



Biology of bacteria producing natural products

VAAM Symposium 2026

Symposium Booklet

Riedberg Campus, Goethe University,
Frankfurt am Main

16th - 18th September 2026

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**Vereinigung für Allgemeine und Angewandte
Mikrobiologie**

Programme of Events

Wednesday, September 16th

Registration and Welcome

at Otto-Stern-Zentrum

Goethe-Universität, Ruth-Moufang-Straße 2, 60438 Frankfurt-Nord-West

12:00-13:20 Registration & Poster Set-Up

13:20-13:30 Eric Helfrich & Max Crüsemann, Goethe University

Welcome and Opening remarks

Session 1

at Otto-Stern-Zentrum

Chaired by Eric Helfrich

13:30-14:30 Keynote Lecture 1: Michelle Chang, Princeton University, USA

Synthetic biology approaches to new chemistry

14:30-15:00 Lecture 1: Xiaoyu Tang, Shenzhen Bay Laboratory, CN

Harnessing Biosynthesis Logic for Natural Product Discovery, Evolution, and Function

15:00-15:30 Coffee break

Session 2: Peptides

at Otto-Stern-Zentrum

Chaired by Lena Barra

15:30-15:50 Lecture 2: Timo Negri, University of Tübingen

Discovery and Heterologous Expression of Soil Metagenome-Derived Lasso Peptides

15:50-16:10 Lecture 3: Christoph Ulbricht, University of Bonn

Investigations on cyanobacterial protease inhibitors with intriguing pharmaceutical potential

16:10-16:30 Lecture 4: Josefine Klerch, University of Leipzig

Unravelling Bestatin Biosynthesis through Genome Mining, Heterologous Expression, and Molecular Networking

16:30-16:50 Lecture 5: Nicolas Müller, Technical University of Munich

Biosynthetic Pathway of Belactosin C Reveals Dual Targeting of Proteasome and ClpP Proteases

Poster session 1

at Otto-Stern-Zentrum

16:50-19:00 Poster numbers: Odd numbers.

Dinner and Conference Party

at Biozentrum

Goethe-Universität, Max-von-Laue-Straße 9, 60438 Frankfurt-Nord-West

19:00- Late *Featuring a range of freshly made pizzas, drinks, games and a DJ.*

Thursday, September 17th

VAAM Hendrik Wolff-Award Lecture

at Otto-Stern-Zentrum

Chaired by Eric Helfrich

08:30-09:00 To be announced

Session 3

at Otto-Stern-Zentrum

Chaired by Eric Helfrich

09:00-10:00 Keynote Lecture 2: Lihan Zhang, Westlake University, Hangzhou, CN
Decoding and Reprogramming Assembly-Line Polyketide Synthase

10:00-10:30 *Coffee break*

Session 4: Biosynthetic Engineering

at Otto-Stern-Zentrum

Chaired by Gerald Lackner

10:30-10:50 Lecture 6: Simon Hunsicker, Goethe University Frankfurt
Engineering Substrate Loading in trans in Modular Polyketide Synthase

10:50-11:10 Lecture 7: Panagiota-Hanna Koutsandrea, University of Tübingen
Uridyl Peptide Antibiotics: Establishing a Production Platform for a Potent Antipseudomonal Drug Candidate

11:10-11:30 Lecture 8: Alison V. Müller, HIPS Saarbrücken
Expanding the genetic toolbox for Myxobacteria: New autonomous shuttle vectors for natural product pathway engineering

11:30-11:50 Lecture 9: Luca Salvi, University of Tübingen
Discovery of novel natural products from a rare actinomycete through heterologous expression

Lunch break

at Pi x Gaumen Cafeteria in the Biozentrum

11:50-13:10

Session 5

at Otto-Stern-Zentrum

Chaired by Max Crüsemann

13:10-14:10 Keynote Lecture 3: Matthew Jenner, University of Warwick, UK

Capturing megasyn(et)ase evolution in action

Session 6: Session New Tools and Methodologies

at Otto-Stern-Zentrum

Chaired by Florian Hubrich

14:10-14:30 Lecture 10: Christopher Whitford, Technical University of Denmark,
Lyngby, DK

Integrating Computational and Experimental Workflows Towards High-Throughput Metabolic Engineering of Streptomyces

14:30-15:00 *Coffee break*

15:00-15:20 Lecture 11: Amay Ajaykumar Agrawal, HIPS Saarbrücken

LoReMINE: Long Read-based Microbial genome mining pipeline

15:20-15:40 Lecture 12: Ondřej Hřebíček, Czech Academy of Sciences, Prague, CZ

CluSeek-Bioinformatic Tool for Gene Cluster Discovery within GenBank

15:40-16:00 Lecture 13: Aleksandra Kushnareva, HIPS Saarbrücken

BGC-QUAST: a quality assessment tool for genome mining software

Poster session 2

at Otto-Stern-Zentrum

16:00-18:00 Poster numbers: Even numbers.

18:00-19:00 *Rest and travel to conference dinner*

Conference Dinner

at Alt-Oberurseler Brauhaus

Ackergasse 13, 61440 Oberursel

19:00-Late *Featuring a course of Brauhaus-style dishes*

Friday, September 18th

Session 7: P450 Monooxygenases

at Otto-Stern-Zentrum

Chaired by Max Crüsemann

-
- | | |
|-------------|---|
| 08:30-08:50 | Lecture 14: Heiner Gerald Weddeling, University of Basel, CH
<i>Non-Canonical Rubromycin Late-Stage Tailoring Involves an Archetypal Internal P450 Monooxygenase</i> |
| 08:50-09:50 | Keynote Lecture 4: Max Cryle, Monash University, Melbourne, AU
<i>Engineered cytochrome P450 enzymes as catalysts for peptide crosslinking</i> |
| 09:50-10:30 | Coffee break |
-

Session 8: Terpenes; Function & Regulation of Natural Products

at Otto-Stern-Zentrum

Chaired by Jethro Hemmann

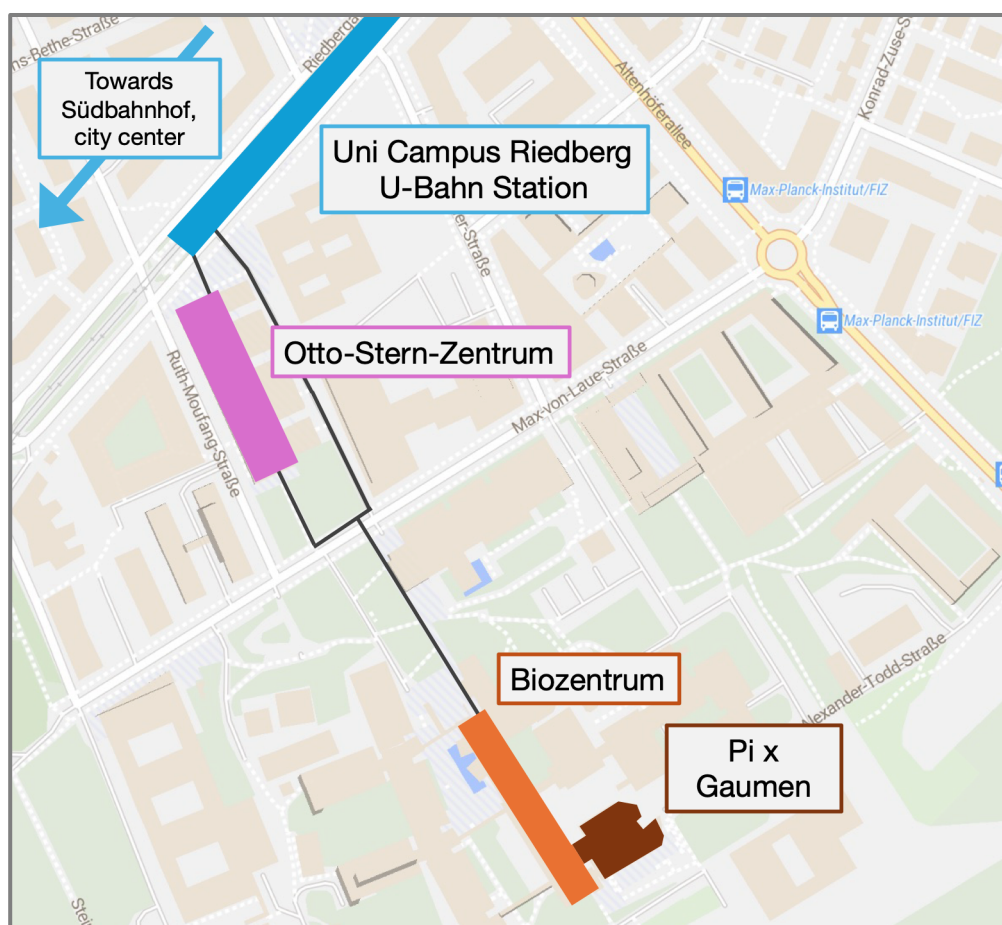
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- | | |
|-------------|---|
| 10:30-10:50 | Lecture 15: Tatjana Reuter, University of Konstanz
<i>Homoterpene biosynthesis widespread in actinobacteria</i> |
| 10:50-11:10 | Lecture 16: Ayesha Ahmed Chaudhri, Goethe University Frankfurt
<i>Genome Mining-Guided Identification and Functional Characterization of Bacterial Terpenoid Biosynthetic Pathways</i> |
| 11:10-11:30 | Lecture 17: Guillermo Cera Ramirez, MPI Marine Microbiology Bremen
<i>Beyond Nutrition: Biosynthetic potential in Chemosynthetic Animal-Microbe Symbioses</i> |
| 11:30-11:50 | Lecture 18: Mufarrah Mehboob, Czech Academy of Sciences, Prague, CZ
<i>Exploring the antibiotic-responsive regulatory function of ABCF proteins in antibiotic-producing bacteria</i> |
-

Poster and Lecture Prizes; Closing Remarks

at Otto-Stern-Zentrum

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- | | |
|-------------|--|
| 11:50-12:10 | Eric Helfrich & Max Crüsemann, Goethe University |
| 12:10 | Exit with pizza and refreshments |
-

Conference Map



Key locations within Riedberg campus.

Google Maps links for key locations

Uni Campus Riedberg U-Bahn station, 60438 Frankfurt-Nord-West	Link	Public transport
Otto-Stern-Zentrum, Goethe-Universität, Ruth-Moufang-Straße 2, 60438 Frankfurt-Nord-West	Link	Registration, Talks, Posters, Coffee breaks
Biozentrum, Goethe-Universität, Max-von-Laue-Straße 9, 60438 Frankfurt-Nord-West	Link	Wednesday social, Lunch (Pi x Gaumen Cafeteria)
Alt-Oberurseler Brauhaus, Ackergasse 13, 61440 Oberursel	Link	Conference dinner

Travel to 'Uni Campus Riedberg' via Public Transport

The nearest U-Bahn station is 'Uni Campus Riedberg' and is served by U-Bahn lines U8 and U9.

The nearest bus stop is also 'Uni Campus Riedberg' and is served by bus line 29.

The Otto-Stern-Zentrum is a large, golden and rectangular building and is less than a 2-minute walk from both the U-Bahn station and the bus stop.

From Frankfurt Airport Regional Train Station (Frankfurt am Main Flughafen Regionalbahnhof)

Approx. 60 minutes

Take either the S8 or S9 via Frankfurt Central Station and Taunusanlage and alight at Hauptwache. At Hauptwache, change to the U8 towards Riedberg and alight at Uni Campus Riedberg.

From Frankfurt Central Station (Frankfurt am Main Hauptbahnhof)

Approx. 40 minutes

Take either the S1 to S6, S8 or S9 via Taunusanlage and alight at Hauptwache. At Hauptwache, change to the U8 towards Riedberg and alight at Uni Campus Riedberg.

From Frankfurt City Centre

Approx. 25 minutes

From city centre U-Bahn stations (e.g. Willy-Brant-Platz, Hauptwache or Eschenheimer Tor), take the U8 towards Riedberg and alight at Uni Campus Riedberg.

From Frankfurt Mertonviertel (e.g. Relaxa Hotel Frankfurt am Main)

Approx. 15 minutes

Take the bus line 29 towards Hohe Brück, and alight at Uni Campus Riedberg.

Additional Information

Contact information

For general inquiries and information, please contact: vaam.frankfurt2026@gmail.com

For specific matters only, please contact Sophie Mektadir (mektadir@bio.uni-frankfurt.de),
Valerie Streek (streek@em.uni-frankfurt.de).

Organisational team and helpers

The symposium is hosted and organised by the Crüsemann group and the Helfrich group at Goethe University Frankfurt. Throughout the conference, organisers and additional helpers from the two groups will be wearing black T-shirts with our conference logo. Please ask if you require any help on the day.

Local organising committee

Max Crüsemann	Eric Helfrich	Sandra Bettenbühl
Michael Brigham	Pia Dockal	Suhan Durusu
Stefanie Lamberth	Sophie Mektadir	Andreas von Recum
Valarie Streek		

Registration

Registration will be open on Wednesday 12:00 till at least 17:00. On other days, the registration area may be closed. Please find a member of the organising committee or email, if you will be late.

Information for short lecture presenters and poster presenters

For short lecture presenters:

- PowerPoints for talks must be submitted at least 2 hours in advance at the submission desk in the foyer, but not during lecture sessions.
- Ensure your PowerPoint presentation is formatted and saved appropriately for Microsoft PowerPoint for Windows 11.
- Please keep to your allotted time slot, including 5 minutes at the end for questions, as we have a tight schedule.

For poster presenters:

- Please set up your poster as soon as possible on Wednesday and take it down on Friday by 13:15.
- There are prizes for the poster presentations, and winners will be chosen by a panel of judges.

Storage rooms and lockers

Storage rooms will be provided in the Otto-Stern-Zentrum on Wednesday afternoon and Friday morning, and in the Biozentrum on Wednesday evening. A cloakroom area will be provided in the Otto-Stern-Zentrum. Approx. 50 electronic lockers are also available in the Otto-Stern-Zentrum. Ensure you remove your items at the end of each day.

Please note that usage of the storage rooms, cloakroom area and lockers is at your own risk, and conference organisers / helpers take no liability for lost or damaged property.

Locker instructions



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1. Press ON
2. Enter any 4-digit code
3. Turn the lever upward

Unlock

1. Press ON
2. Enter your 4-digit code
3. Turn the lever downward

Photography and video

Please do not record videos of any posters or presentations, unless you have explicit permission from the presenter. Only take pictures of lecture presentations during the title and acknowledgement slides, unless you have explicit permission from the presenter to do otherwise.

Internet access

Free WiFi can be found under the WiFi name 'guestGU'. No password is required.

Physical copies of the conference booklet

Paper copies of the programme of events will be provided; however, we will not provide a physical copy of the full conference booklet.

Conference Party

During the conference party, we will have a pizza truck, and we will offer a range of free drinks, games and a DJ. After using your free drink tokens, you will have the opportunity to provide cash donations for additional drinks.

Conference Dinner

Conference participants must make their own way to the restaurant and wear their name badges. You will place your food order upon arrival. The venue consists of two floors, and one floor will offer a pub quiz during the dinner.

We recommend taking the U-Bahn. Please ensure that you own a travel ticket that covers travel via the U-Bahn to Oberursel (e.g. a ticket including VGF Tarif Zone 5100; Deutschland-Ticket; Hessenticket).

U-Bahn Instructions (Approx. 25 minutes total)

1. Take the U-Bahn U8 in the direction Südbahnhof or U9 in the direction Ginnheim Mitte.
2. Alight at the *next* stop, Niederursel, and change to the opposite platform.
3. Take the U-Bahn U3 in the direction Oberursel Hohemark.
4. After 5 stops, alight at Oberursel Altstadt.
5. Walk to Alt-Oberurseler Brauhaus (**Ackergasse 13, 61440 Oberursel**).

To reach the restaurant in time and due to the trains being crowded, we recommend setting off in smaller, separate groups from Uni-Campus Riedberg U-Bahn station at approx. 18:04, 18:13 or 18:28.

Keynote Speaker Abstracts

Wednesday, September 16th

Keynote Lecture 1

Synthetic biology approaches to new chemistry

Michelle Chang

Princeton University, Princeton, NJ, USA

Living systems have evolved the capacity to carry out many chemical transformations of interest to synthetic chemistry if they could be redesigned for targeted purposes. Our group is interested in the discovery, characterization, and engineering of new enzymes for biocatalysis with a focus on metalloenzymes.

