VAAM Workshop

ON THE BIOLOGY OF BACTERIA PRODUCING NATURAL PRODUCTS

Frankfurt, 31.08. - 02.09.2018

TABLE OF CONTENTS

Table of contents 1
Organisation 2
Sponsors and support 3
Program 4
List of lectures 7
List of posters 8
Lecture abstracts 11
Poster abstracts 27
List of participants 69

ORGANISATION

Chairman	Helge B. Bode
Editorial Office	Helge B. Bode
Conference Office	Sophie Moktadir Svenja Simonyi

SPONSORS AND SUPPORT

Organised by

Our Supporters and Sponsors

Poster Awards Supported by

PROGRAM FOR THE VAAM INTERNATIONAL WORKSHOP 2018

L Lecture (Short Talk)

Friday, 31st August 2018

17:00-18:00		Registration
Poster Session		CHAIR: Yi-Ming Shi
18:00-19:00		Short introduction of posters (20 à 2 min) – even numbers
19:00-20:30		Poster session plus snack and drinks

Saturday, 1st September 2018

Lecture Session I		CHAIR: Gerald Lackner
09:00	L1	V. Wiebach *“Lipolanthines – Ribosomally synthesized lipopeptides with anti-staphylococcal activity“*
09:20	L2	R. Hermenau *“Gramibactin a bacterial siderophore with a diazeniumdiolate ligand system“*
09:40	L3	I. Burkhardt *“In vitro characterization of DMATS1 from the plant pathogenic fungus Fusarium fujikuroi“*
10:00	L4	S. Wenski *“Fabclavine biosynthesis: multiple mechanisms for natural product diversification in a peptide, polyketide, polyamine hybrid“*
10:20		Coffee break

Lecture Session II		CHAIR: Yvonne Mast
11:00	L5	P. Michael *“LC-MS based dereplication and isolation of a compound from arctic marine bryozoan Dendrobeania murrayana“*
11:20	L6	C. Bader *“Comparison of DI-FTICR and LC-QTOF for the comprehensive profiling of myxobacterial secondary metabolomes“*
11:40	L7	C. Senges *“The secreted metabolome of Streptomyces chartreusis: implication for bacterial chemistry and metabolite functional analysis“*
12:00		Group picture
12:30		Lunch

Lecture Session III		CHAIR: Jürgen Breitenbach / Helge B. Bode
14:00	L8	J. Watzel *“Structure-function analysis of a new docking domain class identified in the PAX peptide producing NRPS of Xenorhabdus bovienii “*
14:20	L9	F. Ecker *“An insect’s isoprenyl diphosphate synthase controls product specificity through metal ions“*
14:40	L10	M. Schmeing (key lecture) *“Studying bacillamide synthesis by any means necessary“*
15:40		VAAM Business Meeting
16:00		Coffee break

Poster Session	CHAIR: Nicholas Tobias
17:00-18:00	Short introduction of posters (21 à 2 min) – odd numbers
18:00-20:00	Poster session plus snack and drinks

Sunday, 2nd August 2018

Lecture Session IV CHAIR: Markus Nett

09:30	L11	J. Rinkel *“Spinodiene synthase: an unusual diterpene cyclase from Saccharopolyspora spinosa“*
09:50	L12	F. Panter *“Self-resistance guided genome mining uncovers the pyxidicycline class of myxobacterial natural products“*
10:10	L13	D. Dehm *“Systems analysis of orphan natural product biosynthetic gene clusters by reporter-guided screening of symbiotic cyanobacteria“*
10:30	L14	Y.Mast *“SARP-driven activation of antibiotic gene clusters “*
10:50	L15	Y.-M. Shi *“Phenazine antibiotic cocktails via diversity-oriented biosynthesis“*
11:10		Coffee break
12:00		Poster awards and closing remarks

LIST OF LECTURES

L1	V. Wiebach	*“Lipolanthines – Ribosomally synthesized lipopeptides with anti-staphylococcal activity“*
L2	R. Hermenau	*“Gramibactin a bacterial siderophore with a diazeniumdiolate ligand system“*
L3	I. Burkhardt	*“In vitro characterization of DMATS1 from the plant pathogenic fungus Fusarium fujikuroi“*
L4	S. Wenski	*“Fabclavine biosynthesis: multiple mechanisms for natural product diversification in a peptide, polyketide, polyamine hybrid“*
L5	P. Michael	*“LC-MS based dereplication and isolation of a compound from arctic marine bryozoan Dendrobeania murrayana“*
L6	C. Bader	*“Comparison of DI-FTICR and LC-QTOF for the comprehensive profiling of myxobacterial secondary metabolomes“*
L7	C. Senges	*“The secreted metabolome of Streptomyces chartreusis: implication for bacterial chemistry and metabolite functional analysis“*
L8	J. Watzel	*“Structure-function analysis of a new docking domain class identified in the PAX peptide producing NRPS of Xenorhabdus bovienii“*
L9	F. Ecker	*“An insect’s isoprenyl diphosphate synthase controls product specifity through metal ions“*
L10	M. Schmeing	*“Studying bacillamide synthesis by any means necessary“* *Key lecture*
L11	J. Rinkel	*“Spinodiene synthase: an unusual diterpene cyclase from Saccharopolyspora spinosa“*
L12	F. Panter	*“Self-resistance guided genome mining uncovers the pyxidicycline class of myxobacterial natural products“*
L13	D. Dehm	*“Systems analysis of orphan natural product biosynthetic gene clusters by reporter-guided screening of symbiotic cyanobacteria“*
L14	Y. Mast	*“SARP-driven activation of antibiotic gene clusters“*
L15	Y.-M. Shi	*“Phenazine antibiotic cocktails via diversity-orientated biosynthesis“*

LIST OF POSTERS

P1 R. Castillo-Arteaga *“Peroxidase activity as a part of self-resistance mechanisms in cosmomycin D producing strain Streptomyces olindensis DAUFPE 5622“*

P2 K. Tsukada *“Production of diverse diterpenoid pyrones by re-construction and re-designing of fungal biosynthetic pathways in Aspergillus oryzae“*

P3 L. Kreling *“Regulation of anthraquinone biosynthesis in Photorhabdus luminescens“*

P4 A. Popoff *“New DKxanthen-like secondary metabolite from a Myxobacterium shows antimicrobial and cytotoxic activity“*

P5 J. Krumbholz *“Ultrahigh density cultivation enables capturing the secondary metabolite diversities in Nostoc punctiforme PCC 73102“*

P6 J. Diettrich *“Massithiazole, a natural product from Massilia sp.“*

P7 L. Zhao *„Structure and biosynthesis of rhabdopeptide/xenortide-like peptides in Xenorhabdus innexi and Xenorhabdus cabanillasii“*

P8 C. Bader *“Comparison of DI-FTICR and LC-$_{Q}$TOF“ for the comprehensive profiling of myxobacterial secondary metabolomes“*

P9 I. Folger *“Engineering biosynthesis pathways for tailored peptides“*

P10 F. Handel *“Screening for novel protein synthesis inhibitors from uncharacterized Streptomycetes of the ’Tübingen strain collection’ “*

P11 S. Kruth *“Design of vector-based expression systems for myxobacteria“*

P12 D. Abebew *“Uncovering nematicidal natural products from nematode symbiotic bacteria“*

P13 A. Camus *“Biosynthesis of N-Methylated peptides using reprogrammed NRPSs“*

P14 A. Jagels *“Fascinating Stachybotrys – novel cytotoxic meroterpenoids chemically inspired isolation approaches and application of an LC-MS/MS multi-method“*

P15	M. Bader	*“Searching for the acivicin biosynthesis gene cluster“*
P16	K. Ishida	*“Enzyme thioamide formation in bacterial DNA antimetabolite pathway“*
P17	N. Neubacher	*“Global –omics approaches reveal possible key genes of entomopathogenic bacteria required for both sensing of and adaptation to insect hosts“*
P18	T. Bakker	*“Regulation studies of tacrolimus biosynthesis“*
P19	H. Kage	*“A genome streamlining-based bacterial chassis generation“*
P20	H. Zeng	*“An alternative screening approach to discover antimicrobial compounds in myxobacteria“*
P21	J. Hegemann	*“Establishing prochlorosin 2.8 as epitope grafting scaffold“*
P22	F. Wolf	*“Investigating the actinonin biosynthetic gene cluster“*
P23	A. Tietze	*“Dividing condensation domains by half – A new strategy for the engineered biosynthesis of non-ribosomal peptides“*
P24	F. Lünne	*“The ergochrome gene cluster of Claviceps purpurea investigation on further secondary metabolites“*
P25	E. Mingyar	*“Activation of silent secondary metabolite clusters by various regulatory proteins in Actinomycetes“*
P26	J. Hug	*“Genomic investigation and activation of myxobacterial type III PKS gene clusters “*
P27	Y. Morishita	*“Discovery of fungal polyene macrolide by genome mining and heterologous expression of a cryptic HR-PKS cluster“*
P28	J. Krause	*“Phosphonate production in Kitasatospora sp. TÜ4103“*
P29	Y.-N. Shi	*“Three new skeletons from fungus Geomyces auratus and Alternaria alternata“*
P30	F. Panter	*“Self-resistance guided genome mining uncovers the pyxidicycline class of myxobacterial natural products“*
P31	C. Steininger	*“Harnessing fungal nonribosomal cyclodepsipeptide synthetases for mechanistic insights and tailored engineering“*

P32 M. Schmalhofer *“Heterodimeric complex of the dehydratases A_{PE}I/P in the biosynthesis of aryl polyenes“*

P33 P. Michael *“LC-MS based dereplication and isolation of a new alkaloid compound, dendrobeaniamine A, from an artic marine bryozoan Dendrobeania murrayana“*

P34 C. Kegler *“Artificial splitting of a non-ribosomal peptide synthetase by inserting natural docking domains”*

P35 M. Zdouc *“Investigation of Planomonospora as secondary metabolite producer by metabolomic tools“*

P36 F. Wiker *“Identification of novel pyrones from Conexibacter woesi“*

P37 K. Arend *“Production of calcimycin and its analogs in S. chartreusis“*

P38 N. Berghaus *“Bioactivity analysis of different fabclavine classes in ∆hfq background in Xenorhabdus szentirmaii“*

P39 Riyanti *“Co-culture marine spongs-associated bacteria as resource for novel antibiotics“*

P40 D. Kresna *“Heterologous expression of biosynthetic gene cluster“*

P41 A. Engelbrecht *“Proteasome inhibitors from Nocardia spp. and their role in pathogen survival“*

LECTURES ABSTRACTS

L1. LIPOLANTHINES - RIBOSOMALLY SYNTHESIZED LIPOPEPTIDES WITH ANTI-STAPHYLOCOCCAL ACTIVITY

Vincent Wiebach[1], Andi Mainz[1], Mary-Ann J. Siegert[1], Natalia A. Jungmann[1], Guillaume Lesquame[2], Sophie Tirat[3], Assia Dreux-Zigha[2], Jozsef Aszodi[2], Dominique Le Beller[2] & Roderich D. Süssmuth[1]

[1] Technische Universität Berlin, Institut für Chemie/ Biologische Chemie, Berlin, Germany, Email: vincent.wiebach@chem.tu-berlin.de
[2] Deinobiotics, Grabels/ France
[3] ICV-Institute Charles Viollette, Univ. Lille, INRA, ISA, Univ. Artois, Univ. Littoral Côte d'Opale, Lille, France.

Due to the emergence of multiple and fully resistant pathogens, the need for novel lead structures for the development of antimicrobial drugs is of highest importance. During the screening of bacterial extracts, the highly potent anti-staphylococcal lanthipeptide microvionin was isolated from a culture of *Microbacterium arborescens*. Microvionin is a new ribosomally synthesized and post-translationally modified peptide (RiPP) which exhibits the new triamino-dicarboxylic acid avionin and an unprecedented lipidation with an N-terminal fatty acid modification.[1]
The genes encoded in the newly identified microvionin (*mic*) gene cluster suggest a combination of polyketide and ribosomal peptide biosynthetic pathways. The in vitro reconstitution of the central steps of the biosynthesis showed the avionin cyclization to depend on the cooperative action two enzymes, a cysteine decarboxylase and a Ser/Thr kinase-cyclase. Genome mining further revealed various microvionin-related gene clusters in other Actinomycetes, showing a widespread distribution of this novel lanthipeptide class, termed lipolanthines.[1] Besides the intriguing biosynthesis, the lipolanthines have a great potential as antibacterial drugs, not only due to their strong biological activity but also with regard to engineering purposes aiming for a modulation of activity and pharmacokinetic properties.

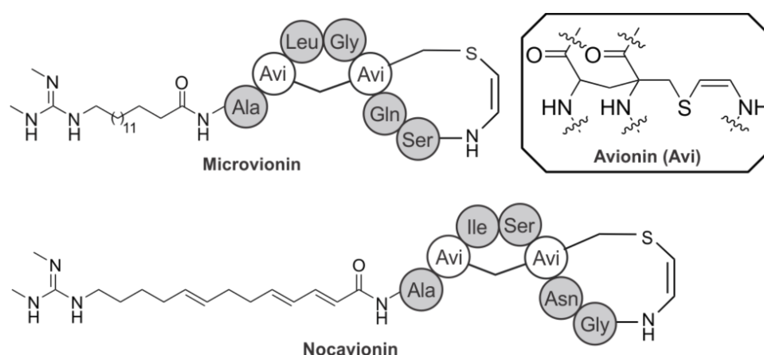

Figure 1: Structures of isolated lipolanthines Microvionin and Nocavionin, containing the characteristic triamino-dicarboxylic acid moiety avionin (Avi).

[1] V. Wiebach, A. Mainz, M.-A. J. Siegert, G. Lesquame, S. Tirat, A. Dreux-Zigha, J. Aszodi, D. Le Beller, R. D. Süssmuth, *Nat. Chem. Biol.* **2018**, *14*, 652–654.

L2. GRAMIBACTIN
A BACTERIAL SIDEROPHORE WITH A DIAZENIUMDIOLATE LIGAND SYSTEM

*Ron Hermenau[1], Keishi Ishida[1], Sofia Gama[2], Bianca Hoffmann[3], Michel Pfeifer-Leeg[3], Winfried Plass[2], Jan Frieder Mohr[2], Thomas Wichard[2], Hans-Peter Saluz[3] and Christian Hertweck[1]**

[1] Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (HKI), 07745 Jena, Germany. Email: Ron.Hermenau@hki-jena.de

[2] Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, 07743 Jena, Germany

[3] Department of Cell and Molecular Biology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), 07745 Jena, Germany

The talk will report the genomics-driven discovery of gramibactin, a new type of siderophore from rhizosphere-associated bacteria (*Paraburkholderia graminis*). Using a combination of bioinformatics, isotope labeling, synthesis, and various spectroscopic methods the structure of gramibactin was elucidated. Highly unusual *N*-nitrosohydroxylamine (diazeniumdiolate) moieties were identified to be part of the iron-binding ligand system. First insight into the ecological significance of gramibactin was gained by metal uptake experiments in corn plants. Homologous biosynthesis gene clusters were found in broadly distributed, plant-associated bacteria.[1]

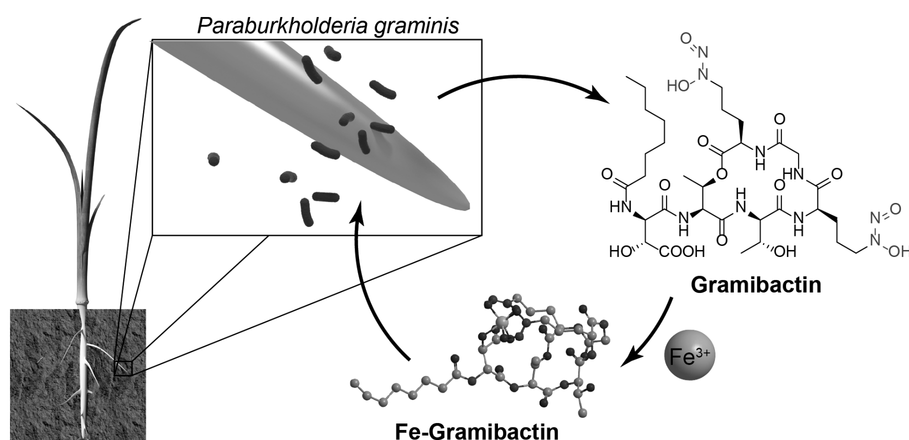

Figure 1. Rhizosphere bacteria *P. graminis* produce a new type of siderophore, gramibactin, that can deliver iron to the host plant.

[1] R. Hermenau, K. Ishida, S. Gama, B. Hoffmann, M. Pfeifer-Leeg, W. Plass, J. F. Mohr, T. Wichard, H. P. Saluz, C. Hertweck, *Nat. Chem. Biol.* [accepted]

L3. In Vitro Characterization of DMATS1 from the Plant Pathogenic Fungus *Fusarium fujikuroi*

Immo Burkhardt[1], *Zhongfeng Ye*[1], *Alexander Babczyk*[1] *and Jeroen S. Dickschat*[1*]

[1] Kekulé-Institut für Organische Chemie und Biochemie, Rheinische Friedrich-Wilhelms-Universität Bonn, Gerhard-Domagk-Straße 1, 53121 Bonn, Germany.
Email: dickschat@uni-bonn.de.

Prenyltransferases of the DMATS superfamily catalyze Friedel-Crafts alkylations of aromatic substrates such as indole-containing molecules using prenyl pyrophosphates as alkylation agents. These enzymes participate in the biosynthesis of a plethora of bioactive natural products like the ergot alkaloids in ascomycetes.[1] The putative prenyltransferase DMATS1 in *Fusarium fujikuroi* was recently shown to be responsible for in vivo production of inversely *N*-prenylated tryptophan (**2**).[2]

In this project, the in vitro characteristics of recombinant DMATS1 were investigated and the selective conversion of tryptophan (**1**) and DMAPP into **2** was proven, which characterizes DMATS1 as the first fungal tryptophan *N*-prenyltransferase. Another accepted substrate is tyrosin (**3**), where a normal prenylation at the phenolic oxygen is observed to produce compound **4**. Understanding of the mechanistic details, leading to the observed products, is addressed by a combination of structural comparison of a DMATS1 homology model to available structural data of another DMATS-type enzyme,[3] incubation with substrate analogs, kinetic assays and site directed mutagenesis of putative key amino acid residues.

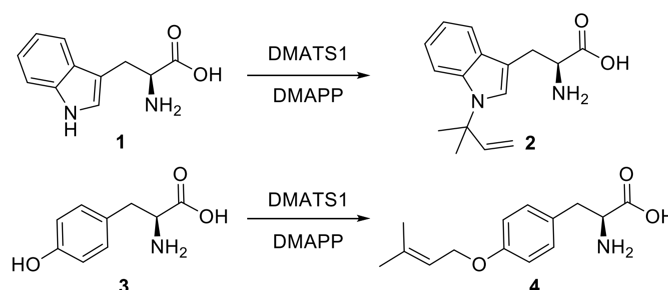

Figure 1. Reaction of L-tryptophan and L-tyrosin with DMAPP catalyzed by DMATS1

[1] S.-M. Li, *Nat. Prod. Rep.* **2010**, *27*, 57–78.

