technische universität
dortmund

International VAAM Workshop 2022
Biology of Microorganisms Producing Natural Products

September 7 - 9, 2022 | Dortmund, Germany

WELCOME ADDRESS

Dear Conference Registrants,

Welcome to the annual VAAM workshop on the “Biology of Microorganisms Producing Natural Products”. My colleagues and me are delighted to be hosting this scientific symposium at TU Dortmund University for the first time.

The Scientific Organizing Committee has assembled an exciting and diverse program. The titles and authors of all presentations as well as the corresponding abstracts can be found in this brochure. The scientific program includes three plenary talks given by internationally renowned scientists, namely Emily Balskus (Harvard University, USA), Alessandra Eustaquio (University of Illinois at Chicago, USA) and Gregory Challis (University of Warwick, UK). Overall, the program remains faithful to the traditional areas of interest to the sponsoring Society, natural products biosynthesis and its regulation, while providing glimpses into areas with strong natural products connections: chemistry of microbial interactions, genome mining, drug discovery and engineering of metabolic pathways.

We are very grateful to the many people who have contributed in some way to make this meeting possible.

We hope you enjoy both the scientific and social program that we have developed.

Yours truly,

Prof. Dr. Markus Nett
Local Organizing Committee Chair

ORGANIZATION

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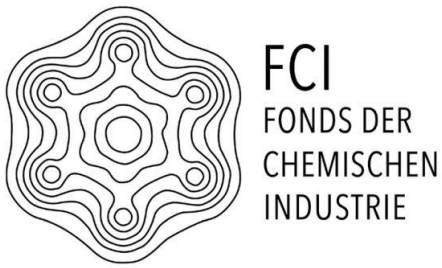
We would like to thank the following partners for their financial support:



Program

Wednesday, September 7th

15:00 – 16:00	Registration (Foyer of Emil-Figge 50)
16:00 – 16:20	Opening remarks and Welcome addresses
16:20 – 17:20	**PL1 - Alessandra Eustaquio, University of Illinois at Chicago** *Reverse genetics and heterologous expression for natural product discovery*
17:20 – 18:30	Poster pitches I Chair: Markus Nett, TU Dortmund University Session "Discovery" (posters **P1-P22**) Session "Evolution, Regulation & Function" (posters **P23-P31**)
18:30 – 20:00	Poster presentation I on site with finger food and drinks as well as online with interactive user interface (Gather T17own)

Thursday, September 8th

	Lecture Session "Discovery" Chair: Hajo Kries, Hans Knöll Institute, Jena
09:00 – 09:15	**SL1 – Therese Horch** *Alternative benzoxazole assembly discovered in anaerobic bacteria provides access to privileged heterocyclic scaffold*
09:15 – 09:30	**SL2 – Sophie Jünger** *Discovery and biosynthesis of methanobactin-like peptides in non-methanotrophic bacteria*
09:30 – 09:45	**SL3 – Pakjira Nanudorn** *Atropopeptides are a novel family of ribosomally synthesized and posttranslationally modified peptides with a complex molecular shape*
09:45 – 10:00	**SL4 – Sebastian Walesch** *Revisiting Angiolams, a class of neglected antibiotics from myxobacteria*
10:00 – 10:15	**SL5 – Elena Seibel** Amycolatopsis *spp. – a neglected source for novel natural products and biosynthetic pathways*

10:15 – 11:00	Coffee break on site and virtual (Gather Town)
11:00 – 12:00	**PL2 - Emily Patricia Balskus, Harvard University** *Chemical discovery in the microbial world*
12:00 – 12:30	VAAM Business Meeting
12:30 – 14:00	Lunch
	Lecture Session "Biosynthesis" Chair: Eric Helfrich, Goethe University Frankfurt
14:00 – 14:15	**SL6 - Sophie Klöppel** *Structural and biochemical insights into the transesterifying thioesterase domain from FR900359 biosynthesis*
14:15 – 14:30	**SL 7 - Mahmudul Hasan** *Evolutionary insights into the biosynthesis of coenzyme 3PG-F_{420}*
14:30 – 14:45	**SL8 - Nancy Magnus** *Identification of non-canonical terpene biosynthetic routes in bacteria*
14:45 – 15:00	**SL9 - Lena Barra** *NAD as a building block in natural product biosynthesis*
15:00 – 15:15	**SL10 - Silja Mordhorst** *Structural and biochemical characterisation of the peptide arginase OspR from landornamide biosynthesis*
15:15 – 15:45	Coffee break on site and virtual (Gather Town)
15:45 – 16:45	**PL3 –Gregory Challis, University of Warwick** *Gladiolin biosynthesis as a model for understanding complex metabolite assembly by trans-AT polyketide synthases*
16:45 – 18:00	Poster pitches II Chair: Stephan Lütz, TU Dortmund University Session "Biosynthesis" (posters **P32-P45**) Session "Engineering & Synthesis" (posters **P46-P64**)
18:00 – 19:30	Poster presentation II on site as well as online with interactive user interface (Gather Town)
19:30	Dinner and Get together

Friday, September 9th

Chair: Helge Bode, MPI for Terrestrial Microbiology, Marburg

Hendrik Wolff Award

08:30 – 09:00 Award lecture

Lecture Session "Engineering & Synthesis"

Chair: Till Schäberle, Justus-Liebig-University Giessen

09:00 – 09:15 **SL11 – Irina Voitsekhovskaia**

Generation of novel glycopeptide antibiotics using mutasynthesis approach

09:15 – 09:30 **SL12 – Huiyun Peng**

Programming nonribosomal peptide synthetases with DNA templates

09:30 – 09:45 **SL13 – Kilan Schäfer**

Biochemical and metabolic engineering of Saccharomyces cerevisiae *for the production of cannabinoid precursors*

09:45 – 10:00 **SL14 – Fabrizio Alberti**

Pleuromutilin biosynthesis and production of its congeners through heterologous gene expression in fungi

10:00 – 10:15 **SL15 – Svetlana Kalinina**

Novel microscale semi-synthetic approach toward biologically active isoindolinones from S. chartarum

10:15 – 11:00 Coffee break on site and virtual (Gather Town)

Lecture Session "Evolution, Regulation & Function"

Chair: Yvonne Mast, Leibniz Institute DSMZ, Braunschweig

11:00 – 11:15 **SL16 – Wiebke Hanke**

Effects of the Gq inhibitor FR900359 on soil nematodes

11:15 – 11:30 **SL17 – Athina Gavriilidou**

Sugar and spice: reclassifying glycopeptides and related antibiotics

11:30 – 11:45	**SL18 – Judith Boldt** *Transcription of BGCs and production of natural compounds in the myxobacterium* Sorangium *sp.*
11:45 – 12:00	**SL19 – Maria Stroe** *On the role of natural products as virulence factors in fungi with a predatory lifestyle*
12:00 – 12:15	**SL20 – Sergii Krysenko** *Polyamine and ethanolamine utilization in* Streptomyces *coelicolor*
12:15 – 12:30	Poster awards and Closing remarks

PL1 Reverse genetics and heterologous expression for natural product discovery

Alessandra S. Eustáquio

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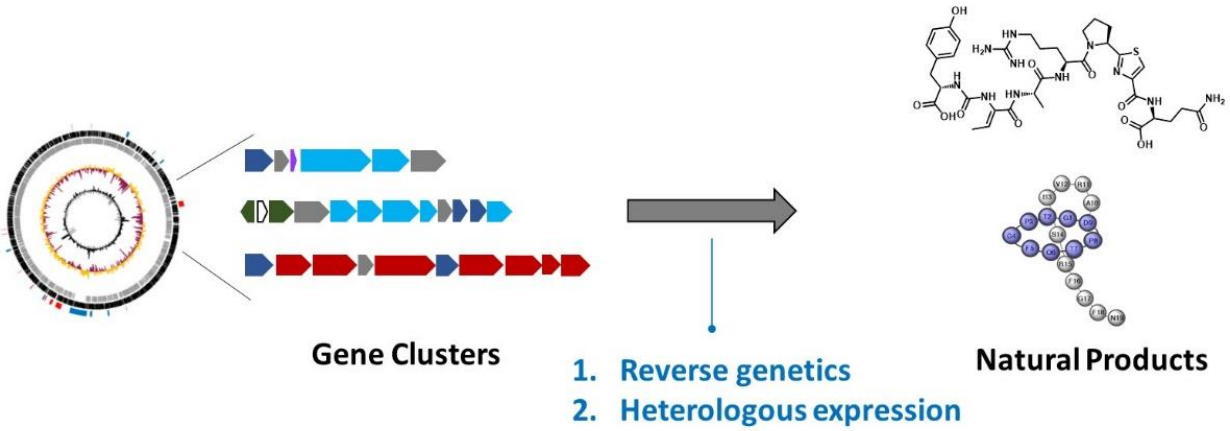
My research group is interested in bacterial natural products for their potential to be used as pharmaceuticals, and to gain insights into the ecological roles they play. We apply bioinformatics tools to identify gene clusters of interest. We then develop either reverse genetics methods or synthetic biology tools to facilitate natural product discovery, to study their biosynthesis and function, and to address supply issues. Two recent examples will be presented.

Reverse genetics. Although natural products have been harnessed as therapeutics to treat human ailments for centuries, their ecological roles are often unknown. We are interested in studying natural products from *Pseudovibrio*, a predominant component of the microbiome of marine sponges that has been associated with sponge health. After establishing reserve genetics for *Pseudovibrio*, we were able to isolate natural products we termed pseudovibriamides and show that they influence motility and biofilm formation, behaviors that are important for host colonization.[1] We are currently investigating how pseudovibriamides affect these behaviors and their relevance to the bacteria-sponge association.

Heterologous expression. Supply issues can hinder natural product development. My laboratory is interested in developing synthetic biology tools to facilitate natural product discovery and production.[2,3] I will present our recent efforts towards exploring *Burkholderia* bacteria as a source of natural products[4] and towards developing a *Burkholderia* host.[5] We obtained promising proof-of-principle data in which our selected *Burkholderia* host produced a model antibiotic in yields that were ~500-fold higher than obtained with the commonly used *Escherichia coli* host.[5] We are currently working on understanding the basis for high-yield production and in applying the knowledge to other products.



[1] Ióca LP, Dai Y, Kunakom S, Diaz-Espinosa J, Krunic A, Crnkovic CM, Orjala J, Sanchez LM, Ferreira AG, Berlinck RGS, Eustáquio AS (2021) A family of nonribosomal peptides modulate collective behavior in *Pseudovibrio* bacteria isolated from marine sponges. *Angew Chem Int Ed Engl* 60: 15891-15898.

[2] Kunakom S, Eustáquio AS (2019) Natural products and synthetic biology – Where we are and where we need to go. *mSystems* 4: e00113-19.

[3] Romanowski S, Eustáquio AS (2020) Synthetic biology for natural product drug production and engineering. *Curr Opin Chem Biol* 58: 137-145.

[4] Kunakom S, Eustáquio AS (2019) *Burkholderia* as a source of natural products. *J Nat Prod* 82: 2018-2037.

[5] Kunakom S, Eustáquio AS (2020) Heterologous production of lasso peptide capistruin in a *Burkholderia* host. *ACS Synth Biol* 9: 241-248.

PL2 Chemical discovery in the microbial world

Emily Patricia Balskus

Department of Chemistry and Chemcial Biology, Harvard University, Cambridge, Massachusetts 02138, USA

Microbes have amazing chemical capabilities, performing reactions unprecedented in organic synthesis and producing complex, biologically active molecules not easily accessed via other approaches. Recent advances in DNA sequencing technologies have delivered a wealth of microbial genomes that encode novel biosynthetic gene clusters. The availability of this genomic data thus represents an unprecedented opportunity for the discovery of new bioactive natural products and new enzymes that have the potential to reveal new principles of catalysis and find use in chemical synthesis. This talk will discuss our recent progress discovering new enzymes that mediate unusual C–X and N–N bond forming reactions in biosynthetic pathways from environmental microbes, and our efforts to leverage an understanding of biosynthetic enzymes to accelerate natural product discovery. Functional and mechanistic characterization of these enzymes is uncovering reactivity with potential applications in biocatalysis and metabolic engineering, as well as revealing previously unappreciated metabolic diversity in the microbial world.

PL3 Gladiolin biosynthesis as a model for understanding complex metabolite assembly by *trans*-AT polyketide synthases

Gregory Challis

Department of Chemistry and Warwick Integrative Synthetic Biology Centre,
University of Warwick, Coventry CV4 7AL, United Kingdom

Polyketides are structurally diverse group of natural products with wide-ranging applications in human medicine, animal health, and crop protection. In bacteria, most structurally complex polyketides are assembled by giant multienzyme assembly lines called type 1 modular polyketide synthases (PKSs), which are divided into two phylogenetically distinct classes termed "*cis*-AT" and "*trans*-AT". While *cis*-AT PKS typically have acyl transferase (AT) domains, which select and load an extender unit onto an adjacent acyl carrier protein (ACP) domain, incorporated into each module, *trans*-AT PKSs employ a single standalone AT that supplies a malonyl extender unit to each module of the assembly line. The mechanisms of polyketide assembly by *cis*-AT PKSs, as exemplified by 6-deoxyerythronolide B synthase, have been extensively studied and are relatively well understood. In contrast, polyketide assembly by *trans*-AT PKSs is still relatively understudied and much remains to be learned about the mechanisms involved. In this lecture, I describe my group's efforts to understand the assembly of gladiolin, a macrolide antibiotic we recently discovered that has promising activity against multi-drug tuberculosis,[1,2] by a *trans*-AT PKS in the cystic fibrosis pathogen *Burkholderia gladioli* BCC0238. The results of these studies indicate that the gladiolin assembly line is an excellent model system for investigating mechanisms of polyketide assembly by *trans*-AT PKSs.[3,4]

[1] L. Song, M. Jenner, J. Masschelein, C. Jones, M. Bull, S. Harris, R. Hartkoorn, A. Vocat, I. Romero-Canelón, P. Coupland, Paul, G. Webster, M. Dunn, R. Weiser, C. Paisey, S. Cole, J. Parkhill, E. Mahenthiralingam and G.L. Challis. Discovery and biosynthesis of gladiolin: a Burkholderia gladioli antibiotic with promising activity against Mycobacterium tuberculosis. J. Am. Chem. Soc. 2017, 139, 7974-7981

[2] C. Perry, J. Sargeant, L. Song and G.L. Challis. Relative stereochemical assignment of C-33 and C-35 in the antibiotic gladiolin. Tetrahedron, 2018, 74, 5150-5155

[3] M. Jenner, S. Kosol, D. Griffiths, P. Prasongpholchai, L. Manzi, A.S. Barrow, J.E. Moses, N.J. Oldham, J.R. Lewandowski and G.L. Challis. Mechanism of intersubunit ketosynthase–dehydratase interaction in polyketide synthases. Nat. Chem. Biol. 2018, 14, 270-275

[4] C. Hobson, M. Jenner, X. Jian, D. Griffiths, D.M. Roberts, M. Rey and G.L. Challis. Diene incorporation by a dehydratase domain variant in modular polyketide synthases. Nat. Chem. Biol. 2022, 18, DOI: 10.1038/s41589-022-01127-y

SL1 Alternative benzoxazole assembly discovered in anaerobic bacteria provides access to privileged heterocyclic scaffold

Therese Horch,[1] *Evelyn M. Molloy*,[1] *Florian Bredy*,[1] *Veit G. Haensch*,[1] *Kirstin Scherlach*,[1] *Kyle L. Dunbar*,[1] *Jonathan Franke*,[1] *Christian Hertweck*[1,2]

[1]*Leibniz Institute of Natural Product Research and Infection Biology, HKI, Jena, Germany*

[2]*Faculty of Biological Sciences, Friedrich Schiller University Jena, Jena, Germany*

Benzoxazole scaffolds feature prominently in diverse synthetic and natural product-derived pharmaceuticals. Our understanding of their bacterial biosynthesis is, however, limited to *ortho*-substituted heterocycles from Actinomycetes. We report an overlooked biosynthetic pathway in anaerobic bacteria (typified in *Clostridium cavendishii*) that expands the benzoxazole chemical space to *meta*-substituted heterocycles and heralds a distribution beyond Actinobacteria. The first benzoxazoles from the anaerobic realm (closoxazole A and B) were elucidated by NMR and chemical synthesis. By genome editing in the native producer, heterologous expression in *Escherichia coli*, and systematic pathway dissection we show that closoxazole biosynthesis invokes an unprecedented precursor usage (3-amino-4-hydroxybenzoate) and manner of assembly. Synthetic utility was demonstrated by the precursor-directed biosynthesis of a tafamidis analogue. A bioinformatic survey reveals the pervasiveness of related gene clusters in diverse bacterial phyla.

SL2 Discovery and biosynthesis of methanobactin-like peptides in non-methanotrophic bacteria

Sophie Jünger, Zhilal Hong, Yanyan Li

Unité Molécules de Communication et Adaptation des Micro-organisms (MCAM), UMR 7245, Muséum National d'Histoire Naturelle (MNHN), CNRS, Paris, France.

Methanobactins (Mbns) are the only known copper-binding peptides (chalkophores) which are produced by methanotrophic bacteria to acquire copper from their environment.[1] Mbns belong to the group of ribosomally synthesized and post-translationally modified peptides (RiPPs) and their characteristic structural feature is a pair of nitrogen-containing heterocycles and two neighbouring thioamide groups.[1] These heterocycles are introduced through the modification of two conserved cysteine residues in the precursor MbnA and enable the coordination of copper by Mbns.[2] To date, Mbns have been isolated only from methanotrophs, although genome mining studies revealed the presence of Mbn-like clusters also in the genomes of non-methanotrophic bacteria.[3] One potential Mbn-like cluster can be found in the environmental strain *Pseudomonas extremaustralis*, which was isolated from a temporary pond located on Antarctic Peninsula. Previous work in our lab indicated however that the biosynthetic gene cluster is silent under tested laboratory conditions. Subsequently, by a promotor exchange approach, we were able to activate *mbn* gene expression in *P. extremaustralis* and LC-MS analysis of the extracted culture medium revealed the presence of the expected Mbn-Cu complex. The identification of a new Mbn peptide from *P. extremaustralis* expands this group of RiPPs and allows us to investigate the biosynthesis as well as gain insights into regulatory mechanisms of copper homeostasis in non-methanotrophic bacteria.

[1] Kim, H. J. *et al*. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria **2004** *Science,* 305, 1612–1615.

[2] Kenney, G. E. *et al*. The biosynthesis of methanobactin **2018** *Science*, 359, 1411–1416.

