technische universität
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Emerging Trends in Natural Product Biotechnology

September 20/21, 2018

Book of Abstracts

50 Jahre

WELCOME ADDRESS

Dear Colleagues,

on behalf of TU Dortmund University, we cordially welcome you to the symposium *Emerging Trends in Natural Product Biotechnology*. In the history of mankind, natural products have long been used for their medicinal, gustatory or other functional properties. In recent years, the rapidly developing fields of metabolic engineering, synthetic biology and systems biology have opened promising new avenues for producing these molecules with mammalian, microbial, or plant cells. On occasion of the 50th anniversary of our university, we are thus very pleased to bring scientists from several countries together to exchange ideas and explore collaborations in this vivid research area.

We would like to express our sincere thanks to the Gesellschaft der Freunde der Technischen Universität Dortmund (GdF), to the Society for Medicinal Plant and Natural Product Research (GA) as well as to our industrial partners for their generous support.

It is wonderful that we have a diverse group of scientists with research interests spanning across so many disciplines represented at this meeting: from natural product biosynthesis and structural biology to pathway refactoring, biocatalysis, bio-processing, and pharmacognosy. We are sure this will make it all together to an exceptional event.

Enjoy the scientific program of this symposium, take the opportunity to exchange experiences, to interact and to network.

We wish you all a fantastic experience.

Prof. Dr. Markus Nett
Laboratory of Technical Biology
TU Dortmund University

Prof. Dr. Dr. Oliver Kayser
Chair of Technical Biochemistry
TU Dortmund University

Prof. Dr. Stephan Lütz
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PROGRAM

Thursday, September 20, 2018

08:00 – 12:00	Registration
09:00 – 09:20	**Opening remarks & Welcome addresses** Prof. Dr. Gabriele Sadowski, Prorector Research, TU Dortmund University Prof. Dr. Dr. Oliver Kayser, Vice-President, Society for Medicinal Plant and Natural Product Research (GA) Organizing Committee
Keynote Lecture I	
09:20 – 10:00	Prof. Dr. Martin Grininger, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt **Engineering fatty acid synthases (FAS) for custom compound synthesis**
Session I: Natural Product Biosynthesis	
10:00 – 10:20	Prof. Dr. Leonard Kaysser, Department of Pharmaceutical Biology, University of Tuebingen **Unusual features in the biosynthesis of β-lactone proteasome inhibitors**
10:20 – 10:40	Prof. Dr. Till F. Schäberle, Institute for Insect Biotechnology, Justus-Liebig-University of Giessen **Alkaloid biosynthesis by marine bacteria**
10:40 – 11:10	COFFEE BREAK
Session II: From Genomics to Drug Development	
11:10 – 11:30	Prof. Dr. Jörn Kalinowski, Center for Biotechnology (CeBiTec), University of Bielefeld **Application of Modern Sequencing Technologies to Natural Product Discovery and Analysis**
11:30 – 11:50	Dr. Charles M. Moore, Global Discovery Chemistry, Novartis Institute for Biomedical Research, Basel **Towards a synthetic biology platform for natural product discovery**
11:50 – 12:10	Dr. Andreas Brunschweiger, Faculty of Chemistry and Chemical biology, TU Dortmund University **Genetically tagged small molecule libraries**
12:10 – 14:00	LUNCH

Thursday, September 20, 2018

Keynote Lecture II

14:00 – 14:40	Prof. Dr. Tilmann Weber, The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby **Integration of informatics and metabolic engineering for the discovery and analysis of natural products**

Session III: Pathway Reconstitution & Metabolic Engineering

14:40 – 15:00	Prof. Dr. Frank Schulz, Faculty of Chemistry and Biochemistry, Ruhr-University Bochum **An enzymatic total synthesis of sesquiterpenes**
15:00 – 15:20	Dr. Anna Kohl, Institute for Molecular Biotechnology, RWTH Aachen **Metabolic engineering for monolignol production in *Escherichia coli***
15:20 – 15:40	Dr. Jan Marienhagen, Institute of Bio- and Geosciences (IBG), Forschungszentrum Jülich **Microbial production of plant polyphenols with *Corynebacterium glutamicum***
15:40 – 17:00	COFFEE BREAK & POSTER EXHIBITION