[2] B. Arndt, S. Janevska, R. Schmid, F Hübner, B. Tudzynski, H.-U. Humpf, *ChemBioChem* **2017**, *18*, 899–904.

[3] U. Metzger, C. Schall, G. Zocher, I. Unsöld, E. Stec, S.-M. Li, L. Heide, T. Stehle, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 14309–14314.

L4. Fabclavine Biosynthesis: Multiple mechanisms for natural product diversification in a peptide, polyketide, polyamine hybrid

Sebastian L. Wenski[1], *Gina L. C. Grammbitter*[1], *Nadine Keller and Helge B. Bode*[1,2]

[1] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt, Germany. E-mail: wenski@bio.uni-frankfurt.de

[2] Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt, 60438 Frankfurt, Germany.

Fabclavines are secondary metabolites described for the enterobacteriaceae *Xenorhabdus szentirmaii* or *X. budapestensis* [1]. Both are living in symbiosis with the entomopathogenic nematodes of the genus *Steinernema*. Fabclavines show a bioactivity against a wide variety of microorganisms like Gram-positive and -negative bacteria, yeast and protozoa [1]. Biochemically they are derived from a non-ribosomal peptide synthetase (NRPS) that produces a hexapeptide, which is elongated with one or two malonate units by a polyketide synthase (PKS) and connected with an unusual polyamine [1]. In this work we analyzed the biosynthesis of the fabclavines in general and further focussed on generating novel fabclavine derivatives.

MALDI-MS analysis of single deletion mutants revealed two parallel assembly lines which act independently. The first assembly line is encoded by the genes *fclCDEFGH*, responsible for the biosynthesis of the polyamine, and the second is encoded by the genes *fclIJK*, responsible for the production of the NRPS/PKS hybrid. Furthermore, we detected a second biosynthesis start which leads to the formation of shortened fabclavine derivatives. This second biosynthesis start occurs also in the wild type which indicates a naturally occuring mechanism to increase the fabclavine diversity. Moreover, we could show that FclL, responsible for the connection of the two assembly lines, is able to integrate externally attached polyamines with varying structures into the fabclavine biosynthesis. In addition, we characterized the proposed thioester reductase FclG by determining *in vitro* its cofactor, possible substrates and corresponding products.

Due to their broad bioactivity the fabclavines are of special interest in order to understand their chemical ecology and as a potential application. For a save and sustainable application it is necessary to increase the bioactivity specifity by altering their chemical structure. Therefore we could elucidate the fabclavine biosynthesis in general and further found promising starting points for biotechnological engineering.

[1] S. W. Fuchs, F. Grundmann, M. Kurz, M. Kaiser, H. B. Bode. *Chembiochem.* **2014**, 15(4): 512-516.

L5. LC-MS BASED DEREPLICATION AND ISOLATION OF A COMPOUND FROM ARCTIC MARINE BRYOZOAN *DENDROBEANIA MURRAYANA*

Priyanka Michael[1]*, Johan Isaksson*[2]*,* Kine Ø. Hansen[1], Marte Albrigtsen[1], Kirsti Helland[1], *Jeanette H. Andersen*[1] *and Espen Hansen*[1]

[1] MARBIO, UiT – The Arctic University of Norway, Brevika, N-9037, Tromsø, Norway. Email: raja.p.siranjeevi@uit.no
[2] Department of Chemistry UiT - The Arctic University of Norway, Brevika, N-9037, Tromsø, Norway.

Abstract: Among marine invertebrates, bryozoans are prolific source of structurally diverse secondary metabolites that can be used as lead compounds in the pharmaceutical industry for drug development [1]. Investigation of natural product chemistry in marine bryozoans are limited. Until 2017, only about 2% of new secondary metabolites (Approximately 225) were described from bryozoan species [2,4]. Most of the secondary metabolites from marine bryozoans are alkaloids such as amathaspiramides, alternamides, aspidostomides convolutamines, flustramines that display antimicrobial, antitrypanosomal and cytotoxic activities [2-5]. Dereplication is an essential process to eliminate re-isolation and re-characterization of previously known compounds. This process accelerates identifying potentially new secondary metabolites in the early stage of Natural Products drug discovery [6,7]. The main aim of this study is identification and isolation of new compounds from the crude extract of Arctic marine bryozoan *Dendrobeania murrayana*, using a combination of ultra-high performance liquid chromatography and high-resolution mass spectrometry (UHPLC-HR-MS) with positive electrospray ionization (ESI^+). In the analysis of the crude extract, a prominent *pseudo-molecular ion* peak with *m/z* 369.2861 was observed. The elemental composition of the compound was calculated, and searches in ChemSpider, SciFinder and Dictionary of Marine Natural Products gave no hits. The target compound was isolated using preparative HPLC. The structure of the compound (**1**) was elucidated by using 1D and 2D nuclear magnetic resonance (NMR) and biological activities of the compound (**1**) was also evaluated.

References:

[1] M.R. Prinsep, Y.B. Nicholson, B.K. Gordon, D.P. *Phytochem. Rev*. **2004,** *3, 3, 325-331*.
[2] J.H. Sharp, M.K. Winson, J.S. Porter, *Nat. Prod. Rep.*, **2007**, *24, 659–673*.
[3] J.W. Blunt, B.R. Copp, R.A. Keyzers, Munro, M.H.G. M.R. Prinsep, *Nat. Pro. Rep*. **2017**, *34, 3, 235-294*.
[4] C. Christophersen, *Acta Chemia Scandinavia* B **1985**, *39, 517-529*.
[5] M.D. Lebar, J.L. Heimbegner, B.J. Baker, *Nat. Prod. Rep.*, **2007**, *24, 774-794*.
[6] G.R. Pettit, C.L. Herald, D.L. Doubek, D.L. Herald, E. Arnold, J.J. Clardy, *Am. Chem. Soc*. **1982**, *104, 6846*.
[7] K.F. Nielsen, M. Månsson , C. Rank, J.C. Frisvad, T.O Larsen, **2011**, *J. Nat. Prod., 74, 11, 2338–2348*.

L6. COMPARISON OF DI-FTICR AND LC-*q*TOF FOR THE COMPREHENSIVE PROFILING OF MYXOBACTERIAL SECONDARY METABOLOMES

Chantal Bader[1], Patrick Haack[1], Fabian Panter[1], Daniel Krug[1] and Rolf Müller[1]

[1] Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research (HIPS), 66123 Saarbrücken, Germany. * Email: Chantal.Bader@helmholtz-hzi.de.

Myxobacteria are a prolific source of natural products featuring chemical novelty as well as promising bioactivites. Follow-up of these bioactivites found in crude extracts using various analytical techniques already led to the discovery of numerous valuable molecules including cystobactamide[1] —a compound showing potent anti Gram-negative activity and epothilone[2] —a cytostatic drug with approval of the FDA. Currently, investigation of the myxobacterial secondary metabolome is based mostly on screening approaches using LC-*hr*MS and subsequent dereplication for the discovery of new natural products from extracts of these promising microbes. However, the genomic revolution strikingly has shown that current methods likely fall short of uncovering the full complement of myxobacterial secondary metabolite diversity. In this study we aim to evaluate the potential of DI-FTICR for extending the scope of myxobacterial metabolites detectable under laboratory conditions. Along these lines we conduct a comprehensive comparison with our LC-*q*TOF platform and characterize the chemical space covered by both methods. This comparison is motivated by the notion that LC-*q*TOF is a robust platform available in most microbial natural product laboratories, whereas high-resolution mass spectra combined with retention time information achieves identification with increased confidence - making fast scan rates during data acquisition irreplaceable. The defining feature of DI-FTICR on the other hand is the capability to generate spectra with a resolution up to 1000000 and thus supporting compound identification solely based on precise mass even from complex mixtures like bacterial crude extracts. Results from the two-platform contest are presented and implications for natural products discovery are discussed.

[1] S. Baumann, J. Herrmann et al., *Angew. Chemie*. **2014**, *126*, 14835-14839.

[2] K. Gerth, N. Bedorf et al., *J Antibiot*. **1996**, *49*(6), 560-563

L7. The Secreted Metabolome of *Streptomyces chartreusis*: Implications for Bacterial Chemistry and Metabolite Functional Analysis

Christoph H. R. Senges[1], *Arwa Al-Dilaimi*[2], *Douglas H. Marchbank*[3], *Daniel Wibberg*[2], *Anika Winkler*[2], *Brad Halli*[3], *Minou Nowrousian*[4], *Jörn Kalinowski*[2], *Russell G. Kerr*[3] *and Julia E. Bandow*[1*]

[1] Applied Microbiology, Ruhr University Bochum, 44780 Bochum, Germany
[2] Center for Biotechnology, Bielefeld University. 33594 Bielefeld, Germany
[3] Department of Chemistry, University of Prince Edward Island, Charlottetown, PE C1A 4P3, Canada
[4] Department of General and Molecular Botany, Ruhr University Bochum 44780 Bochum, Germany

Actinomycetes are known for producing diverse secondary metabolites with often unknown natural functions. Combining genomics with mass spectrometry and molecular networking, we characterized the secreted metabolome of calcimycin producer *Streptomyces chartreusis*. We detected >1,000 secreted metabolites in culture supernatants, including hitherto unknown siderophores and ionophores, specifically tailored to cultivation conditions.[1] Compared with chemically defined medium, in complex medium, total metabolite abundance was higher, structural diversity greater, and the average molecular weight almost doubled. The polyether ionophor calcimycin was produced either in complex medium or in minimal medium in cocultivation with *Bacillus subtilis*. In the literature calcimycin was described to export iron from *B. subtilis in vitro*.[2] In cocultivation *S. chartreusis* extracts the often growth-limiting iron from *B. subtilis* calcimycin-dependently, while also inducing *B. subtilis*' siderophore production. The production of a single metabolite might enable iron pilferage and facilitate siderophore piracy, giving *S. chartreusis* an edge in the competitive habitat soil.

[1] C. H. R. Senges, A. Al-Dilaimi, D. H. Marchbank, D. Wibberg, A. Winkler, B. Haltli, M. Nowrousian, J. Kalinowski, R. G. Kerr, J. E. Bandow, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 2490–2495.
[2] N. Raatschen, M. Wenzel, L. I. O. Leichert, P. Düchting, U. Krämer, J. E. Bandow, *Proteomics* **2013**, *13*, 1358-1370.

L8. STRUCTURE-FUNCTION ANALYSIS OF A NEW DOCKING DOMAIN CLASS IDENTIFIED IN THE PAX PEPTIDE PRODUCING NRPS OF *XENORHABDUS BOVIENII*

Jonas Watzel[1], *Carolin Hacker*[1], *Helge. B. Bode*[1,2], *Jens Wöhnert*[3]

[1] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe University Frankfurt, 60438 Frankfurt, Germany. Email: h.bode@bio.uni-frankfurt.de

[2] Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt, 60438 Frankfurt, Germany.

[3] Zentrum für Biomolekulare Magnetische Resonanz (BMRZ), Fachbereich Biowissenschaften, Goethe University Frankfurt, 60438 Frankfurt, Germany. Email: woehnert@bio.uni-frankfurt.de

Non-ribosomal peptides are structurally diverse natural products often with bioactivities that are interesting for a therapeutic application. One reason for the wide product spectrum of non-ribosomal peptide synthetases (NRPSs), including antibiotics, immunosuppressants and antitumor agents, is their modular architecture in which discrete catalytic domains are linked to modules and these are organized in one or more proteins.

In contrast to single protein NRPS systems, the PAX peptide producing NRPS of the entomopathogenic bacterium *Xenorhabdus bovienii* contains seven modules arranged in three proteins, PaxABC, which interact in a unidirectional way. The predefined order of this three-polypeptide NRPS is partially mediated by N- and C-terminal elements, defined as Docking Domains (DDs). Here we describe the complex structure of an artificial glycine-serine linked DD pair connecting the proteins PaxB and PaxC. This structure reveals a new type of DDs, which is unique in their structural composition in comparison to described DD classes. Additional to the elucidation of the solution structure by NMR spectroscopy, the protein-protein interaction was analysed by NMR titration and isothermal titration calorimetry experiments. Hereby, amino acids were identified which are involved in the binding interface. Furthermore, the quantitative analysis of the binding affinity confirmed that DDs are not the only factor in the functional NRPS assembly, but play a key role in the inter-protein recognition.

This work emphasises how a better characterization of DDs has potential for the successful re-engineering of new polypeptide interactions, which supports rational NRPS design.

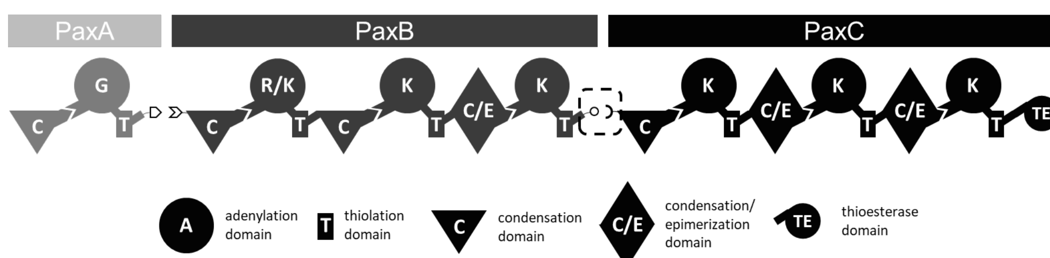

Figure 1. Pax non-ribosomal peptide synthetase. The NRPS comprises three subunits PaxA, PaxB and PaxC, in which the interface between PaxB and PaxC (boxed) was analyzed.

L9. AN INSECT'S ISOPRENYL DIPHOSPHATE SYNTHASE CONTROLS PRODUCT SPECIFICITY THROUGH METAL IONS

Felix Ecker[1], Abith Vattekkatte[2], Michael Groll[1] and Wilhelm Boland[2]

[1]Chair of Biochemistry, Technische Universität München, Lichtenbergstraße 4, 85747 Garching, Germany. Contact: felix.ecker@tum.de

[2]Department Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, 07745 Jena, Germany

The isoprenyl diphosphate synthase from the horseradish leaf beetle *Phaedon cochleariae* (*Pc*IDS1) has been found to alter its product specificity depending on available metal ions.[1] A crystal structure of *Pc*IDS1 in complex with Mg^{2+} and a novel inhibitor termed 3-Br-GPP was obtained at 1.6 Å resolution. A well-ordered cluster of four Mg^{2+}-ions, enzyme residues, and water molecules was observed around the pyrophosphate moiety. An alignment with human farnesyl pyrophosphate synthase (huFPPS) pointed towards a potential binding site for IPP during prenyl elongation.

Further crystallization trials with Co^{2+} and additional ligands could reveal the structural changes that alter product formation. Understanding the terpenoid pathways of insects in general, provides an essential basis for drug development and crop protection.

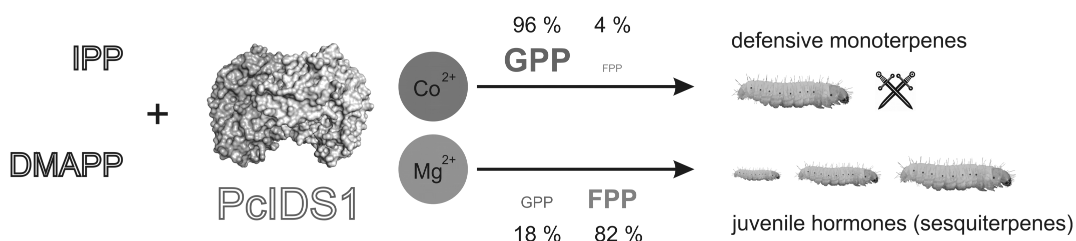

Figure 1. GPP and FPP production by *Pc*IDS1 in *Phaedon cochleariae* depends on different metal ions. A higher affinity for Co^{2+} allows selective production of GPP, needed for defensive monoterpenes, even in presence of Mg^{2+}. When complexed with Mg^{2+}, IDS1 produces predominantly FPP as precursor for developmental hormones.

[1] Frick S., Nagel R., Schmidt A., Bodemann R. R., Rahfeld P., Pauls G., Brandt W., Gershenzon J., Boland W., Burse A., *Proc. Natl. Acad. Sci.* **2013**, *11*, 4194–4199.

L10. Studying Bacillamide Synthesis By Any Means Necessary

T. Martin Schmeing

Department of Biochemistry & Centre for Structural Biology, McGill University, Bellini Life Sciences Building, Office 465, 3649 Promenade Sir William Osler, Montreal, QC, Canada H3G 0B1 email: martin.schmeing@mcgill.ca

Nonribosomal peptide synthetases (NRPSs) are true macromolecular machines, having modular assembly-line logic, a complex catalytic cycle, moving parts and many active sites. We have performed structural and functional analyses of components of the NRPS systems responsible for the syntheses of the anti-algae bacillamide [1,2], the siderophore bacillibactin [3], and the antibiotic linear gramicidin [4]. I will discuss selected results from these studies and the insight they provide into the mechanisms of tailoring in NRPSs, and well as into larger into questions of higher order assembly, superdomain and supermodular architecture and conformational changes in NRPSs.

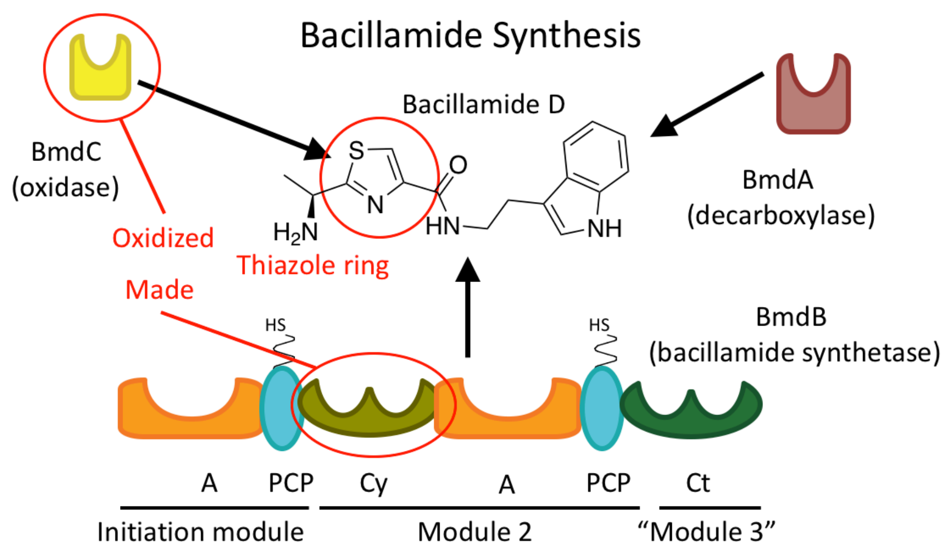

Figure 1. The bacillamide NRPS system

[1] L. Yuwen, F.L. Zhang, Q.H. Chen, S. J. Lin, Y. L. Zhao, Z, Y, Li, *Sci Rep* **2013,** *3*, 1753.

