Abstract Book

VAAM Workshop
"Biology of Bacteria Producing Natural Products"

24–26 September 2025, Berlin, Germany

Organised by
RG Pharmaceutical Biology
Institute of Pharmacy
Freie Universität Berlin

Table of Contents

1 Programme of the VAAM Workshop 2025

AI-generated illustration

Wednesday, September 24th

13:00 – 14:45	**Registration & Poster Set-Up** (Foyer Königin-Luise-Str. 12-16)
14:45 – 15:00	**Welcome and Opening remarks** Timo Niedermeyer, Freie Universität Berlin, Germany
15:00 – 16:00	**Opening lecture (PL1)** Aharon Oren, The Hebrew University of Jerusalem, Israel *Practicing the world's oldest profession*
16:00 – 16:30	Coffee break
16:30 – 18:10	**Poster pitches** Chair: Alexander Weng
18:10 – 19:30	**Poster session I (uneven poster numbers)** with finger food

Thursday, September 25th

8:30 – 9:15	**Lecture Session "Natural Product Ecology & Function"** Chair: Tomáš Galica **PL2: Mitja Remus-Emsermann** Freie Universität Berlin, Germany *Ecological consequences of biosurfactant production on leaf surfaces*
9:15 – 9:30	**SL1:** Alexandra Eckart, Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany *Protective marine symbionts from Hydractinia echinata as source of novel anti-infectives and new biocatalyst*
9:30 – 9:45	**SL2:** Nico Brüssow, Universität Potsdam, Germany *Characterization of a class II lanthipeptide from the cyanobacterium Nostoc punctiforme that is implicated in symbiotic interactions with the liverwort Blasia pusilla*
9:45 – 10:00	**SL3:** Valerie Rebhahn, Freie Universität Berlin, Germany *Aetokthonotoxin uncouples oxidative phosphorylation due to protonophore activity*
10:00 – 10:15	**SL4:** Leo Dumjahn, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany *Dual-use virulence factors of the opportunistic pathogen Chromobacterium haemolyticum mediate haemolysis and colonization*
10:15 – 10:45	Coffee break
10:45 – 11:30	**Lecture Session "Natural Product Chemistry"** Chair: Franziska Schanbacher **PL3: William Gerwick** Scripps Institution of Oceanography, UC San Diego, USA *Biosynthetic and Artificial Intelligence Insights Efficiently Solve Complex Cyanobacterial Natural Product Structures*

11:30 – 11:45	**SL5:** Frederik Weiß, Universität Osnabrück, Germany *Identification of a black pigment produced by Actinosynnema mirum after electoproration*
11:45 – 12:00	**SL6:** Mitja Zdouc, Wageningen University & Research, Netherlands *FERMO: a Dashboard for Automated Prioritization of Molecular Features from Mass Spectral Data*
12:00 – 12:15	**SL7:** Michael Brigham, Goethe University Frankfurt, Germany / University of Leeds, United Kingdom *Discovery and characterisation of biffamycin: a unique, antimicrobial non-ribosomal peptide containing an unprecedented chloro-methoxy-tryptophan moiety*
12:15 – 12:30	**SL8:** Amira Naimi, Philipps Universität Marburg, Germany *Native metabolomics-guided Discovery of a Cell-Permeable Gαi-Inhibitor*
12:30 – 13:30	Lunch break
	Lecture Session "Biosynthesis & Engineering – Part I" Chair: Valerie Rebhahn
13:30 – 13:45	**SL9:** Tetiana Gren, The Novo Nordisk Foundation Center for Biosustainability, Denmark *Genome engineering of Streptomyces species using CASCADE-Cas3 technology*
13:45 – 14:00	**SL10:** Phillip Schlegel, Leipzig University, Germany *Uncovering and Designing a Flexible Amino Acid Ligase from Streptomyces mozunensis*
14:00 – 14:15	**SL11:** Ulrike Tarazona Janampa, Leibniz Institute, DSMZ, Braunschweig, Germany *Exploring the natural product biosynthetic potential of Antarctic actinomycetes*
14:15 – 14:30	**SL12:** Chengzhang Fu, Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany *Unlocking Hidden Chemistry: From Genome Mining to Mechanistic Insights and New Antibiotics*
14:30 – 15:00	Coffee break
15:00 – 16:15	**Poster presentation II (even poster numbers)** with coffee and cookies
16:30 – 20:00	**Special Session: Natural Products from Plants** Botanical Garden of the FU Berlin 18:00 group photo in front of the "Großes Tropenhaus" finger food

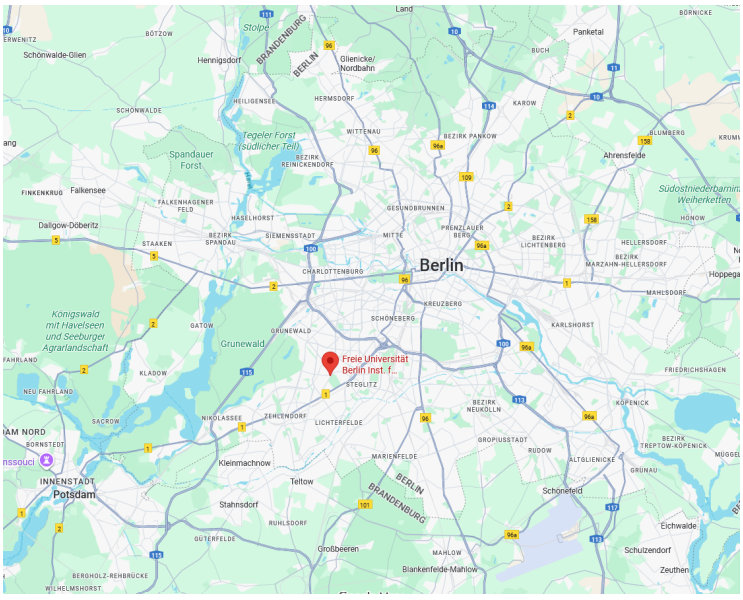
Friday, September 26th

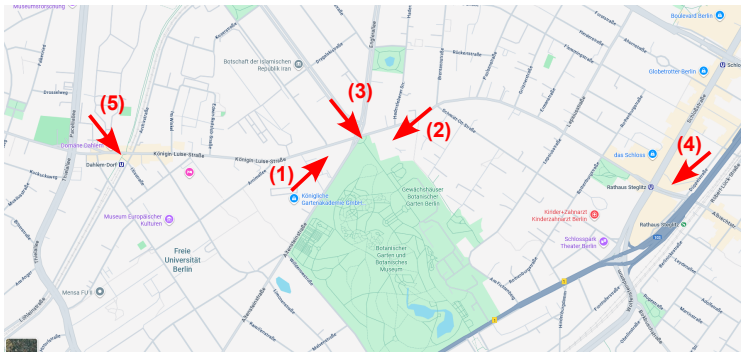
8:30 – 9:00	**Hendrik Wolff Award** Award lecture Chair: Christine Beemelmanns
9:00 – 9:45	**Lecture Session "Biosynthesis & Engineering – Part II"** Chair: Lenka Stenclova **PL4: Muriel Gugger** Institut Pasteur, Paris, France *The Pasteur Cultures of Cyanobacteria: A Treasure for Natural Products*
9:45 – 10:00	**SL13:** Dana Parade, Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany *Advancing heterologous expression of biosynthetic gene clusters in cyanobacterial host organisms*
10:00 – 10:15	**SL14:** Jonas Korb, TU Dortmund, Germany *In Vitro Production of Aminochelin*
10:15 – 10:30	**SL15:** Alena Strüder, Eberhard Karls University Tübingen and Institute for Microbiology and Infection Medicine Tübingen, Germany *Negamycin: Unusual biosynthesis directed by a split biosynthetic gene cluster*
10:30 – 10:45	**SL16:** Florian Hubrich, Saarland University, Germany *Ribosomal peptide prenyltransferases with biocatalytic potential*
10:45 – 11:30	Coffee break
	Lecture Session "Natural Product Evolution & Regulation" Chair: Lukas Koch
11:30 – 11:45	**SL17:** Aleksandra Korenskaia, Interfaculty Institute for Microbiology and Infection Medicine Tübingen, Germany *PhyloNaP: a user-friendly database of Phylogeny for Natural Product–producing enzymes*
11:45 – 12:00	**SL18:** Tim Berger, Philipps Universität Marburg, Germany *The Role of Marine Fungi in Tackling Plastic Waste: A Multiomics Approach*
12:00 – 12:15	**SL19:** Lena Mitousis, University of Tübingen, Germany *CRISPR-Cas systems in antibiotic-producing Actinomycetes: Insights from Streptoalloteichus tenebrariu*
12:15 – 12:30	**SL20:** Luis Linares Otoya, Princeton University, USA *Discovery of the N-acyl-cyclolysine system, a widespread chemical signalling pathway in the Bacteroidota*
12:30 – 13:00	**Poster Awards and Closing remarks** Timo Niedermeyer, Freie Universität Berlin, Germany

2 Conference Information

2.1 Venue

Freie Universität Berlin
Institut für Pflanzenphysiologie
Königin-Luise-Str. 12-16
14195 Berlin





(1) Venue
(2) Institute of Pharmacy
(3) Botanical Garden - Special Session
(4) Rathaus Steglitz (S- and U-Bahn station)
(5) Dahlem-Dorf (U-Bahn station)

2.2 Travel Information/ Getting there

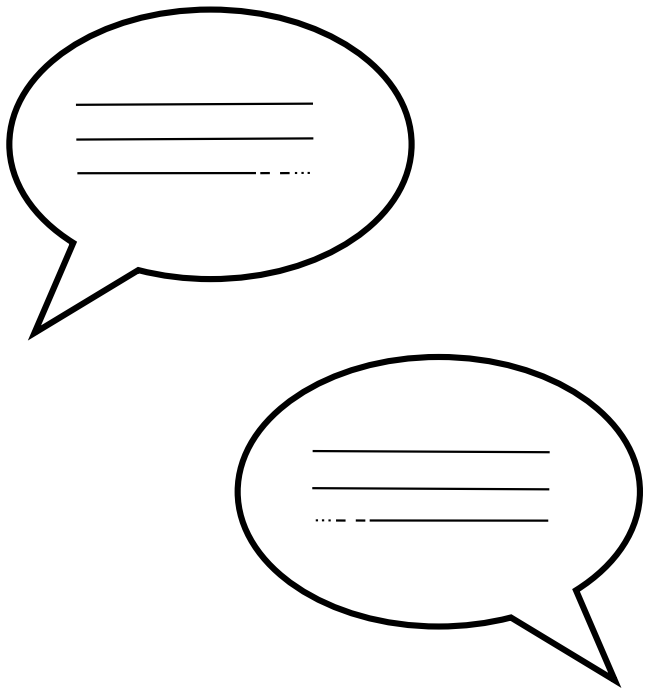
The **venue** and the **Botanical Garden** can be reached by S-Bahn (**S1**) or U-Bahn (**U9**) to **Rathaus Steglitz**, or by U-Bahn (**U3**) to **Dahlem Dorf**. From Dahlem Dorf it is about a 10–15 minute walk to the venue, or you can take the **X83** bus. From Rathaus Steglitz, the **X83** bus also provides a convenient connection, or you can alternatively take a 20–25 minute walk.

2.3 Local Organising Committee

If you have any questions, please feel free to contact us. You can recognise the staff team by their **orange lanyards**. They will also be happy to assist you with practical matters such as where to hang posters, when and where groups meet for the special sessions, and to whom you can hand in your presentations for the oral presentations and short talks.

Name	**Role/ Responsibilities**
Timo Niedermeyer	Conference Host
Valerie Rebhahn	Registration Desk
Franziska Schanbacher	Registration Desk/ Conference Programme
	Guided Tour Botanical Garden - Special Session
Stefan Böttger	Technical Support (Presentation Coordinator)
	Guided Tour Botanical Garden - Special Session
Tomáš Galica	Technical Support (Presentation Coordinator)
Nils Wadehn	"Special Session" Programme
	Guided Tour Botanical Garden - Special Session
Alexander Sonntag	Guided Tour Botanical Garden - Special Session
Philipp Gabor	Guided Tour Botanical Garden - Special Session
Isabella di Stefano	General Organisatorial Support and Coordination
Hardy Mitdank	Guided Tour Botanical Garden - Special Session
Vanessa Ehrentraut	Catering
Fabian Bülow	Conference Photographer

3 Plenary Lectures



PL1

Practicing the world's oldest profession

Aharon Oren[1]

1 Institute of Life Sciences, The Hebrew University of Jerusalem, Edmond J. Safra Campus, 9190401 Jerusalem, Israel

Naming the organisms that live in the world around us is an occupation as old as mankind. However, when Antonie van Leeuwenhoek first observed bacteria in the late 17th century, he did not give names to the 'animalcules' he had discovered. The binomial nomenclature system developed by Linnaeus in the mid-18th century for plants and animals was first applied to non-phototrophic prokaryotes in the beginning of the 19th century, but by then some cyanobacteria had already been named by botanists.

Nomenclature of *Bacteria* and *Archaea* is governed by the rules of the International Code of Nomenclature of Prokaryotes (ICNP) [1], as approved and periodically updated by the International Committee on Systematics of Prokaryotes. As of September 2025, names of over 26,000 species of prokaryotes had been validly published, classified in nearly 4400 genera. In recent years, names of nearly 1100 new species and 200 new genera were validly published annually by publication in the International Journal of Systematic and Evolutionary Microbiology. Yet-uncultivated prokaryotes can be pro-validly published and named as *Candidatus* taxa following the recently approved Section 10 of the ICNP. Detailed information about all those names is found in the List of Prokaryotic names with Standing in Nomenclature (LPSN) database [2]. Practical guidelines have been published to enable colleagues who are not experts in Latin and Greek to propose new names that are compatible with the rules of the ICNP [3-5].

Nowadays, any single culturomic and/or metagenomic study may yield information about hundreds or even thousands of new taxa of prokaryotes, and naming those has become a major challenge. High-throughput creation of names formed by combining Latin, Greek and Neo-Latin word elements in a correct way that satisfies the demands of the ICNP is possible. Thus, genus names such as *Galligastranaerophilus*, *Ornithocaccomicrobium* and *Stercoripulliclostridium* and specific epithets such as *intestinavium*, *gallistercoris*, and *intestinigallinarum* were proposed in a study of the prokaryotic community in chicken dung [6]. An automated approach, employing combinatorial concatenation of roots from Latin and Greek to create linguistically correct names for genera and species has been described [7]. The resulting names are formed according to a standardized pattern, are often long, and are unlikely to arouse general interest in prokaryotic nomenclature. This is even more true for the proposed names of tens of thousands of previously unnamed taxa from the Genome Taxonomy Database such as '*Candidatus* Cofrixana mapaxosa' and '*Candidatus* Sufrabetta lutexaria': meaningless arbitrary names that are allowed under the rules of the ICNP [8]. However, with some effort and creativity it remains possible to propose attractive names for newly described prokaryotes that will boost the interest of the public in prokaryotic nomenclature. Names such as *Dehalogenimonas lycanthroporepellens*, (*Candidatus*) *Desulforudis audaxviator* and '*Candidatus* Vampirococcus lugosii' cannot (yet) be machine-generated [9]. Ideally, there should be an interesting story behind every name proposed for newly characterized *Bacteria* and *Archaea*.

References
[1] Oren, A. *et al.* Int. J. Syst. Evol. Microbiol. 2024, 73, 005585. https://doi.org/10.1099/ijsem.0.005585
[2] Parte, A.C. *et al.* Int. J. Syst. Evol. Microbiol. 2020, 70, 5607–5612. https://doi.org/10.1099/ijsem.0.004332
[3] Trüper, H.G. FEMS Microbiol. Rev. 1999, 23, 231–249. https://doi.org/10.1111/j.1574-6976.1999.tb00397.x
[4] Oren, A. In: Bergey's Manual of Systematics of Archaea and Bacteria. John Wiley & Sons, in association with Bergey's Manual Trust. 2019. https://doi.org/10.1002/9781118960608.bm00008.pub2
[5] Oren, A. In: Li, W.-J. *et al.* (eds.) Modern Taxonomy of Bacteria and Archaea. Springer, Singapore. 2024. https://doi.org/10.1007/978-981-99-5720-0_2
[6] Gilroy, R. *et al.* PeerJ. 2021, 6, e10941. doi: 10.7717/peerj.10941
[7] Pallen, M.J. *et al.* Trends Microbiol. 2021, 29, 289–298. https://doi.org/10.1016/j.tim.2020.10.009
[8] Pallen, M.J. *et al.* Int. J. Syst. Evol. Microbiol. 2022, 72, 005482. https://doi.org/10.1099/ijsem.0.005482
[9] Oren, A. FEMS Microbiol. Lett. 2021, 367, fnaa096. https://doi.org/10.1093/femsle/fnaa096

PL2

Ecological consequences of biosurfactant production on leaf surfaces

Mitja N.P. Remus-Emsermann
Institute of Biology, Freie Universität Berlin, Königin-Luise-Str. 12-16, 14195 Berlin, Germany.

Plant leaf surfaces are colonized by communities of surprisingly diverse bacterial species. These bacteria influence plant health and productivity and have recently attracted increasing attention in both fundamental and applied research. Within these communities, the ability to produce biosurfactants is widespread in different bacterial species. Biosurfactants are biologically produced, amphiphilic molecules that reduce surface tension and mediate interactions between hydrophilic and hydrophobic phases. Leaf surfaces arecovered by a waxy cuticle composed of very long-chain aliphatic compounds, which makes leaves hydrophobic and restricts the diffusion of water and charged solutes from the inside of the leaf to the outside. This hydrophobicity causes water to bead, minimizing contact between water and the leaf surface. Consequently, microbial movement across leaves and access to heterogeneously distributed nutrients are restricted. Surfactants have been shown to increase water spreading on leaves and to enhance diffusion across the cuticular membrane. Beyond these effects, several additional surprising roles of biosurfactants on leaves have been discovered which include increased bacterial survival during fluctuating humidity conditions and hitchhicking of non-biosurfactant-producing bacteria. I will summarize current knowledge on the multiple ecological implications of biosurfactant production by leaf surface bacteria.

PL3

Biosynthetic and Artificial Intelligence Insights Efficiently Solve Complex Cyanobacterial Natural Product Structures

Byeol Ryu[1], Nicole E. Avalon[1,2], Marine Cuau[1,3], Jehad Almaliti[1,4], M. Omar Din[5], Caitriona Brennan[5,6], Evgenia Glukhov[1], Rob Knight[5,7], Lena Gerwick[1] and William H. Gerwick[1,8]. [1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego (UCSD), CA, USA [2]Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University of California, Irvine, CA, USA [3]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Matosinhos, Portugal [4]Department of Pharmaceutical Sciences, College of Pharmacy, The University of Jordan, Amman, Jordan [5]Department of Pediatrics, UCSD, CA, USA [6]Division of Biological Sciences, UCSD, CA, USA [7]Department of Computer Science and Engineering, Department of Bioengineering, Center for Microbiome Innovation, UCSD, CA, USA [8]Skaggs School of Pharmacy and Pharmaceutical Sciences, UCSD, CA, USA

Kahalalide F is a cyclic depsipeptide with demonstrated potent anticancer activity, originally isolated from the marine green alga *Bryopsis* sp. and its sacoglossan mollusk predator *Elysia rufescens*. Although biosynthesis was initially attributed to the host organisms, evidence over the past decade has revealed that the true origin is a bacterial endosymbiont, *Candidatus* Endobryopsis kahalalidefaciens. In this study, we report the discovery of a novel kahalalide F-like compound from a marine cyanobacterium collected from the Las Perlas islands, Panama. To prioritize unknown metabolites, we applied a comprehensive metabolomics workflow that integrated high-resolution LC-MS/MS with *in silico* structural annotation, aided by DeepSAT, an AI-based platform trained to predict chemical structures from HSQC NMR data. This revealed the presence of a kahalalide F-like compound in this cyanobacterial extract, and its structure was subsequently elucidated through rigorous analysis of 1D and 2D NMR spectroscopy, HRMS, and chemical degradation sequences. To determine the biosynthetic origin, we performed whole-genome sequencing of the cyanobacterial strain and identified a putative NRPS Biosynthetic Gene Cluster consistent with the compound's structural framework. This study provides compelling evidence that marine cyanobacteria are also native producers of kahalalide F-like metabolites. Our findings expand the known phylogenetic diversity of kahalalide F-producing microorganisms and underscore the power of integrating AI-driven metabolomics with genome-resolved biosynthetic analysis. This approach accelerates natural product discovery and highlights cyanobacteria as a sustainable source of complex therapeutic leads.

PL4

The Pasteur Cultures of Cyanobacteria: A treasure for natural products

Muriel GUGGER

Institut Pasteur, Collection of Cyanobacteria, France.

Cyanobacteria are a rich source of natural products (NPs). They are well known for their toxins in freshwater bodies and for the closure of recreational sites during summer in Europe. The genetic information and pathways associated to the production of the main cyanotoxins (microcystins, cylindrospermopsins, saxitoxins, anatoxin-a and anatoxin-a(S) renamed guanitoxin) were revealed from 1999 to 2020. Indeed, more than 1100 NPs were characterized from various cyanobacteria.

If we focus on the extent of the cyanobacterial NPs rather than strictly on their toxic aspect, and examine the genetic locus involved, can we detect a trend in these puzzling pathways? In 16 years of collaboration with talented chemists and biochemists, as well as phylogeneticists, we have revealed using the pure strains of the Pasteur Culture of Cyanobacteria collection that we maintain, new pathways and their respective NPs, both chemically known or entirely discovered, new enzymes, and cutting-edge chemistry.

Working with pure cyanobacterial strains from the collection allowed to ascertain the producer of the discovered NP, but also to find natural genetic mutants of the investigated pathway and related compounds. The more PCC strains I sequence, the more new biosynthetic gene clusters I discover. I bet I won’t see the end of these fascinating pathways, and I’ll explain why.

4 Short Lectures

SL1

Protective marine symbionts from *Hydractinia echinata* as source of novel anti-infectives and new biocatalyst

Alexandra Eckart[1], Martinus de Kruijff[1], Emily Feld[12], Tanya L. Decker[1], Rebecca Kochems[1]

[1] Helmholtz Institute for Pharmaceutical Research Saarland, Campus E8 2, 66123 Saarbrücken
[2] Hochschule für Technik und Wirtschaft des Saarlandes, Goebenstraße 40, 66117 Saarbrücken

Hydractinia echinata has been a widely used model organism in biological research for decades. Its life cycle alternates between a mobile, juvenile stage and a sessile, adult colony, with metamorphosis often being induced artificially under laboratory conditions. Our recent studies demonstrate that, in its natural habitat, the hydroid's development is strongly influenced by its associated microbiome. Notably, members of the genus *Pseudoalteromonas* produce bioactive compounds, including phospholipids and rhamnose-containing exopolysaccharides that act as potent inducers of metamorphosis [1, 2].

Given the urgent demand for novel antifungals and antibiotics in the face of rising antimicrobial resistance, we have extended our research to the broader microbial community associated with *H. echinata*. We hypothesize that these bacteria contribute to host defense by producing biologically active secondary metabolites. This is supported by the discovery of metabolically versatile and strong natural product producing strains, such as novel *Micromonospora* spp. - long regarded as "battleships" of natural product research - as well as underexplored genera such as *Mycolicibacterium* spp., which also show promising potential for the production of bioactive compounds. Selected isolates have been nanopore-sequenced and are currently undergoing comprehensive genomic and metabolomic analyses to further assess their biosynthetic capacities.

Our findings highlight the *H. echinata* microbiome as a valuable reservoir for natural product-producing microorgansims, of which many represent novel - yet undescribed species.

References:
[1] Guo, H., Rischer, M., Westermann, M., Beemelmanns, C. (2021). Two Distinct Bacterial Biofilm Components Trigger Metamorphosis in the Colonial Hydrozoan Hydractinia echinata. mBio 12/3, e0040121.
[2] Rischer, M., Guo, H., Beemelmanns, C. (2022). Signalling molecules inducing metamor-phosis in marine organisms. Natural product reports 39/9, 1833–1855.

SL2

Characterization of a class II lanthipeptide from the cyanobacterium *Nostoc punctiforme* that is implicated in symbiotic interactions with the liverwort *Blasia pusilla*

Nico Brüssow[1], Daniel Dehm[1], Elke Dittmann[1]

1 Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Straße 24-25, 14476 Potsdam, Germany.

Cyanobacteria are prolific producers of a broad range of natural products [1]. While classes of RiPPs such as lanthipeptides have been identified in cyanobacteria, their biological functions remain mostly unknown. Additionally, the analysis of lanthipeptides in cyanobacteria presents significant challenges. To date, cyanobacterial lanthipeptides like prochlorosins have predominantly been studied in *E. coli* [2]. To address these issues, we chose to investigate a class II lanthipeptide biosynthetic gene cluster (BGC) from *Nostoc punctiforme* PCC 73102, a terrestrial filamentous cyanobacterium capable of forming symbiotic associations with diverse hosts, including the bryophyte *Blasia*, cycads, and *Gunnera* sp.
Our previous studies demonstrated early upregulation of this BGC during chemical and physical interactions with the liverwort *Blasia pusilla*. Despite the observed upregulation, the lanthipeptide product was not analytically detectable in *N. punctiforme*. Therefore, we heterologously expressed parts of the BGC in *E. coli* and successfully characterized its product, an unusual lanthipeptide harboring two lanthionine rings, a free dehydro amino acid as well as a free cysteine. Apart from the RiPP monomer that is produced when the precursor peptide is coexpressed with the lanthionine synthetase in *E. coli*, we observed a pronounced tendency toward peptide oligomerization (≥200 kDa), especially at elevated pH values. We are currently testing the hypothesis that these oligomers are formed by covalent cross-linking of the lanthipeptides. His-tag labeling of precursor peptide copies in *N. punctiforme* provided evidence that oligomers could be the dominant species in cyanobacteria.
We assume that the biological function is conferred by a lanthipeptide surface layer rather than peptide monomers. Constitutive expression of the lanthipeptide BGC in a second symbiotic *Nostoc* strain lead to a non-motile phenotype and a significant delay in symbiotic interactions particularly affecting the early stages of the symbiosis between *Nostoc* and *Blasia pusilla*. We therefore postulate that the lanthipeptide plays a pivotal role in symbiotic negotiations of *Nostoc* strains.

References

[1] Dittmann, E. *et al*. Trends Microbiol. 2015, 23 (10) 642–652. https://doi.org/10.1016/j.tim.2015.07.008
[2] Li, B. *et al*. Proc. Natl. Acad. Sci. U.S.A. 2010, 107 (23) 10430–10435. https://doi.org/10.1073/pnas.0913677107

SL3

Aetokthonotoxin uncouples oxidative phosphorylation due to protonophore activity

Valerie I. C. Rebhahn[1], Mohamad Saoud[2], Mathias Winterhalter[3], Franziska Schanbacher[1], Maximilian Jobst[4,5], Rebeca Ruiz[6], Alexander Sonntag[1], Johannes Kollatz[1,2], Rieke Sprengel[1], Stephen F. Donovan[7], Giorgia Del Favero[4], Robert Rennert[2], Timo H. J. Niedermeyer[1,*]

1 Institute of Pharmacy, Pharmaceutical Biology, Freie Universität Berlin, 14195 Berlin, Germany
2 Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, 06120 Halle (Saale), Germany
3 School of Science, Constructor University Bremen gGmbH, 28759 Bremen, Germany, and Center for Hybrid Nanostructures, Universität Hamburg, 22761 Hamburg, Germany
4 Department of Food Chemistry and Toxicology, and Core Facility Multimodal Imaging, Faculty of Chemistry, University of Vienna, 1090, Vienna, Austria.
5 University of Vienna, Vienna Doctoral School in Chemistry (DoSChem), 1090 Vienna, Austria
6 Pion Inc., Forest Row Business Park, Forest Row RH18 5DW, United Kingdom
7 Retired
* Part of this work was done at the Department of Pharmaceutical Biology/Pharmacognosy, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, 06120 Halle (Saale), Germany

Wildlife in the south-eastern United States suffer from vacuolar myelinopathy. This disease comprises severe neurological impairment with disruption of myelin sheaths in the white matter and is eventually lethal. We recently determined aetokthonotoxin (AETX) as the causative agent. AETX is a unique natural product synthesized by the epiphytic freshwater cyanobacterium *A. hydrillicola*, and transmitted via the food chain [1]. With the aetiology of the disease being unravelled, we now focused on the mode of action of AETX. The effect of AETX on mammalian cells and bacteria was assessed in various *in vitro* assays using fluorescent probes and a Seahorse XF Pro Analyzer. Effects of AETX on oxygen consumption, mitochondrial membrane potential, reactive oxygen generation and mitochondrial ATP production indicate that the primary mechanism of AETX is the uncoupling of the oxidative phosphorylation in mitochondria. We could further demonstrate that AETX acts as a protonophore.
For the first time, biological activity of AETX was characterized beyond cytotoxicity assays with the aim to elucidate its mode of action. We have found that AETX uncouples the oxidative phosphorylation in mitochondria by acting as a protonophore.