[3] Semrau, J. D., DiSpirito, A. A., Obulisamy, P. K. & Kang-Yun, C. S. Methanobactin from methanotrophs: genetics, structure, function and potential applications **2020** *FEMS microbiology letters* 367, 5.

SL3 Atropopeptides are a novel family of ribosomally synthesized and posttranslationally modified peptides with a complex molecular shape

Pakjira Nanudorn,[1, 2] Sirinthra Thiengmag,[1, 2] Friederike Biermann,[1, 2] Pelin Erkoc,[1,2] Sabrina D. Dirnberger,[1,2] Thao N. Phan,[1,2] Robert Fürst,[1,2] Reiko Ueoka,[3] Eric. J. N. Helfrich[1,2]

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Natural products with ambiguous molecular shape are rare phenomena. Tryptorubin A is a hexapeptide with an extremely rigid three-dimensional shape that can adopt one of two unusual atropisomeric configurations. Initially hypothesized to be a non-ribosomal peptide, we show that tryptorubin A is in fact the first characterized member of a new family of ribosomally synthesized and posttranslationally modified peptides (RiPPs) that we named atropopeptides. Atropopeptide biosynthetic pathways are characterized by single cytochrome P450 monooxygenases that are responsible for the atropo-specific formation of multiple carbon-carbon, carbon-nitrogen and carbon-oxygen bonds. To chart the biosynthetic diversity of the atropopeptides, we developed a machine learning-based genome mining algorithm that identified more than 400 putative atropopeptide biosynthetic gene clusters in publicly available genome sequences. To gain insights into their atropospecific biosynthesis, we characterized a selection of the identified biosynthetic gene clusters. The characterization of the selected pathways revealed an unusual two-step maturation process. Our study establishes atropopeptides as a novel family of RiPPs with unprecedented structural complexity that is introduced by a single tailoring enzyme. Moreover, bioactivity studies showed that atropopeptides promote pro-angiogenic cell functions as indicated by the increase of endothelial cell proliferation and undirected migration. Atropopeptide-modifying P450s expand the biochemical space of RiPP-modifying enzymes and pave the way towards the chemo-enzymatic utilization of the atroposelective transformations.

SL4 Revisiting Angiolams, a class of neglected antibiotics from myxobacteria

Sebastian Walesch,[1,2,3] Janetta Coetzee,[1,2,3] Ronald Garcia,[1,2,3] Sophie Hönig,[1,2,3] Fabian Panter,[1,2,3] Daniel Krug,[1,2,3] Rolf Müller[1,2,3]

[1]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research, University Campus, Saarbrücken, Germany

[2]Department of Pharmacy, Saarland University, Saarbrücken, Germany

[3]German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Germany

For the last decades, antimicrobial resistance (AMR) has been on the rise, leading to an alarming number of almost five million deaths associated with bacterial AMR in 2019.[1] Especially for the treatment of Gram negative bacteria, new antibiotics are needed, as the last new scaffold with activity against those bacteria was introduced more than 35 years ago. One promising source to find new antibiotic scaffolds targeting Gram negative bacteria, are myxobacteria, as they possess a great capability to produce new and diverse bioactive natural products.[2]

The antibiotic angiolam A, which displays activity against *E. coli* TolC and few Gram positive bacteria, was first described from a Brazilian soil myxobacterium *Angiococcus disciformis* An d30 in 1985.[3] However, no follow-up investigations were performed on the proposed mode of action (MoA) and biosynthesis. Recently, angiolam A was observed in the crude extract of two myxobacteria isolated from the citizen science regional sampling project “Sample das Saarland” and could be linked to the activity against *E. coli* JW0451-2. Molecular networking revealed the presence of three new angiolam derivatives (B-D) that were successfully purified. Angiolam resistant mutants of *E. coli* TolC were generated and used to determine its cellular target, frequency of resistance and cross-resistance with other antibiotics. Furthermore, a putative biosynthetic gene cluster (BGC) for biosynthesis of angiolams was observed in both new producers. It was confirmed in a genetically amenable alternative producer as gene disruptions in the putative BGC completely abolished production of angiolams. In this work, we present structures, bioactivities, information about the MoA, and proposed *in silico* biosynthesis of this class of myxobacterial natural products.

[1] Antimicrobial Resistance Collaborators *Lancet* **2022**, *399*, 629.

[2] Herrmann, Abou Fayad, Müller *Nat. Prod. Rep.* **2017**, *34*, 135.

[3] B. Kunze, W. Kohl, G. Höfle, H. Reichenbach, *J. Antibiot.* **1985**, *38*, 1649.

SL5 *Amycolatopsis* spp. – a neglected source for novel natural products and biosynthetic pathways

Elena Seibel,[1] Soohyun Um,[1] Kasun H. Bodawatta,[2] Anna J. Komor,[1] Christine Beemelmanns[1,3]

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[3]Biochemistry of Microbial Metabolism, Institute of Biochemistry, Leipzig University, Leipzig, Germany

Actinobacteria are versatile producers of bioactive natural products and their members are widely distributed in aquatic and terrestrial ecosystems. Besides the well-studied genus *Streptomyces*, members of the genus *Amycolatopsis* are known to produce various types of antibiotics, such as the glycopeptide vancomycin from *Amycolatopsis orientalis*. Recently, our group showed that *Amycolatopsis* sp. M39, isolated from the gut of a fungus-farming termite, is involved in the defense of their termite host against microbial pathogens by producing the antibacterial and antifungal macrolactams macrotermycins A-D. Intrigued by the biosynthetic potential and its putative role as defensive symbiont, we started to explore members of the genus *Amycolatopsis*. Using a combination of ecology-guided metabolomics approach, we identified structurally diverse bioactive natural products, including polyunsaturated PKS-derived macrolactams, ribosomally-synthesized thiopeptides and hydroxylated lipopeptides. Despite the great potential as antibiotic producer, genetic manipulation procedures are established only for a few members of the genus. Hence, our current studies are directed towards the heterologous expression and genetic manipulation of their biosynthetic pathways to unravel novel biosynthetic transformations.

SL6 Structural and biochemical insights into the transesterifying thioesterase domain from FR900359 biosynthesis

Sophie Klöppel,[1] Christian Bonifer,[2] Jonas Mühle,[3] Cornelia Hermes,[1] Gebhard Schertler,[3] Clemens Glaubitz,[2] Gabriele M. König,[1] Max Crüsemann[1]

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[2]Institute of Biophysical Chemistry and Centre for Biomolecular Magnetic Resonance, Goethe University Frankfurt, Germany
[3]Paul Scherrer Institute, Villigen, Switzerland

FR900359 (FR) is a member of the small natural product family of chromodepsins, cyclic depsipeptides with a strong and selective Gαq protein inhibitory effect. Thus, they are an excellent tool to study the Gαq-protein-associated cellular signalling pathways and diseases like asthma and uveal melanoma. The Gram-negative soil bacterium *Chromobacterium vaccinii* is one producer of FR, harbouring the *frs* gene cluster, responsible for its biosynthesis. *frs* encodes two non-ribosomal peptide synthetase (NRPS) assembly lines, composed of eight modules (FrsA, FrsD-G), which activate, modify, and incorporate specific building blocks into the extending peptide chain, by the assistance of an MbtH-like protein (FrsB), giving rise to the complex natural product FR.[1] Aim of this project is the detailed structural and biochemical characterization of the noncanonical FrsA thioesterase (TE) domain, catalyzing intermolecular transesterification of the FR side chain, *N*-propionylhydroxyleucine, onto the cyclic biosynthetic intermediate FR-Core.[2] Through crystallization and X-ray analysis, its structure shall be elucidated. Since initial crystallization were not successful, new protein constructs were generated based on bioinformatical analysis. To investigate interaction between $FrsA_{TE}$ and its substrate FR-Core, NMR measurements were conducted, to obtain detailed insights about its mode of action. For this purpose, $^{[15]}$N-labeled $FrsA_{TE}$ and FR-Core were generated and isolated. Efforts were made to establish an *in vitro* assay to investigate enzyme kinetics and at the same time supplies an opportunity to exchange substrates in order to obtain the substrate scope of the reaction catalyzed by $FrsA_{TE}$. These approaches shall obtain detailed insights about the unusual mode of action, to evaluate its potential for chemoenzymatic syntheses, e.g. the generation of altered, bioactive FR analogues with novel effects.

[1] Crüsemann M. *et al.* (2018). *Angew. Chem., Int. Ed.*, 57, 836–840.

[2] Hermes, C. *et al.* (2021). *Nat. Commun.*, 12, 144.

SL7 Evolutionary insights into the biosynthesis of coenzyme 3PG-F_{420}

Mahmudul Hasan, Daniel Braga, Daniel Last, Gerald Lackner

Junior Research Group Synthetic Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), Beutenbergstr. 11a, 07745 Jena, Germany

Coenzyme F_{420} is a deazaflavin redox cofactor produced by a range of bacteria and archaea. It`s highly negative redox potential permits F_{420} to perform critical reactions in processes like methanogenesis, antibiotic biosynthesis, and xenobiotic degradation. Recently, we discovered 3PG-F_{420}, a novel derivative of the coenzyme, in *Mycetohabitans rhizoxinica*, a symbiotic bacterium of the phytopathogenic fungus *Rhizopus microsporus.* This finding raised the question of how this new derivative might have evolved. We could show that 3PG-F_{420} has evolved from F_{420} by a substrate specificity switch in CofC, an enzyme that controls the entry of metabolites into the F_{420} side-chain biosynthesis pathway.[1] However, the molecular basis behind the specificity switch remained elusive. Structure elucidation and site-directed mutagenesis of CofC revealed residues that are critical for 3PG-F_{420} formation. These residues could even be exploited to mimic the evolution of the cofactor 3PG-F_{420} in the laboratory. Further investigation of CofD homologs for their influence on CofC activity revealed that CofD homologs further control substrate selection during the biosynthesis of F_{420}-derivatives.[2]

These results shed light on the molecular basis underlying the substrate specificity of CofC that led to the evolution of coenzyme 3PG-F_{420}. In addition, we revealed the role of CofD as a second gatekeeper, controlling the incorporation of precursors into the side-chains of F_{420} and its derivatives. Beyond these fundamental insights, these results will facilitate the improved biotechnological applicability of F_{420} derivatives in the future.

[1] Braga *et al.* 2019. *ACS Chem Biol* 14(9): 2088-94

[2] Hasan *et al.* 2022. *ASM mBio* 13(1): 03501-21

SL8 Identification of non-canonical terpene biosynthetic routes in bacteria

Nancy Magnus,[1] *Freya Braack,*[1] *Stephan von Reuß,* [2] *Birgit Piechulla*[1]

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

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

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

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

SL9 NAD as a building block in natural product biosynthesis

Lena Barra,[1,2] *Takayoshi Awakawa*,[1] *Kohei Shirai*,[1] *Ikuro Abe*[1]

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Nicotinamide adenine dinucleotide (NAD) is a pivotal metabolite for all living organisms and functions as a diffusible electron acceptor and carrier in central catabolic processes.[1] During biosynthetic investigations on the structurally unusual anti-cancer compound altemicidin (**1**),[2] we discovered a novel function for NAD as a building block in secondary metabolite biosynthetic pathways (**Fig.1a**).[3] The gatekeeping enzyme of the pathway (SbzP) constitutes a novel family of pyridoxal phosphate (PLP)-dependent proteins, catalyzing formation of the 6-azatetrahydroindane core scaffold *via* a sophisticated (3+2)-cycloaddition reaction, utilizing *S*-adenosyl methionine (SAM) as cosubstrate (**Fig.1a**). Enzymatic reconstitution of the complete downstream pathway revealed the function of several novel dinucleotide-processing enzymes. Intriguingly, functional SbzP homologs were found to be widely distributed in the bacterial kingdom and are encoded in diverse biosynthetic gene clusters (**Fig.1b**). The findings of this study fundamentally expand our understanding of the bacterial secondary metabolite repertoire and will facilitate the discovery and exploitation of a novel class of NAD-derived natural products.[3]

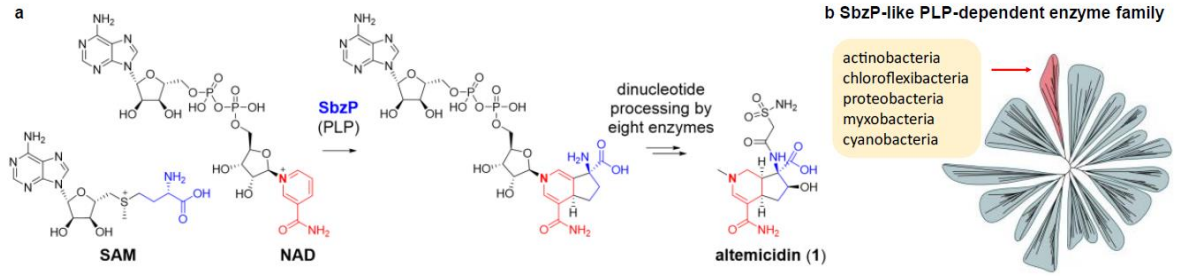

Fig.1 a. *Discovered PLP-mediated 6-azatetrahydroindane scaffold formation by SbzP utilizing NAD and SAM as substrates.* ***b.*** *Phylogenetic tree of the aspartate aminotransferase superfamily and newly discovered SbzP-like family.*

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SL10 Structural and biochemical characterisation of the peptide arginase OspR from landornamide biosynthesis

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Natural products from bacteria make a substantial contribution to the development of pharmaceutically relevant drugs. The cyanobacterium *Kamptonema* sp. PCC 6506 produces the RiPP natural product landornamide A, a cryptic 16mer peptide identified by bottom-up pathway reconstruction in *E. coli*.[1] Landornamide bears several key modifications including lanthionine bridges, d-amino acids, and ornithine residues; features that are crucial to the antiviral activity observed for the mature natural product. The ornithine residues are installed by the enzyme OspR, the first member of the recently discovered RiPP maturase family of peptide arginases. So far, only few representatives are characterised, and they are surprisingly promiscuous and accept a variety of different arginine-containing substrate sequences, including peptide sequences resembling marketed non-ribosomal antibiotics.[2] We solved the first crystal structure of a peptide arginase illuminating the mechanism of posttranslational ornithine formation and providing the molecular basis for substrate binding studies. In addition to the structural characterisation, kinetic analyses of OspR with various substrates provide new insights into the order of biosynthetic reactions during the posttranslational maturation of landornamide A. OspR, and many more promiscuous RiPP maturation enzymes, represent valuable biosynthetic tools for peptide engineering, creating unique opportunities in drug discovery, chemical biology, and synthetic biology.

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SL11 Generation of novel glycopeptide antibiotics using mutasynthesis approach

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

Mutasynthesis is a suitable method to construct new GPA derivatives that also act against resistant pathogens. The prerequisite for this is the construction of a block mutant that is no longer able to produce the Hpg. In *A. balhimycina* this was achieved by the deletion of *hmaS* and *hmaO* genes. *A. balhimycina Δhpg* was cultivated under GPA production conditions supplemented with various Hpg derivatives, which were either commercially available, enzymatically or chemically synthesised by cooperation partners. The production of new balhimycin derivatives was verified by (hr) LC-ESI-MS analysis. Feeding of *A. balhimycina ↗hpg* mutant with fluorinated phenylglycine derivatives resulted in the formation of new balhimycin derivatives. Their biological function is under investigation. The established mutasynthesis approach now enables the production of additional GPA derivatives that are effective against resistant pathogens.

SL12 Programming nonribosomal peptide synthetases with DNA templates

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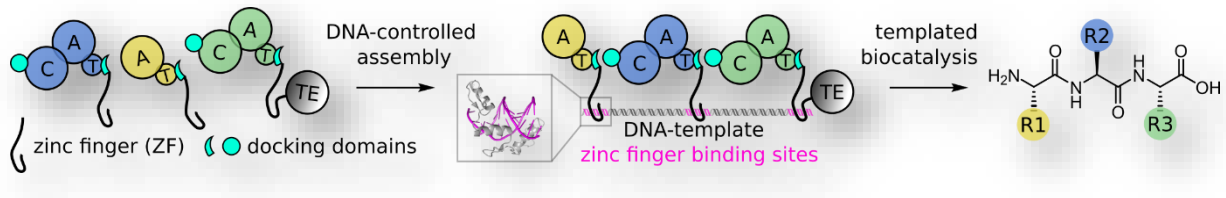
Nonribosomal peptide synthetases (NRPSs) are important enzyme machineries that have supplied the pharmaceutical industry with numerous bioactive natural products.[1] Their modular architecture facilitates production of new compounds through NRPS engineering. However, this strategy faces major challenges because the manipulation of gigantic NRPS genes is time consuming and error-prone.[2,3] To enable fast, targeted rearrangement of NRPS modules, we have established DNA-templated nonribosomal peptide synthetases (DT-NRPSs) by taking advantage of the sequence-specific DNA recognition of fused zinc-finger motifs.[4] DT-NRPSs have been engineered from the gramicidin S (GrS) assembly line in four steps: I) constructing standalone NRPS modules; II) fusing docking domains; III) attaching zinc fingers to each module; and IV) optimizing the system for peptide formation.[4] The production of GrS and peptide intermediates was monitored in each step. NRPS programming with DNA templates has been demonstrated by the enhanced production of the targeted peptides when different DNA templates were added. Lastly, we harnessed DT-NRPSs to produce several new peptides by adding alternative NRPS-zinc-finger bricks. In conclusion, we have successfully reconstituted the GrS assembly line *in vitro* in a DNA-templated format. The engineering potential of DT-NRPSs to rationally produce new peptides has been demonstrated. In the future, DT-NRPSs will be developed into an efficient tool for biocatalytic on-demand peptide synthesis by enhancing enzyme activity and enlarging the collection of suitable DT-NRPS modules.