[2] K. Bloudoff, C. D. Fage, M. A. Marahiel, T. M. Schmeing, *Proc Natl Acad Sci U S A* **2017,** *114* (1), 95-100.

[3] M. J. Tarry, A. S. Haque, K. H. Bui, T. M. Schmeing, *Structure* **2017,** *25* (5), 783-793.

[4] J. M. Reimer, M. N. Aloise, P. M. Harrison, T. M. Schmeing, *Nature* **2016,** *529* (7585), 239-42.

L11. SPINODIENE SYNTHASE: AN UNUSUAL DITERPENE CYCLASE FROM *SACCHAROPOLYSPORA SPINOSA*

Jan Rinkel[1], Simon T. Steiner[1] and Jeroen S. Dickschat[1]*

[1] Kekulé-Institut für Organische Chemie und Biochemie, Rheinische Friedrich-Wilhelms-Universität Bonn, Gerhard-Domagk-Straße 1, 53121 Bonn, Germany.
Email: dickschat@uni-bonn.de.

Terpene cyclases (TCs) represent nature's most efficient way to build up structurally complex small molecules,[1] which can serve as unique signaling agents or as other bioactive secondary metabolites (often after oxidation).[2] The remarkable evolution in overall low conserved TC sequences led to the continued addition of new structures to the already huge group of terpenoid natural products. Facing complex cyclisation mechanisms, isotopic labelling strategies have been and are still a powerful tool to follow TC reactions.[3]

In this talk, the products and the catalysed cyclisation mechanism of a recently investigated bacterial TC will be discussed. The enzyme yields the new structures spinodiene A (**1**) and B (**2**) featuring a tricyclic system as shown in Figure 1. Labelling experiments are focused on the investigation of both structure and cyclisation course revealing some mechanistic surprises in this actinomycetal diterpene cyclase. Moreover, chemical modifications like Diels-Alder reactions on **1** are also possible.

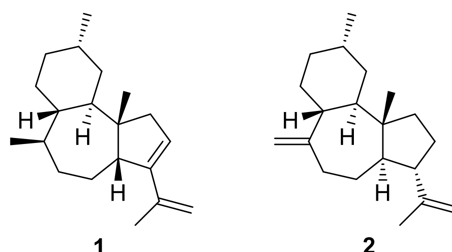

Figure 1. Structures of spinodiene synthase products.

[1] J. S. Dickschat, *Nat. Prod. Rep.* **2016**, *33*, 87–110.

[2] Y. J. Zhao, Q. Q. Cheng, P. Su, X. Chen, X. J. Wang, W. Gao, L. Q. Huang, *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2371–2383.

[3] a) J. S. Dickschat, *Eur. J. Org. Chem.* **2017**, 4872–4882; b) J. Rinkel, L. Lauterbach, P. Rabe, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2018**, *57*, 3238–3241; c) L. Lauterbach, J. Rinkel, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2018**, *57*, 8280–8283.

L12. SELF-RESISTANCE GUIDED GENOME MINING UNCOVERS THE PYXIDICYCLINE CLASS OF MYXOBACTERIAL NATURAL PRODUCTS

Fabian Panter[1], Daniel Krug[1] and Rolf Müller[1]

1 Department of Microbial Natural Products Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany

An astounding discrepancy exists between the genome-inscribed capacity for the production of secondary metabolites and the set of known secondary metabolite classes detected from many microorganisms under laboratory cultivation conditions. Genome-mining techniques are meant to fill this gap. In order to increase chances for finding novel bioactive natural products and connecting them to uncharacterized biosynthetic pathways, genomics-based strategies are reasonably amended with the analysis of associated genetic markers for self-resistance. In this study, we follow a genome-mining approach aimed at discovering inhibitors of topoisomerase, known as target in both cancer therapy and for the treatment of infectious diseases. The so-called pentapeptide repeat proteins in bacteria mediate a common host self-defense mechanism against such inhibitors. Biosynthetic machinery for the production of an alleged topoisomerase inhibitor was found in the genome of the myxobacterium *Pyxidicoccus fallax* And48, as judged by a gene cluster encoding polyketide synthase type II (PKS) enzymes near to a gene predicted to encode a pentapeptide repeat protein, albeit to date no matching compound was reported from the strain. Activation of this biosynthetic gene cluster in the native host as well as heterologous expression following cloning by transformation-associated recombination enabled the structural elucidation of two new natural products called Pyxidicycline A and B. [1] Mutagenesis studies using the heterologous expression system together with *in silico* analysis shed light on Pyxidicycline biosynthesis, while IC50 determinations using human pathogens and cancer cell lines revealed the broad biological activity of the new compounds. Subsequent topoisomerase inhibition assays showed strong affinity to - and inhibition of - unwinding topoisomerases such as *E. coli* topoisomerase IV and human topoisomerase I by Pyxidicyclines, while *E. coli* topoisomerase II (gyrase) was not inhibited at concentrations up to 50 µg/ml.[1]

[1] A. Panter, F. Krug, D. Baumann, S. Müller, R. ***Chem. Sci.***, 2018,**9**, 4898-490

L13. Systems Analysis of Orphan Natural Product Biosynthetic Gene Clusters by Reporter-Guided Screening of Symbiotic Cyanobacteria

Daniel Dehm[1], Julia Krumbholz[1], Anton Liaimer[2], María García-Altares Pérez[3], Christian Hertweck[3], Elke Dittmann[1]

[1] dehm@uni-potsdam.de, elke.dittmann@uni-potsdam.de; University of Potsdam, Institute of Biochemistry and Biology, Department of Microbiology, Potsdam, Germany

[2] anton.liaimer@uit.no; University of Tromsø, Faculty of Biosciences, Fisheries and Economics, Molecular Environments Group, Department of Arctic and Marine Biology, Tromsø, Norway

[3] christian.hertweck@leibniz-hki.de; Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany

Nostoc punctiforme is a cyanobacterium capable of undergoing symbiotic interactions with a variety of different plant partners. [1] Besides that *N. punctiforme* has very complex life cycle and a huge genome that encodes for a large number structurally diverse secondary metabolite gene clusters including NRPS, PKS, RiPPs and hybrids of those. While bioinformatic comparisons revealed this huge potential secondary metabolome in most cases the actual products are not detectable under conventional growth conditions. Those orphan gene clusters seem to be silent and only induced under very specific conditions, such as the interaction with symbiotic partners, changes in population density or varying nutrient supply. For instance, previously conducted MALDI-Imaging studies first time revealed the production of new types of compounds while *N. punctiforme* is in its symbiotic state. [2]

The presented project aims at identifying these specific conditions at which some of the encoded secondary metabolites get induced with the aim to reveal the structure and biological activity of those so far cryptic entities. Therefore, a complete set of transcriptional reporter strains was produced each reporting for different orphan secondary metabolite cluster detected via antiSMASH. The results point out that the largest fraction of these cryptic secondary metabolite gene clusters are not silent but rather show a very distinct but spatially restricted transcription pattern within a single colony. We could also show that cell density and CO_2 concentration in the medium are two factors that trigger an increased transcriptional response of a different subset of secondary metabolite clusters.

[1] Meeks, J. C. American Institute of Biological Sciences 48 1998 (4), 266–276.

[2] Liaimer, A; Helfrich, E. J. N.; Hinrichs, K; Guljamow, A.; Ishida, K.; Hertweck, C.; Dittmann, E. *PNAS* **2015** 112 (6), 1862–1867.

L14. SARP-DRIVEN ACTIVATION OF ANTIBIOTIC GENE CLUSTERS

Yvonne Mast[1,2]*, Janina Krause*[1]*, Ira Handayani*[1] *and Franziska Handel*[1,2]

[1] Mikrobiologie/Biotechnologie, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Universität Tübingen, 72076 Tübingen, Germany. Email: yvonne.mast@biotech.uni-tuebingen.de

[2] Deutsches Zentrum für Infektionsforschung (DZIF), Partner-Standort Tübingen

Actinomycetes, particularly the genus *Streptomyces,* are the most versatile producers of natural compounds. They synthesize about two-thirds of all antibiotics known to date. The antibiotic biosynthesis in these organisms is highly coordinated and subject to diverse environmental and physiological (pre)conditions. Important nodes of regulation are represented by particular pleiotropic, as well as pathway-specific regulators. In my talk, I will give an example how pathway-specific SARP-type regulators can be used in order to exploit the biosynthetic potential of actinomycetes.

PapR2 is a SARP-type regulator from *Streptomyces pristinaespiralis*. Here it acts as an essential activator of pristinamycin biosynthesis, a streptogramin antibiotic, consisting of the two chemically unrelated antibiotics pristinamycin I and pristinamycin II.[1] Overexpression of PapR2 in *Streptomyces lividans* results in the activation of the silent undecylprodigiosin gene cluster. Since SARP-type regulatory genes occur in a lot of different antibiotic gene clusters, especially these regulators represent attractive entities to be used for activation of antibiotic biosyntheses in general. Here, we present an example of a SARP-type overexpression in a novel *Streptomyces* isolate, which leads to the activation of an antibiotic biosynthesis. Furthermore, we will illustrate how bioinformatics efficiently can be applied in order to assign a substance to the observed antibiotic activity.

[1] Mast Y, Guezguez J, Handel F, Schinko E. *Appl Environ Microbiol.* **2015**, *81,* 6621-6636.

L15. Phenazine Antibiotic Cocktails via Diversity-oriented Biosynthesis

Yi-Ming Shi[1], Alexander O. Brachmann[1,3], Margaretha Westphalen[1], and Helge B. Bode[1,2*]

[1]Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, Max-von-Laue-Strasse 9, Frankfurt am Main 60438, Germany. Email: shi@uni-frankfurt.de

[2]Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt, Max-von-Laue-Strasse 15, Frankfurt am Main 60438, Germany.

[3]Current address: Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zurich, Vladimir-Prelog-Weg 4, 8093 Zurich, Switzerland.

Biosynthetic gene clusters (BGCs) bridging genotype and phenotype continuously evolve through gene mutations and recombinations to generate chemical diversity. Phenazine (*phz*) BGCs are widespread in bacteria, and the biosynthetic mechanisms of the formation of the phenazine structural core have been illuminated in the last decade. However, little is known about the complex phenazine post-modification machinery. Here, we report the diversity-oriented modifications of phenazines through two BGCs consisting of one typical *phz* operon and three subclusters. These BGCs are present in the entomopathogenic bacterium *Xenorhabdus szentirmaii* living in symbiosis with nematodes. A previously unidentified aldehyde intermediate is a critical linkage of these BGCs, allowing multiple enzymatic and non-enzymatic modifications. The evaluation of their antibiotic activity suggests a highly effective strategy to convert Gram-positive specific phenazines into broad-spectrum antibiotics, which might help the bacteria-nematode complex to maintain the special environmental niche.

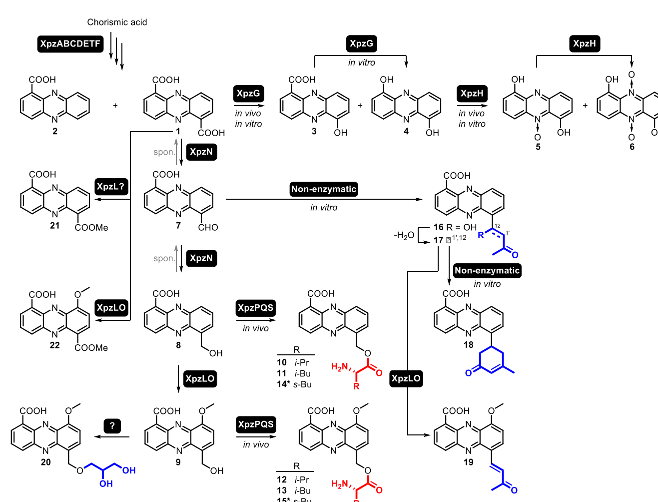

Figure 1. Structures and biosynthetic pathway of phenazines form *X. szentirmaii.*

POSTER ABSTRACTS

P1. Peroxidase activity as a part of Self-resistance mechanisms in cosmomycin D producing strain *Streptomyces olindensis* DAUFPE 5622”

Roger D. Castillo-Arteaga[1,2]*, Leandro Garrido*[2]*, Ruth Ortiz*[2]*, Alejandra Ferreira*[2]*, Manuella Dourado*[2]*, Welington L. Araújo*[2]*, Paul F. Long*[3]*, Bertolt Gust*[1] *and Gabriel Padilla*[2]

[1] Pharmazeutische Biologie, Pharmazeutisches Institut, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Deutschland. Email: roger.castillo@mnf.uni-tuebingen.de

[2] Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, CEP 005508-900, Brazil.

[3] Institute of Pharmaceutical Science, King's College London, 150 Stamford Street SE1 9NH, London, United Kingdom.

Antibiotic producer microorganisms require one or more self-resistance determinants to survival during antibiotic production. The effectors of these mechanisms are proteins that inactivate the antibiotic, facilitate its export or modify the host to render it insensitive to the molecule [1]. *Streptomyces olindensis* DAUFPE 5622 produces the antitumoral Cosmomycin D, a molecule member of the anthracycline family. Here, we identified the self-resistance genes of the Cosmomycin D biosynthetic genecluster including three components: an ATP-dependent efflux pump for transport, a resistance to DNA damage through an UvrA class IIa protein and a response to reactive oxygen species (ROS) by the enzyme Mycothiol peroxidase (MPx). We cloned and expressed the resistance genes from *Streptomyces olindensis* in *Streptomyces albus,* an anthracycline sensitive host, enclosing in the expression plasmid PEM4A with the constitutive promoter ermE*p. We evaluated the capacity of resistance to several concentrations of both anthracyclines Cosmomycin D and the commercial Doxorubicin, widely used for the treatment of human cancers. The expressions of each resistance components enhance the response to anthracyclines.

[1] Sugiyama, M. Structural biological study of self-resistance determinants in antibiotic-producing actinomycetes. *The Journal of Antibiotics*, **2015**, 1–8.

P2. PRODUCTION OF DIVERSE DITERPENOID PYRONES BY RE-CONSTRUCTION AND RE-DESIGNING OF FUNGAL BIOSYNTHETIC PATHWAYS IN *ASPERGILLUS ORYZAE*

Kento Tsukada[1]*, Shono Shinki*[2]*, Akiho Kaneko*[2] *and Teigo Asai*[2*]

[1] Graduate School of Pharmaceutical Sciences, Tohoku University, 9808578 Sendai, Japan. Email: tsukadakento@gmail.com.

[2] Graduate School of Arts and Sciences, The University of Tokyo, 1530041 Komaba, Tokyo. Email: tasai@bio.c.u-tokyo.ac.jp.

Fungal diterpenoid pyrone (DP) is a member of meroterpenoids that comes from polyketide (pyrone) and terpene (C20). The biosynthetic pathways are generally distributed in entomopathogenic fungi and plant pathogenic fungi, and the natural DPs produced by them exhibit a wide range of biological activities. Interestingly, even a small structural differences in each molecule gives them different biological activities such as anti-proliferative activity against cancer cell lines and immunosuppressive activity. Thus we focused on fungal DPs and carried out re-construction and re-designing of their biosynthetic pathways in *Aspergillus oryzae* to generate structurally diverse fungal DPs. We performed genome mining and found putative DP biosynthetic gene clusters in five fungal genera, *Arthrinium*, *Metarhizium*, *Fusarium*, *Macrophomina*, *Colletotrichum*. They all have five common genes that might be responsible for biosynthesis of common intermediate BR-050[1]. However, they are different each other in its accessory genes involved in tailoring steps. The analysis also suggested that those biosynthetic pathways could generate structurally diverse DPs including novel analogs. To reveal all the biosynthetic machineries, we re-constructed each biosynthetic pathway in *A. oryzae*. The results allowed us to reveal all the functions of biosynthetic genes in each pathway and achieve total biosynthesis of known subglutinols[2], higginsianins[3] and novel natural DPs. In the DP biosynthetic pathways, the tailoring enzymes could be divided into two types in modification places at prenyl side chain or pyrone. We hypothesized that they could work at prenyl side chain or pyrone independently. Thus we re-designed DP pathways by expressing them in various combinations, and successfully generated diverse DP derivatives which are not programmed in nature. Now, we are evaluating biological activities of the DPs produced in this study.

[1] Pittayakhajonwut, P. *et al. Tetrahedron* **2009**, *65*, 6069-6073.

[2] Lee, J. C. *et al. J. Org. Chem.* **1995**, *60*, 7076-7077.

[3] Cimmino, A. *et al. J. Nat. Prod.* **2016**, *79*, 116-125.

P3. REGULATION OF ANTHRAQUINONE BIOSYNTHESIS IN PHOTORHABDUS LUMINESCENS

Lukas Kreling[1], Antje K. Heinrich[1], Qiuqin Zhou[1], Eva Huber[2], Michael Groll[2] and Helge B. Bode[1, 3]

[1]Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt, Germany. Email: s8092663@stud.uni-frankfurt.de.

[2]Lehrstuhl für Biochemie, TU München, Lichtenbergstr. 4, 85748 Garching, Germany

[3]Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt, Frankfurt am Main, 60438, Germany.

The Gram-negative, entomopathogenic bacterium *Photorhabdus luminescens* lives in mutualistic symbiosis with nematodes of the genus *Heterorhabdits*[1]. To maintain the symbiotic relationship, the bacteria produce an array of natural products (NP) playing a crucial role in the development of the host and its survival[2]. Strikingly, *Photorhabdus luminescens* is one of a few examples where a PKS II system, normally found in Gram-positive bacteria, synthesizes a set of anthraquinones (AQs), whose function remains mostly unknown[3]. The biosynthesis is controlled by a certain amount of different regulatory factors. While the post-transcriptional factor Hfq acts as a global regulator and is essential for NP production[4], we showed that the pathway-specific transcription factor AntJ increases the AQ production when being overproduced. Furthermore, our investigations revealed a set of homologous methyltransferases being involved in methylation of the precursor AQ-256 resulting in various methylated derivatives. Upon deletion of the corresponding genes, only AQ-256 is detectable via HPLC-MS analysis. The herewith documented experiments are an approach to elucidate the effect of different regulatory factors on AQ production and to generate AQ-overproducing strains.

[1] S. A. Joyce, L. Lango, D. J. Clarke, *Adv. Appl. Microbiol.* **2011**, *76*, 1–25.

[2] E. Duchaud, C. Rusniok, L. Frangeul, C. Buchrieser, A. Givaudan, S. Taourit, S. Bocs, C. Boursaux-Eude, M. Chandler, J.-F. Charles, et al., *Nat. Biotechnol.* **2003**, *21*, 1307.

[3] A. O. Brachmann, S. A. Joyce, H. Jenke-Kodama, G. Schwär, D. J. Clarke, H. B. Bode, *ChemBioChem* **2007**, *8*, 1721–1728.