References
[1] Breinlinger *et al.* 10.1126/science.aax9050 (2021). https://doi.org/10.1126/science.aax9050

SL4

Dual-use virulence factors of the opportunistic pathogen *Chromobacterium haemolyticum* mediate haemolysis and colonization

Leo Dumjahn[1,*], Philipp Wein[1,*], Evelyn M. Molloy[a], Kirstin Scherlach[a], Felix Trottmann[a], Philippe R. Meisinger[1], Louise M. Judd[b], Sacha J. Pidot[b], Timothy P. Stinear[2], Ingrid Richter[1], Christian Hertweck[1,3,4]

1 Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), Jena, Germany;
2 Department of Microbiology and Immunology, Doherty Institute, University of Melbourne, Melbourne, Australia;
3 Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University Jena, Jena, Germany;
4 Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Jena, Germany.

* These authors contributed equally to this work.

Chromobacterium haemolyticum is an environmental bacterium that can cause severe and fatal opportunistic infections in humans and animals [1]. Although *C. haemolyticum* is characterized by its strong β-haemolytic activity, the molecular basis of this phenotype has remained elusive over the more than fifteen years since the species was first described. We report a family of cyclic lipodepsipeptides, the jagaricins, that are responsible for the potent haemolytic activity of *C. haemolyticum* [2]. Comparative genomics of *C. haemolyticum* strains revealed a completely conserved gene locus (*hml*) encoding a nonribosomal peptide synthetase (NRPS). Metabolic profiling of *C. haemolyticum* DSM 19808 identified a suite of cyclic lipodepsipeptides as the products, with the three main congeners (jagaricin A–C) being elucidated by a combination of tandem mass spectrometry, chemical derivatization, and NMR spectroscopy. Significantly, a *C. haemolyticum hml* deletion mutant is devoid of haemolytic activity. Moreover, purified jagaricins are haemolytic at low-micromolar concentrations in an erythrocyte lysis assay. Further bioassays demonstrated that the cyclic lipodepsipeptides are crucial for biofilm-forming and swarming behavior of *C. haemolyticum*. MALDI mass spectrometry imaging showed that primarily jagaricin B and C are involved in these processes *in vitro*. Our data shed light on the bioactivities of jagaricins, specialized metabolites that likely contribute to both successful niche colonization and virulence potential of *C. haemolyticum*.

References
[1] Teixeira P. *et. al.* Mol. Genet. Genomics 2020, 295, 1001–1012. https://doi.org/10.1007/s00438-020-01676-8
[2] Dumjahn L. *et al*. mBio 2025, 16, e03605-24. https://doi.org/10.1128/mbio.03605-24

SL5

Identification of a black pigment produced by *Actinosynnema mirum* after electroporation

Frederik Weiß [1], Janina Krause[1]

1 Biomedizinische Grundlagen, Institut für Gesundheitsforschung und Bildung, Universität Osnabrück, Nelson-Mandela-Str. 13, 49076 Osnabrück, Germany.

Actinosynnema mirum of the genus of Actinomycetes is a known producer of nocardicidin [1]. In the course of our research on this strain, we endeavored to optimize an electroporation protocol for transformation. However, following the successful transfer of the test plasmid pGM1192, *A. mirum* exhibited an unexpected response, characterized by the initiation of black pigment production (Fig. 1). The objective of this study was to isolate and identify the black pigment in question. A genome-wide screening employing the antiSMASH webtool yielded a cluster exhibiting low similarity to that of ochronotic pigment. This finding, in conjunction with the intense black pigmentation, led to the conclusion that the pigment may be melanin, a prevalent actinomycetical metabolite. The extraction of the pigment was achieved through a two-step process involving alkaline extraction, followed by acidic precipitation [2]. The identity of the pigment could be further confirmed by NMR, UV/Vis, and IR spectroscopic measurements. The addition of kojic acid, a known inhibitor of melanin production via the tyrosinase pathway, to the culture medium did not prevent black pigmentation in cultures of *A. mirum* [3]. Furthermore, no antibacterial activity could be detected from either the culture medium or the purified melanin. While melanin production is not uncommon among microorganisms, the circumstances of its activation and the stable production of the pigment over generations require further examination. Consequently, subsequent research endeavors will prioritize the investigation of the genetic underpinnings that precipitated the abrupt onset of melanin production.

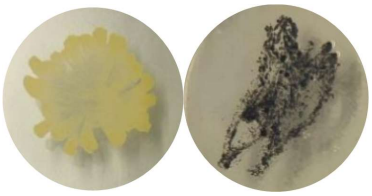


Fig. 1: Yellow and black colonies of *A. mirum*

[1] Land, A. et al. Stand Gen. Sci., 2009, 1, 1, 46-53 https://doi.org/10.4056/sigs.21137
[2] Gadd, G.M., Transact. Brit. Mycolog. Soc, 1982., 87, 1, 115-122 https://doi.org/10.1016/S0007-1536(82)80083-1
[3] Saghaie L et al. Res. Pharm. Sci., 2013; 8,4,233–242.

AI tools: DeepL Write, Deepl SE (https://www.deepl.com/en/write) [01.07.2025]
Reformulation of text

SL6

FERMO: a Dashboard for Automated Prioritization of Molecular Features from Mass Spectral Data

Mitja M. Zdouc[1], Hannah E. Augustijn[1,2], Nataliia V. Machushynets[2], Gilles P. van Wezel[2,4], Marnix H. Medema[1,2] and Justin J. J. van der Hooft[1,5]

1 Bioinformatics Group, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands
2 Institute of Biology, Leiden University, Sylviusweg 72, 2333BE Leiden, the Netherlands
3 Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, Old Aberdeen, AB24 3UE, Scotland, United Kingdom
4 Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, the Netherlands
5 Department of Biochemistry, University of Johannesburg, C2 Lab Building 224, Kingsway Campus, Cnr University & Kingsway Road, Auckland Park, Johannesburg 2006, South Africa

Biological samples, especially bacterial cultures, are chemically complex, with metabolomics analyses using liquid chromatography-mass spectrometry routinely detecting hundreds of metabolites. In natural product discovery, this complexity makes it difficult to identify which molecules are linked to bioactivity or other phenotypic traits, and are most promising for follow-up experimental investigation. Existing computational tools for prioritizing metabolites are often tailored to narrow applications, require advanced expertise, and focus on exploratory data visualization rather than objective, data-driven prioritization. Here, we present FERMO, a freely accessible, interactive dashboard for hypothesis-driven prioritization of metabolites and their corresponding samples. FERMO enables researchers to rapidly connect metabolomics data with phenotypic assays, sample metadata, and genomic context, while also streamlining data organization, annotation, and dereplication. FERMO supports guided prioritization and hypothesis generation, helping users uncover biologically relevant metabolites with greater efficiency. Benchmarking analyses show that FERMO not only recovers experimentally validated bioactive metabolites but also extends beyond the functionality of state-of-the-art tools. Designed for users without computational training, FERMO is interoperable with existing metabolomics tools such as mzmine, accelerating the identification of new bacterial natural products. FERMO is freely available at https://fermo.bioinformatics.nl/.

SL7

Discovery and characterisation of biffamycin: a unique, antimicrobial non-ribosomal peptide containing an unprecedented chloro-methoxy-tryptophan moiety

Michael W. Brigham[1,2,3,4], Edward S. Hems[5,6], Daniel C. L. Van[2,4], Justin E. Clarke[2,4], Sergey Nepogodiev[7], Christian Bassi[2,4], Michael E. Webb[3,4], Glyn R. Hemsworth[2,4], Barrie Wilkinson[5,6], Ryan F. Seipke[2,4]

1. Institute for Pharmaceutical Biology, Goethe University Frankfurt, Max-von-Laue-Straße 9, 60438, Frankfurt am Main, Germany
2. School of Molecular and Cellular Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom
3. School of Chemistry, University of Leeds, Leeds, LS2 9JT, United Kingdom.
4. Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom.
5. Department of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH, United Kingdom.
6. Centre for Microbial Interactions, Norwich Research Park, Norwich NR4 7UG, United Kingdom.
7. NMR Platform, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom.

Nonribosomal peptides (NRPs) frequently possess modified amino acids, and when present, these moieties are often essential for compound activity or biosynthesis. In most cases, these modified amino acids are generated by gene cluster-encoded precursor pathways and post-assembly line tailoring, which can enable bioinformatic strategies to prioritise the investigation of NRPs that may harbour unusual moieties. Genetic engineering was performed to deregulate an otherwise silent NRP gene cluster, which was predicted to encode the production of a glycosylated cyclotetrapeptide that we named biffamycin. Following substantial culture condition optimisation, biffamycin and pathway intermediates have been purified and structurally characterised by nuclear magnetic resonance (NMR) spectroscopy and tandem liquid chromatography high-resolution mass spectrometry (LC-HR-MSMS). The bioactivity of biffamycin has been assessed against a range of organisms, demonstrating a minimum inhibitory concentration ranging between 8 - 16 μg mL^{-1} for bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Mycobacterium smegmatis*. It has also been shown that supplementing media with different halide salts and amino acids can bias biffamycin production to alternative variants.

Notably, biffamycin contains an unprecedented 5-chloro-4-methoxy-tryptophan (5-Cl-4-MeO-Trp) moiety, produced by three enzymes encoded within the corresponding gene cluster: a hydroxylase (BifE), methyltransferase (BifF), and a halogenase (BifK). Absence of the precursor pathway genes resulted in an absence of biffamycin and any potential variants, suggesting the assembly line has specificity against incorporation of standard tryptophan. Meanwhile, placing this precursor pathway in a heterologous host confirmed the minimal gene set required for the precursor's synthesis. Given the unprecedented nature of the 5-Cl-4-MeO-Trp moiety, the order of these biosynthetic steps was investigated further using a combination of genetic, microbiological, biochemical, and computational methods. LC-MS of pathway deletion mutants established that 4-methoxy-tryptophan is generated by BifEF as an intermediate prior to halogenation by BifK. Owing to the product of BifE, 5-hydroxy-tryptophan, not being observed from streptomycete extracts, *in vitro* and *E. coli in vivo* assays were established to demonstrate this enzyme is the first step of the precursor pathway.

In summary, genetic engineering has enabled the production and purification of a novel NRP with a number of unique features, including an unprecedented 5-Cl-4-MeO-Trp residue.

Note to organisers: This work relates to my PhD studies at the University of Leeds, United Kingdom; I have very recently joined the Institute for Pharmaceutical Biology at Goethe University Frankfurt.

SL8

Native metabolomics-guided Discovery of a Cell-Permeable Gαᵢ-Inhibitor

Amira Naimi[1], Tim Berger[1], Marcel Rossol[2], Paolo Stincone[5], Uwe Linne[3], Jens Kockskämper[2], Daniel Hilger[4], Daniel Petras[5], Raphael Reher[1]

1 Institute for Pharmaceutical Biology and Biotechnology, Department of Pharmacy, Marburg University, Robert-Koch-Straße 4, 35037 Marburg, Germany
2 Institute for Pharmacology and Clinical Pharmacy, Biochemical and Pharmacological Centre (BPC) Marburg, Marburg University, Karl-von-Frisch-Str. 2 K|03, 35043 Marburg, Germany
3 Department of Chemistry, Marburg University, Hans-Meerwein-Straße, 35043 Marburg, Germany
4 Department of Pharmaceutical Chemistry, Marburg University, Marbacher Weg 6, Marburg 35037, Germany
5 Department of Biochemistry, University of California Riverside, Riverside

The cost- and time-consuming process of finding a single bioactive molecule for a target protein from multiple complex crude extracts hampers natural product discovery. To accelerate and facilitate this iterative process of fractionating and bioactivity testing, we present a scalable native metabolomics approach, which combines non-targeted liquid chromatography-tandem mass spectrometry with the detection of protein binding by native mass spectrometry (Figure 1).[1,2]

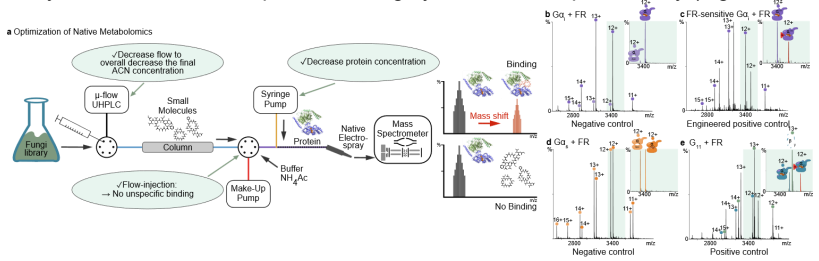


Figure 1a: Workflow of the optimized native metabolomics workflow 1b-e: Proof of concept: Native MS measurements of G proteins from different subfamilies $G\alpha_i$, $G\alpha_{iFR\text{-}sensitive\ mutant}$, $G\alpha_s$, G_{11} against the selective $G_{q/11/14}$ inhibitor FR.

Heterotrimeric G proteins represent an interesting, but so far “undruggable” pharmacological target despite being involved in the regulation of a wide range of physiological and pathophysiological processes in living organisms.[3] Currently, selective small molecule modulators specifically target $G\alpha_q$ and more recently $G\alpha_s$, leaving the remaining Gα subfamilies without such modulators.[4,5]
We selected the $G\alpha_i$ protein for native metabolomics screening against a diverse library of complex extracts from marine fungi due to the high sequence similarity of fungal G proteins to those in the mammalian $G\alpha_i$ superfamily. The marine fungus *Apiospora* sp. 589 was identified as a potential candidate for hit isolation. Structure elucidation of two binders was guided and facilitated by AI-tools.[6] Furthermore, selective binding to $G\alpha_i$ of one natural product (*m/z* 446) and unselective binding of a second molecule (*m/z* 430), as indicated by native mass spectroscopy, was validated with nanoDSF and SPR measurements. HDX-MS analyses revealed that binding of *m/z* 446 weakly stabilizes the nucleotide binding pocket and provided evidence for unselective G protein destabilization caused by *m/z* 430. In addition, bioactivity of the two binders was evaluated using a GTP-turnover assay[7] and G_i-signaling assays in isolated cardiomyocytes, revealing a direct link between molecular binding and functional effects for *m/z* 446 but not for *m/z* 430.
This finding underscores the potential of native metabolomics as a powerful tool for inhibitor discovery and led to the identification of the first small molecule inhibitor for $G\alpha_i$, which paves the way to better understand the role of G_i-signalling in biology and to develop novel therapeutic strategies for $G\alpha_i$-mediated diseases.

References

[1] Reher, R. et al. *Nat Commun* 13, 4619 (2022). https://doi.org/10.1038/s41467-022-32016-6
[2] Stincone, P. & Naimi, A. et. al. *Proteomics* 24 (12–13), 2200533 (2024). https://doi.org/10.1002/pmic.202200533
[3] Dang, C. V. et. al. *Nat. Rev. Cancer* 17, 502–508 (2017). https://doi.org/10.1038/nrc.2017.36
[4] Schrage, R. et al. *Nat Commun* 6, 10156 (2015). https://doi.org/10.1038/ncomms10156
[5] Dai, S. A. et al. *Cell* 185, 3950-3965.e25 (2022). https://doi.org/10.1016/j.cell.2022.09.019
[6] Reher, R. et. al. *J. Am. Chem. Soc.* 142, 4114–4120 (2020). https://pubs.acs.org/doi/10.1021/jacs.9b13786
[7] Hilger, D. et. al. *Science* 369, eaba3373 (2020). https://doi.org/10.1126/science.aba3373

SL9

Genome engineering of *Streptomyces* species using CASCADE-Cas3 technology

Christopher M. Whitford[1], Peter Gockel[1], David Faurdal[1], Tetiana Gren[1], Renata Sigrist[1], Tilmann Weber[1]
1 The Novo Nordisk Foundation Center for Biosustainability, DTU, Søltofts Plads, Kgs. Lyngby, Denmark

Actinobacteria can be considered the most relevant microorganisms, when it comes to production of natural products, with applications ranging from food preservation, agriculture, medicine, personal care. Among them, *Streptomyces* are the most commonly studied species. However, the field of natural products discovery is limited due to the absence of efficient and straightforward *Streptomyces* genome engineering methods.
One of the common methods for discovery of natural products is the expression of cloned biosynthetic gene clusters (BGCs) in heterologous hosts. The development of sophisticated heterologous hosts, the majority of which are genome reduced, remains highly laborious practice. Genome reduction usually focuses on the deletion of entire genetic regions such as BGCs, repetetice elements, transposons, with the aim of reducing the native metabolic background of heterologous hosts and speeding up their growth rate. Such genetic modifications in *Streptomyces* were commonly achieved through PCR targeting. PCR targeting typically involves homologous recombination and double crossover events, where most oft he construction steps are carried out in *Escherichia coli*. This method, though technically sound, requires sequenced fosmid/cosmid genomic libraries of the strains of interest. It is significantly labour/time extensive and cost consuming. Modern approaches typically use CRISPR-Cas9 or Cas12 - based systems (1).
Here, we report a novel CASCADE-Cas3 based system that can facilitate efficient genomic deletions and integrations. This system is based on a compact type I-C CRISPR system. The system was optimized for application in streptomycetes and is expressed from the widely used pCRISPR *Streptomyces* plasmid system. We demonstrate the application of this system for small, mid and large size deletions in several well-established *Streptomyces* heterologous hosts, including *S. coelicolor*, *S. venezuelae* and *S. albidoflavus*, as well as in the novel isolate strain *S. sp*. NBC1270. Finally, we demonstrate streamlined host construction using pCRISPR-Cas3 by performing simultaneous genome deletions and integrations (2).

References
[1] Tong, Y *et al*. Nat. Prot. 2020, 15(8), 2470–2502. https://doi.org/10.1038/s41596-020-0339-z.
[2] Whitford, C.M. *et al*. Res. NAR. 2025, 53(11). https://doi.org/10.1093/nar/gkaf214

SL10

Uncovering and Designing a Flexible Amino Acid Ligase from *Streptomyces mozunensis*

Phillip Schlegel[1], Alexander Zlobin[1], Matheus R. Belisário-Ferrari[1], Clara T. Schoeder[1,3], Leonard Kaysser[1]

1 Institute for Drug Discovery, Leipzig University Medical Faculty, Brüderstr. 34, 04103 Leipzig, Germany
2 Interdisciplinary Center for Bioinformatics, Härtelstr. 16-18, 04107 Leipzig, Germany
3 Center for Scalable Data Analytics and Artificial Intelligence ScaDS.AI, Humboldtstr. 25, 04105 Leipzig, Germany

Phosphoramidon, a widely used metalloprotease inhibitor, is assembled in *Streptomyces* through a biosynthetic pathway that deviates from canonical non-ribosomal peptide synthetase (NRPS) logic. Central to this pathway is the ATP-grasp ligase TalD, which forms the dipeptide backbone but has not been functionally characterized [1]. Here, we investigated TalD to define its substrate specificity, catalytic mechanism, and potential as a flexible biocatalyst. Using a tailored activity assay, we mapped its substrate range and confirmed product formation by mass spectrometry. Enzyme kinetics and mechanism were further examined by isothermal titration calorimetry (ITC), providing detailed mechanistic insight. Building on this understanding, we rationally reprogrammed TalD's substrate scope using computational design tools, including LigandMPNN [2] and Rosetta Enzyme Design [3], achieving precise control over selectivity. These findings establish TalD as a tunable ATP-grasp ligase and highlight its potential as a versatile scaffold for engineering bioactive small molecules.

References
[1] Baulig, A. *et al.* Chem. Sci. 2019, 10, 4486-4490. https://doi.org/10.1039/C9SC00641A
[2] Dauparas, J. *et al.* Nature Methods 2025, 22, 717-723. https://doi.org/10.1038/s41592-025-02626-1
[3] Richter, F. *et al.* PLoS One 2011, 6(5):e19230. https://doi.org/10.1371/journal.pone.0019230

SL11

Exploring the natural product biosynthetic potential of antarctic actinomycetes

Ulrike Tarazona Janampa [1,2], Shabari Girish [3], Karen Harms [3], Frank Surup [3], Raul Olaechea Alejo [2], Yvonne Mast [1,4]

1 Bioresources for Bioeconomy and Health Research, Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Inhoffenstraße 7 B, 38124 Braunschweig, Germany.
2 Comunidades acuáticas, Universidad Científica del Sur, Panamericana Sur Km. 19, 42 Lima, Peru.
3 Microbial Drugs, Helmholtz Centre for Infection Research, Inhoffenstraße 7 B, 38124 Braunschweig, Germany.
4 Institut für Mikrobiologie, Technical University of Braunschweig, Rebenring 56, 38106 Braunschweig, Germany.

Antimicrobial resistance is a growing global health crisis. Despite the advancements in natural product (NP) discovery, the high rate of rediscovery remains a challenge [1]. Recent efforts have focused on exploring extreme ecological niches to identify novel NPs [2]. Among these, Antarctic ecosystems with their unique microbial biodiversity, represent a valuable target for bioprospecting [3]. In particular, actinomycetes are promising sources of novel NPs [4], contributing to nearly two-thirds of clinically used antibiotics. This study aims to investigate the biosynthetic potential of Antarctic actinomycetes for the production of novel bioactive NPs.

Marine sediment samples were collected from six Antarctic locations (140-1150 m depth), and isolation protocols were optimized to selectively recover filamentous actinomycetes. Sixteen strains were selected for further investigation, with a representative subset subjected to PacBio long-read genome sequencing. Among them, strains from different genera were identified, including *Streptomyces, Nocardiopsis, Micromonospora,* and *Pseudonocardia*. The strains were cultivated in ten different media and analysed for antimicrobial activity against a panel of microbial test organisms. Thirteen strains exhibited antimicrobial activity, with *S. fildesensis* DSM 41987, *S. diacarni* DSM 109305, and *S. albidoflavus* DSM 120149, as the most bioactive strains.

A combinatory genome mining and metabolomics analysis approach was employed to correlate gene cluster predictions with compounds production. Strain DSM 41987 produced actinomycin X_2, which was correlated with an actinomycin BGC sharing 89% cluster similarity. Strain DSM 109305 produced various secondary metabolites, including griseusin, dudomycin, tartrolon, and a potential new tartrolon derivative. The latter was correlated with a tartrolon BGC sharing 37% cluster similarity. Strain DSM 120149 produced surugamide A and antimycin A, both of which could be linked to their BGCs, with cluster similarity values of 95% and 100%, respectively. The latter two compounds are known to have antifungal activities and were tested against the fungal Antarctic co-isolate *Metschnikowia australis*. Thereby, it was found that antimycin shows bioactivity against *M. australis* but also against the clinically relevant pathogen *Candida auris*, underscoring the importance of understanding the ecological roles of natural compounds in their native environments for future applications.

References
[1] Walesch, S., *et al*. EMBO Rep. 2023, 24, e56033. https://doi.org/10.15252/embr.202256033
[2] Handayani, I., *et al.* Mar Drugs. 2021, 19(6), 316. https://doi.org/10.3390/md19060316
[3] Medeiros, W., et al. Nat Prod Rep. 2025, 42, 774-787. https://doi.org/10.1039/D4NP00045E
[4] Nouioui, I., *et al*. Curr Res Microb Sci. 2024, 42, 774-787. https://doi.org/10.1016/j.crmicr.2024.100290

SL12

Unlocking Hidden Chemistry: From Genome Mining to Mechanistic Insights and New Antibiotics

Chengzhang Fu[1,2,3]

1 Research Group Genome Mining for Secondary Metabolites (GEMS), Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Saarbrücken 66123, Germany.

2 Helmholtz International Lab for Anti-Infectives, Helmholtz Center for Infection Research, Braunschweig 38124, Germany

3 PharmaScienceHub, Saarbrücken 66123, Germany.

Natural products remain an unparalleled source of bioactive molecules, yet their exploration is often hindered by silent biosynthetic pathways and poorly understood enzymatic mechanisms. Here, we will highlight three recent advances from our group that showcase complementary strategies for advancing natural product research.

First, we developed ACTIMOT, a CRISPR-Cas9–based strategy that mobilizes large chromosomal DNA fragments into self-replicating plasmids within the same cell, enabling efficient activation of cryptic biosynthetic gene clusters. This approach revealed multiple pathways yielding dozens of previously inaccessible natural products across diverse chemical classes.[1] We are now advancing ACTIMOT toward next-generation development and applications, with a particular focus on systematically exploring compound families with therapeutic potential.

Second, we resolved the long-standing enigma of pepstatin biosynthesis, identifying an iterative $F_{420}H_2$-dependent oxidoreductase that catalyzes the post-assembly-line formation of statine residues, thereby uncovering highly unusual enzymatic logic for ketone reduction.[2] Building on this work, we are pursuing complete *in vitro* reconstitution of the pepstatin pathway to investigate additional unique enzymatic steps within the hybrid NRPS-PKS system.

Third, we discovered a rare family of natural products featuring an unusual 3-methoxyl-2,5,6-trialkylpyrazine scaffold with potent activity against *Mycobacterium tuberculosis*. Stable isotope feeding and P450 inhibition studies illuminated their biosynthetic origin, while total synthesis confirmed the structural assignment and provided material for further investigation. Because several congeners exhibit strong anti-tuberculosis activity with low cytotoxicity, we are now evaluating their potential as candidates for further development.

Together, these advances demonstrate how genome mining, enzymology, and metabolite discovery can be integrated to expand our understanding of microbial chemistry and deliver promising candidates for anti-infective drug development.

References

(1) Xie, F.; Zhao, H.; Liu, J.; Yang, X.; Neuber, M.; Agrawal, A. A.; Kaur, A.; Herrmann, J.; Kalinina, O. V.; Wei, X.; Müller, R.; Fu, C. Autologous DNA mobilization and multiplication expedite natural products discovery from bacteria. *Science (New York, N.Y.)* **2024**, *386* (6727), eabq7333. DOI: 10.1126/science.abq7333. Published Online: Dec. 13, 2024.

(2) Mo, J.; Sikandar, A.; Zhao, H.; Bashiri, G.; Huo, L.; Empting, M.; Müller, R.; Fu, C. Tandem ketone reduction in pepstatin biosynthesis reveals an F420H2-dependent statine pathway. *Nature communications* **2025**, *16* (1), 4531. DOI: 10.1038/s41467-025-59785-0. Published Online: May. 15, 2025.

SL13

Advancing heterologous expression of biosynthetic gene clusters in cyanobacterial host organisms

Dana S. Parade[1], T. A. M. Gulder,[1] P. M. D'Agostino[1]

1 Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Department of Natural Product Biotechnology, Helmholtz Centre for Infection Research (HZI) and Department of Pharmacy, PharmaScienceHub (PSH), Saarland University, Campus E8.1, 66123 Saarbrücken, Germany.

Cyanobacteria can be found in nearly every environment on our planet and have been shown to produce a wide variety of bioactive compounds [1]. Nevertheless only a small proportion of cyanobacteria have been investigated for new natural products (NPs) to date, although these organisms show a huge potential to find novel NPs [1].

Investigating the biosynthetic gene clusters (BGCs) responsible for the production of these NPs can be challenging due to slow growth of the native producers, limited genetic tools, and the fact that many of the identified BGCs are silent [2]. Expression of target cyanobacterial BGCs is often achieved by BGC refactoring, using characterized promoters and well-described heterologous hosts [3], of which *E. coli*-based expression systems are the most common [2]. Yet the large metabolic and genetic differences between cyanobacteria and *E. coli* may sometimes lead to failure or production of intermediate products. Therefore, using a cyanobacterial host can be preferable [4]. However, cyanobacterial expression systems currently lack the versatility of expression strategies existing in *E. coli*.

