VAAM Workshop 2021

22.-23.09.2021

Biology of Bacteria Producing Natural Products

jointly organized by

Leibniz-Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig

&

Interfaculty Institute of Microbiology and Infection Medicine, Eberhard Karls University Tübingen, Tübingen

Scientific program

Wednesday, 22.09.2021

08:00-08:30 Technical entry

08:30-08:40 Opening

08:40-08:50 Welcome - Prof. Dr. Jörg Overmann, *Director of the Leibniz-Institut DSMZ - Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, GER*

08:50-09:00 Welcome - Prof. Wolfgang Wohlleben, *Head of Department Mikrobiologie/Biotechnologie; Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Universität Tübingen, GER*

09:00-10:00 **Plenary Talk** - Christophe Corre *(Department of Chemistry, University of Warwick, UK)* Title: Waking-up bacterial genes for natural product discovery

10:00-10:20 Coffee break

Session 1: Biology and Physiology of Natural Compound Producers (Chair: Imen Nouioui)

10:20-10:40 Glycerol alters behaviour and metabolism during *Streptomyces* exploration
Even Shepherdson (McMaster University, Hamilton, CAN)

10:40-11:00 Influence of the Gαq inhibitor FR900359 on the plant-pathogenic nematode *Heterodera schachtii*
Wiebke Hanke (Institute of Pharmaceutical Biology, Bonn, GER)

Session 2: Natural Product Biosynthesis (Chair: Ewa Musiol-Kroll)

11:00-11:20 A directed evolution approach towards improved gramicidin S variants
Philipp Stephan (Leibniz Institute for Natural Product Research and Infection Biology, Jena, GER)

11:20-11:40 The landscape of recombination events that create nonribosomal peptide diversity
Martin Baunach (Universität Potsdam, Potsdam, GER)

11:40-12:00 Genome mining enabled by biosynthetic characterization of benzoxazolinate unravels a widespread class of benzoxazolinate-containing natural products
Jan J. Crames (Max Planck Institute for Terrestrial Microbiology, Marburg; Goethe University Frankfurt, GER)

12:00-13:00 Lunch break

13:00-16:00 **Poster Session**

16:00-16:30 **VAAM Special Section Meeting**

Thursday, 23.09.2021

Session 3: Genetic Engineering and Synthetic Biology (Chair: Juan-Pablo Gomez-Escribano)

09:00-09:20 Engineering of polyketide synthase leads to ring-contracted stambomycins
Li Su (Université de Lorraine, Vandoeuvre-lès-NancyVandoeuvre les Nancy, FRA)

09:20-09:40 Generation of novel pristinamycin derivatives by mutasynthesis approaches
Oliver Hennrich (Leibniz-Institute DSMZ, Braunschweig, GER)

09:40-10:00 Mutasynthesis of physostigmines in *Myxococcus xanthus*
Lea Winand (Technical University of Dortmund, Dortmund, GER)

10:00-10:20 Coffee break

Session 4: Genome Mining/Novel sources for novel natural product discovery (Chair: Martina Adamek)

10:20-10:40 Cochonodin I Is the first Representative of a new lasso peptide subclade frequently associated with human and animal microbiomes
Julian D. Hegemann (Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken, GER)

10:40-11:00 The phylum Planctomycetes as a source of novel natural products with potential biotechnological applications
Nicolai Kallscheuer (Friedrich-Schiller University Jena, GER)

11:00-11:20 What's new in antiSMASH 6 and the antiSMASH database 3
Kai Blin (Technical University of Denmark, Novo Nordisk Foundation Centre for Biosustainability, Kgs. Lyngby, DNK)

11:20-12:00 **Special Workshop on the Nagoya Protocol**
Elizabeth Karger (Leibniz-Institute DSMZ, Braunschweig, GER)
Title: The Nagoya Protocol – what does it mean in practice?

12:00-13:00 Lunch break

Session 5: Regulation and Resistance mechanisms (Chair: Sylvie Lautru)

13:00-13:20 Cystobactamids efficiently kill multi-drug resistant uropathogenic *Escherichia coli* and cystobactamid resistance is mediated through QseBC-regulated LPS modifications
Katarina Cirnski (Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken, GER)

13:20-13:40 **Antimalarial cyclic dipeptides from sponge associated Streptomyces sp. 13-12-16**
Loganathan Karthik (Salem Microbes Private Limited, R and D, Salem, IND)

Session 6: **Bacterial Interactions (Chair: Sergii Krysenko)**

13:40-14:00 The chelating agent EDDS: Natural role and potential applications
Naybel Hernandez Perez (Eberhard Karls Universität Tübingen, Tübingen, GER)

14:00-14:20 Molecular multitasking: inhibition of phage infection by aminoglycoside antibiotics
Aël Hardy (Forschungszentrum Jülich, Jülich, GER)

14:20-14:40 Identification of new prenyl transferases from marine bacteria and elucidation of their substrate specificity
Jamshid Amiri Moghaddam (Hans-Knöll-Institut (HKI), Jena, GER)

14:40-15:00 *Coffee break*

15:00-16:00 **Plenary Talk** - *Marie Elliot (Department of Biology, McMaster University, CAN)*
Title: Exploring (and exploiting) developmental innovations and the metabolic potential of *Streptomyces* bacteria

16:00-16:30 Poster Prizes

16:30-17:00 Farewell

Oral presentations

Waking-up bacterial genes for natural product discovery

Plenary speaker: Christophe Corre

School of Life Sciences, Gibbet Hill Campus, The University of Warwick, Coventry

Abstract

Mining bacterial genomes has revealed a vast number of gene clusters proposed to direct the biosynthesis of novel specialised natural products. However, many of these gene clusters remain silent, or are poorly expressed, in laboratory growth conditions.

Our research group is particularly interested in investigating the molecular mechanisms by which pathway-specific transcriptional regulators control the expression of silent and cryptic biosynthetic gene clusters [1, 2].

By exploiting our structural and functional understanding of specific classes of transcriptional regulators, we have successfully uncovered novel families of natural products and, subsequently, novel biocatalysts [3,4].

This presentation will first introduce some of the fundamental work we have carried out before discussing the specific strategies we have used to access the metabolic products of silent biosynthetic gene clusters.

[1] Corre C *et al.*, Proc. Natl Acad. Sci. USA 2008 ; 105: 17510-17515.
[2] Zhou S *et al.*, Nature 2021 ; 590, 463-467.
[3] Sidda J *et al.*, Chem. Sci. 2014 ; 5: 86-89.
[4] Alberti F *et al.*, Chem. Sci. 2019 ; 10: 453-463.

Exploring (and exploiting) developmental innovations and the metabolic potential of *Streptomyces* bacteria

Plenary speaker: Marie Elliot

Department of Biology, McMaster University, Hamilton, Ontario, Canada

Abstract

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Plenary Talks and Spotlight Topics

The Nagoya Protocol – what does it mean in practice?

Speaker: Elizabeth Karger

German Nagoya Protocol HuB, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures

Abstract

The Nagoya Protocol, which has been in force since 12 October 2014, provides a comprehensive international framework on access to genetic resources, benefit-sharing arising from the use of these resources for research and development, and compliance. There are 130 States have become a Party to the Protocol, many of which have laws regulating access to their genetic resources, and in the European Union (EU), Regulation (EU) No 511/2014 (EU Regulation) is in place to make sure that researchers, including academic and non-commercial researchers, are complying with access and benefit-sharing (ABS) obligations.

All researchers working in the life sciences, who are doing research on non-human biological material like and microorganisms and so-called derivatives, should understand the implications of the Nagoya Protocol, ABS and the EU Regulation for their work.

Compliance matters. The competent authorities across the EU are checking the research community to ensure compliance with the EU Regulation and although measures vary in different countries, researchers could face, for example, orders to stop research, confiscation of their research material and fines in cases of non-compliance. There are other good reasons for being serious about ABS. Non-compliance can lead to negative press and reputational damage, it can make future work difficult in the provider country, and the policies of some scientific journals make compliance with ABS laws necessary before publication is possible.

Although a number of researchers are already well-informed about their obligations and are doing their best to fulfil them, there are still a number of people who have only “heard of” the Nagoya Protocol but do not yet fully appreciate what this means for them in practice. In this presentation, Elizabeth Karger talks about the practicalities of “doing ABS” and complying with the EU Regulation, focusing on the use of microorganisms and biochemical compounds as well as relevant use cases.

About the speaker: Elizabeth Karger is an Australian lawyer and the manager of the German Nagoya Protocol HuB project, or GNP-HuB for short, which is a project funded through the German Federal Agency for Nature Conservation (Bundesamt für Naturschutz, BfN) with funds from the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety that aims to support academic researchers with understanding their Nagoya Protocol obligations. Elizabeth is based at the **Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ) in Braunschweig, Germany.** DSMZ is actively involved in issues relating to the Nagoya Protocol and ABS, participating in various projects and studies as well as bringing the perspective of the science community into relevant policy discussions. As registered collection of genetic resources recognized under the EU Regulation No 511/2014, DSMZ ensures that all of the biological material it holds is compliant with the Nagoya Protocol. Prior to joining DSMZ, Elizabeth worked on ABS and Nagoya Protocol related issues for the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH and the competent authority in Germany, the Federal Agency for Nature Conservation. In 2018 she conducted a study on the use of digital sequence information on genetic resources (DSI) in Germany and in 2019, she co-authored a study for the Secretariat of the CBD on national measures addressing the use of and benefit-sharing arising from the use of DSI.

Biology and Physiology of Natural Compound Producers

Glycerol alters behaviour and metabolism during *Streptomyces* exploration

Authors

Shepherdson	**Evan**	**McMaster University, Hamilton**
Elliot	Marie	McMaster University, Hamilton

Abstract

Exploration represents a new growth strategy in the developmental repertoire of the filamentous *Streptomyces* bacteria. Here, we show that entry into exploration leads to a global reprogramming of gene expression, with notable changes in primary metabolism and the oxidative stress response. In probing different primary metabolic pathways, we discovered that glycerol dramatically enhanced the exploration response in *S. venezuelae,* increasing colony growth rates and architecture. Exploration under these conditions proceeded via a very different transcriptional program than conventional exploration, and led to striking metabolic differences, including the activation of otherwise silent antibiotic and siderophore gene clusters. We identified a critical role for iron when initiating exploration in a competitive environment with yeast. Our results suggest that there may be multiple pathways through which exploration can proceed, and further reveal the flexibility with which *S. venezuelae* can leverage its metabolic repertoire to maximize exploration potential and competitive fitness.