[4] N. J. Tobias, A. K. Heinrich, H. Eresmann, P. R. Wright, N. Neubacher, R. Backofen, H. B. Bode, *Environ. Microbiol.* **2017**, *19*, 119–129.

P4. New DKxanthen-like Secondary Metabolite from a Myxobacterium Shows Antimicrobial and Cytotoxic Activity

Alexander Popoff[1,2], *Ronald Garcia*[1,2], *Rolf Müller*[1,2*]

[1] Helmholtz-Institut für Pharmazeutische Forschung Saarland, 66123 Saarbrücken, Germany. Email: Alexander.Popoff@helmholtz-hzi.de

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

Myxobacteria are an excellent source for new bioactive natural products. We present here a newly isolated Myxobacterium MSr 11954 from the suborder *Soranginae* that shows a Streptomyces-like phenotype. Crude acetone extracts from MSr11954 cultures had good activity against *M. hiemalis* and *M. luteus*. High resolution LC-MS based fractionation reduced the number of molecules which could be responsible for the observed bioactivity down to a group of compounds with similar MS/MS fragmentation pattern. One representative was isolated and characterized applying high resolution 2D NMR techniques. The structure showed intriguing similarity to compounds from the DKxanthen family previously isolated from *Myxococcus xanthus*. However, the newly discovered DKxanthen derivative displays cytotoxic and moderate antimicrobial activity, whereas previously reported DKxanthen congeners were assigned to play a role during *M. xanthus* sporulation. This is a striking example for functional divergence within a structurally-defined class of natural products.[1]

[1] P. Meiser, H. B. Bode, R. Müller, *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 19128.

P5. Ultrahigh Density Cultivation Enables Capturing The Secondary Metabolite Diversities In *Nostoc Punctiforme* PCC 73102

Julia Krumbholz[1], Daniel Dehm[1], Keishi Ishida[2], Christian Hertweck[2,3], Elke Dittmann[1]*

[1] University of Potsdam, Institute for Biochemistry and Biology, Department of Microbiology, 14476 Potsdam-Golm, Germany. Email: julia.krumbholz@uni-potsdam.de, elke.dittmann@uni-potsdam.de

[2] Leibniz Institute for Natural Product Research and Infection Biology (HKI), 07745 Jena, Germany. Email: keishi.ishida@leibniz-hki.de

[3] Friedrich Schiller University, Natural Product Chemistry, 07745 Jena, Germany. Email: christian.hertweck@leibniz-hki.de

Terrestrial cyanobacteria such as *Nostoc punctiforme* show a great capability for the production of diverse secondary metabolites, including ribosomal peptide, non-ribosomal peptide and polyketide peptide classes. With the help of the bioinformatical platform AntiSMASH we detected a large set of orphan biosynthetic gene clusters (BGCs) in the cyanobacterial strain *Nostoc punctiforme* PCC 73102. Yet, the majority of BGCs is silent under standard cultivation conditions.

Recently, we have discovered that the production of the secondary metabolome is upregulated when cultivating *N. punctiforme* at very high cell densities [1]. HPLC analysis of cell pellets and supernatants of a high density (HD) culture compared to a conventional grown culture showed an increase of metabolite production, in both, cell pellet and supernatant. In order to assign novel metabolite families to the specific biosynthetic pathways we utilized a set of *N. punctforme* CFP reporter mutant strains each strain reporting for one of the orphan BGCs. These strains were cultivated conventionally and as high density cell cultures. The analysis of both cultivation approaches with a fluorescence microscope revealed that several gene clusters are upregulated at ultrahigh cell density. Conventionally grown reporter strains corresponding to the upregulated gene clusters were then treated with the supernatant of a wildtype HD culture, to estimate if the accumulated metabolites in the supernatant contribute to an upregulation of BGC expression. This experiment showed that a part of the gene clusters which are upregulated upon HD cultivation are also upregulated when treated with HD supernatant. Currently, we are trying to elucidate the underlying signal cascade with the aim to uncover the quorum sensing network in *N. punctiforme*.

[1] A. Guljamow, M. Kreische, K. Ishida, A. Liaimer, B. Altermark, L. Bähr, C. Hertweck, R. Ehwald, E. Dittmann, *Appl Environ Microbiol.* **2017**, *83*, e01510-17.

P6. MASSITHIAZOLE, A NATURAL PRODUCT FROM *MASSILIA* SP.

Jan Diettrich[1]*, Markus Nett*[1]

[1]Laboratory of Technical Biology, Department of Biochemical and Chemical Engineering, TU Dortmund University, Emil-Figge-Straße 65, 44227 Dortmund

In recent years the number of multidrug-resistant organisms has dramatically increased. In combination with treatment failures these organisms became a considerable threat to public health worldwide. The emergence of resistance to antimicrobial compounds is even more threatening considering that the number of newly approved drugs per year is decreasing within the last decades [1].

The majority of antibiotics in clinical use were originally discovered from Gram-positive bacteria. In recent years Gram-negative bacteria have attracted increasing attention as producers of antimicrobial metabolites [2].

Massilia sp. 4-1 is a Gram-negative, aerobic, non-spore forming rod-shaped Betaproteobacterium which is known to produce the antibiotic agent violacein [3]. According to the OSMAC concept (One strain-many compounds) microbial strains often have the potential to produce a variety of secondary metabolites under different cultivation conditions [4]. We investigated the metabolome of *Massilia* sp. 4-1 under different cultivation conditions and were able to identify a new compound. Its structure was elucidated on the basis of extensive spectroscopic analyses, including mass spectrometry as well as 1D and 2D NMR measurements.

[1] Magiorakos, a, Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Hindler, J. F. (2011). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria : an international ixpert proposal for interim standard definitions for acquired resistance. *Microbiology*, *18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

[2] Masschelein, J., Jenner, M., & Challis, G. L. (2017). Antibiotics from Gram-negative bacteria: a comprehensive overview and selected biosynthetic highlights. *Nat. Prod. Rep.*, *34*(7), 712–783. https://doi.org/10.1039/C7NP00010C

[3] Agematu, H., Suzuki, K., & Tsuya, H. (2011). Massilia sp. BS-1, a Novel Violacein-Producing Bacterium Isolated from Soil. *Bioscience, Biotechnology, and Biochemistry*, *75*(10), 2008–2010. https://doi.org/10.1271/bbb.100729

[4] Bode, H. B., Bethe, B., Höfs, R., & Zeeck, A. (2002). Big effects from small changes: possible ways to explore nature's chemical diversity. *Chembiochem*, *3*(7), 619–627. https://doi.org/10.1002/1439-7633(20020703)3:7<619::AD-CBIC619>3.0.CO;2-9

P7. Structure and Biosynthesis of Rhabdopeptide/Xenortide-like Peptides in *Xenorhabdus innexi* and *Xenorhabdus cabanillasii*

Lei Zhao[1], *Xiaofeng Cai*[1], *Marcel Kaiser*[2,3] *and Helge B. Bode*[1,4]

[1] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany. Email: zhao@bio.uni-frankfurt.de.

[2] Parasite Chemotherapy, Swiss Tropical and Public Health Institute, 4051 Basel, Switzerland

[3] University of Basel, 4003 Basel, Switzerland

[4] Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany

Rhabdopeptide/xenortide-like peptides (RXPs) are a unique class of peptides exclusively found in entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*.[1,2] They are derived from monomodular non-ribosomal peptide synthetases (NRPSs) and are structurally diverse.[1,2] Here we report the structure and biosynthesis of RXPs from two NRPS systems, namely InxABC and CabABCD, in *Xenorhabdus innexi* and *Xenorhasbdus cabanillasii*, respectively. InxABC is flexible to make diverse RXPs with putrescine or ammonia as the C-terminal amines, which are the most active RXP derivatives against protozoan parasites known to date. While CabABCD is relatively specific and could be used as a tool to make new defined RXPs via exchange of adenylation or adenylation-methyltransferase domains.

[1] D. Reimer, K. N. Cowles, A. Proschak, F. I. Nollmann, A. J. Dowling, M. Kaiser, R. Ffrench-Constant, H. Goodrich-Blair, H. B. Bode, *ChemBioChem* **2013**, *14*, 1991−1997.

[2] X. Cai, S. Nowak, F. Wesche, I. Bischoff, M. Kaiser, R. Fürst, H. B. Bode, *Nat. Chem.* **2017**, *9*, 379−386.

P8. Comparison of DI-FTICR and LC-qTOF for the comprehensive profiling of myxobacterial secondary metabolomes

Chantal Bader[1], *Patrick Haack*[1], *Fabian Panter*[1], *Daniel Krug*[1] *and Rolf Müller*[1]

[1] Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research (HIPS), 66123 Saarbrücken, Germany. * Email: Chantal.Bader@helmholtz-hzi.de.

Myxobacteria are a prolific source of natural products featuring chemical novelty as well as promising bioactivites. Follow-up of these bioactivites found in crude extracts using various analytical techniques already led to the discovery of numerous valuable molecules including cystobactamide[1] —a compound showing potent anti Gram-negative activity and epothilone[2] —a cytostatic drug with approval of the FDA. Currently, investigation of the myxobacterial secondary metabolome is based mostly on screening approaches using LC-*hr*MS and subsequent dereplication for the discovery of new natural products from extracts of these promising microbes. However, the genomic revolution strikingly has shown that current methods likely fall short of uncovering the full complement of myxobacterial secondary metabolite diversity. In this study we aim to evaluate the potential of DI-FTICR for extending the scope of myxobacterial metabolites detectable under laboratory conditions. Along these lines we conduct a comprehensive comparison with our LC-*q*TOF platform and characterize the chemical space covered by both methods. This comparison is motivated by the notion that LC-*q*TOF is a robust platform available in most microbial natural product laboratories, whereas high-resolution mass spectra combined with retention time information achieves identification with increased confidence - making fast scan rates during data acquisition irreplaceable. The defining feature of DI-FTICR on the other hand is the capability to generate spectra with a resolution up to 1000000 and thus supporting compound identification solely based on precise mass even from complex mixtures like bacterial crude extracts. Results from the two-platform contest are presented and implications for natural products discovery are discussed.

[1] S. Baumann, J. Herrmann et al., *Angew. Chemie*. **2014**, *126*, 14835-14839.

[2] K. Gerth, N. Bedorf et al., *J Antibiot*. **1996**, *49*(6), 560-563

P9. ENGINEERING BIOSYNTHETIC PATHWAYS FOR TAILORED PEPTIDES

Ines Folger, David Niquille, Anna Camus and Donald Hilvert[*]

Laboratory of Organic Chemistry, ETH Zurich, 8093 Zurich, Switzerland, E-mail: hilvert@org.chem.ethz.ch

Nonribosomal peptide synthetases (NRPS) produce a wide range of biologically active natural products that constitute a valuable source of therapeutic agents.[1] Although redesign of NRPS pathways for the biosynthesis of tailored peptides represents a promising alternative to laborious and limited semisynthetic approaches, such engineered pathways often suffer from low product yields.[2] Here we show that tyrocidine synthetase can be effectively reprogrammed to produce modified cyclic peptides *in vitro* in yields comparable to those observed with the natural system. Specifically, we reengineered an adenylation domain in an elongation module to activate and incorporate para-substituted Phe analogues, including bioorthogonal “click” handles, enabling facile functionalization of the tyrocidine scaffold as well as generation of peptide libraries.

[1] R. D. Süssmuth, A. Mainz, *Angew. Chem. Int. Ed.* **2017**, *56*, 3770-3821.
[2] a) M. J. Calcott, D. F. Ackerley, *BMC Microbiol.* **2015**, *15*, 162;
b) H. Kries, D. L. Niquille, D. Hilvert, *Chem. Biol.* **2015**, *22*, 640-648;
c) K. Bloudoff, T. M. Schmeing, *Biochim. Biophys. Acta, Proteins Proteomics* **2017**.

P10. Screening for novel protein synthesis inhibitors from uncharacterized streptomycetes of the 'Tübingen strain collection'

Franziska Handel[1], *Katharina Wex*[2], *Wolfgang Wohlleben*[1] *and Yvonne Mast*[1]

[1] Interfacultary Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbiology / Biotechnology, University of Tübingen, 72076 Tübingen, Germany. Email: franziska.handel@student.uni-tuebingen.de

[2] Interfacultary Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbial Bioactive Compounds, University of Tübingen, 72076 Tübingen, Germany.

Antibiotics are our most important weapon in the treatment of bacterial infections, including life-threatening hospital infections. Approximately 70% of all known antibiotics are produced by actinomycetes, whereat streptomycetes make up the largest part of it. Over time, antibiotic resistances have become a huge major threat to public health and thus it is urgently needed to find new effective antibiotics. The bacterial ribosome is a hotspot for the action of numerous successful antibiotics. However, not all promising binding sites are therapeutically exploited. The aim of project TTU 09.812, funded by the German Center for Infection Research (DZIF), is to identify and characterize new protein synthesis inhibitors.

The Tübingen Streptomyces strain collection harbors >2.000 natural product producers, mostly actinomycetes. For ~100 of these strains genome sequence data are available. In order to find new protein synthesis inhibitors, these genome-sequenced strains have been pre-screened by a protein synthesis inhibitor-sensitive reporter assay in the group of Prof. Heike Brötz-Oesterhelt (University Tübingen). The identified protein synthesis inhibitor producers are analyzed with the bioinformatic tools AntiSMASH1 and ARTS2 in order to identify the corresponding antibiotic gene clusters. The candidate strains are grown in different culture media following the OSMAC strategy and culture extracts are analyzed by HPLC-MS/MS for determination of antibiotics. Producers of unknown substances will be analyzed in detail.

Our strategy is to use different bioinformatic tools in order to identify novel protein synthesis inhibitors from uncharacterized streptomycetes of the Tübingen strain collection.

[1] Tilmann Weber, Kai Blin, Srikanth Duddela, Daniel Krug, Hyun Uk Kim, Robert Bruccoleri, Sang Yup Lee, Michael A. Fischbach, Rolf Müller, Wolfgang Wohlleben, Rainer Breitling, Eriko Takano & Marnix H. Medema. *Nucleic Acids Res.* **2015** doi: 10.1093/nar/gkv437.

[2] Alanjary,M., Kronmiller,B., Adamek,M., Blin,K., Weber,T., Huson,D., Philmus,B. and Ziemert,N. *Nucleic Acids Res.* **2017**, 10.1093/nar/gkx360.

P11. Design of vector-based expression systems for Myxobacteria

Sebastian Kruth[1] & *Markus Nett*[1]

[1]Laboratory of Technical Biology,
Department of Biochemical and Chemical Engineering,
TU Dortmund University, Emil-Figge-Straße 65, 44227 Dortmund
Email: sebastian.kruth@tu-dortmund.de; markus.nett@tu-dortmund.de

Myxobacteria are generally known for the production of a wide range of bioactive secondary metabolites. Many among these metabolites have potent antibacterial or cytotoxic effects, making them valuable candidates for drug development.[1,2] Regardless of their biosynthetic potential, there are still few tools available for the genetic engineering of myxobacteria. In particular, gene overexpression is hindered due to the lack of appropriate plasmids that can autonomously replicate in myxobacterial cells. Therefore, any new genetic information has to be chromosomally integrated.

Here, we will outline our strategy for the construction of myxobacterial expression vectors. The corresponding plasmids feature the origin of replication from pMF1, which was found in 2008 in *Myxococcus falvus* 124B02 and, up to now, represents the only known naturally occurring plasmid from myxobacteria.[3] We will describe the selection of useful promoter, ribosomal binding site and terminator sequences, as well as the general assembly strategy for the expression plasmids.

[1.] Schäberle, T. F. *et al.* Antibiotics from myxobacteria. *Nat. Prod. Rep.* **31**, 953-972 (2014).

[2.] Herrmann J. *et al.* Natural products from myxobacteria: novel metabolites and bioactivities. *Nat. Prod. Rep.* **34**, 135-160 (2017).

[3.] Zhao, J. Y. *et al.* Discovery of the autonomously replicating plasmid pMF1 from *Myxococcus fulvus* and development of a gene cloning system in M*yxococcus xanthus*. *Appl. Environ. Microbiol.* **74,** 1980–1987 (2008).

P12. UNCOVERING NEMATICIDAL NATURAL PRODUCTS FROM NEMATODE SYMBIOTIC BACTERIA

Desalegne Abebew[1], Fatemeh Sayedain[1], Christian Pohl[2] and Helge. B. Bode[1]

[1] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt, Germany. Email: abebewsyit@bio.uni-frankfurt.de.

[2] Buchmann Institute for Molecular Life Sciences (BMLS), Goethe University Frankfurt, Max-von-Laue-Str. 15, 60438 Frankfurt, Germany.

Nematodes are a major burden for human health especially in the non-western countries. Additionally, they cause major losses of crop and animals by infecting roots or intestines, respectively [1], [2]. Therefore, it is important to have new nematicidal compounds. During our analysis of natural products from the entomopathogenic bacterial genera *Xenorhabdus* and *Photorhabdus*, we found that *Xenorhabdus* strains displayed promising nematicidal activity against the root-knot nematode *Meloidogyne incognita* and free-living nematode *Caenorhabditis elegans*. The cell-free and heat-treated culture filtrates of some *Xenorhabdus* strains killed almost all the second stage juveniles of *M. incognita and C. elegans* within 48 hours. When supernatants from Xenorhabdus strains were used that did only produce one desired natural product, we could identify fabclavine and other natural products as nematicidal compounds. Among them, fabclavine is the most efficient compound that initially paralyzes the nematodes. Finding of such nematicidal natural products may provide template for uncovering effective and environmentally safe alternatives to the presently used agricultural toxic synthetic nematicides and might result also in nematocidal natural products with less side effects in human treatment.

[1] S. Bulgheresi. All the microbiology nematodes can teach us. *FEMS Microbiol. Ecol.*, 2016, **92**, fiw007.

[2] L. A. Lacey, D. Grzywacz, D. I. Shapiro-Ilan, R. Frutos, M. Brownbridge and M. S. Goettel. Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol.*, 2015, **132**,1–41.

P13. BIOSYNTHESIS OF *N*-METHYLATED PEPTIDES USING REPROGRAMMED NRPSS

Anna Camus, David Niquille, Ines Folger, Douglas Hansen and Donald Hilvert[*]

Laboratory of Organic Chemistry, ETH Zurich, 8093 Zurich, Switzerland. Email: donald.hilvert@org.chem.ethz.ch.

Nonribosomal peptides are a clinically important class of natural products that display a wide range of antimicrobial, immunosuppressive and anticancer activities. Despite their therapeutic potential, nonribosomal peptides often suffer from proteolytic instability and low membrane permeability. Such shortcomings can be overcome by backbone *N*-methylation as illustrated by the nonribosomal peptide cyclosporin A. The corresponding nonribosomal peptide synthetase (NRPS) contains dedicated methylation (M) domains responsible for the online modification of the peptide backbone. Here, we demonstrate M domain-independent incorporation of *N*-methylated amino acids by reprogramming a gatekeeper NRPS adenylation (A) domain using a combination of yeast surface display and fluorescence-activated cell sorting.[1] Importantly, the engineered A domain functions with downstream domains to produce backbone-modified peptides *in vitro*. Furthermore, the engineered *N*-methyl phenylalanine activating A domain is compatible with a previously engineered A domain for *O*-propargyl tyrosine[2] as demonstrated by the nonribosomal biosynthesis of a doubly modified peptide. Our results highlight the great potential of A domain engineering for the strategic incorporation of novel building blocks into nonribosomal peptides.