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SL13 Biochemical and metabolic engineering of *Saccharomyces cerevisiae* for the production of cannabinoid precursors

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Cannabinoids belong to a class of bioactive compounds found in the plant species *Cannabis sativa* and have gained a significant amount of research attention in recent decades. Consequently, the huge pharmacological and therapeutic potential of the main cannabinoid species, ↗[9]-tetrahydrocannabinol (THC) and cannabidiol (CBD), has been uncovered and this, together with the identification of a broad range of novel species, has prompted an exponential increase into both the research and commercialization of these compounds. However, due to the extensive and cost intensive processes involved in directly extracting the cannabinoids from plants and the difficulty of yielding significant amounts of rarer cannabinoid species, biotechnological approaches aiming at synthesizing these compounds in a pure and economic manner have been the center of much research and investment. In recent years, the use of various microbial species as cell factories has been presented for the synthetic production of THC and CBD or their precursors, olivetolic acid (OA) and cannabigerolic acid (CBGA). However, limitations remain within the low titers achieved or the requirement of externally supplementing precursor molecules such as hexanoate. Here, we present approaches to implement and combine a range of biochemical strategies to overcome such limitations using the chassis organism, *Saccharomyces cerevisiae*. Through extensive genetic and metabolic engineering using the CRISPR/Cas9 technology as well as other gene editing strategies, we engineered recombinant strains leading to the *de novo* production of up to 120 mg L^{-1} of hexanoic acid. Through the subsequent expression of the *C. sativa* genes, olivetol synthase (*OLS*) and olivetolic acid cyclase (*OAC*), we successfully demonstrate the production of OA, the central aromatic polyketide precursor for cannabinoid biosynthesis, thus preventing the need for hexanoate supplementation.

SL14 Pleuromutilin biosynthesis and production of its congeners through heterologous gene expression in fungi

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Pleuromutilins are a class of semisynthetic antibiotics derived from a fungal natural product, and they have been used for over 40 years in veterinary medicine, as well as for over 10 years in human medicine.[1] In order to understand the biosynthesis of pleuromutilin, we identified its gene cluster using a combination of genome sequencing and transcriptomics analysis. Heterologous expression of the genes responsible for production of pleuromutilin allowed us to increase the production titre of this antibiotic by a tenfold.[2] Using a combination of synthetic chemistry and synthetic biology, the biosynthetic pathway to pleuromutilin was elucidated, and novel derivatives with increased antimicrobial activity were produced.[3] Using the heterologous host *Aspergillus oryzae* as the expression platform for pleuromutilin biosynthetic genes, we were able to produce novel pleuromutilin congeners and semisynthetic derivatives, demonstrating the relaxed substrate specificity of some of the pathway enzymes.[4] This study expands our knowledge on the biosynthesis of pleuromutilin and provides avenues for the development of novel pleuromutilin analogues by combining synthetic biology and synthetic chemistry.

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SL15 Novel microscale semi-synthetic approach toward biologically active isoindolinones from *S. chartarum*

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Stachybotrys chartarum is a fungus of the genus *Stachybotrys* whose occurrence is described in dead plant materials and buildings affected by water. These fungi produce a diverse group of secondary metabolites including macrocyclic trichothecenes, atranones, and phenylspirodrimanes (PSDs). Some known PSD representatives possess highly reactive *o*-dialdehyde groups at their aryl fragment. These phenylspirodrimanes including stachybotrydial, stachybotrydial acetate and acetoxystachybotrydial acetate were used as starting materials for the synthesis of their corresponding isoindolinones. To access the library of the PSDs derivatives and screen them against physiologically relevant serine proteases, a microscale semi-synthetic approach was developed. The generated library of 35 lactams was tested for the inhibitory activity toward thrombin (IIa) FXIIa, FXa, and trypsin. Among them, the agmatine-derived lactam showed the highest inhibitory activity. Subsequently, the lead compound was shown to demonstrate the anticoagulant properties in two plasma coagulation tests. Moreover, it has been demonstrated that semi-synthetic isoindolinones were significantly less toxic compared to their parental natural PSDs. Additional efforts were undertaken to investigate the water-mediated lactonization of isolated PSDs utilizing quantum chemical DFT-calculations corroborated by LC-MS and NMR experiments.

SL16 Effects of the G_q inhibitor FR900359 on soil nematodes

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The non-ribosomal peptide FR900359 (FR) is a member of the chromodepsins, a small family of potent and specific G_q protein inhibitors.[1] FR was first isolated from the leaves of the plant *Ardisia crenata*, and later found to be produced by the unculturable endosymbiotic bacterium "*Candidatus* Burkholderia crenata" living in nodules located at the leaf margin. Natural products, like FR, are specialized metabolites, that show unique biological effects under laboratory conditions. Their ecological role, however, is in many cases not further investigated. G_q signalling is important for eukaryotic organisms and we verified that FR has an insecticidal effect, and may thus protect the plant from insect feeding.[2]

We recently discovered that the cultivable soil bacterium, *Chromobacterium vaccinii*, harbours the *frs* gene cluster and produces FR under laboratory conditions.[3] In this work the ecologically relevant bioactivity of FR produced by *C. vaccinii* was further investigated using *Caenorhabditis elegans*, model organism and free-living soil nematode, and *Heterodera schachtii* as plant parasitic nematode. *In silico* and *in vitro* experiments presented evidence that nematodic G_q proteins are FR sensitive, which was strengthened by *in vivo* tests. Experiments with *C. elegans* depicted inhibition of locomotion and egg laying by FR. These findings align with our experiments on *H. schachtii*, where FR decreases activity and inhibits hatching of juvenile *H. schachtii* at stage 2. Together, our findings point towards a plant-protecting effect of *C. vaccinii* in soil. Further comprehension of plant-protecting mechanisms might provide new opportunities for the development of ecological agriculture.

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SL17 Sugar and spice: reclassifying glycopeptides and related antibiotics

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Antibiotics have been essential to modern medicine since their initial discovery. The continuous exploration of new antibiotic candidates is a necessity, since there is increasing emergence of resistance to antimicrobial compounds among pathogens. Since the majority of known antibiotics are manufactured by bacteria, we need to understand which of them are capable of production and how they manage it. We chose glycopeptides as a model system since their biosynthesis is well known and they show diversity in both compound structure and involved genes. Known glycopeptides typically comprise 7 residues with a variable number of sugar moieties and other modifications. These glycopeptide antibiotics (GPAs) have been classified into 4 main types based on their structural characteristics. Lately, some unusual GPAs have been discovered, whose structure is so different that a 5th type was introduced, that has a different mode of action and does not include sugar moieties. We studied the evolution of all sequences related to the biosynthesis of such compounds and we observed a clear evolutionary diversification of the original GPA types and the latest one. Here we are suggesting a phylogeny-driven reclassification and separation of glycopeptides from glycopeptide related peptides (GRPs).

SL18 Transcription of BGCs and production of natural compounds in the myxobacterium *Sorangium* sp.

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The ever-increasing spread of multidrug-resistant pathogenic bacteria demands the discovery of novel antibiotics. Myxobacteria are a valuable source of novel natural products, including antibiotics. While genome studies of myxobacteria indicate a large number of biosynthetic gene clusters (BGCs) with unknown natural products, many of these compounds are not detected under standard laboratory conditions. Improved understanding of the regulation of natural product synthesis will facilitate the targeted induction and manipulation of natural compound production and hence could accelerate the discovery of novel antibiotics.

To this end, we have investigated the time course of BGC transcription in the myxobacterium *Sorangium* sp. Soce836 and related it to natural compound production. We combined genome mining with time-course RNA-seq and LC/MS approaches over five growth times, covering all growth phases in batch culture.

As a result, we found that both, natural compound production and BGC transcription varied dynamically over time. The normalized transcription levels of BGC genes encoding core features for the production of non-ribosomal peptides and polyketides correlated strongly with the production rate per cell of the natural compound. Surprisingly, transcription of many BGCs and natural compound production rates were maximal in the exponential growth phase.

SL19 On the role of natural products as virulence factors in fungi with a predatory lifestyle

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An intriguing and fascinating inter-kingdom interaction occurs between nematodes and fungi that trap and prey on them. Nematode-trapping fungi are soil microbes which can switch from a saprotrophic lifestyle to a predatory behavior in the presence of nematodes.[1,2] Among them, *Arthrobotrys flagrans* is a typical member of the soil microbiome and is able to form adhesive, three-dimensional trap networks to catch nematodes. Using *Caenorhabditis elegans* as model, we previously showed that polyketides and volatiles produced by *A. flagrans* are crucial for attracting the nematodes into fungal colonies and traps and for controlling trap formation, together with nematode-derived ascarosides.[3] Bioinformatic analysis has revealed that *A. flagrans* only encodes 3 PKSs, 3 NRPSs and 3 NRPS-like biosynthetic gene clusters, which is a relatively small collection of clusters compared to other fungi. Therefore, the *A. flagrans* - *C. elegans* interaction offers the perfect system and opportunity to elucidate the entire secondary metabolism of *A. flagrans* and the importance of the produced compounds for nematode predation. Here we focus on the two uncharacterized PKS-encoding genes of *A. flagrans*, *pksB* and *pksC*. We show that the two genes are expressed in the fungal traps and in fungal hyphae inside the nematode. Chemical analysis revealed that the two PKSs work in a concerted fashion and biosynthesize a statin natural product, indicating an unprecedented role for an HMG-CoA reductase inhibitor as virulence factor. Studies on the molecular nematode targets and the exact role of the fungus-derived statin are under way.

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SL20 Polyamine and ethanolamine utilization in *Streptomyces coelicolor*

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Naturally occurring mono- and polyamines are required for cell growth; however they can be very toxic when present in excess. Such compounds can interact with negatively charged DNA, RNA and other components of the cell and under high concentrations they may lead to deregulation of the cell metabolism and unbalanced homeostasis. Our studies revealed that *S. coelicolor* can utilize polyamines (putrescine, cadaverine, spermine and spermidine) and the monoamine ethanolamine as a sole nitrogen source and is able to grow in the presence of high concentrations of these compounds.[1] However, their uptake and utilization pathways are largely not characterized neither in streptomycetes nor in other Actinomycetales. Transcriptional analysis was used to identify genes that encode uptake systems and enzymes involved in polyamine and ethanolamine utilization in *S. coelicolor*. Our investigations showed a large number of genes involved in assimilation of polyamines and ethanolamine that allow the survival of *S. coelicolor* under nitrogen limitation conditions.[1] Furthermore, we analyzed the function of three proteins annotated as glutamine synthetase-like enzymes (GS-like): GlnA2$_{Sc}$, GlnA3$_{Sc}$ and GlnA4$_{Sc}$ and demonstrated that in *S. coelicolor* GlnA2$_{Sc}$ and GlnA3$_{Sc}$ are involved in the catabolism of polyamines and that GlnA4$_{Sc}$ is involved in the catabolism of ethanolamine. Transcriptional, phenotypical, structural and biochemical analysis of these enzymes demonstrated a dual function of GlnA2$_{Sc}$, GlnA3$_{Sc}$ and GlnA4$_{Sc}$ in ensuring both nutrients availability (*C*- and *N*-source) and resistance against high poly- or ethanolamine concentrations.[1] Further analysis revealed the presence of GlnA2, GlnA3 and GlnA4 enzymes also in other actinobacteria including human pathogens like *M. tuberculosis*. Since GS-like enzymes, e.g. like GlnA3$_{Mt}$, ensure survival of mycobacteria in their host, inhibition of these enzymes might be an effective therapeutic strategy. Our current studies aim further characterization of polyamine and ethanolamine metabolism in Actinobacteria as well as the development of novel drugs based on the validation of GS-like enzymes as targets.

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P1 Genome sequence-based screening for novel phosphonate producers

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Phosphonates are a unique class of natural products with diverse chemical structures and bioactivities. Numerous phosphonate natural compounds found their way into the market as for example the herbicide bialaphos, the antimalarial agent fosmidomycin or the antibiotic fosfomycin. The structural unity of all phosphonates is the characteristic C-P bond, which is formed in an initial biosynthetic reaction step catalysed by the enzyme phosphoenolpyruvate phosphomutase PepM, which converts phosphoenolpyruvate (PEP) to phosphonopyruvate (PnPy).[1] Due to the conservation of the PepM enzymatic reaction in the vast majority of phosphonate producers, the respective biosynthetic gene *pepM* is well suited to be used as a molecular marker to screen for potential phosphonate producer strains.

In our study, we aim to screen for novel phosphonate producers from the DSMZ strain collection based on genome-sequences. The DSMZ strain collection harbours >3.600 actinomycetes, many of which have already been genome-sequenced. A bioinformatic analysis of ~600 genome sequences revealed 33 strains containing a *pepM* gene and thus a potential phosphonate biosynthetic gene cluster (BGC). Out of these, 15 showed antimicrobial activity against the phosphonate-sensitive *E. coli* strain WM6242.[2] Phylogenetic analysis of the PepM amino acid sequences revealed a BGC-specific cladding. Cluster networking analysis are performed in order to prioritize strains with unique clusters for further analysis. Phosphonates will be identified and characterized by ^{31}P NMR spectroscopy and mass spectrometry in cooperation with AG Chambers Hughes (Universität Tübingen) and BGC of selected hits will be characterised by mutagenesis.

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P2 Functional characterization of bacterial type III polyketide synthases from bacterial symbionts of higher eukaryotes

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Type III polyketide synthases (PKS) are multi-functional biocatalysts that produce a variety of polyketide scaffolds including pyrones, resorcinols, resorcylic acids and chalcones. Type III PKS biosynthesis is based on repetitive decarboxylative Claisen-like condensations of coenzyme A-activated short carboxylic acids. Moreover the multifunctional PKSs catalyse a small set of cyclization reactions. Type III PKSs have predominantly been characterized from plants, but in the course of the last two decades many bacterial type III PKS biosynthesis gene clusters (BGCs) have been identified. The characterization of bacterial type III PKS BGCs revealed a product spectrum consisting mainly of pyrones or resorcinols.

In this work, meta genomes that were obtained as “contaminations” from sequencing projects of higher eukaryotes were mined for natural product BGCs, leading to the identification of a high number of bacterial type III PKS BGCs. Since the assembly of bacterial sequencing contaminations results in fragmented genome sequences, the comparably small size of type III PKS BGCs renders these systems ideal candidates for functional characterization studies. Remarkably, the identified BGCs harbour many additional genes that encode potential tailoring enzymes suggesting that the final polyketide product is highly decorated. A selection of bacterial type III PKSs were heterologous expressed in *Escherichia coli* and the corresponding cell extracts analysed. An unusual type III PKS showing an unprecedented product spectrum was selected for characterization. To do so, an *in vitro* assay to yield higher polyketide titers was established. The identification of multiple functional BGCs established bacterial “contaminations” that are obtained during sequencing of higher eukaryotes as a promising source for the identification of unusual bacterial natural products.

P3 Identification and characterization of potential novel glycopeptides

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Glycopeptide antibiotics (GPAs) such as vancomycin and teicoplanin are clinically used antibiotics that are produced as secondary metabolites by actinomycete genera such as *Streptomyces* and *Amycolatopsis*. The biosynthesis of these metabolites is very complex and is encoded in large biosynthetic gene clusters (BGCs) that exceed more than 60 kb. The BGCs possess genes required for peptide assembly and modification, genes encoding pathway-specific regulators, exporters, the amino acid precursor supply and in some cases resistance determinants.

We aim to analyze how the BGCs of GPAs are distributed throughout the bacterial kingdom, to identify strains harboring unusual GPA BGCs and characterize the mode of action of the corresponding GPAs.

Therefore, we performed a BigSCAPE analysis based on a dataset of more than 90 GPA producers, resulting in the identification of numerous unusual GPA BGCs. The structures, predictable from the genetic information, differ from classical GPAs mainly in the composition of the peptide backbone, which makes their classification into previous type categorization of GPAs difficult.[1] Among them, we identified a type III GPA BGC in *Amycolatopsis azurea* and a GPA BGC completely different from known GPA BGCs in a *Streptomyces* strain. As a proof of concept to optimize the production of GPA, we introduced the gene encoding the GPA pathway specific regulator (Bbr) heterologously under the control of a constitutive promoter into the genome of *A. azurea* and cultivated the recombinant strain *A. azurea_bbr* under GPA production conditions. HPLC/MS analysis revealed that the strain produces 20-fold GPA compared to the wild type. Currently, we are purifying various GPA derivatives produced by *A. azurea_bbr* by a series of chromatography steps in order to elucidate their structure by NMR. Analogous approaches are also applied to the *Streptomyces* strain to identify and purify the corresponding GPA and to characterize its mode of action.

[1] Xu, Min. *et al.* Phylogeny-Informed Synthetic Biology Reveals Unprecedented Structural Novelty in Type V Glycopeptide Antibiotics. ACS Central Science 2022 *8* (5), 615-626

P4 Investigations on structural diversity and biosynthesis of biarylitides - a novel class of minimal RiPPs

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A recently discovered, novel class of ribosomally-synthesized and post-translationally modified peptides (RiPPs) are the biarylitides.[1] In their biosynthesis, the smallest coding gene ever described across the tree of life plays a decisive role. This 18 bp gene encodes a precursor pentapeptide, which is enzymatically modified and cleaved into a tripeptide with a biaryl bond linking two aromatic amino acids. This biaryl bond is generated by a downstream-encoded, specific cytochrome P450 monooxygenase. This minimal biosynthetic gene cluster (BGC) was discovered in a strain of the less explored actinobacterial genus *Planomonospora*. Global bioinformatic analyses revealed biarylitide BGCs in at least 200 bacterial genomes, in some cases encoding further modifying enzymes, indicating a large, undiscovered structural diversity of biarylitides. This project focuses on the isolation and characterisation of structurally modified biarylitides, which is aimed to be achieved by heterologous expression of selected BGCs. We report the detection of four novel biarylitides, one of which appears to be the first modified member described. Additionally, systematic mutation experiments of the biarylitide precursor gene resulted in heterologous production of peptides with altered sequences. Among these is the first *in vivo* produced biarylitide with a tryptophan at the crosslinking position.

[1] Zdouc MM et al. (2021) Cell Chem Biol 28 (5), 733-739

P5 Functional characterization of a non-canonical sesquarterpene biosynthetic gene cluster from a bacterial symbiont of leaf cutting ants

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Leaf cutting ants are social insects that farm fungal gardens that they use as their primary food source. It is hypothesized that these ants engage in symbiotic relationships with actinobacteria that biosynthesize selective antifungal metabolites to protect their fungal food source from the invasion of pathogenic fungi. We characterized an unconventional terpene produced by a *Pseudonocardia* sp. that was isolated from an ant nest in Panama that showed anti-fungal activity specifically against the fungal pathogens but no activity against the fungal cultivar. Moreover, the isolated terpene showed strong activity against the human pathogen *Candida albicans* that is the most common source for invasive candidiasis. Structural characterization revealed a sesquarterpene (C35) with an unprecedented sugar moiety, epoxide, ether and unusual thiourea residues. Mining the genome of the producer revealed a putative BGC responsible for the biosynthesis of the isolated sesquarterpene. In this work, we are functionally characterizing the putative BGC for the biosynthesis of the unusual sesquarterpene. To do so, we have optimized *Streptomyces* host strains to produce high terpenoid titers. We heterologously expressed the genes required for the biosynthesis of the terpene hydrocarbon backbone, the gene cassette that is putatively responsible for the production of the unusual sugar moiety, and the entire BGC. The ecology-guided discovery of the unusual sesquarterpene deepens our understanding of the multipartite interaction between the ant host, its bacterial symbionts, the fungal cultivar and pathogenic fungi. Moreover, the functional characterization of the corresponding biosynthetic pathway will pave the way towards a biosynthetic model for the biosynthesis of one of the most complex bacterial terpenoids.