Biology and Physiology of Natural Compound Producers

Influence of the $G\alpha_q$ inhibitor FR900359 on the plant-pathogenic nematode *Heterodera schachtii*

Authors

Hanke	**Wiebke**	**Institute of Pharmaceutical Biology, Bonn**
Gutbrod	Philipp	BioZentrum, Molekulare Biotechnologie/Biochemie, Bonn
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Crüsemann	Max	Institute of Pharmaceutical Biology, Bonn
König	Gabriele	Institute of Pharmaceutical Biology, Bonn

Abstract

Natural products are specialized metabolites, that show unique and specific biological effects under laboratory conditions. Their ecological role, however, is in many cases not further investigated.

FR900359 (FR) is a complex non-ribosomal peptide and a potent, as well as a specific inhibitor of $G\alpha_q$ proteins, which are important transducers of manyfold eucaryotic signalling pathways.[1] FR was first isolated from the leaves of the plant *Ardisia crenata*, and later found to be produced by the unculturable endosymbiont *Candidatus* Burkholderia crenata living in nodules located at the leaf margin. We verified that FR has an insecticidal effect, and may thus protect the plant from insect feeding.[2] We recently discovered that the cultivable soil bacterium, *Chromobacterium vaccinii*, isolated from the soil associated with the Cranberry plant harbours the FR gene cluster and produces FR under laboratory conditions.[3]

In this work the ecological relevant bioactivity of FR produced by *C. vaccinii* is further investigated. The effect of FR on the plant-pathogenic cyst nematode *Heterodera schachtii* was explored in cooperation with the IMBIO (Dr. Philipp Gutbrod). The results demonstrate that FR inhibits hatching of the juveniles from their cyst. Ongoing experiments focusing the model organism *Caenorhabditis elegans* were performed in cooperation with the Scholz Lab (Dr. Jun Liu and Dr. Monika Scholz) to provide additional insights into the effects of FR on nematodes. As soil bacteria play an important role for the health of associated plants, comprehension of the plant-protecting mechanisms might provide new opportunities for the development of ecological agriculture.

[1] Schrage, R. *et al*. (2015). *Nature Communications.* 6 (10156). p. 1-17.
[2] Crüsemann, M. *et al*. (2018). *Angewandte Chemie International Edition*. 57. p. 836–840.
[3] Hermes, C. *et al.* (2021). *Nature Communications*. 12 (1).

Natural Product Biosynthesis

A directed evolution approach towards improved gramicidin S variants

Authors

Stephan	**Philipp**	**Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), Jena**
Winkler	Daniela	Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), Jena
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Abstract

The increasing prevalence of multi-resistant bacteria and fungi makes their treatment with common antibiotics increasingly difficult and may soon become one of the biggest healthcare issues. To combat resistance one can either search for new structures with new modes of action or modify existing molecules to bypass resistances or increase therapeutic properties. The second option often includes laborious organic synthesis steps making it expensive for industrial production. For instance, gramicidin S (GrS) is a non-ribosomal peptide antibiotic with activity against Gram(+) bacteria and several fungi. Its clinical application is limited due to a small therapeutic window caused by a high haemolytic activity. GrS is a cyclic decapeptide with the structure $(fPVOL)_2$ produced by the non-ribosomal peptide synthetases GrsA and GrsB. Analysis of variants produced by organic synthesis showed that replacement of Pro against cationic amino acids results in reduced haemolysis while maintaining the antibiotic activity.

Here, we describe an approach to improve the therapeutic window of GrS through a combination of protein engineering, biosynthesis, and chemical modification. We have subjected module GrsB1, which is responsible for the incorporation of Pro in the peptide chain, to repeated cycles of directed evolution to alter its specificity from Pro to the cyclic amino acid piperazic acid (Piz). Mutations were chosen based on sequence alignments with Piz activating NRPS modules or based on our experience with positions that influence substrate specificity in other NRPSs. The exchange is tolerated by all down-stream steps in the biosynthesis resulting in ^{Piz}GrS. A hydrazine in the side chain of Piz can be reductively cleaved to yield ornithine (Orn). After five cycles of directed evolution the initial specificity of GrsB1 for Piz of 0.5% when compared to native Pro could be changed so that Piz is the only substrate. The reductive cleavage of Piz to Orn could be achieved in Piz containing sample peptides with low-valent titanium (Ti^0) as reactive species. Our results show that directed evolution of nonribosomal substrate specificity can lead to improved peptide antibiotics.

Natural Product Biosynthesis

The landscape of recombination events that create nonribosomal peptide diversity

Authors

Baunach	**Martin**	**Universität Potsdam, Potsdam**
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Stallforth	Pierre	Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Jena
Dittmann	Elke	Universität Potsdam, Potsdam

Abstract

Nonribosomal peptides (NRP) are crucial molecular mediators in microbial ecology and provide indispensable drugs. Nevertheless, the evolution of the flexible biosynthetic machineries that correlates with the stunning structural diversity of NRPs is poorly understood. Our results show, that recombination is a key driver in the evolution of bacterial NRP synthetase (NRPS) genes across distant bacterial phyla that has guided structural diversification in a plethora of NRP families by extensive mixing and matching of biosynthesis genes [1]. The systematic dissection of a large number of individual recombination events did not only unveil a striking plurality in the nature and origin of the exchange units but allowed deducing overarching principles that enable efficient exchange of adenylation (A) domain substrates whilst keeping the functionality of the dynamic multienzyme complexes. In the majority of cases, recombination events have targeted variable portions of the A_{core} domains, yet domain interfaces and the flexible A_{sub} domain remained untapped. Our results strongly contradict the widespread assumption that adenylation and condensation (C) domains coevolve and significantly challenge the attributed role of C domains as stringent selectivity filter during NRP synthesis. Moreover, they teach valuable lessons on the choice of natural exchange units in the evolution of NRPS diversity, which may guide future engineering approaches.

[1] Baunach M., Chowdhury S., Stallforth P., Dittmann E., *Mol Biol Evol.* 2021; 38(5):2116-2130.

Natural Product Biosynthesis

Genome mining enabled by biosynthetic characterization of benzoxazolinate unravels a widespread class of benzoxazolinate-containing natural products

Authors

Crames	**Jan Johannes**	**Max Planck Institute for Terrestrial Microbiology, Goethe University Frankfurt, Molecular Biotechnology, Department of Biosciences, Frankfurt am Main**
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Abstract

Benzoxazolinate is a rare bis-heterocyclic moiety in natural products, being able to interact with protein and DNA and thereby conferring extraordinary bioactivity on natural products, exemplified by the most cytotoxic compound C-1027. Benzoxazolinate is derived from chorismic acid that is processed by amination, dehydrogenation, and cyclization. Here, we first mine putative benzoxazolinate biosynthetic gene clusters (BGCs) in the genomes of entomopathogenic bacteria, *Xenorhabdus* and *Photorhabdus*. In *Xenorhabdus szentirmaii*, we found that the benzoxazolinate pathway borrows 2-amino-2-deoxyisochorismic acid (ADIC) synthesized by the phenazine pathway and that a putative acyl AMP-ligase mediates the cyclization of 3-O-enolpyruvoylanthranilic acid (OPA) to afford benzoxazolinate. Using the putative acyl AMP-ligase as a probe for mining diverse BGCs that encode benzoxazolinate-containing natural product biosynthesis, we discover that the orphan type I-type II hybrid non-ribosomal peptide synthetase (NRPS) BGC in *Xenorhabdus* and *Photorhabdus* encoding the biosynthesis of benzobactin A is prevalent across *Proteobacteria* (particularly abundant in *Pseudomonas*) and *Firmicutes*. It turns out that *Pseudomonas chlororaphis* subsp. *piscium* DSM 21509 produces a plethora of benzobactins, thereby offering an opportunity to investigate their biosynthesis. A combination of sequence-similarity-based bioinformatic analysis, gene deletions, and in vitro enzymatic reconstruction provides biosynthetic insights into benzobactins. The benzobactin biosynthesis in *P. chlororaphis* subsp. *piscium* DSM 21509 are highlighted by a synergistic positive effect of the anthranilate synthase component I from the benzobactin pathway itself and the ADIC synthase from the phenazine pathway, which boosts the production level of benzobactins; a serine hydroxymethyltransferase catalyzes mono- and bis-hydroxymethylation of d-/l-serine and glycine, respectively, into 2-hydroxymethylserine; and functional potential of the free-standing NRPS gene architecture for generating structural diversity.

Genetic Engineering and Synthetic Biology

Engineering of polyketide synthase leads to ring-contracted stambomycins

Authors

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Abstract

Bacterial polyketide secondary metabolites exhibit a wide range of useful biological activities, including antibiotic, antifungal, anti-tumor and immunosuppressive properties. However, it is often necessary to modify the native polyketide structures to make them suitable for therapeutic application. In this work, we aimed to establish a strategy towards accessing structurally-simplified polyketide analogues [1] via internal truncation of the polyketide synthase (PKS) machinery responsible for biosynthesis of the core structure.

For this, we selected the stambomycin PKS of *Streptomyces ambofaciens* ATCC23877 [2, 3], which encompasses 9 distinct polypeptide subunits, and gives rise to a small family of 51-membered macrolides (stambomycins A−F), which are among the largest of all known polyketides. Using an approach combining engineering of docking domains and a set of key interdomain interactions contributing to polyketide chain translocation and elongation, we successfully produced a series of 37-membered mini-stambomycins [4]. However, the low yields of the target compounds demonstrate that even strategies based upon strong literature precedent remain to be optimized.