Thursday, September 17th

Keynote Lecture 2

Decoding and Reprogramming Assembly-Line Polyketide Synthase

Lihan Zhang

Department of Chemistry, Westlake University, Hangzhou 310030, China; zhanglihan@westlake.edu.cn

Modular polyketide synthases (PKSs) are multidomain assembly line enzymes that biosynthesize diverse natural products, making them an outstanding platform for custom production of designer molecules. However, rational reprogramming of PKS assembly lines has long been hampered by unpredictable losses in catalytic efficiency upon domain or module swapping. To address this challenge, we recently developed a plug-and-play engineering strategy that enables modular, combinatorial assembly of PKS pathways, hinted by natural evolutionary mechanism of modular recombination. Through extensive engineering campaigns applying this strategy across diverse PKS systems, we found that domain-domain interactions at module and domain boundaries are a critical, often underappreciated determinant of successful reprogramming. Particularly, interdomain interactions between acyl carrier protein (ACP) and its downstream catalytic domain were identified as key factors in governing substrate channeling and catalytic communication, highlighting the need to take an ACP-centric approach in rationalizing modular PKSs. In this talk, I will summarize our recent progress in modular PKS redesign and discuss how to better understand these assembly lines based on both evolutionary and mechanistic approaches.

Keynote Lecture 3

Capturing megasyn(et)ase evolution in action

Matthew Jenner¹

1: Department of Chemistry, University of Warwick, Coventry, CV4 7AL, United Kingdom.

Modular polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) generate a vast array of bioactive natural products, and are thought to evolve through gene duplication, deletion, and recombination events. However, direct evidence of these processes in action has been limited. Here, we analysed more than 200 genome-sequenced *Burkholderia gladioli* strains and identified multiple naturally occurring PKS and NRPS variants generated by rare recombination events. By linking these genetic changes to their corresponding metabolites, we captured evolutionary diversification at the intraspecies level. In addition, read-mapping analyses revealed a sliding recombination zone capable of generating functionally equivalent enzymes and products through distinct recombination events, providing a striking example of the "blind watchmaker" process of evolution.

Friday, September 18th

Keynote Lecture 4

Engineered cytochrome P450 enzymes as catalysts for peptide crosslinking

Max Cryle

Monash University, Melbourne, Australia

Cytochrome P450s are capable of a wide range of oxidative transformations across diverse biosynthetic processes. Given their latent synthetic utility and combination of oxidative power and regiochemical precision, it is little surprise that P450s have been widely implicated as potential biocatalysts. Here, I will report on our recent investigations into peptide crosslinking P450 enzymes from glycopeptide antibiotic (GPA) biosynthesis and from biarylittide biosynthesis. Here, biarylittide biosynthesis serves both as an important model system to better understand the more complex crosslinking process that is essential for the activity of the glycopeptide antibiotics, and as a source of potential biocatalysts to generate cyclic tripeptides as building blocks for complex peptide natural products.

In this presentation, I will detail the results of our ongoing research to characterise the effects of substrate modifications on the activity of both native and engineered P450s, and the extent to which chemical modifications and amino acid isosteric replacements are tolerated by both these classes of peptide crosslinking P450 enzymes. I will also discuss the results of our recent investigations into how subtle modifications to crosslinking P450s can lead instead to peptide nitration, a process exemplified in the nitration of tyrosine during rufomycin biosynthesis. This further underlines the diversity of catalytic outcomes possible from such P450s, and the importance of understanding their reactivity if we are to harness these enzymes as future biocatalysts.

Lecture Abstracts

Wednesday, September 16th

Short Lecture 1

Harnessing Biosynthesis Logic für Natural Product Discovery, Evolution, and Function

Xiaoyu Tang

Shenzhen Bay Laboratory, China; xtang@microbechembio.org

Natural product biosynthesis has generated a wealth of knowledge that not only enables the discovery of new molecules but also provides a conceptual framework for understanding their origins, evolution, and biological functions. In this report, I will discuss recent progress from my laboratory in exploring new paradigms in microbial natural product research guided by biosynthetic logic. Specifically, I will present (1) the targeted genome mining of novel antimicrobial macrocyclic peptides (Nature Communications, 2024), (2) the discovery of an evolutionary repurposed thiolase complex that catalyzes Friedel–Crafts C-acylation in antibiotic biosynthesis (Nature Communications, 2026), and (3) the elucidation of the physiological functions of microbiota-derived natural products in host health and disease (Nature, in revision). Together, these studies demonstrate how biosynthetic logic can serve as a powerful framework for discovering new molecules, uncovering new enzymatic chemistry, and understanding the biological functions of microbial metabolites.

Discovery and Heterologous Expression of Soil Metagenome-Derived Lasso Peptides

Timo Negri, Giovanni Andrea Vitale, Christian Geibel, Martina Adamek, Caner Bağcı, Daniel Petras, Chambers C. Hughes, Nadine Ziemert

Interfaculty Institute of Microbiology and Infection Medicine (IMIT), University of Tübingen; negri0811@gmail.com

The rise of multidrug-resistant bacteria underscores the urgent need for new antibiotics, yet the pace of new compound discovery has slowed in recent decades. Although advances in microbial cultivation have expanded access to previously elusive taxa, many biosynthetic gene clusters (BGCs) encoding promising natural products remain hidden within the genomes of uncultivated microbes. Culture-independent metagenomic approaches offer a route to access these resources by capturing and expressing environmental BGCs in tractable hosts. Lasso peptides are a subclass of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products with diverse bioactivities. Recently, we reported the discovery and heterologous expression of the first lasso peptide BGC recovered directly from a soil metagenome. Expression of the refactored pathway in *Escherichia coli* yielded metanodin, a lasso peptide featuring an unprecedented asparagine-based macrolactam ring linkage.

Here, we address the common bottleneck of low production yields by combining additional pathway refactoring, alternative heterologous expression hosts, and the investigation of further metagenomic lasso peptide BGCs. These approaches enabled the detection of three additional products with masses matching those predicted from their corresponding metagenomic BGCs. One candidate exhibited a considerably higher signal intensity than metanodin, providing a promising target for purification, structural characterization, and bioactivity testing. Our results demonstrate that metagenome mining, coupled with pathway refactoring and heterologous expression, can unlock natural products from previously inaccessible microbial lineages and expand the chemical diversity of even well-studied natural product classes.

Investigations on cyanobacterial protease inhibitors with intriguing pharmaceutical potential

Christoph Ulbricht¹, Teresa Marina Dreckmann¹, Stefan Kehraus¹, Amira Naimi², Tung Lam Trinh¹, Kaya Törkel¹, Uwe Linne³, Kornelia Hardes⁴, Raphael Reher², Martin Baunach¹

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.; 3: Department of Chemistry, Philipps-Universität Marburg, Hans-Meerwein-Straße 4, 35043 Marburg, Germany; 4: Fraunhofer-Institut für Molekularbiologie und Angewandte Oekologie IME, Ohlebergsweg 12, 35392 Gießen, Germany; s6schulbr@uni-bonn.de

Cyanobacteria produce a wide variety of specialized metabolites [1]. These metabolites have evolved and been refined over billions of years, resulting in a vast treasure trove from a pharmacological perspective [2,3]. Our studies describe the identification, purification, and structure elucidation of protease inhibitors from *Nostoc* sp. KVJ20 and *Scytonema hofmannii*. In addition to the well-known suomilides and hofmannolin, novel congeners of these compounds have been discovered and investigated. Members of the suomilide family, in particular, are showing promising results in initial anti-cancer and anti-viral testing due to their role as highly potent trypsin inhibitors [4].

References

- [1] E. Dittmann, M. Gugger, K. Sivonen, D. P. Fewer, Natural Product Biosynthetic Diversity and Comparative Genomics of the Cyanobacteria, *Trends in Microbiology*, **2015**, 23, 642-652.
- [2] B.A. Neilan, L.A. Pearson, J. Muenchhoff, M.C. Moffitt, E. Dittmann, Environmental conditions that influence toxin biosynthesis in cyanobacteria. *Environmental Microbiology*, **2013**, 15, 1239-1253.
- [3] S. Vijayakumar, M. Menakha, Pharmaceutical applications of cyanobacteria - A review, *Journal of Acute Medicine*, **2015**, 5, 15-23.
- [4] A. Naimi, C. Ulbricht, T.L. Trinh, Native Metabolomics Unveils Suomilide Analogs with Potent Trypsin Inhibitory Activity, *Journal of Natural Products*, **2026**, 89, 1148-1160.

Unravelling Bestatin Biosynthesis through Genome Mining, Heterologous Expression, and Molecular Networking

Josefine Klerch, Phillipp Fink, Raimund Nagel, Severin Sasso, Leonard Kaysser

Universität Leipzig, Germany; josefine.klerch@medizin.uni-leipzig.de

The α -hydroxy- β -amino acid motifs are important pharmacophores in inhibitors of metalloproteases, particularly aminopeptidases. A great variety of α -hydroxy- β -amino acid-containing peptides have been isolated from different Actinomycetes over the last decades. Among them, bestatin is the best-characterized and is approved in Japan as an adjunct drug in the therapy of acute myeloid leukemia (Ubenimex®).

In this study, we used antiSMASH-based genome mining to identify a putative biosynthetic gene cluster (BGC) in the bestatin producer *Streptomyces olivoreticuli* ATCC31159. This BGC encodes a hybrid polyketide synthase/non-ribosomal peptide synthetase (PKS/NRPS) assembly line, where a putative aminotransferase and a putative oxidase domain were also predicted. To verify its function, the BGC was cloned and heterologously expressed in *Streptomyces coelicolor* M512. LC-MS analysis showed the specific production of bestatin in the heterologous mutant, validating the identified gene cluster.

Intriguingly, the amastatin producer *Streptomyces* sp. ATCC31318 contains an almost identical bimodular NRPS/PKS with the same distinct domain architecture. Amastatin is another α -hydroxy- β -amino acid-containing peptide, but in contrast to the dipeptide bestatin consists of four amino acids. This raises the question of whether these bimodular pathways are able to assemble tri- or tetrapeptides.

Indeed, analysis of LC-MS/MS data from the bestatin producer strains with GNPS molecular networking uncovered several uncharacterized congeners. Feeding experiments with isotope-labelled amino acids confirmed the production of novel bestatin-like di- and tripeptides. This suggests an unusual biosynthetic pathway with relaxed substrate specificity, allowing incorporation of diverse amino acid precursors and potentially involving an iterative mechanism.

Biosynthetic Pathway of Belactosin C Reveals Dual Targeting of Proteasome and ClpP Proteases

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β -lactone natural products comprise a structurally diverse class of metabolites with broad biological activity. Among these, bacterial β -lactones such as omuralide, salinosporamide A, and chemically optimized belactosin derivatives are recognized as highly potent 20S proteasome inhibitors. As the major eukaryotic protease, the proteasome is vital for protein homeostasis and cell-cycle regulation. However, the molecular basis of target engagement by the naturally occurring compound belactosin C has remained unresolved.

Here, we report the first crystallographic characterization of unmodified belactosin C in complex with the 20S yeast proteasome. The structure reveals an unexpected binding mode in which belactosin C occupies the non-primed substrate-binding channel, distinct from previously described synthetic derivatives. To assess how biosynthetic maturation influences proteasome affinity, we chemically prepared and systematically investigated every β -lactone intermediate en route to belactosin C. Crystal structures of each intermediate in complex with the proteasome, combined with fluorescence-based activity measurements, provide the first comprehensive view of how biosynthetic progression modulates proteasome inhibition.

The observed binding mode prompted an investigation of alternative biological targets. We demonstrate that belactosin C inhibits the bacterial caseinolytic protease ClpP, a key regulator of bacterial stress responses and persistence. Our high-resolution crystal structures of belactosin C covalently bound to ClpP from *Staphylococcus aureus* and *Aquifex aeolicus* confirm target engagement, supported by enzymatic activity assays and LC-MS analysis.

Taken together, these findings establish belactosin C as a dual β -lactone protease inhibitor with activity against both the eukaryotic proteasome and bacterial ClpP. This promiscuity challenges the traditional view of belactosins as selective proteasome inhibitors and suggests that their ecological function in the competitive environment of *Streptomyces* species remains largely unexplored.

Short Lecture 6

Engineering Substrate Loading *in trans* in Modular Polyketide Synthase

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Multidomain type I polyketide synthases (PKSs) are complex biosynthetic machineries organized into modules that assemble structurally diverse, often therapeutically relevant natural products. During biosynthesis, PKSs stepwise elongate a growing polyketide chain, with each elongation cycle relying on an acyltransferase (AT) domain to select and load the corresponding extender substrate. Engineering AT domains is a powerful approach for generating novel polyketide analogs with improved bioactivity, stability, and pharmacological properties. Yet, extensive modifications frequently compromise protein stability and catalytic efficiency, limiting this approach. Here, we present an alternative, minimally invasive strategy: rather than extensively modifying the native AT domain itself, we recruit a stand-alone acyltransferase *in trans* to a PKS module via genetically encoded recruitment tools, allowing it to take over substrate loading.

Specifically, we harness the malonyl/acetyltransferase domain (MAT) from murine fatty acid synthase to achieve directed loading of PKS modules with extender substrates. Using modules from the erythromycin synthase (DEBS) and pikromycin synthase (PIKS) as established *in vitro* testbeds, we show that recruitment mediated by synthetic zippers (SYNZIPs) or the SpyTag:SpyCatcher system yields stable, catalytically active PKS:MAT complexes. Moreover, both recruitment strategies were implemented simultaneously within the same assembly line, opening a route toward directing distinct substrates to distinct modules of a single PKS pathway. Together, these results establish recruitment-based *trans*-complementation as a minimally invasive framework for engineering substrate loading in modular PKSs that is broadly adaptable to other PKS biosynthetic pathways.

Uridyl Peptide Antibiotics: Establishing a Production Platform for a Potent Antipseudomonal Drug Candidate

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The compound class of uridyl peptide antibiotics (UPAs) is characterized by a uridyl moiety, which is linked through a 4',5'-enamide linkage to the core *N*-methyl-2,3-diaminobutyric acid, located at the center of a (pseudo)tetra- or pentapeptide backbone [1]. Originating in different streptomycetes, UPAs display potent antibiotic activity against the nosocomial pathogen *Pseudomonas aeruginosa* [1, 2]. UPAs inhibit the underexplored target phospho-*N*-acetylmuramyl-pentapeptide translocase, which is involved in bacterial cell wall biosynthesis [3]. Four subclasses have been described: pacidamycins, mureidomycins, napsamycins and sansanmycins, which mainly vary in the peptide backbone [1].

Mureidomycins A and C have been identified as the most bioactive UPAs, but production in the native producer and heterologous hosts was low. In the present work, the pacidamycin (*pac*) and napsamycin (*nps*) gene clusters were engineered to establish a heterologous production platform for mureidomycin A, which can then be converted to mureidomycin C by a semisynthetic approach. For this purpose, a two-pronged strategy was devised. The *pac* gene cluster was modified by replacing key non-ribosomal peptide synthetase genes with their orthologs of the *nps* gene cluster to facilitate mureidomycin formation. While the native *pac* cluster only produces pacidamycins, mureidomycin A formation of ~190 mg/L was detected in the engineered strain. Simultaneously, the *nps* gene cluster was modified by gene deletions and promoter tuning to enhance mureidomycin A production, increasing the yield by approximately two orders of magnitude (up to ~1.7 g/L). Thus, two bacterial strains suited for the large-scale isolation of mureidomycin A were generated, facilitating future hit-to-lead development.

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Expanding the genetic toolbox for Myxobacteria: New autonomous shuttle vectors for natural product pathway engineering

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Myxobacteria are an important source of bioactive secondary metabolites, yet their vast biosynthetic potential remains underexploited, partly due to the lack of suitable autonomously replicating plasmids 1–3. To overcome this limitation, we conducted a systemic bioinformatic analysis across high-quality myxobacterial genomes and identified conserved RepA/ParAB replication-partitioning modules originating from diverse extrachromosomal elements - previously unrecognized for vector design. These findings formed the basis for the construction of synthetic *myxoplasmids*, minimal dual-host plasmids combining *Escherichia coli* and myxobacterial replication systems. This autonomously replicating platform enables stable maintenance of large inserts and heterologous expression of complex biosynthetic gene clusters in *Myxococcus xanthus* (*M. xanthus*) DK1622, providing the first reproducible non-integrative alternative to chromosomal engineering in myxobacteria. Our *myxoplasmids* facilitate rapid, flexible biosynthetic pathway optimization and extend genetic accessibility to myxobacterial strains beyond commonly used hosts such as *M. xanthus* DK1622, accelerating discovery of novel natural products and investigation of underlying biosynthetic machinery.

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Discovery of novel natural products from a rare actinomycete through heterologous expression

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Rare actinomycetes are known producers of structurally and biologically relevant natural products, yet they have remained largely under-explored due to their low isolation frequency and the challenges associated with their cultivation and genetic manipulation [1]. Moreover, the spectrum of compounds produced in the laboratory does not reflect the vast biosynthetic potential encoded in their genomes [2].