P6 Ecology-driven and genomics-guided discovery of novel bioactive natural products

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Bacteria produce a wide range of natural products (NPs) to interact with their environment and to engage in “metabolic small talk” with coexisting organisms. Due to their chemical diversity and biological activities, NPs provide a rich source for antibiotic and antimycotic lead structures. As drug resistance develops rapidly, different approaches have been applied to facilitate the discovery of truly novel bioactive NPs. Traditional bioactivity-guided approaches frequently result in the rediscovery of known compounds. Genome mining, on the other hand, often results in the detection of “silent” biosynthetic gene clusters (BGCs). In other cases, it might be difficult to assign a bioactivity to a natural product that was identified by genome mining. To circumvent these problems, we use an ecology-driven approach that is based on a bioactivity-based strain prioritization step, followed by the genomics-guided dereplication of BGCs that are associated with known NPs. As an example for our approach, we have isolated the antifungal nonribosomal peptide attinimicin from bacterial symbionts of leaf-cutting ants. Leaf-cutting ants cultivate fungal gardens as their primary food source. The mutualism between ant and fungus is threatened by pathogenic fungi that can invade the ant nest. It is believed that bacterial symbionts of the ants produce antifungal compounds, such as attinimicin, to selectively inhibit the growth of the fungal pathogens while leaving the fungal cultivar uneffected. In a similar approach, we are exploring several other ecological niches that are likewise shaped by the production of NPs with a certain bioactivity. Our studies not only provide a deeper understanding of the multipartite interactions within an ecological niche, but also pave the way towards the identification of novel bioactive NPs.

P7 Identification and characterization of ClpP targeting molecules

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Streptomyces hawaiiensis NRRL 15010 is the producer of the natural product ADEP (acyldepsipeptide 1, "factor A"). ADEP derivatives represent a promising new class of antibiotics which have potent antibacterial activity against Gram-positive bacteria by deregulating ClpP, the proteolytic core of the bacterial caseinolytic protease.

The identification and characterization of the ADEP biosynthetic gene cluster (BGC) revealed a cluster-associated *clpP* homologous gene ($clpP_{ADEP}$). Heterologous expression of $clpP_{ADEP}$ in different ADEP-sensitive *Streptomyces* species confirmed its role in conferring ADEP resistance and revealed a novel type of antibiotic resistance.

In this project, we aim to identify and characterize novel ClpP-targeted natural products. Based on the localization of the resistance gene $clpP_{ADEP}$ in the ADEP BGC, a bioinformatic screening procedure was established. By combining antiSMASH and ARTS, the bacterial NCBI database was screened for BGCs in actinomycetes harboring potential *clpP* homologous genes. Thirteen interesting BGCs were selected encoding the synthesis of different types of natural products. The corresponding strains were grown in different media. The culture extracts were used in bioassays against *B. subtilis* 168 WT and a *B. subtilis* 168 Δ*clpP* mutant to investigate their potential of targeting ClpP. One of the culture extracts exhibited differential activities against the two test strains. HPLC/MS analyses and comparison of the masses with the masses in the natural product database suggest production of a new compound.

P8 Omics- and resistance-guided discovery of novel antibiotic leads from phyllosphere-associated bacteria

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Natural products have been used for medicinal purposes for millennia and are well known for their diversity in structure and function. One important application of natural products is their use as anti-infectives. Antibiotic resistance is a global concern in different areas and urges the development of new and effective antibiotics to fight human diseases.

Gnotobiotic *Arabidopsis* plants can be colonized by microbiota culture collections obtained from *Arabidopsis* leaf and root in a way resembling the natural microbiota found in their host organs, thus serving as an excellent study model. Our previous study revealed over hundreds of previously unknown biosynthetic gene clusters (BGCs) from 258 strains, thus revealing the potential of the phyllosphere as an environment for discovering novel antibiotics and natural product scaffolds.[1]

The aim of this research project is to investigate selected novel bacterial strains isolated from the phyllosphere of *Arabidopsis thaliana* strategy, which showed inhibitory activity against *Acinetobacter baumannii* and *Staphylococcus aureus,* by a paired omics and resistance-guided approach for the discovery of novel antibiotics.

State-of-the-art bioinformatic tools are employed to identify and predict natural products of interest (structural, biosynthetic novelty, bioactivity, and resistance genes) from biosynthetic gene clusters via “resistance-guided genome mining”.

Co-culturing and OSMAC (one strain, many compounds) approaches are applied as well to induce the production of secondary metabolites of interest. Moreover, an advanced direct cloning strategy[2] is being employed to express candidate cryptic BGCs in heterologous hosts.

[1] Qi *et al.*, *Environ. Microbiol.*, **2021**, 23(4), 2132–51.

[2] Enghiad *et al., Nat. Commun.*, **2021,** 12(1), 1171.

P9 Genomic and molecular information of siderophores and biosurfactants

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Siderophores and especially biosurfactants play a major role in different industrial fields. Due to the amphiphilic nature of biosurfactants and marine siderophores they are especially interesting for the cleaning and pharmaceutical industry. Usually, these compounds are obtained from the petrochemical or oleochemical sector, and therefore have a negative impact on our environment. The increased demand for these compounds incremented the interest in acquiring surfactants and siderophores from a renewable source in order to lower the impact on our environment and the dependency on fossil resources. Normally, all genes needed to produce these natural products are located in BGCs (**B**iosynthetic **G**ene **C**lusters), which are part of the secondary metabolism. The overreaching goal of the EU funded project SECRETed (**S**ustainable **E**xploitation of bio-based **C**ompounds **R**evealed and **E**ngineered from na**T**ural sources) is to produce new-to-nature tailor-made compounds with specific properties by combining different components of genomic clusters together. The combination of clusters has the potential to increase the diversity of these molecules and increment the possible use cases in different industrial sectors. To reach this goal a database containing all known biosurfactants and siderophores was build, for which the BGC is known. This database includes the basic chemical class and producing BGC of the compound as well as the predicted biosynthetic pathway. Currently, it contains 65 siderophores and 101 biosurfactants. These compounds where analysed to identify common grounds and sub-clusters. Information about similarities between the clusters and the function of sub-clusters can then be used down the line to synthetically design a molecule by "Mixing and Matching" the sub-clusters together. Furthermore, available genomes of producer strains were analysed using antiSMASH where no cluster is linked to the produced compound. Already collected data and defined cluster rules were then used to identify the possible cluster which could be responsible for the production of the compound. Using the rules

defined during the comparison, an additional 87 possible clusters were linked to a compound. All collected data in this part of the project will be utilized to feed a machine learning algorithm which will then be used to further increase the database and collect more biosynthetic gene clusters, molecular structures and chemical properties of biosurfactants and siderophores.

P10 Identification and isolation of savacyclins, novel peptides belonging to a recently discovered class of RiPPs

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RP-66453,[1] isolated from *Streptomyces* 9738 is used as a lead structure for the development of antipsychotic pharmaceuticals. It binds specifically to the neurotensin receptor and is claimed to be useful for treating psychosis, Alzheimer's and Parkinson's diseases. From a synthetic perspective, RP-66453 is an interesting molecule because of the inherent ring strain associated with its two-bridged macrocyclic ring systems.[2] Although its chemical synthesis was established, nothing was known so far on its biosynthesis in the natural producer. In order to identify further RP-66453 like compounds and their biosynthetic gene clusters (BGCs), we performed a genome mining approach. Genome analysis of *Streptomyces violaceusniger* led to the identification of a RiPP-BGC, encoding a peptide precursor, a cytochrome P450 monooxygenase, a N-acetyltransferase, five ABC transporters and a S9 family peptidase. Heterologous expression of the precursor peptide gene and the P450 monooxygenase gene in *Streptomyces coelicolor* M1146 resulted in the production of a crosslinked tetrapeptide consisting of Tyr-Tyr-Ser-Tyr. Surprisingly, the P450 monooxygenase catalyses the formation of both, the biaryl and aryl-oxygen-aryl ether crosslinks. A second diacetylated variant was detected in heterologous host, indicating that a homologous N-acetyltransferase in *S. coelicolor* is able to acetylate the tetrapeptide.

[1] G. Helynck, C. Dubertret, D. Frechet, J. Leboul, J. Antibiot. 1998, 51, 512–514.

[2] M. Bois-Choussy, P. Cristau, J. Zhu, Angew. Chem. 2003, 35, 4238-4241

P11 Design of tetrazine probes for the discovery of novel isonitrile-containing natural products

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Isonitrile-containing natural products (NPs) have been isolated from marine sponges, cyanobacteria, bacteria, fungi and nudibranchs, with their first discovery dating back to as early as the 1970s. These NPs have recently sparked interest following the realization that a diisonitrile NP called SF2768 is a biologically active chalkophore.[1] Chalkophores are able to chelate and transport extracellular copper, similar to the more extensively studied class of siderophores that transport iron. Diisonitrile SF2768 is completely unrelated to methanobactins, which thus far remain the only well-studied class of chalkophores, and like most isonitriles it exhibits antibacterial and antifungal activity. As such, the discovery of new isonitriles with putative antibacterial properties is of great interest.

In terms of their chemical properties, isonitriles exhibit very specific reactivity toward tetrazines with little to no side-reactivity with other naturally occurring functionalities, which means that tetrazines provide for a means to label isonitriles in complex extracts. A study describing the application of tetrazines toward the detection of isonitriles in extracts is reported,[2] but the method utilizes simple commercially-available tetrazines in well-characterized extracts and is low throughput because each labeling reaction must be carefully examined by LC-MS to determine whether an isonitrile is present in any given extract.

This work seeks to exploit the unique reactivity between isonitriles and tetrazines in order to establish a robust, fast and high-throughput method for the discovery and isolation of novel isonitrile-containing natural products. This is achieved by the use of a synthetic fluorogenic “sensing” tetrazine probe for the detection of isonitriles in complex extracts, and an “anchoring” tetrazine probe for the straightforward isolation thereof. A proof of concept is achieved through the re-discovery of hazimycin in a bacterial extract.

[1] Wang, L.; Zhu, M.; Zhang, Q.; Zhang, X.; Yang, P.; Liu, Z.; Deng, Y.; Zhu, Y.; Huang, X.; Han, L.; Li, S. and He, J.; ACS Chem. Biol., 2017, 12, 3067.

[2] Huang, Y. B.; Cai, W.; Flores, A. D. R.; Twigg, F. F. and Zhang, W.; Anal. Chem., 2020, 92, 599-602.

P12 13,14-*seco*-derivatives of hygrocins from *Streptomyces rapamycinicus* IMET 43975

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Ansamycins, represented by antituberculosis drugs rifamycins, are an important family of natural products. To obtain new members of ansamycins, *Streptomyces rapamycinicus* IMET 43975 harboring an ansamycin biosynthetic gene cluster was fermented in a 50 L scale, chromatography led to the isolation of one new analogue of divergolides (divergolide P) in addition to divergolide O and three new derivatives of hygrocin A (4, 5 and 6) together with three other ansamycins, which differ in the configuration at C-2 and ansa ring cleavage. The structures of hygrocins (**1–8**) were determined by the analysis of the 1D and 2D NMR spectroscopy as well as high-resolution mass spectrometry data. The structures broadened the diversity of ansamycins. The bioactivites will be investigated for structure activity relationships.

P13 Cultivation systems impacts formation of secondary metabolite profiles in *Streptomyces griseochromogenes*

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The primary sources of novel lead compounds to treat diseases are secondary metabolites (SMs), produced by bacteria, plants and fungi. SM production are often cryptic or silent and can be activated through altering growth condition in the method referred as one strain many compounds (OSMAC) approach. OSMAC is usually performed in small scale cultivation vessels and face therefore low throughput and poor reproducibility, especially during scale up to stirred tank bioreactors. Here, we assessed whether SM profiles in *Streptomyces griseocromogenes* is responsive to cultivation systems aside from cultivation conditions.

To analyze the dependency and reproducibility of SM profiles obtained in microscale culture systems and stirred bioreactors, the actinobacterium *S. griseochromogenes* was cultivated in a DASbox mini bioreactor system (DB) and in the five different microscale cultivation systems, 96 deep well plates (DWP), 24 deep well plates (DWP), 48 flower shaped deep well plates (FP), baffled (SFS) and non-baffled shakeflasks (SF). The culture extracts were analyzed using a non-targeted metabolomics workflow. We found that *S. griseochromogenes* was responsive to different cultivation system. Desferrioxamine derivatives like desferrioxamine Et1 was exclusively found after cultivation in FP (55 %) and 24 DWP (45 %) and Leupeptin chemicals such as leupeptin Pr hydrat was only detected during cultivation in 24 DWP. Certain masses (e.g. 9.99_546.4884) were only formed during cultivation in DB. The highest reproducibility of SM profile between cultivation systems and the bioreactor was found in 24 DWP with 18 of 24 SMs in common with DB.

This work demonstrates the influence of cultivation systems in SM discovery of *S. griseochromogenes*. Aside of the OSMAC conditions, an optimal cultivation system has to be chosen to successfully elucidate the SM profiles for novel lead compounds. A most promising cultivation system as screening platform for OSMAC experiments were 24 DWP and FP, showing a diverse SM profile, possibility of testing multiple conditions simultaneously and good reproducibility in bioreactors.

P14 The disorazole Z family of highly potent anticancer natural products from *Sorangium cellulosum*

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Myxobacteria serve as a treasure trove for secondary metabolites. In the course of our ongoing search for bioactive natural products, a novel subclass of disorazoles termed disorazole Z was discovered. Ten disorazole Z family members were purified from a large-scale fermentation of the myxobacterium *Sorangium cellulosum* So ce1875, and characterised by ESI-*hr*MS, X-ray, NMR, and Mosher ester analysis. The main component disorazole Z1 shows comparable antitumor activity to disorazole A1[1] via binding to tubulin. Morever, the disorazole Z biosynthetic gene cluster (BGC) was identified and characterised from the alternative producer *S. cellulosum* So ce427 and compared to the known disorazole A BGC,[2] followed by heterologous expression in the host *Myxococcus xanthus* DK1622. Pathway engineering paves the way for further biosynthesis studies and efficient heterologous production of disorazole Z congeners.

[1] Yasser A. Elnakady, Florenz Sasse, Heinrich Lünsdorf, Hans Reichenbach *Biochem. Pharmacol.* **2004**, *67*, 927-935.

[2] Ruby Carvalho, Ralph Reid, Nina Viswanathan, Hugo Gramajo, Bryan Julien *Gene*. **2005**, *359*, 91-98.

P15 Flow plate cultivation - a novel method to generate bioactive extracts with mixed secondary metabolite profiles

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Bacteria have been observed to exhibit distinct secondary metabolite profiles based on the method of cultivation, namely liquid and solid cultivation. A novel flow plate prototype was designed to link these established cultivation methods and generate mixed secondary metabolite profiles using myxobacteria as an example. Flow plate cultivation was shown to increase the spectrum of secondary metabolites compared to established cultivation methods. Additionally, flow plate cultivation simplifies and accelerates the process of extract production enormously, when compared to solid cultivation. For instance, flow plate cultivation of the strain *Cystobacter* SBCb004 yielded crude extracts with antibacterial activity, which had previously only been found using extracts obtained from solid plate cultivation.

P16 Establishing an alternative bioactivity screening method for myxobacteria

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Identification, isolation and screening of new myxobacteria over the last three decades have led to the discovery of many new compounds. This implies that the probability of finding uncharacterized metabolites increases with isolation and screening of novel families, genera or species of myxobacteria.[1] Some myxobacteria strains produce anti-gram negative compounds only when they growth on solid media. In this study, we aimed to develop an alternative screening method in order to overcome the difficulties experienced in the production of bioactive molecules caused by cultivation type and condition. For this purpose, different myxobacteria strains were grown in solid media and their bioactivities were tested against gram-negative bacteria using a newly developed solid-liquid screening method. This method was performed for various cultivation conditions such as different incubation time and incubation in presence or absence of light. Preliminary data reveal gram-negative bioactivity from myxobacterial strains, which do not exhibit this activity when grown in liquid media. Thus, variation of cultivation type is an important tool to discover novel bioactive natural products from myxobacteria.

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P17 University X Industry: A new paradigm for screening large collections of microorganisms for development of the next generation crop protection biologicals

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Smarter AgroBiological Screening (SABS) is developing an automated pipeline for the high throughput (HTP) screening of microbial collections for the development of biological crop protection. A proof-of-concept platform is being established to screen a DTU-owned strain collection for anti-fungal properties in an industrial collaboration with FMC. A diverse set of liquid handling robots is used to screen over 40,000 bacterial and fungal strains for activity against two economically important phytopathogens: *Fusarium graminearum* and *Zymoseptoria tritici*, causing Fusarium head-blight and leaf blotch disease respectively. We focus on the disease prevalence in wheat, the main European cereal crop (120M t/yr.), where the dependency on chemical pesticides is high and there are very limited options for organic disease control. Our HTP-design combines state-of-the-art laboratory automation, carefully designed biological assays, reflecting field condition, as well as LC-MS/MS-based metabolomics to investigate the mode-of-action and identify known and novel bioactive metabolites. By funneling increasing numbers of strains through our workflow, we carefully select candidates for greenhouse and field trials, based on diverse parameters (e.g., antifungal activity and metabolomics). Artificial intelligence will furthermore be used to predict the success in upcoming field trials and improve screening efficiency. Thus, through DTU Bioengineering's expertise within microbiology, metabolomics, automation, and AI, as well as FMC's deep understanding of plant health, product application, and marketing of agricultural products, SABS brings together academia and industry to identify and develop microbial candidates that can combat one or both of these fungal diseases and provide the first biological option for wheat crop protection.