1. Guo, Z. (2017) The modification of natural products for medical use. *Acta Pharm. Sinica B* **7**, 119−136.
2. Laureti L. *et al.* (2011) Identification of a bioactive 51-membered macrolide complex by activation of a silent polyketide synthase in *Streptomyces ambofaciens. Proc. Natl. Acad. Sci. USA* **108**, 6258−6263.
3. Aigle B. *et al.*, Stambomycins and derivatives, their production and their use as drugs. Patent WO/2011/009938.
4. Su L. *et al*., (2021) Successes, surprises and pitfalls in modular polyketide synthase engineering: generation of ring-contracted stambomycins, DOI:10.21203/rs.3.rs-222743/v1, *Nat. Commun.,* ***in revision***

Genetic Engineering and Synthetic Biology

Generation of novel pristinamycin derivatives by mutasynthesis approaches

Authors

Hennrich	**Oliver**	**Leibniz-Institute DSMZ, Braunschweig, Germany**
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Abstract

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

[1] Mast et. al (2014)
[2] Al Toma et. al (2015)
[3] Mast et. al (2011)

Genetic Engineering and Synthetic Biology

Mutasynthesis of physostigmines in *Myxococcus xanthus*

Authors

Winand	**Lea**	**TU Dortmund University, Department of Biochemical- and Chemical Engineering, Dortmund**
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Pietruszka	Jörg	Heinrich-Heine-University Düsseldorf at Forschungszentrum Jülich, Institute of Bioorganic Chemistry, Jülich
Nett	Markus	TU Dortmund University, Department of Biochemical- and Chemical Engineering, Dortmund

Abstract

The alkaloid physostigmine is an approved cholinesterase inhibitor for the therapy of anticholinergic poisoning and glaucoma.[1] In recent years, the interest in physostigmine has gained momentum due to its beneficial effects in the treatment of the Alzheimer's disease.[2] We set out to reconstitute the physostigmine pathway in the myxobacterium *Myxococcus xanthus* and to generate derivatives of this drug in a mutasynthetic approach. For this purpose, we used a myxobacterial expression plasmid, which had been endowed with the necessary biosynthesis genes. By means of pathway refactoring and the selection of an appropriate expression strain, the physostigmine titer could be significantly improved in *M. xanthus* from <1 to 72 mg/L. Subsequently, we used this system to produce analogues of the naturally occurring compound via mutasynthesis. Synthetically prepared precursors were successfully introduced into the physostigmine pathway, leading to the formation of various analogues. The produced physostigmine derivatives were tested for their inhibitory efficiency on acetyl- and butyrylcholinesterase. One analogue exhibits improved bioactivity and reduced toxicity compared to the parental natural product. In addition, we present the first biocatalytic concept for the synthesis of the drug candidate phenserine.

[1] Andrade, O. A.; Zafar Gondal, A., Physostigmine, *In*: StatPearls, **2020**. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK545261

[2] Greig, N. H.; Pei, X. F.; Soncrant, T. T.; Ingram, D. K.; Brossi, A., *Res. Rev.* **1995**, *15*, 3-31.

Genome Mining/Novel Sources for Natural Product Discovery

Cochonodin I is the first representative of a new lasso peptide subclade frequently sssociated with human and animal microbiomes

Authors

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Abstract

Lasso peptides are fascinating natural products belonging to the superfamily of ribosomally synthesized and post-translationally modified peptides (RiPPs). As such, their biosynthesis starts with the ribosomal assembly of a precursor that is then processed by biosynthetic enzymes. Members of the lasso peptide RiPP subfamily are defined by a characteristic fold reminiscent of a lariat knot, where an N-terminal macrolactam ring is threaded by a linear C-terminal tail.

Here, it will be described how the lasso peptide cochonodin I was isolated and studied after its heterologous production in *E. coli*. The corresponding biosynthetic gene cluster originated from *Streptococcus suis* LSS65 and belongs to a unique lasso peptide subclade defined by the fusion of a leader peptidase (B2) with an ABC transporter (D) domain. Bioinformatic analysis furthermore revealed that these clusters also encode homologs of the NisR/NisK regulatory system and the NisF/NisE/NisG immunity factors, which are usually associated with the clusters of antimicrobial class I lanthipeptides like nisin; another, distinct RiPP subfamily. It will be demonstrated how the threaded structure of cochonodin I was evidenced through extensive MS analysis and if the ABC transporter domain from the SsuB2/D fusion protein is essential for lasso peptide maturation. Through extensive genome mining dedicated exclusively to other gene clusters featuring B2/D fusion proteins, it was further revealed that especially many bacteria associated with human or animal microbiomes hold the biosynthetic potential to produce cochonodin-like lasso peptides, implicating that these natural products might play roles in human and animal health, which makes their study particularly relevant.

In summary, the discovery of the novel lasso peptide cochonodin I will be presented, which represents the first studied member of a unique lasso peptide subclade. Of particular interest is not only its implied function in mammalian microbiomes, but also that these lasso peptide systems apparently acquired specific immunity and regulatory elements that are normally associated with the lanthipeptide RiPP subfamily.

Genome Mining/Novel Sources for Natural Product Discovery

The phylum *Planctomycetes* as a source of novel natural products with potential biotechnological applications

Authors

Kallscheuer Nicolai Friedrich-Schiller University Jena, Department for Microbial Interactions, Jena

Abstract

Members of the phylum *Planctomycetes* occur ubiquitously, but can be the dominant phylum on the surface of macroscopic aquatic phototrophs, e.g. algae or seagrasses. An observed abundance of planctomycetes in the range of 60-85% of the epiphytic bacterial community on such surfaces is quite astonishing when taking into account that planctomycetes grow considerably slower than other heterotrophic bacteria occupying the same ecological niches. The dominance is suggested to result from complex phototroph-planctomycete allelopathic interactions that have not yet been studied on the molecular level. The interaction probably involves the production of bioactive compounds by planctomycetes. In my talk I will present the outcome of a recently completed sampling and strain isolation campaign targeting the phylum *Planctomycetes*. The current collection of more than 120 characterized members provides genome- and lifestyle-based evidence for the role of planctomycetes as yet for the most part untapped source of bioactive compounds with potential health-promoting activities. The current transition phase from the pure description of novel isolates to natural product discovery and potential biotechnological applications is reinforced by the discovery of the first two natural products in planctomycete cultures in the last two years.

Genome Mining/Novel Sources for Natural Product Discovery

What's new in antiSMASH 6 and the antiSMASH database 3

Authors

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Abstract

Since 2011, antiSMASH has assisted researchers in their microbial genome mining tasks. Earlier this year we released the updated version 6 of antiSMASH. This version updates and adds detection rules for different biosynthetic classes, bringing the total number up to 71. For multimodular NRPS and PKS clusters, antiSMASH now detects and displays the modules explicitly in addition to just showing individual enzyme domains. To improve the accuracy of our comparisons of identified regions with similar regions in other genomes, we added a new ClusterCompare algorithm that, in addition to the sequence-based similarity and gene synteny scoring already provided by ClusterBlast, considers the absence/presence of biosynthetic components and module counts for multimodular enzymes, giving a more accurate score. antiSMASH also utilises the RiPP recognition element (RRE) detection from RRE-Finder, making it easier to confidently identify tailoring enzymes associated with RiPP clusters. A new *RRE-containing* detection rule makes use of RRE-Finder"s detection profiles to potentially pick up novel RRE-containing cluster types that antiSMASH does not have specific detection rules set up for. To make it easier to integrate antiSMASH's manually curated cluster predictions with cluster predictions from machine learning based tools like ClusterFinder, DeepBGC, or GECCO, a new *sideloading* system specifies a JSON-based file format these tools can use to have their table-based results shown within the antiSMASH HTML output.

Last year we also released version 3 for the antiSMASH database, for the first time providing archaeal and fungal genomes along with the bacterial genomes. The database now contains 147,517 high quality BGC regions from 388 archaeal, 25,236 bacterial, and 177 fungal genomes. This dataset is also used for the general ClusterBlast in antiSMASH 6. The database allows for easy searches on all those results. Version 3 adds a new *module query* search feature allowing users to search for NRPS/PKS clusters that contain modules with a given domain layout.

In this presentation, we will give an overview of the new features along with practical tips on how to make use of them.

Regulation and Resistance mechanisms

Cystobactamids efficiently kill multi-drug resistant uropathogenic *Escherichia coli* and cystobactamid resistance is mediated through QseBC-regulated LPS modifications

Authors

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Abstract

Due to steadily increasing antimicrobial resistance and the shortage of novel antimicrobials reaching the market, the need to evaluate and develop new antibacterial agents is higher than ever. Extensive microbiological and mechanistic evaluation of the novel natural compound class cystobactamids revealed that they act as potent inhibitors of bacterial gyrase and topoisomerase IV with IC_{50} values for particular cystobactamids in the sub-µM and µM range, respectively. Cystobactamids efficiently and with high potency kill multi-drug resistant *Escherichia coli*, an important nosocomial pathogen. *In vitro* studies revealed a bactericidal mechanism with a rapid onset of action. Based on their mode of action, cystobactamid-resistant *E. coli* mutants developed at a low frequency of resistance, 10^{-8}-< 10^{-10}. Intriguingly, no mutations were found in *gyrA* or *parC* target genes and instead, all mutations were observed in QseBC, a virulence-regulating, quorum-sensing *E. coli* two-component system. Resistance to cystobactamids did not induce a fitness cost in the *E. coli* mutants, however, isothermal microcalorimetry revealed a significant change of heat release during metabolic processes. RT-qPCR showed that exposing wildtype *E. coli* to cystobactamid did not result in a significant change in *qseBC* and *pmrAB* transcript levels. However, transcriptome analyses of resistant mutants revealed that the two-component systems QseBC and PmrAB are strongly interconnected and together they initiate a cascade of events leading to lipopolysaccharide (LPS) modifications. In case of cystobactamids, we observed two major lipid A modifications connected to the *arn* operon: addition of either one or two 4-amino-4-deoxy-L-arabinose units.
The QseBC system of *E. coli* has been mostly studied in the context of virulence regulation, and cystobactamids are the first antibacterial compound class for which resistance mutations were found in *qseBC*. Importantly, although the resistance phenotype comprises modifications of LPS, cystobactamids were found to lack cross-resistance with other antibiotic classes such as the polymyxins.