To explore novel chemistry from rare actinomycetes, we performed genome mining, prioritized and investigated an uncharacterized BGC in *Actinoallomurus* sp., predicted to encode the production of a hybrid PKS-NRPS compound containing multiple cysteine residues. Initial cultivation of *Actinoallomurus* sp. did not lead to the identification of any compound that could be associated with this BGC. Moreover, the strain is slow-growing and not genetically accessible using standard procedures. To overcome these limitations, the BGC was cloned using CAPTURE cloning procedure [3] and transferred into different heterologous hosts. However, metabolite production attributable to the BGC was not observed under the tested conditions.

To enable expression, the BGC was refactored by replacing the putative native promoter region with the constitutive promoter *SP44* [4]. Transcriptional activation of the refactored BGC was confirmed by RT-PCR analysis. Cultivation of the resulting strains led to the appearance of distinct additional peaks in HPLC-MS analysis. The compounds are currently being purified for further structural elucidation.

Overall, this work highlights rare actinomycete strains as an underexploited source of natural product diversity and shows that BGC cloning and refactoring can enable access to novel chemistry while overcoming the limitations associated with native producer strains.

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Integrating Computational and Experimental Workflows Towards High-Throughput Metabolic Engineering of Streptomyces

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Streptomyces are among the most prolific producers of complex specialized metabolites. However, up to 97 % of the biosynthetic potential of streptomyces remains untapped. Compared to sequencing and genome mining workflows, methods for metabolic engineering of streptomyces have not progressed at the same pace and are increasingly turning into the main bottleneck towards exploration of this vast biosynthetic potential. Here, we present how a combination of advanced new experimental methods, as well as tight integration with computational workflows, can significantly increase the throughput of metabolic engineering experiments in streptomyces, and enable the discovery of new chemistry.

First, we show that the type I CRISPR system CASCADE-Cas3 can facilitate highly efficient genome engineering in streptomyces. CASCADE-Cas3 achieved up to 100 % efficiencies without further modifications in several Streptomyces species and enabled rapid construction of genome reduced hosts for expression of heterologous BGCs. Next, we developed StreptoCAD to automate the entire design process of genome engineering experiments in streptomyces. StreptoCAD enables high throughput and robust experimental design with traceable design parameters.

Finally, we show how overexpression libraries designed by StreptoCAD can be used to activate silent BGCs. Using LC-MS/MS analysis and feature based molecular networking, we discovered several putative new molecules, which are currently being further investigated. The presented research highlights how integrating advanced experimental and computational workflows can bring us one step closer to streamlined exploration of the vast untapped biosynthetic potential of streptomyces.

LoReMINE: Long Read-based Microbial genome mining pipeline

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For decades, microbial natural products (NP) have been a major source of medicines, with most of the clinically used antibiotics being their derivatives. Recent advances in DNA sequencing technologies enable the reconstruction of more complete and continuous microbial genomes, revealing a vast and largely untapped diversity of biosynthetic gene clusters (BGC) responsible for NP biosynthesis. Despite the increasing availability of microbial genome sequences, large-scale NP discovery still remains challenging because the existing genome mining approaches lack integrated workflows for rapid dereplication of known compounds and prioritization of novel candidates, forcing researchers to rely on multiple tools that require extensive manual curation and expert intervention at each step. To address these limitations, we introduce LoReMINE (**Long Read-based Microbial genome mining pipeline**), an automated end-to-end pipeline designed to simplify microbial genome mining directly from long-read sequencing data. The pipeline consists of four submodules: (i) assemble, which generates high-quality genome assemblies using strategies optimized for different sequencing read types; (ii) taxonomy, which identifies taxonomy; (iii) identify_bgcs, which detects BGCs; and (iv) bgc_clustering, which clusters predicted BGCs into gene cluster families (GCFs) by optionally incorporating reference BGCs from the MIBiG database. Application of LoReMINE to a diverse set of bacterial strains demonstrated its ability to generate high-quality genome assemblies and accurately characterize their biosynthetic potential. By integrating state-of-the-art tools into a unified workflow, LoReMINE enables scalable, reproducible, and comprehensive genome mining across diverse microbial taxa, thereby accelerating the discovery of novel natural products. More details about pipeline are available in a preprint (<https://www.biorxiv.org/content/10.64898/2026.02.02.703231v2>).

CluSeek: Bioinformatics Tool for Gene Cluster Discovery within GenBank

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We present CluSeek, a Python-based bioinformatics tool designed to identify, visualize, and analyze gene clusters, i.e., co-localized genes of interest and their neighbourhoods. Rather than annotating individual genomes using pre-defined criteria, CluSeek searches all GenBank data for any gene clusters which encode homologs of user-specified genes. This, for instance, allows for the targeted discovery of all gene clusters, which encode biosynthesis of a specific structural motif. This makes CluSeek's use case distinct from other genome mining tools like antiSMASH1. I will demonstrate CluSeek and its versatility using a case study of genome mining of specialized metabolites with a 4-alkyl-L-proline motif. Applying CluSeek to this case study led to the discovery of 16 putative new classes of metabolites incorporating this motif, compared with only three classes known to date, all of which were discovered during the Golden Era of Antibiotics. CluSeek is open-source software available via cluseek.com. It has a graphical user interface, and is designed to be launched and operated by IT non-experts.

BGC-QUAST: a quality assessment tool for genome mining software

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Biosynthetic gene clusters (BGCs) encode microbial natural products, many of which have important ecological and biomedical roles. Genome mining tools enable large-scale BGC prediction, but their outputs differ substantially, complicating comparison and interpretation. We present BGC-QUAST, a framework for evaluating and comparing BGC predictions across three analysis modes: comparison across samples, assessment of BGC recovery in draft assemblies relative to reference genomes, and comparison of predictions from different tools using overlap analysis. BGC-QUAST provides standardized metrics, interactive visualizations, and integrated outputs for joint inspection of predictions, enabling the comprehensive comparison of genome mining results and facilitating sample prioritisation based on biosynthetic potential.

Short Lecture 14

Non-Canonical Rubromycin Late-Stage Tailoring Involves an Archetypal Internal P450 Monooxygenase

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Rubromycins are a class of bacterial aromatic polyketides with cytotoxic and antimicrobial activities that were shown to inhibit multiple proteins interacting with nucleic acids ^[1]. In their biosynthesis the formation of the central [5,6]-spiroketal moiety is followed up by various late-stage tailoring enzymes like reductases, transferases, oxidases and oxygenases which lead to the naturally observed rubromycin variants ^[2]. Our goal was the reconstitution of the final rubromycin tailoring steps *in vitro* and to elucidate the underlying reaction mechanisms.

We now show that the eastern methyl group of a central late-stage intermediate is hydroxylated by the distinct cytochrome P450 (CYP) HyalU. The cryptic quinone oxidoreductase-like HyalO7 promotes the full oxidation of the introduced hydroxyl group to the carboxylic acid. Detailed analysis of *in vitro* reactions as well as cocrystallization revealed that HyalO7 functions as a quinone reductase of the the rubromycin backbone. Remarkably, the reduced HyalO7-product is then taken up by HyalU and re-oxidized, involving the transfer of electrons to the heme cofactor and thereby enabling subsequent CYP oxygenation chemistry. This substrate-based heme reduction represents a novel strategy for heme-dependent monooxygenases to achieve O₂ activation. Following biosynthetic steps contain reduction of the hydroxyl group of the central spiroketal moiety to a methylene by a non-canonical flavoenzyme, as well as carboxylic acid methylation or amidation in case of γ -rubromycin and hyaluromycin, respectively.

In conclusion this study highlights multiple unconventional enzymatic reactions in the tailoring of rubromycin polyketides, featuring the first “internal” cytochrome P450 operating in conjunction with a quinone oxidoreductase.

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Homoterpene biosynthesis widespread in actinobacteria

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Terpenes constitute the largest class of natural products and are of major pharmaceutical and industrial relevance.^[1] Most terpenes follow the classical “biogenic isoprene rule”, consisting of carbon skeletons assembled from C₅ units.^[2] In contrast, homoterpenes containing an additional C₁ unit represent only a small fraction of the approximately 180,000 known terpenoids.^[3] However, their biosynthetic pathways provide unique opportunities to uncover enzymatic strategies for targeted backbone modifications.

We recently discovered a widespread actinobacterial biosynthetic pathway toward homoterpenes representing methyl analogs of germacrene and eudesmane sesquiterpenes.^[4] The pathway features unprecedented enzymatic transformations catalyzed by two novel enzyme families. A family of class I methyltransferases with dual transferase and double bond isomerase activities converts the canonical precursor farnesyl diphosphate to (2*E*,7*E*)-6-methyl-farnesyl diphosphate. This intermediate serves as the specific substrate for a family of type I terpene cyclases that operate through a distinct “inverse” cyclization mechanism. Functional characterization of enzyme pairs from diverse actinobacterial strains demonstrates that this pathway is broadly conserved.

Considering the profound impact of strategically placed methyl groups on pharmaceutical activity, an observation known as the “magic methyl effect”^[5], these findings raise intriguing questions regarding the biological significance and pharmaceutical potential of terpene backbone methylations. Beyond expanding our understanding of terpene biosynthesis, the newly discovered enzymology provides a foundation for exploring the diversity of terpene methylations in nature and for developing biocatalytic and genetically programmable strategies for the generation of tailored homoterpene backbones, potentially enabling fine-tuning, altering, or even introducing novel properties into bioactive terpenoids.

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Genome Mining-Guided Identification And Functional Characterization Of Bacterial Terpenoid Biosynthetic Pathways

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Terpenoids constitute the largest and structurally most diverse natural product class. Although terpenoids have traditionally been isolated from plants and fungi, bacterial genome sequencing has revealed a remarkable untapped repertoire of orphan terpenoid biosynthetic gene clusters (BGCs), highlighting bacteria as an underexplored reservoir of terpenoid diversity.¹

In this work, we mined an in-house bacterial genome database to identify terpenoid BGCs. We characterized three cryptic terpenoid BGCs from *Streptomyces* spp. with unusual gene architectures and terpene cyclases with low sequence identity to characterized homologs. The first BGC encodes a diterpenoid pathway, harboring an unusual didomain terpene cyclase fused to a truncated reductase, a hypothetical protein and multiple cytochrome P450s, including a rare split P450 encoded by three separate genes.² The other two BGCs encode rare bacterial sesterterpenoid pathways, one of which harbors two terpene cyclases, a type II terpene cyclase and a transmembrane-associated terpene cyclase. Heterologous expression in a *Streptomyces* heterologous host yielded new structurally complex di- and sesterterpenoids. To elucidate their biosynthesis, we constructed single-gene deletion mutants for one gene at a time in the pathway and structurally characterized the accumulating biosynthetic intermediates and shunt products. Our gene disruption studies revealed an unprecedented dual role for the hypothetical protein as an isomerase-oxygenase in diterpenoid biosynthesis.² In vivo characterization of the type II terpene cyclase from the third BGC revealed a unique monocyclic C₂₅ scaffold containing a centrally positioned cyclohexane ring. Overall, the functional characterization and bioinformatic analysis of these BGCs expand the chemical and enzymatic space of bacterial terpenoids and provide new insights into bacterial terpenoid biosynthesis.

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Short Lecture 17

Beyond Nutrition: Biosynthetic potential in Chemosynthetic Animal–Microbe Symbioses

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Little to nothing is known about biosynthetic gene clusters (BGCs) in chemosynthetic symbioses. Chemosynthetic symbioses are partnerships in which bacteria oxidize reduced compounds (e.g., H₂S) to generate energy for fixing CO₂, supplying their animal hosts with organic carbon. Although these associations are classically viewed as purely nutritional, BGC-encoded metabolites may confer key ecological advantages (e.g., mediate host-symbiont specificity, exclude competing microorganisms, or defend against predators and viruses).

Here, we characterize the biosynthetic potential of *Candidatus Thiosymbion*, a genus of closely related, unculturable, marine chemosynthetic bacteria that has formed obligate symbioses with three very distantly related host groups from two animal phyla: Stilbonematinae nematodes, *Astomonema* nematodes, and gutless marine oligochaetes. Notably, *Thiosymbion* has different lifestyles in each host group: they occur as ectosymbionts on Stilbonematinae, as extracellular endosymbionts in gutless oligochaetes, and within the gut lumen of *Astomonema* nematodes.

Genome mining revealed pronounced differences in BGC richness between endosymbiotic and ectosymbiotic lineages. Endosymbionts contained only few BGCs, primarily terpene and RiPP recognition elements, or none at all, whereas several more BGCs were predicted in the ectosymbionts, including non-ribosomal peptide synthetases and polyketide synthases. Our results suggest that BGCs may play important ecological roles in ectosymbiotic chemosynthetic symbionts, and may also represent an unexplored source of biosynthetic novelty, as many remained unannotated. To further investigate the roles of the ectosymbiont-derived BGCs, we are evaluating their in-situ expression and heterologously expressing a selection of them to characterize their products. Altogether, our study offers the first view into the biosynthetic potential in chemosynthetic symbionts.

Exploring the antibiotic-responsive regulatory function of ABCF proteins in antibiotic-producing bacteria

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ABCF proteins are cytosolic ATPases of the ABC superfamily that include factors involved in translation regulation and ribosome protection against antibiotics. Despite their diverse biological functions, all characterized ABCF proteins share the ability to modulate the peptidyl transferase centre in an ATP-dependent manner. However, the vast majority of the ~30 bacterial ABCF subfamilies remain functionally uncharacterized. The previous work of our lab demonstrated that the ABCF protein LmrC activates lincomycin biosynthesis in response to the sub-inhibitory concentrations of lincosamides^[1], suggesting a previously unrecognized role of ABCF proteins in antibiotic-dependent signalling. Furthermore, bioinformatic analyses reveals that ABCF proteins are frequently encoded within biosynthetic gene clusters (BGCs) for ribosome-targeting antibiotics. Specifically, the ABCF subfamily present in a given BGC correlates with the mode of antibiotic binding to the ribosome. Based on these observations, we hypothesize that BGC-encoded ABCF proteins act as sensors that couple the presence of ribosome-binding antibiotics in the environment to the modulation of secondary-metabolite production.

To test this hypothesis, we selected candidate strains harbouring representatives of the ARE4, AAF1, and AAF2 ABCF subfamilies in BGCs and evaluated the effect of various ribosome binding antibiotics on the production of antibiotics targeting ribosome. Initial screens revealed distinct regulatory phenotypes: while tylosin and spiramycin biosynthesis remained unaffected by external antibiotic cues, the accumulation of griseoviridin and viridogrisein was suppressed upon exposure to sub-inhibitory concentrations of tylosin. Interestingly, all these clusters encode ABCF factors belonging to the ARE4 subfamily. We particularly focused on Hyg28, an ABCF protein located within the hygromycin ABGC. Hyg28 belongs to the AAF1 subfamily, a group that remains poorly understood. Our goal is to characterise Hyg28 by genetic, functional and structural studies.

By establishing the biochemical activity, resistance spectrum, and ribosome-bound architecture of an AAF1-type ABC-F factor, this work will fill a major knowledge gap in the evolutionary landscape of ABC-F proteins. It will also provide significant understanding of both the resistance and potential regulatory capacities of Hyg28. These insights offer a foundation for understanding ABCF proteins' role in ribosomal protection or in developing biosensors for the discovery of novel bioactive compounds.

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Poster Abstracts

Poster 1

A Platform to Study *Streptomyces* Chemical Ecology

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Soil microbiomes are complex and functionally important parts of our planet's ecosystems, involved in nutrient cycles, plant health, or soil detoxification. [1] Secondary/specialized metabolites production and chemical interactions shape these soil microbial communities through cooperation, competition and communication [2]. Understanding these processes remains challenging due to the complexity and heterogeneity of natural environments. Here, we applied a hydrogel bead-based soil-mimetic synthetic community [3] (SynCom) framework and modified it to investigate interspecies chemical interplay between *Streptomyces* and faster-growing heterotrophic bacteria. All 7 strains (*Pedobacter* sp. D749, *Rhodococcus globerulus* D757, *Stenothrophomonas indicatrix* D763, *Chryseobacterium* sp. D764, *Streptomyces* sp. P8-A1, *Streptomyces* sp. P8-A4, *Streptomyces* sp. P8-A7) [3] used in this study were isolated from soil samples and characterized through genomics and metabolomics in monoculture. After optimization, the SynCom system allowed for temporal tracking of microbial dynamics through an integrated multi-omics approach. Metabolites that appeared differentially expressed in the SynCom relative to monocultures were selected for detailed follow-up analyses. Furthermore, a semi-automated platform for bead generation and SynCom inoculation was built around this project to increase throughput and scale-up the study of soil bacteria chemical ecology. Overall, this platform provides an experimental model for investigating the chemical ecology underlying *Streptomyces* and other soil bacterial interactions and community dynamics.