Within this work, we aim to develop a flexible heterologous expression platform compatible with the Direct Pathway Cloning strategy [5, 6] to facilitate the discovery process. The model-strains *Synechoccocus elongatus* PCC 7942 and *Synechocystis* sp. PCC 6803 as well as the marine *Synechoccocus* sp. PCC 7002 and the fast-growing *Synechoccous* sp. PCC 11901 were selected as host organisms. Further, four constituitive and five inducible promoters previously described in literature were investigated. The promoters are tested for function by YFP-fluorescence and subsequently analysed for their capacity for NP production using BGCs of different lengths and classes resulting in a toolbox adaptable for analysing different compounds.
This platform will provide an additional tool to characterize BGCs using unicellular cyanobacteria as the host organism.

References

[1] D'Agostino, P.M. *Nat. Prod. Rep.*, **2023**, *40*, 1701-1717, https://doi.org/10.1039/D3NP00011G.
[2] Dhakal, D. *et al.*, *J. Ind. Microbiol. Biotechnol.* **2021**, *48*, kuab003, https://doi.org/10.1093/jimb/kuab003.
[3] Li, L. *et al.*, *Curr. Opin. Biotechnol.* **2021**, *69*, 145–152, https://doi.org/10.1016/j.copbio.2020.12.011.
[4] Zhang, J. J. *et al.*, *Nat. Prod. Rep.* **2019**, *36*, 1313–1332, https://doi.org/10.1039/C9NP00025A.
[5] Greunke, C. *et al.*, *Metab. Eng.* **2018**, *47*, 334–345, https://doi.org/10.1016/j.ymben.2018.03.010.
[6] D'Agostino, P.M. *et al. ACS Synth. Biol.* **2018**, *7* (7), 1702-1708, https://doi.org/10.1021/acssynbio.8b00151.

SL14

In Vitro Production of Aminochelin

Jonas Korb[1], Tanja Becker[1], David Kempel[1], Markus Nett[1]

[1]Technical Biology, TU Dortmund University, August Schmidt Straße 1, 44227 Dortmund, Germany.

Introduction
Aminochelin is a catechol siderophore produced by *Azotobacter vinelandii* that is able to bind both molybdenum and iron, which makes it a promising candidate for bioremediation with potential applications in heavy metal detoxification [1, 2]. Up to now, the biosynthesis of aminochelin has not been experimentally investigated. Yet, a plausible biosynthetic model can be postulated based on functional and structural relatedness to the siderophores vibriobactin from *Vibrio cholerae* and myxochelin from *Myxococcus xanthus* [3, 4, 5, 6]. According to this model 2,3-dihydroxybenzoic acid is activated by the ligase AvCA_21210 and loaded on the phosphopantetheine arm of the aryl carrier protein (ArCP) AvCA_21200 [7]. The condensation domain AvCA_21160 then catalyzes the condensation with putrescine to form aminochelin [7].
Goals
To validate the role of AvCA_21160 in aminochelin biosynthesis, we reconstituted the entire pathway in vitro. Furthermore, we aimed to optimize this enzyme cascade utilizing a Bayesian optimization approach and to investigate the substrate specificity of the cascade.
Results
For the reconstruction of aminochelin biosynthesis we utilized enzymes from three different bacteria. *A. vinelandii* provided the condensation domain AvCA_21160, while homologs of AvCA_21210 and AvCA_21200 were initially obtained from *M. xanthus*. The enzyme, which is necessary for the phosphopantetheinylation of the ArCP originated from *Bacillus subtilis*. Aminochelin could be generated from 2,3-dihydroxybenzoic acid and putrescine with the aforementioned enzymes and the required cofactors (ATP, coenzyme A, Mg^{2+}), thereby validating the proposed pathway. After replacing the ArCP domain from *M. xanthus* with AvCA_21200 from *A. vinelandii* the production of aminochelin was improved. Bayesian optimization was performed by varying temperature, enzyme concentration, pH and precursor concentrations. Bayesian optimization led to a major increase in product concentration by a factor of 68.
Outlook
Future studies will focus on evaluating the substrate specificity of the cascade by testing various diamines and benzoic acid derivatives under the optimized reaction conditions. This could enable broader applications for engineering siderophores for bioremediation and industrial biotechnology.

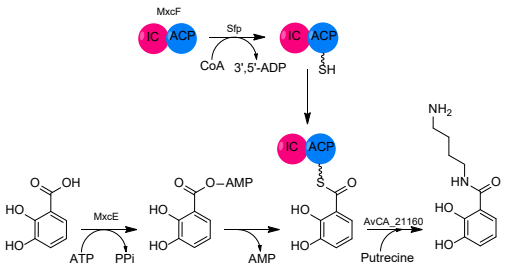


Figure 1. Enzyme cascade for aminochelin production utilizing AvCA_21160 from *A. vinelandii*, MxcF and MxcE from *M. xanthus* and Sfp from *B. subtilis*.

References
[1] Page W.J. *et al.* Microbiology 1988, 134, 453–460. https://doi.org/10.1099/00221287-134-2-453
[2] Hofmann M. *et al.* Nat. Prod. Rep. 2020, 37,1262-1283. https://doi.org/10.1039/C9NP00058E
[3] Keating T.A. *et al.* Biochemistry 2000, 39, 15513–15521. https://doi.org/10.1021/bi001651a
[4] Keating T.A. *et al.* Biochemistry 2000, 39, 15522–15530. https://doi.org/10.1021/bi0016523
[5] Blin K. *et al.* Nucleic Acids Res. 2023, 51, W46-W50. https://doi.org/10.1093/nar/gkad344
[6] Li Y. *et al.* J. Am. Chem. Soc. 2008, 130, 7554–7555, https://doi.org/10.1021/ja8025278
[7] Baars O. *et al.* Appl. Environ. Microbiol. 2016, 82, 27–39. https://doi.org/10.1128/AEM.03160-15

SL15

Negamycin: Unusual biosynthesis directed by a split biosynthetic gene cluster

Alena Strüder[1]; Saad Touzi[2]; Heike Brötz-Oesterhelt[1]; Emmanuelle Darbon[2]; Paul Frankenreiter[1]; Chambers C.Hughes[1]; Sylvie Lautru[2]; Evi Stegmann[1]

1 Eberhard Karls University of Tübingen, IMIT, Microbial bioactive compounds, Institute for Microbiology and Infection Medicine Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany
2 Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France

The alarming rise of antimicrobial resistance, particularly among Gram-negative ESKAPE pathogens, represents one of the most urgent global health challenges. To counteract this threat, the discovery of natural products with novel mechanisms of action is essential. Negamycin, a hydrazide antibiotic originally isolated from *Kitasatospora purpeofusca*, exhibits broad-spectrum activity against both Gram-positive and Gram-negative bacteria by targeting helix 34 of the 16S rRNA at a site distinct from other ribosome-targeting antibiotics [1, 2, 3]. In addition, negamycin promotes premature stop codon readthrough, extending its therapeutic potential beyond infectious disease [4, 5, 6].

Despite its unique features, the development of negamycin has long been hindered by low natural yields and synthetic intractability owning to its unusual N-N bond. Here, we identified and characterized the genetic basis of its biosynthesis and uncovered an uncommon split pathway architecture encoded by two distant chromosomal loci, *neg1* and *neg2*. Functional studies demonstrated that *neg1* provides nitrite as an essential precursor, while *neg2* governs β-lysine biosynthesis and scaffold assembly. Targeted deletions and complementation confirmed the essentiality of both loci, and isotope-labelling experiments verified the incorporation of nitrite, glycine, and lysine into the final structure.

Most importantly, full pathway reconstruction was achieved by co-expressing *neg1* and *neg2* in *Streptomyces albidoflavus* Del14 [7]. Strikingly, this heterologous host produced negamycin at levels comparable to the native producer. The successful heterologous production not only establishes a robust platform for yield optimization but also provides new opportunities to generate novel derivatives as a potential weapon against multidrug-resistant pathogens.

References:

[1] Hamada, M. *et al*. J. Antibiot (Tokyo) 1970, 23, 170–171. https://doi.org/10.7164/antibiotics.23.170
[2] Raju, B. *et al*. Patent application No : US 20030109583A1 2003.
[3] Polikanov, Y.S. *et al*. Mol. Cell 2014, 56, 541–550. https://doi.org/10.1016/j.molcel.2014.09.021.
[4] Malik, V. *et al*. Ther. Adv. Neurol. Disord. 2010, 3, 379–389. https://doi.org/10.1177/1756285610388693
[5] Bordeira-Carriço, R. *et al*. Trends Mol. Med. 2012, 18, 667–678. https://doi.org/10.1016/j.molmed.2012.09.004
[6] Kerem, E. *et al*. The Lancet 2008, 372, 719–727. https://doi.org/10.1016/S0140-6736(08)61168-X
[7] Myronovskyi, M. *et al* Metab. Eng. . 2018, 49, 316–324. https://doi.org/10.1016/j.ymben.2018.09.004

SL16

Ribosomal peptide prenyltransferases with biocatalytic potential

Leon Schwarz,[1] Ajay Nimbalkar,[1] Alexander Major,[1] Olga Kalinina,[1] Florian Hubrich[1,2]

1 Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus E8.1, 66123 Saarbrücken, Germany.
3 Institute of Pharmaceutical Biology, Saarland University, Campus C2.3, 66123 Saarbrücken, Germany.

Lipidation enhances peptide pharmacokinetics and bioactivity. FDA-approved lipopeptide antibiotics, e.g., daptomycin and polymyxin are fatty acylated. Unfortunately, acyl chain elongation of lipopeptide antibiotics often increases bioactivity and cytotoxicity, limiting drug development.[1] Alternative lipid moieties such as isoprenoid-based lipidation represents a promising alternative, recently reported to show more beneficial bioactivity to cytotoxicity relationships when compared with acyl chain length with similar size.[2]

Peptide prenyltransferases (PTs) catalyze isoprenoid attachment, but only a few have been characterized, including cyanobactin PTs, which post-translationally modify ribosomal peptides.[3] Recently, the Piel lab characterized the prenylated ribosomal peptide steromaze featuring a pseudo-steroid scaffold installed by geranylgeranylation of tryptophan and subsequent cyclization;[4] the key biosynthetic enzyme of steromaze biosynthesis is NctPC, a fusion enzyme consisting of an N-terminal PT and a C-terminal terpene cyclase domain. Characterization of the standalone PT NdnP and terpene cyclase NdnC showed geranylation and cyclization at the C-terminal Trp of the precursor peptide NdnA and variants thereof, suggesting potential for chemo-enzymatic peptide late-stage functionalization using NctP-like PTs.[5] Genome mining and structural modeling indicate NctP-like PTs belong to a distinct peptide PT family sharing an ABBA-fold with cyanobactin PTs and dimethylallyl tryptophan synthases.[3] To expand this family, we analyzed over 1,000 candidate NctP-like PTs sequences, selected the cyanobacterial PT NcbP and showed *O*-prenylation of Tyr by heterologous expression in *Escherichia coli*. Using bioinformatics-guided active site conservation analysis, and experimental validation, we identified a sequence motif likely determining prenyl donor specificity of NctP-like PTs, predicted the prenyl donor specificity of uncharacterized homologs and validated our predictions experimentally. Our discovery enables us to perform bioinformatics-based peptide PT function predictions to establish a peptide PT library for peptide drug development.

References
[1] Muttenthaler, M. *et al*. Nat. Rev. Drug. Discov. 2024, 20, 309-325. https://doi.org/10.1038/s41573-020-00135-8
[2] Ozawa, H. *et al*. J. Am. Chem. Soc. 2025, 147, 25642-25651. https://doi.org/10.1021/jacs.5c06850
[3] Zhang, Y. *et al*. Trends Biochem. Sci. 2023,48, 360-374. https://doi.org/10.1016/j.tibs.2022.11.002
[4] Hubrich, F. *et al*. *Chem* 2024, 10, 3224-3242. https://doi.org/10.1016/j.chempr.2024.07.026
[5] Magyari, S. *et al*. Manuscript in preparation.

SL17

PhyloNaP: a user-friendly database of Phylogeny for Natural Product–producing enzymes

Aleksandra Korenskaia[1], Judit Szenei[2], Kai Blin[2], Nadine Ziemert[1]

1 Translational Genome Mining for Natural Products, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), Interfaculty Institute for Biomedical Informatics (IBMI), University of Tübingen, Tübingen, Germany
2 The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby, Denmark

Natural products (NPs) are highly valuable in medicine and industry, and advances in genome mining have enabled large-scale identification of biosynthetic gene clusters (BGCs) responsible for production of NPs. Predicting the structures of NPs from BGCs is central to their targeted discovery but remains challenging. Automated methods typically capture only the biosynthesis of a core structure, and even then only when core enzymes' specificity and evolutionary relationships are well understood. In contrast, predicting modifications introduced by tailoring enzymes requires manual effort: relevant proteins and their functional annotations must be collected from scattered sources, and their evolutionary relationships reconstructed through phylogenetic analysis. This process is labor-intensive, inefficient, and partly redundant across studies, highlighting the need for resources that aggregate, annotate, and organize phylogenetic data to support functional prediction.

We present PhyloNaP, a web-based database of over 18,000 annotated phylogenetic trees covering biosynthetic enzymes, with a focus on tailoring enzymes from bacterial BGCs. PhyloNaP stores both published annotated trees and newly generated trees created by integrating data from MiBiG [1], MITE [2], UniProt SwissProt [3], and antiSMASH-DB [4], linking protein sequences to chemical, functional, and taxonomic metadata. The platform supports interactive exploration, including options to display structures and reactions directly on a tree. Users can automatically classify their sequences on a tree to facilitate functional inference in an evolutionary context. The website also provides a submission form for manually annotated phylogenetic trees and metadata, encouraging community contributions.

PhyloNaP fills a critical gap in natural product biosynthesis studies by providing a unique, centralized repository of annotated phylogenetic data. By assisting in the functional analysis of biosynthetic enzymes, PhyloNaP accelerates both structure prediction for individual clusters and the development of reliable structure prediction models for valuable natural products.

References

[1] Zdouc MM, et al. *Nucleic Acids Res.* 2024:gkae1115. doi:10.1093/nar/gkae1115
[2] Zdouc MM, et al. *ChemRxiv*. 2024. doi:10.26434/chemrxiv-2024-78mtl-v2
[3] Boutet E, et al. *Methods Mol Biol Clifton NJ*. 2007;406:89-112. doi:10.1007/978-1-59745-535-0_4
[4] Blin K, et al. *Nucleic Acids Res*. 2024;52(D1):D586-D589. doi:10.1093/nar/gkad984

SL18

The Role of Marine Fungi in Tackling Plastic Waste: A Multiomics Approach

Tim Berger[1,2], Syrena Whitner[2], Raphael Reher[1], Anthony Amend[2]

1 Institute for Pharmaceutical Biology and Biotechnology, Marburg University, Robert-Koch-Str. 4, 35037 Marburg, Germany;
2 Pacific Biosciences Research Center, University of Hawaii at Mānoa, 1800 East-West Rd, 96822 Honolulu, Hawai'i, USA.

Marine-derived fungi are widely recognised as prolific producers of bioactive natural products.[1,2] For survival in ecological niches (high salt content, tropical or arctic temperatures, respectively, endosymbiotic relationships), a multitude of enzymatic reactions, which can be both highly specific and complex in nature, occur within these organisms. In consideration of the enormous annual global plastic production, which inevitably results in a problematic accumulation of plastic in the oceans, there is potential for investigation of the ability of fungal enzymes to degrade plastic polymers. In this study, we want to test whether native organisms of the marine environment, such as marine-derived fungi, can offer a fruitful source for plastic degradation.
It has previously been demonstrated that a number of marine fungi collected in the waters off the Hawaiian island of O'ahu have exhibited a visible clearance effect on polyurethane (PU)-containing agar plates, hinting towards a degradation process.[3] To procure more detailed information regarding this process, the present study is implementing a multiomics approach, including genomics, metabolomics, and transcriptomics experiments (**Figure 1**).

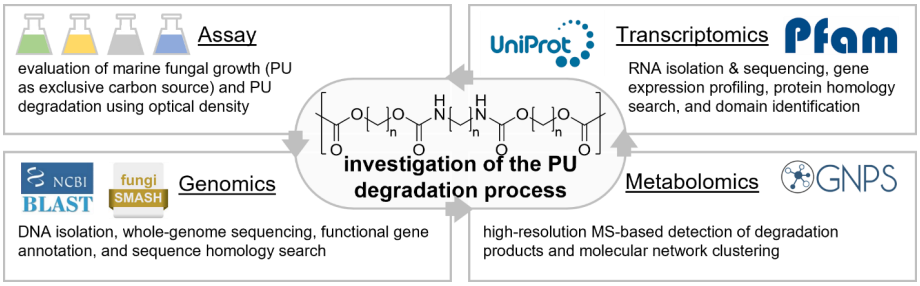


Figure 1 Overview of the applied multiomics workflow

This approach is designed to facilitate the identification of the implicated enzymes and enable their correlation with the detected metabolic products. Therefore, the genomes of the investigated marine fungi were analyzed to ascertain the possible presence of any enzymes that have previously been identified as capable of degrading plastic polymers, as documented in the PAZy Database.[4] In addition, high-resolution mass spectrometry facilitates the identification of degradation products through the utilization of MS-based molecular networking, which employs a clustering approach to organize features according to their analogous fragment patterns.[5] Finally, the exploration of overexpressed genes during RNA sequencing analysis ultimately led to the correlation of specific modifications in the molecular structure of the PU polymer to specific present enzymes and their associated functions.
In summary, the results of the study provide valuable insights into the process of enzymatic degradation of PU by marine-derived fungi. Moreover, the findings of this research establish a solid foundation for the development of novel strategies for the management of plastic waste.

References
[1] Carroll, A. R. *et al. Nat. Prod. Rep.* 2024, *41* (2), 162–207. https://doi.org/10.1039/D3NP00061C.
[2] Berger, T. *et al. J. Nat. Prod.* 2024. https://doi.org/10.1021/acs.jnatprod.3c00750.
[3] Steinbach, R. M. *et al. Mycologia* 2025, *117* (1), 1–8. https://doi.org/10.1080/00275514.2024.2422598.
[4] Buchholz, P. C. F. *et al. Proteins: Struct., Funct., Bioinf.* 2022, *90* (7), 1443–1456. https://doi.org/10.1002/prot.26325.
[5] Wang, M. *et al. Nat. Biotechnol.* 2016, *34* (8), 828–837. https://doi.org/10.1038/nbt.3597.

SL19

CRISPR-Cas systems in antibiotic-producing *Actinomycetes*: Insights from *Streptoalloteichus tenebrarius*

Lena Mitousis[1], Ewa M. Musiol-Kroll[1,2], Wolfgang Wohlleben[1]

1 Interfaculty Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbiology/ Biotechnology, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany
2 present address: Symrise AG, Mühlenfeldstraße 1, 37603 Holzminden

Many Actinomycetes are important producers of valuable secondary metabolites with potent properties like antimicrobial, anti-tumor or immunosuppressive activity. However, their genetic engineering is often prohibited by mechanisms that prevent the stable establishment of foreign DNA. CRISPR-Cas systems are known as adaptive prokaryotic immunity systems. They play an important role in the defence against foreign mobile genetic elements like plasmids or phages. Recently, also the involvement of CRISPR-Cas in other cellular processes including DNA-repair or cell-differentiation was described. But so far the knowledge about the function of CRISPR-Cas in Actinomycetes is still very limited [1].
The goal of this project is the functional analysis of CRISPR-Cas systems in antibiotic-producing actinomycetes. This includes adaptive immunity as well as functions beyond immunity, such as a potential role of CRISPR-Cas in regulation of antibiotic production. For this, we use the industrial aminoglycoside antibiotic producer *Streptoalloteichus tenebrarius* as model strain. It is particularly interesting, as its genome encodes three CRISPR-Cas systems (Region 1-3) and 71 CRISPR-arrays, containing in total over 300 spacers. Bioinformatic sequence analysis on the origin of the spacers did not result in matches to known sequences from databases.
The immunity function can be investigated with DNA-uptake assays based on transformation, conjugation and/or phage infection. To identify a phage that infects *S. tenebrarius*, an extensive screening of newly isolated phages as well as known actinomycetes phages was conducted. It was shown, that *S. tenebrarius* is not included in the host range of all tested phages.
Since the genetic manipulation of *S. tenebrarius* is challenging, the CRISPR-Cas systems of *S. tenebrarius* were heterologously expressed in *Escherichia coli* and *Streptomyces coelicolor*. The DNA-Uptake assays performed with *E. coli* expressing CRISPR-Cas Region 1 did not reveal activity as adaptive immunity system. However, using *S. coelicolor* as heterologous host, an increased plasmid loss was observed, suggesting CRISPR-Cas activity.
Furthermore, CRISPR-Cas deletion mutants in *S. tenebrarius* were generated. Deletion of CRISPR-Cas Region 1 led to a sporulation-deficient phenotype with influence on the aminoglycoside antibiotic production. This suggests an involvement of CRISPR-Cas in cell-differentiation processes in *S. tenebrarius*.

References
[1] Mitousis, L. *et al*. microLife. 2025, 6, uqaf010. https://doi.org/10.1093/femsml/uqaf010

SL20

Discovery of the *N*-acyl-cyclolysine system, a widespread chemical signalling pathway in the Bacteroidota

Luis Linares-Otoya[1,2], Jaden D. Shirkey[1], Bhuwan Khatri Chhetri[1], Amira Mira[1,3], Abhishek Biswas[1], Samuel L. Neff[4], Maria V. Linares-Otoya[2], Ye Chen[1], Julio V. Campos-Florian[2], Mayar L. Ganoza-Yupanqui[2], Philip D. Jeffrey[1], Frederick M. Hughson[1] & Mohamed S. Donia[1,5,6]

[1] Department of Molecular Biology, Princeton University, USA
[2] Faculty of Pharmacy and Biochemistry, National University of Trujillo, Peru
[3] Faculty of Pharmacy, Mansoura University, Egypt
[4] Lewis-Sigler Institute for Integrative Genomics, Princeton University, USA
[5] Department of Chemical and Biological Engineering, Princeton University, USA
[6] Department of Ecology and Evolutionary Biology, Princeton University, USA

Considerable advances have been made in characterizing bioactive molecules secreted by bacteria, yet the regulatory elements controlling their production remain largely understudied. Here we identify and characterize the N-acyl-cyclolysine (ACL) system—a cell-density-dependent chemical signalling system specific to and widespread in the phylum Bacteroidota (formerly Bacteroidetes)—and show that it regulates the expression of co-localized operons encoding diverse secreted molecules.

Using genetic and biochemical analyses, combined with structural studies of a key biosynthetic enzyme, AclA, we elucidate the molecular structure of various ACLs and their complete biosynthetic pathway involving l-lysine acylation and ATP-dependent cyclization. Furthermore, we find that secreted ACLs are sensed by a dedicated transcription factor, AclR, resulting in the expression of associated operons and the autoinduction of ACL biosynthesis. Moreover, we show that different Bacteroidota strains produce structurally diverse ACLs and encode transcription factors with varying ligand specificities. Finally, we find that the acl circuit is widely distributed and transcribed in human gut and oral microbiome samples, with clear evidence for an active role in regulating associated operons under host colonization conditions.

Understanding the function of the ACL system in different contexts has the potential to reveal details about the biology, ecology and chemistry of the Bacteroidota and how members of this phylum interact with their environments and hosts.

References
[1] Linares-Otoya, L. *et al*. Nature 2025. Discovery of a widespread chemical signalling pathway in the Bacteroidota. https://doi.org/10.1038/s41586-025-09418-9

5 Posters

AI-generated and modified illustration

P1

Metabolic Engineering of *Streptomyces tendae* Tü 4042 to Boost Lysolipin I Biosynthesis

Samia Mohamed[1], Dumitrita Iftime[1, 2], Alina Burov[1], Andreas Kulik[1] and Wolfgang Wohlleben[1,2]

1 Interfaculty Institute of Microbiology and Infection Medicine (IMIT) Microbiology/Biotechnology, Eberhard Karls Universität Tübingen, Germany
2 DZIF, Tübingen, Germany.

Polyketide antibiotics are a diverse and clinically significant group of natural products that includes macrolides, tetracyclines and anthracyclines. Despite their relevance in the pharmaceutical industry, production yields in native hosts are often low due to tight regulatory mechanisms and metabolic limitations.

Lysolipins are bioactive polyketides produced by the bacterium *Streptomyces tendae* [1] that exhibit potent antimicrobial properties [2]. The biosynthesis of a single lysolipin molecule requires 12 malonyl-CoA molecules.
In this study, we engineered *S. tendae* to overexpress the acetyl-CoA carboxylase (ACC), a key enzyme complex in the conversion of acetyl-CoA to malonyl-CoA — a critical precursor in the biosynthesis of the lysolipin polyketide. Increased inhibition of bacterial growth against *B. subtilis* indicate enhanced lysolipin production in the recombinant strain overexpressing the ACC.
Quantitative analysis by HPLC confirmed a remarkable increase in lysolipin titers in the engineered strain compared to the wild type. These results demonstrate that targeted metabolic engineering of central carbon metabolism can effectively increase the production of secondary metabolites in *Streptomyces*, providing a promising strategy for improving the yield of valuable bioactive compounds.

References
[1] Lopez *et al*. Gene 2010, 5-14. https://doi.org/10.1016/j.gene.2010.03.016
[2] Drautz et al., Arch Microbiol. 1975, 106(3):175-90. https://doi.org/10.1007/BF00446521.

P2

Expanding the RiPP Toolbox: Biochemical Characterization of Peptide Arginases

Maria-Paula Schröder[1], Isabel P.-M. Pfeiffer[1], Silja Mordhorst[1]

[1] Department of Pharmaceutical Biology, University of Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a diverse class of natural products whose biosynthesis includes the installation of a broad array of post-translational modifications (PTMs). RiPP-modifying enzymes often show a relaxed substrate specificity offering opportunities for structural and functional diversification in peptide-based drug discovery.
A few years ago, a novel family of RiPP-modifying enzymes has been described: peptide arginases (pfam 12640) hydrolyse arginine residues in RiPP precursors to ornithine.[1] Ornithine residues can be used for further functionalization, such as lipidation or hydroxylation, which are interesting structural features for the design of peptide therapeutics.[2,3]
To further investigate the deguanidination as a PTM, we characterized two novel peptide arginases (OhkR and FlmR), which are associated with uncharacterised RiPP precursors.[4] First *in vivo* co-expressions in *E. coli* were used to investigate the PTMs on the native precursors. OhkR completely converted both precursors (OhkA1 and OhkA2) in overnight co-expression, while FlmR showed about 50 % conversion of its native precursors (FlmA1, FlmA2, and FlmA3) in overnight co-expressions. Since all core peptides contain two arginine residues, the directionality of arginine-to-ornithine modifications of FlmR and OhkR on their native substrates was explored, employing tandem mass spectrometry. In addition, the cross-reactivity was examined *in vivo* by precursor swapping, whereby the co-expression of OhkR with FlmA1-3 and FlmR with OhkA1-2 was analysed. It was observed that both OhkR and FlmR exhibited partial conversion of the non-native precursors. *In vitro* kinetic assays were used to determine the Michaelis–Menten parameters. As the precursors show high sequence similarity, the corresponding A1 precursors were selected for detailed kinetic analyses. Relative rates were determined for all other precursors.[4] The resulting kinetic parameters are comparable to those of OspR, the only other characterised peptide arginase.[5]
Our study contributes to the biochemical understanding of peptide arginases and supports their integration into peptide-based drug discovery platforms.

References
[1] Mordhorst, S. *et al. Angew. Chem. Int. Ed.* **2020**, *59*, 21442–21447. doi:10.1002/anie.202008990
[2] Padhi, C. *et al. J. Am. Chem. Soc.* **2025**. doi:10.1021/jacs.5c06044
[3] Hubrich, F. *et al. PNAS* **2022**, *119*, e2113120119. doi:10.1073/pnas.2113120119
[4] Pfeiffer, I. P.-M. *et al.* 2025, submitted
[5] Mordhorst, S. *et al. ACS Chem. Biol.* **2023**. doi:10.1021/acschembio.2c00879

P3

Predictive Biocatalyst Selection for Metabolite Synthesis

Jacqueline Wistuba-Hamprecht[1], Alexander Fejzagic[2], Marco Santagostino[2], Nico Pfeifer[3], Nadine Ziemert[1]

1 Translational Genome Mining, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Germany
2 Global Development CMC NCE, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany
3 Methods in Medical Informatics, Department of Computer Science, University of Tübingen, Germany

In the synthesis of new building blocks for pharmaceutical substances, biocatalysis offers a viable alternative to the conventional chemocatalytic methods used in the pharmaceutical industry. The high abundance, diversity and intrinsic chirality of enzymes make them a powerful tool for catalysing a wide range of chemical transformations. Despite their potential, current biocatalytic strategies often rely on empirical “brute-force” screening of extensive enzyme libraries derived from various biological sources. These approaches are typically time- and resource-intensive and depend exclusively on experimental data. With the advent of next-generation sequencing, high-throughput screening techniques and growing protein database availability, coupled with machine learning (ML), a shift towards data-driven enzyme engineering is emerging as a promising solution [1].