Regulation and Resistance mechanisms

Antimalarial cyclic dipeptides from sponge associated *Streptomyces* sp 13-12-16

Authors

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Abstract

Marine actinobacteria genome mining has revealed that it is rich source of natural products which play a major role in the drug industries but still most of the genes are silent under laboratory conditions. Several methods were developed for inducing the cryptic gene from marine actinobacteria but still it had several limitations. Therefore, newer and innovative approaches are required to maximize the diversity of natural products from marine actinobacteria. To date, nanotechnology has grown rapidly and has been applied in diverse fields. Here, we show the activation of the cryptic gene from *Streptomyces* sp. 13-12-16 by silver nanoparticles (AgNPs). Using the RT-PCR and UPLC-QTOF-MS technologies, we demonstrated that the interaction between *Streptomyces* sp. 13-12-16 and AgNPs leaded to the specific activation of macrolactum polyketide compound (ML-449). In addition, the *Streptomyces* sp. 13-12-16 and the AgNPs interactions have shown in using fluorescence staining method (aggregation of the cells) and the reactive oxygen species (ROS) assay (generation of the intracellular ROS). In the present work, the induction of the cryptic natural product biosynthetic gene clusters in the absence of the molecular alternations in a normal laboratory conditions has been confirmed.

Bacterial Interactions

The chelating agent EDDS: Natural role and potential applications

Authors

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Abstract

Actinomycetes are filamentous soil bacteria that produce not only antibiotics but also a variety of secondary metabolites, which are used in different fields, such as agriculture, medicine, and industrial biotechnology. For optimal growth, microbes need to acquire essential metal ions from the environment, in particular iron and zinc. However, in many habitats, the availability of zinc and iron is limited due to low solubility. In order to respond to this nutrient deficiency, microbes synthesize chelating compounds to sequester metal ions from the environment. These chelators have high affinities for metal ions, making them accessible to microorganisms. They can be taken up either by the producers themselves or by other organisms in the environment. In addition to their function in the provision of metal ions, ionophores are also known to mediate signaling among strains in the microbial community, playing an important role in the ecology of microorganisms. An example of a bacterial ionophore is the zincophore ethylene diamine-disuccinate (EDDS), which is produced by *Amycolatopsis japonicum,* particularly under zinc-limited growth conditions. EDDS is a biodegradable EDTA isomer. Its biodegradability and excellent chelating properties make EDDS attractive for many industrial applications.

In this project, we analyze the ecological role of EDDS in the soil community. For this purpose, actinomycetes were isolated from soil samples, and pairwise interactions with the EDDS-producer were made. The influence of EDDS on e.g. growth or production of secondary metabolites in the isolated strains is investigated by HPLC, HPLC/MS, metabolomics, and transcriptome analyses. We also study the biosynthetic pathway of EDDS in *A. japonicum* using metabolic engineering approaches and feeding experiments. Altogether, this work will deliver novel information about the interaction of EDDS with the microbial community from the soil and insights on EDDS biosynthesis. This knowledge will allow to increase the EDDS production for further applications and replace the environmentally harmful EDTA with EDDS.

Bacterial Interactions

Molecular multitasking: inhibition of phage infection by aminoglycoside antibiotics

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Abstract

Bacterial populations face the constant threat of viral predation exerted by bacteriophages (or phages). In response, bacteria have evolved a wide range of defense mechanisms against phage challenges. The currently known antiphage defense repertoire relies on a wide range of inhibitory mechanisms but is mainly mediated by protein effectors and acts primarily at the cellular level. Here, we show that aminoglycosides, a well-known class of antibiotics produced by *Streptomyces*, are potent inhibitors of phage infection. We observed a broad phage inhibition by aminoglycosides, as diverse phages infecting the Gram-positive bacteria *Corynebacterium glutamicum* and *Streptomyces venezuelae* as well as the model Gram-negative bacterium *E. coli* were impacted. We demonstrate that aminoglycosides do not prevent the injection of phage DNA into bacterial cells but instead block an early step of the viral life cycle, prior to genome replication. Importantly, inhibition of a *Streptomyces* phage was also achieved using the supernatants from a natural aminoglycoside producer, hinting at a broad physiological significance of the antiviral properties of aminoglycosides. Altogether, this study expands the potential functions of aminoglycosides in bacterial communities. It further suggests that aminoglycosides are not only used by their producers as toxic molecules for their bacterial competitors but could also serve as a protection against the threat of phage predation at the community level.

Bacterial Interactions

Identification of new prenyl transferases from marine bacteria and elucidation of their substrate specificity

Authors

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Abstract

Marine bacteria habour a diverse set of secondary metabolite-related biosynthetic gene clusters. Our recent comparative analysis of the collected genomes using the antiSMASH pipeline revealed the presence of more than 7480 BGCs and similarity network analysis revealed that BGCs related to terpene (24%) and RiPPs (21%) biosynthesis were amongst the most common BGCs within marine bacteria followed by PKS, NRPS and betalactone-related pathways, as well as the biosynthesis of the global osmolyte ectoine [1]. However, most of the encoded structures are yet unknown and their functions not elucidated.

Based on our genome-mining driven studies, we identified a novel class of prenyl transferases conserved within certain bacterial lineages known to be involved in several marine symbioses. We then characterized the conserved prenyl transferases using molecular biological tools, evaluated the substrate scope and promiscuity of the enzyme, and analyzed whether or not the wild type produced the identified products. Results of our studies will shed light on the biosynthesis of an abundant group of marine natural products that might serve as chemical signal within the marine symbioses.

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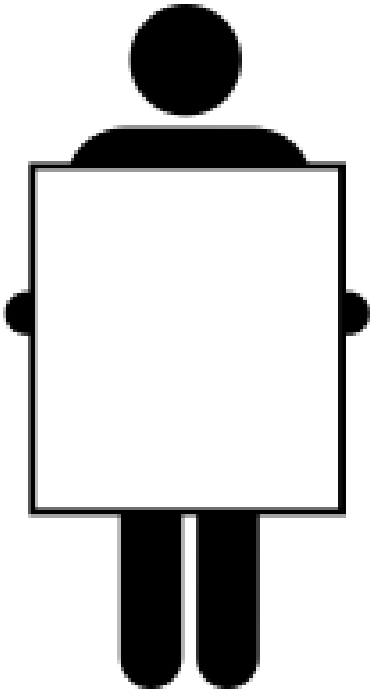
Poster presentations



Biology and Physiology of Natural Compound Producers

The evolution of glycopeptide antibiotics

Authors

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Abstract

Glycopeptide antibiotics (GPAs), like vancomycin or teicoplanin, are important last resort antibiotics that are used for treatment of critical MRSA infections. They are produced by Actinobacteria. The peptide backbone of the glycopeptides is assembled by NRPS (non-ribosomal peptide synthetase) genes. The GPAs (type I-IV) differ in their amino acid building blocks, crosslinking, and acylation patterns. The peptide backbone is further modified by varying tailoring enzymes, which leads to a high structural variability.

Our aim was to shed light on the evolutionary processes that led to such a diversification. Therefore, we studied the phylogenetic history of the main glycopeptide biosynthesis genes. We were able to show that GPA biosynthetic gene clusters were undergoing extensive horizontal gene transfer (HGT). This concerns the entire biosynthesis gene cluster as such, but also various tailoring enzymes and functional domains. The GPA scaffold evolved from a more complex to a simpler structure, meaning the GPA ancestor had seven aromatic amino acids with four crosslinks encoded on four NRPS genes, while the type I GPAs as seen today have five aromatic and two aliphatic amino acids with only three crosslinks on three NRPS genes. This happened through recombination of functional domains and complete modules around the same evolutionary timescale as the loss of the crosslinking monooxygenase. Furthermore, the GPA ancestor molecule was glycosylated and halogenated. Reconstruction of the evolutionary history of the tailoring enzymes showed that they evolved through duplication and were acquired via HGT mostly from other Actinobacteria. Gene gain or loss happened much more often than alterations of the NRPS backbone. Finally, we were able to reconstruct the sequence of the ancestral glycopeptide NRPS genes, which then were synthesized and cloned into a heterologous host (ongoing work).

The knowledge how ancient genes looked and which changes in the genetic composition of the NRPS genes led to successful structure alterations can be used to guide genetic engineering and synthetic biology approaches for NRPS genes and polypeptide structures.

Biology and Physiology of Natural Compound Producers

Glutamine synthetase-like enzymes in *Streptomyces coelicolor* as novel drug targets

Authors

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Abstract

The actinobacterial model organism *Streptomyces coelicolor* is able to use nitrogen from a variety of sources including unusual compounds originating from the decomposition of dead plant and animal material, such as polyamines or monoamines (like ethanolamine). Assimilation of nitrogen from these sources in *S. coelicolor* remains largely unstudied. Our investigations showed that *Streptomyces coelicolor* M145 is able to metabolize polyamines and ethanolamine as an *N*-source. We analyzed the function of three proteins annotated as glutamine synthetase like-enzymes (GS-like): GlnA2, GlnA3 and GlnA4 and demonstrated that GlnA2 (SCO2241) and GlnA3 (SCO6962) are involved in the catabolism of polyamines and that GlnA4 (SCO1613) is involved in the catabolism of ethanolamine (1). Transcriptional, phenotypical, structural and biochemical analysis of these enzymes demonstrated a dual function of GlnA2, GlnA3 and GlnA4 in ensuring both nutrients availability (*C*- and *N*-source) and resistance against high poly- or ethanolamine concentrations, which are toxic for bacteria (1). *In silico* analysis revealed the presence of these enzymes also in human pathogenic actinobacteria, such as *Mycobacterium tuberculosis*. Structural and biochemical studies demonstrated the glutamylation of the polyamine spermine by the mycobacterial GlnA3 and its involvement in the polyamine catabolism in *M. tuberculosis* while proliferating in macrophages during the infection. Thus, GlnA3*Mt* ensures survival of Mycobacteria in their host. Inhibition of GlnA3*Mt* might be an effective therapeutic strategy that is safe, since these kinds of enzymes do not occur in eukaryotes. Therefore these enzymes represent a promising drug target for novel antibiotic drugs.