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Lipolanthines: Biosynthesis and Mode of Action

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Lipolanthines are a class of ribosomally synthesized and post-translationally modified peptides (RiPPs) produced by Actinobacteria. Most known lipolanthines exhibit potent antibacterial activity against gram-positive bacteria; however, their mode of action remains unclear ^{[1][2]}.

The ribosomally produced precursor peptides, LanA, can be divided into a leader and a core peptide region. The leader peptide region is important for recognition by the modification enzymes, which install their modifications in the core peptide region. The lipolanthine core peptide is dehydrated and cyclized by LanKC enzymes and decarboxylated by LanD enzymes. These modifications generate the characteristic avionin motif: a bicyclic octapeptide featuring a central quaternary carbon atom and an aminovinylthioether bridge. Following modification by LanKC and LanD, the leader peptide is cleaved, and an N-terminal lipid modification consisting of an N-terminal methylated guanidino fatty acid (MGFA) is attached, contributing to the distinctive architecture of lipolanthines ^{[1][2]}.

This poster provides an overview of lipolanthine biosynthesis and antibacterial activity, with a particular focus on the biosynthetic machinery involved in peptide maturation. We aim to elucidate the structural mechanism underlying the mutual regulation of the modifying enzymes LanKC and LanD during the maturation of the precursor peptide LanA, as well as to contribute to a better understanding of the mode of action of lipolanthines.

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Vancoresmycin targets Cell Wall Biosynthesis

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Antimicrobial resistance represents a major global health threat, as treatment options for infections caused by multidrug-resistant pathogens are becoming increasingly limited. The vast majority of antibiotics currently in clinical use are derivatives of molecules discovered during the early to mid-20th century, highlighting the critical need for novel compounds with new modes of action or molecular targets^[1]. Bacterial cell wall biosynthesis remains a highly effective antibiotic target, and complex natural products, such as the hybrid polyketide-nonribosomal peptide vancoresmycin, offer promising scaffolds to address this challenge.

In this project, we investigate the mode of action of vancoresmycin and assess a series of defined molecular fragments. Whole-cell reporter assays in *Bacillus subtilis*, combined with *in vitro* studies, revealed that vancoresmycin interferes with cell wall biosynthesis. Evaluation of individual substructures, including the tetramic acid core, eastern and western polyketide segments, and the mycosamine residue, showed that key isolated motifs lack significant antibacterial activity^[2]. Only the western fragment exhibited weak antibacterial activity, accompanied by indications of membrane-associated stress.

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Genome mining of the moss microbiome for natural product discovery

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Moss plants harbor a diverse microbiome, which contributes to their resilience and high adaptability to a range of different environments. However, moss microbiomes remain largely understudied, despite representing a promising source of novel natural products with potential ecological and biotechnological relevance. In this study, we look at the biosynthetic potential of moss microbiomes using both short and long read sequencing to recover novel biosynthetic gene clusters. The phyllosphere microbiome of *Hypnum cupressiforme* growing on four different substrates (soil, rock, tree and deadwood) was sequenced and hybrid assemblies were constructed, resulting in over 300 metagenome-assembled genomes (MAGS), including 194 good to high quality MAGS. The bacterial microbiome was found to be dominated by Sphingomonadales, Pseudomonadales, Rhizobiales and Burkholderiales bacteria, which are commonly associated with phyllosphere environments. Additionally, 539 unique Gene Cluster Families (GCFs) containing full-length BGCs were recovered across all substrate types, dominated by terpenes, RiPPs and NRPS GCFs. 36% of these GCFs are distinct from known BGCs described in the MiBIG and BiG-FAM databases, and thus potentially novel. These results confirm the potential of the moss microbiome for natural product discovery. This is further exemplified by the identification of a novel hybrid NRPS-PKS-RiPP BGC from a *Streptomyces* isolate obtained from the moss *Orthotrichum anomalum*. This unusual cluster, harboring biosynthetic enzymes from three different natural product families, is conserved across several *Streptomyces* species. Using yeast-mediated homologous recombination, the BGC was cloned into several *Streptomyces* hosts. Work is underway to characterize the product of this BGC.

CycloPe – Biosynthesis of bicyclic peptides with antibiotic properties

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The rapid emergence of antibiotic resistance has created an urgent need for novel antimicrobial compounds. The promising new antimicrobial compound Darobactin A (DAR A), a ribosomally synthesised and post-translationally modified peptide (RiPP), has been shown to be active against Gram-negative bacteria.^[1] DAR A is synthesised from a precursor peptide encoded by *darA*, which is post-translationally modified by the radical S-adenosylmethionine (rSAM) enzyme DarE, encoded by *darE*, creating the bicyclic heptapeptide.^[2] Since the discovery of DAR, multiple putative DAR variants have been identified in other organisms.^[3]

The CycloPe project aims to identify novel rSAM tailoring enzymes capable of producing previously unknown bicyclic peptides. To this end, a genome mining platform has been developed to identify previously unknown DarE-DarA-like protein pairs based on sequence similarity and local alignment. The platform has additionally been designed to predict their potential core peptides. Candidate biosynthetic gene clusters (BGCs) were then grouped into families using BiG-SCAPE (Biosynthetic Gene Similarity Clustering and Prospecting Engine) and then further analysed with CORASON (Core Analysis of Syntenic Orthologs to prioritise Natural products). In order to identify promising candidates from these, a multi-parameter method was applied, which considers both biosynthetic-relatedness and the potential for novelty. Two candidate DarE-DarA-like pairs, differing to various extents from the already characterised darobactin system, were selected for heterologous expression. These two potential BGCs will be produced *in vivo*, and their products and biosynthetic activities will be characterised. Ultimately, this approach aims to facilitate the biosynthesis of novel bicyclic peptides that possess enhanced antibiotic potential.

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Phylogeny-informed prioritization of chassis strains for heterologous biosynthetic gene cluster Expression

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Selecting a suitable host for the heterologous expression of a biosynthetic gene cluster (BGC) remains a major challenge in natural product discovery. We developed a phylogeny-informed method to prioritize candidate hosts for a given gene cluster family (GCF) based on their evolutionary distance to strains carrying members of that family.

Using a large collection of *Streptomyces* genomes, we calculated the phylogenetic distance between each candidate strain and the nearest carrier of each GCF. GCFs were also grouped by biosynthetic class, including nonribosomal peptide synthetase (NRPS), polyketide synthase (PKS), and hybrid NRPS–PKS pathways.

Because this approach assumes that GCF distributions are phylogenetically structured, we tested this using two complementary statistical analyses. We compared observed distances among GCF carriers with randomized null expectations and quantified the phylogenetic signal of GCF presence across the reference tree.

Both analyses indicated that most GCFs were significantly clustered among related strains, although the strength of clustering varied across GCFs and biosynthetic classes. Hybrid NRPS-PKS GCFs showed particularly strong clustering, but comparisons among classes should be interpreted cautiously due to unequal sample sizes. A smaller subset showed weak or dispersed patterns, potentially reflecting horizontal gene transfer, differential gene loss, or limited statistical power.

These results indicate that phylogeny captures meaningful information about GCF distributions in *Streptomyces* and may provide a rational first-pass criterion for chassis prioritization. Experimental validation is needed to determine whether phylogenetically closer hosts are more suitable for heterologous expression and how phylogeny should be combined with other genomic and laboratory-based criteria.

EXPLORA: Unlocking the Hidden Treasures of Aquatic Extremophiles – Identification and Heterologous Expression of Novel RiPP Gene Clusters

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Extremophiles inhabit ecologically underexplored niches in extreme aquatic habitats and thus yield an unusually high number of gene clusters lacking known relatives in reference databases. Therefore, they are considered a promising source for bioactive compounds. One of the aims of the EU-funded project “EXPLORA” is to discover and validate such compounds, including molecules with antimicrobial or antioxidative effects as well as sialylated extracellular polymeric substances and acidic polyesterases with possible industrial applications in different sectors. In our study, we analysed a dataset of about 1000 genomes of psychrophilic and acidophilic organisms as well as one metagenomic sample from Tinto river.

We focused on four classes of ribosomal synthesized and postranslationally modified peptides (RiPPs): Lanthipeptides, lassopeptides, azol-containing RiPPs and triceptides, due to their compact biosynthetic gene clusters (BGCs), high structural diversity and high antimicrobial potential. The analysis consisted of the following steps: 1) BGC detection with antiSmash, 2) clustering based on similarity with BiG-SCAPE to evaluate the novelty, 3) scoring based on characteristics like evidence for bioactivity, structural diversity, completeness of the BGC and practical factors. This resulted in 17 promising BGCs that have been cloned into a suitable expression vector for *E. coli*. Further steps will be heterologous expression, LC-MS analysis, purification, characterization and upscaling of the production.

Exploring the biosynthetic potential of Bacteroidota: from nonribosomal peptides to NIS-derived siderophores

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Bacteroidota are widespread across diverse ecological habitats but remain comparatively underexplored as producers of specialized metabolites. Genome mining has revealed a remarkably diverse biosynthetic repertoire, with nonribosomal peptide synthetase (NRPS) pathways representing a particularly prominent source of unexplored chemical diversity.

Extending these studies, we investigated NRPS pathways associated with the biosynthesis of sulfonated monobactams. Screening approximately 600 Bacteroidota genomes identified three candidate biosynthetic gene clusters in *Pedobacter*, *Flavobacterium*, and *Chitinophaga* closely related to the sulfazecin pathway, the first characterized biosynthetic system for a naturally occurring sulfonated monobactam. These compounds are of particular interest as unusual NRPS-derived β -lactams and as natural-product scaffolds relevant to β -lactam resistance and the development of the clinically used antibiotic aztreonam.

Candidate pathways were investigated by systematic cultivation profiling, biosynthetic gene-expression analysis, and biological response testing. In parallel, a targeted LC-MS/MS workflow exploiting the characteristic SO_3 neutral-loss signature was established together with extraction strategies tailored to highly polar metabolites. Candidate compounds are currently undergoing isolation and purification for NMR-based structural elucidation. In the future, the identified pathways provide a basis for genetic modification and pathway engineering to produce diversified monobactam derivatives.

Beyond monobactam biosynthesis, the pronounced NRPS potential of Bacteroidota contrasts with the scarcity of canonical NRPS-dependent metallophore pathways. We therefore investigated NRPS-independent siderophore synthetase (NIS) pathways in *Pedobacter*. Comparative genomic, transcriptional, and metabolomic analyses support iron-responsive NIS-mediated siderophore biosynthesis as an alternative strategy for iron acquisition.

Together, these two examples highlight the substantial potential of Bacteroidota for natural product discovery, with their largely unexplored NRPS-derived compounds and alternative siderophore pathways providing opportunities for the development of novel bioactive molecules while offering insights into the chemical strategies underlying ecological adaptation and microbial competition within this widespread phylum.

Paenidepsins constitute a new family of Lipopeptides from *Paenibacillus*

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Lipopeptides (LPs) represent a structurally diverse group of specialized metabolites that consist of a peptide moiety attached to a fatty acid. Bacteria from the genera *Bacillus* and *Paenibacillus* are notable producers of LPs, including prominent examples such as surfactin, fengycin, and polymyxins; these compounds display a wide spectrum of biological activities, including antimicrobial, antifungal, and anticancer effects.^[1]

In the search for novel bioactive metabolites, we isolated paenidepsin A, a macrocyclic lipododecapeptide with antifungal activity, from the culture broth of *Paenibacillus apiarius* MW-14.^[2] Its chemical structure was elucidated by extensive tandem mass spectrometry (MS/MS) and nuclear magnetic resonance (NMR) experiments. Further paenidepsin analogs B–F were detected through global natural product social (GNPS) 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.^[3] We performed comprehensive phylogenetic and comparative analyses to demonstrate that paenidepsin-like BGCs constitute a novel gene cluster family from *Paenibacillus*. Moreover, detailed NRPS domain-level analyses provided bioinformatic evidence for recombination-driven evolutionary diversification of this BGC.^[4] Our findings illustrate the utility of integrating mass spectrometric and genomic tools to discover novel specialized metabolites and highlight the significant biosynthetic potential of members of the genus *Paenibacillus*.

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Discovery of natural products from the bryophyte microbiome

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Bryophytes are the evolutionary oldest plant lineage. Because of their remarkable resilience towards environmental stresses and their important role in many ecosystems, they are interesting model systems to study plant biology and plant-microbe interactions. The bryophyte microbiome contributes to host fitness by enhancing stress tolerance, promoting plant growth and potentially protecting the host against pathogens. While secondary metabolites are known to contribute to interactions between microorganisms and their hosts, they remain largely unexplored in the context of the bryophyte microbiome. Studying secondary metabolites from bryophyte microbiomes will thus improve our understanding of their ecological functions and may uncover an untapped source of novel natural products with pharmaceutical relevance.

Using the moss *Funaria hygrometrica*, we characterized its microbiome through 16S rDNA sequencing, revealing a community dominated by Alphaproteobacteria, Cyanobacteria and Gammaproteobacteria. From the same plants, a collection of diverse bacterial strains was isolated, and specific isolation of cyanobacterial members is ongoing.

To investigate the biosynthetic potential of our isolates we use genome mining and cultivation-based approaches, focusing on RiPP BGCs and metallophores. We have further established a co-cultivation system for *F. hygrometrica* and its associated bacteria to analyze the interaction-induced production of secondary metabolites from the bacteria. Additionally, we are investigating the secondary metabolome of *Paenibacillus amylolyticus*, a putative symbiont of the liverwort *Marchantia polymorpha*.

Together, these approaches provide first insights into the metabolite diversity of bryophyte-associated bacteria, laying the foundation for the discovery of novel natural products and the elucidation of their ecological functions.

Characterization of novel linear peptides from *Xenorhabdus mauleonii*

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Xenorhabdus species encode numerous biosynthetic gene clusters that produce specialized metabolites, yet many of these pathways remain silent or poorly expressed under laboratory conditions. Here, we activated biosynthesis gene clusters (BGCs) encoding non-ribosomal peptide synthetases (NRPS) in *Xenorhabdus mauleonii* by replacing their native promoter with arabinose inducible PBAD promoter. Metabolites produced by the engineered strain were extracted and analyzed by LC-MS and compared to those of the wild type strain. Promoter activation resulted in the appearance of several peptide that were absent in the wild type. HRMS together with NMR supported the incorporation of arginine related analogues and the presence of N-methylated citrulline within a family of structurally related linear peptides. Feeding experiments with L-[methyl-D3]methionine produced a mass shift consistent with the incorporation of a deuterium labelled methyl group, supporting the presence of an N-methylation event in the peptide and suggesting the involvement of a SAM-dependent methyltransferase during biosynthesis. Future work will employ synthetic zipper based strategy to split and co-express the NRPS into separately expressed modules that are reassembled through high affinity zipper interactions in order to generate additional peptides carrying the unusual amino acid.

Streptomyces natural products discovery

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Actinobacteria such as *Streptomyces* produce over 70% of known antibiotics through biosynthetic gene clusters (BGCs), but most remain silent due to complex and dormant regulation including gamma-butyrolactone quorum sensing. This PhD project focuses on discovering new natural products with high therapeutic potential from the soil bacterium *Streptomyces* sp. BBFR2, isolated from the bumblebee *Bombus terrestris*, which exemplifies the primary global source of secondary metabolites providing over two-thirds of known antibiotics.

Natural products are classified into major groups: PKS (polyketides like erythromycin), NRPS (non-ribosomal peptides like vancomycin), RiPP (post-translationally modified ribosomal peptides), siderophores (metal chelators like iron binders), and butyrolactone signals. A cosmid, a 35-45 kb plasmid-phage hybrid vector with cos sites derived from lambda phage, enables cloning of complete large genomic BGC fragments. A cosmid library from *S. BBFR2* was constructed via partial fragmentation, ligation, and *E. coli* transformation, followed by biparental conjugation into the optimized heterologous host *S. albus* Δ14-a minimal strain lacking 1 Mb of endogenous BGCs and sporulation genes for clean expression and reduced metabolic impurities.

The priority cosmid AB-ID2AS5 encodes two complementary orphan BGCs with low identity (<5%): Region 7 (11.5 kb) features a RiPP-like cluster with DUF692 iron-dependent oxygenase and TIGR04222 membrane precursor bearing a cysteine/serine-rich tail, predicting an oxidized macrocyclic defense peptide (4-8 kDa) with antibacterial/anticancer potential; Region 27 (10.9 kb) harbors a classic butyrolactone cluster including ScbA/BarX synthase and dual ScbR repressors, yielding SCB-like gamma-butyrolactones (250-350 Da) from AlkB- and glucose-1-dehydrogenase-derived hydroxy acid precursors as QS signals, BGC activators, and PPAR/HDAC candidates.

The main objective is to activate these BGCs via heterologous expression, detect metabolites by LC-MS/GC-MS, purify via HPLC/NMR, validate antimicrobial activities and elucidate biochemical mechanisms for pharmaceutical applications against antibiotic resistance.