P18 Investigation of natural products as treatment option for plants infected by *Xylella fastidiosa* through a microfluidic *in vivo*-mimicking assay

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Xylella fastidiosa is an insect vector-transmitted plant pathogen, which induces various crop diseases with enormous economic impact around the world. Inside the host plants, this Gram-negative bacterium blocks the sap flow in the xylem vessels by biofilm formation. The biofilm disrupts water and nutrients transportation, which eventually kills the host plants.[1] Despite numerous efforts and control measures conducted, there is currently no sustainable solution available to treat or prevent the infections caused by this pathogen.

This project aims to implement an *in vivo*-mimicking assay utilizing microfluidic technology[2,3] and adapt it to investigate treatment options for *X. fastidiosa* infections in greater detail. This technology allows dynamic real-time analysis of bacterial cell adhesion and biofilm formation under conditions, which imitate the vascular flow in the plant. The assay can be integrated into our running natural product discovery pipeline and will be used to evaluate bioactive compounds (actives) identified against *X. fastidiosa.* The *in vivo*-mimicking assay might help to enhance the transfer rate of actives discovered in a primary assay to *in planta* experiments. Thereby, we hope to increase the probability to successfully discover a treatment option for *X. fastidiosa*, which is urgently needed.

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[2] Yawata, Y., Nguyen, J., Stocker, R. & Rusconi, R. Microfluidic Studies of Biofilm Formation in Dynamic Environments. *J. Bacteriol.* **198**, 2589–2595 (2016).

[3] Straub, H. *et al.* A microfluidic platform for *in situ* investigation of biofilm formation and its treatment under controlled conditions. *J. Nanobiotechnology* **18**, 166 (2020).

P19 Sample das Saarland: Expanding myxobacterial phylogeny for chemical diversity

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Myxobacteria contribute significantly to natural product discovery from bacterial sources. The HZI-HIPS strain collection is constantly extended by newly isolated strains, which are channeled into the natural products discovery workflow. Comprehensive statistical analysis of myxobacterial secondary metabolomes has previously underpinned a correlation between phylogenetic distance and the production of distinct compound families.[1] We are thus seeking to expand the taxonomical coverage of our strain collection with strategic focus on the isolation of new myxobacterial species, genera, and families. Here, we present results from soil samples obtained by the "Sample das Saarland" citizenscience campaign aimed at mining the biodiversity accessible on a regional scale for novel natural products. From 649 soil samples over 1000 myxobacterial strains were already isolated, among them candidates for novel species, genera, and families. Feedback was given to the citizen scientists in the form of microbiological portraits of myxobacterial strains found in their samples. The project is currently being extended to operate on a nationwide scale in Germany.

[1] Hoffmann T, Krug D, Bozkurt N, Duddela S, Jansen R, Garcia R, Gerth K, Steinmetz H, Müller R. Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria. Nat Commun. 2018 Feb 23;9(1):803

P20 Deciphering the transvalencin biosynthesis

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Natural Products (NPs) are considered to be nonessential metabolites for bacterial growth and reproduction but rather providing evolutionary fitness under certain environmental conditions. In consequence, they are not constitutively produced, rather expressed as adaptive response to changing environmental conditions.

Utilizing genomic prediction tools, we implemented a targeted strategy to correlate biosynthetic gene clusters (BGC) with regulons responsive to known environmental stimuli. We screened the genomes of actinomycetes for the presence of the zinc-responsive Zur-binding sites in yet unknown BGCs.

This approach revealed a predictively zinc-regulated NRPS-BGC family in eleven strains of the genus *Nocardia*. Based on the BGC composition, we postulated that this BGC family encodes the biosynthesis of transvalencin A, a thiazolidine exhibiting antifungal activity. Transvalencin was exclusively detected under zinc-deficient growth conditions while completely repressed in the presence of 6.25 µM zinc. Furthermore, we observed a heterogeneity within the transvalencin BGC family, possibly relating to a broader structural variation within this compound class. A comparative metabolomics study showed a strain specific transvalencin analogue profile, including di-halogenated and desmethoxy-transvalencins.

To confirm that this putative BGC is corresponding to the biosynthesis of transvalencin A, we disrupt the NRPS genes by plasmid-mediated homologous recombination. Mutant strains are selected by their resistance towards apramycin. Thereby, the *sacB* gene is used as a counter-selection marker. The mutant will be cultured and compared to *Nocardia* wild type in zinc-deficient growth conditions.

P21 Exploration of the natural product diversity in *Photorhabdus temperata* using the easyPACId approach

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The genera *Photorhabdus* and *Xenorhabdus* are Gram-negative gammaproteobacteria and form entomopathogenic symbioses with *Steinernema* and *Heterorhabditis* nematodes, respectively. Bacteria and nematodes cooperate for the infection of the insect and rapidly kill the prey together. Meanwhile, the bacteria produce several degradative enzymes to break down the insect carcass and provide nutrients for the nematode, as well as different natural products (NPs) including some broad spectrum antibiotics. Nowadays, the exponentially increasing number of genome sequences has revealed plenty of uncharacterized BGCs for NPs hidden in the microbial genomes. For *Photorhabdus* and *Xenorhabdus*, over 6.5% of their genome sequences are dedicated to secondary metabolite (SM) biosynthesis. Therefore, they represent promising SM producers. Herein, we aim to exploit their chemical potential *via* the easyPACId approach (easy Promoter Activated Compound Identification) to gain more bioactive NPs from the entomopathogenic bacteria *P. temperata* K122. In addition to the known GameXPeptides, phototemtides and mevalagmapeptides, more than 20 NRPS-derived new compounds were successfully identified by activation of six BGCs with the arabinose-inducible P_{BAD} promoter, which also highlights the easyPACId approach could be a rapid and efficient way to discover new products from cryptic BGCs. Moreover, we expect to get a better understanding of these metabolites as well as their distribution and regulatory mechanism, which may provide new insights into their role in the environment in the future.

P22 RESIST- resolving infection susceptibility - heterologous expression approach for a new potential ranthipeptide from *Veillonella* spp.

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The composition of the lung microbiome of chronic obstructive pulmonary disease (COPD) patients was determined via metagenomics sequencing approaches in cooperation with the medical school Hanover (MHH). The results indicate that the lungs of COPD patients harbor a plethora of bacterial species, which build the microbiome. In several metagenome profiles, *Moraxella catarrhalis* was determined as distinguishing biomarker and subsequently bacterial species varying in their α- and β diversity were analyzed. In order to probe the potential influence of the secondary metabolome of individual microbiome constituents, one family of bacteria, *Veillonellaceae*, came into focus for additional metabolomic studies. As mass spectrometry revealed no secondary metabolite candidates in extracts from the wildtype strain *Veillonella parvula*, an alternative biotechnological approach was taken. We report here the results from heterologous expression experiments. Preliminary data suggest the production of a potential ranthipeptide, a RiPP, which is now targeted for purification and structure elucidation.

P23 Bacterial endosymbionts protect beneficial soil fungus from nematode attack

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Healthy soil is indispensable for agriculture, important for clean water and air, and the nutritional source for plants and animals. It is also a complex and competitive environment with concurrent soil-dwelling microorganisms, animals and plants, all requiring nutrients, minerals and carbon sources. Especially saprophytes and decomposer, like fungi of the genus *Mortierella*, play pivotal roles in carbon cycling, xenobiont degradation and plant growth promotion. Despite their important role in healthy soils, the knowledge on their protective traits is limited.

We have found that *Mortierella verticillata* NRRL 6337 shields itself from nematodal micropredators with the help of a toxin-producing endobacterial symbiont. We provide evidence that the highly cytotoxic macrolactones (CJ-12,950 and CJ-13,357, syn. necroxime C and D), which were believed to be fungal metabolites, are in fact produced by a previously overlooked bacterial endosymbiont, *Candidatus* Mycoavidus necroximicus. Using the model organism *Caenorhabditis elegans* and the fungivorous nematode *Aphelenchus avenae*, we probed the anthelmintic activity of the necroximes and demonstrated the effective host protection in cocultures of nematodes with symbiotic and chemically complemented aposymbiotic fungal strains. This study reveals an important function for endofungal bacteria as producer of protecting agents and opens the possibility for the development of new biocontrol agents.

P24 The role of secondary metabolites in plant associated microbial communities

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The progressive climate change is threatening plant growth all over the planet. Since plants represent the most important nutrient source for humans, their health is of great interest for our food supply. To increase the environmental stress tolerance of plants in the future, the natural plant microbiome is gaining increasing attention. The plant microbiome is essential and several positive effects on growth, pathogen resistance and nutrient uptake have been attributed to certain microbes culturing the plant phyllosphere.[1]

These microorganisms not only interact with the plant but furthermore strongly affect each other. Recent studies found that the leaf microbiome of *Arabidopsis thaliana* shows a huge potential for the production of secondary metabolites.[2] Since these compounds are fundamental units for microbes to sense and respond to their environment, we hypothesized, that they play a major role in mediating interactions in the leaf microbiome. To investigate secondary metabolite-based interactions within plant microbiomes, we use a beneficial synthetic community (SynCom) assembled from *Arabidopsis thaliana* leaves. In first experiments, we investigated the potential of our SynCom to produce secondary metabolites *in sillico* and on standard media in the lab. Using co-cultivation experiments and HPLC-analysis, we identified *Pseudomonas koreensis* and *Bacillus altitudinis* as potential interaction drivers. *Bacillus altitudinis* was identified as the producer of an antibacterial compound, inhibiting almost all SynCom bacteria. *Pseudomonas koreensis* was detected to produce a siderophore, which we identified as pseudobactin. In growth experiments we saw, that pseudobactin strongly inhibits other SynCom bacteria. The results indicate that inhibiting interactions play a major role in shaping the plant leaf microbiome. In future experiments, we aim to investigate the identified compounds in gnotobiotic experiments on plant leaf surfaces.

[1] Chaudhry, V., et al., *Shaping the leaf microbiota: plant-microbe-microbe interactions.* J Exp Bot, 2020.

[2] Helfrich, E.J.N., et al., *Bipartite interactions, antibiotic production and biosynthetic potential of the Arabidopsis leaf microbiome.* Nat Microbiol, 2018. **3**(8): p. 909-919.

P25 Regulatory mechanism for natural product biosynthesis in entomopathogenic bacteria

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Natural products (NP), also called specialized metabolites (SM), represent important source of therapeutics such as antibiotics and anticancer agents. Beyond clinical therapeutics these NPs can have agricultural and biotechnological applications. *Photorhabdus* and *Xenorhabdus* bacteria produce numerous NPs that regulate mutualistic associations with entomopathogenic nematodes affecting symbiosis and virulence. It was previously shown that a LysR type regulator HexA and a small RNA ArcZ regulate NP biosynthesis depending on a RNA chaperon Hfq and deletion of both *hfq* and *arcZ* resulted in loss of production of all NPs. In search of further global regulators for NP biosynthesis we have conducted a genome wide screening with transposon mutagenesis. This approach resulted in the identification of the regulatory repertoire for NP biosynthesis in *Photorhabdus* and *Xenorhabdus* based on loss of NP-dependent pigmentation. Deletion of an identified candidate gene resulted in loss of the complete bacterial NP profile that was further used as a tool to overproduce desired target NPs in a cleaner background thus allowing direct bioactivity testing and NP isolation. A global transcriptomic and proteomic approaches was performed to decipher the biology behind the regulation of NP biosynthesis.

"WITHDRAWN"

P26 Characterization of a class II lanthipeptide from the cyanobacterium *Nostoc punctiforme* and investigation of its role in symbiotic interaction

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The terrestrial cyanobacterium *Nostoc punctiforme* is a versatile microorganism that can live freely in the soil and in symbiosis with various partners, such as mosses, cycads, lichen or *Gunnera* sp. The *Nostoc* symbioses can be classified as nutritional symbioses, whereby its role is nitrogen fixation. *N. punctiforme* is a promising producer of bioactive compounds as it harbours a multitude of cryptic biosynthetic gene clusters (BGCs) for secondary metabolites. A ribosomally synthesized and post-translationally modified peptide (RiPP) of the model strain *N. punctiforme* PCC 73102 that has shown to play a role in symbiosis with mosses is the class II lanthipeptide RiPP4. Its biosynthetic gene cluster (BGC) showed an early upregulation in chemical and physical interaction studies with the moss *Blasia pusilla*.

Since the product of the RiPP4 BGC could not yet be analytically detected in *N. punctiforme* itself, we aim to express the peptide heterologously in *E. coli* or to reconstitute the peptide *in vitro* using chemoenzymatic synthesis. One of these approaches will be used to characterize the involved class II lanthipeptide synthetase and can provide an avenue to design bioassays to further understand the role of RiPP4 in symbiotic interactions of *N. punctiforme* and *B. pusilla*.

P27 Ubiquitous bacterial polyketides induce cross-kingdom microbial interactions

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Although the interaction of prokaryotic and eukaryotic microorganisms is critical for the functioning of ecosystems, urgently needed information about the processes driving microbial interactions within communities remains scarce. We previously reported that the soil bacterium *Streptomyces iranensis* specifically triggers the production of natural products in the fungus *Aspergillus nidulans*.[1,2] The trigger, however, remained obscure. Recently, we discovered that the compound family of arginine-derived polyketides including azalomycin F produced by S*. iranensis* serve as the long sought-after bacterial signals for this induction.[3]

To analyse whether these compounds play a crucial role in the soil community, we randomly collected soil and isolated bacteria.[3] To test their ability to induce the *ors* gene cluster we generated an *A. nidulans* reporter strain which has the *orsA* gene translationally fused to a nanoluciferase and eGFP. With this reporter strain we were able to identify several bacterial species that induced green fluorescence in the fungus. Interestingly, extracted soil also led to an increased nanoluciferase activity indicating that arginine-derived polyketides are indeed present in the soil. Arginine-derived polyketides can be found around the world and seem to play an important role in shaping microbial interactions in the soil.

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[2] Fischer J, *et al*. eLife 2018

[3] Krespach MKC, Stroe MC, *et al.* bioRxiv 2022

P28 Biological evaluation of biomimic enterobactin analogues in *E. coli* and *P. aeruginosa*

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Resistance development to antimicrobial drugs is inevitable and this, in turn, necessitates the development of novel drugs to overcome resistance among human pathogens. However, antimicrobial drug development against Gram-negative bacteria is challenging due to drug exporters and the outer membrane permeability barrier. Many compounds fail to translocate over the cell envelope barrier, which prevents activity. An innovative approach to overcome this is the conjugation with siderophores, also known as the Trojan horse approach. Siderophores, such as enterobactin, are iron chelators that are biosynthesized by bacteria to acquire metal ions for growth. Inspired by natural siderophores, artificial siderophore drug conjugates can be synthesized in order to enhance antibacterial activity compared to the parental free antibiotics. In this work, we present the biological evaluation of the artificial enterobactin analogue Ent_{KL} and fluorophore-conjugates that were designed and synthesised by R. Zscherp and P. Klahn at the Institute of Organic Chemistry, Technische Universität Braunschweig, Germany. Growth recovery assays under iron-limiting conditions revealed a concentration-dependent growth effect of Ent_{KL} comparable to natural enterobactin. Further, imaging studies utilizing $BODIPY_{FL}$-conjugates, demonstrated the ability of Ent_{KL} to overcome the Gram-negative outer membrane permeability barrier to deliver molecular cargos *via* the bacterial iron transport machinery of *E. coli* and *P. aeruginosa*.

P29 Natural products of *Aster tataricus* endophytes

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Aster tataricus is a plant used in Traditional Chinese Medicine with antitussive and anticancer activity. Halogenated non-ribosomal cyclopeptides, called astins, have been identified as the main cytotoxic compounds in the plant. The astins are a family of 13 compounds (astin A to P), differing e.g. in the presence or absence of hydroxyl groups or halogens.[1] These compounds have been discussed as potential anticancer drugs. Recently, it has been found that some of the astins are not produced by the plant, but by an endophytic fungus called *Cyanodermella asteris*,[2] isolated from *A. tataricus*. However, as not all known astins could be detected in the lab-cultivated endophyte, it has been suggested that some plant-fungus interaction is vital for the production of the complete astin series.[3] To study this interaction, we performed cultivation studies with the fungus and plants, and used mass spectrometry imaging (MSI) to visualize the astin distribution in different plant tissues. An *A. tataricus* plant was harvested, and various tissues were embedded, sectioned, and after sample preparation analyzed by AP-MALDI-MSI. We found nonhomogeneous astins distribution in the different sections and differing relative abundance in each tissue. In extensive fungus and tissue cultivation experiments, we found in NaCl-supplemented media, all known astins are produced by *C. asteris*. This suggests that the local environment inside the plant results in the production of the whole astin series, rather than a plant metabolic contribution that results in the observed astin diversity. Initial results in *A. tataricus* callus experiments indicate *C. asteris* might help the plant to tolerate increased soil salt concentrations.

Additional *A. tataricus* endophytic fungi were isolated. From a yet unidentified fungus, a series of 4 new azaphilones were isolated.

[1] Tan N-H, Zhou J. Plant cyclopeptides. Chemical Reviews 2006; 106: 840–895; DOI: 10.1021/cr040699h

[2] Vassaux A, Tarayre C, Arguëlles-Arias A, Compère P, Delvigne F, Fickers P, Jahn L, Lang A, Leclère V, Ludwig-Müller J, Ongena M, Schafhauser T, Telek S, Théatre A, van Berkel WJH, Vandenbol M, van Pée K-H, Willems L, Wohlleben W, Jacques P. Astin C Production by the Endophytic Fungus *Cyanodermella asteris* in Planktonic and Immobilized Culture Conditions. Biotechnol J 2019; 14: e1800624; DOI: 10.1002/biot.201800624

[3] Jahn L, Schafhauser T, Pan S, Weber T, Wohlleben W, Fewer D, Sivonen K, Flor L, van Pée K-H, Caradec T, Jacques P, Huijbers M, van Berkel W, Ludwig-Müller J. *Cyanodermella asteris* sp. nov. (Ostropales) from the inflorescence axis of *Aster tataricus*. mycotaxon 2017; 132: 107–123; DOI: 10.5248/132.107

P30 Comparison of the global regulation of two similar heterologously expressed antibiotic gene clusters

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Heterologous expression and activation of orphan biosynthetic gene clusters from bacterial genomes is routinely applied for a detailed examination of the corresponding compound. This project aims at a better understanding of the interplay between the host and the cluster-specific regulatory networks. We employed comparative DNA affinity capturing assays (DACA) to identify proteins from the host organism *Streptomyces coelicolor* M512 that bind to putative promoter regions within the biosynthetic gene clusters (BGC) encoding for the antibiotics caprazamycins (Cpz) and liposidomycins (Lpm). Both liponucleoside antibiotics share a high degree of similar structural moieties and similarities in the genetic organization of their BGCs. In total, 2241 proteins were identified with the vast majority (80%) of them binding unspecifically to several of the tested promoter regions. For each promoter, the 50 most abundant specific proteins were analysed in more detail revealing some interesting candidates: two TetR- and a MarR-regulator. Additionally, a CRP-type regulator, Sco3571, which Gao *et al*. described to influence the expression of a gene cluster involved in precursor supply for Cpz and Lpm, was included.[1] Upon overexpression, a significant change in liponucleoside antibiotic production was observed for one TetR- and the CRP- regulator. Simultaneously, we perform RNA sequencing to compare transcriptional profiles of the strain with and without the BGCs, focusing on regulatory genes that exhibit a strong differential expression.