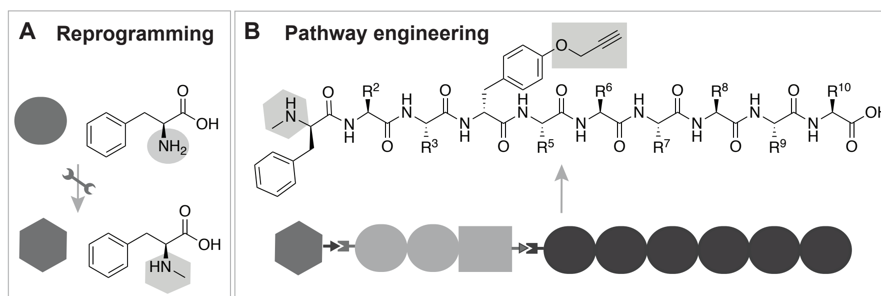

Figure 1. A. Reprogramming of an NRPS A domain (circle) for the incorporation of *N*-methylated phenylalanine (hexagon). **B.** *In vitro* biosynthesis of a doubly modified peptide by a modified NRPS containing two engineered A domains (hexagon and square).

[1] D. L. Niquille, D. A. Hansen, T. Mori, D. Fercher, H. Kries, D. Hilvert, *Nature Chemistry* **2018**, *10*, 282-287.

[2] H. Kries, R. Wachtel, A. Pabst, B. Wanner, D. Niquille, D. Hilvert, *Angewandte Chemie International Edition* **2014**, *53*, 10105-10108.

P14. Fascinating *Stachybotrys* – Novel Cytotoxic Meroterpenoids, Chemically Inspired Isolation Approaches and Application of an LC-MS/MS multi-method

Annika Jagels[1], Viktoria Lindemann[1], Yannick Hövelmann[1], Alexa Zielinski[1], Melanie Esselen[1], Jens Köhler[2], Florian Hübner[1], Hans-Ulrich Humpf[1]

[1] Institute of Food Chemistry, Westfälische Wilhelms-Universität Münster, Germany

[2] Institute of Pharmaceutical and Medicinal Chemistry, Westfälische Wilhelms-Universität Münster, Germany

The genus *Stachybotrys* is commonly associated with indoor water damage and has become increasingly popular since the 1990s within cases of idiopathic pulmonary hemosiderosis in infants [1]. There are still controversial discussions, whether the fungal growth was correlated with the diseases. *Stachybotrys* produces a broad variety of secondary metabolites including macrocyclic trichothecenes, atranones and phenylspirodrimanes. In the past, investigations concerning the phenylspirodrimanes have been neglected, although they occur in even higher levels and in a remarkable multitude of different compounds. Moreover, they are known to be cytotoxic as well as immunosuppressant and additionally, combinatory effects among them have never been considered. For this purpose, a series of phenylspirodrimanes occurring in all common *Stachybotrys* species were isolated in order to be used as reference standards for the development of an LC-MS/MS quantitation multi-method. The general objective of this multi-method is to enable the determination in fungal extracts and various matrices, such as building materials, dust, feedstuff, and physiological samples. A noteworthy representative of the phenylspirodrimane family is stachybotrydial. Its structural properties led to the development of chemically inspired isolation approaches, either based on supplementation of the culture media or semisynthesis. Both strategies could potentially be utilized to simplify access to certain reference standards from *Stachybotrys*.

In the course of gaining new insights into the secondary metabolite profile, three novel cytotoxic meroterpenoids, namely stachybotrychromenes A-C, were discovered [2]. Their structures were elucidated by extensive spectroscopic analysis (NMR, HRMS, CD). First cytotoxic effects have been determined and the new compounds clearly contribute to the overall toxicity profile of this fungus.

Consequently, the genus *Stachybotrys* offers an enormous potential for future investigations to conclusively determine the role of this fungus within specific building-related case studies as described above and to clarify the relationship between human health and fungal contamination in indoor environments in general.

[1] R. A. Etzel, E. Montaña, W. G. Sorenson, G. J. Kullman, T. M. Allan, D. G. Dearborn, *Arch Pediatr Adolesc Med* **1998**, *152*.

[2] A. Jagels, Y. Hövelmann, A. Zielinski, M. Esselen, J. Köhler, F. Hübner, H.-U. Humpf, *Mycotoxin research* **2018**.

P15. SEARCHING FOR THE ACIVICIN BIOSYNTHESIS GENE CLUSTER

Marius Bader[1,2], *Ghazaleh Jahanshah*[1], *Patrik Stange*[3], *Harald Gross*[1] *and Leonard Kaysser*[1,2 *]

[1] Department of Pharmaceutical Biology, University of Tübingen, 72076 Tübingen, Germany. Email: marius.bader@uni-tuebingen.de

[2] German Centre for Infection Research (DZIF), partner site Tübingen, 72076 Tübingen, Germany

[3] Institute of organic Chemistry, University of Tübingen, 72076 Tübingen, Germany

Natural products, especially from the genus *Streptomyces* are one of the main sources of lead structures in pharmaceutical drug design. Isoxazoles are widely used in medicinal chemistry as building blocks for the development of diverse synthethic small molecule therapeutics. However, they are rarely found in bioactive natural products. Acivicin, produced by *S. sviceus* ATCC 29083 is an isoxazole-containing modified amino acid[1]. It is an antimetabolite of glutamine- and asparagine-dependent enzymes and it has a wide spread activity against bacteria, fungi and other eukaryotic cells. Its warhead, a 4-chloro-dihydroisoxazole ring system, is attacked by a reactive catalytic residue in the active site of target enzymes, e.g. a cysteine moiety in glutamine amidotransferases. After the nucleophilic attack, displacement of the chloro substituent generates a stable enzyme-inhibitor complex. The unique structure and mechanism of action makes this compound an interesting lead for drug design.

For the better understanding of the biosynthesis, previous feeding experiments have shown, that L-ornithine is the precursor of acivicin[2]. Furthermore, 3-hydroxy-ornithine was postulated as a likely intermediate in acivicin biosynthesis. Based on this information we screened the genome of the acivicin producer for enzymes, which could be involved in hydroxylation, halogenation or ring closure of the molecule. Thereby, three putative biosynthetic gene cluster were found. We are currently evaluating these gene clusters by heterologous pathway expression.

[1] L. J. Hanka, A. Dietz, *Antimicrobial agents and chemotherapy* **1973**, *3*, 425-431.

[2] S. J. Gould, S. Ju, *Journal of the American Chemical Society* **1989**, *111*, 2329-2331.

P16. Enzyme Thioamide Formation in Bacterial DNA Antimetabolite Pathway

*Agnieszka Litomska[1], Keishi Ishida[1], Kyle L. Dunbar[1], Marco Boettger[1], Sébastien Coyne[1], Christian Hertweck[1,2]**

[1]Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; [2]Natural Product Chemistry, Friedrich Schiller University, Jena, Germany. E-mail: keishi.ishida@leibniz-hki.de.

Sulfur is an element that had played a key role in the evolution of life and is essential to all known living species. The replacement of oxygen with sulfur in biomolecules, such as thioamides, may dramatically alter their functions. An important example of a thioamide-containing compound is the rationally designed DNA-targeting antimetabolite 6-thioguanine (6TG), which is in clinical use for the treatment of various severe diseases including chronic and acute leukemias.[1] Surprisingly, 6TG is also a critical virulence factor of pathogenic bacteria (*Erwinia amylovora*) responsible for fire blight, a devastating apple tree disease.[2,3] Mechanistic and phylogenetic analyses revealed that 6TG biosynthesis evolved from ancient tRNA modifications that support translational fidelity in all domains of life. We identified a specialized YcfA-YcfC bipartite system consisting of an ATP-dependent sulfur transferase (YcfA) and a PLP-dependent carbon-sulfur (C-S) lyase (YcfC) responsible for the peculiar oxygen-by-sulfur substitution reaction. The successful *in vitro* reconstitution of the enzymatic thioamidation showed that YcfA employs a specialized sulfur shuttle that markedly differs from universal RNA-related systems.[4] Deciphering the key biosynthetic steps of a pivotal virulence factor may set the basis for controlling one of the top 10 plant pathogens.

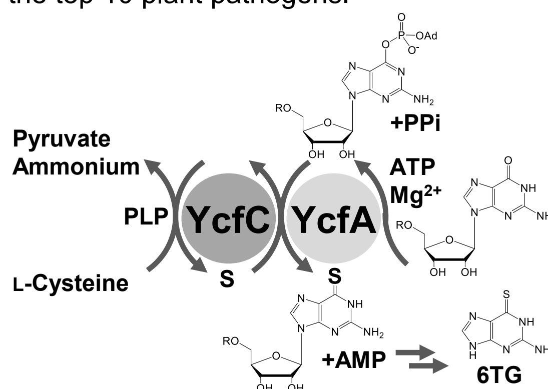

Figure 1. Proposed 6TG biosynthetic pathway.

[1] Karran, P., Attard, N. *Nat. Rev. Cancer* **2008**, *8*, 24-36.
[2] Coyne, S., Chizzali, C., Khalil, M. N. A., Litomska, A., Richter, K., Beerhues, L., Hertweck, C. *Angew. Chem. Int. Ed.* **2013**, *52*, 10564-10568
[3] Coyne, S., Litomska, A., Chizzali, C., Khalil, M. N., Richter, K., Beerhues, L., Hertweck, C. *ChemBioChem* **2014**, *15*, 373-376.
[4] Litmska, A., Ishida, K., Dunber, K., Boettger, M., Coyne, S., Hertweck, C., *Angew. Chem. Int. Ed.* **2018**, *published online.*

P17. GLOBAL -OMICS APPROACHES REVEAL POSSIBLE KEY GENES OF ENTOMOPATHOGENIC BACTERIA REQUIRED FOR BOTH SENSING OF AND ADAPTATION TO INSECT HOSTS

Tien Duy Vo[1], Nick Neubacher[1] & Helge B. Bode[1,2]*

[1] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany. *E-Mail: vo@bio.uni-frankfurt.de.
[2] Buchmann Institute for Molecular Life Sciences, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany.

Associations between microbes and other organisms (e.g. animals, plants) are ubiquitous and can be found everywhere in nature. These relationships can either be beneficial, neutral or harmful for the respective host. The lifecycle of entomopathogenic *Xenorhabdus* and *Photorhabdus* bacteria contains a mutual symbiotic as well as a pathogenic phase in their nematode- and insect hosts, respectively, and therefore represents a particularly suitable model for studying microbe-host interactions[1].

In this work, we tried to simulate the *in vivo* environment using insect homogenate made from *Galleria mellonella* larvae. The aim was to identify changes in the production of secondary metabolites specifically to the addition of insect homogenate to the cultures. Indeed, the addition of insect homogenate to cultures of *Photorhabdus* and *Xenorhabdus* led to a significant change in the production of some of the analyzed secondary metabolites for all tested strains.

RNA-sequencing was used to determine the genome-wide quantitative changes on the transcriptional level comparing the WT of *X. szentirmaii*, *X. nematophila* and *P. luminescens* in their mid-exponential growth phase with and without the addition of insect homogenate. Using this approach, we were able to identify about 30 possible key genes for recognition of and/or adaptation to the insect hosts.

On top, a proteomic approach with cells harvested in the stationary phase was accomplished in order to compare with the RNA-sequencing data set. Even though in many cases transcripts and their respective product do not correlate in a linear manner, we were able to identify a set of proteins whose relative change matched the results of the transcriptome data[2][3].

[1] Shi, Yi-Ming; Bode, Helge B. (2018): Chemical language and warfare of bacterial natural products in bacteria-nematode-insect interactions. *Natural product reports* 35 (4), 309–335. DOI: 10.1039/c7np00054e.
[2] Haider, Saad; Pal, Ranadip (2013): Integrated analysis of transcriptomic and proteomic data.*Current genomics* 14 (2), 91–110. DOI: 10.2174/1389202911314020003.
[3] Yeung, Edward S. (2011): Genome-wide correlation between mRNA and protein in a single cell. *Angewandte Chemie Int Ed.* 50 (3), 583–585. DOI: 10.1002/anie.201005969.

P18. REGULATION STUDIES OF TACROLIMUS BIOSYNTHESIS

Tomke Bakker[1], Dr. Mirita Franz-Wachtel[2], Andreas Kulik[3], Prof. Dr. Boris Macek[2], PD Dr. Alexander Kristian Apel[1], Prof. Dr. Harald Groß[1] and PD Dr. Bertolt Gust[1]

[1] Pharmaceutical Biology, Pharmaceutical Institute, University of Tübingen, 72076 Tübingen, Germany. Email: tomke.bakker@uni-tuebingen.de

[2] Proteome Center Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, 72076 Tübingen, Germany.

[3] Interfaculty Institute for Microbiology and Infection medicine, University of Tübingen, 72076 Tübingen, Germany.

Tacrolimus is a 23-membered polyketide macrolide lactone produced by *Streptomyces* species, which shows efficient immunosuppressive properties. So far, the regulation of tacrolimus biosynthesis has been poorly investigated and the insights gained until now are in part conflicting. [1,2] The aim of our work is to identify putative regulatory proteins of tacrolimus biosynthesis using a DNA affinity capturing assay (DACA). Based on dRNA-seq results [3] we chose four promotor regions located within the tacrolimus biosynthetic gene cluster for capturing of proteins extracted from *Streptomyces tsukubaensis* NRRL18488. Promotor sequences were amplified and coupled to magnetic Dynabeads to be used in the DACA. Proteins that had bound specifically to a promotor fragment were identified and quantified via label free mass spectrometry. This approach allowed us to detect several regulatory or putative regulatory proteins binding within the tacrolimus biosynthetic gene cluster. After application of different selection criteria, we now focus on 42 proteins showing specific binding properties. To further examine the role of these proteins in tacrolimus biosynthesis, we created mutants of *Streptomyces tsukubaensis* NRRL 18488 overexpressing the respective proteins. Initial results of the DACA experiments will be presented.

[1] Mo *et al.,* Roles of fkbN in positive regulation and tcs7 in negative regulation of FK506 biosynthesis in *Streptomyces sp*. strain KCTC 11604BP, **2012**, *Appl. Environ. Microbiol.* 78(7), 2249–2255.

[2] Goranovic *et al*., FK506 biosynthesis is regulated by two positive regulatory elements in *Streptomyces tsukubaensis*, **2012**, *BMC Microbiol.* 12, 238.

[3] Bauer *et al.,* dRNA-seq transcriptional profiling of the FK506 biosynthetic gene cluster in *Streptomyces tsukubaensis* NRRL18488 and general analysis of the transcriptome, **2017**, *RNA Biol.*, 0(0), 1-10.

P19. A Genome Streamlining-Based Bacterial Chassis Generation

Hirokazu Kage[1*]

[1] Department of Biochemical and Chemical Engineering, Laboratory of Technical Biology, TU Dortmund University, Emil-Figge-Strasse 66, 44227 Dortmund, Germany. Email: hirokazu.kage@tu-dortmund.de.

Heterologous production of secondary metabolites is a powerful method for obtaining secondary metabolites from cryptic biosynthetic gene clusters. Despite the apparent simplicity, there are challenges in the heterologous reconstitution of secondary metabolite biosynthesis, such as (1) the diversity of codon usage patterns between donor and host organisms, (2) limitation of precursor supply and (3) the lack of broadly applicable host systems. To address these problems, the present study attempts to generate a novel chassis system, which derives from the model bacterium *Agrobacterium tumefaciens* C58. Prominent studies on this unique bacterium have revealed several useful traits such as 1) fast growth, 2) compatibility with standard recombineering tools and 3) the ability to produce both endogenous and exogenous secondary metabolites. [1,2,3,4] Based on the genome streamlining concept,[5] we currently modify a genome of the candidate bacterium by targeting non-essential genes for its survival, namely megaplasmids. The generation of a new bacterial chassis system will expand a selection of chassis systems, promoting both the discovery and the production of secondary metabolites.

[1] I. K. Leth, K. A. McDonald, *Appl. Microbiol. Biotechnol.*, **2017**, *101*, 4895-4903.

[2] S. Hu, J. Fu, F. Huang, X. Ding, A.F. Stewart, L. Xia, Y. Zhang, *Appl. Microbiol. Biotechnol.,* **2014**, *98*, 2165-2172.

[3] S. Hu, Z. Liu, X. Zhang, G. Zhang, Y. Xie, X. Ding, X. Mo, A. F. Stewart, J. Fu, Y. Zhang, L. Xia, *Sci. Rep.*, **2016**, *6*, 29087.

[4] J.J. Zhang, X. Tang, M. Zhang, D. Nguyen, B.S. Moore, *MBio.*, **2017**, *8*, e01291-17

[5] A. Leprince, M. W. J. van Passel, V. A. P. Martins dos Santos, *Curr. Opin. Biotechnol.*, **2012**, *23*, 651-658.

P20. An alternative screening approach to discover antimicrobial compounds in Myxobacteria

Hu Zeng[1], *Carsten Volz*[1] *and Rolf Müller*[1*]

[1] Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology, Saarland University, 66123 Saarbrücken, Germany. rolf.mueller@helmholtz-hzi.de.

Myxobacteria are ubiquitous Gram$^-$ bacteria, which produce prolific secondary metabolites. However, the whole genome sequencing revealed a striking discrepancy between the number of novel antimicrobials and the number of encoded secondary metabolite gene clusters [1]. The conventional screening procedures include cultivation in liquid culture and activity-guided isolation. As soil-dwelling bacteria, some gene clusters in Myxobacteria may not express in liquid culture. Besides, some low producing compounds may be neglected during screening due to the complicated metabolite background. Here, we tried to establish a screening approach by using solid-phase cultivation of putative producer strains in the presence and absence of putative pathogenic target bacteria (Figure 1). Many strains considered not to produce an antibiotic based on previous conventional screening procedures are indeed able to form unknown bioactive compounds.