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Biology and Physiology of Natural Compound Producers

Exploring the antimicrobial, antibiofilm and antiviral potential of a Saharan saline soil-derived actinobacteria, *Nocardiopsis dassonvillei* GSBS4 strain

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Abstract

The emergence and rapid spread of multidrug resistant bacteria such as the ESKAPE pathogens, represents a major problem for the bacterial infections treatment. Screening of novel actinobacteria from the extremobiosphere is one of the main strategies to obtain novel bioactive molecules. During our screening program, a total of 273 actinobacteria strains were isolated from a Saharan soil sample (El-Oued locality, South Algeria) and subjected to antagonistic activity test against human pathogenic germs. The strain GSBS4 was selected for its potent antimicrobial activity and 16S rRNA gene sequence analysis and polyphasic taxonomy approach identified the strain as *Nocardiopsis dassonvillei* with high sequence similarities to *Nocardiopsis dassonvillei* T X97886.1 (99%). The ethyl acetate crude extract of the GSBS4 strain presents a strong inhibitory activity against *E. coli*, *P. aeruginosa*, *S. aureus*, MRSA, *V. cholerae*, *L. inocua* and *C. albicans* pathogenic microorganisms. It exhibited strong inhibitory effect against the biofilm formation by *S. aureus* and MRSA strains (Biofilm inhibitory concentration) displaying a susceptibility with MICs estimated at 1.44. 102 mg/L and 11.5. 102 mg/L, respectively. A 44% biofilm reduction was obtained for *S. aureus* and 61% for *P. aeruginosa*. The antiviral effect of the crude extract was also evaluated on human coronavirus HCoV 229E and on model enterovirus (poliovirus 1, PV1), a dose-dependent increase in cell viability of HCoV 229E-infected cells was observed as the viability went from 21% to 37%, in presence of 25 µg/ mL and 60 µg/ mL of the extract, respectively. The chemical profile of the active fraction was analyzed by the online HPLC-ESIMS technique.

Biology and Physiology of Natural Compound Producers

Effect of carbon source and cultivation conditions on bacterial cellulose produced by *Komagataeibacter xylinus* K2G30-UMCC 2756

Authors

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Abstract

Question

Bacterial cellulose (BC) is an attractive industrial biopolymer due to its chemical and mechanical properties. Among bacterial cellulose producers, *Komagataeibacter* genus is considered the most relevant due to its high BC yield. Production of BC varies by the strains used, the carbon source supplied, the cultivation system and conditions.*Komagataeibacter* strains are mainly isolated from food matrices, as fermented beverages, and fruits. Considering the isolation matrices, probably they possess the ability in metabolic remodulation based on the carbon source availability [1].In this study, the strain *K. xylinus* K2G30 (UMCC 2756) previously tested in glucose, mannitol and glycerol based media, was used [2]. Although, K2G30 can use multiples carbon sources, the pattern of utilization and time of adaptation to a new sugary environment is still unclear.

Methods

Here, this strain was tested for the ability to produce BC production in mannitol medium according to time of adaptation. Cultivation was performed in mannitol medium and refreshed every 4 days (each cycle) for 20 days. BC yield was quantified comparing to glucose medium as control.

Results

BC yield was observed lower (0.65 g/L) in mannitol medium compared to glucose control (1.63 g/L) in the first cycle, whereas it showed approximately similar rate of production at 5th cycle. Carbon source consumption was increased by time, which shows adaptation of the strain in mannitol medium. pH was not affected and gluconic acid production was very low (0.06-0.5 g/L).

Conclusion

Results suggest that *K. xylinus* K2G30 can remodulate the biochemical metabolism based on the carbon source, increasing the BC production. Further studies are aimed at evaluating the time of adaptation and phenotypic and genotypic changes during metabolic shift from glucose to mannitol.

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Natural Product Biosynthesis

Exploring the Functional Sequence Space of SrfAC Adenylation by Hydroxamate Profiling

Authors

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Abstract

Enzyme promiscuity is a key characteristic required for evolutionary innovation since activation of noncognate substrates can serve as a springboard toward novel activities. We aim to recapitulate this process in the laboratory to generate custom-made catalysts by directed evolution. Ideal candidate enzymes for studying enzyme promiscuity should be functionally diverse and have a large pool of substrates. Both criteria are fulfilled by nonribosomal adenylation (A-)domains. Nonribosomal peptide synthetases, with their enormous repertoire of building blocks, have great potential to be repurposed as a source of tailored peptides. In nonribosomal peptide synthesis, the A-domain is a main checkpoint for the identity of the incorporated substrate. The discovery of the 10-residue "specificity code" in the binding pocket enabled the prediction of A-domain substrate specificity from sequence data. However, rational modifications of the specificity code achieve mostly conservative changes accompanied by losses of catalytic activity. To improve our understanding of A-domain substrate selection, we take advantage of the hydroxamate specificity assay (HAMA) to determine the relative contribution of specificity code and second shell residues to adenylation promiscuity. First, we use the FuncLib algorithm to develop VSA, a promiscuous, ancestor-like A-domain variant of SrfAC, the termination module of surfactin synthetase. Second, we fully randomize the binding pocket residues of VSA and determine specificity profiles for each single mutant. Thereby, we determine the weight of each position, identify invariable residues, and show a high malleability of adenylation specificity at high catalytic rates. Additionally, we demonstrate that both promiscuity- and specificity-promoting mutations occur at only a few selected specificity code positions. Together, our data reveal the unexplored functional flexibility of A-domains, provide insights for more streamlined A-domain engineering and confirm the evolutionary potential of promiscuous enzymes.

Natural Product Biosynthesis

Cellulase and Ethylene production by phytopathogenic fungus *Plectosphaerella melonis* 502 *in vitro*

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Abstract

In the last time, many countries around the world recorded the cases of the new plants disease of the Cucurbitaceae family, the agent of which was *Plectosphaerella melonis* (*Acremonium cucurbitacearum*). All the responses of host-plant (symptoms of the disease) are associated with phytopathogen effectors. Their action can be multiple: some take part in degradation of the cellular wall (hydrolytic enzymes), other (phytohormones) are involved in host-plant metabolism and induce a cycle of biochemical reactions that in turn contribute to disease manifestation. Pathogenesis is induced by primary effectors that are the pathogenicity factors and stipulate the ability of microorganism to induce symptoms of the disease. The secondary effectors are the next involving in this process, and they provide pathogen virulence. The objectives were to study the ability of synthesing exo-, endoglucanase, β-glucosidase and ethylene by *P. melonis* 502 *in vitro*.

We revealed that *P. melonis* 502 is capable to synthesize exo-, endoglucanase and β-glucosidase. The highest cellulolytic activity was detected after 6 weeks of mold cultivation at pH 8.5. In the process, the total cellulolytic activity was 0.326 U/ml^{-1}, exoglucanase activity – 0.539 U/ml^{-1}, endoglucanase activity – 0.950 U/ml^{-1} and β-glucosidase activity – 0.795 U/ml^{-1}. The ability of *P. melonis* 502 to biosynthesize ethylene was revealed, and the highest level of it was registered after 5 weeks of cultivation (111.78 nmol/h/g).

Therefore, we have shown the ability of *P. melonis* 502 to synthetize cellulase enzymes (exo-, endocellulases and β-glucosidases) and ethylene, which may be virulence factors. Under the influence of cellulase enzymes, the cell wall of the plant degrades, and the hypersynthesis of ethylene causes necrosis of the tissues of the leaf blade. Furthermore, synthesis of ethylene by the fungus affects the activity of cellulases and accelerates the aging of leaves.

Natural Product Biosynthesis

Selection marker recycling by using split marker approach for gene deletion in fungi

Authors

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Abstract

Natural products isolated from microorganisms are well-known for their biological activities and are promising candidates for drug development. Recent advances in targeted gene manipulation, such as gene disruption and heterologous expression, allowed the analysis of gene functions and lead to the elucidation of many natural product biosynthetic pathways. The *pyrG* gene, coding for the orotidine 5'-phosphate decarboxylase in the biosynthesis of nucleobases, is usually used as selection marker for genetic manipulation in fungi. Based on bidirectional selection, *pyrG* can be easily recycled and used for multiple gene deletion or overexpression. While *pyrG* deficient strains are auxotrophic for uracil and uridine, fungi with the intact *pyrG* gene cannot grow on 5-FOA supplemented media due to the conversion of 5-FOA to the toxic intermediate 5-fluoro-UMP.[1]

In our study, we identified and deleted the *pyrG* gene in *P. crustosum* and used it as selection marker for gene deletion.[2] For convenient recycling of the *pyrG* marker to allow multiple gene disruptions in one strain, we developed a split-marker based deletion construct, in which a 0.3 kbp fragment of the downstream region was cloned directly in front of the *pyrG* gene. Transformants were selected by uracil/uridine auxotrophy. For marker recycling, the mutants were subsequently cultivated on medium supplemented with 5-FOA, uracil and uridine. Recombination between the 0.3 kbp regions before and after the *pyrG* gene resulted in the deletion of the *pyrG* gene, which could then be used for further rounds of gene deletion experiments. By using the split-marker approach for our gene deletion constructs, we successfully deleted the genes for non-homologous end joining events and reduced significantly ectopic DNA integration, which often remains a big challenge for the genetic manipulation of fungi. Our gene deletion strategy is therefore applicable to improve gene targeting and to facilitate the elucidation of natural product biosynthetic pathways.

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Natural Product Biosynthesis

Structural expansion and assembly line promiscuity in the biosynthesis of actinochelins, a widespread family of siderophores from Actinobacteria

Authors

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Abstract

We report the discovery of actinochelins, a new group of non-ribosomal peptide siderophores produced under specific stress culture conditions by *Actinomadura atramentaria* DSM43919 and *Saccharopolyspora* sp. KY21. Actinochelins feature an acylated pseudotripeptide backbone (2,3-dihydroxybenzoic acid/threonine/N-hydroxyornithine) akin to the rhodochelins (previously described in *Rhodococcus jostii* HA1), but they differ in the rich structural diversification of the N-hydroxyornithine moiety and in their alternative tailoring. Genome analysis led to the identification of putative actinochelin BGCs both in *A. atramentaria* and *Sacchopolyspora* sp. KY21, showing a progressive clustering compared to the three subclusters reported as required for the biosynthesis of rhodochelins. CRISPR/Cas9 genomic editing in *Sacchopolyspora* sp. KY21 and heterologous gene complementation experiments were performed to confirm the function of the BGC. Pan-genomic analysis indicated the presence of similar BGCs in multiple species belonging to *Actinomadura* and *Saccharopolyspora*. Actinochelins are essential for the growth of *Sacchopolyspora* sp. KY21 under iron depletion conditions, but strikingly the gene deletion of the standalone adenylation domain responsible for the incorporation of 2,3-dihydroxybenzoic acid to the assembly line resulted in a mutant strain still able to grow under iron depletion. Untargeted metabolomics revealed that this mutant produces high quantities of low iron-affinity actinochelin congeners to support its growth, thus suggesting an unusual assembly line promiscuity phenomenon that ensures the redundancy of an essential ecological function.