[1] Gao, C., *et al.* Crp is a global regulator of antibiotic production in *Streptomyces*. mBio. 3:1-12 (2012).

P31 Genome-wide prediction of small non-coding RNAs in *Streptomyces clavuligerus* ATCC 27064 by comparative genomics and conservation of RNA secondary structures

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Introduction: *Streptomyces clavuligerus* ATCC 27064 produces clavulanic acid, a β-lactamases suicide inhibitor. Small RNAs regulate an enormous variety of metabolic processes in diverse prokaryotic organisms, including secondary metabolites biosynthesis. Despite its importance, knowledge about small RNAs (sRNA) in *Streptomyces clavuligerus* is limited.

Methods: Detection of conserved Intergenic Regions (IGRs) in the genome of *S. clavuligerus* ATCC 27064 was performed with Biopython scripts.[1] We used these IGRs to detect promoters,[2] terminators[3] and to predict structurally conserved and thermodynamically stable RNA secondary structures.[4] These structures were visualized in RNAfold and their possible targets were defined using IntaRNA 2.0.[5]

Results and Conclusions: We performed a comparative genomic analysis of the IGRs between *S. clavuligerus* ATCC 27064 and the closely related strains *S. clavuligerus* F1D-5, *S. clavuligerus* F613-1, *S. pristinaespiralis* HCCB 10218 and *S. lunaelactis* MM109. In these strains, 247 IGRs were conserved in at least 4 strains. Of these, 57 putative small RNAs were detected by RNAz with an RNA-class probability above 0.9, indicating a high conservation of their secondary structure. In addition, the presence of promoters and terminator signals was detected in 213 and 17 IGRs, respectively. The analysis of their possible targets showed that these sRNAs interact with multiple mRNAs which suggest they might be involved in the regulation of diverse cellular activities, e.g., central metabolism processes and secondary metabolites production. This study showed that less explored genomic regions, e.g., IGRs, can harbour novel regulatory elements in *Streptomyces* species which lay the groundwork for future studies aimed to elucidate the role of small RNAs, mainly in secondary metabolites production.

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[4] Lorenz, R., et al. Algorithms Mol Biol. 2011, 6:26. doi:10.1186/1748-7188-6-26

[5] Mann, M., et al. Nucl. Acids Res. 2017, 45:W1. doi:10.1093/nar/gkx279

P32 Investigation of RiPPs originating from two-domain precursors

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Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a diverse class of secondary metabolites with a wide range of bioactivities. They all share a ribosomal origin, as the peptide sequence is encoded in a so-called precursor gene. Often, precursor peptides are short and lack clear structural features. As an exception, the family of the "nitrile hydratase leader peptides" (NHLP) are characterized by unusually long leader sequences that show similarity to the enzyme nitrile hydratase.[1] In certain species of the order Burkholderiales, these NHLP precursors further appear as tandem genes, i.e., two copies of the precursor are present in a row. Intriguingly, in a few strains, these genes are fused into a single two-domain precursor, resulting in a ~270 amino acid precursor protein. The function of the two leader domains as well as the nature of the resulting RiPP(s) is currently unclear.

Here, we investigated these so-far uncharacterized RiPP clusters in order to identify the produced metabolite(s), characterize the biosynthetic enzymes, and explore the role of the two leader domains in the precursors. Following a bottom-up approach, we heterologously expressed the biosynthetic gene cluster containing the fused two-domain precursor in *Escherichia coli* and analyzed the modifications in the precursor peptide using LC-MS.

The activity of the cyclodehydratase and methyltransferase present in the gene cluster could be successfully reconstituted in *E. coli*. The resulting modifications were localized at the *C*-terminus of the precursor and a dehydrated cysteine and a methylated threonine were observed. Size-exclusion chromatography unexpectedly revealed that the precursor assembles into a tetramer of ~120 kDa. Such multimeric assemblies are surprising for a RiPP precursor and we are currently investigating the role of these structures. We further aim to characterize the remaining proteins in the gene cluster and to isolate the RiPP from the native producer.

[1] Haft et al., *BMC Biology* **8**, 70 (2010).

P33 PreFerrP450 – A machine-learning driven platform for the heterologous expression of cytochrome P450s

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Cytochrome P450s are ubiquitously distributed monooxygenases that play a key role in the modification of all classes of natural products. They are capable of catalyzing a large variety of biochemical transformations ranging from the prototypical hydroxylation of non-activated carbon atoms to complex rearrangement reactions. P450s require a redox system that transfers electrons from NADPH to the central iron of the P450's heme-cofactor for their activity. These redox systems that usually comprise a ferredoxin and a ferredoxin reductase, are usually not encoded in a biosynthetic gene cluster. Since the redox partners are required for the activity of the gene cluster-encoded P450, the absence of the corresponding redox system, results in non-functional P450s if the heterologous host does not encode the required redox partners. To facilitate the efficient heterologous expression of biosynthetic gene clusters that require these auxiliary gene products for the full maturation of a natural product, I developed PreFerrP450 – a machine learning-based algorithm for the prediction of P450 – ferredoxin pairs. Based on our predictions, the corresponding redox partners can be co-expressed with the biosynthetic gene cluster of interest to achieve P450 functionality. As a proof of principle, we selected the five most commonly required ferredoxin/ferredoxin reductase pairs and put each of them under the control of a different small-molecule inducible promoter of the *Escherichia coli* Marionette system. We will be using small model biosynthetic gene clusters of all natural product classes to functionally validate our PreFerrP450 algorithm. The combination of PreFerrP450 and the inducible ferredoxin/ferredoxin reductase platform will contribute to the success of heterologously expressing biosynthetic gene clusters that harbor P450 genes.

P34 Spatial and temporal cellular assembly of the glycopeptide biosynthetic complex

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Glycopeptide antibiotics (GPAs) are clinically important natural compounds, produced by various actinomycetes. The biosynthesis of the vancomycin-type GPA balhimycin synthesized by Amycolatopsis balhimycina, is the best studied GPA production pathway in vivo. The biosynthesis of GPAs involves enzymes responsible for the synthesis of the precursors, which are assembled into a peptide by non-ribosomal peptide synthetases (NRPS; BpsA-C) and modified by several enzymes like a halogenase (BhaA), P450-monooxygenases (OxyABC), glycosyltransferases (BgtfABC) and a methyltransferase (Bmt). In previous studies we demonstrated that BhaA and the OxyABC display their activity when the peptide is bound to the NRPS, suggesting that the central steps of the biosynthesis are carried out at a multi-enzyme enzyme complex. To investigate the composition of the multi-enzyme complex, we applied genetic manipulation and state-of-the-art methods to analyze the protein-protein interactions. Experiments using the bacterial two-hybrid system (B2H) confirmed previously known and revealed yet unknown interactions occurring at the NRPSs. Through genetic manipulation, we analyzed the functional role of specific domains in vivo, which displayed the important functions within the biosynthetic complex. In addition, separation of protein complexes by blue native PAGE and subsequent MS analyses provided important insights into the composition of the multi-enzyme complex.

P35 The SAM-dependent methyltransferase MftM is responsible for methylation of the redox cofactor mycofactocin

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Actinomycetota are known for their capabilities to produce a wide range of natural products, such as antibiotics or unusual cofactors. An example of the latter is the recently discovered redox cofactor mycofactocin (MFT). In our first *in-vivo* study,[1] we showed that this ribosomally synthesized and posttranslationally modified cofactor is produced by MftABCDEF, including the radical SAM maturase MftC, and the glycosyltransferase MftF. The latter can decorate the mycofactocin redox core premycofactocin with up to nine glucose moieties in a β-1,4-linkage. However, we also found that the second glycosyl moiety can be 2-*O*-methylated, albeit no putative methyltransferase is encoded in proximity to the gene cluster. In this study, we discovered the responsible methyltransferase gene *mftM* in the model organism *Mycolicibacterium smegmatis* and confirmed its activity by targeted gene inactivation and metabolic profiling. We also provide evidence that a variety of predicted mycofactocin producers, such as *Mycobacterium tuberculosis*, indeed biosynthesize the redox cofactor and that its methylation also depends on the presence of *mftM* homologs in these organisms. Furthermore, it was shown that deletion of *mftM* in *M. smegmatis* causes an elongated lag phase upon cultivation on ethanol as the sole carbon source, which is known to induce the biosynthesis of mycofactocin. Finally, we could link the methylation of mycofactocin to resistance against cellulase-degradation *in vitro*.

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P36 Sequential allylic alcohol formation by a multifunctional cytochrome P450 monooxygenase with rare redox partners

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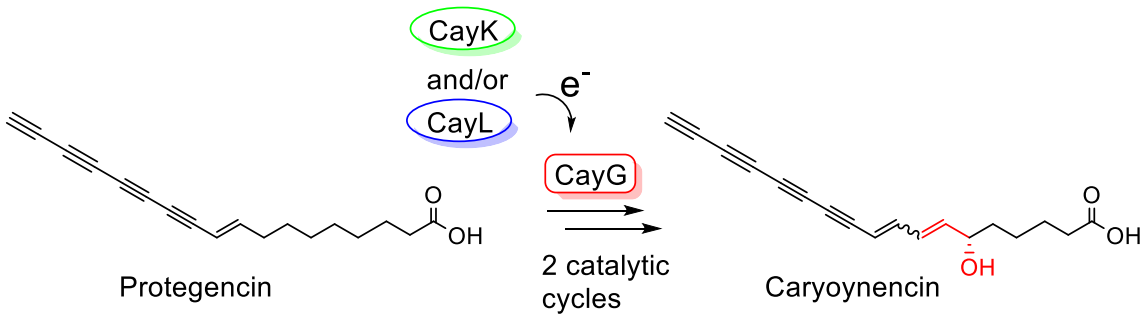
Caryoynencin is a toxic and antifungal fatty acid derivative produced by a number of plant-pathogenic and insect-protective bacteria (*Trinickia caryophylli* and *Burkholderia* spp.).[1–4] Structure-activity studies showed that – in addition to the reactive terminal tetrayne – the presence of an allylic alcohol moiety is critical for antimicrobial activities. Gene cluster analyses pointed to a gene (*cayG*) coding for a cytochrome P450 monooxygenase (CYP) that could play a key role in the formation of this pharmacophoric unit, yet the biochemical basis of this multi-step biotransformation has remained elusive.[5] By a combination of mutational analyses, heterologous expression and *in vitro* reconstitution experiments we show that CayG catalyzes the complex transformation of a saturated carbon backbone into an allylic alcohol. Unexpectedly, we found that CayG employs a ferritin-like protein (CayK) or a rubredoxin (CayL) component for electron transport.[6] Furthermore, we deduced the timing and course of the desaturation-hydroxylation sequence from a time-course study and *in vitro* biotransformations with pathway intermediates and substrate analogues, protegencin congeners isolated from *Pseudomonas protegens* Pf-5, and synthetic derivatives. We report a novel enzymatic reaction sequence by a multifunctional oxygenase, which not only sheds light on an important biosynthetic transformation but also may inspire future biocatalytic applications.



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P37 MftG catalyzes oxidation of mycofactocinols to mycofactocinones in *Mycolicibacterium smegmatis*

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The mycofactocin (MFT) biosynthetic gene cluster, encoding a ribosomally synthesized and posttranslationally modified peptide, was postulated by *in-silico* analysis to produce a redox cofactor in mycobacteria[1] Phenotypic analysis of MFT gene knock-outs revealed an important role in mycobacterial ethanol metabolism[2] and interferences on *Mycobacterium tuberculosis* growth in infection models.[3] *In-vitro* analysis elucidated the role of the *mftABCDE* genes in the biosynthesis of a redox-active core moiety.[4] Metabolomics studies further revealed that MFT is decorated with a *b*-1,4-glucan chain by the glycosyltransferase MftF and exists in both reduced (mycofactocinol) and oxidized (mycofactocinone) forms in the cell.[5]

However, the mechanism of cofactor regeneration, i.e., the re-oxidation of mycofactocinols to mycofactocinones, was still elusive. The genomic deletion of a glucose-methanol-choline oxidoreductase gene (*mftG*) exhibited an impairment of growth on ethanol as the sole carbon source. Metabolomic profiling showed an accumulation of mycofactocinols in the ↗*mftG* strain suggesting the function of MftG as a MFT dehydrogenase or oxidase. Confirmation of the oxidase activity was accomplished by cell-free activity assays with mycofactocinol as a substrate.

This study closes an important knowledge gap of the mycofactocin system and fosters further analyses regarding the function of MFT in bacteria.

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P38 Insights into the biosynthesis of a hybrid RiPPs system

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Bacteria producing natural products represent a valuable source of novel and complex bioactive molecules. *Streptomyces griseus* S4-7 was isolated from a wilt-suppressive soil as the representative strain producing anti-*Fusarium* metabolites.[1,2] Genomic analyses revealed the presence of biosynthetic gene clusters (BGCs) encoding ribosomally synthesized and post-translationally modified peptides (RiPPs) with no chemical structure connected.

Our project aims to characterize the structure and biosynthesis of a hybrid RiPPs system involved in the plant-protective effect of *S. griseus* S4-7.

Through an *in vitro* reconstitution approach, we have found that the system is constituted by two labionin-containing lanthipeptides which might be further modified by a rSAM protein in a leader-dependent manner. Additionally, a new member of the recently discovered S8 family of proteases was found to partially remove the leader peptide.

The system is completed by a third peptide which is modified by a class II lanthipeptide synthetase (LanM). However, as this peptide does not contain cysteines in the core region, no thioether crosslinks can be formed. This peptide containing four dehydroamino acids is then modified by an oxidoreductase, yielding a linear peptide representing a new RiPP variant that has not been experimentally described.

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P39 Functional characterization of a non-canonical diterpene biosynthetic pathway

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Terpenoids are the largest and structurally most diverse class of natural products. Complex terpenoids have traditionally predominantly been isolated from plants and fungi. Bacteria, on the other side, have only recently been identified as an almost untapped treasure trove of biosynthetic blueprints for the production of complex terpenoids. Terpenes are formally biosynthesized from isoprene units, that are first oligomerized, followed by complex cyclization and rearrangement reactions that convert achiral oligoprenyl precursors into complex cyclic hydrocarbon scaffolds with multiple stereocenters. Additional structural diversity is introduced through a variety of tailoring reactions, many of which are carried out by cytochrome P450 monooxygenases (P450s). We mined bacterial genome sequences for sesqui- and diterpene biosynthetic pathways with unusual gene architecture. A diterpene biosynthetic pathway was selected that features an unusual terpene cyclase that is fused to a reductase, multiple P450s, one of which is split into three fragments that act together to form a functional P450 and a hypothetical protein which is involved in terpene biosynthesis. Heterologous expression of the selected gene cluster in a *Streptomyces* host that was optimized to produce high terpene titers resulted in the identification of three novel diterpenes. Functional characterization of each gene product by gene disruption and bioinformatic analyses paved the way towards a model for the biosynthesis of the characterized diterpenes. The functional characterization of the diterpene biosynthetic gene cluster revealed several peculiarities that, based on bioinformatic analyses of other, cryptic terpene biosynthetic gene clusters, seem to me more widespread than initially anticipated.

P40 Discovery and biosynthetic origin of cyclopropanol substituted toxins in human pathogenic bacteria

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The *Burkholderia* species *B. mallei* and *B. pseudomallei* are notorious human pathogens that are also considered potential biowarfare agents. Besides the danger of exposure through deliberate release, 165,000 cases of melioidosis, the disease caused by *B. pseudomallei*, are estimated to occur annually. Treatment of the often lethal infections is complicated by the pathogens' high resistance to clinically used antibiotics. Despite this eminent threat, the knowledge of effector molecules by which these bacteria induce pathology is limited.

This presentation will discuss the discovery and biosynthetic origin of toxic secondary metabolites (malleicyprols) produced by bacteria belonging to the *B. pseudomallei* complex. Malleicyprols are assembled by an unusual polyketide synthase encoded by the virulence-associated biosynthetic gene cluster *bur*. A hallmark of the polyketide metabolites is a highly strained cyclopropanol "warhead" that is essential for their detrimental effect. The biosynthetic route to the cyclopropanol warhead starts from L-methionine to produce sulfonium-metabolites known from global sulfur cycling. The key step in cyclopropanol formation is catalysed by a member of the KARI enzyme family, which is widespread among bacterial primary metabolism and plays an important role in branched amino acid biosynthesis. In the case of pathogenic *Burkholderia* spp. however, this enzyme fold has been repurposed to build the virulence-conferring cyclopropanol structure. The enzymatic mechanism that includes a concealed redox reaction and leads to ring formation will be discussed.

P41 Deciphering the biosynthetic pathway to massinidine

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Recently, the antiplasmodial alkaloid massinidine was isolated from the Gram-negative bacterium *Massilia* sp. NR 4-1.[1] Massinidine features a guanidine-containing, 5,5-fused bicyclic ring system that is rarely found in nature. Isotope labelling studies indicated that L-arginine, L-phenylalanine and acetate are precursors of massinidine biosynthesis. A bioinformatic analysis revealed a putative biosynthetic gene cluster in the genome of *Massilia* sp. NR4-1. This gene cluster comprises four genes, *mssA-D*, which presumably encode an aldolase, a dehydratase, a cyclodeaminase and an amino acid C-acyltransferase. To verify if the enzymes MssA-D indeed are involved in the biosynthesis of massinidine, we performed a heterologous expression in the myxobacterial model organism *Myxococcus xanthus*, which had previously been shown to be well-suited for the heterologous production of alkaloids.[2] Plasmid-based expression of the genes *mssA-D* in *M. xanthus* conferred the myxobacterium the ability to produce massinidine. Accordingly, we propose that MssA catalyzes an aldol reaction of phenylacetaldehyde with an acyl-CoA. The resulting β-hydroxy thioester is subject to a dehydration reaction by MssB. Subsequently, MssD attaches L-arginine in a decarboxylative Claisen-like condensation to assemble the α-aminoketone scaffold. Lastly, we propose that the cyclodeaminase MssC is involved in the generation of the characteristic 5,5-fused bicyclic guanidine motif. *In vitro* testing of the enzymes MssA-D revealed new insights into the biosynthetic route to massinidine.