One area of application of biocatalysts is in the development of active pharmaceutical ingredients (API). These are considered to be the core components of drugs, and the evaluation of their metabolic degradation products for toxicity and biological activity is a critical step in the pharmaceutical development process. In this context, ML approaches offer the potential to identify promising biocatalysts which can be used for the *in vitro* synthesis of the desired metabolites by detecting latent patterns in existing datasets. These models can predict enzyme–substrate compatibility and suggest novel or optimized enzyme variants resulting in a reduced experimental workload and accelerating the discovery of environmentally friendly biocatalysts.

The aim of this project is to apply ML methods to enhance the selection of biocatalysts for the synthesis of next-generation therapeutic building blocks. Our focus lies on the cytochrome P450 monooxygenase family, a group of bacterial enzymes (bac CYP450) that are known to catalyse a diverse range of substrates and play a central role in the biosynthesis of many natural products. We aim to establish a benchmark dataset of bac CYP450 protein sequences and develop a robust ML workflow capable of reliably predicting (I) substrate properties or (II) discovering biocatalysts.

References

[1] Siedhoff, Niklas E., Ulrich Schwaneberg, and Mehdi D. Davari. "Machine learning-assisted enzyme engineering." Methods in enzymology 643 (2020): 281-315.

P4

Unfolding Peptide Arginases: Bioinformatic Insights into RiPP Precursor-Maturase Interaction

Isabel P.-M. Pfeiffer[1], Maria-Paula Schröder[1], Silja Mordhorst[1]

1 Pharmaceutical Biology, Pharmaceutical Institute, University of Tübingen

Many peptide natural products produced by microbes exhibit antibacterial, antifungal, or cytotoxic activities. Small peptide or peptide-derived drugs have been used to treat various diseases since the last century [1].
Ribosomally synthesised and post-translationally modified peptides (RiPPs) represent a large group of peptide natural products [2]. The RiPP precursor peptide is synthesised at the ribosome, and thus it contains solely the 20 canonical amino acids [3]. Extensive post-translational modifications (PTMs) of the core peptide by maturases – enzymes performing a wide range of different PTMs – lead to the highly diverse chemical structures of RiPP natural products [4].
One example of an interesting RiPP-PTM reaction was identified in the maturation of the antiviral peptide landornamide A: the hydrolysis of arginine residues to ornithine catalysed by the peptide arginase OspR [5]. Further biosynthetic gene clusters encoding peptide arginase homologs (pfam 12640) were identified by genome mining, revealing a so far uncharacterised RiPP precursor type, defined by a highly conserved DD(I/V)LF sequence motif. The peptide arginases FlmR and OhkR which have been identified in the distantly related bacteria *Flagellimonas meridianipacifica* and *Ohtaekwangia koreensis* have been found to be associated with this novel precursor type. The predicted structures of these proteins exhibit a high degree of similarity to the OspR structure (PDB 8BRP). *In vivo* and *in vitro* activity of FlmR and OhkR on their native substrates (FlmA1-3 and OhkA1-2) was shown experimentally [6].
To gain deeper understanding of the precursor-maturase binding and interaction, bioinformatics was employed including AlphaFold (AF) modelling and PISA (Proteins, Interfaces, Structures and Assemblies) analysis [7, 8]. The impact of a modified precursor peptide sequence and the number of protein entities on the AF model were investigated, as well as the confidence of AF models by repeated modelling of the identical input. The majority of the obtained AF models align well with the experimental data. However, no reliable predictions were generated for a few models, which demonstrates the limitations of AF predictions. Nonetheless, the obtained bioinformatic data provides insights into the binding of precursors to the maturase, the positioning of catalytic residues within the active site, and offers clues about the directionality of arginine-to-ornithine conversion, if more than one arginine residue is present in the core peptide [6]. Taken together, these elements can provide a foundation for the development of maturase and peptide substrate engineering.

References
[1] Dang, T. *et al.* Acc. Chem. Res. 2017, 50, 1566-1576. https://doi.org/10.1021/acs.accounts.7b00159
[2] Dell, M. *et al.* Nat. Prod. Rep. 2022, 39, 453-459. https://doi.org/10.1039/D1NP00019E
[3] McIntosh, J. A. *et al.* Nat. Prod. Rep. 2009, 26, 537-559. https://doi.org/10.1039/B714132G
[4] Montalbán-López, M. *et al.* Nat. Prod. Rep. 2021, 38, 130-239. https://doi.org/10.1039/D0NP00027B
[5] Bösch, N. M. *et al.* Angew. Chem. 2020, 132, 11861-11866. https://doi.org/10.1002/ange.201916321
[6] Pfeiffer, I. P.-M. *et al.* 2025, submitted
[7] Abramson, J. *et al.* Nature 2024, 630, 493–500. https://doi.org/10.1038/s41586-024-07487-w
[8] Krissinel, E. *et al.* J. Mol. Biol. 2007, 372, 774-797. https://doi.org/10.1016/j.jmb.2007.05.022

P5

Tailoring fungal leucine-rich non-ribosomal peptides by mutasynthesis in a heterologous *Aspergillus* host

Julia Bischof[1], Hannes Maier[1] Andreas Kulik[1], Harald Groß[2], Wolfgang Wohlleben[1]

1 University of Tübingen, Dep. Biotechnology/Microbiology, Tübingen Germany
2 University of Tübingen, Dep. Pharmacy, Tübingen Germany

The marine filamentous fungus *Asteromyces cruciatus* 763 (*Pleosporaceae, Ascomycota*), collected from the coastal waters of La Jolla (San Diego, USA), was identified as the natural producer of the cyclic non-ribosomal pentapeptide lajollamide A, whose structure was previously elucidated by HPLC-MS and NMR [1].
Although structurally intriguing, leucine-rich cyclic pentapeptides like lajollamide A remain largely underexplored for their antimicrobial properties. Given its weak but promising antibacterial activity, this study aimed to establish a heterologous production platform for lajollamide A and its derivatization via precursor-directed biosynthesis (PDB) in *Aspergillus*.
Genome mining in *A. cruciatus* 763 revealed a putative biosynthetic gene cluster (BGC) encoding a single non-ribosomal peptide synthetase (NRPS), predicted to assemble lajollamide A from L-leucine (3x), N-methyl-L-leucine (1x) and L-valine (1x).

In order to achieve efficient lajollamide A production we used the model fungus *Aspergillus nidulans* and cloned the *nrps* gene from *A. cruciatus* 763 under the strong constitutive *tef1* promoter and introduced it into the auxotrophic GR5 strain via PEG-mediated protoplast transformation [2] [3].
This resulted in prototrophic transformants generated by multiple random ectopic integration of the introduced plasmid-DNA. Small-scale chemical screening of 50 transformants, analyzed by HPLC-MS and MS/MS confirmed lajollamide A production in 30% of the prototrophic strains along with the identification of a naturally occurring congener, demethyllajollamide. This successful reconstruction of the lajollamide A biosynthetic pathway in *Aspergillus* represents the first report of a heterologously expressed BGC from the genus *Asteromyces* and sets the foundation for further derivatization.

Feeding various leucine analogues resulted in the biosynthesis of novel lajollamide derivatives highlighting broad substrate tolerance of all leucine-incorporating A-domains. Simultaneous incorporation of up to four leucine analogues led to the biosynthesis of chemically highly functionalized derivatives with presumably enhanced or altered antimicrobial activities.

References
[1] Gulder, T., H. Hong, J. Correa, E. Egereva, J. Wiese, J. Imhoff and H. Gross (2012). "Isolation, Structure Elucidation and Total Synthesis of lajollamide A from the Marine Fungus *Asteromyces cruciatus*." Marine Drugs 10: 2912-2935.
[2] Tilburn, J., Scazzocchio, C., Taylor, G. G., Zabicky-Zissman, J. H., Lockington, R. A., & Davies, R. W. (1983). Transformation by integration in *Aspergillus nidulans*. Gene,26(2-3), 205-221
[3] Yelton, M. M., Hamer, J. E., & Timberlake, W. E. (1984). Transformation of *Aspergillus nidulans* by using a trpC plasmid. Proceedings of the National Academy of Sciences, 81(5), 1470-1474

P6

Protective bacterial symbionts from toxic passerine birds from Papua New Guinea

Lena Schweizer [1,2], Kasun H. Bodawatta[3], Knud A. Jønsson[4], Christine Beemelmanns[1,2]

1 Department Anti-infectives from Microbiota, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Saarbrücken, Germany
2 Universität des Saarlandes, Saarbrücken, Germany.
3 Section for Molecular Ecology and Evolution, Globe Institute, University of Copenhagen, Copenhagen, Denmark
4 Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark.

It has been demonstrated that avian species from Papua New Guinea constitute a fascinating source of protective symbionts residing within the uropygial gland (UG) [1,2]. It has been hypothesised that the compounds produced by such symbionts function in the elimination of ectoparasites in the plumage of birds, with a particular emphasis on feather-degrading bacteria [3]. This assertion is supported by the findings of *Amycolatopsis* sp. PS_44, which was isolated from the UG of *P. schelegelii* and was found to produce two new compound families that demonstrated activity against feather-degrading bacteria [1]. This suggests that toxic passerine birds from Papua New Guinea could serve as a valuable source for the development of novel anti-infectives.

A screening process was undertaken in order to identify avian microbes that have the capacity to produce compounds that may be of benefit. The process involved conducting an interaction assay, the objective of which was to investigate their capacity to combat potentially harmful bacteria within the bird itself by growing them in close proximity. The identification of the bacteria was achieved by means of 16S rRNA sequencing.

It was demonstrated that all strains exhibited activity against at least one other strain, with seven strains displaying activity against over 30% of the tested combinations. The majority of these strains belonged to the genus *Curtobacterium*. Furthermore, five strains were classified within the rare genera *Calidifontibacter*, *Tersicoccus* and *Frondihabitans*.

The findings of this study suggest that toxic passerine birds from Papua New Guinea may serve as a valuable source for the discovery of novel anti-infectives and the exploration of rare and under-explored genera. This observation enhances the prospects of identifying bioactive natural products.

References

[1] Seibel, E., Um, S., Bodawatta, K. H., Komor, A. J., Decker, T., Fricke, J., Murphy, R., Maiah, G., Iova, B., Maus, H., Schirmeister, T., Jønsson, K. A., Poulsen, M., & Beemelmanns, C. (2024). Bacteria from the Amycolatopsis genus associated with a toxic bird secrete protective secondary metabolites. *Nature communications*, *15*(1), 8524. https://doi.org/10.1038/s41467-024-52316-3

[2] Bodawatta, K. H., Hu, H., Schalk, F., Daniel, J. M., Maiah, G., Koane, B., Iova, B., Beemelmanns, C., Poulsen, M., & Jønsson, K. A. (2024). Multiple mutations in the Nav1.4 sodium channel of New Guinean toxic birds provide autoresistance to deadly batrachotoxin. Molecular ecology, 33(9), e16878. https://doi.org/10.1111/mec.16878

[3] Alt, G., Mägi, M., Lodjak, J., & Mänd, R. (2020). Experimental study of the effect of preen oil against feather bacteria in passerine birds. *Oecologia*, *192*(3), 723–733. https://doi.org/10.1007/s00442-020-04599-8

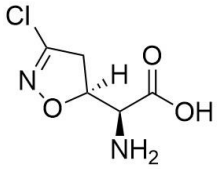
P7

Identification of the biosynthetic gene cluster of acivicin

Mathilde Büttner, Florian Marzec, Matheus Regis Belisário-Ferrari, Leonard Kaysser

Department of Pharmaceutical Biology, Institute for Drug Discovery, University of Leipzig, Germany

Acivicin was firstly described in 1972 after isolation from *Streptomyces sviceus* by Haňka and Dietz [1]. It is a glutamine analog with anti-tumor activity that has been widely studied and has shown activity against various types of cancer [2, 3]. Despite these promising properties it can not be used for therapy due to its high neurotoxicity [3]. Nevertheless, the compound remains interesting due to its unusual structure and likely unique biosynthetic pathway.



Acivicin

Feeding experiments by Gould and co-workers in the 1980s revealed that L-ornithine serves as the starter unit for acivicin, along with potential intermediates, establishing the foundation for a first biosynthetic model [4, 5]. However, further details on the formation of this unique compound is not yet known. Here, we describe an approach to identify and characterise the biosynthetic gene cluster of acivicin, including an in silico genome analysis, preparation of a fosmid library, development of an extraction method for LC-MS analysis, and heterologous expression of the compound.

References

[1] Hanka LJ, Dietz A. U-42, 126, a new antimetabolite antibiotic: production, biological activity, and taxonomy of the producing microorganism. Antimicrob Agents Chemother 1973; 3: 425–431; DOI: 10.1128/aac.3.3.425

[2] Kreuzer J, Bach NC, Forler D, Sieber SA. Target discovery of acivicin in cancer cells elucidates its mechanism of growth inhibition†Electronic supplementary information (ESI) available: Synthesis, cloning, protein expression, purification and biochemical assays. See DOI: 10.1039/c4sc02339k. Chem Sci 2014; 6: 237–245; DOI: 10.1039/c4sc02339k

[3] Earhart RH, Neil GL. Acivicin in 1985. Advances in Enzyme Regulation 1985; 24: 179–205; DOI: 10.1016/0065-2571(85)90076-7

[4] Gould SJ, Ju S. Biosynthesis of acivicin. 3. Incorporation of ornithine and N.delta.-hydroxyornithine. J Am Chem Soc 1992; 114: 10166–10172; DOI: 10.1021/ja00052a012

[5] Gould SJ, Ju S. The biosynthesis of acivicin and 4-hydroxyacivicin from N.delta.-hydroxyornithine. J Am Chem Soc 1989; 111: 2329–2331; DOI: 10.1021/ja00188a072

P8

Exploring the Role of Ribosomal Peptides in the Human Lung Microbiome

Florian Semmler[1], Maria Kulosa[1], Leonard Kaysser[1]

1 Department of Pharmaceutical Biology, Institute for Drug Discovery, University of Leipzig, Eilenburger Straße 14, 04317 Leipzig, Germany.

Following initial difficulties in detecting microorganisms, the human lung has become a well-established bacterial colonisation site with a distinct microbiome that differs from that of other sites in the respiratory tract [1–4]. However, research into bacterial metabolites, which are often involved in the complex interactions within these bacterial communities and with the host, is lacking, despite existing for many other microbiota. Ribosomally synthesised and post-translationally modified peptides (RiPPs) have already proven to be natural products with a variety of biological activities, and are therefore a key factor in microbiome regulation and interaction. [5–8]

In this study, we utilised both precursor-centric and PTM-centric tools to detect a total of 4544 ribosomal peptides from the metagenome data of the lung microbiome. We provided a more detailed description of the distribution of the different RiPP classes and demonstrated that putative RiPP candidates expand the chemical space. Furthermore, we examined two specific groups — ranti-peptides and autoinducing peptides (AIPs) — in greater detail, investigating their potential role in lung microbiome communication. We used an established synthetic bioinformatic natural product (syn-BNP) strategy [5] to predict 14 possible AIPs, five of which we then chemically synthesised and tested for activity using an artificial lung microbiome model. Thus, the study makes an important contribution to our understanding of the natural function of RiPPs in the human microbiota, establishing the lung microbiome as a prolific source of promising secondary metabolites.

References

[1] Stubbendieck RM, Zelasko SE, Safdar N, Currie CR. Biogeography of Bacterial Communities and Specialized Metabolism in Human Aerodigestive Tract Microbiomes. Microbiol Spectr 2021; 9: e0166921; DOI: 10.1128/Spectrum.01669-21
[2] Li Y, Pan G, Wang S, Li Z, Yang R, Jiang Y, Chen Y, Li SC, Shen B. Comprehensive human respiratory genome catalogue underlies the high resolution and precision of the respiratory microbiome. Brief Bioinform 2024; 26; DOI: 10.1093/bib/bbae620
[3] Dickson RP, Erb-Downward JR, Freeman CM, McCloskey L, Falkowski NR, Huffnagle GB, Curtis JL. Bacterial Topography of the Healthy Human Lower Respiratory Tract. mBio 2017; 8; DOI: 10.1128/mbio.02287-16
[4] Dickson RP, Erb-Downward JR, Freeman CM, McCloskey L, Beck JM, Huffnagle GB, Curtis JL. Spatial Variation in the Healthy Human Lung Microbiome and the Adapted Island Model of Lung Biogeography. Ann Am Thorac Soc 2015; 12: 821–830; DOI: 10.1513/AnnalsATS.201501-029OC
[5] Zhang J, Zhang D, Xu Y, Zhang J, Liu R, Gao Y, Shi Y, Cai P, Zhong Z, He B, Li X, Zhou H, Chen M, Li Y-X. Large-scale biosynthetic analysis of human microbiomes reveals diverse protective ribosomal peptides. Nat Commun 2025; 16: 3054; DOI: 10.1038/s41467-025-58280-w
[6] Telhig S, Ben Said L, Zirah S, Fliss I, Rebuffat S. Bacteriocins to Thwart Bacterial Resistance in Gram Negative Bacteria. Front Microbiol 2020; 11: 586433; DOI: 10.3389/fmicb.2020.586433.
[7] Bjarnsholt T, Ciofu O, Molin S, Givskov M, Høiby N. Applying insights from biofilm biology to drug development - can a new approach be developed? Nat Rev Drug Discov 2013; 12: 791–808; DOI: 10.1038/nrd4000.
[8] Benjdia A, Berteau O. Sulfatases and radical SAM enzymes: emerging themes in glycosaminoglycan metabolism and the human microbiota. Biochem Soc Trans 2016; 44: 109–115; DOI: 10.1042/BST20150191.

P9

Analysis of the CRISPR-Cas Region 1 in *Streptoalloteichus tenebrarius*

Annika Bruntner[1], Lena Mitousis[1], Wolfgang Wohlleben[1]

1 Interfaculty Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbiology/Biotechnology, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany

CRISPR-Cas is primarily known as an immunity defence system against foreign DNA, but the involvement in processes like cell-differentiation and DNA-repair has also been described. In approximately 50% of all Actinobacteria genomes CRISPR-Cas systems are present. However, to date, these systems have been experimentally characterized in only two actinomycete strains and a lot still remains unknown [1]. During the optimization process of the industrial strain *Streptoalloteichus tenebrarius* for enhanced tobramycin production, the strain could only be genetically manipulated with an extremely low frequency [2]. Subsequent bioinformatic analysis revealed that *S. tenebrarius* harbors three CRISPR-Cas systems (Region 1-3) in its genome.

This project focuses on the analysis of the CRISPR-Cas Region 1 in *Streptoalloteichus tenebrarius*. This region is categorized as a Class 1 Type I-E system, characterized by possessing multi-subunit crRNA-effector complexes with multiple Cas proteins. Previous experiments involving the deletion of the entire CRISPR-Cas Region 1 showed that *S. tenebrarius* lost its ability to sporulate. Furthermore, also the ability to produce antibiotics was affected. To characterize the CRISPR-Cas Region 1 in more detail, the *cas3* gene, predicted to encode an enzyme with helicase and DNase activity, was specifically deleted. The deletion resulted in the isolation of five independent mutants with a sporulation-deficient phenotype and the loss of antibiotic production. Genomes of various deletion mutants as well as the parental strain were sequenced, assembled, annotated and further analyzed with comparative genomics tools in order to identify genes responsible for the unexpected phenotype.

In summary, this study aims to elucidate the role of the CRISPR-Cas Region 1, with a particular focus on Cas3, in the regulation of sporulation and antibiotic production in *Streptoalloteichus tenebrarius* and to gain further insights on the function of CRISPR-Cas systems beyond their immunity functions.

References
[1] Mitousis, L. *et al.* microLife. 2025, 6. https://doi.org/10.1093/femsml/uqaf010
[2] Mitousis, L. *et al.* Molecules 2021, 26(14), 4343. https://doi.org/10.3390/molecules26144343

P10

Unexpected occurrence of marine secondary metabolites in inland waters of post-mining lakes in north-west of Czechia

Lenka Štenclová[1,2*], Kumar Saurav[3], Jan Mareš[1,3], Petra Urajová[3], Andreja Kust[4], Kateřina Čapková[1], Klára Řeháková[1]

[1] Institute of Hydrobiology, Czech Academy of Sciences, České Budějovice, Czech Republic
[2] Institute of pharmacy, Free University of Berlin, Berlin, Germany
[3] Institute of Microbiology, Czech Academy of Sciences, Třeboň, Czech Republic
[4] Innovative Genomics Institute, University of California, Berkeley, USA

**lenka.stenclova@fu-berlin.de*

Tropical oceans are well-known hotspots of mat-forming filamentous cyanobacteria from the order Oscillatoriales (sensu lato), the producers of a wide range of secondary metabolites. Among them bioactive oligopeptides and depsipeptides are in the special interest. The dolastatins and their derivatives, a group of highly cytotoxic oligopeptides were as well for forty years regarded to be only of marine origin. Recently, dolastatin 10 derivatives aetokthonostatins were discovered to be produced by the invasive cyanobacterium *Aetokthonos hydrillicola* occurring in fresh-water reservoirs of sub-tropical United States[1]. The discovery triggered the question, whether can be other producers of such compounds inhabiting further fresh-water ecosystems.
Our metabolomic investigation (HPLC-HRMS/MS with GNPS comparison) of algal mats of inland waters of post-mining lakes in the north-west of Czechia determined production of a rich spectrum of cyanobacterial secondary metabolites. The most prominent group were oligopeptides known to be produced by tropical marine Oscillatoriales, including dolastatin 1, 14 and G derivatives. Their presence was partly partially confirmed by the MS/MS spectra (fragmentation analysis).
Originally, dolastatins were described from community samples and producers identified as taxa of the genera *Lyngbya* and *Symploca.* However, the genera are highly polyphyletic due to frequent morphological convergence in the oscillatorialean group. By recent metagenomic research the actual producers of dolastatin 10 – *Caldora penicillata*[2] and dolastatin 11 derivatives lyngbyastatins – *Okeania* sp.[3] were identified with the phylogenetic position retrieved. Such information for the dolastatin 1, 14 and G and their derivatives is still missing. Complementary analysis of V4-V5 16S rRNA amplicons was conducted here and identified 30 amplicon sequence variants (ASVs) belonging to the order Oscillatoriales. Their phylogenetic analysis found previously undetected lineages e.g. from the marine groups Sirenicapillariaceae and Vermifilaceae. Present and following research can lead to the determination of the actual producers of less examined dolastatin derivatives and tracing of their impact on the fresh-water environment.

Keywords: algal mats, cyanobacteria, cytotoxicity, dolastatins, oligopeptides

References
1. Schwark., Martínez-Yerena., *PNAS,* **2023**, 120(40). 10.1073/pnas.2219230120
2. Kallifidas., Dhakal., *Organic letters*, **2024**, 26(7), 1321-1325. 10.1021/acs.orglett.3c04083
3. Dhakal., Kokkaliari., *Journal of Natural Products*, **2023**, 86(1), 85-93. 10.1021/acs.jnatprod.2c00782

Acknowledgements and Funding
Thanks are due to Czech Science Foundation (GAČR 19-05791S) and Free University of Berlin (Rising Star – Junior Fellowship in the Department of Biology, Chemistry, Pharmacy).

P11

Direct Cloning of Metagenomic BGCs for Broad-Host Expression and Natural Product Discovery

Shreyash Pramod Borkar[1,2], Lena Schilling[1], Rico Czaja[1]

1 c-LEcta GmbH, Alte Messe 3, 04103, Leipzig, Germany
2 University of Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany

We present a scalable method for direct cloning of biosynthetic gene clusters (BGCs) from genomic and metagenomic DNA, enabling targeted recovery and expression of cryptic natural product pathways in different hosts. Traditional metagenomic approaches rely on random library construction and functional screening, which are inefficient, costly, and often yield fragmented or non-functional BGCs.

Our method adapts the CAPTURE protocol (*1*), originally developed for targeted BGC cloning from cultured strains, to work directly with metagenomic DNA. Unlike CAPTURE, which relies on CRISPR-based excision, our approach uses PCR amplification, more suitable for metagenomic samples where DNA complexity and uneven BGC distribution make CRISPR impractical. We optimized PCR conditions to accommodate high-GC content and complex templates, ensuring high fidelity and successful amplification of long PCR fragments.

Using next-generation sequencing and genome mining, we generate in-silico libraries of predicted BGCs from metagenomic samples. These clusters are selectively amplified and cloned into a modified broad-host vector system. In addition to the BAC ori, which enables cloning and maintenance of large inserts, the vector allows parallel expression in *E.coli*, *Bacillus sp.*, *Streptomyces sp.* and various gram-negative organisms. . Dual antibiotic markers support selection across hosts.

We have successfully cloned over 20 BGCs from genomic and metagenomic DNA, including a 20 kb cluster. We next plan to in parallel clone around 50 BGCs in a medium-throughput format. The method also allows stitching together multiple ~10 kb fragments, enabling cloning of larger BGCs (≥40kb),extending applicability to diverse BGC classes.

We next aim to test expression in *E.coli, Burkholderia sp.*, *Bacillus sp.*, and *Streptomyces sp.*as examples of both Gram-negative and Gram-positive bacteria. This approach streamlines functional metagenomic mining, enabling mid-throughput cloning and expression of BGCs from environmental DNA, and accelerating the discovery of novel bioactive compounds.

References

1. Enghiad, B.; Huang, C.; Guo, F.; Jiang, G.; Wang, B.; Tabatabaei, S. K.; Martin, T. A.; Zhao, H. Cas12a-assisted precise targeted cloning using in vivo Cre-lox recombination. *Nat Commun [Online]* **2021,** *12* (1), 1171. https://www.nature.com/articles/s41467-021-21275-4.

P12

From Genes to Molecules – Predicting the Structure of Glycopeptides from Biosynthetic Gene Clusters

Noel Kubach[1], Nadine Ziemert[1,2]

1 Translational Genome Mining for Natural Products, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), Interfaculty Institute for Biomedical Informatics (IBMI), University of Tübingen, Auf der Morgenstelle 24, 72076 Tübingen, Germany
2 German Center for Infection Research (DZIF), Partner Site Tübingen, Germany

An important field for discovering new drugs and antibiotics is genome mining for natural products (NPs). A variety of bioinformatics tools were developed around the concept of biosynthetic gene clusters (BGCs), in which most genes for the production of NPs are organized [1]. Tools like antiSMASH [2], ARTS [3], and PRISM [4] can identify BGCs (antiSMASH), predicting their products (PRISM), and prioritizing potential new antibiotics by searching for resistance genes (ARTS). The new tools complement traditional screening methods and enable searching for potential drugs even in metagenomes of uncultured organisms.

Glycopeptides (GPs) are a class of natural products with mostly antibacterial activity. GPs consist of a peptide backbone, synthesized by modular non-ribosomal peptide synthetases (NRPS), which is modified by *tailoring enzymes* that introduce crosslinks between aromatic side chains and add various functional groups, including the eponymous glycosyl residues [5]. The tailoring enzymes include P450 monooxygenases that introduce biaryl or biaryl ether crosslinks, methyltransferases that N-, O-, or C-methylate amino acids, halogenases that chlorinate aromatic residues, acyltransferases that introduce various acyl chains to the molecules, and glycosyltransferases which perform glycosylation with different glycosyl residues.

As part of the AVOGADRO project funded by JPIAMR, a tool for predicting the products of glycopeptide biosynthetic gene clusters is being developed in the project presented here. Within AVOGADRO, the predicted structures will be tested in the future in molecular docking simulations to prioritize BGCs that produce novel antibiotics for expression in wet lab experiments. The development of the prediction tool is divided into three stages: (1) setting up a database for BGCs, orthologous groups of enzymes, and known reactions. (2) Annotating the orthologous groups of enzymes in the database with the reactions they perform. (3) Using *in silico* reactions to infer the products of clusters without a known product.