A multi-layered annotation workflow improves genome mining of secreted and carbohydrate-active enzymes in myxobacteria

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Myxobacteria are prolific producers of specialized metabolites and extracellular enzymes, yet the functional interpretation of their large and complex genomes remains limited by incomplete annotation of accessory and lineage-restricted genes. In particular, many proteins potentially involved in nutrient acquisition, environmental sensing, and ecological competition remain annotated as hypothetical proteins by standard genome annotation pipelines.

To address this gap, we analyzed more than 1.3 million coding sequences from 155 myxobacterial genomes. Orthogroup inference resolved 58,223 protein families and enabled family- and strain-level comparisons of gene repertoire composition. By integrating orthology, functional-domain evidence, secretion prediction, and biosynthetic gene cluster context, we added functional evidence for 56.50% of family-specific orthogroups (6,265/11,089). More specifically, representative proteins from 3,673 orthogroups carried predicted signal peptides related to secretion, translocation, or membrane association. Across the dataset, 41 strains were significantly enriched and 45 were significantly depleted in predicted exported proteins (FDR-corrected $p < 0.05$), revealing substantial strain-level variation in predicted extracellular repertoires. Analysis of candidate extracellular carbohydrate-active enzymes further revealed substrate-degradation potential toward plant-associated polymers, alginate, and host-associated glycans. In addition, biosynthetic gene cluster context identified candidate exported proteins associated with specialized-metabolite loci.

Together, these results show that integrative annotation substantially improves functional recovery from myxobacterial genomes and enables more targeted genome mining. Through functional diversification, individual strains appear to assemble distinct combinations of surface-associated enzymes, carbohydrate-degrading proteins, secretion-related systems, and biosynthetic gene cluster-associated exported proteins. These combinations may shape nutrient acquisition, prey interaction, and competition through specialized metabolites.

From Prediction to Function: Unlocking RiPP Diversity in the Human Lung Microbiome

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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. However, while microbiome-derived metabolites are increasingly recognised as important mediators in cell communication, the metabolic potential of the lung microbiome remains comparatively unexplored. Ribosomally synthesised and post-translationally modified peptides (RiPPs) represent a structurally diverse class of natural products with a broad range of biological functions. Here, we establish a workflow that bridges genome-based prediction of RiPP biosynthetic potential with experimental production and functional characterisation.

Genome mining of lung-associated microorganisms revealed a diverse repertoire of putative RiPP biosynthetic gene clusters (BGCs) and precursor peptides. From these predictions, representatives of three distinct RiPP classes—lasso peptides, lanthipeptides, and autoinducing peptides (AIPs)—were selected for experimental investigation. Putative lasso peptide and lanthipeptide BGCs were transformed into heterologous expression systems to enable experimental validation and production of the predicted compounds. In parallel, a synthetic bioinformatic natural product (syn-BNP) approach was applied to predict 14 putative AIPs, five of which were selected for chemical synthesis, thereby enabling their investigation independently of the corresponding biosynthetic pathways. Selected peptides are subsequently subjected to functional assays addressing potential antimicrobial, antibiofilm, and microbiome-modulating activities.

By integrating computational discovery with heterologous expression, chemical synthesis, and biological testing, this workflow provides a framework for the experimental validation of previously unexplored RiPPs and for investigating their potential roles in microbial interactions within the human respiratory tract.

Core Enzyme-Independent Biosynthetic Gene Cluster: Repurposing Ergosterol Metabolism

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Fungal-derived steroidal specialised metabolites are poorly studied, except for the key membrane building block ergosterol. Their biosynthesis is unusual, as most proposed pathways start with ergosterol or other primary metabolism steroidal intermediates, and the pathways therefore lack a backbone-building core enzyme. This means the typical architecture of associated biosynthetic gene clusters (BGC) also lack identifiable genes encoding core biosynthetic enzymes, making them impossible to find by standard genome mining methods (e.g. AntiSmash).

This project revolves around elucidating the biosynthesis of rearranged steroids (abeosteroids), in this case cyclocitrinol. This includes genome-mining for oxidative enzymes, e.g., cytochrome P450s (CYPs), gene expression analysis under producing and non-producing conditions, and knock-out and heterologous expression experiments of the most promising candidates.

After identifying cyclocitrinol production in the fungi *Penicillium citrinum* and *Penicillium chrysogenum*, all CYP-encoding genes (197 in total) were identified by CDD-dependent BLAST searches. Phylogenetic analysis and the bioinformatic tool OrthoFinder yielded a putative cluster of three CYP genes adjacent to a gene supposedly involved in ergosterol metabolism. The expression of the respective CYPs was correlated with cyclocitrinol production in a qPCR-like experiment. Finally, heterologous expression of the three CYPs in *Aspergillus oryzae* NSAR1 resulted in the production of a new compound with appropriate chemical and physical properties.

Further analysis of the putative CYP cluster revealed an adjacent gene encoding a reductase, which shows high sequence similarity to ERG4 (C-24(28) sterol reductase) from ergosterol metabolism. Future work will focus on including the ERG4 homologue in the heterologous expression system and verifying the putative production of cyclocitrinol.

Unlocking the functional potential of natural products in *Blautia* species from the human gut

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The human gut is inhabited by a diverse community of microorganisms that have a profound impact on host physiology. The gut microbiota has been shown to produce a variety of natural products (NPs) with, e. g., cytotoxic, antimicrobial and immunomodulatory properties.^[1] However, our mechanistic and functional understanding of NPs produced by many gut microbial representatives remains limited.

The *Blautia* genus was described in 2008 and makes up 2-8 % of the human gut microbiome.^[2] A recent study highlighted the potential of *Blautia* sp. to produce bioactive NPs.^[3] Up to date only two NPs from these bacteria have been experimentally characterized – blauticin and nisin O.^[4,5] The goal of this project is to systematically explore the NP diversity from *Blautia* sp. And to study their role in the chemical interplay within the human gut.

We first performed bioinformatic analyses to identify the diversity of biosynthetic gene clusters (BGCs) potentially encoding NPs in *Blautia* sp. Then, the BGCs were prioritized according to different criteria, such as their novelty or completeness. The selected BGCs were cloned using the Direct Pathway Cloning strategy (DiPaC),^[6] heterologously expressed and the encoded compounds isolated for in-depth chemical and biological characterization. Using this pipeline, a lanthipeptide was already successfully heterologously expressed and is currently being further analyzed. This project will foster our understanding of the influence of *Blautia* sp. on gut microbial composition and human health.

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Metagenome Mining of a Soil Microbiome for Antibiotic Lasso Peptide Biosynthetic Gene Clusters

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The rapid emergence of antibiotic-resistant bacteria has created an urgent need for new antimicrobial agents, while the discovery of new antibiotic classes has stagnated for decades. Natural products, particularly secondary metabolites from soil microorganisms, represent a promising source of bioactive compounds. Among these, ribosomally synthesized and post-translationally modified peptides (RiPPs), such as lasso peptides, are of particular interest because of their defined biosynthetic pathways and potential antibacterial activity.

In this study, we aimed to establish a targeted workflow for the identification and analysis of lasso peptide biosynthetic gene clusters (BGCs) with potential antimicrobial activity from soil metagenomes. Soil samples were collected from the A horizon of a cambisol in the Schönbuch forest near Tübingen, Germany, and processed for metagenomic DNA extraction followed by Nanopore long-read sequencing. Subsequent bioinformatic analysis using antiSMASH, BLASTp, and ARTS enabled the identification of numerous complete BGCs, including more than 3000 lasso peptide clusters. Cluster selection for potential antibacterial activity was based on the presence of putative resistance genes. Selected clusters were then subjected to cluster-specific primer design, PCR amplification from metagenomic DNA, and assembly into heterologous expression vectors.

Here, we show the identification and prioritization of lasso peptide BGCs from a soil metagenome, their successful amplification from environmental DNA, and their reconstruction in heterologous expression vectors. We further present initial heterologous expression experiments and discuss the opportunities and challenges associated with the functional characterization of metagenome-derived lasso peptide clusters.

Mining microbial contamination sequences in public genome databases reveals Desulfobacterales as a treasure trove of RiPP biosynthetic gene clusters

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The widespread adoption of high-throughput sequencing has generated vast genomic datasets from both prokaryotic and eukaryotic organisms. A considerable proportion of bacterial sequences is routinely classified as contamination. Since these sequences are erroneously attributed to the target organism, they are typically removed from genome assemblies to ensure accurate taxonomic assignment and downstream analyses. Surprisingly, 36.8 Gbp in the NCBI database which represents 0.16% of total bases were identified as bacterial contaminations of the target genomes. The contaminating sequences range from bona fide symbionts of the sequenced hosts to likely methodological artefacts. We analyze the biosynthetic potential of these bacterial contamination sequences. We screened the GenBank-and RefSeq-derived contamination sequences for biosynthetic gene clusters (BGCs) and identified a strong enrichment of ribosomally synthesized and posttranslationally modified peptide (RiPP) BGCs. Taxonomic profiling showed that contaminations predominantly occur within the Pseudomonadota, with a notable enrichment of uncharacterised Desulfobacterales-derived BGCs, many of which exhibit low similarity to characterised BGCs. Multiple yet uncharacterised RiPP families were identified among the contamination-derived BGCs. Overall, our findings highlight the untapped potential of putative contaminations from publicly accessible genomic databases for natural product discovery. At the same time, they underscore the importance of accurately identifying and removing contaminating sequences from genome assemblies. Failure to do so may result in the erroneous assignment of BGCs to the wrong species, thereby confounding the interpretation of biosynthetic potential and the distribution of natural product diversity.

Discovery and Production of Ancient Lasso peptides

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Ancient DNA preserves microbial biosynthetic information across archaeological timescales and represents an important resource for natural product discovery.^[1,2] Phylogeny-guided genome mining of ancient human dental calculus metagenomes revealed a distinct lasso peptide biosynthetic lineage associated with oral *Actinomyces*, comprising a family of related palbiosynthetic gene clusters (BGCs). We reconstructed a *pal* BGC from an approximately 30,000-year-old oral microbiome and demonstrated its functionality by heterologous expression in *Streptomyces* strains and *Escherichia coli* BL21(DE3), leading to the discovery of two lasso peptides, palassoptides A1 and A2. These peptides contain chemically distinct residues at core position 1, Phe and Glu, respectively, demonstrating that the same biosynthetic machinery can process precursors bearing markedly different N-terminal core residues. Mutational analysis showed that the lasso cyclase PalC exhibits exceptionally broad tolerance at core position 1, accepting all proteinogenic amino acids except Pro. This represents the broadest tolerance at this position reported for a lasso cyclase to date. The two palassoptides also exhibited combination-dependent antibacterial activity against selected Gram-positive bacteria. Homologous BGCs are widespread among modern oral *Actinomyces*, indicating long-term preservation of this biosynthetic lineage within the human oral microbiome.^[3]

Together, these findings highlight the potential of ancient DNA as a resource for natural product discovery and reveal PalC as a lasso cyclase with unusually broad substrate tolerance at core position 1. Ongoing investigation of PalC using additional precursor variants may provide insight into macrolactam ring formation and possible determinants of substrate recognition.

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Discovery of quinolone-based meroditerpenoids biosynthesized through oxidative indole rearrangement and unusual terpene cyclizationYuya Kakumu¹, Eric J. N. Helfrich^{1,2}

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Terpenoids and meroterpenoids are the structurally most diverse class of natural products, characterized by densely functionalized, C(sp³)-rich polycyclic architectures. In canonical (mero)terpenoid biosynthesis, these molecular frameworks are constructed through carbocation-mediated cyclizations catalyzed by two types of terpene cyclases (TCs), followed by tailoring enzyme-mediated modifications. Over the past decades, however, evolutionarily distinct transmembrane proteins, termed Pyr4-like TCs, have been shown to catalyze complex scaffold formation in the biosynthesis of fungal (mero)terpenoids. In contrast to the well-defined functions of fungal Pyr4-like TCs, their roles in bacterial (mero)terpenoid biosynthesis remain largely unknown.

In this study, we comprehensively charted the biosynthetic space of bacterial Pyr4-like TCs and functionally characterized the *zig* biosynthetic gene cluster (BGC). Heterologous expression of the *zig* BGC led to the discovery of zignalone A, a novel pyranoquinolone diterpenoid featuring a 6/6/6/5/6/6/6-heptacyclic scaffold. Its biosynthetic pathway was established by a combination of gene inactivation, structural characterization of pathway intermediates, biotransformation assays, and bioinformatics analyses. Key transformations include an oxidative indole ring expansion catalyzed by a thiamine pyrophosphate-dependent enzyme to form a 4-quinolone. Subsequent cytochrome P450-mediated epoxidation and hydroxylation prime carbo- and oxacyclizations catalyzed by the Pyr4-like TC to forge a bilobed architecture comprising a pyranoquinolone and a 6/6/6-tricyclic ring system. Further cytochrome P450-mediated oxidations and a nonenzymatic reaction generate a spiro-fused tetrahydrofuran, completing the heptacyclic scaffold of zignalone A. Moreover, zignalone A congeners show potent antimicrobial activity against *Staphylococcus aureus*. This work provides new insights into bacterial Pyr4-like TC-associated pathways, expanding the biosynthetic logic underlying the assembly of complex bioactive meroterpenoids.

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

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Cyanobacteria are prolific producers of secondary metabolites and hold promise as sustainable cell factories for a wide range of chemical compounds.² Currently, unicellular cyanobacteria of the genera *Synechococcus* and *Synechocystis* serve as the primary chassis for the production of fine chemicals and commodity compounds. However, these organisms are not well suited for biosynthesis of complex NRPS-type natural products. Filamentous cyanobacteria of the genus *Nostoc*, however, offer strong potential for high-titer production of nonribosomal peptides (NRPs), due to their high number of encoded NRPS biosynthetic gene clusters (BGCs). Among these, the filamentous cyanobacterium *Anabaena* sp. PCC 7120 has emerged as the most effective heterologous host for cyanobacterial natural products to date.³

The pyreudione synthetase is a monomodular NRPS responsible for the biosynthesis of pyreudione, an amoebicidal alkaloid.¹ Recently, we achieved heterologous expression of the NRPS-derived natural product pyreudione A from *Pseudomonas fluorescens* at high titers in *Nostoc* sp. KVJ2. Notably, our expression platform not only enabled high-titer production but also induced a broad shift in the specialized metabolism of the mutant strain.

Based on these findings, the present study aims to establish a new filamentous cyanobacterium as a heterologous host for NRP production. Different plasmid-based expression strategies will be evaluated using constitutive and inducible promoters. Spatial imaging will further be employed to visualize and compare metabolic phenotypes between wild-type and mutant strains. Collectively, this work seeks to expand and optimize our heterologous expression toolkit across multiple *Nostoc* strains, advancing production of bioactive NRPS-type peptides from cyanobacterial chassis.

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Investigating the biosynthesis of illudins and engineering the biosynthesis of cytochalasans

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Project 1

Illudins are secondary metabolites with important biological properties. Illudin M, a member of this group, was proven to have anticancer activity. Even though Illudin M has been successfully synthesized by chemists, its biosynthetic pathway remains unknown. To understand the biosynthetic pathway of illudin M a potential biosynthetic gene cluster of illudin M was identified in the genome of the fungus *Omphalotus nidiformis*. The fungal strain *A. oryzae* NSAR1 was transformed with vectors containing the five essential genes for the biosynthesis of illudin M. After the production of illudin M was observed, via LCMS analysis, the same fungal strain was transformed with vectors containing four out of the five, essential for the biosynthesis of illudin M, genes. Some intermediates of the illudin M biosynthesis were observed after the LCMS analysis of the transformants.

Project 2

Cytochalasans are secondary metabolites with the ability to bind on the barbed end of the actin filaments, thus inhibiting their polymerisation. A cytochalasan expression platform needs to be created for the utilization of cytochalasans for pharmaceutical reasons among others. To create an *M. grisea* NI980 strain which produces vanilla pyrichalasin H, a large fragment of the pyrichalasin H biosynthetic gene cluster, containing the genes which encode the tailoring enzymes and three essential genes, including the transcription factor gene for the biosynthesis of vanilla pyrichalasin H, was knocked out. The three essential genes were reintroduced into the genome after the knock out. The genes were proven to be successfully knocked out of the biosynthetic gene cluster but no production of vanilla pyrichalasin H was observed after the gene reintroduction. Upon further investigation it was determined that the genes were underexpressed. A longer fragment of the transcription factor gene was introduced in the genome after the knock out, an improvement in the gene expression levels has been observed.