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P42 Clickable microcystins as payloads for antibody-drug conjugates

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Microcystins (MCs) are nonribosomal cyclic heptapeptides produced by freshwater cyanobacteria like *Microcystis* and *Planktothrix*. Microcystins are well studied cyanotoxins, and known for their inhibition of the eukaryotic serine/threonine protein phosphatases 1 and 2a with IC_{50} values in the pico- to nanomolar concentration range.[1] Unlike many other cytotoxic agents that enter cell by passive diffusion, MCs are dependent on an active uptake via organic anion transporting polypeptides (OATP) 1B1 and 1B3, which are expressed especially by liver cells.[2] The transportability strongly depends on the structure of the MCs and can dramatically differ by the exchange of one single amino acid in the core structure.[3]

Because of the high potency, the yet unexploited mode-of-action of MCs, the unlikely resistance development, and the prospect of lower side effects compared to known payloads of antibody-drug conjugates (ADCs), we strive to develop MC derivatives with optimized properties that can be used as cytotoxic payloads for ADCs. Here, we present the semi-synthesis of MC analogues bearing different properties and their *in vitro* characterization. Easily derivatizable, "clickable" MCs were produced in distinct *Microcystis* sp. strains by precursor-directed biosynthesis,[4] followed by extraction of biomass and isolation of these unnatural MCs by flash chromatography and HPLC. The obtained MCs were modified with a library of small molecules with different properties (e.g. charge, lipophilicity, size) using copper-catalyzed cycloaddition ("click chemistry"). The structures of the synthesized MC derivatives were confirmed by $HRMS^2$. To study structure-activity as well as structure-transportability relationships of the compounds, both cell viability studies as well as phosphatase inhibition assays have been performed.

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P43 Identification of the biosynthetic pathway of chloszentirazine in *Xenorhabdus szentirmaii*

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Nature is a prolific producer of biologically active natural products. Organisms producing bioactive compounds must be resistant to their biological effects in order to survive from production/accumulation process. Microbes, especially bacteria, have developed different strategies to prevent self-toxicity, such as self-resistance, chemical modification and efflux pumps. Following the self-resistance guided approach, together with genome mining, we found a new compound chloszentirazine from *Xenorhabdus szentirmaii* DSM 16338, which could serve as a putative topoisomerase inhibitor.

Chloszentirazine is a new chlorinated derivative of szentirazine, a modified diketopiperazine reported in 2019. As it is a rare halogenated diketopiperazine from bacteria, we aim to investigate its biosynthetic pathway. We successfully identified the biosynthetic gene cluster of chloszentirazine, and elucidated its biosynthetic pathway based on heterologous expression and gene deletion experiments. Feeding experiment proved that the halogenase CszA showed high substrate promiscuity towards different halogens, which provided a potential enzyme to generate further halogenated derivatives.

P44 Precursor-directed biosynthesis of massinidine derivatives in *Massilia* sp. NR 4-1

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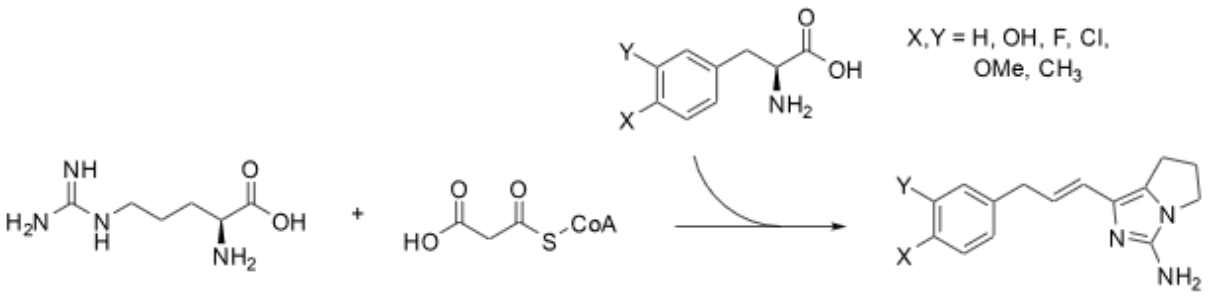
New therapeutic agents are urgently needed to counter drug resistance of pathogenic bacteria and protozoa. The genomic era led to the realization that the biosynthetic potential of microorganisms is much larger than expected. Especially underexplored taxa possess a considerable potential for the biosynthesis of novel bioactive compounds. An illustrative example is given by the β-proteobacterium *Massilia* sp. NR 4-1, which was recently shown to produce two novel metabolites, massiliachelin and massinidine.[1,2] The latter is a guanidine alkaloid with antiplasmodial activity.

The aim of this study was to generate unnatural derivatives of massinidine, which can be used for structure-activity relationship studies in the future. As derivatization through total synthesis is often laborious, we decided to exploit the plasticity of the massinidine pathway in *Massilia* sp. NR 4-1 in order to introduce artificial building blocks into the biosynthesis. Here, we will present the results of the feeding studies involving various phenylalanine derivatives.



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P45 Biosynthesis study of a microviridin-class peptide from a *Bacteroidetes* strain with serine protease inhibitory activity

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Microviridins are a class of ribosomally synthesized and post-translationally modified peptides originally discovered from cyanobacteria, featured by the intramolecular ω-ester and ω-amide bonds catalyzed by two ATP-grasp ligases. In this study, 104 biosynthetic gene clusters of microviridins from *Bacteroidetes* were analyzed, which unveiled unique features of the precursor peptides. The analysis of core peptides unearthed a microviridin-like biosynthetic gene cluster from a *Bacteroidetes* strain consisting of two potential precursors, ChiA1 and ChiA2. Unexpectedly, the core peptide sequence of ChiA1 is consistent with the backbone of a reported elastase-inhibiting peptide, while ChiA2 is likely to be a precursor of an unknown product. ATP-grasp ligases ChiB and ChiC were biochemically characterized to be responsible for the intramolecular ester and amide bond formation, respectively. *In vitro* reconstitution of the pathway showed the three-fold dehydrations of ChiA1 while unusual four-fold dehydrations were observed for ChiA2. Furthermore, *in vivo* gene co-expression facilitated the production of chitinoviridin A1 and two novel microviridin-class compounds chitinoviridin A2A and chitinoviridin A2B with an extra macrolactone ring. These peptides all showed potent inhibitory effects against elastase and chymotrypsin, independently.

P46 Making efforts to bring eco-friendly chelators into the market: Optimization of the [*S,S*]-EDDS production

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Under nutrient deficiency conditions, bacteria produce a variety of chelating compounds to acquire essential metal ions from the environment, in particular iron and zinc. The actinomycete *Amycolatopsis japonicum* produces the chelating compound [*S,S*]-EDDS (EDDS). Because EDDS production is tightly inhibited by traces of zinc, it is considered that EDDS is functioning as a zincophore. Structurally, EDDS represents an EDTA isomer. EDTA is a chemically synthesized complexing agent used in many industrial and agricultural applications. Unlike EDTA, which accumulates in the environment causing toxicity, EDDS is highly biodegradable. The combination of excellent chelating properties and biodegradability make EDDS a promising alternative to EDTA and other environmentally threatening chelating agents for many applications.

Our work focuses on the establishment of an industrial EDDS bioprocess. In order to improve the production yield by metabolic engineering approaches it is necessary to elucidate the stepwise biosynthesis. Genetic manipulation of EDDS biosynthetic gene cluster, biochemical characterization of the encoded enzymes and feeding experiments with labeled precursors are used to determine the precursors and intermediates of biosynthesis. These results will be exploited to specifically target the metabolism of *A. japonicum*.

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P47 Heterologous production of a two-component Lantibiotic that originates from a human skin commensal

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The rise and spread of multi drug-resistant bacteria is becoming a serious threat for human health that demands the development of new antimicrobial drugs. Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a superfamily of peptidic natural products, whose members often exhibit promising antimicrobial activities and thus, they are an interesting source for potential new drug leads. Lanthipeptides belong to the largest group of RiPPs and their defining feature is the presence of β-thioether crosslinks that are installed during the post-translational modification process. Lanthipeptides with antimicrobial activity are often also referred to as lantibiotics. The focus of the project presented here lies on a set of two-component lantibiotics that were discovered from a beneficial human commensal isolated from the skin microbiota of a healthy human. These lantibiotics show potent activity against Staphylococcus aureus (including clinical MRSA isolates), while not affecting other beneficial commensals, and it was previously demonstrated how these compounds prevent S. aureus colonization of the skin. Hence, these lantibiotics might be interesting new drug leads for the selective treatment of S. aureus. The biosynthetic gene clusters of two-component lantibiotics typically encode two orthogonal precursor peptide/lanthipeptide synthetase pairs and only correct pairings are productive. Additionally, an ABC-transporter with an N-terminal peptidase domain is encoded that removes the leader and exports the mature lanthipeptides. Here, we will present our work towards identifying the correct precursor peptide/lanthipeptide synthetase pairings, how to establish and optimize heterologous co-expression systems with them, and how the cytosolic leader peptidase domain of the peptidase-ABC transporter fusion can be utilized for in vitro removal of the leader peptide. These efforts will pave the way for performing in-depth structure-activity-relationship studies and for investigating the mode-of-action of these lantibiotics.

P48 Biosynthetic incorporation of fluorinated amino acids into the nonribosomal peptide gramicidin S

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Introduction of fluorine atoms is an excellent method to fine-tune the chemical properties of drug molecules.[1] Biosynthesis of fluorinated natural products has largely relied on the tolerance of biosynthetic enzymes for fluorinated precursor analogs,[2,3] which limits product yields. For instance, the nonribosomal peptide trifluorosurfactin has been produced through feeding of trifluorovaline to *Bacillus subtilis*.[3] However, the impact of substrate fluorination on the molecular recognition by nonribosomal adenylation domains, which determine amino acid specificity of nonribosomal peptide synthetases (NRPSs), has not been investigated. Here, we have observed that feeding of fluorinated phenylalanine analogues does not lead to incorporation into gramicidin S by the natural producer organism. We have determined saturation kinetics of the NRPS module GrsA, which has revealed a surprisingly strict differentiation between fluorinated amino acids and their natural counterparts. These findings are supported by binding studies using isothermal titration calorimetry (ITC). By using an *in vitro* platform for the synthesis of fluorinated gramicidin S analogues, we have successfully overcome the selectivity of GrsA. A better understanding of fluorine recognition by NRPSs will pave the way for the efficient synthesis of valuable peptide drugs in the future.

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P49 One-pot chemoenzymatic synthesis of microviridin analogs containing functional tags

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Microviridins are a family of ribosomally synthesized and post-translationally modified peptides (RiPPs) featuring characteristic lactone and lactam rings. Their high activities as potent serine protease inhibitors, whereat individual variants specifically inhibit different types of proteases, are of pharmacological interest. Functional tags, that provide attractive properties additional to the posttranslational modifications, which are crucial for the stability and bioactivity of RiPPs, can be introduced in the structure of RiPPs. So far neither microviridins containing such functional tags nor a method generating bioactive microviridins containing functional tags has been reported. In this study, a chemoenzymatic *in vitro* platform is used to introduce functional tags in various microviridin variants yielding biotinylated, dansylated or propargylated congeners. Notably, the modified compounds retained full or at least partial activity against trypsin and elastase, respectively, allowing their immediate use as molecular tools. As a proof of concept, we used dansylated microviridin B as a diagnostic tool to selectively label elastase in protease mixtures. This straightforward approach paves the way for customized protease inhibitors with built-in functionalities that can help to unravel the still elusive ecological roles and targets of this remarkable class of compounds and to foster applications based on protease inhibition.

Additionally, we investigated an aminopeptidase suitable for microviridin leader peptide cleavage.

P50 Heterologous Production of the Antifungal Lanthipeptide Pinensin

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Pinensin is the first and thus far only reported lanthipeptide known to exhibit antifungal properties. Its potential use in a clinical setting has however been hampered due to its cytotoxic side effects. Therefore, it would be of interest to perform Structure-Activity-Relationship (SAR) and Mode-of-Action (MoA) studies with pinensin. The goal of these efforts would be the identification of variants of this natural product with an enhanced selectivity profile for its antifungal over the cytotoxic activity.

As lanthipeptide biosynthesis starts with the ribosomal synthesis of a genetically-encoded precursor peptide, lanthipeptide variants can be easily accessed via the mutation of the precursor encoding gene once a heterologous production system has been established. Hence, it is an important goal to find strategies that enable the heterologous production of pinensin (and subsequently variants thereof) in E. coli. Here, the work towards this goal will be presented. It will be shown how the combination of a series of plasmids carrying genes essential for the post-translational modification of a His6-tagged precursor peptide (His6-PinA) provides access to fully modified PinA and how the lanthipeptide can be released in vitro through a peptidase specific for this purpose.

In addition to these efforts, the results of a genome mining campaign will be shown. By search for clusters producing homologs of pinensin and analysis of the sequences of the identified precursor peptides, three distinct clades of pinenins-like lanthipeptides can be defined, one of which is closely associated with known pathogens implying an additional role of such compounds in virulence.

P51 Genome mining of cryptic natural products via cloning and heterologous expression of biosynthetic gene clusters

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The demand for novel small-molecule drugs is on a continuous rise regarding a number of different pathologies. Bacteria are a prolific source of secondary metabolites with diverse structures and biological activities. However, the numbers and diversity of biosynthetic gene clusters (BGCs) predicted in the genomes of conventional drug-producing actinobacteria and myxobacteria far outnumber currently known natural products discovered from these bacteria. To explore the underexploited biosynthetic potential, promising myxobacteria and actinobacteria strains were whole-genome sequenced with PacBio sequencing technology. All BGCs were prioritized based on self-resistance and intriguing biosynthetic genes for heterologous expression. Depending on the BGCs organization diversity in the genome of these strains, distinctive cloning strategies were utilized. For those with more than three interested BGCs, cosmid genomic DNA libraries were constructed to obtain multiple expression constructs with different BGCs in parallel. RecET direct cloning and Gibson assembly-based BGCs capture were employed to clone the BGCs from the strains which contain only one or two prioritized BGCs. The BGCs covered by more than one cosmid are assembled by gene recombineering technologies. Promoter exchange or transcription regulator engineering were applied to improve the success rate of BGC heterologous expression.

The genetic modification of promoters replacement and integrase cassette insertion within the abovementioned BGCs containing self-resistance genes, e.g. *trpRS* and *gyrase B*, as well as key biosynthetic genes, such as F420-dependent oxidordeuctase *pepI* are transferred into different suitable hosts for efficient heterologous expression. The novel compounds are produced, isolated and structure elucidated from the heterologous expression systems with the help of State-of-the-art analytic methods and instruments.

P52 Implementation of activity and structure-guided darobactin biosynthetic pathway engineering to generate novel derivatives with improved antibacterial activity against Gram-negative pathogens

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Alarming mortality rates, connected to bacterial infections, combined with rising numbers of multidrug-resistant bacteria require the development of new antibiotics. Especially, Gram-negative pathogens are emphasized in the WHO priority list. The recently discovered darobactin A, a ribosomally produced and post-translationally modified bicyclic heptapeptide, shows promising bioactivity against a broad range of Gram-negative pathogens by targeting a site not exploited by known antibiotics, the outer-membrane protein BamA. We first analysed darobactin biosynthesis by independent deletions of genes from the biosynthetic gene cluster (BGC), revealing that only *darA* and *darE*, encoding a radical forming S-adenosyl-L-methionine-dependent enzyme, are required for formation [Groß et al., *ChemSci*, 2021]. Subsequently, activity and structure guided engineering of darobactin BGC in *Escherichia coli* was initiated. Heterologously expressed and purified non-native derivatives exhibit superior antibacterial activity compared to native darobactins. Some of the non-natural compounds, show, up to 8-fold enhanced, sub-µM activity against e.g. *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii* and clinical carbapenem-resistant *A. baumannii* isolates compared to native darobactin A.

P53 Generation of a *Myxococcus xanthus* chassis strain for the production of natural products

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Myxobacteria are Gram-negative deltaproteobacteria that are well-studied with regard to their motility, social behavior and the production of a variety of secondary metabolites. These molecules can exhibit different bioactivities and, therefore, are of high interest for the discovery and development of new drugs. However, often they are characterized by a complex chemical structure and are synthesized non-ribosomally. This impedes their heterologous biosynthesis in frequently-used prokaryotic hosts like *Escherichia coli* or *Bacillus subtilis*. For this reason, the myxobacterium *M. xanthus* is gaining increasing attention as host for the production of these compounds.

Our aim is to construct a *M. xanthus* strain that is optimized for the plasmid-based expression of natural product biosynthesis genes. For this purpose, genes that code for proteins known to decrease plasmid stability (e.g. RecA) or to inhibit the production will be deleted from the chromosome with classical gene targeting. Furthermore, native secondary metabolite gene clusters will be targeted to decrease the metabolic burden and simplify the purification of the product of interest.

The resulting strain will be then compared to *M. xanthus* laboratory strains (e.g. NM, FB, DK1622, DZ2) concerning its ability to produce alkaloids, non-ribosomal peptides, polyketides or ribosomally-synthesized and posttranslationally-modified peptides. Since the nonmotile strain *M. xanthus* NM was superior to its direct motile ancestor FB in the recombinant production of secondary metabolites,[1] NM will be used as starting point to generate the *tabula rasa* organism. Our hypothesis is that the loss of motility is responsible for the different strain performance. Therefore, the inactivation of motility and its impact on the production titer of different natural product classes will be part of this work.