Publicly available resources, such as the MIBiG database for experimentally characterized BGCs [6] and the recently developed database for characterized tailoring enzymes MITE [7], as well as data from the AVOGADRO collaboration partners are used to annotate homologous groups of tailoring enzymes. The reactions catalyzed by the enzymes are stored in the database in the machine readable format SMILES, which can be used with the cheminformatics platform RDKit to simulate *in silico* reactions and synthesis pathways. Clustering the enzymes of uncharacterized BGCs with annotated enzymes from the database will be used to predict the structure(s) of products that might be produced. For the reaction annotation, the prediction of products, and the design of new to nature BGCs with novel products, an interactive web frontend is being developed, which will facilitate reaction annotations and visualize possible synthesis pathways of a BGC.

Within AVOGADRO, the predicted structures will be confirmed by wet lab experiments and subject to molecular docking simulations to discover the antibiotics of tomorrow.

References

[1] Ziemert, Nadine et al. 2016. *Natural Product Reports* 33 (8): 988–1005. https://doi.org/10.1039/C6NP00025H
[2] Blin, Kai et al. 2025. *Nucleic Acids Research*, April, 1–7. https://doi.org/10.1093/nar/gkaf334
[3] Mungan, Mehmet D. et al. 2020. *Nucleic Acids Research* 48 (W1): W546–52. https://doi.org/10.1093/nar/gkaa374
[4] Skinnider, Michael A . et al. 2017. *Nucleic Acids Research* 45: W49. https://doi.org/10.1093/NAR/GKX320
[5] Yim, Grace et al. 2013. *The Journal of Antibiotics 2014 67:1* 67 (1): 31–41. https://doi.org/10.1038/ja.2013.117
[6] Zdouc, Mitja M. et al. 2025. *Nucleic Acids Research* 53 (D1): D678–90. https://doi.org/10.1093/NAR/GKAE1115
[7] Zdouc, Mitja M. et al. 2025. *ChemRxiv (Preprint)*, August. https://doi.org/10.26434/chemrxiv-2024-78mtl-v2

P13

Discovery and in vitro reconstitution of closoxazole biosynthesis from *Pyxidicoccus fallax*

Lucia Lernoud[1], Rimonda Jakob[1], Lars Warnick[1], Marie Luisa Marx[1], Lea Winand[1]

1 Department of Biochemical and Chemical Engineering, Laboratory of Technical Biology, TU Dortmund University, Emil-Figge-Str. 66, 44227 Dortmund, Germany

Benzoxazoles are crucial structural motifs found in numerous bioactive natural products and pharmaceutical compounds, such as the insomnia medication suvorexant or the transthyretin stabilizer tafamidis.[1,2] In recent years, several benzoxazole natural products have been discovered. In this study, a putative benzoxazole biosynthetic gene cluster from the myxobacterium *Pyxidicoccus fallax* is investigated. Sequence homology analysis suggests that this cluster is responsible for the biosynthesis of closoxazoles, a class of 3,4-AHBA derived compounds previously identified in the anaerobic bacterium *Clostridium cavendishii*.[3] To verify this hypothesis, we reconstituted the closoxazole biosynthetic pathway of *P. fallax* in vitro. Our results confirm the involvement of the indentified gene cluster in closoxazole. The pathway includes two AMP-ligases, which catalyze the adenylation and ligation of aryl carboxylic acid building blocks, and a condensing amidohydrolase that performs the heterocyclization delivering the benzoxazole scaffold. Functional characterization of these enzymes revealed substrate flexibility, enabling the generation of several novel closoxazole derivatives through precursor directed biosynthesis in an engineered *Escherichia coli* strain.[4]
These findings not only validate the function of the gene cluster in *P. fallax* but also shed light on the enzymatic mechanisms underlying benzoxazole formation. The widespread occurrence of benzoxazole biosynthesis across diverse bacterial phyla suggests convergent evolutionary solutions for constructing the benzoxazole scaffold. Our work provides a foundation for future structural and mechanistic studies of the involved enzymes and paves the way for biocatalytic or synthetic biology approaches to access novel benzoxazole-based compounds.

References
[1] L. P. H. Yang, *Drugs* **2014**, *74*, 1817.
[2] J. Park, U. Egolum, S. Parker, E. Andrews, D. Ombengi, H. Ling, *Ann. Pharmacother.* **2020**, *54*, 470.
[3] T. Horch, E. M. Molloy, F. Bredy, V. G. Haensch, K. Scherlach, K. L. Dunbar, J. Franke, C. Hertweck, *Angew. Chem., Int. Ed. Engl.* **2022**, *61*, e202205409.
[4] L. Lernoud, R. Jakob, L. Warnick, M. L. Marx, L. Winand, *ChemBioChem* **2025**, *26*, e202500126.

P14

Antimycobacterial Lasso Peptides – Identification, Production, and Opportunities in Drug Development & Synthetic Biology

Lorenz Thost[a], Dr. Panagiota-Hanna Koutsandrea[a], Jun.-Prof. Dr. Silja Mordhorst[a]

[a] Department of Pharmaceutical Biology, University of Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany

Lasso peptides are ribosomally synthesized and post-translationally modified peptides (RiPPs), which exhibit high metabolic stability and bioavailability [1]. These properties make them promising drug candidates. Some lasso peptides, such as lassomycin [2] and kitamycobactin [3], demonstrate potent activity against *Mycobacterium tuberculosis*, a pathogenic bacterium causing over one million deaths each year [4]. The aim of this project is to identify novel lassomycin-like lasso peptides and explore ways to modify them for enhanced effectivity against mycobacteria. For this purpose, a broad methodology is utilized, including bioinformatic tools, molecular cloning strategies as well as biochemical analytics.

In the present work, several actinobacteria encoding lassomycin-like biosynthetic gene clusters (BGCs) were identified through extensive genome mining. Production of lasso peptides in the native host is often limited; therefore, a more promising approach is the heterologous expression of these BGCs. Selected BGCs from different actinobacteria were cloned into appropriate plasmids and transferred to suitable expression hosts for cultivation and lasso peptide production.

Beside genes typically found in lasso peptide BGCs, which encode a precursor peptide, a macrolactam synthetase, a peptidase and an ABC transporter, lassomycin-like BGCs contain a gene that encodes an *O*-methyltransferase [2]. These enzymes methylate the *C*-terminal carboxyl group of precursor peptides, but the exact function of the resulting methyl ester and the substrate scope of *O*-methyltransferases remain unknown. Thus, the investigation of *O*-methyltransferases associated with lasso peptide BGCs is another main focus of this project, as its potential in increasing the activity of antimycobacterial lasso peptides can be expanded as a general tool in synthetic biology.

References

[1] Montalbán-López, M. *et al.* Nat. Prod. Rep. 2021, 38, 130–239. https://doi.org/10.1039/D0NP00027B

[2] Gavrish, E. *et al.* Chem Biol 2014, 21, 509–518. https://doi.org/10.1016/j.chembiol.2014.01.014

[3] Quigley, J. *et al.* mBio 2020, 11, 01516-20. https://doi.org/10.1128/mbio.01516-20

[4] Global tuberculosis report 2024. Geneva: World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO.

P15

Discovery and Biosynthesis of Celluxanthenes, Antibacterial Arylpolyene Alkaloids From Diverse Cellulose-Degrading Anaerobic Bacteria

Keishi Ishida[1+], Jana Krabbe[1+], Philippe R. Meisinger[1], Gulimila Shabuer[1], Sebastian Schieferdecker[1], Michael Cyrulies[2], Cedric Tank,[2] Emma Barnes[1], Christian Paetz[3], and Christian Hertweck[1,4]

1 Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), 07745, Jena, Germany
2 BioPilot Plant, Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), 07745, Jena, Germany
3 Research Group Biosynthesis/NMR, Max Planck Institute for Chemical Ecology 07745, Jena, Germany
4 Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University Jena 07743, Jena, Germany

+ these authors have contributed equally to this work

Cellulose degradation by anaerobic bacteria plays an eminent role in the global carbon cycle and is a critical step in biofuel production.[1,2] The anaerobic thermophile Clostridium thermocellum (now: Acetivibrio thermocellus) is particularly efficient at breaking down biomass and produces a “yellow affinity substance” (YAS), a pigment that has been implicated in signaling and conferring higher affinity of the cellulosome to YAS-loaded cellulose.[3] However, the nature and biosynthetic origin of YAS have remained elusive. Here, we show by isolation and structure elucidation that YAS is a complex of unusual arylpolyene alkaloids (celluxanthenes). Stable isotope labeling experiments reveal all biosynthetic building blocks for celluxanthene assembly. Through a targeted gene deletion, we identify the celluxanthene (cex) biosynthesis gene cluster and propose a biosynthetic model in which an arylpolyene generated by an iterative type I polyketide synthase (PKS) undergoes a head-to-head fusion with a tryptophan-derived ketoacid to form a tetronate. Genome mining and metabolic profiling revealed that diverse cellulolytic anaerobes harbor cex gene loci and produce celluxanthene congeners. Celluxanthenes show antibiotic activity against Gram-positive bacteria including clinically relevant strains. This study solves the long-standing enigma surrounding the nature of YAS and lays the groundwork for elucidating the precise biological roles of these intricate pigments.

References

[1] D. P. Delmer, C. H. Haigler, Metab. Eng. 2002, 4, 22–28. https://doi.org/10.1006/mben.2001.0206
[2] G. P. Robertson, S. K. Hamilton, B. L. Barham, B. E. Dale, R. C. Izaurralde, R. D. Jackson, D. A. Landis, S. M. Swinton, K. D. Thelen, J. M. Tiedje, Science 2017, 356, eaal2324. DOI: 10.1126/science.aal2324
[3] L. G. Ljungdahl, M. P. Coughlan, F. Mayer, Y. Mori, H. Hon-nami, K. Hon-nami, Methods Enzymol. 1988, 160, 483–500. https://doi.org/10.1016/0076-6879(88)60158-3

P16

Engineering NP discovery – Miniaturization and Parallelization for Multivariate OSMAC

Till Steinmetz[1], Anton Lindig[1], Georg Hubmann[1], Stephan Lütz[1]

1 Chair for Bioprocess Engineering, TU Dortmund University, Emil-Figge-Strae 66, 44227 Dortmund, Germany.

The discovery of bacterial natural products (NPs) is often hindered by the repeated isolation of known compounds and the challenge of activating silent biosynthetic gene clusters. Cultivation conditions are a critical factor influencing secondary metabolite (SM) production, yet an in-depth analysis and comparison of cultivation systems for NP discovery has not been carried out so far. With this in mind, we evaluated the metabolic footprints of four bacterial species, *Bacillus amyloliquefaciens*, *Corallococcus coralloides*, *Streptomyces griseochromogenes*, and *Streptomyces cattleya*, grown in microtiter plate cultivation systems (MPCS), shake flasks, and stirred tank bioreactors (STR). Using untargeted LC-MS/MS metabolomics and molecular networking, we assessed the generated mass features (MFs) as proxies for SM production across cultivation modes.

While biomass formation was comparable in most cultivation systems, a considerable difference in secondary metabolite production was observed. Unicellular bacteria such as *B. amyloliquefaciens* showed only minor effects from the cultivation system, whereas filamentous actinobacteria displayed stronger differences, with maximal MF overlap between systems reaching only 31%. MPCS, particularly 48-well flower plates, supported the broadest SM diversity and showed the closest similarity to STR profiles, outperforming traditional shake flasks in both quantity and diversity of metabolites detected. The detected SMs and their derivatives exhibited structural modifications depending on the cultivation system. A comparison of the *S. griseochromogenes* NP profile revealed that MPCS yielded less divergent SM formation than shake flasks.

Our comprehensive assessment is the first to demonstrate the impact of cultivation systems on the bacterial metabolic footprint, confirming that MPCS provide a robust platform for parallelized bacterial cultivation, enhancing the discovery of NPs and expanding access to chemical diversity.

[1] Lindig, A. *et al*. Biotechnol. Bioeng. 2025; 122:2021–2036 https://doi.org/10.1002/bit.29002

P17

Adapting Versatile Opportunities for Glycopeptide Antibiotics against Drug-Resistant Organisms

Anna Katharina Siegert[1], Noel Kubach[2], Nadine Ziemert[2], Evi Stegmann[1],

1 Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, 72076 Tübingen, Germany
2 Translational Genome Mining for Natural Products, IMIT, Interfaculty Institute for Biomedical Informatics (IBMI), University of Tübingen, 72076 Tübingen, Germany

Glycopeptide antibiotics (GPAs), such as vancomycin, are crucial for treating infections caused by Gram-positive bacteria. However, the emergence of resistance - often through modification of the lipid II target D-Ala-D-Ala to D-Ala-D-Lac to evade antibiotic binding - demands the development of new therapeutic strategies.[1]
Produced by actinomycetes, GPAs exhibit high structural diversity and undergo complex biosynthesis involving non-ribosomal peptide synthetases (NRPSs), oxidative cascades, and diverse post-synthetic modifications. Based on their structures, GPAs are classified into four main types, with recently discovered atypical type V members displaying unique architectures and mechanisms.[2]
NRPSs generate the linear peptide backbone from both proteinogenic and non-proteinogenic amino acids. P450-mediated oxidative cyclization establishes a rigid, cross-linked scaffold[3]. Additional modifications can include halogenation, sulfation, glycosylation, and acylation.[1]

Sulfation, a rare GPA modification, can significantly expand the antibacterial spectrum by circumventing resistance mechanisms.[4] Notably, two recently identified sulfated GPAs showed selective activity against Gram-negative pathogens.[5]
The project aims to develop next-generation GPAs with improved and expanded activity against resistant Gram-positive and Gram-negative bacteria. Key objectives include genome mining for novel GPA biosynthetic gene clusters (BGCs) and modification enzymes, production and isolation of candidate compounds, and detailed structural and functional characterization. As chassis for generating novel GPAs, we focus on *Amycolatopsis balhimycina* and *Amycolatopsis japonicum*, which produce the type I GPA balhimycin and the type III GPA ristomycin, respectively. Our synthetic biology approach involves introducing targeted mutations in both producers and evaluating their effects on peptidoglycan biosynthesis, lipid II affinity, and antibacterial activity.
Preliminary genome mining has identified three BGCs encoding putative novel GPA structures, including sulfotransferases. Sulfotransferases require the cofactor 3'-phosphoadenosine-5'-phosphosulfate (PAPS), which serves as a sulfate donor.[6] To generate a platform for producing novel sulfated GPAs, we introduce PAPS biosynthesis genes into both producer strains. For this purpose, we utilize PAPS encoding genes from *S. toyocaensis*, which are responsible for sulfate transfer.

References
[1] Stegmann, E. et al. Curr. Opin. Microbiol. 13, 595–602 (2010) https://doi.org/10.1016/j.mib.2010.08.011
[2] Gavriilidou, A. et al. bioRxiv (2023) https://doi.org/10.1101/2023.02.10.526856
[3] Hansen, M. H. et al. Curr. Opin. Biotechnol. 77, 102767 (2022) https://doi.org/10.1016/j.copbio.2022.102767
[4] Oluwole, A. O. et al. Nat. Commun. 13, 2278 (2022) https://doi.org/10.1038/s41467-022-29836-x
[5] Öster, C. et al. Chem. Sci. 9, 8850–8859 (2018) https://doi.org/10.1039/C8SC03655A
[6] Burkart, M. et al. J. Org. Chem. 65, 5565-5574 (2000) https://doi.org/10.1021/jo000266o

P18

Enhancing natural products extraction: using metabolomics for evaluating analytical methods

Elisa de Lannoy Guerra[1], Simon Cremers[2], Felix Penth[3], Daniela Becher[3], Ronald Garcia[2], Alexander Voltz[2], Sebastian Walesch[2], Chantal Bader[2], Daniel Krug[1,2], Rolf Müller[1,2]

1 Universität des Saarlandes, Department of Pharmacy, Campus E8.1, 66123 Saarbrücken, Germany
2 Department of Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI) and Department of Pharmacy at Saarland University, Campus E8.1, 66123 Saarbrücken, Germany
3 MyBiotech GmbH, Industriestraße 1b, 66802 Überherrn, Germany

Microbial natural products are historically known for their role in antibiotic discovery and potential for other drugs development [1]. The success of isolating new microbial natural products depends not only on enhancing biotechnology cultivation techniques, but also on extraction and analytical methods [2]. From this perspective, alternative methods are being explored both to maximize the extraction efficiency of secondary metabolites and to achieve environmentally friendly processes. To assess if a greener technique can substitute or complement traditional approaches, we employ here a metabolomics-based approach for evaluating alternative methodologies from the natural products perspective. In this context, techniques that aim to diminish the quantity of solvent use, and therefore being eco-friendlier, were chosen for the current study. This poster outlines two experimental setups in which metabolomics is used to evaluate extraction and analytical analysis of bacterial crude extracts, respectively. To address the extraction process, a microreactor is being tested for facilitating the extraction of endometabolites by disrupting cells without the need of solvent use. For the analytical step, a supercritical fluid chromatography method – UltraPerformance Convergence Chromatography (UPC²) – coupled to mass spectrometry (MS) is compared to a conventional Ultra-High Performance Liquid Chromatography (UHPLC) coupled to mass spectrometry-system for analysing myxobacterial crude extracts.

This poster is part of a project that has received funding from the European Union Horizon Europe MSCA Doctoral Network programme under EC Grant Agreement 101120061 and by UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee [grant number EP/Y032039/1].

References

[1] Hutchings MI, Truman AW, Wilkinson B. Antibiotics: past, present and future. Curr Opin Microbiol. 2019;51:72–80. doi:10.1016/j.mib.2019.10.008

[2] Bader CD, Neuber M, Panter F, Krug D, Müller R. Supercritical fluid extraction enhances discovery of secondary metabolites from myxobacteria. Anal Chem. 2020;92(23):15403–11. doi:10.1021/acs.analchem.0c02995

P19

Identification of novel secondary metabolites from rare actinomycetes through genome mining and heterologous expression approaches

Mareike Danne[1], Luca Salvi[1], Christian Geibel[1], Margherita Sosio[2], Stefano Donadio[2], Chambers Connor Hughes[1], Evi Stegmann[1]

1 Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany
2 NAICONS Srl, Milan, Italy

Actinomycetes have long been known as prolific producers of bioactive secondary metabolites, particularly antibiotics [1]. While the biosynthetic capacity of the genus *Streptomyces* is well characterized [2], comparatively little attention has been given to “rare” actinomycetes. Recent advances in genome sequencing and bioinformatics have revealed that these taxonomically diverse genera harbor remarkable potential for natural product discovery and chemical diversity [3], [4]. However, the genetic intractability of rare actinomycetes often hampers the activation of biosynthetic gene clusters (BGCs) in the native producers, leaving much of their metabolic capacity unexplored. This highlights the need for innovative genetic tools and heterologous expression strategies.

In this study, we employed the CAPTURE cloning procedure [5] to clone two previously uncharacterized BGCs from the rare actinomycete *Nonomuraea* sp. and introduced them into four different heterologous hosts. AntiSMASH analysis predicted one BGC to encode the synthesis of an NRPS/T1PKS hybrid compound and the other of an azole-containing RiPP. We are currently screening and analysing the metabolomic profiles of the recombinant strains.

This approach facilitates the access to BGCs that remain silent in their native producers, and it paves the way for the discovery of novel bioactive natural compounds, including new antibiotics, from underexplored microbial sources.

References

[1] Hutchings M.I. *et al.* Curr Opin Microbiol 2019, 51, 72–80, https://doi.org/10.1016/j.mib.2019.10.008

[2] Gavriilidou A. *et al.*, Nat Microbiol 2022, 7, 5, 726–735, https://doi.org/10.1038/s41564-022-01110-2

[3] Parra J. *et al.,* Curr Opin Microbiol 2023, 76, 102385, https://doi.org/10.1016/j.mib.2023.102385

[4] Ezeobiora C.E *et al.*, Futur J Pharm Sci 2022, 8, 1, 23, https://doi.org/10.1186/s43094-022-00410-y

[5] Enghiad B. *et al.*, Nat Commun 2021, 12, 1, 1171, https://doi.org/10.1038/s41467-021-21275-4

P20

Improved biopolymer production in the genetically recombinant filamentous cyanobacterium *Nostoc* sp. PCC7120 without nitrogen starvation by utilizing an inducible biosynthesis platform

Phillipp Fink[1], Alisa Klemm[1], Kim Gaupp[1], Karl Forchhammer[1]

1 Organismic Interactions Department, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany.

The importance of developing sustainable, carbon dioxide (CO_2)-neutral alternatives to plastic has become evident in the context of global pollution levels. In the last decades, the utilization of heterotrophic bacteria for biodegradable polymer production has greatly contributed to the reduction of fossil fuel-derived plastic [1,2]. A promising candidate for replacing conventional plastic is polyhydroxybutyrate (PHB), a biodegradable polyhydroxyalkanoate produced by a plethora of microorganisms, including heterotrophic bacteria as well as certain cyanobacteria. Phototrophic cyanobacteria convert sunlight and CO_2 to PHB, thereby providing an alternative, eco-friendly, and sustainable production platform [3,4]. However, a major drawback is the need for a two-stage production process, since relevant amounts of PHB are mainly produced after transferring the culture to conditions of nutrient limitation, such as nitrogen starvation. This tedious process hinders large-scale production [5,6].

We have previously shown that *Nostoc* sp. PCC7120, a filamentous, diazotrophic cyanobacterium, constitutes a genetically amenable chassis strain suitable for continuous PHB production. The integration of *phaP1*, encoding an important phasin of the carbonosome in the heterotrophic PHB producer *Cupriavidus necator* H16, contributed to stabilizing PHB production, whilst simultaneously improving cell viability of the recombinant *Nostoc* strain [7].

Nonetheless, by introducing the heterologous biosynthetic pathway into *Nostoc* sp. PCC7120, the metabolic burden of PHB overproduction became more apparent. Prolonged, constitutive PHB overproduction led to the formation of recurring, unwanted, distinguishable filaments linked to decreased PHB granule accumulation in the recombinant *Nostoc* strains.

To reduce the metabolic burden of PHB production and enhance the overall biosynthetic productivity, the chassis strain *Nostoc* sp. PCC7120 was transformed with an enhanced PHB biosynthetic gene cluster, which harbours an inducible hybrid promotor system. This system enables an improved cultivation strategy: first, biomass accumulation can be maximized by favourable growth conditions; subsequently, PHB production is induced by the simple addition of an inducer eliminating the need for nitrogen starvation. Overall, this improved cultivation approach enhances the potential for sustainable, industrial-scale PHB production in cyanobacteria.

References

[1] Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, et al. Plastic waste inputs from land into the ocean. Science. 2015 Feb 13;347(6223):768–71

[2] Reinecke F, Steinbüchel A. Ralstonia eutropha Strain H16 as Model Organism for PHA Metabolism and for Biotechnological Production of Technically Interesting Biopolymers. Journal of Molecular Microbiology and Biotechnology. 2008 Oct 29;16(1–2):91–108

[3] Z. Naser A, Deiab I, M. Darras B. Poly(lactic acid) (PLA) and polyhydroxyalkanoates (PHAs), green alternatives to petroleum-based plastics: a review. RSC Advances. 2021;11(28):17151–96

[4] Koch M, Forchhammer K. Polyhydroxybutyrate: A Useful Product of Chlorotic Cyanobacteria. Microbial Physiology. 2021 May 12;31(2):67–77.

[5] Panda B, Mallick N. Enhanced poly-β-hydroxybutyrate accumulation in a unicellular cyanobacterium, Synechocystis sp. PCC 6803. Letters in Applied Microbiology. 2007 Feb 1;44(2):194–8

[6] Carpine R, Olivieri G, Hellingwerf KJ, Pollio A, Marzocchella A. Industrial Production of Poly-β-hydroxybutyrate from CO2: Can Cyanobacteria Meet this Challenge? Processes. 2020 Mar;8(3):323

[7] Fink, P., Menzel, C., Kwon, JH., Forchhammer K. A novel recombinant PHB production platform in filamentous cyanobacteria avoiding nitrogen starvation while preserving cell viability. *Microb Cell Fact* 24, 43 (2025). https://doi.org/10.1186/s12934-025-02650-y

P21

Native Mass Spectrometry as a High-Throughput Platform for Protein-Ligand Interaction Screening

Kai Schließmann[1], Alexander Voltz[1], Daniel Krug[1], Rolf Müller[1]

1 Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) and Helmholtz Centre for Infection Research (HZI), Campus E8.1, 66123, Saarbrücken, Germany.

Native mass spectrometry (MS) is a versatile high-throughput method for screening protein–ligand interactions in a near-native environment. With this analytical method, ligand binding can be identified directly by mass shifts corresponding to the mass of the protein–ligand complex, providing fast insight into non-covalent binding events across the protein surface. The minimal sample requirements and swift data acquisition render native MS especially efficient for identification of potential binders to the target protein in early-stage drug discovery.[1, 2]

This project is designed to establish a native MS workflow for screening our in-house compound library against a range of protein targets, aiming to identify potential binders and prioritziting hits for further evaluation. We developed an autoflow injection setup that enables continuous measurements, followed by automated data evaluation using Visual Basic and Python. As an example, we will present data from the screening experiments with the non-structural protein NSP10 (SARS CoV2) from initial screening to data evaluation.

References

[1] Gavriilidou A. F. M., Sokratous K., Yen H.-Y., de Colibus L. Front. Mol. Biosci., 2022, 9, 1–12. https://doi.org/10.3389/fmolb.2022.837901

[2] Benesch J. L. P., Ruotolo B. T. Curr. Opin. Struct. Biol., 2011, 21, 641–649. https://doi.org/10.1016/j.sbi.2011.08.002

P22

Novel Bestatin Derivatives from a PKS/NRPS Hybrid Pathway

Josefine Klerch [1], Phillipp Fink [1], Raimund Nagel [2], Severin Sasso [2], Leonard Kaysser [1]

1 Institute for Drug Discovery, Department of Pharmaceutical Biology, University of Leipzig, Eilenburgerstr. 14, 04317 Leipzig, Germany.

2 Institute for Biology, Department of Plant Physiology, University of Leipzig, Johannisallee 23, 04103 Leipzig, Germany.

Protease inhibitors play a vital role in treating viral infections and cancers, such as multiple myeloma [1-3]. One such compound is the PKS/NRPS hybrid Bestatin, which is derived from *Streptomyces olivoreticuli* (ATCC 31159) [4] and has been studied for its use in treating acute myeloid leukaemia [5-7]. Its structural similarity to the natural substance amastatin, as well as the same domain constellation of the biosynthetic gene cluster (BGC), raises intriguing questions for further research.

In this study, LC/MS-MS data from the wild type producer and the heterologous host were used to generate a molecular network by GNPS, facilitating the identification of novel derivatives for bestatin. These derivatives indicate an unusual putative biosynthetic pathway where disparate starting units and diverse amino acids are accepted, which could suggest an iterative mechanism. In order to verify this hypothesis, further studies, including the feeding of labelled precursors, are required. Nevertheless, our investigation of the bestatin biosynthesis contributes to the understanding of protease inhibitors and enables new opportunities for drug development.

References

[1] Mendez-Lopez, M. et al. HIV protease inhibitors for the treatment of multiple myeloma. *Clin Adv Hematol Oncol*, 2019, 17. Jg., Nr. 11, S. 615-623. PMID: 31851164

[2] An, H. et al. PHF19 before and post induction treatment possess favorable potency of reflecting treatment response to protease inhibitors, event-free survival, and overall survival in multiple myeloma patients. *Hematology*, 2024, 29. Jg., Nr. 1, S. 2331389. DOI: 10.1080/16078454.2024.2331389

[3] Barchielli, G.; Capperucci, A.; Tanini, D. Therapeutic cysteine protease inhibitors: a patent review (2018–present). *Expert Opinion on Therapeutic Patents*, 2024, 34. Jg., Nr. 1-2, S. 17-49. DOI: 10.1080/13543776.2024.2327299

[4] Umezawa, H. et al. Bestatin, an inhibitor of aminopeptidase B, produced by actinomycetes. The Journal of antibiotics, 1976, 29. Jg., Nr. 1, S. 97-99. DOI: 10.7164/antibiotics.29.97

[5] Hu, X. et al. Efficacy of Standardised Treatments Combined with Ubenimex in Patients with Malignant Tumors. *J. Coll. Physicians Surg. Pak*, 2021, 31. Jg., S. 206-209. DOI: 10.29271/jcpsp.2021.02.206

[6] Xi, Y., et al. Quantitative profiling of CD13 on single acute myeloid leukemia cells by super-resolution imaging and its implication in targeted drug susceptibility assessment. *Nanoscale*, 2019, 11. Jg., Nr. 4, S. 1737-1744. DOI: 10.1039/c8nr06526h

[7] Ota, K. Review of ubenimex (Bestatin): clinical research. *Biomedicine & pharmacotherapy*, 1991, 45. Jg., Nr. 2-3, S. 55-60. DOI: 10.1016/0753-3322(91)90123-b

P23

Development of an expression platform for nonribosomal peptides in symbiotic cyanobacteria of the genus *Nostoc*

Sofia Kerestetzopoulou[1], Arthur Guljamow[1], Lena Hochrein[1], Min Qiu[2], Nicole Strittmatter[2], Elke Dittmann[1]

1 Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Straße 24-25, 14476 Potsdam, Germany.
2 TUM School of Natural Sciences, Technical University of Munich, Lichtenbergstraße 4, 85748 Munich, Germany.