Keynote Lecture III

17:00 – 17:40	Prof. Dr. Dr. Birger Lindberg Møller, Plant Biochemistry Laboratory, Center for Synthetic Biology, University of Copenhagen **P450 driven production of plant natural products**

Session IV: Redirecting Plant Biosynthesis

17:40 – 18:00	Dr. Jakob Franke, Center of Biomolecular Drug Research (BMWZ), Leibniz University Hannover **Developing a plant-based withanolide synthetic biology platform**
18:00 – 18:20	Dr. Felix Stehle, Laboratory of Technical Biochemistry, Department of Biochemical and Chemical Engineering, TU Dortmund University **Deciphering the effect of phytohormones and microRNAs in glandular trichome development and distribution**
19:00 – 21:00	DINNER

PROGRAM

Friday, September 21, 2018

Keynote Lecture IV	
09:00 – 09:40	Prof. Dr. Jason Micklefield, School of Chemistry and Manchester Institute of Biotechnology, The University of Manchester **Diversification of natural and non-natural products using engineered biosynthetic pathways and enzymes**
Session V: Pathway Engineering	
09:40 – 10:00	Prof. Dr. Matthias Brock, School of Life Sciences, University of Nottingham **One enzyme, two products: Recombinant production of NRPS-like enzymes in fungal expression platforms**
10:00 – 10:20	Prof. Dr. Frank Hahn, Department of Chemistry, University of Bayreuth **In vitro studies on biosynthetic key enzymes and their applicability for chemoenzymatic synthesis**
10:20 – 10:50	COFFEE BREAK
Keynote Lecture V	
10:50 – 11:30	Prof. Dr. Frank Hollmann, Department of Biotechnology, Delft University of Technology **Are peroxygenases the new P450s?** **Scope and current challenges of peroxygenases for selective oxyfunctionalisation chemistry**
Session VI: Biocatalysis	
11:30 – 11:50	Prof. Dr. Tanja Gulder, Department of Chemistry and Catalysis Research Center, Technical University Munich **Effectively combining biocatalysis and photochemistry: green and mild *C,C*-functionalizations**
11:50 – 12:10	Dr. Silja Mordhorst, Department of Pharmaceutical and Medicinal Chemistry, Albert-Ludwigs-University Freiburg **Modular enzyme systems for cofactor regeneration**
12:10 – 12:30	Dr. Katrin Rosenthal, Laboratory of Bioprocess Engineering, Department of Biochemical and Chemical Engineering, TU Dortmund University **Enzymatic synthesis of cyclic dinucleotides**
12:30 – 12:45	POSTER AWARDS & CLOSING REMARKS
12:45	END OF SYMPOSIUM

K 1

Engineering fatty acid synthases (FAS) for custom compound synthesis

Jan Gajewski, Manuel Fischer, Alexander Rittner, Christina Heil, Karthik Paithankar, Martin Grininger

Institute of Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany

Multienzyme type I fatty acid synthases (FAS) provide compartmentalized reaction space for C-C bond formation. While FAS naturally perform the biosynthesis of C_{16} and C_{18} fatty acids, we have demonstrated in recent studies that these proteins can also be employed for the custom synthesis of commodity products, among them short fatty acids, methylketones and a lactone[1,2]. The production of the lactone was achieved by the coupling of two fungal FAS constructs in a way that construct 2 starts synthesis only when diffusion-loaded with the product of construct 1. Key to the successful design was the well-established understanding of these proteins, and the combined *in vitro* protein analysis and *in silico* description of the relevant enzymatic functions.

In spite of the successful production of new compounds, our work also disclosed the limitation of fungal FAS as a scaffold for custom compound synthesis. We have recently shown that the mammalian FAS is a better-suited scaffold, since the open architecture of the fold allows the direct communication of FAS constructs ([3]). Mammalian FAS may be arranged to assembly lines in programmable manner, which sets out the perspective to harness a similar large chemical space as achieved with the related polyketide synthases (PKS).

(1) Gajewski, J.; Buelens, F.; Serdjukow, S.; Janssen, M.; Cortina, N.; Grubmuller, H.; Grininger, M., Engineering fatty acid synthases for directed polyketide production. *Nat Chem Biol.* **2017,** *13*, 363-365.

(2) Gajewski, J.; Pavlovic, R.; Fischer, M.; Boles, E.; Grininger, M., Engineering fungal de novo fatty acid synthesis for short chain fatty acid production. *Nat Commun.* **2017,** *8*, 14650.