A New Class of Terpene Antibiotics from Actinobacteria: Discovery, Characterization, and Biosynthesis

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The current situation with drug-resistant pathogens is critical, underscoring an urgent need for novel, efficient antibiotics. Herein, we report the discovery and characterization of novel terpene antibiotics, gromomycins and darumycins. Isolated via actinomycete library screening and heterologously produced in *Streptomyces* chassis strains,¹ gromomycins represent a new class of guanidine-containing pentacyclic triterpenes and exhibit potent antimicrobial activity.² The corresponding biosynthetic gene cluster was identified by transposon mutagenesis after bioinformatic tools failed to detect it. Using gene cluster engineering, feeding experiments, and LC-MS and NMR analyses, we proposed the biosynthetic pathway for gromomycins, which are the first bacterial triterpenes synthesized independently of the squalene pathway. Furthermore, gromomycins represent a new type of biosynthetic logic in secondary metabolism, utilizing a unique cyclization path based on a hexaprenylguanidine precursor. Identification, cloning, and heterologous expression of related clusters uncovered new gromomycin derivatives, expanding their phylogenetic distribution. Furthermore, using the genome-mining approach, we have discovered a new family of guanidine-containing sesterterpene antibiotics, named darumycins. Targeted gene deletions, along with LC-MS and NMR analyses, complemented by in vitro biochemical characterization, enabled us to propose a darumycin biosynthetic pathway.

Mechanistic studies revealed that gromomycins disrupt bacterial membrane integrity, ultimately causing cell lysis. Scanning and transmission electron microscopy further confirmed membrane damage and cytoplasmic leakage in *S. aureus*, supporting a membrane-targeting mode of action.³ Overall, these findings expand our understanding of terpene diversity and reveal new potential for antimicrobial discovery.

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Nanmadolamide biosynthesis reveals the sequential basis of leader-dependent RiPP prenylation

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Lipidation enhances the therapeutic properties of peptides.[1] Many FDA-approved lipopeptide drugs are acylated. However, longer acyl chains can increase cytotoxicity, motivating the search for alternative lipid modifications.[2] Prenylation offers comparable therapeutic benefits while avoiding the limitations.[3,4] Recent studies have expanded the chemical space of prenylated peptides through the discovery of prenylated cyanobactins, a class of ribosomally synthesised and post-translationally modified peptides (RiPPs).[5] However, genome mining suggests that the diversity of prenylated RiPPs remains far from fully explored, as exemplified by the characterisation of steromaze, a RiPP-derived natural product of cyanobacterial origin, which features the polycyclic amino acid, heptophan, along with three macrocyclic dl-methylanthionine rings.[5,6] The characterized steromaze-like prenyltransferases (PTs) share characteristic biosynthetic features, including a dependence on the lanthionine rings as a prerequisite for efficient modification as well as leader region dependency.[7] Understanding these features in more detail could help to reveal how prenylation is integrated into more complex RiPP maturation pathways.

To answer the question if modification and leader dependency are general features of steromaze-like PTs, we aimed to expand steromaze-like PTs beyond tryptophan prenylation. Using targeted genome mining, we identified the previously uncharacterized PT NcbP within the *ncb* biosynthetic gene cluster. This cluster comprises four Nif11-type precursor peptides (NcbA1–NcbA4), the PT NcbP, a radical SAM enzyme (NcbS), and an asparagine synthetase (NcbN). Pathway reconstruction of this cluster revealed leader-dependent tyrosine prenylation, in which a short sequence within the N-terminal leader of the precursor peptide appears to enable prenylation by this ABBA-type PT. Our results discovery expands the known catalytic potential of ABBA-type PTs and provide further insights into substrate recognition and scope while providing a foundation for future enzyme engineering.

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Expanding glycopeptide antibiotic diversity through sulfotransferase-mediated modification

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Glycopeptide antibiotics (GPAs), such as vancomycin and teicoplanin, are crucial for treating infections caused by Gram-positive bacteria. However, the emergence of resistance through replacement of the terminal d-alanyl-d-alanine moiety of the target lipid II with d-alanyl-d-lactate, which reduce antibiotic binding, highlights the need for 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 proteinogenic and non-proteinogenic amino acids. P450-mediated oxidative cyclization establishes a rigid, cross-linked scaffold. Additional modifications can include halogenation, sulfation, glycosylation, and acylation.^[3]

Sulfation, a rare GPA modification, can significantly expand the antibacterial spectrum by circumventing resistance mechanisms. Sulfotransferases, such as StaL, utilize 3'-phosphoadenosine 5'-phosphosulfate (PAPS) as a sulfate donor.^[4]

In this project, we aim to modify the type IV GPA A47934 produced by *Streptomyces toyocaensis* (*S. toyocaensis*), which already carries a sulfate moiety, by introducing three sulfotransferase genes *sut_{AC1}*, *sut_{AC2}*, and *sut_{AC3}* identified within a GPA biosynthetic gene cluster from a strain of the MEDINA strain collection. In addition, we will delete the native sulfotransferase gene *staL* in *S. toyocaensis* and complement the resulting mutant with genes *sut_{AC1-3}* individually and in combination. This approach will provide insights into the substrate specificity and catalytic activity of sulfotransferases in GPA biosynthesis.

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Negamycin: Unusual Biosynthesis Directed By A Split Biosynthetic Gene Cluster

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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 owing 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.

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Docking domain based combinatorial biosynthesis of novel peptides

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Natural products (NPs) represent a rich source of bioactive compounds with a wide use in modern medicine^{1,2}. The need to full explore the diversity of chemical space with new-to-nature structures that harbor a wide range of bioactivity is indispensable. NPs are derived mainly from enzymatic pathways catalyzed by non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs). These enzymes represent, multi-modular enzymes, which often build multi-enzymatic complexes involving more than two polypeptides. In NRPSs, one module incorporates a specific amino acid which is linked in a growing peptide chain. It requires a coordinated interaction between the proteins, allowing a defined communication, which facilitates substrate shuttling between donor and acceptor modules. This interaction is mediated by the so-called docking domains (DDs)³. DDs share a similar functional framework but can differ in their structural properties. So far, there are two types of DDs, which occur solely in NRPSs: COM domains⁴⁻⁶ and β -hairpin docking domains (β -HDs)⁷.

Herein, we explore docking domains as a tool to mediate communication between non-cognate NRPSs. For this purpose, we investigate several biosynthetic gene clusters (BGCs) found in species of the *Xenorhabdus* genus, which based on sequence homology show the presence of β -HDs. We chose BGCs encoding for NRPSs and expressed them heterologously and separately in *E. coli* *DH10B::mtaA* by using a two-plasmid system. This allowed a combinatorial approach to screen NRPSs from distinct BGCs producing new-to-nature cyclic peptides. The results indicate that functional NRPS complexes are formed, despite their distinct origin. However, based on structure predictions, we found an unprecedented dual docking domain type, including COM domains and the β -HDs, providing an additional layer of interaction specificity and putatively domain orientation during the biosynthesis. This analysis, lays the foundation of additional engineering strategies to unlock the full potential of controlling NRPS-NRPS communication for the production of cyclic peptides.

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An Inducible Biopolymer Biosynthesis Platform in the Recombinant Filamentous Cyanobacterium *Nostoc* sp. PCC 7120 without Nitrogen Starvation

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The importance of developing sustainable, carbon dioxide (CO₂)-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 CO₂ 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. PCC 7120, 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. PCC 7120, 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. PCC 7120 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.

Unravel the biosynthesis of an antifungal compound produced in *Burkholderia cepacia* complexes

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An antifungal compound AFC-BC11 is a natural secondary metabolite produced in the strain *Burkholderia pyrrocinia* BC11 (formerly *B. cepacia* BC11).^[1] In general, these strains belong to the *Burkholderia cepacia* complex (BCC) and are known to be human pathogens that cause respiratory problems in patients with cystic fibrosis (CF).^[2] However, it is also well known that *Burkholderia* produces various secondary metabolites with antibacterial, antifungal, insecticidal and herbicidal effects.^[3] For example, *B. pyrrocinia* is an effective biological control agent against *Rhizoctonia solani*-induced root rot in poinsettias.^[4,5]

The recently published study by Zhong and colleagues provided the first structural characterization of the natural product AFC BC11.^[1] It consists of *O*-methylated malic acid-fatty acid (MMFA) featuring a fatty acyl chain with a double bond coupled to a dehydro-tetrapeptide, consists of three dehydro- β -alanine (DBA) residues and one γ,δ -dehydro-lysine (DHLys) residue. In a series of different in vitro reconstitution experiments the assembly of the backbone of AFC-BC11 was elucidated and the role of various enzymes associated with the biosynthetic gene cluster were characterized. The BGC of AFC-BC11 contains over 25 different genes involved in the biosynthesis and transport of the compound. Despite the peptide-like structure, features of a classic modular non-ribosomal peptide synthetase (NRPS), including a condensation domain (C) that catalyzes peptide bonds, are not present. In one of the various in vitro reconstitutions, a ketoacyl synthase (KAS) III-like protein was shown to induce peptide formation and release of the peptide chain from the protein itself.

Engineering of the Xenocoumacin biosynthesis and NRPS acylation modules

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Xenocoumacins (XCNs) are key secondary metabolites from the entomopathogenic bacterium *Xenorhabdus nematophila*, exhibiting potent bioactivity.^[1] They are synthesized as inactive, N-terminally acylated prodrugs by a hybrid of non-ribosomal peptide synthetase and polyketide synthase (NRPS/PKS). Activation requires sequential proteolytic cleavage to yield the antimicrobial XCN1, followed by further enzymatic modification to generate XCN2, a bioactive compound with promising anti inflammatory properties.^[2-4] However, since the conversion of XCN1 to XCN2 is inefficient and multi-step, we aim to unlock the therapeutic potential of XCN2 by reprogramming the XCN biosynthetic machinery to directly and efficiently produce XCN2 based on NRPS engineering.

The N-terminal acylation step, catalyzed by the initial acylation modules of NRPS or PKS systems, critically determines the prodrug activation mechanism of XCNs and biological activity of other lipopeptides.^[5] However, starter domains such as condensation starter (C_s) or acyl-CoA ligase (CAL) domains frequently exhibit substrate promiscuity and their specificity remains poorly understood, posing significant challenges for predictable pathway engineering. To address this, we aim to develop a high throughput applicable screening assay to systematically evaluate starter domain specificity toward diverse acyl substrates. Combining in vivo reconstitution with mass spectrometry, these insights will advance our understanding of N-terminal acylation in NRPS/PKS systems and enable the rational design of lipopeptides with tailored lipid moieties.

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Towards a sustainable bio-based route for production of tetrahydroisoquinoline scaffolds

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Trabectedin (Ecteinascidin 743, ET-743) is a natural product marketed under the name Yondelis® for the treatment of soft tissue sarcoma.^[1] It was first identified from the tunicate *Ecteinascidia turbinata* and, more precisely, made by a symbiotic bacterium '*Candidatus Endoecteinascidia frumentensis*'.^[2] ET-743 is part of a diverse family of compounds characterized by the presence of a bis-tetrahydroisoquinoline (bis-THIQ) core.^[3,4] However, it is the only member to be marketed as an approved drug. Due to low yields from the current production methods,^[5] we aim to develop a biocatalytic production system for ET-743 to increase production efficiency and allow for facile access to therapeutically relevant analogs. In this regard, the biosynthetic development falls into three categories: i.) early-stage modification of amino acid precursors, ii.) bis-THIQ core formation, and iii.) late-stage diversification..

Across the bis-THIQ family, the early-stage modification of the tyrosine precursor is consistent, and we have validated enzymatic reactions catalyzing both C- and O-methylation, as well as hydroxylation by a unique class of heme proteins.^[6] We also deepened our understanding of the order of these early-stage transformations via kinetics studies, and found them to be variable among members of the bis-THIQ family.

The formation of the bis- THIQ core depends on a non-ribosomal peptide synthetase (NRPS) that catalyzes two iterative Pictet-Spengler reactions combining the modified tyrosine with an additional amino acid. In the case of EtuA2 from ET-743 biosynthesis, cysteine incorporation is proposed to facilitate a late-stage cyclization to form a thioester bond in a mechanism under investigation in this work. This reconfigured bis-THIQ then undergoes a final Pictet-Spengler reaction to incorporate an additional modified tyrosine, thereby building the characteristic tri-THIQ scaffold of ET-743. In this work, we are bypassing the challenges of working with microbial symbiont-derived enzymes by applying microfluidics-based multiplex protein screening for the production of soluble Pictet-Spenglerase NRPSs, which were otherwise intractable to traditional heterologous expression methodologies. With these enzymes in hand, we performed in vitro enzymology experiments to validate cell-free protein expression as a viable method for functional megasynthetase production, while simultaneously pursuing the molecular-level insights into bis-THIQ core formation. We envision that these newly characterized enzymes will pave the way toward a biotechnology platform for the sustainable production of the anticancer therapeutic ET-743 and its previously inaccessible analogs.

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Sustainable Bioproduction of Tetrahydroisoquinoline Alkaloids: Mining and Engineering Gene Clusters from Uncultured Microbial Communities

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Nature is an incredible source of therapeutic compounds.^[1] Many are produced by large megaenzymes such as non-ribosomal peptide synthetases (NRPS). One notable class of NRPS-derived natural products are the antitumour bis-tetrahydroisoquinoline (bis-THIQ) alkaloids [2]. These compounds share a common core structure that is decorated by a suite of tailoring enzymes that vary widely between different microbial producers.^[3–5] We recently discovered two new putative bis-THIQ biosynthetic pathways, which we aim to heterologously express in *P. putida* AG5577. The committed step in all bis-THIQ biosynthesis is the methylation and hydroxylation of tyrosine. These initial tyrosine modifying enzymes have been characterized in vitro and in cellulo. Building on this foundation, our goal is to develop a sustainable biosynthesis platform for producing bis-THIQ analogues in cellulo, utilizing a broad range of known and engineered enzymes for core and late-stage diversification. We will apply metabolic engineering to remove bottlenecks such as precursor and co-factor availability, while pathway engineering of the core formation will increase yields. This project will enable the production of novel bis-THIQs through sustainable, modular methods that expand beyond traditional synthetic chemistry. Ultimately, this work will showcase the viability of enzymatic late-stage functionalisation of highly complex natural products.

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Revealing prenylated natural products from lichen cyanobiont genus *Rhizonema*

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Peptide-based natural products are a major source of therapeutic leads, and prenylation, the attachment of isoprenoid groups, is a modification known to increase hydrophobicity, membrane interactions, and metabolic stability of potential peptide drugs.^[1, 2] The prenyltransferases (PT) that catalyze these modifications are known to vary considerably in their regioselectivity, substrate specificity, and type of prenyl donor, underscoring the value of characterizing novel representatives from underexplored biosynthetic sources.^[3] In particular, cyanobacteria are prolific sources of prenylated peptides and thus, are important targets for bioprospecting.

Metagenomes containing the lichen cyanobacterial symbiont *Rhizonema* were mined for the presence of PT-encoding BGCs. Four PT-encoding ribosomally synthesized and post-translationally modified peptide (RiPP) biosynthetic gene clusters (BGCs) were identified, each encoding distinct core peptides suggesting broad substrate scope and structurally varied products. All four were obtained by gene synthesis and cloned into an *E. coli*-based heterologous expression host, with the precursor peptide gene and tailoring genes distributed across separate plasmids. Heterologous expression and high-resolution LC-MS of digested modified peptides showed a mass shift consistent with dimethylallyl pyrophosphate-derived prenylation in one of the detected precursors. Structural modeling using AlphaFold3 proposed Tyr94 as the likely site of prenylation. Subsequent MS/MS analysis of the prenylated precursor was consistent with modification at hydroxyl-bearing residues (Ser91, Tyr94 or Ser100) within the core peptide. Overall, this work establishes a platform for the biochemical investigation of prenylated RiPP biosynthesis from lichen cyanobionts, highlighting their potential as a source of novel natural products.

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A Biosynthetic Pathway Towards an Improved Polymyxin

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Polymyxins are potent nonribosomal peptide antibiotics, yet their clinical use is limited by a narrow therapeutic window and nephro-/neurotoxicity. Conventional efforts to improve these agents rely on the incorporation of non-natural scaffolds that cannot be accessed biosynthetically and therefore must be introduced by total synthesis. Here we report a biosynthetic route to a next generation polymyxin that circumvents this limitation. By engineering the substrate binding pocket of a nonribosomal adenylation (A-)domain, we created an expanded pocket that selectively activates the fatty amino acid 2-aminodecanoic acid (Ada). Incorporation of this engineered A-domain into the native polymyxin biosynthesis enabled the production of a 2-Ada-containing polymyxin directly in *Paenibacillus polymyxa* fermentation with the high potential for lowered toxicity. Then we turned to *in vivo* supply of the 2-Ada precursor by conversion of the cellular metabolite capric acid, with the aim of reducing costs and increasing product yield through improved bioavailability of 2-Ada. For this purpose, we have developed a synthetic pathway consisting of a P450 monooxygenase, an oxidoreductase, and an *E. coli* aminotransferase with promising preliminary results both *in vitro* and in *E. coli*. This work demonstrates that rational redesign of NRPS A-domains can redirect natural product biosynthesis toward chemically diverse, therapeutically superior antibiotics, opening new avenues for combating drug resistant bacteria, with future prospect of producing these compounds cheaply in large scale fermentation.