Cyanobacteria are a diverse group of oxygenic phototrophic bacteria that are prolific producers of secondary metabolites. They hold great promise as sustainable producers of a wide range of chemical compounds [1]. Currently, mainly unicellular cyanobacteria of the genera *Synechococcus* and *Synechocystis* are established as chassis for the production of fine chemicals and commodities. However, these model organisms are not perfectly suited as hosts for the production of complex NRPS-type natural products. In contrast, filamentous cyanobacteria of the genus *Nostoc* have the potential for high-titer production of NRPs as they naturally encode a high number of NRPS BGCs. The filamentous cyanobacterium *Anabaena* sp. PCC 7120 has emerged as the most effective heterologous host for cyanobacterial natural products [2].
In this study, we aim to establish a new filamentous cyanobacterium as a heterologous host for the production of NRPs. In our recent work we were able to heterologously express the NRPS-derived natural product pyreudione A from *Pseudomonas fluorescence* in high titers in *Nostoc* sp. KVJ2. We now aim to compare the production titers of pyreudione between *Nostoc* sp. KVJ2 and *Anabaena* sp. 7120. Additionally, spatial imaging will be employed to visualize metabolic phenotypes between wild-type and mutant strains. Different strategies will be compared for the expression of the peptides and both inducible and constitutive promoters as well as plasmid-based and genomic integration strategies will be evaluated. Our goal is to expand and optimize our expression strategy with more sophisticated NRPSs and expand the heterologous expression toolkit in *Nostoc* strains.

References
[1] Knoot, C. J. *et al*. J. Biol. Chem. 2018, 293, 5044-5052. https://doi.org/10.1074/jbc.R117.815886
[2] Taton, A. *et al*. ACS Synth. Biol. 2020, 9, 3364-3376. https://doi.org/10.1021/acssynbio.0c00431

P24

Modeling cyanotoxin production in Lake Mendota

Pablo Barrancos[1], Ferdi L. Hellweger[1]

1 Technical University of Berlin, Strasse des 17. Juni 135, 10623 Berlin, Germany.
barrancos.garrigos@tu-berlin.de, ferdi.hellweger@tu-berlin.de

Toxins produced by cyanobacteria in freshwaters may lead to several problems such as dog deaths [1, 2], human intoxications [3] or drinking water supply interruptions [4]. Mechanistic models may help us to understand the environmental processes driving this toxin production and to evaluate management measurements.

In order to understand better the toxin production, we have used an existing mechanistic model [5] that simulates the growth of toxigenic and non-toxigenic cyanobacteria *Microcystis* and the production of toxin Microystin (MC). The synthesis of MC depends on the variable nitrogen quota within the cells, light availability and temperature. In this model, the intracellular MC might become extracellular due to excretion, grazing or death of the cells. A general benefit provided by MC to *Microcystis* is included by making the growth rate a function of MC quota.

Here we present the extension and application of this model to Lake Mendota (Wisconsin, USA), where we simulate *Microcystis* and MC concentration over a 6 year time period. This application includes several extensions\improvements to the previous model applications:

-Dynamic simulation of the sediment bed flux
-Simulation of grazing based on the herbivore zooplankton concentration
-Sedimentation as a function of water temperature
-Death function due to viruses infection
-Simulation of additional non-*Microcystis* phytoplankton species
-Establishment of a dormancy concentration

References

[1] Johnston, L.H., Huang, Y., Bermarija, T.D., et al. Sci. Total Environ. 2024, 917, 170476. https://doi.org/10.1128/AEM.01397-20
[2] McCarron, P., Rafuse, C., Scott, S., et al. Toxicon 2023, 227, 107086 https://doi.org/10.1016/j.toxicon.2023.107086
[3] Giannuzzi, L., et al. Mar. Drugs 2011, 9(11), 2164–2175 https://doi.org/10.3390/md9112164
[4] McKindles, K.M., et al. Appl. Environ. Microbiol. 2020, 86(19), e01397- https://doi.org/10.1128/AEM.01397-20
[5] Schampera, C.; Hellweger, F.L. Ecol. Model. 2025, 504, 111094. https://doi.org/10.1016/j.ecolmodel.2025.111094

P25

Paenidepsins: a novel family of lipopeptides from *Paenibacillus*

Lukas Zimmer[1], Daniel Torres-Püschel,[2] Stefan Kehraus[2], Aurélien Carlier[3], Max Crüsemann[1]

1 Institute of Pharmaceutical Biology, Goethe-University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany.
2 Institute of Pharmaceutical Biology, University of Bonn, Nußallee 6, 53115 Bonn, Germany.
3 LIPME, Université de Toulouse, INRAE, CNRS, Castanet-Tolosan 31326, France.

Lipopeptides represent a structurally diverse group of secondary metabolites that are composed of a peptide moiety attached to a fatty acid chain. Bacteria from the genera *Bacillus* and *Paenibacillus* are notable producers of lipopeptides including prominent examples such as surfactin, fengycin, and polymyxins; compounds that exhibit a broad spectrum of biological activities with antimicrobial, antifungal, and anticancer effects [1].

In the search for novel bioactive metabolites, we isolated paenidepsin A from the culture broth of *Paenibacillus apiarius*, a 12-membered macrocyclic lipopeptide that contains a 3-hydroxy-14-methylpalmitoyl moiety. Further paenidepsin analogs were detected through molecular networking and structurally characterized via MS/MS analyses. Peptidogenomic analysis revealed the corresponding nonribosomal peptide synthetase (NRPS) biosynthetic gene cluster (BGC) in the genome of the producer strain. We performed comprehensive phylogenetic and comparative analyses to demonstrate that paenidepsin-like BGCs constitute a novel gene cluster family from *Paenibacillus*. Furthermore, detailed NRPS domain-level analyses provided bioinformatic evidence for recombination-driven evolutionary diversification of this BGC. Our findings illustrate the utility of integrating mass spectrometric and genomic tools to discover diverse natural products, and highlight the significant biosynthetic capacity of *Paenibacillus* species.

References
[1] Cochrane, S.A. & Vederas, J.C. Med. Res. Rev. 2016, 36, 1. https://doi.org/10.1002/med.21321

P26

Discovery of natural products from moss-associated bacteria

Carlotta Hecker[1], Malin Kretzschmar[1], Sophie Jin[1], Jethro Hemmann[1]

1 Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Junior Research Group Metabolomics-guided Natural Product Discovery, Adolf-Reichwein-Straße 23, 07745 Jena, Germany

Mosses thrive in diverse and often harsh environments and are extremely resilient against desiccation and other environmental stresses. They play important roles in many ecosystems and, as the evolutionary oldest plant lineage, they maintain several ancestral features, making them interesting model systems in plant biology and to study plant-microbe interactions. Their robustness can partly be attributed to their microbiome, which enhances stress tolerance, promotes plant growth, and defends their host against pathogens. However, the secondary metabolites from the moss microbiota as well as their roles in microbial interactions remain largely unexplored and represent a unique source for novel natural products with ecological and pharmaceutical relevance.

To investigate the biosynthetic potential of moss-associated bacteria and to identify the natural products they produce, we analyzed three different moss species from two different environments. We characterized the microbial constitution of these mosses through 16S rDNA sequencing and isolated 86 bacterial strains across all three mosses. Initial community profiling revealed a strong overlap between the microbiomes of the two epilithic mosses *Orthotrichum anomalum*, and *Grimmia pulvinata*, both dominated by Actinobacteria, Alphaproteobacteria, and Bacteroidia. In contrast, the microbiome of the soil-dwelling *Funaria hygrometrica* was dominated by Alphaproteobacteria, Cyanobacteria and Gammaproteobacteria, but was distinct from the soil community. These differences suggest that the microbiomes of the three mosses are influenced primarily by environmental conditions rather than the host species. The isolated strains largely reflect these microbiomes, as they were found to represent the most abundant taxonomic classes identified through sequencing.

To understand the role of natural products in ecological interactions in the moss microbiome and to activate cryptic biosynthetic gene clusters, we are currently developing co-cultivation approaches to study both bacteria-bacteria as well as moss-bacteria interactions of our isolates. Using untargeted metabolomics, we identify novel metabolites arising from these interactions. In addition, we mined the genomes of our isolates for novel types of biosynthetic gene clusters, focusing especially on ribosomally synthesized and post-translationally modified peptides. Ongoing analyses will link metabolites to specific interactions and biosynthetic gene clusters, ultimately aiming to identify novel natural products from moss-associated bacteria.

P27

Diversification of the myxobacterial metabolome by cultivation-dependant approaches

Simon Cremers[1,2], Sebastian Walesch[1,2], Markus Neuber[1,2] ,Chantal D. Bader[1,2] ,Daniel Krug[1,2] and Rolf Müller[1,2]

1 Department of Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Campus E8 1, 66123 Saarbrücken, Germany.
2 German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Inhoffenstraße 7, 38124 Braunschweig, Germany.

The investigation of microbial secondary metabolites remains one of the cornerstones in the search for urgently needed novel antibiotic classes [1]. However, the majority of biosynthetic pathways in microbial genomes cannot be linked to the matching natural products in standard laboratory cultivation settings [2]. This also holds true for Gram-negative myxobacteria, which caught the interest of researchers for their sophisticated lifestyles and remarkable capabilities to produce biologically active metabolites [3]. One powerful approach to better access the unrealised biosynthetic potential of microbes is the variation of different aspects of the cultivation conditions, known as the OSMAC strategy [4].
In the past years, we have applied different cultivation-dependent approaches to diversify the myxobacterial secondary metabolism. Media screening and co-cultivation experiments with *Pendulispora brunnea* MSr12523^T, member of a newly described myxobacterial family, led to the discovery and investigation of several biologically active natural products, belonging to known and new chemical scaffolds in myxobacteria [5-7]. In order to better access the secondary metabolites that are only produced during cultivation on solid surfaces, the “flow-plate”, a mixed solid-liquid cultivation platform, was developed. Cultivation of *Myxococcus xanthus* DK1622 on the “flow-plate” increased its metabolome coverage with simultaneous production of features typically observed in solid and liquid cultivation [6]. Moreover, we could demonstrate that cultivating myxobacteria in presence of elicitors can reproducibly alter their secondary metabolome [7]. Based on our current experiments, we are in the process of developing a standardised elicitor screening platform for myxobacteria.
On this poster, we give an overview of the effects of different cultivation-dependent approaches on the secondary metabolome of myxobacteria on a feature-level and highlight three natural product classes that were investigated during our studies.

References
[1] Walesch, S. *et al.* EMBO Rep. 2023, 24, 1. https://doi.org/10.15252/embr.202256033
[2] Nett, M. *et al*, Nat. Prod. Rep. 2009, 26, 1362. https://doi.org/10.1039/B817069J
[3] Bader, C. *et al*, Biotechnol. Adv. 2020, 39, 107480. https://doi.org/10.1016/j.biotechadv.2019.107480
[4] Bode, H.J. *et al*, ChemBioChem. 2002, 3, 619. https://doi.org/10.1002/1439-7633(20020703)3:7%3C619::aid-cbic619%3E3.0.co;2-9
[5] Garcia, R. *et al*, Chem. 2024, 10, 8. https://doi.org/10.1016/j.chempr.2024.04.019
[6] Neuber, M. PhD thesis UdS. 2022. http://dx.doi.org/10.22028/D291-37296
[7] Walesch, S. PhD thesis UdS. 2023. http://dx.doi.org/10.22028/D291-41491

P28

OSMAC-Driven Investigation of Marine-derived Myxobacteria: Insights from Strain MSr13327

Martin Gepperth[1,2], *Joy Birkelbach*[1,2], *Ronald Garcia*[1,2], *Daniel Krug*[1,2], *Rolf Müller*[1,2]

[1] Department of Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI) and Department of Pharmacy at Saarland University, Germany
[2] German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Germany

Myxococcota are a Gram-negative bacterial phylum renowned for their prolific production of diverse secondary metabolites, which exhibit a wide range of bioactivities and hold significant potential for pharmaceutical applications. To date, over 800 myxobacterial natural products from approximately 170 chemical scaffolds have been reported [1]. Intriguingly, within this diversity, natural products derived from halotolerant and halophilic Myxococcota account for only nine families of secondary metabolites described in literature recently. Notably, all of the marine-derived natural products reported so far have been isolated from six strains belonging to the *Nannocystineae* suborder. The scarcity of natural product discoveries from only few halophilic Myxococcota highlights their untapped phylogenetic and chemical potential, which is partly attributed to the limited experience in cultivating this group of bacteria [2].

In order to expand the insight into secondary metabolite production from marine Myxococcota, in this work the “One Strain-Many Compounds” (OSMAC) approach is applied to marine-derived myxobacterial strains. The OSMAC strategy is based on the observation that one strain is able to produce different secondary metabolites when grown under different cultivation conditions, thus providing a promising strategy for the discovery of novel natural products [3]. In this investigation, halophilic Myxococcota are cultivated in ten different media followed by extraction of the resulting cultures. The extracts are analyzed via LC-MS, and MS/MS spectra are recorded to enable molecular networking and to facilitate the dereplication process. Additionally, crude extracts are submitted to our in-house bioactivity screening platform.

On this poster, we demonstrate this OSMAC-driven investigation with a marine-derived *Sorangiineae* strain, MSr13327, as no natural products from halophilic members of this suborder have been reported so far. Detailed comparative analysis of chromatograms and molecular networks as well as dereplication with in-house and publically available databases, revealed a promising target mass that was observed in significant quantities in only one of the ten tested media. Therefore, this compound was selected for purification, as a potentially novel and first natural product derived from halophilic *Sorangineae*.

References
[1] Wang C.-Y. et al. Nat. Prod. Rep. 2024, 41, 905–934. https://doi.org/10.1039/D3NP00062A
[2] Gemperlein, K et al. Mar. Drugs 2018, 16, 314. https://doi.org/10.3390/md16090314
[3] Bode HB et al. Chembiochem. 2002 Jul 2;3(7):619-27. https://doi.org/10.1002/1439 7633(20020703)3:7<619::AID-CBIC619>3.0.CO;2-9

P29

Uncovering Hidden Biosynthetic Potential with ACTIMOT

Haowen Zhao[1,2], Feng Xie[1,2], Wanqi Qin[1,2],Shuqin Liu[1,2], Rolf Müller[1,2], Chengzhang Fu[1,2]

1 Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Campus E8 1, 66123 Saarbrücken, Germany
2 German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Germany

Bacterial genomes harbor a wealth of biosynthetic gene clusters (BGCs) that produce specialized metabolites, many with potential pharmaceutical applications [1]. However, many BGCs remain silent under standard laboratory conditions. To access this hidden potential, we developed ACTIMOT (Advanced Cas9-mediaTed In vivo MObilization and mulTiplication of BGCs), a CRISPR-Cas9-based method that enables targeted BGCs to be mobilized and relocated onto multicopy plasmids directly within the native hosts [2]. This strategy facilitates BGC activation and enhances expression through gene dosage effects, while also facilitating subsequent heterologous expression, thereby accelerating the discovery of novel natural products. In our previous work, ACTIMOT successfully activated silent or weakly expressed BGCs from well-studied *Streptomyces* strains, leading to the discovery of four previously unknown families of natural products.
Currently, we are applying ACTIMOT to systematically explore the biosynthetic potential of diverse *Streptomyces* strains with the aim of discovering new bioactive compounds. A major focus is the discovery of lipopeptides, a class of molecules known for their wide range of biological activities. As an example of our systematic endeavor, we have activated three silent lipopeptide BGCs, producing three new families of compounds.
ACTIMOT also enabled the discovery of actimotin, a group of benzoxazole-containing natural products featuring rare meta-substituted benzoate units. Notably, actimotin J showed transthyretin (TTR) amyloidogenesis inhibition activity, a process linked to TTR amyloidosis, a progressive and potentially fatal disease [3]. A bottom-up strategy was adopted to investigate actimotin biosynthesis, which also allowed the establishment of a platform for future expression of benzoxazole natural products identified through genome mining.
Together, these results demonstrate that ACTIMOT is a powerful and versatile tool for activating biosynthetic pathways, thereby enabling the discovery of structurally and functionally diverse natural products.

References
[1] Gavriilidou, A. *et al*. Nat. Microbiol. 2022, 7, 726–735, https://doi.org/10.1038/s41564-022-01110-2
[2] Xie, F. *et al*. Science. 2024, 386, 6727, https://doi.org/10.1126/science.abq7333
[3] Ando, Y. *et al*. J. Neurol. Sci. 2016, 362, 266–271, https://doi.org/10.1016/j.jns.2016.01.046

P30

Chemoenzymatic synthesis of lasso peptides for the modulation of the glucagon receptor

Antonio Sáenz-Castillo[1], Leonard Kaysser[1], Georg Künze[1]

[1]Institute for Drug Discover, Faculty of Medicine, Universität Leipzig, Liebigst. 21, 04103 Leipzig, Germany.

Ribosomally synthesized and post-translationally modified pepetides (RiPPs) represent a class of bioactive natural products which are generated through the translation of a precursor peptide-encoding gene by the ribosome and the subsequent processing by dedicated enzymes that introduce a variety of post-translational modifications.[1] Lasso peptides are one of the largest known classes of RiPPs, characterized by a mechanically interlocked structure.[2] These compounds offer valuable starting points for drug discovery efforts due to their remarkable stability, which is notable for its combination of enzymatic, thermal, and chemical stability, along with a variety of biological activities.[3]

Lasso peptides such as the previously described BI-3216[4] have been demonstrated to function as antagonists for G-protein coupled receptors (GPCRs), an example of which is the glucagon receptor (GCGR). The glucagon receptor has been identified as a possible drug target for the treatment of hyperglycemia in diseases such as type 1 and type 2 diabetes. This makes BI-32169 a pharmacologically important compound; nevertheless, its structural basis for interaction with the GCGR remains to be understood. Furthermore, there is a lack of effective workflows to synthesize and chemically modify BI-32169, while preserving its functionally critical lasso structure. The purpose of this investigation is to develop a combined chemical and enzymatic strategy that has the advantages of solid-phase peptide synthesis for the production of modified precursor peptides of BI-32169 and the lasso peptide formation with the responsible enzymes.

Our studies have led to the identification of the enzymatic machinery and precursor peptides necessary for the synthesis of BI-32160. This includes the RiPP recognition element (B1), the cysteine protease (B2), and lasso cyclase (C), which were identified through genome mining. The enzymes have been designed with a solubility tag, such as a maltose binding protein, to enhance their solubility. The successful expression and purification of two enzymes of the reaction complex, the RRE element and the protease, has been achieved. The following steps are to be taken: first, the third enzyme responsible for lasso formation must be obtained in a soluble form so that the *in vitro* reaction can be tested and lasso peptide formation can be verified.

References

1. M. Jangra *et al.*, *Nature* **640**, 1022–1030, ISSN: 1476-4687, (https://doi.org/10.1038/s41586-025-08723-7) (26 Mar. 2025).
2. S. E. Barrett *et al.*, *Nature Chemical Biology* **21**, 412–419, ISSN: 1552-4469, (https://doi.org/10.1038/s41589-024-01727-w) (11 Sept. 2025).
3. K. Schiefelbein *et al.*, *Journal of the American Chemical Society* **146**, 17261–17269, ISSN: 15205126, (https://pubs.acs.org/doi/full/10.1021/jacs.4c03898) (25 June 2024).
4. O. Potterat *et al.*, *Journal of Natural Products* **67**, PMID: 15387654, 1528–1531, (https://doi.org/10.1021/np040093o) (4 Aug. 2004).

P31

Characterization of natural products with anti-infective properties originating from human microbiota

Lilu Liu[1], Leidy Alejandra Gonzalez Molano[2], Andreas Keller[1,2,4], Rolf Müller[1,3,4,5]

1 Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus E8.1, 66123 Saarbrücken, Germany.
2 Chair for Clinical Bioinformatics, Saarland University, 66123 Saarbrücken, Germany.
3 Helmholtz Centre for Infection Research (HZI), Inhoffenstraße 7, 38124 Braunschweig, Germany.
4 PharmaScienceHub (PSH), 66123 Saarbrücken, Germany.
5 Department of Pharmacy, Saarland University, 66123 Saarbrücken, Germany.

With the development of human microbiota research, more “dark matter” from human microbiota has been gradually investigated. Natural products from human microbiota have been discovered by interdisciplinary strategies, including culture based isolation, metagenomics and heterologous expression, bioinformatics and chemical synthesis [1]. The reported human microbiota natural products show high structural diversity and a wide range of bioactivities [2,3], which suggests human microbiota are promising resources for drug and probiotics discovery. However, metabolites found to date are only the tip of the iceberg. In this study, we therefore aim to explore and characterize bioactive secondary metabolites from human microbiota using combined methods. By applying a culture based isolation method, species from clinical oral plaque samples were isolated and initially identified by 16S rRNA sequencing. Analysis of their metabolomes indicates candidate compounds for further investigation. Meanwhile, comprehensive analysis based on metagenomics sequences from oral samples was performed to explore the connection between biosynthetic gene clusters (BGCs) families and diseases. The significant differences of BGCs distribution were observed across healthy and diseased cohorts [4]. The product of one health associated BGC in our dataset has been characterized [5] and proven to have antibacterial activity, while others show low similarity with known BGCs. Consequently, two ribosomally synthesized and post-translationally modified peptides (RiPPs) BGCs that are correlated with a healthy phenotype were selected for heterologous expression. Taken together, our study will expand the diversity of human microbiota natural products, and contribute to a better understanding of the connection between small molecules from human microbiota and human health.

References
[1] Dai, H. *et al*. Nat Prod Rep. 2023, 40, 1078-1093. https://doi.org/10.1039/d2np00075j
[2] Torres Salazar, B.O. *et al*. Nat Microbiol. 2024, 9, 200-213. https://doi.org/10.1038/s41564-023-01544-2
[3] Li, Z. *et al*. Nat Chem Biol. 2021, 17, 576-584. https://doi.org/10.1038/s41589-021-00745-2
[4] Schmartz, G.P. *et al*. Nat Commun. 2024, 15, 8261. https://doi.org/10.1038/s41467-024-52598-7
[5] Bushin, L.B. *et al*. Nat Chem Biol. 2022,18, 1135-1143. https://doi.org/10.1038/s41589-022-01090-8

P32

Genomic insights into antimicrobial resistance genes in Myxobacteria

Huixin Xu[1], Alper Yurtseven[1], Olga Kalinina[1], Rolf Müller[1], Chengzhang Fu[1],

1 Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) and Helmholtz Centre for Infection Research (HZI), Campus E8.1, 66123, Saarbrücken, Germany.

Myxobacteria are a unique group of predatory, Gram-negative bacteria known for their complex life cycles and secondary metabolite production especially of antimicrobial compounds[1, 2]. Despite their ecological importance and pharmaceutical potential, little is known about their role in the spread and evolution of antimicrobial resistance. Antimicrobial resistance genes (ARGs) and associated mobile genetic elements (MGEs) are key drivers of resistance dissemination across microbial communities, yet their diversity and prevalence in myxobacteria remain largely underexplored[3, 4].

This project aims to characterize the myxobacterial resistome through comprehensive genome analyses combined with functional screening based on our in-house Myxobacteria library. By systematically identifying and annotating ARGs and MGEs, we seek to unravel their diversity, distribution, and evolutionary origins within this taxon. In addition to known resistance determinants, we will also investigate genomic contexts to identify potential novel ARGs that may represent previously unrecognized resistance mechanisms.

In summary, this study will provide the first detailed overview of ARGs and MGEs in myxobacteria, highlighting their significance in microbial ecology and resistance evolution. Findings from this project may expand our understanding of how myxobacteria contribute to the global resistome and guide future efforts to uncover novel resistance mechanisms.

References

[1] Herrmann J, Abou Fayad A, Müller R. *Nat. Prod. Rep.,* 2017,34, 135-160. https://doi.org/10.1039/C6NP00106H
[2] Reichenbach H, Höfle G. *Biotechnol Adv*. 1993, 11(2):219-77. https://doi.org/10.1016/0734-9750(93)90042-L
[3] Deng Z. *et al*. *Environ Res*. 2025, 122495. https://doi.org/10.1016/j.envres.2025.122459
[4] Partridge SR. *et al*. 2018. *Clin Microbiol Rev*. 31(4), 10-1128.https://doi.org/10.1128/cmr.00088-17

P33

Optimizing the [*S,S*]-EDDS precursor L-aspartate in *Amycolatopsis japonicum* by metabolic engineering.

Thomas Heschele*, Vida Röhm Peralta, Naybel Hernández Pérez, Evi Stegmann

Microbial Bioactive Compounds, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), University of Tübingen, Germany

*Corresponding author: thomas.heschele@student.uni-tuebingen.de

Actinomycetes are filamentous soil bacteria well known for producing antibiotics and a wide range of secondary metabolites. Like many microorganisms, they require essential metal ions such as iron, zinc, and copper for growth and metabolism, yet these are often scarce in natural environments. To overcome this limitation, bacteria have evolved strategies for metal acquisition, including the synthesis of specialized metabolites.

Under zinc-limited conditions, *Amycolatopsis japonicum* produces the zincophore [*S,S*]-ethylenediamine-disuccinate (EDDS), a structural isomer of EDTA. While EDTA is widely used in detergents, agriculture, and industrial cleaning, its poor biodegradability causes environmental persistence, particularly in aquatic systems. In contrast, EDDS combines strong metal-chelating capacity with biodegradability, making it a promising sustainable alternative.

Because aspartate is a biosynthetic precursor of [*S,S*]-EDDS [1,2], this project aims to optimize aspartate metabolism to boost EDDS production and establish a cost-effective biotechnological process for industrial application. A previously engineered producer strain already achieved 9.8 g/L EDDS, a significant improvement over the wild type (0.3 g/L) [3]. To further enhance production, genes influencing L-aspartate availability were targeted. Overexpression of genes encoding L-asparaginase, aminotransferase, and aspartate ammonia-lyase did not improve yields. However, deletion of the aspartate kinase gene (*lysC*) increased EDDS titers by 1.57-fold compared to the wild type.

Together, these findings represent a significant step toward efficient [*S,S*]-EDDS production, advancing the replacement of environmentally harmful EDTA with a more sustainable alternative for industrial use.

[1] Cebulla, I. (1995). Gewinnung komplexbildender Substanzen mittels Amycolatopsis orientalis. (Eberhart-Karls Universität, Tübingen)

[2] Hernández Pérez, N. (2025). Investigation on [*S,S*]-EDDS biosynthesis and its applications. doi:10.15496/publikation-84582.

[3] Edenhart, Simone et al. (2020). “Metabolic engineering of Amycolatopsis japonicum for optimized production of [S,S]-EDDS, a biodegradable chelator”. In: Metabolic engineering 60, pp. 148–156. doi: 10.1016/j.ymben. 2020.04.003.

P34

Discovery of novel suomilides from *Nostoc* sp. KVJ20.

Christoph Ulbricht[1], Amira Naimi[2], Tung Lam Trinh[1], Stefan Kehraus[1], Raphael Reher[2], Martin Baunach[1]

1 Institute of Pharmaceutical Biology, University of Bonn, Nußallee 6, 53115 Bonn, Germany.
2 Institute of Pharmaceutical Biology and Biotechnology, University of Marburg, Robert-Koch-Str. 4, 35037 Marburg, Germany.

Suomilides are a family of aeruginosin-type non-ribosomal peptides. Of these, suomilide A has been shown to be highly effective at inhibiting human trypsin and the invasion of PC-3M prostate cancer cells [1]. Here, we unveil yet-unknown suomilide derivatives, suomilide F and G (Fig.1), which were identified through native metabolomics and molecular networking. Initial trypsin inhibition assays suggest that also suomilides other than suomilide A are potent trypsin inhibitors.