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P54 NRPS design guided by adenylation promiscuity

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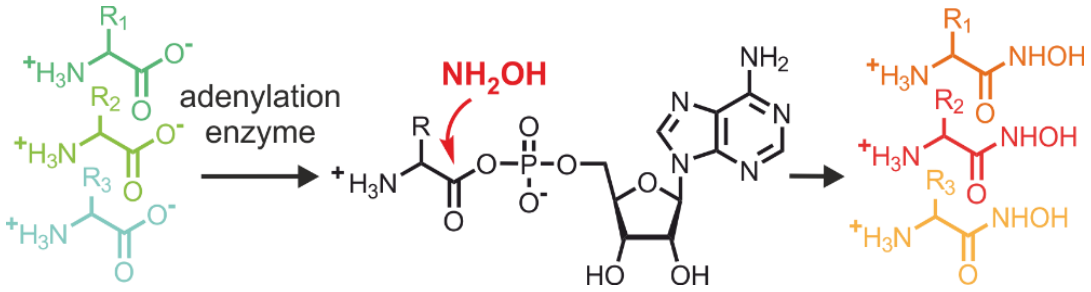
Adenylation domains control the specificity of nonribosomal peptide synthetases (NRPSs), an important group of enzymes synthesizing numerous bioactive natural products. Despite great efforts invested in adenylation domain engineering in the past, progress has been restrained by the lack of suitable assays for the screening and characterization of mutants.[1] We have developed a hydroxamate assay (HAMA) that detects multiple quenched products in a single reaction under substrate competition as in the cellular environment (Figure).[2] Our assay takes advantage of hydroxylamine to quench activated carboxylates to form hydroxamic acids which are sensitively and specifically detected by UPLC-MS/MS in a multiplexed fashion. HAMA provides a fast and reliable method for simultaneously recording adenylation profiles with dozens of substrates. HAMA screening has been used to generate a promiscuous mutant of module SrfA-C from the surfactin NRPS and to profile hundreds of variants obtained by site-saturation mutagenesis (unpublished data). Dramatic specificity changes with several mutants are consistent with an important role of promiscuous enzymes for evolution. Harnessing promiscuity during directed evolution of adenylation domains will open avenues for engineering NRPS specificity towards biosynthesis of new bioactive peptides.



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P55 Biosynthesis and heterologous production of myxovalargins

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The myxovalargins (MXV) are NRPS-derived peptide antibiotics first discovered in *Myxococcus fulvus* Mx f65 in 1982. They show strong anti-Gram-positive activity (MIC 0.3-5 µg/mL) and at higher concentrations also anti-Gram-negative activity (MIC 6-100 µg/mL) by inhibition of the protein synthesis through blocking the A site of ribosomes. A heterologous expression platform was highly desirable to gain insights into the biosynthesis of the MXVs and allow biosynthetic pathway engineering for the production of new derivatives. After significant cloning efforts, an expression construct harboring the entire biosynthetic gene cluster was obtained and transformed into *Myxococcus xanthus* DK1622. The heterologous expression driven by a vanillate-inducible promoter was successful, leading to competitive production titers of the main derivative MXV A. The here established heterologous production platform for MXV was utilized to initiate in-depth analysis of the involvement of the putative β-hydroxylase MxvH and the single PCP domain MxvB in the MXV biosynthesis, as well as attempts to increase the MXV A production titer.

P56 Efforts to increase production levels of the uridyl peptide antibiotic mureidomycin C by promoter swap experiments

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Uridyl Peptide Antibiotics (UPAs) are a group of highly-specific anti-pseudomonal compounds which address the translocase MraY, an unexploited target in bacterial cell wall biosynthesis. In previous screening experiments, mureidomycin C was identified as a promising UPA, exhibiting the lowest MIC and IC_{50} values of all tested candidates out of the four subgroups mureidomycins, napsamycins, pacidamycins and sansanmycins. Since production in the original producer and the heterologous host *S. albus* J1074 are low, the aim of this study is to increase mureidomycin C production by enhancing the expression of the pacidamycin gene cluster, which has been genetically modified to produce mureidomycin C. Pacidamycins display significantly higher production levels than the closely-related mureidomycins. For the present study, bidirectional promoter cassettes containing strong, constitutive promoters were constructed in order to activate the modified pacidamycin gene cluster. Subsequently, the cassettes were inserted into the bidirectional promoter sites of the cluster by λRed-mediated homologous recombination. The resulting constructs containing either one, two or both promoter swaps were conjugated with the heterologous host *S. albus* J1074, *S. albus* J1074/pRS02 (containing two additional genes crucial for mureidomycin C production) and *S. albus* J1074/pRS04 (containing the genes of pRS02 and, additionally, the cluster-specific positive regulator *npsM*). Thus, mutants of each combination were cultivated and production levels of all observed UPAs examined by LC-MS and compared. The complete cessation of UPA production was observed for one promoter cassette, while the other increased production levels of most UPAs.

P57 Systems bioengineering gives insight into heterologous cannabinoid metabolism in *Saccharomyces cerevisiae* CEN.PK2-1C

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We present the production of cannabinoids from *Cannabis sativa* L. using Yeast Cell Factories (YCF's). YCF's are used to produce novel agricultural, pharmaceutical, and industrial chemical portfolios. We produced a cannabinoid biosynthetic strain of CEN.PK2-1C by manipulating the endogenous mevalonate pathway, integrating polyketide pathway synthases, and finally, incorporating the bacterial prenyltransferase NphB and THCAS from *C. sativa*. Under this cloning strategy, an evaluation of cannabinoid production in our cell strain indicates that < 1 ppm cannabinoid is produced, despite a titer of almost 70 ppm olivetolic acid. In this report, we model and probe whole-cell metabolism of our strains by building strain-specific genome assemblies, curating strain-specific genome-wide metabolic (GEM) models and investigating cannabinoid flux optimization using flux-balance analysis (FBA). Preliminary FBA was performed using the genome of CEN.PK113-7D, accounting for our manipulated pathways, which has indicated that allele substitutions of the endogenous yeast acyl-activating enzyme FAA2 with *fadD* from *Escherichia coli* or *pclA* from *Penicillium chrysogenum* may result in the accumulation of acetaldehyde by increasing the reaction fluxes of the yeast pathways ethanol degradation II and pyruvate fermentation to ethanol II, where *fadD* substitution yields an additional 10.177 mmol/gDW/hour acetaldehyde and *pclA* substitution yields an additional 5.876 mmol/gDW/hour acetaldehyde. Taken together, the model shows that we could manipulate acetaldehyde metabolism in our acyl-activating-enzyme substituted strains to increase the production of acetyl-coA using *adhE* from *E. coli* and overall, increase production of cannabinoids.

P58 Design of novel surfactin derivatives via synthetic biology

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Cyclic lipopeptides like surfactin, iturin or fengycine are a group of natural products, produced by Bacilli, with a wide range of promising properties, including antimicrobial, antitumor, antiviral and surface tension lowering activity. This is reflected in the various possible applications of these compounds which range from their usage as antibiotics via their use in cleaning formulations to their usage as biological oil dispersant.

Structurally, cyclic lipopeptides usually consist of a peptide ring and a lipid moiety. The peptide portion is produced by non-ribosomal-peptide synthetases (NRPSs), while the lipid residue is occasionally generated by a polyketide synthetase (PKS). NRPSs and PKSs exhibit a modular structure, which is reflected in their genetic organization. This modular structure genetic organization could be utilized to create new derivatives of cyclic lipopeptides. Modules can be either deleted, added or replaced by others to create new cyclic lipopeptides derivatives.

We are using the surfactin NRPS assembly line in *Bacillus subtilis* 168 to create novel surfactin derivatives. The peptide ring of surfactin consists of seven amino acids and its lipid chain varies in length (C_{13} to C_{16}) and in the type of branching (iso, anteiso). Our focus lies on the modification of the peptide ring. The amino acid composition and order of surfactin is (L)Glu1-(L)Leu2-(D)Leu3-(L)Val4-(L)Asp5-(D)Leu6-(L)Leu7. In our work, we focused on the modification of the charged amino acids at position one and five, the D-configured amino acids at position three and six and the leucine at position seven. The charged amino acids should be deleted or replaced to increase the surfactin stability in seawater. The stereochemistry of surfactin could be modified with epimerase domains within the NRPS. Derivatives with more D-amino acids should be more stable towards ubiquitous proteases compared to derivatives with L-amino acids. Modifications on the leucin at position seven could be interesting because they can offer new insights into the ring formation process in surfactin. So far, we were able to delete the aspartic acid at position five and the leucine at position seven. The analysis of the properties of the derivatives is in process.

P59 Darobactin derivatization – expanding the class of bicyclic BamA inhibitors

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Darobactin A is a natural product (NP) with resistance-breaking antibacterial activity *in vitro* and *in vivo*. It is a ribosomally synthesized and post-translationally modified peptide (RiPP), which consists of seven amino acids. The modifying radical SAM enzyme DarE is responsible to catalyse the formation of two intramolecular rings on the peptide precursor. Darobactin A inhibits the attractive antibiotic target BamA. As a central component of the β-Barrel Assembly Machinery (BAM) complex, this protein is required for viability and bacterial pathogenesis. The BamA location in the outer membrane is responsible for darobactin specificity towards Gram-negative bacteria.

To further evaluate the potential of the new class of bicyclic heptapeptides, we aimed to identify additional natural variants and to create a comprehensive derivatives library. Here, we will report the first marine-derived darobactins and provide insights into the flexibility of the essential modifying enzyme DarE to generate the derivatives library. To address the latter and to improve production yields, a heterologous expression platform was generated, which provided access to a bioengineered library. In addition, during generation of the heterologous expression platform, the minimal biosynthetic gene cluster was determined. The biotechnological access to a high number of variants and high throughput screening strategies will form a basis for the future generation and analysis of BamA inhibitors. A novel target open ways to develop new drugs and the path towards lead nomination of a bicyclic heptapeptide will be presented.

P60 Biocatalytic synthesis routes for novel cyclic dinucleotides

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The second messenger cyclic GMP-AMP (cGAMP) is relevant in immunology research as inducer for the production of type I interferons and is a promising candidate for clinical-stage immuno-oncology studies. The biocatalytic cGAMP synthesis with cGAMP synthase (cGAS) offers several advantages compared to the chemical synthesis, such as shorter synthesis time and the avoidance of complex protecting group chemistry.[1] We therefore tested various cGAS homologs for their activity towards the conversion of nucleotides into cGAMP.[2] All tested variants were expressible and, interestingly, most of them catalyzed cGAMP-synthesis, albeit with a vast difference in their activity. One of these cGAS homologs was further characterized with regard to its substrate acceptance of a broad spectrum of nucleotide derivatives (Figure 1).[3] The products, cyclic dinucleotide (CDN) derivatives, are very valuable for pharmaceutical applications, as a few of them are known to be more resistant to hydrolysis or to have a higher affinity to the STING receptor. Human cGAS proved to be promiscuous catalyzing the synthesis of a variety of novel CDN derivatives.

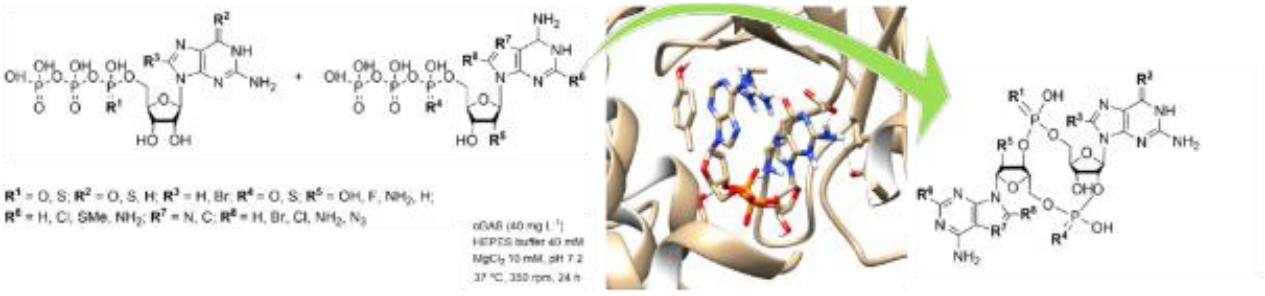

Figure 1. cGAS-catalyzed biotransformation of nucleotides into cyclic dinucleotides.

This enzyme was also studied in combination with three kinases to develop a one-pot multi-enzyme cascade for cGAMP synthesis starting from the less expensive adenosine instead of ATP.[4] The four-enzyme cascade was successfully established and optimized, achieving synthesis rates comparable to those of the one-step synthesis with ATP as the

starting substrate. Thus, the cascade ran close to its optimum, demonstrating the successful application of enzyme cascades for the synthesis of pharmaceutically relevant CDNs.

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P61 Uridyl Peptide Antibiotics (UPA): Genetic engineering of the pacidamycin gene cluster to produce mureidomycin

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Uridyl peptide antibiotics (UPAs) are a complex class of highly specific anti-pseudomonal compounds, produced by *Streptomyces*. They target phospho-MurNAc-pentapeptide translocase (MraY), catalysing the first membrane bound step of peptidoglycan biosynthesis. Biosynthetic gene clusters of pacidamycins, napsamycins, mureidomycins and sansanmycins were identified and their biosynthesis studied to some extent. The structure of all UPAs consists of a 3`-deoxyuridine moiety connected to an *N*-methyl-2,3-diaminobutyric acid (DABA) via a 4´,5´-enamide linkage. However, they differ in their peptide structure, where variations at four positions are possible. From the different variations of the peptidyl core structure of UPAs, mureidomycin C shows the highest anti-pseudomonal activity. Mureidomycins are produced at lowest yield, therefore, the biosynthetic gene cluster of the highly-produced pacidamycins was converted to a mureidomycin-like gene cluster by multiple NRPS domain swaps and the replacement of a tRNA-dependent aminoacyl transferase. The impact of the strain modifications was evaluated by LC-MS measurements of pacidamycins vs. mureidomycins production. With some more genetic engineering steps to do, we want to establish a platform for the exclusive production of mureidomycins at high yields.

P62 Engineering the heterologous production of cannabinoids in *S. cerevisiae*

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The interest in cannabinoids derived from the secondary metabolism of the plant *Cannabis sativa* L. for pharmaceutical purposes has been growing in recent years. The most prominent example is Δ^9-tetrahydrocannabinolic acid (THCA), the carboxylated form of the psychoactive tetrahydrocannabinol (THC). Biotechnological approaches for the production of cannabinoids are a promising alternative for common production methods, as they offer the opportunity to use simple carbon sources to produce high quantities with reliable quality, even of rare cannabinoids like cannabichromenic acid (CBCA). Functional implementation of the complete cannabinoid biosynthesis in *S. cerevisiae* was first reported in 2019.[1] However, the resulting product titers are too low to set up an efficient bioprocess. This outlines the need to improve the heterologous biosynthesis of cannabinoids through strategies combining pathway, protein and process engineering.

In this project the soluble aromatic prenyltransferase NphB from *Streptomyces* sp. strain CL190 is used to produce cannabigerolic acid (CBGA), the central intermediate of the cannabinoid pathway, in *S. cerevisiae* as an alternative to the corresponding membrane bound prenyltransferase *Cs*PT4 from *C. sativa*. In former studies NphB was shown to produce CBGA from geranyl diphosphate and olivetolic acid *in vitro*.[2,3] We demonstrate the production of CBGA from glucose and hexanoic acid catalyzed by NphB *in vivo* for the first time. Synthesis of THCA and CBCA from CBGA was also achieved after introducing tetrahydrocannabinolic acid synthase from *C. sativa* (THCAS) to the heterologous pathway in *S. cerevisiae*.

[1] Luo *et al.*, *Nature* **2019**, 567, 123-126

[2] Zirpel *et al.*, *Journal of Biotechnology* **2017**, 259, 204-212

[3] Valliere *et al., Nature Communications* **2019**, 10, 565

P63 Investigation of ECO-0501 – A polyene antibiotic with unknown mode of action

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ECO-0501 (Fig.1; molecular formula: $C_{46}H_{68}N_4O_{10}$), is a polyene antibiotic, which was first discovered in *Amycolatopsis orientalis* ATCC 43491.[1] Interestingly, it was shown to have strong antibacterial activity against a number of broadly resistant Gram-positive bacteria including vancomycin-resistant *Enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA),[1] while having low toxic effects.[2] However, the mode of action of ECO-0501 has not been elucidated yet.

In order to analyze the mode of action in detail, ECO-0501 must be isolated in sufficient amounts. We have chosen *Amycolatopsis japonicum* as the producer, as it is a strain that also contains the ECO-0501 biosynthetic gene cluster. This strain has the advantage of being fast growing and easy to genetically manipulate. To optimize the production process, we introduced the pathway-specific regulator under the control of a strong, constitutive promoter in *A. japonicum*. Since ECO-0501 is unstable, we are currently establishing an effective purification method.

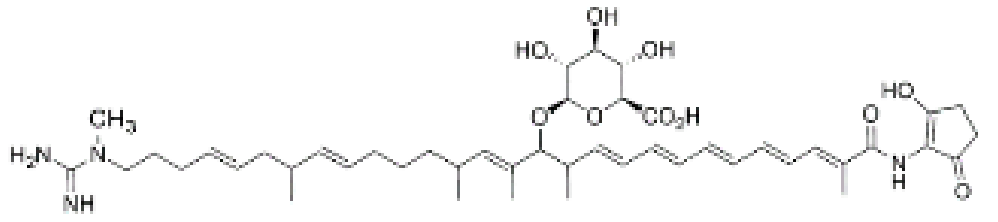

Fig. 1: Structural formula of ECO-0501 according to [2].

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P64 Identification of novel synthetic cannabinoids using the promiscuity of the aromatic prenyltransferase NphB

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Cannabinoids are an important class of plant secondary metabolites which can be applied in a diverse spectrum of diseases such as epilepsy, schizophrenia or multiple sclerosis.[1] The biosynthesis of cannabinoids can be transferred to other organisms like *Saccharomyces cerevisiae*.[2] An essential step during the biosynthesis is the formation of the intermediate cannabigerolic acid (CBGA) using olivetolic acid and geranyl diphosphate as substrates. Next to the natural plant prenyltransferase, alternative enzymes from different organisms were successfully identified to catalyze the formation of CBGA. A promising candidate enzyme is NphB, an aromatic prenyltransferase with high promiscuity for the aromatic acceptor substrate including flavonoids, isoflavonoids and plant polyketides like olivetolic acid.[3] With the exchange of substrates used in the biosynthesis of cannabinoids it is easily possible to form a library of novel synthetic cannabinoids, which can be used as future drugs.

In the present study the potential of novel olivetolic acid analogs, which differ in their hydrocarbon chain connected to the C6 of the core structure, were examined for the formation of novel synthetic cannabinoids within the biosynthesis in yeast. The formation of CBGA analogs is crucial for the subsequent steps in the cannabinoid biosynthesis pathway. As a first step, the substrate specificity of NphB regarding the novel olivetolic acid analogs was determined. By using in silico experiments including docking a promising molecule library was created, and differences in catalytic activity and substrate acceptance were predicted. Through different synthesis routes the novel analogs were synthesized and via in vitro assays the conversion was monitored with structure elucidation of the newly formed products.

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