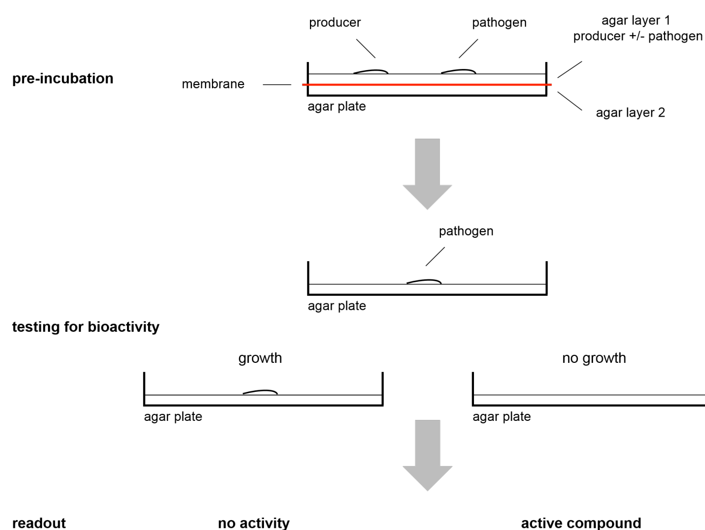

Figure 1. Screening workflow

[1] S. C. Wenzel, R. Müller, *Molecular BioSystems* **2009**, *5*, 567–74

P21. ESTABLISHING PROCHLOROSIN 2.8 AS EPITOPE GRAFTING SCAFFOLD

Julian D. Hegemann[1], *Silvia C. Bobeica*[1], *Mark C. Walker,*[1] *Ian R. Bothwell,*[1] *and Wilfred A. van der Donk*[1,*]

[1] Department of Chemistry, University of Illinois at Urbana-Champaign, 600. South Mathews Avenue, Urbana, Ilinois 61801, USA. *E-mail: vddonk@illinois.edu

Lanthipeptides belong to the class of ribosomally-synthesized and posttranslationally-modified peptides (RiPPs) and are characterized by their typical α-thioether cross links.[1] There are four classes of lanthipeptides, which are discriminated by their respective processing enzymes.[1] Amongst the class II lanthipeptides are the prochlorosins (Pcn) from *Prochloroccocus* MIT9313. This strain encodes 30 different precursor peptides with ~70 aa long, highly conserved leader regions and highly diverse core peptides.[1,2] Remarkably, the whole genome contains only one single gene encoding a class II lanthipeptide processing enzyme, ProcM, which is hypothesized to carry out the maturation of all 30 ProcA precursor peptides.[1,2]

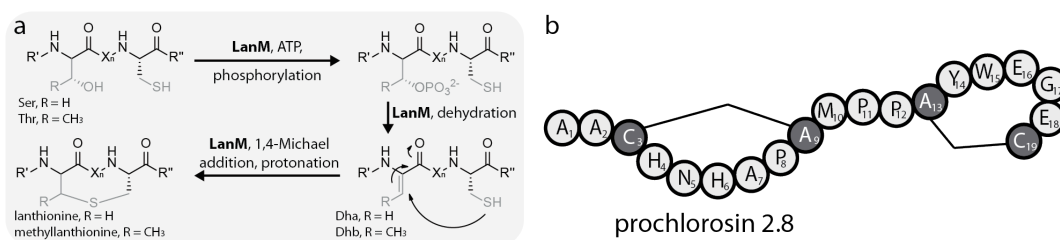

Figure 1. a) Biosynthesis of class II lanthipeptides. b) Schematic depiction of Pcn2.8.

Here, we chose the ProcA2.8 precursor, which yields the bicyclic Pcn2.8 lanthipeptide, and performed a thorough mutational analysis of single residues and structural elements and investigated how these exchanges affected processing by ProcM. Based on the findings of these experiments, we wanted to assess if Pcn2.8 would be a suitable scaffold for epitope grafting and introduced the RGD integrin binding epitope at different positions in Pcn2.8. Amongst the generated peptides, one high affinity (K_i = 1.6 nM) binder of the $\alpha v\beta 3$ integrin was identified. This integrin is important for angiogenesis and is a promising target to trigger growth inhibition of certain tumor types.

[1] L. M. Repka, J. R. Chekan, S. K. Nair, W. A. van der Donk, *Chem. Rev.* **2017**, *117*, 5457-5520.

[2] B. Li, D. Sher, Y. Shi, K. Huang, P. J. Knerr, I. Joewono, D. Rusch, S. W. Chisholm, W. A. van der Donk, *Proc. Natl. Acad. Sci.* **2010**, *107*, 10430-10435.

P22. INVESTIGATING THE ACTINONIN BIOSYNTHETIC GENE CLUSTER

Felix Wolf[1, 2], *Franziska Leipoldt*[1, 2], *Andreas Kulik*[3], *Daniel Wibberg*[4], *Jörn Kalinowski*[4] *and Leonard Kaysser**[1, 2]

[1] Department of Pharmaceutical Biology, University of Tübingen, 72076 Tübingen, Germany. Email: leonard.kaysser@uni-tuebingen.de

[2] Centre for Infection Research (DZIF), partner site Tübingen

[3] Microbiology/Biotechnology, University of Tübingen, 72076 Tübingen, Germany

[4] Center for Biotechnology (CeBiTec), Bielefeld University, 33594 Bielefeld, Germany

Actinonin is produced by *Streptomyces* sp. ATCC 14903 and features a rare *N*-hydroxy-alkyl-succinamic acid warhead connected to a dipeptide consisting of valine and a hydroxymethyl pyrrolidinyl moiety.[1] It shows inhibitory activity towards peptide deformylase (PDF), human mitochondrial PDF, CD13/aminopeptidase N, leucine aminopeptidase and various metalloproteinases. Actinonin relies on its hydroxamate-containing hydroxy-alkyl-succinamic acid residue which coordinates with divalent cations in the catalytic center of metalloproteases and peptide deformylases.[2] We recently elucidated the biosynthesis of another group of hydroxamate-containing molecules, the matlystatins.[3] To support the biosynthetic model of matlystatins, we sequenced the producer strain of actinonin, *S.* sp. ATCC 14903. Subsequently, a NRPS gene cluster was identified, containing five genes highly homologous to genes of the matlystatin pathway that were assigned to the formation of the *N*-hydroxy-alkyl-succinamic acid moiety, that is common in both compounds.[4]

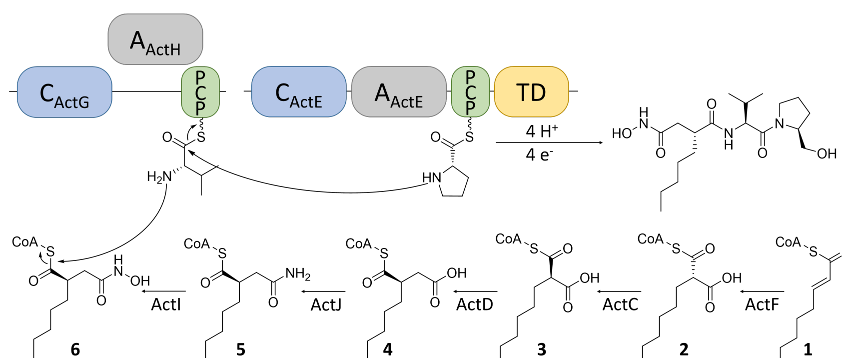

Figure 1. Model for the biosynthesis of actinonin from *Streptomyces* sp. 14903.

[1] J. J. Gordon, B. K. Kelly, G. A. Miller, *Nature* **1962**, *195*, 701-702.

[2] J.-P. Guilloteau, M. Mathieu, C. Giglione, V. Blanc, A. Dupuy, M. Chevrier, P. Gil, A. Famechon, T. Meinnel, V. Mikol, *Journal of Molecular Biology* **2002**, *320*, 951-962.

[3] F. Leipoldt, J. Santos-Aberturas, D. P. Stegmann, F. Wolf, A. Kulik, R. Lacret, D. Popadic, D. Keinhorster, N. Kirchner, P. Bekiesch, H. Gross, A. W. Truman, L. Kaysser, *Nature communications* **2017**, *8*, 1965.

[4] F. Wolf, F. Leipoldt, A. Kulik, J. Kalinowski, D. Wibberg, L. Kaysser, *Chembiochem* **2018**.

P23. Dividing condensation domains by half – A new strategy for the engineered biosynthesis of non-ribosomal peptides

Andreas Tietze[1]*, Janik Kranz[1]*, Kenan A. J. Bozhüyük[1], Annabell Linck[1] & Helge B. Bode[1,2]

[1] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany.
*E-Mail: a.tietze@bio.uni-frankfurt.de, kranz@bio.uni-frankfurt.de.
[2] Buchmann Institute for Molecular Life Sciences, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany.

Peptides derived from non-ribosomal peptide synthetases (NRPS) show a high structural diversity, resulting in many properties of biological relevance.[1] Non-ribosomally synthesized peptides (NRP) with antiviral, anticancer, anti-inflammatory, immunosuppressant, surfactant and antibiotic qualities have been identified.[2] In the field of antibiotics there is a growing demand for new compounds as the world is facing a global public health crisis with an increasing number of antimicrobial resistances emerging. In this context, NRPs are of great pharmaceutical interest and one possible source of new antibacterial agents.[3]

Due to the modular character of NRPSs scientists strived to engineer these systems but to date most attempts yielded impaired or non-functional enzymes.[4] Recently, we published the concept of eXchange Units (XU) enabling the efficient and reproducible reprogramming of NRPSs and therefore the production of new artificial NRPs.[5]

Here, we will describe an improved version of our concept of XU which uses a specific assembly point within the condensation (C) domains of NRPSs. This new technology for NRPS reprogramming called $XU_{2.0}$ bypasses the so far limiting C domain specificity. This simplifies and broadens the possibilities of engineering NRPSs for the biotechnological production of tailor-made NRPs. Furthermore, we will show the application of the $XU_{2.0}$ concept for the generation of random natural product-like peptide libraries. This can be used for the future identification of bioactive compounds for pharmaceutical and other applications.

[1] R. D. Süssmuth, A. Mainz, *Angew Chem Int Ed* **2017**, *56*, 3770-3821.
[2] E. A. Felnagle, E. E. Jackson, Y. A. Chan, A. M. Podevels, A. D. Berti, M. D. McMahon, M. G. Thomas, *Mol Pharm* **2008**, *2*, 191.211.
[3] E. Oldfield, X. Feng, *Trends Pharmacol Sci* **2014**, *35*, 664-674.
[4] M. J. Calcott, D. F. Ackerley, *Biotechnol Lett* **2014**, *36*, 2407-2416.
[5] K. A. J. Bozhüyük, F. Fleischhacker, A. Linck, F. Wesche, A. Tietze, C.-P. Niesert, H. B. Bode, *Nat Chem* **2018**, *3*, 275-281.

P24. The ergochrome gene cluster of *Claviceps purpurea*: Investigation on further secondary metabolites

Friederike Lünne[1], Eva-Maria Niehaus[1], Svetlana A. Kalinina[1] and Hans-Ulrich Humpf[1]*

[1] Westfälische Wilhelms-Universität Münster, Institut für Lebensmittelchemie, Corrensstr. 45, 48149 Münster, Germany. Email: humpf@wwu.de

Claviceps purpurea is known as a plant pathogenic fungus infecting a wide range of grasses especially rye. The fungus is renowned for its production of toxic ergot alkaloids. Besides this, only little is known about further secondary metabolism of the fungus. Recently, the genome of *C. purpurea* was sequenced and bioinformatics analysis showed the potential of the fungus to produce new secondary metabolites.[1]

Analyses of the gene clusters in *C. purpurea* indicated homology of Polyketide-Synthase 4 (PKS4) to the key enzyme of the monodictyphenone cluster in *A. nidulans*. Overexpression (OE) of the cluster-specific transcription factor (TF) yielded in the elucidation of the gene cluster encoding for ergot pigments, which belong to different groups: anthraquinonecarboxylic acids and dimeric hydroxanthone derivatives (ergochromes).[2] The latter are known for their biological activity.

In the current project *C. purpurea* and the mutant with the overexpressed transcription factor (OE::*TF*) were cultivated in axenic cultures for two weeks. After extraction, the metabolites secalonic acid A-C, endocrocin and clavorubin were successfully isolated, the structures elucidated and the cytotoxicity checked on HepG2 cells. Comparison of the metabolic profiles of the wild type and the OE::*TF* mutant showed new upcoming peaks in the profile of the mutant. To rise concentration of these new metabolites, the medium was supplemented with sodium bromide. Currently, the focus is on the isolation of these new metabolites built by the ergochrome gene cluster. The chemical structures will be elucidated via NMR and HPLC-HRMS experiments. Furthermore, the influence of salt stress on the gene expression within the ergochrome gene cluster will be outlined.

[1] C.L. Schardl, C.A. Young, U. Hesse, S.G. Amyotte *et al.*, Plant-symbiotic fungi as chemical engineers: multi-genome analysis of the Clavicipitaceae reveals dynamics of alkaloid loci. *PLoS Genet.*, 2013, 9(2).

[2] L. Neubauer, J. Dopstadt, H.-U. Humpf, P. Tudzynski, Identification and characterization of the ergochrome gene cluster in the plant pathogenic fungus *Claviceps purpurea*. *Fungal Biol. Biotechnol.*, 2016, 3(1): 2.

We would like to thank the Deutsche Forschungsgemeinschaft for funding (HU 730/11-2)

P25. ACTIVATION OF SILENT SECONDARY METABOLITE CLUSTERS BY VARIOUS REGULATORY PROTEINS IN ACTINOMYCETES

Erik Mingyar[1,2], Wolfgang Wohlleben[1,2], Evi Stegmann[1,2]*

[1] Microbial Biotechnology, Interfaculty Institute of Microbiology and Infection Medicine, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany. *E-mail: erik.mingyar@uni-tuebingen.de
[2] German Centre for Infection Research (DZIF)

Actinomycetes are producers of many secondary metabolites with various applications as medical, agricultural and industrial. The biosynthesis of secondary metabolites is encoded in their genomes and is usually organized in clusters. The continuously increasing genome sequencing data have revealed the presence of numerous clusters in Actinobacteria, which might encode novel secondary metabolites with interesting biological activities. However, utilization of this hidden potential has been hindered by the observation that many of these gene clusters remain „silent“ (or cryptic or poorly expressed) under laboratory conditions. Different ways of silent secondary metabolite clusters activation were described, such as using of constitutive promoters, genetic manipulation of regulators, optimalization of culture conditions or ribosome engineering [1, 2].

In our approach we are focusing on the activation of silent secondary metabolite clusters by using a set of regulatory proteins from different *Streptomyces* strains. We have constructed four integrative plasmids containing different classes of regulatory genes under the control of *ermE*p* promotor (Gamma-butyrolactones – GBLs – pEM1, Global regulators – GR – pEM2, Cluster situated regulators – CSR – pEM3, and *Streptomyces* antibiotic regulatory proteins – SARPs – pEM4).

These plasmids were introduced via conjugation into strains from Tuebingen strain collection and the metabolic profiles of the recombinant strains were compared to the metabolic profile of the corresponding wild type (WT) strains.

The analyses of the MS-data revealed that the recombinant strains produced metabolites, which are not produced by the WT, and the production of certain metabolites is increased in the recombinant strains. In order to avoid dereplication the obtained masses were compared with the masses of the natural product database.

Based on this proof of concept we now apply this strategy to screen the Tuebingen strain collection in a high throughput manner.

[1] G.Liu, K.F. Chater, G. Chandra, G. Niu, H. Tan *MMBR* **2013**, 77, 112-143.
[2] M. Spohn, N. Kirchner, A. Kulik, A. Jochim, F. Wolf, P. Muenzer, O. Borst, H. Gross, W. Wohlleben, E. Stegmann *Antimicrob Agents Chemother* **2014**, 58 (10):6185-96

P26. GENOMIC INVESTIGATION AND ACTIVATION OF MYXOBACTERIAL TYPE III PKS GENE CLUSTERS

Joachim J. Hug[1,2], *Fabian Panter*[1,2], *Daniel Krug*[1,2], *Rolf Müller*[1,2*]

[1] Department Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI) and Department of Pharmaceutical Biotechnology Saarland University, Campus E8.1, 66123 Saarbrücken, Germany

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

Type III polyketide synthases (PKSs) are comparatively small homodimeric enzymes affording natural products with various structures and functions [1]. Plant type III PKSs are widely distributed and have been studied thoroughly in the past, in contrast to their underinvestigated homologues from bacteria and fungi. Despite the fact that myxobacteria are prolific producers of structurally diverse and biologically active natural products, no type III polyketide has been isolated so far from these underexplored microorganisms [2]. Bioinformatic investigation of myxobacterial genome sequences highlighted numerous type III PKSs of unknown function. However so far none of these genes could be connected to any metabolite found in the correlated metabolome, suggesting that these biosynthetic genes are remaining silent under laboratory cultivation conditions. Unlike type I and II PKSs, type III PKSs generally accomplish an entire series of decarboxylative condensations and cyclization reactions in a single active site impeding *in silico* structural prediction of the natural product. We present here results from analysis of type III PKSs from myxobacterial genomes and report the isolation of a type III polyketide from the myxobacterial model host *Myxococcus xanthus* DK1622, using promoter-induced gene expression and heterologous expression of biosynthetic type III PKS genes.

[1] Y. Shimizu, H. Ogata, S. Goto, *ChemBioChem* **2017**, *18*, 50.

[2] J. Herrmann, A. A. Fayad, R. Müller, *Nat. Prod. Rep.* **2017**, *34*, 135.

P27. DISCOVERY OF FUNGAL POLYENE MACROLIDE BY GENOME MINING AND HETEROLOGOUS EXPRESSION OF A CRYPTIC HR-PKS CLUSTER

Yohei Morishita[1] *and Teigo Asai*[1*]

[1] Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 153-0041 Tokyo, Japan. Email: ymorishita@g.ecc.u-tokyo.ac.jp

Fungi possess a huge number of cryptic biosynthetic gene clusters (BGCs) that would be attractive source for drug seeds, and we have applied genome mining and heterologous expression to discover novel natural products from those cryptic BGCs. During our genome mining of fungi collected from plants and insects, we found a series of cryptic BGCs containing highly reducing polyketide synthase (HR-PKS) and putative thioesterase (TE). Recently, HR-PKS and TE were demonstrated to be responsible for the biosynthesis of brefeldin A[1], which is a member of fungal macrolide with a 16-membered ring. This suggested that the cryptic BGCs with HR-PKS and TE would generate macrolide metabolites. Therefore, we tried to discover novel fungal macrolide metabolites by heterologous expression of those cryptic BGCs in *Aspergillus oryzae*.

Analyzing draft genome of *Arthrinium phaeospermum* isolated from a hairy caterpillar, we found two cryptic BGCs with HR-PKS (KS-AT-DH-ER-KR-ACP) and TE. One of the gene cluster (kemushi632) was introduced in *Aspergillus oryzae*, and the transformants produced **1** and **2** (Figure 1). The 2D structures were determined to be a novel 34/32-membered macrolide with a conjugated hexaene by the analyses of UV spectrum and 1D and 2D NMR spectra (Figure 1). This is the first example of polyene macrolide from fungi. Furthermore, 34-membered ring system in **1** is the largest size in fungal macrolide compounds. In addition to kemushi632, other gene clusters in several species of fungi were heterologously expressed, and successfully we obtained some macrolides of different ring sizes.

