however been notoriously difficult and frequently unsuccessful. In the present study, we report on the isolation of a novel *Acidimicrobiia* strain Hal371^T from the Baltic Sea sponge *Halichondria panicea*. The strain forms very small, nearly translucent colonies on Baltic Sea agar plates that can easily be overlooked. Based on phylogenetic, genomic, chemotaxonomic, and physiological features, strain Hal317^T was described as member of a novel genus of the family *Iamiaceae,* for which the name *Actinospongicola halichondria* is proposed. The genome of strain Hal317^T exhibited diverse characteristics, ranging from the utilization of diverse carbohydrates, transport of amino acids, osmoprotectants synthesis, defense mechanisms against

reactive oxygen species (ROS), chaperones, and toxin antitoxin systems, that may reflect an adaptation to a lifestyle within marine sponges. Strain Hal317^T displays further unique genome features that set it apart from other type strains of *Iamiaceae,* such as a non-phosphorylated L-fucose pathway, a substrate-specific phosphonate pathway, and a glutamate-uptake system. By metabolizing of taurine as well as by uptaking and utilizing of urea and ammonia the isolate might be involved in detoxification processes of sponge-derived metabolic products.

LP-07

Functional characterisation of the [FeFe] hydrogenase SoHydAB from *Shewanella oneidensis*

*F. Riemer[1], E. Hofmann[1]
[1]*Ruhr-Universität Bochum, AG Proteinkristallographie, Bochum, Germany*

Hydrogenases are metalloenzymes that catalyse the interconversion between two protons and two electrons on one hand and molecular hydrogen (H2) on the other hand. Diverse members of this enzyme family fulfil a wide variety of metabolic functions. The [FeFe] hydrogenase of *Shewanella oneidensis* presents an intriguing case.

This bacterium is well known for its metabolic versatility, allowing it to grow on and reduce an astounding variety of substrates, including heavy metals and highly toxic chemicals. These abilities have made *Shewanella* a popular choice for the application in microbial fuel cells and wastewater treatment. Due to these properties, the physiology and metabolism of *Shewanella* has been studied intensively and is well characterised. Biochemically however, the hydrogen metabolism of *Shewanella* remains understudied.

The [FeFe] hydrogenase of *Shewanella oneidensis* is located in the periplasm, but presumably anchored to the inner membrane. Its activity is completely abolished by the deletion of formate dehydrogenase, but unlike *E. coli* for example, *Shewanella* does not appear to form a formate-hydrogen-lyase complex. Furthermore, it has been shown that hydrogenase activity is also fully abolished in the absence of menaquinone.

Taken together, the properties of this enzyme and its metabolic context suggest that it either forms a scientifically interesting complex or has a variety of interaction partners, which is likely to lead to further research questions regarding the mechanisms and regulation of these interactions. The first step in elucidating the hydrogen metabolism in *Shewanella* is a full investigation into the properties of the hydrogenase itself.

This poster presents the first characterisation of the [FeFe] hydrogenase SoHydAB utilising an array of chromatographic, electrochemical and spectroscopic methods.

LP-08

From random mutagenesis to genome editing: Targeting *spo0A* in *Bacillus thuringiensis* with CRISPR-Cas9

*I. Chouaibi[1], S. Beirami[2], F. Benjedou[1], S. M. Diaz Valerio[2], A. Poehlein[2], S. Tounsi[1], R. Zribi Zghal[1], H. Liesegang[2]
[1]*University of Sfax, Biopesticides laboratory, Center of Biotechnology of Sfax, Sfax, Tunisia*
[2]*University of Göttingen, Department of Genomic and Applied Microbiology, Goettingen, Germany*

***Bacillus thuringiensis (Bt)*,** a Gram-positive spore former, is a reference biopesticide, largely due to its production of insecticidal 6-endotoxins. However, introducing high quantities of a sporulating organism may prolong its environmental persistence and pose ecological concerns. This study combines random mutagenesis and CRISPR-Cas9 genome editing to obtain more efficient, eco-friendly *Bt* strains, focusing on the inactivation of the master sporulation regulator, *spo0A*.

Experimental Setup

Our approach comprised two phases:

(I) Random mutagenesis generated hyperproductive and oligosporogenic variants of the *Bt* strain BUPM98, which were screened for toxin production and sporulation.

(II) The most promising mutant was chosen for CRISPR-Cas9 genome editing, targeting *spo0A* to modulate sporulation without undermining toxin output. This strategy pinpoints crucial genomic changes that affect production and sporulation.

Results

Five hyperproductive mutants were identified, three of which displayed markedly Reduced sporulation. Genomic analyses revealed multiple chromosomal rearrangements, frequently mediated by prophages and transposons. Mutant N12 exhibited a 112% surge in toxin production relative to the wild type, with sporulation rates approximating the parental strain (log = 0.04). This outcome correlates with expanded copies of the *ylmC* gene, likely integral to hyperproduction. In contrast, mutant Nl3 lost several sporulation-related genes, resulting in a reduced sporulation rate (1og2 = -1.34). Guided by these observations, we designed a *spo0A*- targeting RNA to engineer a non-sporulating strain from N12 while preserving its productivity.

Conclusion

These findings highlight the impact of genomic rearrangements and targeted editing on *Bt* phenotypes. Notably, N12 combines enhanced toxin yield with stable sporulation, making it a promising candidate for sustainable biocontrol solutions. The *spo0A* guide RNA further

underscores CRISPR-Cas9"s potential for refining *Bt* strains in line with ecological pest management. Overall, merging classical mutagenesis with modem genome editing can tailor *Bt* to meet specifıc agricultural and environmental needs.

Keywords

Bacillus thuringiensis, random mutagenesis, genomic rearrangements, sporulation genes, CRISPR-Cas9

ISBN 978-3-948023-51-5