Biosynthetic and structure-activity investigations on translation-inhibiting cyclic peptide natural products

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Nonribosomal peptides (NRPs) are a class of natural products assembled by large multi-modular enzymes with high structure complexity and bioactive diversity, investigations of their biosynthesis allow using bioengineering approaches to generate novel peptides with altered bioactivity that can be exploited as pharmaceuticals are therefore of great interest [1]. GE82832 and its analog Dityromycin are complex and highly modified macrocyclic depsipeptides antibiotics targeting the protein S12 at the 30S unit of the bacterial ribosome thereby inhibiting elongation factor G-catalyzed tRNA translocation, the unique mechanism outlined a region of the ribosome that can be a target of future structure-based antibiotic design. [2] Through bioinformatic analysis of their genomes, we identified the candidate biosynthetic gene clusters of GE82832 and Dityromycin, which suggested an unconventional nonribosomal peptide synthetase assembly line for these two antibiotics. The isolation, purification, and structure elucidation of GE82832, stable-isotope feeding experiments supporting isoleucine as a biosynthetic precursor and in vivo functional assays of two cluster-encoded cytochrome P450 oxygenases will be presented.

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Cyanobacterial Siderophore Associated with Novel Class of Acyl Hydrolases

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Iron is a key micronutrient that constrains microbial growth and productivity in many aquatic and terrestrial environments due to its limited bioavailability. Microorganisms evolved sophisticated acquisition strategies, including the production of siderophores, high-affinity iron-chelating molecules that facilitate iron solubilisation and uptake. Cyanobacteria, photosynthetic prokaryotes and major contributors to global primary production, also depend on iron as a cofactor to their core metabolic enzymes. However, very few cyanobacterial siderophores were described so far, and cyanobacteria remain an underexplored source of possibly novel siderophores. Here we report a novel cyanobacterial siderophore, cyanochelin C, that employs two β -hydroxyaspartate residues for iron chelation. We provide extensive nuclear magnetic resonance (NMR) and mass spectrometry (MS) evidence on the molecular structure and identify the corresponding biosynthetic gene cluster (BGC). Bioinformatic analysis of the BGC further revealed the presence of an acylase CcsQ clustering with a broader cyanobacteria-specific family of acylases associated with predicted siderophore-encoding BGCs. Discovery of cyanochelin C and its deacylation by CcsQ expands the known structural diversity of cyanobacterial siderophores and improves the understanding of important enzymatic reactions.

Isolation and Derivatization of Mureidomycin A & C

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Uridyl peptide antibiotics like mureidomycin A & C (MrdA & MrdC) are promising natural compounds due to their inhibitory capabilities. They target the transmembrane-enzyme MrdY (translocase I), which catalyses the first membrane associated step in peptidoglycan biosynthesis. For MrdY two different binding sites, intracellularly and on the periplasmic side of the enzyme have been identified [1]. In addition, they also inhibit a key enzyme of the LPS biosynthetic pathway [2]. This specific triple binding at two different targets leads to the inhibition of *Pseudomonas aeruginosa*, a Gram-negative bacterium that causes difficult-to-treat infections. Among the mureidomycin compound family, MrdC is the most promising member, since it showed the strongest activity towards *P. aeruginosa* (IC₅₀=0.1 – 3.13 μ M)[3,4]. During previous work, a genetically engineered *S. albus* del 14 strain was created to produce MrdA at gram scale. Regrettably, MrdC is only produced by the native producer strains but inefficiently produced by heterologous hosts. However, a semisynthetic approach to transform MrdA into MrdC was developed, which makes the compound now available for further investigations. We therefore established an isolation and purification process for MrdA with the addition of a semisynthetic step to finally obtain MrdC.

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Turning Natural Products into Water-soluble Crystalline Salts for X-ray Crystallography

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Small-molecule structure elucidation is one of the most important aspects of natural product discovery. In addition to other techniques available for structure elucidation, such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), X-ray crystallography is considered one of the most direct methods for elucidating the structure of natural products, providing the three-dimensional structure of molecules.

X-ray crystallography can reveal the atomic or molecular structure of crystals by detecting the diffracted beams generated by the crystalline structure. To perform this analysis, a crystalline molecule and an X-ray-quality single crystal are required. However, growing a single crystal suitable for analysis is challenging, even though various methods for crystallizing molecules are available. Derivatization is one such method, and although p-nitrobenzoyl and p-bromobenzoyl chloride are well established, they are not generally effective.

Our research aims to identify highly reactive and crystalline derivatizing agents to address this challenging problem of crystallizing natural products. Salts readily crystallize; therefore, organic salts are considered promising candidates for the crystallization of natural products. By derivatizing natural products to Girard's reagent P, the natural products become more water-soluble, while the salt form of the derivatives enhances their crystallinity. This reagent allows natural products bearing a ketone or aldehyde group to be converted into crystalline derivatives, making X-ray crystallographic analysis of the derivatives possible.

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

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Ribosomally synthesized and post-translationally modified peptides (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. Lasso peptides are one of the largest known classes of RiPPs, characterized by a mechanically interlocked structure. 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.

Lasso peptides such as the previously described BI-321694 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-32169. This includes the RiPP recognition element (RRE), 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 for the successful in vitro cleavage of the precursor for core peptide release confirmed by mass spectrometry and fusion protein in vivo cleavage. Nevertheless, lasso peptide formation has not been observed with subsequent lasso cyclase incubation. Further efforts in lasso cyclase cyclase engineering will be performed for improved protein expression and activity towards formation of lasso peptides.

Phylogeny-guided discovery of a promiscuous P450 macrocyclase for the production of diverse atropopeptides

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Cyclic peptides exhibit diverse bioactivities and are distinguished by their enhanced cell permeability, improved proteolytic stability, and increased binding affinity due to their conformational rigidity. Despite significant advancements in peptide synthesis, the production of complex cyclic peptides remains a challenge. Nature has evolved diverse strategies for peptide cyclization, with an ever-growing repertoire of characterized cyclases involved in the biosynthesis of ribosomally synthesized and post-translationally modified peptides (RiPPs). These enzymes convert linear precursor peptides into complex (poly-)cyclic structures. The discovery of the atropopeptides has significantly expanded the chemical diversity of RiPPs with unique (poly-)cyclic structures.^[1-3] In this study, we employed a phylogeny-guided approach to identify a substrate-promiscuous cytochrome P450 macrocyclase that catalyzes the formation of cyclic peptides through atropospecific C–N or C–C bond formation between aromatic amino acid side chains. Combinatorial biosynthetic studies revealed that ScaB encoded in the scabirubin biosynthetic gene cluster efficiently cyclizes a wide range of atropopeptide precursors. Furthermore, extensive site-directed mutagenesis studies of the tetrapeptide core sequence further expanded the diversity of atropopeptides. Notably, three tested atropopeptides show antiviral activity and one of the non-natural atropopeptides displays anti-inflammatory activity. Our findings establish a broadly substrate-tolerant atropopeptide-modifying P450 as a versatile biocatalyst for the synthesis of bioactive, biaryl-bridged macrocyclic peptides.

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Characterization and Design of a Flexible Amino Acid Ligase from *Streptomyces mozunensis*

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Talopeptin is a natural dipetide-derived metalloprotease inhibitor produced by *Streptomyces mozunensis* via a biosynthetic pathway that deviates from canonical non-ribosomal peptide synthetase (NRPS) logic. Central to this pathway is TalD, a ATP-grasp ligase responsible for assembling the L-Leu-L-Trp backbone. Although its potential as a promising biocatalyst for sustainable synthesis of bioactive peptidic compounds and crucial role in the biosynthetic pathway, the enzyme has not been functionally characterized yet. In this study, we systematically investigated TalD to delineate its substrate profile, catalytic efficiency, and synthetic utility. Using tailored in vitro activity assays, we mapped its substrate scope, determined its kinetic parameters, and confirmed product formation by mass spectrometry. Building on these insights, we applied computational protein design methods to rationally reprogram TalD's active site, achieving precise control over its substrate specificity. Together, these findings establish TalD as a highly tunable amide ligase and a versatile protein scaffold for the biocatalytic synthesis of structurally diverse peptide derivatives.

***In vivo* MAT recruitment enables non-native extender-unit incorporation in modular cis-AT polyketide synthases**

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Polyketides are a structurally diverse class of natural products, many of which show clinically relevant antibacterial, antifungal, immunosuppressive or anticancer activities. Many complex polyketides are assembled by modular cis-acyltransferase polyketide synthases (cis-AT PKSs). Each module contains a ketosynthase that catalyses chain elongation, an acyltransferase (AT) that selects and loads the extender unit, and an acyl carrier protein that tethers the growing chain. Modules can additionally harbour facultative accessory domains that further modify the growing chain. Diversity arises from module number and composition, AT-selected extender units, and overall PKS architecture, making PKSs attractive targets for site-selective structural diversification. Module swapping and exchange of accessory domains are established engineering strategies. Reprogramming AT selectivity is a more recent approach and remains difficult to control.

Three AT engineering strategies exist: active-site engineering, AT-domain swapping, and native AT inactivation with trans-AT complementation. The latter is the least invasive, preserving native domain-domain interactions and folding. However, freely diffusing trans-ATs cannot distinguish the inactivated module from the remaining active ATs, so extender-unit loading lacks site-selectivity. Recruitment tags tethering the trans-acting AT to the target module were therefore tested *in vivo*, using the promiscuous acyl-CoA transferase (MAT) domain of the murine fatty acid synthase, accepting both malonyl- and methylmalonyl-CoA.

In *Streptomyces venezuelae*, the cis-AT PKS involved in venemycin biosynthesis incorporates almost exclusively malonyl-CoA despite a high endogenous methylmalonyl-CoA pool. Complementing the inactivated AT domain of one of its extending modules with a trans-acting MAT, recruited non-covalently via SYNZIP17/18 or covalently via SpyTag/SpyCatcher, yielded the methylated 3-methylvenemycin analogue. Both recruitment strategies shifted the product distribution toward 3-methylvenemycin (~53% for SYNZIP17/18; ~16% for SpyTag/SpyCatcher), compared with ~4% for unrecruited MAT and ~2% in the wild type.

These results provide the first *in vivo* demonstration of directed non-native extender-unit incorporation into a cis-AT PKS by trans-MAT recruitment, offering a possible route to broader polyketide diversification.

Potential Degradation of Microplastics by Human Lung-Associated Bacteria

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The presence of micro- and nanoplastics (MNPLs) in the human lung poses potential health risks, while the ability of the respiratory microbiome to transform these particles remains largely unexplored. This study investigates the enzymatic potential of lung-associated bacteria to hydrolyze synthetic polyesters, with a particular focus on polyethylene terephthalate (PET).

Bioinformatic screening identified a candidate enzyme from *Microbulbifer hydrolyticus* containing conserved PET hydrolase motifs and a putative catalytic triad. *M. hydrolyticus* showed esterase activity when cultured with PET and other polyesters at physiologically relevant temperatures. Following recombinant expression, biochemical characterization demonstrated hydrolysis of polycaprolactone and a preference for medium-chain p-nitrophenyl ester substrates. PET weight-loss assays further revealed measurable degradation at physiologically relevant temperatures.

These findings highlight the functional diversity of lung-associated bacteria and their potential as a source of enzymes capable of hydrolyzing synthetic polymers. The identified putative PETase may contribute to polyester degradation in the respiratory environment and provides insights into interactions between the lung microbiome and inhaled plastic particles. Beyond its potential physiological relevance, the enzyme may represent an interesting microbial biocatalyst for further biochemical characterization and engineering.

Prenylation as a Key Modification in the (Bio)Synthesis of Antimicrobial Peptides

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The increasing prevalence of multidrug-resistant bacterial pathogen emphasizes the development of novel antimicrobial agents. Peptide-based antibiotics such as murepavadin possess considerable therapeutic potential. However, their clinical application is limited by undesirable side effects.¹ Targeted late-stage functionalization of established lead compounds offers an promising strategy to optimize their biological and pharmacological properties without fundamentally altering their underlying mechanism of action.² Prenylation represents a particularly promising approach in this context.³ The dimethylallyl tryptophan synthase (DMATS) family of ABBA-fold prenyltransferases employed in this project catalyze the regio- and chemoselective transfer of prenyl groups to tryptophan and indole derivatives.⁴ Within this project, selected DMATS prenyltransferases are applied for the enzymatic prenylation of antimicrobial peptides. The target substrates include murepavadin and the antimicrobial hexapeptide MP196 (RWRWRW-NH₂). Given its pronounced antibacterial activity against gram-positive pathogens, its membrane-active mechanism of action, and its low cytotoxicity, MP196 represents a potent lead structure for structural modification.⁵ In addition, a chemoenzymatic approach is being pursued in which prenylated tryptophan building blocks are synthesized and subsequently incorporated into antimicrobial peptides via solid-phase peptide synthesis. The long-term objective of this project is the development of novel antimicrobial agents with improved efficacy and tolerability for the treatment of infections caused by multidrug-resistant bacterial pathogens.

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In vitro reconstitution of the [S,S]-EDDS biosynthetic pathway.

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[S,S]-ethylenediamine disuccinate ([S,S]-EDDS) is a biodegradable metal chelator produced by the actinomycete *Amycolatopsis japonica*. As a structural isomer of the industrial non-degradable chelator EDTA, it is of interest as an environmentally friendly alternative. The biosynthetic gene cluster (BGC) responsible for its biosynthesis encodes three enzymes (AesA-C) and an exporter (AesD) [1]. In previous work, we established that [S,S]-EDDS biosynthesis starts from *L*-aspartate and O-phosphoserine [2]. To elucidate the biosynthetic pathway, we investigated the metabolites associated with individual pathway steps. The key intermediate ([S]-2-amino-2-carboxyethyl)-*L*-aspartate (ACEAA) was identified in the supernatant of a mutant containing only *aesA*, *aesC* and *aesD*. Based on this finding, we proposed a pathway in which ACEAA is decarboxylated by AesB and subsequently converted to [S,S]-EDDS by AesA. *In vitro* enzymatic reactions were carried out and analyzed with HPLC-MS. AesB catalyzes the proposed decarboxylation of ACEAA. Coupling the AesB reaction with AesA resulted in the formation of [S,S]-EDDS. The AesA reaction incorporates one molecule of oxaloacetic acid and requires NADPH for the final reduction. Together, these results reconstitute the enzymatic steps of [S,S]-EDDS biosynthesis from ACEAA *in vitro* and support the proposed pathway. This provides new insights into the biosynthesis of [S,S]-EDDS and lays the foundation for optimization of its biotechnological production.

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Thermophilic SAM hydrolases counter epimerisation

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S-Adenosyl-L-methionine (SAM) dependent chlorinase, fluorinases and SAM hydrolases (SAMHs) constitute an enzyme superfamily present in bacteria and archaea. In a SN₂ reaction, they produce L-methionine and 5'-chloro-5'-deoxyadenosine, 5'-fluoro-5'-deoxyadenosine or adenosine (Ado), respectively [1-3].

First SAMHs were identified in marine bacteria of the genus *Salinispora*, and were proposed as a salvage pathway for the inactive SAM-diastereomer with (*R*)-configuration at the sulfonium-centre [(*RS*,*SC*)-SAM] [4]. This diastereomer is formed by non-enzymatic epimerisation of the canonical diastereomer (*Ss*,*Sc*)-SAM (**Figure 1**) [5].

In this work, enzymes from the acidophilic thermophilic archaeon *Sulfolobus acidocaldarius* and the halophilic archaeon *Haloferax volcanii* were recombinantly produced, purified and their activity as (*RS*,*SC*)-SAMHs was confirmed. Their physiological role was investigated using knock-out strains. Phenotype and intracellular SAM-content of wild-type and KO-strains were compared, showing that (*RS*,*SC*)-SAMH's activity prevents the accumulation of the inactive diastereomer in thermophiles [6].

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Structural and Functional Siderophore Remodeling by Enzymatic Delipidation

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Bacteria employ specialized metabolites called siderophores to acquire scarce iron, but these molecules may serve additional ecological roles. Here we reveal that *Pandoraea* species, including environmental isolates and opportunistic pathogens typically acquired from the environment, produce bifunctional lipopeptides that undergo enzymatic remodeling to switch from promoting bacterial motility to optimizing iron capture. Through genome mining and metabolic profiling, we discovered pandorachelins, diazeniumdiolate-containing siderophores.^[1] Comprehensive NMR analysis, derivatization, and isotope labeling established that pandorachelin A is a head-to-tail-fused homodetic cyclopeptide, revising a recently proposed structure.^[2] We identified the elusive biosynthetic precursor, pandorachelin B, as a lipocyclopeptide with a lactone moiety and N-terminal fatty acid. A specialized acylase (PdnM) cleaves the lipid tail of pandorachelin B, triggering an O→N acyl shift that contracts the ring and transforms the biological function: the lipopeptide enables bacterial swarming through surfactant activity, while the delipidated product exhibits enhanced iron-chelating capacity but no motility promotion. Genetic knockouts, enzyme reconstitution, and phenotypic assays confirm this maturation sequence. The functional switch correlates with ecological niches across *Pandoraea* species, revealing a sophisticated strategy for sequential niche colonization and nutrient acquisition. These findings identify PdnM as a potential antivirulence target and expand the functional repertoire of bacterial siderophores.