Natural Product Biosynthesis

Aetokthonostatins: Highly cytotoxic dolastatins-derivatives produced by the "eagle-killer" cyanobacterium *Aetokthonos hydrillicola*

Authors

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Abstract

The cyanobacterium *Aetokthonos hydrillicola* recently became famous for the production of a novel toxin causing the fatal neurological disease Vacuolar Myelinopathy (VM): aetokthonotoxin (AETX).1 Interestingly, extracts of *A. hydrillicola* were found to be highly cytotoxic, although AETX itself shows only weak cytotoxicity (IC50 10 µM). Microfractionation of an *A. hydrillicola* extract and subsequent cytotoxicity assays showed that not AETX, but the main compound in this extract, which is also present in extracts not inducing VM, was highly cytotoxic. As dereplication suggested this compound to be novel, we set out to isolate and characterize it. One- and two-dimensional NMR as well as MS^2 experiments allowed us to elucidate the planar structure of the new natural product aetokthonostatin (AEST). AEST, a linear pentapeptide, is chemically closely related to the dolastatins, symplostatins, and lyngbyastatins. It differs from symplostatin 1 only in the C-terminus, which in the case of AEST features a hitherto unknown modified phenylalanine-derived amino acid. Similar to the dolastatins and other compounds from this family, AEST is extremely cytotoxic (IC50 10 pm). Using molecular networking of untargeted mass spectrometry data (GNPS), we could identify AEST-derivatives in the *A. hydrillicola* extract, differing mainly in the methylation pattern and the presence or absence of the C-terminal amino acid. Some of these derivatives have already been isolated to study structure-activity relationships of the aetokthonostatins. Genome sequencing of *A. hydrillicola* allowed the identification of the putative AEST biosynthetic gene cluster, which we currently characterize. *A. hydrillicola* is the first freshwater cyanobacterium found to produce highly cytotoxic dolastatin-like metabolites. As *A. hydrillicola* is able to produce two harmful substances (AETX and AEST), monitoring and further studies are mandatory to protect wildlife and human health.

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Natural Product Biosynthesis

Structural insights into the noncanonical TE domain from FR900359 biosynthesis

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Abstract

The cyclic depsipeptide FR900359 (FR) is a strong and selective Gαq protein inhibitor making it an excellent tool to study the influence of Gα proteins on cellular signaling pathways and for the therapy of Gαq-protein-associated diseases like asthma and uveal melanoma. FR is produced by the Gram-negative soil bacterium *Chromobacterium vaccinii*, harboring the *frs* gene cluster, responsible for FR biosynthesis. It encodes for two non-ribosomal peptide synthetase (NRPS) assembly lines, composed of eight modules (FrsA, FrsD-G), which activate and modify specific building blocks, by the assistance of an MbtH-like protein (FrsB) and two modifying enzymes (FrsC, FrsH), to give rise to the complex natural product FR. [1]

Aim of this project is the detailed structural and biochemical characterization of the noncanonical FrsA thioesterase (TE) domain, which catalyzes the intermolecular transesterification of the FR side chain, *N*-propionylhydroxyleucine, onto the cyclic biosynthetic intermediate FR-Core.[2] Structural characterization approaches are planned through crystallization and subsequent X-ray analysis . Since initial crystallization approaches were not successful, optimized crystallization constructs of the protein were generated by partial proteolysis. NMR investigations will be conducted with 15N-labeled FrsA TE. For this purpose, protein expression is established in chemically defined M9 minimal medium. The interaction of enzyme and substrates will then be investigated by comparative measurements with and without labeled FR-Core to obtain detailed insights about the unusual mode of action, to evaluate its potential for chemoenzymatic syntheses, e.g. the generation of altered, bioactive FR analogues with novel effects.

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Genetic Engineering and Synthetic Biology

Exploring the biosynthetic potential of epoxyketone synthase EpnF

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Abstract

Current treatments for different types of cancer include natural product derived epoxyketones which act as proteasome inhibitors.[1] The chemical syntheses of epoxyketone drugs are very inefficient due to loss of half of the material in the unselective formation of the epoxyketone warhead.[2,3] Epoxyketone natural products are found in a variety of natural organism and are biosynthesized by epoxyketone synthases, which enantio-selectively form the epoxyketone from α-dimethyl-β-keto acids.[3,4] To enable a chemoenzymatic and more sustainable production of the epoxyketone in proteasome inhibitors, the biosynthetic potential of known epoxyketone synthase EpnF from the eponemycin gene cluster was explored.[4] A variety of chemically stable derived α-dimethyl-β-keto methyl ester precursor was synthesized and converted into the epoxyketone synthase substrate by pig liver esterase (PLE) *in situ*. EpnF was expressed and purified and coupled *in vitro* assays with PLE and EpnF showed production of the desired epoxyketone, indicating that EpnF accepts different amino acids next to the α-dimethyl-β-keto acid. Enzyme assays with synthetic epoxyketone precursors will reveal the full biosynthetic potential of EpnF for the chemoenzymatic production of potential proteasome inhibitor drugs. Genetic engineering and A-domain swap in epoxyketone biosynthetic gene clusters could enable de novo construction of bacterial pathways for natural product derived epoxyketone proteasome inhibitors.

Genetic Engineering and Synthetic Biology

Reconstitution of Aurachin Biosynthesis in *Myxococcus xanthus* and *Escherichia coli*

Authors

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Abstract

The aurachins are a family of prenylated quinolone antibiotics, which were first discovered in the myxobacterium *Stigmatella aurantiaca*.[1] Bioactivity testing revealed that these natural products possess potent antiplasmodial activities, but also high cytotoxicity.[2,3] Therefore, there is a great interest in the generation of unnatural aurachin derivatives with a larger therapeutic window. While several research groups employ chemical synthesis in the derivatization process,[4] our aim is to redirect the biosynthetic pathway and to produce the desired compounds by fermentation.

A recent study indicated that the aurachin pathway is amenable to precursor-directed biosynthesis.[5] However, the titers of the generated analogues were low, which was due to a competition with natural precursors and due to the diversion into shunt pathways.[5] To circumvent these issues, we set out to reconstitute the biosynthesis in a heterologous host.

Aurachins biosynthesis starts with anthranilic acid that is converted by a type II PKS to 4-hydroxy-2-methylquinoline.[6] Subsequently, the quinoline nucleus is prenylated with a farnesyl moiety by a membrane-bound prenyltransferase to give the aurachin scaffold.[7] Since quinoline derivatives are easily synthetically prepared,[8] we decided to use the corresponding intermediate as entry point to reroute the biosynthesis. Here, we will present our recent findings from this project.

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Genetic Engineering and Synthetic Biology

Marker-free genome engineering in *Amycolatopsis* using the pSAM2 site-specific recombination system

Authors

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Abstract

Actinobacteria belonging to the genus *Amycolatopsis* are important for antibiotic production and other valuable biotechnological applications such as biodegradation or bioconversion. Despite their industrial importance, tools and methods for the genetic manipulation of *Amycolatopsis* are less developed than in other actinobacteria such as *Streptomyces*. Moreover, most of the existing methods do not support convenient marker-free genome engineering. Here, we report the use of the pSAM2 site-specific recombination system for the efficient deletion of marker genes or large DNA regions in *Amycolatopsis*. For this purpose, we constructed a shuttle vector, replicating in *Escherichia coli* and *Amycolatopsis*, expressing the Xis and Int proteins from the *Streptomyces* integrative and conjugative element pSAM2. These proteins are sufficient for site-specific recombination between the attachment sites *attL* and *attR*. We also constructed two plasmids, replicative in *E. coli* but not in *Amycolatopsis*, for the integration of the recombination sites *attL* and *attR* on each side of a region targeted for deletion. We exemplified the use of these tools in *Amycolatopsis mediterranei* DSM 40773 by obtaining with high efficiency (>95%) a marker-free deletion of one single gene in the rifamycin biosynthetic gene cluster or of the entire 90-kb cluster.

Genome Mining/Novel Sources for Natural Product Discovery

Genome-Based Discovery of Enantiomeric Pentacyclic Sesterterpenes Catalyzed by Fungal Bifunctional Terpene Synthases

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Abstract

Bifunctional terpene synthase (BFTS) is one kind unique enzyme, which contains both prenyltransferase (PT) domain and terpene cyclase (TC) domain and can only be found in fungi, generate products usually have unique molecular skeletons. Based on our phytopathogenic fungi database, using BFTS as a probe to screen homologous genes in fungi at the gene level. Genome-based discovery of two previously unreported fungal BFTSs enzymes from phytopathogenic fungi, FoFS catalyzing the formation of fusoxypenes A–C (**1**–**3**) and (–)-astellatene (**4**), and AtAS capable of synthesizing preaspterpenacid I (**6**). Interestingly, FoFS and AtAS catalyzed the formation of enantiomeric 5-6-7-3-5 ring system sesterterpenes. C22-oxidative modification of preaspterpenacid I by AtP450 was characterized as well. The plausible cyclization pathways of fusoxypenes was illustrated by DFT calculations.

Genome Mining/Novel Sources for Natural Product Discovery

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

Authors

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Abstract

Most of the antibiotic biosynthetic gene clusters (BGCs) in *Streptomyces* are not expressed under laboratory conditions, however these clusters are maintained in the genomes of these strains, therefore indicating that they must play important roles in adaptation and survival within their ecological niches. Understanding the global regulation patterns that affect transcription of antibiotic BGCs in these strains under soil conditions versus laboratory conditions allows us to identify novel routes for up- or down- regulation in order to trigger expression of these BGCs and therefore production of these antibiotics under controlled laboratory and fermentation conditions.