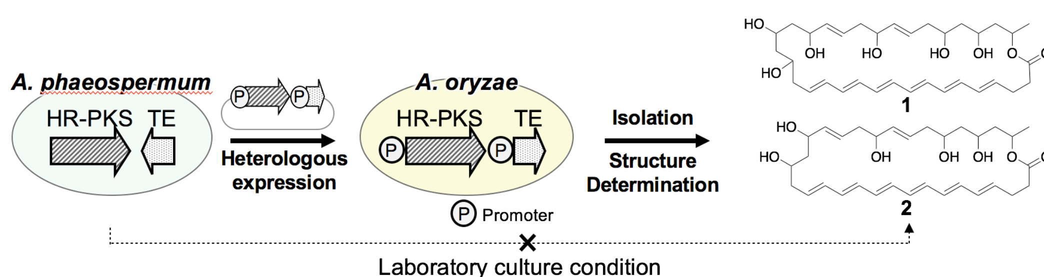

Figure 1. Discovery of polyene macrolide **1** and **2** by using heterologous expression system.

[1] A. O. Zabala, Y. H. Chooi, M. S. Choi, H. C. Lin, Y. Tang, *ACS Chem. Biol.* **2014**, *9*, 1576-1586.

P28. PHOSPHONATE PRODUCTION IN KITASATOSPORA SP. TÜ4103

Janina Krause[1], *Wolfgang Wohlleben*[1], *Yvonne Mast*[1]

[1]Microbial Biotechnology, Interfaculty Institute of Microbiology and Infection Medicine, Eberhard Karls Universität Tübingen, 72074 Tübingen Germany
E-Mail: janina.krause@uni-tuebingen.de

Indonesia, as a “biodiversity hotspot” [1], harbours unknown microbial species, which may produce novel antibiotics [2] Our project aims to identify new antibiotics from Indonesian actinomycetes. A Javanese strain (Tü4103) has been selected as potent antibiotic producer and its genome was sequenced by the Pacbio sequencing technology. To find novel substances we pursue a genome-mining-driven approach.

16S phylogenetic analysis revealed that TÜ4103 belongs to the genus *Kitasatospora*. The strain produces a substance with antimicrobial activity against gram+ bacteria. In a *Bacillus subtilis* promoter-based reporter assay (AG Heike Brötz-Oesterhelt) the TÜ4103 product induced a promoter, which reacts on substances targeting DNA synthesis. AntiSMASH analysis of the TÜ4103 genome sequence revealed that one of the predicted antibiotic gene clusters may encode a phosphonate. Bioactivity tests with the phosphonate-sensitive strain WM6242 [3] support that the TÜ4103 product is a phosphonate. The expression of the putative phosphonate-biosynthetic gene under production conditions was verified via RT-PCR. Detailed cluster analysis, as well as target specific-bioassays indicates that the phosphonate antibiotic affects DNA synthesis and/or SOS response. Altogether our data suggest that TÜ4103 may produce a novel type of phosphonate.

[1] N. Myers *Environmentalist* **1988,** 8, 187–208.
[2] A. Lazzarini, L. Cavaletti, G. Toppo, F. Marinelli, A. Van Leeuwenhoek *J. of Micobiology* **2000,** 78, 399–405.
[3] A. C. Eliot, B. Griffin, P. Thomas, et al. *Chemistry & Biology* **2008**, 15, 766-770.

P29. Three new skeletons from fungi *Geomyces auratus* and *Alternaria alternata*

Yan-Ni Shi[1], *Christian Richter*[2], *Yi-Ming Shi*[1] *and Helge B. Bode*[1,3]

[1]Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany

[2]Institut für Organische und Chemische Biologie, Zentrum für Biomolekulare Magnetische Resonanz (BMRZ), Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany

[3]Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany

Georatusin (**1**), featuring a highly reduced, methylated polyketide moiety fused to a tryptophan by amide and ester bonds forming a 13-membered ring, was produced by the soil fungus *Geomyces auratus*. An HMQC–COSY spectrum was measured to establish the connectivities despite the overlapping proton signals. DQF-COSY, HETLOC, J-HMBC, and ROESY were implemented to determine the relative configuration of the flexible moiety. Georatusin (**1**) shows specific antiparasitic activities against *Leishmania donovani* and *Plasmodium falciparum* without obvious cytotoxicity. The biosynthesis of georatusin (**1**) was also proposed.

Additionally, two new highly quaternary dimeric compounds of the alternariol class of natural products, verrulactones D (**2**) and E (**3**), were obtained from the fungus *Alternaria alternata* which was isolated from roots of the halophlic plant *Salicornia* sp. in a salt marsh in Santa Pola, SE Spain. Their structures were elucidated on the basis of extensive NMR, MS spectrum and calculated methods.

P30. SELF-RESISTANCE GUIDED GENOME MINING UNCOVERS THE PYXIDICYCLINE CLASS OF MYXOBACTERIAL NATURAL PRODUCTS

Fabian Panter[1], Daniel Krug[1] and Rolf Müller[1]

1 Department of Microbial Natural Products Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany

An astounding discrepancy exists between the genome-inscribed capacity for the production of secondary metabolites and the set of known secondary metabolite classes detected from many microorganisms under laboratory cultivation conditions. Genome-mining techniques are meant to fill this gap. In order to increase chances for finding novel bioactive natural products and connecting them to uncharacterized biosynthetic pathways, genomics-based strategies are reasonably amended with the analysis of associated genetic markers for self-resistance. In this study, we follow a genome-mining approach aimed at discovering inhibitors of topoisomerase, known as target in both cancer therapy and for the treatment of infectious diseases. The so-called pentapeptide repeat proteins in bacteria mediate a common host self-defense mechanism against such inhibitors. Biosynthetic machinery for the production of an alleged topoisomerase inhibitor was found in the genome of the myxobacterium *Pyxidicoccus fallax* And48, as judged by a gene cluster encoding polyketide synthase type II (PKS) enzymes near to a gene predicted to encode a pentapeptide repeat protein, albeit to date no matching compound was reported from the strain. Activation of this biosynthetic gene cluster in the native host as well as heterologous expression following cloning by transformation-associated recombination enabled the structural elucidation of two new natural products called Pyxidicycline A and B. [1] Mutagenesis studies using the heterologous expression system together with *in silico* analysis shed light on Pyxidicycline biosynthesis, while IC50 determinations using human pathogens and cancer cell lines revealed the broad biological activity of the new compounds. Subsequent topoisomerase inhibition assays showed strong affinity to - and inhibition of - unwinding topoisomerases such as *E. coli* topoisomerase IV and human topoisomerase I by Pyxidicyclines, while *E. coli* topoisomerase II (gyrase) was not inhibited at concentrations up to 50 µg/ml.[1]

[1] A. Panter, F. Krug, D. Baumann, S. Müller, R. ***Chem. Sci.***, 2018,**9**, 4898-490

P31. Harnessing Fungal Nonribosomal Cyclodepsipeptide Synthetases for Mechanistic Insights and Tailored Engineering

Charlotte Steiniger[1], Sylvester Hoffmann[1], Andi Mainz[1], Marcel Kaiser[2], Kerstin Voigt[3], Vera Meyer[4] and Roderich D. Süssmuth[1]*

[1] Biological Chemistry, Institute of Chemistry, Technische Universität Berlin, 10623 Berlin, Germany

[2] Swiss Tropical and Public Health Institute, 4051 Basel, Switzerland

[3] Leibniz-Institute for Natural Product Research and Infection Biology, 07745 Jena, Germany

[4] Applied and Molecular Microbiology, Institute of Biotechnology, Technische Universität Berlin, 13355 Berlin, Germany

Nonribosomal peptide synthetases represent potential platforms for the design and engineering of structurally complex peptides. While previous focus has been centered mainly on bacterial systems,[1] fungal synthetases assembling drugs like the antibacterial cephalosporins or the anthelmintic cyclodepsipeptide (CDP) PF1022 await in-depth exploitation. As various mechanistic features of fungal CDP biosynthesis are only partly understood,[2] effective engineering of NRPSs has been severely hampered. By combining protein truncation, *in trans* expression and combinatorial swapping, we assigned important functional segments of fungal CDP synthetases and assessed their *in vivo* biosynthetic capabilities.[3] Hence, full length artificial assembly lines comprising of up to three different synthetases were generated. Using *Aspergillus niger* as a heterologous expression host, we obtained new-to-nature octa-enniatin (4 mg/L) and octa-beauvericin (10.8 mg/L), as well as high titers of hexa-bassianolide (1.3 g/L) with an engineered ring size. The hybrid CDPs showed up to 12-fold enhanced antiparasitic activity against *Leishmania donovani* and *Trypanosoma cruzi* compared to the reference drugs miltefosine and benznidazole, respectively. Our findings thus contribute to a rational engineering of iterative nonribosomal assembly lines.

[1] R. D. Süssmuth, A. Mainz, *Angew Chem Int Ed Engl.* **2017**, *56*, 3770-3821.

[2] Yu *et al.*, *Nat Commun.* **2017**, *8*, 15349.

[3] Steiniger *et al.*, *Chem. Sci.* **2017**, *8*, 7834-7843.

P32. HETERODIMERIC COMPLEX OF THE DEHYDRATASES APEI/P IN THE BIOSYNTHESIS OF ARYL POLYENES

Maximilian Schmalhofer[1], *Gina Grammbitter*[2], *Michael Groll*[1] *and Helge Bode*[2,3*]

[1] Lehrstuhl für Biochemie, Technische Universität München, 85748 Garching bei München, Lichtenbergstraße 4, Germany. Email: M.Schmalhofer@tum.de.

[2] Fachbereich Molekulare Biowissenschaften, Molekulare Biotechnologie, Goethe-Universität Frankfurt, Max-von-Laue-Straße 9, 60483 Frankfurt am Main, Germany

[3] Buchmann Institute for Molecular Life Sciences (BMLS), Goethe-Universität Frankfurt, Max-von-Laue-Straße 15, 60483 Frankfurt am Main, Germany

A recently identified aryl polyene compound (APE) in the Gram-negative bacterium *Xenorhabdus doucetiae* is produced by a new subfamily of type II polyketide synthases.[1,3] The main characteristic of these natural products is their carotenoid-like structure, which is in contrast to the typical aromatic ring systems.[2]

An important key step of the aryl polyene biosynthesis is the dehydration reaction from the β-hydroxy group to the α-β trans double bond catalyzed by the dehydratases 1 and 2 (ApeI/P) in an iterative manner. Interestingly, ApeI/P are capable to convert all intermediates up to the final product with a chain-length of 22 carbon atoms.

We performed the structural analysis and confirmed a heterodimeric complex of the dehydratases (DHs) ApeI/P with high similarity to bacterial DHs involved in fatty acid biosynthesis.

[1] D. Du, Y. Katsuyama, K. Shin-Ya, Y. Ohnishi, *Angewandte Chemie (International ed. in English)* **2018**, *57*, 1954.
[2] T. A. Schöner, S. Gassel, A. Osawa, N. J. Tobias, Y. Okuno, Y. Sakakibara, K. Shindo, G. Sandmann, H. B. Bode, *Chembiochem : a European journal of chemical biology* **2016**, *17*, 247.
[3] G. Grammbitter, H. Bode *et al.* (unpublished)

P33. LC-MS BASED DEREPLICATION AND ISOLATON OF A NEW ALKALOID COMPOUND, DENDROBEANIAMINE A, FROM AN ARCTIC MARINE BRYOZOAN DENDROBEANIA MURRAYANA

Priyanka Michael[1], *Kina Ø Hansen*[1], *Johan Issakson*[2], *Jeanette H Andersen and Espen Hansen*[*1]

[1] MARBIO, UiT – The Arctic University of Norway, Brevika, N-9037, Tromsø, Norway. Email: raja.p.siranjeevi@uit.no

[2] Department of Chemistry UiT - The Arctic University of Norway, Brevika, N-9037, Tromsø, Norway.

Abstract: Coldwater marine bryozoans are a source of structurally diverse compounds including macrocyclic bryostatins, halogenated alkaloids, sterols and monoterpenes [1-4]. Dereplication is an essential process to eliminate re-isolation and re-characterization of previously known compounds. This process accelerates identifying potentially new secondary metabolites in the early stage of Natural Products drug discovery [5]. The main aim of this study is identification and isolation of new compounds from the crude extract of Arctic marine bryozoan *Dendrobeania murrayana*, using a combination of ultra-high performance liquid chromatography and high-resolution mass spectrometry (UHPLC-HR-MS) with positive electrospray ionization (ESI^+). In the analysis of the organic crude extract, a prominent *pseudo-molecular ion* peak with *m/z* 369.2861 was observed. The elemental composition of the compound **1** was calculated, and searches in ChemSpider, SciFinder and Dictionary of Marine Natural Products gave no hits. The target compound **1** was isolated using preparative HPLC. The structure of the compound **1** was elucidated by using 1D and 2D nuclear magnetic resonance (NMR). We have named the compound **1** as ''Dendrobeaniamine A'' and the biological activities of Dendrobeaniamine A was evaluated based on cellular and biochemical assays *in vitro*. It is the first report, a guainidine based alkaloid compound, isolated from marine bryozoan.

References:

[1] G.R. Pettit, C.L. Herald, D.L. Doubek, D.L. Herald, E. Arnold, J.J. Clardy, *Am. Chem. Soc*. **1982**, *104, 6846*.
[2] C. Christophersen, *Acta Chemia Scandinavia* B **1985**, *39, 517-529*.
[3] J.H. Sharp, M.K. Winson, J.S. Porter, *Nat. Prod. Rep*., **2007**, *24, 659–673*.
[4] M.D. Lebar, J.L. Heimbegner, B.J. Baker, *Nat. Prod. Rep*., **2007**, *24, 774-794*.
[5] K.F. Nielsen, M. Månsson , C. Rank, J.C. Frisvad, T.O Larsen, **2011**, *J. Nat. Prod.*, *74, 11, 2338–2348*.

P34. ARTIFICIAL SPLITTING OF A NON-RIBOSOMAL PEPTIDE SYNTHETASE BY INSERTING NATURAL DOCKING DOMAINS

Carsten Kegler[1] and Helge B. Bode[1,2]*

[1] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany. *E-Mail: kegler@bio.uni-frankfurt.de
[2] Buchmann Institute for Molecular Life Sciences, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany.

Non-ribosomal peptide synthetases (NRPS) multienzymes, involving more than one NRPS polypeptide, have to interact with the correct biosynthesis partner(s) polypeptide(s) to facilitate a successful biosynthesis. Connecting the correct NRPS polypeptides with each other in the cell, while excluding every other possible connection to other NRPS polypeptides, is brought about by regions referred to as 'docking domains' (DD). Typically, a docking domain can be found at the C terminus (^{C}DD) of the NRPS^{x} following the last domain. This ^{C}DD then in turn interacts specifically with the N terminally positioned docking domain (^{N}DD) partner of the NRPS^{x+1} of a given biosynthesis.

In this work we try to shed light on the functional role of DD-pairs by importing DD-pairs into the three module containing, one polypeptide NRPS XfpS, which synthesizes the nonribosomal peptide (NRP) xefoampeptide (XFP) (Tobias et al, Nat Microbiol, 2017). In so doing we created artificial two- and three-polypeptide NRPS biosyntheses. The amount of Xefoampeptide produced was analysed qualitatively and quantitatively via LC-MS. The Xefoampeptide production data was used to assess the success of connecting the NRPS modules via the DD-pairs in a non-covalent fashion and, at the same time, maintaining the biosynthesis capabilities.

The xefoampeptide producing NRPS XfpS, originating from the bacterium *Xenorhabdus bovienii,* was heterologously produced in *E. coli* and all subsequent work, probing the DD efficiency to connect the corresponding NRPS-Xfp parts, was conducted in *E. coli* as host. To substantiate the deduced assumptions based on the different introduced DDs, we compared the amount of the produced peptides to the amount of the NRPS subunits in *E. coli* protein extracts.

Introducing DDs, thereby creating a two protein NRPS synthesis, led to xefomapeptide production. Quantification of XfpS proteins corroborated the notion that the abundance of XFP peptide and XfpS protein concentrations do not correlate in all cases but are a function of multienzyme efficiency.

We believe this work represents a worthwhile approach in probing aspects of the DD-mode of action and function. We are fully aware of the fact that this approach can be used for NRPS combinatorial approaches potentially creating new NRP's. Further-more, we would like to stress the value of correlation NRPS protein amounts in the cell to peptide production, rarely done in the PKS/NRPS scientific endeavour.

NJ Tobias et al. Nat Microbiol. 2017, vol 2, 1676-1685; doi: 10.1038/s41564-017-0039-9

This study was supported by the LOEWE program (Landes-Offensive zur Entwicklung wissenschaftlich-ökonomischer Exzellenz) of the state of Hesse and was conducted within the framework of the MegaSyn Research Cluster.

P35. Investigation of Planomonospora as Secondary Metabolite Producer by Metabolomic Tools

Mitja Zdouc[1,2], Margherita Sosio[1], Sonia Maffioli[1], Matteo Simone[1], Tanneke den Blaauwen[2] and Stefano Donadio[1]*

[1] Naicons Srl., Viale Ortles 22/4, 20139 Milano, Italy. E-mail:mzdouc@naicons.com

[2] Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

The increasing resistance of pathogens to commonly used antibiotics is an alarming threat that has to be addressed by provision of new classes of anti-microbial agents. The limited success of synthetic chemistry to fill this gap has renewed interest in natural product drug discovery. One strategy to find novel scaffolds is the screening of underexploited and rare genera of Actinomycetes. In this study, the genus *Planomonospora* was investigated for its capacity to yield secondary metabolites by the cultivation of 80 diverse strains from NAICONS's library, following the OSMAC-approach (one strain, many compounds). A total of 640 extracts from four different cultivation conditions was analysed by LC-MS/MS and the data visualised, using the Global Natural Product Social Molecular Networking (GNPS) platform. Following this method, we were able to identify several families of diverse natural products, some of which also demonstrated antimicrobial properties. To our knowledge, this is the first instance of a systematic evaluation of the genus *Planomonospora* by means of molecular networking. Furthermore, combination of the molecular network with phylogenetic information allowed for strain prioritisation and selection of interesting molecular clusters, of which specific examples will be presented. These preliminary results confirm the value of this unusual genus for antibiotic drug discovery and give insight into its secondary metabolom.

P36. IDENTIFICATION OF NOVEL PYRONES FROM *CONEXIBACTER WOESI*

Franziska Wiker[1], *Irina Helmle*[1], *Harald Groß*[1] *and Bertolt Gust*[1*]

[1]University of Tübingen, Pharmaceutical Institute, Department of Pharmaceutical Biology, 72076 Tübingen, Germany.

*Email: franziska.wiker@uni-tuebingen.de.

Polyketide-derived pyrones are a group of structurally diverse secondary metabolites that have been identified in plants, fungi and bacteria. One important class are the α-pyrones considering their broad spectrum of biological activities, such as cytotoxic[1], antimicrobial[1], antifungal[2] and anticancer[3] properties. Furthermore, it has been shown that they can act as signaling molecules in bacterial communication[4]. Recently, their function as a sulfate shuttle in the sulfation process of caprazamycins in *Streptomyces* sp. MK730–62F2 could be demonstrated[5]. Biosynthesis of molecules belonging to this class of compound can occur via different pathways including type I and type III PKSs.