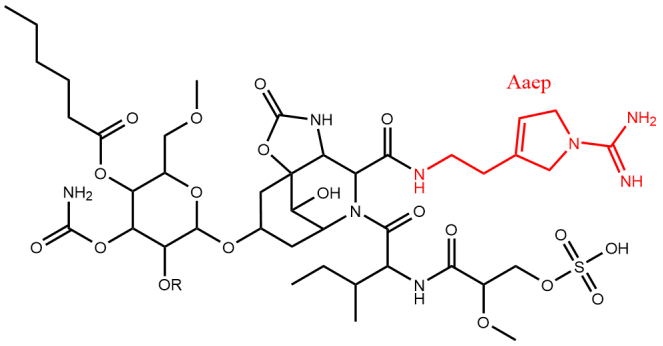


Figure 1: Structures of suomilide F and G. The Aaep moiety, which addresses the trypsin specificity pocket as an arginine mimetic is marked in red. Aaep – 1-amidino-3-(2-aminoethyl)-3-pyrroline.

References
[1] Muhammad, N.A. *et al*. ACS Chemical Biology 2021 16 (11), 2537-2546. https://doi.org/10.1021/acschembio.1c00611

P35

Microbelix – exploring microbial diversity in soil by a citizen science approach

Anna-Maria Kastner[1,2], Daniel Krug[1,2,3], Leidy-Alejandra G. Molano[1,3], Laxmi Neupane[1,2], Guangyi Chen[1], Alexander Becker[1], Ronald Garcia[1,2,3], Andreas Keller[1,3], Olga Kalinina[1,3], Rolf Müller[1,2,3]

1 - Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken, and Helmholtz Center for Infection Research, Braunschweig, Germany
2 - German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Germany
3 - Universität des Saarlandes, Saarbrücken, Germany

The Microbelix citizen science campaign aims to explore the microbial communities in different habitats with the help of environmental samples collected by citizen scientists. Its main focus is set on the diversity of the so called Myxobacteria (phylum *Myxococcota*). They can be found in many different environments, such as soil or marine environments, and are known as an important source of bioactive natural products.

A clear correlation between phylogenetic distance of Myxobacteria and production of distinct secondary metabolite families could be shown [1]. This is why constant screening for and isolation of more and new Myxobacteria for the HZI-HIPS strain library is important to access a highly diverse set of natural products and therefore yet unseen anti-infective compounds.

One approach to support more effective isolation of novel strains from soil samples is gDNA-based metagenomics analysis. Done ahead of strain isolation, it may help to prioritize soil samples, enable more targeted isolation efforts and allow first insights into available biosynthetic potential. Here we present our current wet-lab pipeline, first preliminary results of the bioinformatics analysis and some recently isolated strains from our Microbelix soil samples.

References

[1] Hoffmann, T., Krug, D., Bozkurt, N. *et al.* Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria. *Nat Commun* **9**, 803 (2018). https://doi.org/10.1038/s41467-018-03184-1

P36

Mining functional natural products from the gut microbiome

Dëborë Zenelaj,[1] Tobias Füssel,[1] Lisa Göbner,[1] Maximilian Hohmann,[2] Tobias Gulder[1]

1 Department of Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Natural Product Biotechnology, Helmholtz Centre for Infection Research (HZI) and Department of Pharmacy, PharmaScienceHub (PSH), Saarland University, Campus E8.1, 66123 Saarbrücken, Germany.
2 Chair of Technical Biochemistry, TUD Dresden University of Technology, Bergstraße 66, 01069 Dresden, Germany.

The human body is host to a variety of microorganisms, with the largest diversity of bacterial cells found in the gastrointestinal tract, known as the gut microbiota. There is a substantial degree of variation in the gut microbiota composition among healthy individuals, subject to host and environmental selective pressures [1]. Nevertheless, bacterial communities of the human gut have been associated with a range of roles beneficial to the host, such as nutrient metabolism, host immunity, reduction of pathogen colonization etc. The disruption of the normal host-associated microbiota population (dysbiosis) can strongly impact the pathogenesis of intestinal and extraintestinal diseases [2]. Although bacterial natural products (NPs) are actively studied due to their pronounced bioactivities, the number of known NPs that play a role in the human gut is limited.

Within our laboratory, we are thus interested in further investigating such NPs and their potential roles in health and disease. To this end, a structure-based approach was developed to select the most promising biosynthetic gene clusters (BGCs), particularly by prioritizing heterocyclic structural elements. Recently, through structure-based genome mining, Direct Pathway Cloning (DiPaC) [3,4] of BGCs, and recombinant expression in *E. coli*, aureusimines from *Staphylococcus xylosus* and cytotoxic bacillamides from a *Bacillus* sp. gut isolate were discovered. Following a similar approach, antibiotic bilothiazoles encoded by a BGC found in metagenomic data of the human gut were identified [5,6].

Additionally, targeted genome mining was employed to discover NPs of *Desulfovibrio* sp. bacteria, connected to the pathogenesis of inflammatory bowel disease. Although the exact cause of this disease is not known, it is hypothesized to be related to a reduced microbial diversity and significant microbial imbalance, with an upregulation of *Desulfovibrio* sp. [7,8]. A number of strains of this genus contain highly conserved NRPS/PKS hybrid systems that do not resemble any NRPS/PKS BGCs characterized in literature, likely encoding NPs of unknown structure and function. Supported by the bioinformatic prediction of structural motifs otherwise associated with biologically active compounds [9], these NPs may have important functions in the specialized habitat of the gut. Recent advances in heterologously expressing, structurally and functionally characterizing these NPs will be presented.

References
[1] Huse, S. M. *et al*. PLoS Genet. 2008, 4. DOI: 10.1371/journal.pgen.1000255
[2] Carding, S. *et al*. JMicrobial Ecol. Heal. Dis. 2015, 26. DOI: 10.3402/mehd.v26.26191
[3] Greunke, C. *et al*. Metab. Eng. 2018, 47, 334-345. DOI: 10.1016/j.ymben.2018.03.010
[4] D'Agostino, P. M.; Gulder, T. A. M. ACS Synth. Biol. 2018, 7, 1702-1708. DOI: 10.1021/acssynbio.8b00151
[5] Hohmann, M. *et al*. Nat. Comm. Biol. 2024, 7, 655. DOI: 10.1038/s42003-024-06208-3
[6] Hohmann, M. *et al*. ACS Synth. Chem. 2025, 14, 3, 967-978. DOI: 10.1021/acssynbio.5c00042
[7] Ott, S. J. *et al*. Gut 2004, 53, 685-693. DOI: 10.1136/gut.2003.025403
[8] Manichanh, C. *et al*. Gut 2006, 55, 205-2011. DOI: 10.1136/gut.2005.073817
[9] Gupta, V.; Kant, V. Sci. Int. 2013, 1, 253-260. DOI: sciintl.2013.253.260

P37

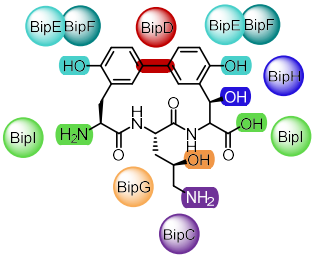
Biosynthesis of the Biphenomycin Family of Potent Antibiotics

Alfred Lobert,[1] Elisabeth Strunk,[2] Tatiana Khorovich,[1,2] Katia M. Guzman Lucio,[1,2] René Richarz,[3] Maximilian Hohmann,[2] Paul M. D'Agostino[1,2] Tobias A. M. Gulder[1-3*]

[1] A. Lobert, T. Khorovich, K. M. Guzman Lucio, Dr. P. M. D'Agostino, Prof. Dr. T.A.M. Gulder
Department of Natural Product Biotechnology, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI) and Department of Pharmacy at Saarland University, PharmaScienceHub (PSH), Campus E8.1, 66123 Saarbrücken, Germany.
E-mail: tobias.gulder@helmholtz-hips.de

[2] E. Strunk, T. Khorovich, K. M. Guzman Lucio, Dr. M. Hohmann, Dr. P. M. D'Agostino, Prof. Dr. T.A.M. Gulder
Chair of Technical Biochemistry, Department of Chemistry and Food Chemistry, Technical University of Dresden, Bergstraße 66, 01069 Dresden, Germany.

[3] Dr. R. Richarz, Prof. Dr. T.A.M. Gulder
Biosystems Chemistry, Faculty of Chemistry, Technical University of Munich, Lichtenbergstraße 4, 85748 Garching, Germany.



Biphenomycins A and B exhibit potent antibacterial activity against Gram-positive pathogens, highlighting their therapeutic potential. However, the biosynthetic origin of this natural product family has remained elusive for over four decades.
Recent investigations have finally uncovered critical enzymatic functions within the biosynthetic pathway, revealing previously uncharacterized activities in ribosomally synthesized and post-translationally modified peptides (RiPPs). [1, 2]
A total of seven genes from *S. griseorubiginosus* No. 43708 encode the enzymes responsible for biphenomycin biosynthesis. In our work, we identified and characterized the enzymatic maturation steps both *in vivo* and *in vitro*. These include an MNIO-catalyzed double *ortho*-hydroxylation of two phenylalanine residues, followed by a B12-dependent rSAM-mediated *C,C*-cross-coupling. Additional post-translational modifications are carried out by a highly regioselective arginase and distinct *β*- and *γ*-hydroxylases. In a last step, the matured core peptide is cleaved by stepwise proteolytic processing to yield the active natural product.
Additional comparative genomic analysis has identified putative additional biphenomycin producers, including *P. simplex*_D NPDC058078, for which we successfully confirmed biphenomycin production. Further investigations did not only discover novel organisms but also show unexplored and divergent enzymes within biphenomycin-type biosynthetic gene clusters as well as variations within the core peptides as shown by cBlaster analysis. These findings expand the biochemical potential of the biphenomycin family, offering opportunities to explore new congeners as antibiotic leads, addressing the urgent demand for new antimicrobial agents.

References

[1] Strunk, E.; Lobert, A.; Khorovich, T.; Guzman Lucio, K.; Richarz, R.; Hohmann, M.; D'Agostino, P. M.; Gulder, T.; ChemRxiv. 2025; https://doi.org/10.26434/chemrxiv-2025-vkzfb
[2] Padhi, C.; Zhu, L.; Chen, J. Y.; Huang, C.; Moreira, R.; Challis, G. L.; Cryle, M. J.; van der Donk, W. A.; J. Am. Chem. Soc. 2025, 147, 23781-23796, https://doi.org/10.1021/jacs.5c06044

P38

Development of a Cascading Biorefinery Concept for Medium-Chain Fatty Acid Production from Sugar Beet Pulp

Mourice Wölbeling[1,2], Hilke Würdemann[1], Flávio C. F. Baleeiro[1,2]

1 Department of Engineering and Natural Sciences, University of Applied Science Merseburg, Germany
2 Department of Microbial Biotechnology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

Achieving net-zero CO_2 emissions requires innovative strategies that valorise existing resources more effectively. Agricultural by-products, particularly from the sugar industry, offer untapped potential for generating high-value products. This research proposes a cascading biorefinery approach using sugar beet pulp as a feedstock to produce medium-chain fatty acids (MCFA) – in particular caproic and caprylic acids – as local and sustainable alternatives to products currently derived from tropical oils such as palm and coconut.

Germany, as the largest sugar producer in the EU, generates substantial volumes of sugar beet pulp (~ 2.78 Mt/year) and molasses (~ 0.88 Mt/year) as co-products. Currently, sugar beet pulp is primarily used as animal feed, while future strategies outlined in the German sugar industry's roadmap aim to valorise it for energy production via biomethane or solid biofuels. However, these applications represent low-value endpoints and do not fully exploit the resource's potential. Our proposed cascade utilization framework seeks to increase value creation through microbial fermentation for MCFA production, followed by nutrient recovery and, finally, energy generation.
Chain elongation using naturally enriched microbial consortia enables the conversion of sugar beet-derived short-chain fatty acids into MCFA. Electron donors such as ethanol and lactate are formed naturally during the fermentation process, eliminating the need for external supplementation. A key challenge in this process is product inhibition caused by the accumulation of MCFA, which can negatively affect microbial activity and limit yields. To address this, an in-line extraction and purification strategy will be implemented to enhance product recovery and potentially improve process stability. The effects of this approach on microbial activity, product yields, and overall process dynamics will be systematically investigated. The study will also examine how different bases used for pH regulation affect microbial activity, product yields, and the stability of the chain elongation process. Local MCFA production from sugar beet pulp could significantly reduce the environmental impacts associated with tropical oil-based routes, including deforestation, biodiversity loss, and long-distance transport emissions.

The research is structured into four work packages: (1) investigation of fermentation yields and parameters for MCFA production from sugar beet pulp at lab and pilot scale; (2) development of an in-line product recovery method to improve fermentation efficiency and stability; (3) analysis of extract impurities and identification of contaminants or valuable by-products; and (4) conversion of the post-extraction fermentation sludge, in combination with phosphorus-rich sludge from the Bio-P process in wastewater treatment, into a high-value recycled fertilizer, with final energy recovery via biogas. The project aims to close carbon and nutrient loops within a regional circular bioeconomy, offering a scalable and sustainable model for industrial transformation in the sugar sector.

P39

Exploring the specialized metabolome of the cyanobacteria *Nostoc punctiforme*

María Miguel-Gordo[1], Barabara Matic[4], Sofia Kerestetzopoulou[1], Julia Krumbholz[1], Keishi Ishida[2], Roderich D. Süssmuth[3], Nicole Strittmatter[4] and Elke Dittmann[1]

1 Institute of Biochemistry and Biology, Universität Potsdam, Karl-Liebknecht Str. 24/25, 14476 Potsdam-Golm, Germany
2 Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Adolf-Reichwein-Straße 23, 07745 Jena, Germany
3 Institut für Chemie, Technische Universität, Müller-Breslau-Str. 10, 10623 Berlin, Germany
4 School of Natural Science, Technische Universität München, Lichtenbergstraße 4, 85748 Garching bei München, Germany.

The cyanobacterium *Nostoc punctiforme* PCC73102 is a fascinating organism with an extensive biosynthetic capability for specialized metabolites and the amenability to being engineered for the production of valuable products. All of these in a sustainable manner, as their autophototrophic ability makes them perfect candidates for bioproduction. However, understanding the activation of the biosynthetic gene clusters (BGCs) and pairing the still cryptic ones with their products remains challenging. To overcome these difficulties, various strategies can be employed to elucidate their complex metabolome.

In this study, we utilized the genetically modified strain AraC_PKS1, based on the overexpression of the AraC regulatory gene of the *pks1* BGC. This genetic modification facilitated the discovery of the *pks1* products, nostovalerolactone and nostoclides, using high-density cultivation conditions [1]. Remarkably, these metabolites function as cell density mediators, influencing the transcriptional regulation of other secondary metabolite BGCs. Furthermore, high-density cultivation is also capable of reprogramming the secondary metabolites of *N. punctiforme* [2]. By comparing the metabolome between the wild-type and mutant strain across different cultivation methods, and the difference in the transcription levels, we can detect metabolites whose genes are silent under conventional conditions and investigate the distribution of the specialized metabolites.

This research integrates genome mining, transcriptomic data, and untargeted metabolomic analysis, including MS/MS feature-based molecular networking and spatial mass spectrometry. We explored the metabolome of *Nostoc punctiforme*, characterizing specialized metabolites to facilitate the study of their biosynthetic pathways and potential activities.

References
[1] Krumbholz J. et al. Angew. Chem. Int. Ed. 2024, 61, 26. https://doi.org/10.1002/anie.202204545
[2] Guljamow, A. et al. AEM. 2017, 83, 23. https://doi.org/10.1128/AEM.01510-17

P40

Spatial organization of the glycopeptide antibiotic biosynthetic machinery

Dardan Begaj[1*], Nicola Gericke[4], Thomas Hamm[1], Nina Pfahler[5], Boris Macek[2], Mirita Franz-Wachtel[2], Wolfgang Wohlleben[3], Samuel Wagner[4] and Evi Stegmann[1]

1 University of Tübingen. Department of Microbial Active Compounds, Tübingen, Germany.
2 University of Tübingen. Proteome Center Tübingen, Tübingen, Germany.
3 University of Tübingen. Department of Microbiology/Biotechnology, Tübingen, Germany.
4 University of Tübingen. Department of Cellular and molecular microbiology, Tübingen, Germany.
5 University of Tübingen. Interfaculty institute of biochemistry. Tübingen, Germany.

Glycopeptide antibiotics (GPAs) are an important class of natural products, including key clinical drugs such as vancomycin and teicoplanin. These antibiotics are predominantly produced by actinomycetes. *Amycolatopsis balhimycina*, the producer of the vancomycin-type GPA balhimycin, serves as an ideal model system for investigating GPA biosynthesis. The biosynthetic pathway of GPAs involves the coordinated action of enzymes responsible for synthesis of the non-proteinogenic amino acids, which are assembled into a peptide backbone by non-ribosomal peptide synthetases (NRPS; BpsA-C). Subsequently, the peptide backbone undergoes modifications facilitated by various enzymes, including a halogenase (BhaA), three P450-monooxygenases (OxyA/B/C), three glycosyltransferases (BgtfABC), and a methyltransferase (Bmt)[1,2,3]. Finally, the GPA is released into the extracellular environment via a specific ABC transporter (Tba)[4]. Although previous studies have uncovered some modification processes occurring while the peptide is bound to the NRPS machinery[1,2,5], the protein-protein interactions (PPI) between the enzymes orchestrating GPA biosynthesis, along with the influence of the exporter on this process, remain unclear.

To investigate the interaction partners within the multi-enzyme complex, including the interconnection between the biosynthetic machinery and the GPA exporter, we employed an artificial intelligence-based approach to predict interactions and validated these predictions experimentally using various methods. Genetic inactivation experiments provided compelling evidence for the crucial role of docking domains in mediating interactions between NRPSs. Bacterial two-hybrid studies revealed interactions between NRPSs and modification enzymes, as well as with small accessory proteins known as MbtH-like proteins (MLPs). Although MLPs are not directly involved in the biosynthesis, they indirectly support the NRPS process. Additionally, Blue native-PAGE and proximity dependent labelling experiments combined with MS/MS-based analysis provided further insights into the interactome of the multi-enzyme complex. These studies highlighted the important role of the specific exporter Tba, acting as an anchor for the multi-enzyme complex. These findings significantly improve our understanding of the GPAs biosynthetic processes and shed light on the complex molecular interactions of the biosynthetic machinery.

1. Stegmann, Evi et al. “Glycopeptide biosynthesis in the context of basic cellular functions.” Current opinion in microbiology vol. 13,5 (2010): 595-602.
2. Kittilä, Tiia et al. “Halogenation of glycopeptide antibiotics occurs at the amino acid level during non-ribosomal peptide synthesis.” Chemical science vol. 8,9 (2017): 5992-6004.
3. Bischoff, Daniel et al. “The biosynthesis of vancomycin-type glycopeptide antibiotics--a model for oxidative side-chain cross-linking by oxygenases coupled to the action of peptide synthetases.” Chembiochem 6,2 (2005)
4. Menges, R et al. “The ABC transporter Tba of *Amycolatopsis balhimycina* is required for efficient export of the glycopeptide antibiotic balhimycin.” Applied microbiology and biotechnology vol. 77,1 (2007): 125-34.
5. Haslinger, Kristina et al. “X-domain of peptide synthetases recruits oxygenases crucial for glycopeptide biosynthesis.” Nature vol. 521,7550 (2015): 105-9.

P41

Precursor-Directed Biosynthesis in Cyanobacteria:

Incorporation of Clickable Amino Acids into Cyanobacterial Peptides

Fritsch, Annika N.[1], Ehrentraut, Vanessa[1], Schanbacher, Franziska[1], Enke, Heike[2] Niedermeyer, Timo H.J.[1]

1 Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str. 2+4, 14195 Berlin, Germany

2 Simris Biologics GmbH, Magnusstraße 11, 12489, Berlin, Germany

Cyanobacteria produce a variety of specialized metabolites, among them many peptides with pronounced biological activities [1,2]. Several of these are promising leads for the development of drugs against e. g. cancer. Natural product leads often need derivatization before they can be used in therapy. Introduction of new functional groups into those leads that make further derivatization possible is one strategy that can be used for this purpose. The Copper-catalyzed azide–alkyne cycloaddition (CuAAC) [3] is a simple way to add other molecules, e. g. antibodies or small fragments, to the lead. Precursor-directed biosynthesis is a rather simple way to alter the structure of peptides and has already been shown to be effective with microcystins [4] – the largest family of cyanobacterial peptides.

In this project, we used different amino acids containing functional groups able to undergo click reactions for precursor-directed biosynthesis. The amino acids were added under controlled conditions to investigate if they would be accepted by the non-ribosomal peptide biosynthesis enzymes of different cyanopeptides. The cultures were extracted and analyzed with HPLC-MS/MS, and the data were evaluated with classical molecular networking (GNPS).

The results show that in every chosen group of peptides (e.g. aetokthonostatins, cryptophycins, microginins) some of the ‘clickable’ amino acids could be incorporated. This approach might be an easy way to alter peptides with interesting modes of action to make them more accessible for pharmaceutical purposes.

[1] Baunach, M. et al. J. Nat. Prod. Rep., 2024, 41, 347-369. https://doi.org/10.1039/D3NP00045A
[2] Tan, L. T. et al. Molecules 2020, 25(9), 2197. https://doi.org/10.3390/molecules25092197
[3] Presolski, S. I. et al. Curr. Protoc. Chem. Biol., 2011, 3(4):153–162. https://doi.org/10.1002/9780470559277.ch110148
[4] Moschny, J. et al. J. Nat. Prod. 2020, 83, 6, 1960–1970. https://doi.org/10.1021/acs.jnatprod.0c00251

P42

Cyanobacteria-microbe interactions influenced by photolytic cyanobacterial siderophores

Berness Peter Falcao[1,2], Tomáš Galica,[1,3] Viviana Di Matteo,[4] Jose Martinez Yerena,[1,5] Štenclová Lenka,[3] Eliška Kozlíková,[5] Jan Mareš,[1,5] Valeria Costantino,[4] Pavel Hrouzek[1]

1 Institute of Microbiology, CAS Centre Algatech, Laboratory of algal biotechnology, Třebon, Czech Republic.
2 University of South Bohemia, České Budějovice, Czech Republic.
3 Freie Universität, Berlin, Germany.
4 Department of Pharmacy, Università degli Studi di Napoli Federico II, 80131 Napoli, Italy.
5 Biology Centre CAS Institute of Hydrobiology, Department of Aquatic Microbial Ecology (AME), České Budějovice, Czech Republic.

Siderophores are iron binding secondary metabolites secreted by microbes, when their internal iron reserves are exhausted. Cyanochelins are recently discovered amphiphilic lipopeptidic siderophores synthesized by cyanobacteria. They bind insoluble Fe^{3+} precipitates inaccessible to microbes; and facilitate iron diffusion to siderophore producers by specific transporter uptake. Iron-cyanochelin complexes are also photolytic and upon exposure to UV light reduce the bound Fe^{3+} to more soluble Fe^{2+}, that can be taken up by almost any microbe. Biosynthetic gene clusters that encode production of cyanochelin-like siderophores are spread across cyanobacteria that generally live in sun-exposed environments and hence the photolytic properties of cyanochelins are likely to offer some benefits to the producer and its partners in the microbial community. [1]
Our latest investigation yielded a detailed structure of cyanochelin B that was elucidated by a combination of Liquid chromatography–mass spectrometry and Nuclear magnetic resonance. Along with the structure, we also determined the kinetics of photolysis and the prominent fragments formed after lysis. The photolysis follows 0-order kinetics and the iron-cyanochelin B are lysed rapidly with a half-life of 2.3 minutes in natural sunlight. To better understand the biological role of cyanochelin, we designed a co-cultivation setup consisting of *Leptolyngbya* sp. NIES-3755 as a cyanochelin producer and *Synechocystis* sp. PCC6803 as a siderophore deficient cohabitant and exposed the test co-cultures to naturally relevant levels of photolytic UV-A light. [2] The reduced iron released by the photolysis of iron-cyanochelin complexes in UV-exposed cultures benefited the acceptor strain and supported their growth two-fold compared to controls. Furthermore, our field campaign delivered cyanobacteria-rich soil crusts from which we have isolated additional cyanochelin B producer - several strains of *Phormidesmis* - as well as 2 strains of heterotrophic bacteria capable of utilizing cyanochelin for iron import.
These findings offer us valuable insights into the role of photolytic siderophores in cyanobacterial soil crusts and how their role may vary depending on the natural light the cyanobacteria and its inhabitants are exposed to.

References

[1] Galica, T. *et al*. J. Applied and Environmental Microbiology. 2021, 87(17), e0312820. https://doi.org/10.1128/AEM.03128-20
[2] Falcao, B. *et al*. bioRxiv. 2025, p. 2025.01.08.631963. https://doi.org/10.1101/2025.01.08.631963

P43

Prenylation of bioactive peptides for drug delivery

Leon Frederic Schwarz[1], Claus-Michael Lehr,[2] Florian Hubrich[1]

1 Helmholtz Institute for Pharmaceutical Research Saarland, Campus E8.1, 66123 Saarbücken, Germany,
2 Pharmacy Department, Saarland University, Campus, 66123 Saarbrücken, Germany.

The discovery of novel and the optimization of characterized antibiotics is crucial to address the escalating challenges of bacterial resistance. Drug lipidation often increases the drug potency, including the efficacy of peptide drugs. All market lipopeptide drugs are acylated, including FDA-approved antibiotics daptomycin and pneumocandin. However, fatty acid lipidation is often associated with an increase in cytotoxicity, corresponding to longer acylchains. Recently, another form of lipidation, the prenylation, has been shown to express similar potency in drug improvement as acylation while cytotoxic effects were observed to increase not or less. [1,2] Thus, prenylation provides a viable alternative to established fatty acylation to expand the current lipopeptide drug diversity.

Natural products (NPs) are a valuable resource for drug discovery, considering their potential as drug leads. The NP superfamily of ribosomally synthesized and post-translationally modified peptides (RiPPs) is highly accessible with targeted genome mining. Hence, the discovery of new peptides is facilitated accompanied with involved modifying enzymes, including a recently discovered novel class of ABBA-type prenyltransferases (PTs), the NctP-like PTs. [3] The recently characterized member NdnP is highly promiscuous, providing a biosynthetic and biocatalytic tool for peptide prenyaltion of different peptides. [4] The prenylation is postion-specific for a determinated amino acid. Genome minig efforts and *in silico* structural modeling resulted in the identification of the active center and the key amino acids for isoprenoid donor specificity, opening up possible modifications to change the prenylated chain length. Ultimately, the prenylated peptides will be used as self-assembling drug delivery particles to achieve an active drug carrier system to be loaded and synergize with established drugs.

References
[1] Elshahawi S.I. *et al*. Nat. Chem. Biol. 2017, 13, 366-368. https://doi.org/10.1038/nchembio.2285
[2] Scull E.M. *et al*. Appl. Microbiol. Biotechnol. 2020, 104, 7853-7865. https://doi.org/10.1007/s00253-020-10790-x
[3] Hubrich F. *et al*. Chem. 2024, 10, 3224-3242. https://doi.org/10.1016/j.chempr.2024.07.026
[4] Magyari S. *et al*. in preparation

P44

From a Single Toxin to a Toxin Network: New Insights into Aetokthonotoxin Derivatives

Schanbacher, Franziska[1*], Rebhahn, Valerie I.C.[1], Niedermeyer, Timo H.J.[1]
1 Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str. 2+4, Berlin, Germany

Cyanotoxins exhibit a remarkable chemical diversity, showing most distinct structures.[1] This diversity, driven by different environmental and ecological factors, plays a critical role in their impact on aquatic ecosystems and human health.[1,2] The cyanobacterium *Aetokthonos hydrillicola* has recently become famous as the "eagle killer" producing the biindole alkaloid aetokthonotoxin (AETX), a pentabrominated neurotoxin causing Vacuolar Myelinopathy.[3] The toxin is transported via the food chain, posing risks to wildlife and potentially human health.[3] HPLC-HRMS^2 analysis of extracts of environmental samples of the cyanobacterium revealed the presence of AETX derivatives and biosynthetic intermediates. Mass spectrometry, with its high-throughput capabilities and enhanced by computational approaches, is the optimal method for streamlining and accelerating the discovery of natural products and their derivatives.[4] We aimed to use these approaches, e.g. Classical Molecular Networking for obtaining an overview over the chemical space around AETX, MassQL for in-depth analysis,[6] and Feature-Based Molecular Networking for studying structural diversity.[7] We identified in total 43 biosynthetic intermediates and derivatives of AETX. Intriguingly, we detected several iodinated derivatives, a rare halogenation in specialized metabolites of freshwater organisms. Supplementation studies with KBr, KI or both were utilized to investigate the production of these derivatives. After large scale cultivation, five AETX derivatives were isolated, and their structures were elucidated using NMR spectroscopy. Based on these data, the chemical structures of the additional derivatives detected in the extracts by HPLC-HRMS^2 could be proposed. We conclude that in addition to bromide, iodide is also accepted as a substrate by the halogenases involved in AETX biosynthesis, AetF and AetA. Moreover, cytotoxicity assays of the isolated derivatives showed that they differ markedly in their cytotoxicity.
This study demonstrates the diversity of natural AETX derivatives, and the indisputable advantages of the MS-based tools mentioned.