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High diversity but limited expression of biosynthetic gene clusters in a peatland microbial community

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The *in situ* relevance of biosynthetic gene clusters (BGCs) remains poorly understood. We applied meta-omics to characterize BGC diversity and activity along a peatland redox gradient. From seven metagenomes, we recover 9,694 BGCs, spanning diverse taxa, most lacking close relatives in reference databases, indicating extensive novelty. Only 9-27% of this potential is expressed *in situ*, with Acidobacteriota, despite moderate repertoires, accounting for over half of all BGC transcription. Talented producers with up to 24 clusters are largely silent, and expression is inversely related to BGCs per genome. Acidobacteriota, specialized in complex carbon processing potential expressed a larger proportion of their BGC repertoires than BGC-rich Pseudomonadota, which had faster doubling times and lower potential for complex carbon processing. At the genome level, BGC expression intensity is most strongly coupled to carbohydrate-active enzyme expression, particularly glycoside hydrolases, linking secondary metabolism to active carbon processing in peatland microbes. Thus, although BGCs are widespread, BGC expression *in situ* is integrated into the carbon-cycling activity of individual taxa rather than a shared physiological state.

Uncovering the Hidden Diversity of Cyanobacterin Analogues

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Cyanobacterial specialized metabolites (SMs) possess chemically diverse structures and a broad range of biological activities, affecting aquatic ecosystems and human health while offering opportunities for new drug discovery.^[1, 2]

However, traditional research methods for discovering new SMs remain resource intensive and time-consuming. Mass spectrometry, with its high-throughput capabilities and enhanced by computational approaches, provides an efficient strategy to accelerate SM discovery.^[3] We aimed to use computational approaches, including Haloseeker^[4] and MassQL^[5], for targeted screening for (poly)halogenated ions. HPLC-HRMS2 analyses of a cyanobacterial extract library comprising 547 samples revealed several extracts containing halogenated SMs. An extract of *Tolypothrix* sp. PCC 9009, a known producer of cyanobacterin (CB),^[6] was found to be particularly rich in these compounds. Classical Molecular Networking^[7] was used to explore the chemical space of natural CB analogues, while Feature-Based Molecular Networking^[8] was employed to investigate and uncover the structural diversity of previously unknown analogues of the known chlorinated, herbicidal SM.

In total, 15 previously unknown CB analogues were identified, six of which were isolated and structurally elucidated using 1D and 2D NMR and HRMS data. Among these, a [2+2] photocycloaddition dimer and two additional analogues corresponding to the non-chlorinated precyanobacterin I and II, previously reported from heterologous expression in *E. coli*,^[9] were identified.

Based on the known furanolide core assembly^[9] and the findings of this study, a biosynthetic pathway is proposed that may explain the tailoring enzyme reactions leading to cyanobacterin formation, with the newly discovered structures incorporated into the pathway.

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High-Throughput Bioactivity Screening and Iron-Binding Metabolite Mapping against *Phytophthora* Using LC–HRMS-Coupled Microfractionation

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Oomycete pathogens of the genus *Phytophthora* cause severe crop losses worldwide, highlighting the need for new microbial biocontrol agents and bioactive natural products. Plant-associated microorganisms are known to produce diverse secondary metabolites that can contribute to plant health and antagonism against phytopathogens. Iron availability is a key factor shaping plant–microbe interactions, as microbial siderophores can influence both iron acquisition by plants and competition with phytopathogens. In order to identify bioactive natural products from *Serratia marcescens* against *Phytophthora*, we developed an LC–HRMS/MS-coupled microfractionation workflow for high-throughput screening of bacterial metabolites.

Dual-culture assays confirmed the antagonistic activity of *S. marcescens* against *Phytophthora*. Extracellular crude extracts were subsequently evaluated against multiple *Phytophthora* species and further fractionated by solid-phase extraction (SPE). Minimum inhibitory concentrations were determined for both crude extracts and SPE fractions, with the strongest activity localized to the 80% MeOH fraction.

A high-frequency microfractionation protocol using a microspotter enabled parallel testing of narrow chromatographic fractions and direct alignment of biological activity with LC–HRMS features. Orthogonal iron-chelation assays and iron-supplemented LC–HRMS analysis were used to map iron-binding metabolites within the chromatographic profile. Activity-associated features included known *S. marcescens* metabolites, such as prodigiosin and serratiochelins, together with additional unresolved metabolite candidates.

This integrated workflow enables rapid prioritization of bioactive and iron-binding metabolites from complex bacterial extracts and provides a flexible platform for investigating microbial natural products targeting phytopathogenic oomycetes.

Biosensor-based genome mining for the discovery of premature termination codon-readthrough inducing compounds in a large actinomycetal strain collection

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The increasing availability of high-quality genome sequences and bioinformatic tools have leveraged the genome mining field. While it opens a gate for great potential of discovery, it also poses the challenge to prioritize BGCs for further exploration. Methods for choosing a BGC to work with go from bioactivity tests, previous knowledge to random picking. Random selection of BGCs may lead to non-interesting, difficult, or cryptic BGCs, wasting time and resources. Furthermore, classical inhibition assays may lead to rediscovery and false positives. Overcoming these challenges is crucial in large-scale genome mining projects. In this project we decided to target aminoglycosides and follow a biosensor-based strategy to achieve so. We employed a yeast biosensor for premature termination codon (PTC) readthrough^[1], an unusual bioactivity produced by aminoglycoside antibiotics in eukaryotes^[2]. We inspected the NBC collection^[3], an actinomycetal strain collection of more than 1000 strains owned by the Natural Products Genome Mining group at DTU, which contains around 30,000 BGCs. Using BGCFlow^[4], we identified 106 regions predicted to produce aminocyclitols, 2-deoxystreptamine aminoglycosides, or both, gathered in 54 gene cluster families. We tested 95 strains in 4 different media; we found 9 strains giving positive signals for the biosensor. Following metabolomics analyses we found two putative novel molecules from non-aminoglycoside origin. This research expands the repertoire of molecules known to induce PTC readthrough and showcases the advantages of using biosensors for natural products discovery pipelines.

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From metabolic plasticity to biosynthetic potential: exploring natural products from *Acidobacteriota*

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Introduction: *Acidobacteriota* are among the most abundant bacterial phyla in soils, yet their natural product chemistry remains poorly characterised. Notably, most natural products reported from this phylum to date originate from marine environments. To broaden our understanding of the chemical potential of the phylum across different ecological contexts, we investigated newly isolated terrestrial Acidobacteriaceae for their metabolic and biosynthetic potential.

Methods: We combined cultivation-based OSMAC approaches with untargeted metabolomics, bioactivity-guided fractionation, and comparative genome mining to investigate condition-dependent metabolite production and its potential functional relevance.

Results: OSMAC profiling revealed pronounced cultivation-dependent metabolic variation, with tryptophan supplementation triggering extensive metabolic reprogramming and indole-derived metabolite production. Linking metabolomics with antifungal activity enabled the identification of malassezindoles and pityriacitrins associated with activity against *Zymoseptoria tritici*. Genome mining further revealed a diverse but unevenly distributed repertoire of biosynthetic and putative plant-associated traits across *Acidobacteriota*.

Conclusion and outlook: Building on these findings, current work focuses on strain FHG110511 (*Edaphobacter* sp.) as a model for connecting cultivation-dependent chemistry with predicted biosynthetic potential. Its genome encodes an occidiofungin-like biosynthetic gene cluster alongside additional putative plant growth-promoting traits. Ongoing work investigates cultivation dependent metabolite production and expression of the occidiofungin-like cluster, aiming to experimentally connect predicted pathways with their chemical products. This targeted approach provides a next step towards accessing and characterising the still largely unexplored natural product repertoire of *Acidobacteriaceae*.

Metabolomic consequences of second messenger modulation in genetically engineered *Cyanobacteria*

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Cyanobacteria are photoautotrophic microorganisms that are capable to produce a large variety of specialized metabolites. ^[1] Second messenger are small molecules that occur in living organisms and regulate physiological processes in response to environmental changes. In cyanobacteria they can influence photosynthesis, nitrogen fixation, light perception and more. ^[2] We investigated whether genetic modifications of enzymes that are responsible for generating and degrading second messengers had an influence on the specialized metabolome of *cyanobacteria*. Cultures of genetically modified *Trichormus* sp. PCC 7120 were induced with copper and after cultivation extracted and analysed with HPLC-MS/MS. The data were processed with mzmine for feature finding, evaluated with GNPS, and annotated with SIRIUS. The results show that there are indeed changes in the specialized metabolome when enzymes, generating or degrading second messengers are influenced by genetic modifications. Further investigations will help identifying these differentially regulated specialized metabolites.

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Untargeted metabolite profiling of novel *Streptomyces* species from Anatolia, Türkiye

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Actinobacteria are well known for their outstanding ability to produce bioactive natural products. Notably, the genus *Streptomyces* has been extensively investigated for the discovery of new bioactive metabolites. In search of novel streptomycete species with bioactivity in *Anatolia*, strains AK11H and K1A1-R1 were isolated from red-colored forest soil and a lichen sample, respectively. Phylogenomic analysis confirmed that both strains are representatives of two novel species for the genus *Streptomyces*. Genome mining for gene clusters of secondary metabolites on the antiSMASH server revealed that both strains have the potential to produce novel terpenes, siderophores, non-ribosomal peptides, non-alpha poly-amino acids, and ribosomally synthesized and post-translationally modified peptide products. Moreover, strain K1A1-R1 encodes genes for novel type 1 and type 3 polyketides, lanthipeptides (class I, II, and III), and butyrolactones. Both strains were cultivated in ISP 2 and N-Z-Amine broth media, followed by extraction with ethyl acetate. The antimicrobial activity of the extracts against *Bacillus subtilis* and *Escherichia coli* was assessed using the disc diffusion method. Both strains inhibited the growth of *B. subtilis*, while no bioactivity was detected against *E. coli*. The metabolites present in the ethyl acetate extracts of fermentation broths were examined through an untargeted Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) approach coupled with Global Natural Product Social Molecular Networking (GNPS). The GNPS analysis revealed that both strains exhibit variations in metabolite profiles across growth media, and most metabolites did not match any known compounds, suggesting that both strains may produce novel chemical entities with antimicrobial activity.

Pigment formation by the food-spoilage bacterium *Pseudomonas carnis*

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The genus *Pseudomonas* comprises a diverse group of bacteria, some of which are among the most important bacterial contributors to food spoilage, particularly in protein- and fat-rich foods including meat and dairy products^[1]. Among these, the recently described species *Pseudomonas carnis* B4-1^[2] has been associated with intriguing spoilage phenomena, notably black discoloration of chilled meat^[3] and the “blue mozzarella” defect^[4]. Comparative genomic studies revealed a conserved Blue Associated Region (BAR) biosynthetic gene cluster (BGC) in pigment-producing *Pseudomonas* strains. This cluster contains duplicated tryptophan biosynthesis genes together with accessory enzymes implicated in pigment formation^[5,6]. Metabolomic analysis of the culture supernatant from pigment producing *P. carnis* B4-1 revealed a major ion at m/z 290.1131, which clustered in a GNPS feature-based molecular network with related ions at m/z 218.0920, 276.0750, and 246.1233. Isotopic pattern analysis, combined with spectroscopic characterization of the purified metabolite, enabled the assignment of the molecular formula $C_{14}H_{15}N_3O_4$ and suggested its classification as a substituted aminodiazanaphthalene derivative. Comparative exometabolomic analysis further showed that the feature at m/z 218.0920 was present in the wild-type strain but absent in the *P. carnis* $\Delta trpB^{**}$ mutant, whereas the remaining congeners were retained. These findings provide strong evidence that these metabolites are directly associated with the BAR locus and likely represent biosynthetic intermediates or shunt products of the pigment biosynthetic pathway.

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Translating genes into molecules: ML-based prediction of natural products

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Rapidly growing databases of biosynthetic gene clusters (BGCs) highlight the vast untapped potential of natural products, yet experimental characterization remains slow and resource-intensive. Computational approaches that infer molecular structures directly from genomic information could enable downstream analyses such as similarity search and bioactivity prediction; however, few such tools are currently available. Here, we investigate a modular transformer-based framework for translating BGC representations into molecular structures. Instead of training a complete sequence-to-sequence model from scratch, the approach combines separately pretrained genomic and molecular architectures through a trainable bridge, thereby restricting the main learning task to the translation between their latent representations.

Identification and characterization of novel metallophores from *Nostoc Cyanobacteria*

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Metallophores are specialized metabolites secreted by microorganisms to facilitate the acquisition of essential metallic elements under nutrient limitation or to detoxify the environment by chelating excess metals. These metal-binding properties can be exploited for diverse applications, including clinical use as components of antimicrobial drug conjugates [1]. Genome mining studies predict that approximately 41% of cyanobacterial taxa contain at least one non-ribosomal peptide metallophore; however, only a few have been thoroughly described, indicating that their full potential remains undiscovered [2]. Consequently, the genus *Nostoc* has revealed numerous cryptic hybrid polyketide synthase and non-ribosomal peptide synthetase (PKS/NRPS) biosynthetic gene clusters (BGCs) that are predicted to encode innovative metallophore pathways.

Following studies aimed at uncovering the specialized metabolism of *N. punctiforme* PCC73102, we selected the orphan metallophore PKS4 BGC for further investigation. This BGC exhibits a unique combination of tailoring enzymes, featuring a fatty acid acyl-CoA ligase domain, genes encoding β -hydroxyaspartate and an expected modified histidine, as well as an acylase and a glycosyltransferase. Comparative genomic analysis also led us to select the *Nostoc* sp. KVJ3 strain, which contains a similar BGC. The KVJ3 strain showed robust production of a major compound under iron-deprived conditions, which we predict to be the KVJ3 metallophore. In contrast, *N. punctiforme* did not show a major change in its metabolome under these same conditions. While several candidate peaks showed potential to be the PKS4 product, a clear major compound was not observed. This distinct lack of production in *N. punctiforme* suggests that the PKS4 BGC may be silent or activated by alternative environmental triggers, indicating that additional regulatory mechanisms are involved in its expression.

This research integrates a multi-omics platform to discover novel *Nostoc* metallophores. Current efforts focus on culture optimization, isolation, and structural characterization. Future experiments will elucidate the enzymatic steps of this pathway. Understanding these specific biosynthetic mechanisms may enable the awakening of dormant BGCs with analogous enzymatic mechanisms in related or distant microorganisms.

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Integrating untargeted metabolomics and bioactivity correlation to explore Cameroonian bacteria for antimicrobials leads discovery

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The rising tide of antimicrobial resistance (AMR) necessitates the discovery of novel chemical scaffolds to develop the next generation of medicines. While actinomycetes remain the premier source of antibiotics, traditional bioprospecting is often hampered by the frequent rediscovery of known compounds. However, recent advances in metabolomic and genomic technologies offer a promising path to revisit these microbial resources and uncover untapped chemical diversity. We conducted an ecological survey across underexplored Cameroonian diverse environments, including forests, mountains, rivers, landfills, ant and termite nests, zoological parks, swamps, and ponds, yielding over 200 diverse bacterial isolates belonging to over 20 genera. Utilizing an OSMAC approach across eight media, over 700 crude extracts were generated from 90 selected isolates. These crude extracts were subjected to LC-MS/MS untargeted metabolomics and screened against a panel of five WHO-priority multi resistant pathogens (*Staphylococcus aureus* N315, *Enterococcus faecium* V583, *Acinetobacter baumannii* Z13, *Klebsiella pneumoniae* E1267 and *Pseudomonas aeruginosa* K109). Data were processed via MZmine 4.8.0 and dereplicated against GNPS2 and our in-house library of 10,000+ compounds. By correlating bioactivity with the global metabolomics dataset, we successfully distilled an initial pool of 40,000 features down to 195 bioactive leads. Extensive dereplication annotated 39 features effectively isolating 161 features as high-priority candidates for novel antibiotic discovery. Large-scale fermentation and targeted isolation of these leads are currently ongoing. By integrating ecological sampling with a robust prioritization workflow, we aim to expand the curated repository of Cameroonian microbial metabolites and provide new weapons in the fight against AMR.