Here we present the isolation and structure elucidation of new α-pyrones obtained by heterologous expression of a *Conexibacter woesi* type III PKS gene in *Streptomyces coelicolor*. The new structures were verified by one- and two-dimensional NMR experiments and supported by HR MS analyses. Additionally, feeding studies with C^{13}-labeled precursors allow us to propose a biosynthetic pathway.

[1] I. J. S. Fairlamb, L. R. Marrison, J. M. Dickinson, F.-J. Lu, J. P. Schmidt, *Bioorganic & Medicinal Chemistry* **2004**, *12*, 4285-4299.

[2] N. Claydon, M. Allan, J. R. Hanson, A. G. Avent, *Transactions of the British Mycological Society* **1987**, *88*, 503-513.

[3] D. Liu, X.-M. Li, L. Meng, C.-S. Li, S.-S. Gao, Z. Shang, P. Proksch, C.-G. Huang, B.-G. Wang, *Journal of Natural Products* **2011**, *74*, 1787-1791.

[4] A. O. Brachmann, S. Brameyer, D. Kresovic, I. Hitkova, Y. Kopp, C. Manske, K. Schubert, H. B. Bode, R. Heermann, *Nat Chem Biol* **2013**, *9*, 573-578.

[5] X. Tang, K. Eitel, L. Kaysser, A. Kulik, S. Grond, B. Gust, *Nat Chem Biol* **2013**, *9*, 610-615.

P37. Production of calcimycin and its analogs in *S. chartreusis*

Leonie Sagurna[1], *Christoph H. R. Senges*[1], *Kirstin I. Arend*[1], *and Julia E. Bandow*[1*]

[1] Applied Microbiology, Ruhr University Bochum, 44801 Bochum, Germany. Email: Kirstin.arend@rub.de

The genus *Streptomyces* is known for its production of biologically active substances in order to compete with other microorganisms. One of these substances are ionophores, which transport ions across biological membranes. The polyether ionophore calcimycin, produced by *Streptomyces chartreusis*, transports divalent cations like Fe, Mn and Ca[1], and is already widely used as biochemical tool to study calcium-dependent processes.[2] Besides calcimycin, *S. chartreusis* produces precursors and derivatives of calcimycin, originating from the same biosynthetic pathway.[3] These analogs reveal different ion transportation efficiencies[4] like the precursor cezomycin, which lacks a methylamino group and has a ten-fold lower binding affinity to calcium than calcimycin. [5] Since the synthesis of calcimycin and its analogs depends on growth conditions[6], we analyzed the production in different media. More complex media lead to the synthesis of a wide range of analogs, however, calcimycin was the predominant species. In contrast, when *S. chartreusis* was cultivated in minimal medium no calcimycin was produced. In co-cultivation with *B. subtilis*, the calcimycin production was nearly abolished and high amounts of cezomycin were found. Only minor structural differences distinguish calcimycin from most of its known analogs, indicating that the absence of complex precursor is not the reason for low calcimycin production. Instead, it is conceivable that *S. chartreusis* synthesizes specific analogs according to the ionic strength and nutrient availability. Thus, further analysis of calcimycin analogs is crucial to elucidate their function in S*. chartreusis*.

[1] N. Raatschen, M. Wenzel, L. I. Ole Leichert, P. Düchting, U. Krämer, J. E. Bandow, *Proteomics* **2013**, *13*, 1358.
[2] H. Tateno, D. Krapf, T. Hino, C. Sánchez-Cárdenas, A. Darszon, R. Yanagimachi, P. E. Visconti, *Proceedings of the National Academy of Sciences of the United States of America* **2013**, *110*, 18543.
[3] Q. Wu, J. Liang, S. Lin, X. Zhou, L. Bai, Z. Deng, Z. Wang, *Antimicrobial agents and chemotherapy* **2011**, *55*, 974.
[4] W. L. Erdahl, C. J. Chapman, E. Wang, R. W. Taylor, D. R. Pfeiffer, *Biochemistry* **1996**, *35*, 13817.
[5] A. M. Albrecht-Gary, S. Blanc, L. David, and G. Jeminet.
[6] C. H. R. Senges, A. Al-Dilaimi, D. H. Marchbank, D. Wibberg, A. Winkler, B. Haltli, M. Nowrousian, J. Kalinowski, R. G. Kerr, J. E. Bandow, *Proceedings of the National Academy of Sciences of the United States of America* **2018**, *115*, 2490.

P38. Bioactivity Analysis of Different Fabclavine Classes in a ∆*hfq* Background in *Xenorhabdus szentirmaii*

Natalie Berghaus[1], *Sebastian Wenski*[1], *Helge B. Bode*[1,2],

[1] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt, Germany. E-mail: s4256010@stud.uni-frankfurt.de.

[2] Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt, 60438 Frankfurt, Germany.

Entomopathogenic bacteria of the genus *Xenorhabdus* live in mutualisitic symbiosis with nematodes of the genus *Steinernema*. After they have together invaded an insect larva the bacterium produces a variety of different secondary metabolites.[1] These structurally diverse compounds fulfill different roles such as killing the insect larvae or protecting the dead prey from food competitors. Many of these small molecules are biosynthesized by non-ribosomal-peptide-sythetases (NRPS), poly-ketide-synthetases (PKS) or hybrids of both.
Secondary metabolites have been reported to be under the control of global regulators. In *Xenorhabdus*, the regulation appears to be mediated by the RNA-chaperone Hfq. The deletion of *hfq* results in a nearly complete loss of production of secondary metabolites. It is also reported, that *hfq* deletion has an influence on the virulence, enhances biofilm and pigment formation.
The elimination of interfering background signals from other natural products during analysis of specific compounds can be achieved by a deletion of *hfq*. Furthermore, we established a protocol to utilize the inducible P_{BAD}-promotor to selectively reactivate the production of the compound of interest.[2]
Fabclavines are a class of hybrid peptide-polyketide-polyamino natural products, characterized in *X. szentirmaii*. They have been reported to exhibit a broad bioactivity against different organisms.[3] To allow further investigations of fabclavine derivative classes like short fabclavines or only the polyamino parts, they were produced in an ∆*hfq* background with a promotor exchange, that allowed controlled gene expression. Additionally, bioactivity assays with these mutants have been conducted.

[1] 1] Y.M. Shi, H.B. Bode, Chemical language and warfare of bacterial natural products in bacteria-nematode-insect interactions. *Nat Prod Rep*. **2018**, *35(4)* ,309-335
[2] E. Bode, A.O. Brachmann, C. Kegler, R. Simsek, C. Dauth, Q. Zhou, M. Kaiser, P. Klemmt, H.B. Bode, Simple "On-Demand" Production of Bioactive Natural Products. *Chembiochem*. **2015**, *16(7)* ,1115–1119.
[3] S.W. Fuchs, F. Grundmann, M. Kurz, M. Kaiser, H.B. Bode, Fabclavines: bioactive peptide-polyketide-polyamino hybrids from Xenorhabdus. *Chembiochem*. **2014**, *15* ,512-516.

P39. CO-CULTURE MARINE SPONGE-ASSOCIATED BACTERIA AS RESOURCE FOR NOVEL ANTIBIOTICS

Riyanti[1,2], Zerlina G. Wuisan[1], Nils Böhringer[1], Walter Balansa[5], Frans Gruber Ijong[5], Heike Wägele[4], Gabriele M. König[3], Till F. Schäberle[1]*

[1]Institute of Insect Biotechnology, Justus Liebig University of Gießen, Germany
[2]Faculty of Fisheries and Marine Science, Jenderal Soedirman University, Purwokerto, Indonesia
[3]Institute for Pharmaceutical Biology, University of Bonn, Bonn, Germany
[4]Zoological Research Museum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Bonn, Germany
[5]Politeknik Nusa Utara, Sangihe Islands, Indonesia

Marine sponges, a group of filter feeders, represent a reservoir for bacterial microorganisms and are a proven source of bioactive natural products. Numerous natural products originally isolated from marine sponges show structural similarities or even resemble to metabolites of microbial origin, thereby suggesting that the microorganisms are the true source of the biologically active metabolites. To trigger the biosynthesis of metabolites, in this project co-culture experiments of bacteria isolated from sponges are performed.

This study aims to isolate and identify antibacterial compounds from marine sponge-associated bacteria. Starting from 10 sponge samples, 835 bacterial strains were isolated using 10 different isolation media and different antibiotics. All isolates were fermented in the appropriate solid medium and subsequently tested for their antibacterial activity against *Escherichia coli* and *Micrococcus luteus*. Of all isolates, 101 showed activity against test strains and were identified by 16S rRNA gene sequencing. Most of the active isolates belonged to the genera *Bacillus, Pseudomonas, Serratia, Verrucosispora, Brevibacterium, Cellulosimicrobium, Micromonospora,* and *Solwaraspora*. To answer basic questions about the *inter* species relation between these marine bacteria, we have tested inhibitory effects of both, strains originating from one sponge and between different sponges. LC/MS analysis was used to evaluate specialized metabolite production in co-culture. The strongest competitors and promising co-culture combinations were analyzed by MALDI-imaging.

Competition by co-cultivation led to shifts in the metabolome. Production of present compounds was significantly enhanced and novel metabolites were detected. Co-culture of *Verrucosispora* sp. 325 and *Bacillus* sp. 454 increased the production of an unknown compound (853.552 m/z). This metabolite will be isolated in the future.

P40. HETEROLOGOUS EXPRESSION OF BIOSYNTHETIC GENE CLUSTER

I Dewa M. Kresna[1], Zerlina G. Wuisan[1], *Nils Böhringer*[1] *and Till F. Schäberle*[1*]

[1] Institute for Insect Biotechnology, Justus-Liebig-University, Giessen, Germany

Most bioactive specialized metabolites known to date were discovered from cultivable bacteria. However, the cultured bacteria only represent a small fraction of the total bacterial diversity. Moreover, many biosynthetic gene clusters (BGCs) produce the corresponding metabolite only under certain conditions, which are often different to the ones applied in the laboratory.

The aim of the project is to discover novel compounds with antibacterial activity from Ophistobranch-associated microorganisms by heterologous expression of putative BGCs. The latter are beforehand identified by *in silico* analysis (Anti-SMASH[1]). Thereafter, first, a (meta)genomic library[2] is generated and screened towards the presence of the BGC of interest. Second, the BGC – if separated on different vectors – must be assembled. This is achieved by cutting the region of interest from the vector using either common restriction enzymes, or CRISPR-Cas9[3]. Subsequently, TAR cloning[4] or Gibson assembly are used to assemble the BGC in an expression vector. Third, to enhance the expression of the BGC, promoter(s) have to be introduced. This can be achieved either by **λ**-red recombineering and subsequent flp-*FRT* recombination[5], or by CRISPOmyces recombineering[6]. Fourth, transfer of the expression construct into a suitable expression host by conjugation[4].

The complete methodology will be presented on the poster.

[1] T. Weber, K. Blin, S. Duddela, D. Krug, H. U. Kim, R. Bruccoleri, S. Y. Lee, M. A. Fischbach, R. Müller, W. Wohlleben, et al., *Nucleic Acids Res* **2015**, 43, W237–W243.

[2] C. Gurgui, J. Piel, in Metagenomics: Methods and Protocols (Eds.: W.R. Streit, R. Daniel), *Humana Press, Totowa, NJ,* **2010**, pp. 247–264.

[3] K. E. Kistler, L. B. Vosshall, B. J. Matthews, *Cell Reports* **2015**, 11, 51–60.

[4] The Moore Lab, Direct Cloning and Heterologous Expression of Biosynthetic gene cluster. (n.d.). *Scripps Institution of Oceanography.* **(n.d.)** , UCSD.

[5] B. Gust, G. Chandra, D. Jakimowicz, T. Yuqing, C. J. Bruton, K. F. Chater, *Adv. Appl. Microbiol.* **2004**, 54, 107–128.

[6] R. E. Cobb, Y. Wang, H. Zhao, *ACS Synth. Biol.* **2015**, 4, 723–728.

P41. PROTEASOME INHIBITORS FROM *NOCARDIA SPP.* AND THEIR ROLE IN PATHOGEN SURVIVAL

Alicia Engelbrecht[1], *Nicole Staudt*[1], *Leonard Kaysser*[1]

[1] Pharmaceutical Biology, Auf der Morgenstelle 8, 72076 Tübingen, E-Mail: alicia.engelbrecht@uni-tuebingen.de

The proteasome regulates numerous cellular processes, such as mitosis, inflammation, apoptosis and immune responses. Exploitation of the ubiquitin-proteasome degradation machinery is therefore a developed strategy by human bacterial pathogens to evade the host immune system. *Nocardia* spp. are gram positive, filamentous Actinobacteria, that can cause localized and systemic Nocardiosis, mostly in immunosuppressed patients. It was previously shown that the highly virulent *N. cyriacigeorica* GUH-2 is capable of inducing a small molecule-mediated inhibition of the proteasome [1]. Interestingly, we identified the first two biosynthetic gene clusters of the proteasome inhibitors of the epoxyketone-family, Epoxomicin and Eponemycin, and a homologous pathway was also found in *N. cyriacigeorica* GUH-2 [2]. We know raise the question if this orphan gene cluster of *N. cyriacigeorgica* GUH-2 encodes for a proteasome inhibitor and which role this small molecule plays in the pathogenicity of this strain. Identification and characterisation of the putative proteasome inhibitor will be accomplished by RED/ET recombineering mediated gene-cluster knock-outs, as well as heterologous pathway expression of the whole candidate gene-cluster. Metabolite production will be followed by LC-MS. For the biological characterization of the PI, we just recently established a proteasome inhibition assay and additionally plan to do various mammalian cell-based assay, e.g. infection experiments with live *N. cyriacigeorgica* GUH-2.

[1] D. P. Barry, B. L. Beaman, *Research in microbiology* **2007**, *158*, 86-96.
[2] M. Schorn, J. Zettler, J. P. Noel, P. C. Dorrestein, B. S. Moore, L. Kaysser, *ACS chemical biology* **2013**, *9*, 301-309.

LIST OF PARTICIPANTS

Arend	Kristin	Ruhr Universität Bochum
Abebew	Desalegne	Goethe Universität Frankfurt
Awori	Ryan	Goethe Universität Frankfurt
Bader	Chantal	Helmholtz Institut Saarbrücken
Bader	Marius	Universität Tübingen
Bakker	Tomke	Universität Tübingen
Balansa	Walter	Universität Gießen
Berghaus	Natalie	Goethe Universität Frankfurt
Breitenbach	Jürgen	Goethe Universität Frankfurt
Bode	Helge	Goethe Universität Frankfurt
Bode	Edna	Goethe Universität Frankfurt
Bohn	Monika	Universität Tübingen
Burkhardt	Immo	Universität Bonn
Cai	Xiaofeng	Goethe Universität Frankfurt
Camus	Anna	ETH Zürich
Castillo Arteaga	Roger David	Universität Tübingen
Dehm	Daniel	Universität Potsdam
Diettrich	Jan	TU Dortmund
Dittmann	Elke	Universität Potsdam
Drechsler	Moritz	Goethe Universität Frankfurt
Ecker	Felix	TU München
Engelbrecht	Alicia	Universität Tübingen
Folger	Ines	ETH Zürich
Frank	Nicolas	Helmholtz Institut Saarbrücken
Grammbitter	Gina	Goethe Universität Frankfurt
Groll	Michael	TU München
Handel	Franziska	Universität Tübingen
Hegemann	Julian	University of Illinois
Hennrich	Oliver	Universität Tübingen
Hermenau	Ron	Hans Knöll Institut Jena
Hug	Joachim	Helmholtz Institut Braunschweig
Ishida	Keishi	Hans Knöll Institut Jena
Jagels	Annika	Universität Münster
Kage	Hirokazu	TU Dortmund
Kegler	Carsten	Goethe Universität Frankfurt
Keller	Nadine	Goethe Universität Frankfurt
Kranz	Janik	Goethe Universität Frankfurt
Krause	Janina	Universität Tübingen
Kreling	Lukas	Goethe Universität Frankfurt
Kresna	I Dewa Made	Universität Gießen
Krumbholz	Julia	Universität Potsdam
Kruth	Sebastian	TU Dortmund
Lackner	Gerald	Universität Jena
Leis	Benedikt	Fraunhofer Institut Gießen
Lünne	Friederike	Universität Münster
Mast	Yvonne	Universität Tübingen
Mettal	Utel	Universität Gießen
Michael	Priyanka	University of Tromsø , Norway
Metsä-Ketalä	Mikko	University of Turku, Finland
Mihajlovic	Sanja	Fraunhofer Institut Gießen
Mingyar	Erik	Universität Tübingen

t

Morishita	Yohei	University of Tokyo
Nett	Markus	TU Dortmund
Neubacher	Nick	Goethe Universität Frankfurt
Panter	Fabian	Helmholtz Institut Braunschweig
Popoff	Alexander	Helmholtz Institut Saarbrücken
Rinkel	Jan	Universität Bonn
Riyanti		Universität Gießen
Schmalhofer	Maximilian	TU München
Schmeing	Martin	McGill University, Montreal, Kanada
Schneider	Florian	Goethe Universität Frankfurt
Senges	Christoph	Ruhr Universität Bochum
Shi	Yan-Ni	Goethe Universität Frankfurt
Shi	Yi-Ming	Goethe Universität Frankfurt
Steiniger	Charlotte	TU Berlin
Tietze	Andreas	Goethe Universität Frankfurt
Trottmann	Felix	Hans Knöll Institut Jena
Tsukada	Kento	Tohoku University, Japan
Tobias	Nicholas	Goethe Universität Frankfurt
Vidaurre	Daniela	Goethe Universität Frankfurt
Vural	Helena	Goethe Universität Frankfurt
Watzel	Jonas	Goethe Universität Frankfurt
Weber	Tilmann	Technical University of Denmark
Wemakor	Emmanuel	Universität Tübingen
Wenski	Sebastian	Goethe Universität Frankfurt
Wesche	Frank	Goethe Universität Frankfurt
Westphalen	Margaretha	Geothe Universität Frankfurt
Wiebach	Vincent	TU Berlin
Wiker	Franziska	Universität Tübingen
Wohlleben	Wolfgang	Universität Tübingen
Wolf	Felix	Universität Tübingen
Wuisan	Zerlina	Universität Gießen
Zdouc	Mitja	NAICONS Srl Milan, Italy
Zeng	Hu	Helmholtz Institut Braunschweig
Zhao	Lei	Goethe Universität Frankfurt
Zumkeller	Celine	Fraunhofer Institut Gießen

Goethe Universität Frankfurt
Institut für Molekulare Biowissenschaften
Biozentrum
Campus Riedberg
Gebäudeteil 250, Raum 0.04
Max-von-Laue-Str. 9
60438 Frankfurt am Main

T +49 69 798-29558
F +49 69 798-29527
E-mail: VAAM-Workshop-2018@bio.uni-frankfurt.de
http://www.bio.uni-frankfurt.de/55740469/VAAM-Workshop-2018