References
[1] Buratti, F.M., Manganelli, M. *et al.*, Archives of Toxicology, 2017, 91, 1049-1130, https://doi.org/10.1007/s00204-016-1913-6
[2] Janssen, E.M.L., Water Research, 2019, 151, 488-499, https://doi.org/10.1016/j.watres.2018.12.048
[3] Breinlinger S., Phillips T.J., *et al*., Science. 2021, 371, https://doi.org/10.1126/science.aax9050
[4] Jarmusch, S.A., van der Hooft, J.J.J. *et al.*, Nat. Prod. Rep., 2021, 38, 2066-2082. https://doi.org/10.1039/d1np00040cC
[5] Wang, M., Carver, J.J. *et al.*, Nat. Biotechnol., 2016, 34, 828-37. https://doi.org/10.1038/nbt.3597
[6] Jarmusch A.K., Aron A.T., *et al*., bioRxiv., 2022, 2022.08.06.503000. https://doi.org/10.1101/2022.08.06.503000
[7] Nothias L-F, Petras D, *et al*., Nat. Methods. 2020, 17, 905-8, https://doi.org/10.1038/s41592-020-0933-6

P45

Exploring biosynthetic gene clusters from *Nostocales* cyanobionts

C. Kollten[1], Y. Kriukova[1], F. Hubrich[1], T. A. M. Gulder[1], P. M. D'Agostino[1]

1 Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Department of Natural Product Biotechnology, Helmholtz Centre for Infection Research (HZI) and Department of Pharmacy, PharmaScienceHub (PSH), Saarland University, Campus E8.1, 66123 Saarbrücken, Germany.

Cyanobacteria are photosynthetic microorganisms that exhibit exceptional adaptability in almost all environmental niches [1]. Bioactive natural products (NPs) from symbiotic cyanobacteria (cyanobionts), particularly those in terrestrial hosts like lichens and cycads, remain largely unexplored [1]. Among cyanobacteria producing compounds with potentially beneficial activities, the order *Nostocales* represents the predominant source [2]. Within this order, the genera *Nostoc* has in the past already proven to be a promising target due to their habitat diversity and biosynthetic potential. Examples include the lichen *Peltigera canina* hosting *Nostoc* cyanobionts from which the anti-infective NPs Nostoclide I and II were isolated, or cryptophycin isolated from *Nostoc* sp. ATCC 53789, one the most potent tubulin-destabilizing molecules ever discovered. These examples illustrate the metabolic potential of cyanobionts [2, 3] and make them a promising target for further exploration of novel bioactive compounds with potential anti-infective effects. Other *Nostocales* cyanobiont genera such as *Rhizonema* represent completely unexplored targets for NP discovery on both, genetic and chemical levels.
Within this work, we investigated 408 *Nostocales* genomes of the order *Nostocales* for the identification of novel interesting biosynthetic gene clusters (BGCs). A phylogenetic analysis was performed, placing the cyanobionts into five clades. The symbiotic clades 1 to 3 contain different species of cyanobionts and free-living cyanobacteria, whereas the other two strictly symbiotic clades contained *Rhizonema* or *Nostoc azollae*. Based on these results, genome mining was performed including an antiSMASH analysis of all genomes for BGC detection and further investigation of all 81 cyanobiont genomes derived from cycads or lichens [4]. This was completed with a visualization of the 2007 detected BGCs in a BiG-SCAPE-generated similarity network, in which the BGCs were grouped into 938 gene cluster families (GCFs) including 630 singletons [5]. Genome analysis of *Rhizonema* revealed BGCs encoding prenylated ribosomally synthesized and post-translationally modified peptides. Prenylation, a post-translational modification commonly associated with secondary metabolism, increases peptide lipophilicity, thereby facilitating membrane interactions and improving bioavailability and stability [6]. Selected BGCs will further be cloned into a heterologous host utilizing Direct Pathway Cloning (DiPaC) for expression and subsequent elucidation of NP structures and functions [7, 8]. This study shows that *Nostocales* cyanobionts harbour a huge diversity of cryptic BGCs, thereby establishing a foundation for further exploration of cyanobacterial NPs with prospective bioactivities, including anti-infective properties.

References
[1] Rikkinen, J., Mod. Topics Phototrophic Prokaryotes 2017, 243-294. https://doi.org/10.1007/978-3-319-46261-5_8
[2] D'Agostino, P.M. Nat. Prod. Rep. 2023, 40, 1701-1717. https://doi.org/10.1039/D3NP00011G
[3] Magarvey, N.A., *et al.* ACS Chem Biol. 2006, 1(12), 766-779. https://doi.org/10.1021/cb6004307
[4] Medema, M. H., *et al.* Nucleic Acids Res. 2011, 39 (suppl 2), W339–W346. https://doi.org/10.1093/nar/gkr466
[5] Navarro-Munoz, J.C. *et al*. Nat. Chem. Biol. 2020 16(1), 60–68. https://doi.org/10.1038/s41589-019-0400-9
[6] Xhang, Y. *et al*. Trends Biochem. Sci. 2023, 48 (4), P360-374. https://doi.org/10.1016/j.tibs.2022.11.002
[7] Greunke, C., *et al.* Metab. Eng. 2018, 47, 334-345. https://doi.org/10.1016/j.ymben.2018.03.010
[8] D'Agostino, P.M. *et al.* ACS Synth Biol. 2018, 7(7), 1702-1708. https://doi.org/10.1021/acssynbio.8b00151

P46

Mixing and Matching of Hybrid Megasynthases is a Hub for the Evolution of Metabolic Diversity in Cyanobacteria

Keishi Ishida[1], Qi Sun[2], Jonna Teikari[3], Christian Hertweck[1,4], Elke Dittmann[5], Masahiro Murakami[2], Martin Baunach*[6]

1 Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Beutenbergstr. 11a, 07745 Jena, Germany.
2 Department of Marine Biochemistry, Graduate School of Agricultural Life Sciences, The University of Tokyo 1-1-1 Yayoi, Bunkyo-ku, 113-8657 Tokyo, Japan.
3 Institute for Atmospheric and Earth System Research, University of Helsinki, P.O. Box 56 (Viikinkaari 9), FI-00014 University of Helsinki, Finland.
4 Institute of Microbiology, Friedrich Schiller University Jena, Neugasse 25, 07743 Jena, Germany.
5 Department of Microbiology, Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24/25, 14476 Potsdam-Golm, Germany.
6 Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, 53115 Bonn, Germany.

Modular megasynthases, such as polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs), are molecular assembly lines that biosynthesize many pharmaceutically and ecologically important natural products. Understanding how these compounds evolve could inspire the artificial evolution of compound diversity by metabolic engineering. Over the past two decades, a number of seminal studies have significantly contributed to our understanding of natural product evolution. However, the evolution of NRPS and PKS assembly lines remains poorly understood, especially for NRPS/PKS hybrids. Here, we provide substantial evidence for a remarkable cluster-mixing event involving three cyanobacterial biosynthetic gene clusters, resulting in the emergence of novel peptide-polyketide hybrids that were named minutumamides.[1] By combining retro-evolutionary analysis with structure-guided genome mining we could discover a potential evolutionary ancestor that links nostopeptolide and minutumamide biosynthesis. In addition, we were able to trace nostopeptolide-related module and domain blocks in various other biosynthetic pathways indicating a surprisingly vivid mixing and matching of biosynthesis genes in the evolution of NRPS and *cis*-acyltransferase PKS/NRPS pathways, which was previously regarded as a unique feature of *trans*-acyltransferase PKS. These remarkable insights into the evolutionary plasticity of NRPS-PKS assembly lines provide valuable guidance for pathway engineers looking for productive combinations that yield "non-natural" hybrid natural products.

Reference

[1] K. Ishida, Q. Sun, J. Teikari, C. Hertweck, E. Dittmann, M. Murakami, M. Baunach. *Angew. Chem. Int. Ed.* **2025**, *64*, :e202502461. doi: 10.1002/anie.202502461.

P47

MS-integrated Reactivity-Based Genome Mining: A Novel Approach for Natural Product Discovery

Bastian Brand[1], Martin Baunach[2], Chambers Hughes[3] , Raphael Reher[1]

1 Institute of Pharmaceutical Biology and Biotechnology, Marburg University, Robert-Koch-Str. 4, 35037 Marburg, Germany.
2 Institute of Pharmaceutical Biology, University Bonn, Nussallee 2, 53115 Bonn, Germany
3 Department of Microbial Bioactive Compounds, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), University Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany

Marine fungal specialized metabolites (SMs) are an important source of bioactive small molecules. However, their discovery is hampered by frequent re-isolation of known compounds, low or silent expression of biosynthetic gene clusters (BGCs), and the difficulty of linking BGCs to their corresponding metabolites.

Here, we introduce **MS-integrated Reactivity-based Genome Mining (MS-RGM)**. This approach employs reactive targeted and untargeted chemical probes to capture (i) SMs with defined functional groups and (ii) volatile metabolites and biosynthetic intermediates using broadly reactive probes. The resulting adducts are detected through MassQL-based MS queries and subsequently prioritized for isolation and structural elucidation (Fig. 1).[1] In addition, silent BGCs can be activated by ecological interactions in co-culture, OSMAC strategies, or probe-induced chemical stress. Compared to classical genome mining, MS-RGM enables more efficient BGC–SM connections and expands accessible chemical space by generating “unnatural” pseudo-SMs for bioactivity screening.

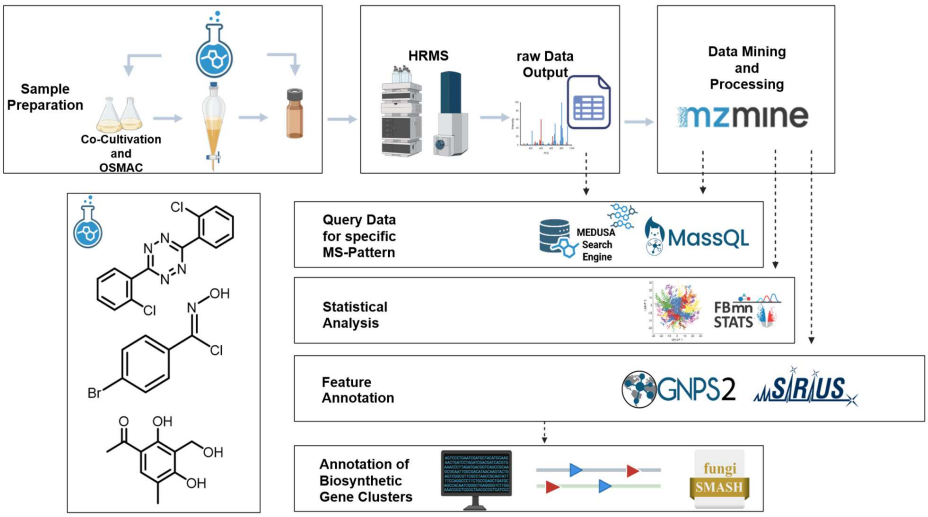


Figure 1 Workflow: MS-integrated Reactivity-based Genome Mining

As a proof of concept, we focus on isonitrile-containing metabolites. We used a trifunctional probe, bearing a chlorooxime moiety.[2] Application of this isonitrile-specific probe to a known cyanobacterial extract led to the re-discovery of established isonitrile SMs.

References

[1] Damiani, T., Jarmusch, A.K., Aron, A.T. *et al. Nat Methods* **22**, 1247–1254 (2025)
[2] Schäfer, R. J. B. *et al. Chemistry A European J* **29**, e202203277 (2023)

P48

Functional and Ecological Characterization of Specialized Peptides in *Bacillota* from the Gut Microbiome

Johanna Bosch[1], Qingin Pu[2], Thomas C. A. Hitch[1], David Wylensek[1], Lauren Boak[3], Liam Cassidy[4], Andreas Tholey[4], Klaus Winzer[3], Tobias A. M. Gulder[2], and Thomas Clavel[1]

[1]*Functional Microbiome Research Group, University Hospital RWTH Aachen, Germany*
[2]*Chair of Technical Biochemistry, Technical University of Dresden, Germany*
[3]*Synthetic Biology Research Centre, University of Nottingham, United Kingdom*
[4]*Systematic Proteomics and Bioanalytics, University of Kiel, Germany*

Natural products like ribosomally synthesized and post-translational modified peptides are structurally and functionally very diverse. Whilst many are being discovered *in silico* using pattern recognition tools such as antiSMASH[1], functional studies of those produced in the human gut microbiome are needed. In previous work, we identified a novel group of ranthipeptides in cultured bacteria from the pig gut microbiome[2]. Genomic analysis of 675 isolates from multiple host species (human, n = 340; mouse, n = 212; pig, n = 123), revealed that 177 strains encoded related corresponding biosynthetic gene clusters (BGCs) and all of them belonged to the phylum *Bacillota*. Prevalence of the precursor peptides across 9,634 human gut metagenomes was >10 % for 109 strains, with up to 66.8 % for the peptide encoded by *Maccoyia intestinihominis*. Amino acid sequence comparison revealed major peptide clusters, one of which included none of the so-far described sacti- and ranthipeptides and only isolates of the genus *Clostridium* (*sensu stricto*). These peptides shared a conserved motif, with 6 cysteines within 27 amino acids. To investigate their function in targeted manner, we selected the ranthipeptide encoded by *Clostridium beijerinckii* DSM 105335 as a model. qPCR data showed the precursor peptide of *C. beijerinckii* to be mainly expressed during mid-exponential growth phase, similar to other antimicrobial peptides like steptosactin[3]. A knockout variant of this strain unable to produce the mature ranthipeptides due to deletion of the maturation enzyme (ΔrSAM) was created by ClosTron mutagenesis[4]. To find possible microbiome members affected by the specialized peptide, the growth of commensal isolates was tested in the presence of culture supernatant from the wildtype and mutant strains. The gut commensal *Roseburia intestinalis* was found to be sensitive, i.e. its growth was significantly reduced by cell-free supernatants from the wildtype but not the ΔrSAM strain. Co-cultivation experiments confirmed these findings. A defined community of cultured bacteria was developed to study the relevance of the novel ranthipeptide in gnotobiotic mice. Preliminary batch fermentaiton experiments revealed a difference in relative abundances of *C. beijerinckii* WT and ΔrSAM within the community (25.6% vs. 6.8% after 5 daily transfers). Samples from both, the *in vitro* and *in vivo* experiments will undergo metagenomic and proteomic analysis, which will enable detailed analysis of community dynamics and test whether the ranthipeptide may provide a competitive advantage or alter community composition in the gut.

References:

[1] Medema *et al.* (2011), **NAR,** https://doi.org/10.1093/nar/gkr466
[2] Wylensek *et al.* (2020), **Nat Commun., https://doi.org/10.1038/s41467-020-19929-w**
[3] Bushin *et al.* (2020), **J. Am. Chem. Soc., https://pubs.acs.org/doi/10.1021/jacs.0c05546**
[4] Kuehne & Minton (2012),**Bioengineered., https://doi.org/10.4161/bioe.21004**

P49

Introducing the research activities and services of the BCCM/ULC cyanobacteria public collection.

Maria Christodoulou[1,2*], Haifaa Savora[1], Guillaume Dumoulin[1], Luc Cornet[1,3], Annick Wilmotte[1]

1 BCCM/ULC, InBios–Molecular Diversity and Ecology of Cyanobacteria, University of Liège,Chemin de la Vallée 4, 4000 Liège, Belgium
2 Cyanobacteria Research Group, Department of Microbiology, University of Helsinki, Viikinkaari 9, 00790, Helsinki, Finland
3 InBioS–PhytoSYSTEMS, Eukaryotic Phylogenomics, University of Liège,Chemin de la Vallée 4, 4000 Liège, Belgium

Cyanobacteria are a distinctive group of oxyphototrophic bacteria characterized by extensive morphological and metabolic diversity. Their long and complex evolutionary history has contributed to the successful colonization of a wide range of habitats from polar to temperate and tropical regions.
The BCCM/ULC is a young public collection funded by the Belgian Science Policy Office (BELSPO). The collection is currently hosting more than 500 cyanobacterial strains, of which approximately 140 derive from polar, subpolar, and alpine environments, followed by tropical, subtropical and mediterranean biotopes as well as strains of Belgian origin. An ISO certificate covers the public deposition and distribution of strains, as part of a multi-site certification for the BCCM consortium.
All strains are studied by applying a polyphasic approach workflow (i.e., morphological, molecular, and ecological data). Furthermore, the collection includes more than 20 strains that are the reference (or 'type') for newly described taxa including *Plectolyngbya*, *Shackletoniella*, *Timaviella, Parakomarekiella*, *Petrachloros, Leptochromothrix, Vermifilum*, *Tigrinifilum, Affixifilum, Sirenicapillaria, Ophiophycus* and *Floridanema*.
Cyanobacteria are known for producing a large number of bioactive metabolites. To date, there have been more than 2000 functionally diverse and structurally complex bioactive metabolites identified from cyanobacteria, including alkaloids, cyclic and linear peptides, polyketides and nonribosomal peptides. These metabolites range from toxins to biologically active molecules showing promise as potential drug leads. As part of an ongoing effort to discover new molecules with potential pharmaceutical applications, the strains are being evaluated for their antibacterial and antifungal activities.
Additionally, an integrated approach that combines whole-genome sequencing, metagenomics, phylogenomics and phylogenetic placement is applied to study taxonomically interesting morphotypes and bioactive metabolite-producing strains. Recently, the collection has developed a suite of highly reproducible genomic workflows (GEN-ERA; https://github.com/Lcornet/GENERA), coded in Nextflow and based on Singularity containers, that meet the requirements of open science and FAIR practices.

P50

Aetokthonotoxin uncouples oxidative phosphorylation due to protonophore activity

Valerie I. C. Rebhahn[1], Mohamad Saoud[2], Mathias Winterhalter[3], Franziska Schanbacher[1], Maximilian Jobst[4,5], Rebeca Ruiz[6], Alexander Sonntag[1], Johannes Kollatz[1,2], Rieke Sprengel[1], Stephen F. Donovan[7], Giorgia Del Favero[4], Robert Rennert[2], Timo H. J. Niedermeyer[1,*]

1 Institute of Pharmacy, Pharmaceutical Biology, Freie Universität Berlin, 14195 Berlin, Germany
2 Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, 06120 Halle (Saale), Germany
3 School of Science, Constructor University Bremen gGmbH, 28759 Bremen, Germany, and Center for Hybrid Nanostructures, Universität Hamburg, 22761 Hamburg, Germany
4 Department of Food Chemistry and Toxicology, and Core Facility Multimodal Imaging, Faculty of Chemistry, University of Vienna, 1090, Vienna, Austria.
5 University of Vienna, Vienna Doctoral School in Chemistry (DoSChem), 1090 Vienna, Austria
6 Pion Inc., Forest Row Business Park, Forest Row RH18 5DW, United Kingdom
7 Retired
* Part of this work was done at the Department of Pharmaceutical Biology/Pharmacognosy, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, 06120 Halle (Saale), Germany

Wildlife in the south-eastern United States suffer from vacuolar myelinopathy. This disease comprises severe neurological impairment with disruption of myelin sheaths in the white matter and is eventually lethal. We recently determined aetokthonotoxin (AETX) as the causative agent. AETX is a unique natural product synthesized by the epiphytic freshwater cyanobacterium *A. hydrillicola*, and transmitted via the food chain [1]. With the aetiology of the disease being unravelled, we now focused on the mode of action of AETX. The effect of AETX on mammalian cells and bacteria was assessed in various *in vitro* assays using fluorescent probes and a Seahorse XF Pro Analyzer. Effects of AETX on oxygen consumption, mitochondrial membrane potential, reactive oxygen generation and mitochondrial ATP production indicate that the primary mechanism of AETX is the uncoupling of the oxidative phosphorylation in mitochondria. We could further demonstrate that AETX acts as a protonophore.
For the first time, biological activity of AETX was characterized beyond cytotoxicity assays with the aim to elucidate its mode of action. We have found that AETX uncouples the oxidative phosphorylation in mitochondria by acting as a protonophore.

References
[1] Breinlinger *et al.* 10.1126/science.aax9050 (2021). https://doi.org/10.1126/science.aax9050

6 Poster List

Presenting Author	Poster ID	Title
Samia Mohamed	P01	Metabolic Engineering of *Streptomyces tendae* Tü 4042 to Boost Lysolipin I Biosynthesis
Maria-Paula Schröder	P02	Expanding the RiPP Toolbox: Biochemical Characterization of Peptide Arginases
Jacqueline Wistuba-Hamprecht	P03	Predictive Biocatalyst Selection for Metabolite Synthesis
Isabel P.-M. Pfeiffer	P04	Unfolding Peptide Arginases: Bioinformatic Insights into RiPP Precursor-Maturase Interaction
Julia Bischof	P05	Tailoring fungal leucine-rich non-ribosomal peptides by mutasynthesis in a heterologous *Aspergillus* host
Lena Schweizer	P06	Protective bacterial symbionts from toxic passerine birds from Papua New Guinea
Mathilde Büttner	P07	Identification of the biosynthetic gene cluster of acivicin
Florian Semmler	P08	Exploring the Role of Ribosomal Peptides in the Human Lung Microbiome
Annika Bruntner	P09	Analysis of the CRISPR-Cas Region 1 in *Streptoalloteichus tenebrarius*
Lenka Štenclová	P10	Unexpected occurrence of marine secondary metabolites in inland waters of post-mining lakes in north-west of Czechia

Shreyash Pramod Borkar	P11	Direct Cloning of Metagenomic BGCs for Broad-Host Expression and Natural Product Discovery
Noel Kubach	P12	From Genes to Molecules – Predicting the Structure of Glycopeptides from Biosynthetic Gene Clusters
Lucia Lernoud	P13	Discovery and in vitro reconstitution of closoxazole biosynthesis from *Pyxidicoccus fallax*
Lorenz Thost	P14	Antimycobacterial Lasso Peptides – Identification, Production, and Opportunities in Drug Development & Synthetic Biology
Philippe R. Meisinger	P15	Discovery and Biosynthesis of Celluxanthenes, Antibacterial Arylpolyene Alkaloids From Diverse Cellulose-Degrading Anaerobic Bacteria
Till Steinmetz	P16	Engineering NP discovery – Miniaturization and Parallelization for Multivariate OSMAC
Anna Katharina Siegert	P17	Adapting Versatile Opportunities for Glycopeptide Antibiotics against Drug-Resistant Organisms
Elisa de Lannoy Guerra	P18	Enhancing natural products extraction: using metabolomics for evaluating analytical methods
Mareike Danne	P19	Identification of novel secondary metabolites from rare actinomycetes through genome mining and heterologous expression approaches
Jakob Philipp Fink	P20	Improved biopolymer production in the genetically recombinant filamentous cyanobacterium *Nostoc* sp. PCC7120 without nitrogen starvation by utilizing an inducible biosynthesis platform
Kai Schließmann	P21	Native Mass Spectrometry as a High-Throughput Platform for Protein-Ligand Interaction Screening
Josefine Klerch	P22	Novel Bestatin Derivatives from a PKS/NRPS Hybrid Pathway

Sofia Kerestetzopoulou	P23	Development of an expression platform for nonribosomal peptides in symbiotic cyanobacteria of the genus *Nostoc*
Pablo Barrancos Garrigós	P24	Modeling cyanotoxin production in Lake Mendota
Lukas Zimmer	P25	Paenidepsins: a novel family of lipopeptides from *Paenibacillus*
Carlotta Hecker	P26	Discovery of natural products from moss-associated bacteria
Simon Cremers	P27	Diversification of the myxobacterial metabolome by cultivation-dependant approaches
Martin Gepperth	P28	OSMAC-Driven Investigation of Marine-derived Myxobacteria: Insights from Strain MSr13327
Haowen Zhao	P29	Uncovering Hidden Biosynthetic Potential with ACTIMOT
Antonio Sáenz-Castillo	P30	Chemoenzymatic synthesis of lasso peptides for the modulation of the glucagon receptor
Lilu Liu	P31	Characterization of natural products with anti-infective properties originating from human microbiota
Huixin Xu	P32	Genomic insights into antimicrobial resistance genes in Myxobacteria
Thomas Heschele	P33	Optimizing the [S,S]-EDDS precursor L-aspartate in *Amycolatopsis japonicum* by metabolic engineering
Christoph Ulbricht	P34	Discovery of novel suomilides from *Nostoc* sp. KVJ20
Daniel Krug	P35	Microbelix – exploring microbial diversity in soil by a citizen science approach

Dëborë Zenelaj & Tobias Füssel	P36	Mining functional natural products from the gut microbiome
Alfred Lobert	P37	Biosynthesis of the Biphenomycin Family of Potent Antibiotics
Mourice Wölbeling	P38	Development of a Cascading Biorefinery Concept for Medium-Chain Fatty Acid Production from Sugar Beet Pulp
María Miguel-Gordo	P39	Exploring the specialized metabolome of the cyanobacteria *Nostoc punctiforme*
Dradan Beqaj	P40	Organization and functional complex formation of the glycopeptide antibiotic biosynthetic machinery
Annika Fritsch	P41	Precursor-Directed Biosynthesis in Cyanobacteria: Incorporation of Clickable Amino Acids into Cyanobacterial Peptides
Berness Peter Falcao	P42	Cyanobacteria-microbe interactions influenced by photolytic cyanobacterial siderophores
Leon Frederic Schwarz	P43	Prenylation of bioactive peptides for drug delivery
Franziska Schanbacher	P44	From a Single Toxin to a Toxin Network
Yelyzaveta Kriukova & Cassandra Kollten	P45	Exploring biosynthetic gene clusters from Nostocales cyanobionts
Keishi Ishida	P46	Mixing and Matching of Hybrid Megasynthases is a Hub for the Evolution of Metabolic Diversity in Cyanobacteria
Bastian Brand	P47	MS-integrated Reactivity-Based Genome Mining: A Novel Approach for Natural Product Discovery
Johanna Bosch	P48	Functional and Ecological Characterization of Specialized Peptides in Bacillota from the Gut Microbiome

Maria Christodoulou	P49	Introducing the research activities and services of the BCCM/ULC cyanobacteria public collection.
Valerie I. C. Rebhahn	P50	Aetokthonotoxin uncouples oxidative phosphorylation due to protonophore activity

7 Food & Dining Options Nearby

If you still feel hungry after the programme, would like to end the evening in a relaxed atmosphere, or simply enjoy a drink nearby, we have put together a few recommendations within walking distance of the venue. For everything else, Berlin offers countless opportunities for going out. Several colleagues from Berlin are part of our team and will be happy to share further tips.

"**Alter Krug**" Dahlem – 900 m from venue / approx. 13 minutes on foot (traditional German cuisine, mid-range) Königin-Luise-Straße 52, 14195 Berlin – Zehlendorf

"**Luise**" Dahlem – 700 m from venue / approx. 10 minutes on foot (traditional German and Italian cuisine, mid-range) Königin-Luise-Straße 40–42, 14195 Berlin – Zehlendorf

"**Hee Lam Mun**" – 750 m from venue / approx. 11 minutes on foot (traditional Chinese cuisine, mid-range) Grunewaldstraße 23, 12165 Berlin – Steglitz

Ristorante Pizzeria "**Piaggio**" – 800 m from venue / approx. 11 minutes on foot (traditional Italian cuisine, mid-range) Königin-Luise-Straße 44, 14195 Berlin – Zehlendorf