These cryptic pathways represent an untapped resource in terms of new metabolites and novel chemistry that could be very useful in the clinic if we can awaken their expression and production under large scale production conditions. We are using RNA sequencing to analyse global transcriptional patterns of *S. venezuelae* and *S. rimosus* grown in sterile soil, non-sterile soil and standard laboratory solid medium. We will also use an unbiased metabolomics approach to identify compounds produced in soils. These results will enable the identification of global regulatory pathways that activate cryptic clusters in nature and lead to the production of these compounds in soil. This knowledge will enable us to generate genetically modified strains engineered to increase the yield of both well characterised and cryptic clusters which encode potentially novel compounds under industrial fermentation conditions. This will ultimately increase the number of compounds which will enter the clinical trial pipeline. It is worth noting that the transcriptional data may also help shed light on the complex regulation of the life cycle of these *Streptomyces* strains in an ecologically relevant environment.

Genome Mining/Novel Sources for Natural Product Discovery

Mycofactocin: a novel redox cofactor of mycobacteria

Authors

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Abstract

A decade ago, bioinformatic analysis revealed the mycofactocin (MFT) system, a gene cluster encoding a putative novel redox cofactor predominantly present in mycobacteria(1). The ribosomally synthesized and post-translationally modified peptide (RiPP) cluster (*mftA-F*) was shown to be crucial for ethanol consumption in *Mycobacterium tuberculosis* and *Mycolicibacterium smegmatis*(2). *In-vitro* studies with biosynthetic enzymes demonstrated the cyclization of the precursor peptide MftA by the radical SAM maturase MftC and subsequent liberation of the core (AHDP) by the peptidase MftE. Furthermore, a redox-active keto amide (PMFT) was revealed to be produced via the oxidative deamination of AHDP by the deaminase MftD (3).

This information guided our efforts towards the discovery of the natural mycofactocin *in vivo*(4). Through comparative metabolomics using 13C-labeling we discovered for the first-time products of MFT biosynthesis in *M. smegmatis*. Structure elucidation by enzymatic treatment, NMR, MS/MS, and GC-MS disclosed the decoration of MFT with up to nine *β*-1,4-linked glucose residues including 2-*O*-methylglucose. By analyzing the metabolomes of mutants deficient in single MFT genes we showed that the oligoglycosylation of MFT is dependent on the glycosyltransferase MftF. An activity-based metabolomic assay with the carveol dehydrogenase LimC enzyme confirmed the redox activity of the glycosylated MFT congeners.

The confirmation of the mycofactocin pathway *in vivo* as well its structure elucidation opens the path for further physiological and biochemical studies of the mycofactocin system in *M. smegmatis* as well as in the deadly pathogenic *M. tuberculosis*.

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Genome Mining/Novel Sources for Natural Product Discovery

Genome Sequence-based Screening for novel phosphonate producers

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Abstract

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

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

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Genome Mining/Novel Sources for Natural Product Discovery

A novel cyclic lipopeptide LP-17 expands the metabolic and antimicrobial versatility of the *Pseudomonas putida* group

Authors

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Abstract

Among the *Pseudomonas* genus, the *P. putida* group is a major group which occupies diverse ecological niches and possesses beneficial agricultural and bioremediation properties. Interestingly, several *P. putida* strains produce secondary metabolites such as Cyclic Lipopeptides (CLPs) which contribute to their ecological and antimicrobial functions. We characterized a novel CLP, LP-17, from a cocoyam rhizosphere isolate, *P. putida* COR35. For chemical characterization of the novel CLP, we combined High Performance Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR) methods whereas, whole genome mining and site-directed mutagenesis was employed for elucidation of the CLP biosynthetic gene cluster and functional analyses, respectively. HPLC and NMR analyses revealed the production of a major compound, LP-17A, and a minor compound, LP-17B. LP-17 differed from previously described CLPs by the composition of 17 amino acids with eight in the macrocycle. Furthermore, mining the whole genome of COR35 showed that LP-17 is assembled as LP-17A, LP-17B and LP-17C, three co-linear non-ribosomal synthetases (NRPS) with closest similarity to the xantholysin NRPSs of *Pseudomonas* sp. BW16M2. Analyses of an LP-17 biosynthesis deletion mutant showed that LP-17 is important for the swarming motility of COR35 and the formation of a white line-in-agar phenotype in interaction with a sessilin-producing strain, *Pseudomonas* sp. CMR12a. However, an LP-17 deficient mutant formed substantially more biofilm on polypropylene tubes. Additionally, *in vitro* antagonism assays using strain COR35, and its LP-mutant showed that LP-17 mediates inhibition against *Rhizoctonia solani*, *Pythium myriotylum* and mycophagy in interaction with *Fusarium oxysporum f. sp*. *lycopersici*. Our study contributes to insights regarding ecological and plant-beneficial roles of secondary metabolites in their producing strains.

Genome Mining/Novel Sources for Natural Product Discovery

Novel bioactive secondary metabolite from *Burkholderia* strain Bmkn7 associated with saline affected inland rice variety

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Abstract

Plant growth-promoting rhizobacteria (PGPR) benefit the host in different ways including prevention of the deleterious effects of phytopathogenic microorganisms. These antagonistic rhizobacteria are the bona fide source of the farming sector as they can be subverted to extract pesticidal metabolites. The plant probiotic bacteria studies to obtain novel bioactive secondary metabolite producing cultures from underexploited ecosystems such as saline-affected inland rice plants unveiled a potential rhizobacterium designated as Bmkn7. This culture shows broad spectrum antimicrobial activity against plant and animal pathogens. Notably, it possessed antifungal activity in agar but not in broth. While a majority of the bacteria in this genus is known for antimicrobial activity due to siderophore production, the antifungal activity of this strain proves to be non-siderophore mediated. In addition to its non-phytotoxic nature confirmed through in-planta studies, this strain also exhibited phosphate solubilisation which is an important PGPR trait. Strikingly, the extraction of the antifungal metabolite from agar using a highly polar solvent marks its specific extraction process and the presence of more than one metabolite. Interestingly, the initial data obtained from HRMS and LC-MS analysis summarize the possibility of a novel bioactive compound, as the obtained masses from this culture did not correspond to any of the antimicrobial compounds existing in related literature. These findings, led us to sequence the genome of Bmkn7. The 16S rRNA gene base phylogeny confirmed the strain to be a member of the *Burkholderia cenocepacia* complex. Finally, data obtained from Antibiotics and Secondary Metabolite Analysis Shell (antiSMASH) analysis provided a concrete insight into possibilities of the strain to produce novel antimicrobial compounds, as several unknown clusters of Non-ribosomal Peptide Synthetase (NRPS) genes were identified. Further studies are underway to characterize the extracted antifungal compound.

Genome Mining/Novel Sources for Natural Product Discovery

Investigations on Structure and Biosynthesis of Biarylitides - a novel class of minimal RiPPs

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Abstract

A recently discovered, novel group of RiPPs are the biarylitides. [1] In their biosynthesis, the smallest coding gene ever described plays a decisive role. The sequence, which is only 18 base pairs short, codes for a precursor pentapeptide which is enzymatically modified and cleaved into a tripeptide with two aromatic amino acids linked by a biaryl bond. A downstream-encoded, specific P450 monooxygenase is postulated to link the biaryl bond. This minimal gene cluster was discovered in the bacterial genus *Planomonospora*, the first known producer of biarylitides. Bioinformatic analyses showed that in at least 200 other bacterial strains related P450 monooxygenases are encoded in close proximity to equally short precursor sequences, and in some cases further modifying enzymes, indicating a large, undiscovered structural diversity of biarylitides. This project focuses on the isolation and characterisation of structurally modified biarylitides, as well as the detailed elucidation of the biosynthesis and substrate specificity of the modifying P450 monooxygenases. In addition, the bioactivity of the biarylitides obtained will be determined in existing biological test systems.

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

Genome Mining/Novel Sources for Natural Product Discovery

From the bottom of the sea: insights into a new member of the tetrahydroisoquinoline family antibiotics

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Abstract

Tetrahydroisoquinoline (THIQ) molecules exhibit potent antitumor antibiotic activities. Several members of this family have been reported, such as saframycin, safracin and ecteinascidin; isolated from soil bacteria and marine invertebrates. Despite their different isolation source, they share common structural features like a moiety derived from a modified L-tyrosine (mTyr). THIQs are biosynthesized by a nonribosomal peptide synthase (NRPS), which catalyses their skeleton through a highly unusual pathway, involving multiple reductions and the Pictet-Spengler (PS) reaction. Based on bioinformatic, genetic engineering and chemical analyses, we report for the first time a marine *Streptomyces* taxonomically related to *S. albidoflavus* producing a new member of the THIQ family. Comparative bioinformatic analysis of *S. albidoflavus* was performed, including strains from our marine culture collection plus all the available ones from Refseq. Pangenome analysis retrieved three clades, where our collection" strains grouped separately. Genome-mining revealed a novel NRPS that harbour low similarity to the saframycin BGC, present in this clade but absent in the others. From our culture collection, *Streptomyces* sp. G11C, isolated from a South Pacific marine sediment, was selected to study the THIQ biosynthesis. The saframycin-like BGC was targeted for genetic engineering and chemical analysis (LC-HRMS and MS/MS). Molecular modifications consisted of *nrps* disruption and native promoter exchange, together with the overexpression of a positive regulator. The *nrps* overexpression produced a metabolite showing THIQs characteristic features, such as the PS domain and incorporation of the mTyr into the assembly line. However, chemical analyses display insights into several novelties, suggesting that this NRPS from strain G11C is involved in producing a new member of the THIQ family.

Regulation and Resistance mechanisms

Activation and identification of a griseusin cluster in *Streptomyces* sp. CA-256286 by employing transcriptional regulators and multi-omics methods

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Abstract

Streptomyces are well-known producers of antibiotics and other bioactive compounds, including secondary metabolites belonging to the type II polyketides. Recently, it has been demonstrated that "silent" biosynthetic gene clusters (BGCs) can be activated by overexpressing transcriptional regulators. Here, we activate a silent BGC in *Streptomyces* sp. CA-256286 by overexpression of SARP family transcriptional regulators. Employing a combination of multi-omics and metabolic engineering techniques, we identify the responsible BGC and propose a biosynthetic pathway. The product structure is elucidated by NMR and found to be an AcCys adduct of 3´-O-α-D-forosaminyl-(+)-griseusin A. This compound is known and structurally very similar to other pyranonaphtoquinone polyketides, the BGC however, is quite different from the one initially described for griseusin A. The formation of AcCys adducts by recruitment of mycothiol detoxification enzymes, suggests a conserved pathway for modifying bioactive compounds, toxic to the producer. This workflow demonstrates how silent BGCs can be activated, followed by identification and characterization of the produced compound, responsible BGC and biosynthetic pathway.

Regulation and Resistance mechanisms

The role of soil saprotrophic fungal metabolites in the formation of the endophytic association *Chaetomium cochliodes* / *Fagopyrum exculentum*

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Abstract

The aim of our study was to establish the features of the formation endophytic association *Chaetomium cochliodes* 3250 and buckwheat plants.

Chaetomium cochliodes Palliser 3250 is a fungus strain taken from useful soil micro-organisms collection at The Institute of Agricultural Microbiology and Agro-Industrial Production NAAS. Auxins and cytokines determination were carried out using method of spectrodensitometric thin-layer chromatography. The quantitative content of gibberellins and brosinosteroids was made via high-pressure efficient chromatography method. Exoglucanase, endoglucanase, β-glucosidase, polygalacturonase activiti study conventional biochemical methods (Bilay, 1982; Gamayurova, 2010).

We established the capability of *C. cochliodes* 3250 for growth-regulating substances synthesis. It has been shown that C. cochliodes 3250 can produce 2.4-epibrassinolide, being crucial for plants" resistance to pathogens, and ergosterol. Synthesized indoly-l,3-acetic acid and ergosterol synthesized can serve as the main mediator molecules in the process of formation of *C. cochliodes* 3250 symbiotic systems with plants. We found cellulase complex enzymes activity in the culture liquid of *C. cochliodes* 3250. Exogluconase activity in the fungus culture liquid was 0.67 units/ml of cultivation, meaning that *C. cochliodes* 3250 can degrade the cellulose crystalline state. Endogluconases ensure hydrolysis of amorphous cellulose to cellobiose (exogluconase activity of *C. cochliodes* 3250 was 0.52 units/ml). β-glucosidase completes the breakdown of cellulose and provides hydrolysis of celibiosis to glucose (β-glucosidase activity of *C. cochliodes* 3250 was 1.02 unit/ml). Polygalactorous activity of the culture liquid of *C. cochliodes* 3250 predetermines the process of depolymerization of the adherent layer of pectin between the adjacent walls of plant cells. The highest polygalacturonase activity was recorded on the 9th day of cultivation of micromycete and was 2.95 units/ml.

Metabolites of the fungus played a major role in the formation of the endophytic association of the fungus *C. cochliodes* / buckwheat plants.

Regulation and Resistance mechanisms

Comparative genomics of van-genes in glycopeptide producing *Actinobacteria* and beyond

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Abstract

Glycopeptide antibiotics (GPAs) are last resort drugs produced by filamentous actinobacteria mainly from genera *Amycolatopsis, Actinoplanes, Nonomuraea* and *Streptomyces.* GPAs are divided into five types, and among them types I-IV are clinically relevant. They are active against Gram-positive bacteria, binding the d-Ala-d-Ala termini of lipid II pentapeptide side chains. This interferes with extracellular stage of peptidoglycan biosynthesis leading to the cell death. GPA-resistant pathogens and GPA producers alike, utilize so-called *van*-genes for cell-wall remodeling to achieve GPA resistance.

It was long noticed that GPA biosynthetic gene cluster (BGCs) carry *van*-genes within. The biological ratio behind seems obvious: GPA producers are avoiding suicide in this way. However, some GPA non-producers (like *Streptomyces coelicolor*) are also known to carry *van*-genes, as well as non-harmful soil-inhabitant low G-C Gram-positives do. Consequently, GPA-producers were considered a probable primary source of *van*-genes for either GPA-non-producers, low G-C Gram-positives or pathogens. Alternative hypothesis assumed that *van*-genes evolved independently in soil-dwelling low G-C Gram-positives and then spread to pathogens.

In current work we have decided to test these hypotheses. We screened 7108 genome assemblies of the phylum *Actinobacteria*, either complete or drafts, searching in parallel for *van*-genes and GPA BGCs. We have discovered *van*-genes in at least 900 assemblies, coming from 22 orders of *Actinobacteria*. Only 39 assemblies contained type I-IV GPA BGCs. Peculiarly, genomes with GPA BGCs still tended to have multiple additional *van*-genes beyond the borders of BGCs. Then we have analyzed 2379 full genomes of the order *Bacillales* (phylum *Firmicutes*) and found *van*-genes only in six species. Finally, we have performed a set of phylogenetic reconstructions to gain insights into the evolution of *van*-genes from *Actinobacteria.* Our results suggest that *Actinobacteria* represent the main source of *van*-genes. Importantly, *van*-genes are not a unique feature of GPA producers, being widely distributed in different actinobacteria orders.

Regulation and Resistance mechanisms

N-Acetyltransferase deactivates paenilamicin as a self-resistance mechanism

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Abstract

Paenibacillus larvae, the causative agent of the devastating honey bee disease American Foulbrood, produces the unusual cationic polyketide-peptide hybrid paenilamicin that displays high antibacterial and antifungal activity.[1] Its biosynthesis gene cluster contains the acetyl-CoA-dependent *N*-acetyltransferase PamZ, a self-resistance factor that deactivates paenilamicin. Using tandem mass spectrometry and two-dimensional nuclear magnetic resonance, we determined the *N*-acetylation site of paenilamicin at the N-terminal amino group of the agmatinamic acid. A derivative bearing an inversion of the stereoconfiguration at this amino group is not acetylated and thus shows the high substrate specificity of PamZ. We further eludicated the crystal structure of the binary PamZ:acetyl-CoA complex at a resolution of 1.34 Å. An unusual tandem-domain architecture of PamZ provides a well-defined substrate-binding groove that is decorated with negatively-charged residues to specifically attract the cationic peptide paenilamicin. Bioinformatic analysis further indicated that the biosynthetic gene cluster also encodes for genes indicative of a prodrug resistance mechanism[2] which masks the very same N-terminal amino group of Aga with an *N*-acyl-D-Asn cap. Hence, we propose a dual-resistance strategy of *P. larvae* against its own antibiotic agent. These findings will help to understand the mode-of-action of paenilamicin as well as its role in pathogenicity of *P. larvae*.

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Bacterial Interactions

Endophytic bacteria & fungi: A new source of potential anticancer agents

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Abstract

In recent years, high throughput screening systems, genomics and bioinformatics tools, rational design, and combinatorial chemistry have been invested by pharmaceutical industries for the discovery of the latest bioactive compounds. Plants have served as a source of medicinal bioactive compounds against numerous kinds of ailments for hundreds of years. Ironically, in recent years, microorganisms related to plants instead of plants themselves have demonstrated to offer products with high therapeutic anticancer potential. Endophytes, an endosymbiotic group of microorganisms often bacteria or fungi that colonize within the tissues of living plants are potential sources of novel natural products for exploitation in modern medicine. So far, a great number of novel natural products possessing anticancer activities have been isolated from endophytes. It"s believed that screening for anticancer compounds from endophytes is a promising way to overcome the increasing threat of drug resistance and chemotherapy-driven treatment pathways. These secondary metabolites isolated from endophytes are categorized into diverse functional classes: alkaloids, peptides, steroids, terpenoids, phenols, quinones, and flavonoids. There is an urgent need for new anticancer drugs because tumor cells are developing resistance against currently available drugs, such as vinca alkaloids and taxanes, which is believed to be a significant reason behind the failure in the chemotherapeutic treatment of cancers. That"s why several derivatives of Camptothecine (obtained from endophytic bacteria) and Cryptophycins from cyanobacteria, Polyketide, and lactone, quinone derivatives from endophytic fungus are used as potential anticancer agents for their cytotoxic activities. Besides, genetic engineering, improved cultivation, and fermentation techniques can allow researchers to isolate new endophytic microorganisms producing antitumor compounds. Research focuses on molecular characterization of endophytes and optimization of culture conditions that may improve the probabilities of success in new drug discovery efforts.

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Bacterial Interactions

Antifungal lanthipeptides involved in mutualistic plant-microbe interactions

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Abstract

The microbial characterization of a suppressive soil to *Fusarium* wilt identified *Streptomyces griseus* S4-7 as the representative strain protecting strawberry plants [1]. Further studies described a tripartite mutualism between this strain, plant, and pollinator partners. S4-7 is transferred from the rhizosphere to the roots, from where it can travel via the plant vascular system to above-ground tissues; then, pollinator bees carry it among flowers and other plants.

Ribosomally synthesized and post-translationally modified peptides (RiPPs) produced by S4-7 play a crucial role in the strong antifungal and thus important ecological activity of the strain, and likely act synergistically, as shown by complete loss of antifungal activity by individual mutants.

Our project focuses on the characterization of a novel lanthipeptide BGC encoded in *S. griseus* S4-7 which has been clearly related to the antifungal activity. Unlike two-component lanthipeptide gene clusters, this BGC encodes three precursor peptides with unusual sequences, two lanthipeptide synthetases, and additional modifying enzymes.

The lanthipeptide locus is investigated via an *in vitro* approach. For this, all predicted peptides and biosynthetic genes have already been cloned into pET expression vectors and purified by Ni-based affinity chromatography. Expression protocols have already been established in optimized *E. coli* strains, and purification of precursor peptides and enzymes has been accomplished.

Preliminary results show that the class II synthetase modifies a precursor peptide that does not contain cysteines in the core region. The other precursors are likely modified by a class III enzyme giving two lanthipeptides containing labionin structures. Additional experiments to determine the modifications installed by a methyltransferase and an oxidoreductase found in the BGC will be conducted.

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More information can be found on:

- https://www.dsmz.de/events/vaam-workshop-2021

- https://vaam.de/die-vaam/fachgruppen/biologie-bakterieller-naturstoffproduzenten/termine/