(3) Rittner, A.; Paithankar, K. S.; Vu Huu, K.; Grininger, M., Characterization of the polyspecific transferase of murine type I fatty acid synthase (FAS) and implications for polyketide synthase (PKS) engineering. *ACS Chem Biol.* **2018**, *13*, 723-732

L 1

Unusual Features in the Biosynthesis of β-Lactone Proteasome Inhibitors

Felix Wolf[1,2], Judith S. Bauer[1], Theresa M. Bendel[1,2], Andreas Kulik[3], Jörn Kalinowski[4], Harald Gross[1], Leonard Kaysser[1,2,]*

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[4] Center for Biotechnology (CeBiTec), University of Bielefeld, 33615 Bielefeld, Germany

Belactosins and cystargolides are natural product proteasome inhibitors from Actinobacteria. Both consist of a dipeptide connected to a unique β-lactone building block. Herein, we present a detailed investigation of their biosynthesis. Identification and analysis of the corresponding gene clusters indicate that both compounds are assembled by rare single enzyme amino acid ligases. Isotope-labeled precursor feeding experiments and in vitro biochemistry showed that the formation of the β-lactone warhead is unprecedented reminiscent of leucine biosynthesis and involves the action of isopropylmalate synthase homologs. Furthermore, recent result adressing the origin and biosynthesis of the rare aminocyclopropyl mojety in belactosin A are presented.

Alkaloid Biosynthesis by Marine Bacteria

Till F. Schäberle
Institute for Insect Biotechnology, Justus-Liebig-University of Giessen
Department of Bioresources of the Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany

Elucidating marinoquinoline biosynthesis

Bacterial aminophenylpyrrole-derived alkaloids (APPA) like the antifungal pyrrolnitrin, which was already discovered in the 60ies, represent high value lead compounds that were developed into globally important fungicides. More recently, diverse APPAs were discovered from bacteria belonging to the *Cytophagales* order (phylum *Bacteroidetes*).[1-4]

In the present work, a bioinformatics and phylogenetic approach enabled the elucidation of the genetic basis, as well as the non-enzymatic processes involved in the biosynthesis of these natural products showing diverse bioactivities. Initially, our strain collection[5] was screened for potential APPA producers. Subsequently, a bioinformatic analysis of the producer genomes allowed the identification of the putative APPA biosynthetic gene cluster (BGC). The biosynthesis was experimentally validated by heterologous expression of the BGCs in *E. coli* and revealed that first, one enzyme-dependent biosynthetic step yields the tryptophan-derived precursor 3-(2'-aminophenyl)-pyrrole. Second, a spontaneous Pictet-Spengler-like coupling reaction enables the producer strains to create a library of tricyclic alkaloids. Which alkaloids are formed is highly dependent on the chemical environment, since several aldehydes can be applied as substrates. The diversity of these natural products class is further enlarged by the catalytic action of a methyltransferase (MT), which adds a methyl group to the aminophenyl intermediate. This *N*-MT is encoded in some of the bacterial BGCs, and was investigated in detail, using *in vivo* and *in vitro* experiments.

References:

[1]) Y. Sangnoi, O. Sakulkeo, S. Yuenyongsawad, A. Kanjana-Opas, K. Ingkaninan, A. Plubrukarn, K.A. Suwanborirux, *Mar Drugs* **2008**, 6, 578-586.
[2] Y. Sangnoi, A. Plubrukarn, V. Arunpairojana, A. Kanjana-Opas, *World J. Microbiol. Biotechnol.* **2014**, *30*, 1135-1139.
[3] P.W. Okanya, K.I. Mohr, K. Gerth, R. Jansen, R. Müller, *J. Nat. Prod.* **2015**, *74*, 603-608.
[4] E.J. Choi, S.J. Nam, L. Paul, D. Beatty, C.A. Kauffman, P.R. Jensen, W. Fenicall, *Chem. Biol.* **2015**, *22*, 1270 -1279.
[5] L. Linares-Otoya, V. Linares-Otoya, L. Armas-Mantilla, C. Blanco-Olano, M. Crüsemann, M.L. Ganoza-Yupanqui, J. Campos-Florian, G.M. König, Schäberle T.F. **2017**, *15*, pii: E308.

Application of Modern Sequencing Technologies to Natural Product Discovery and Analysis

Christian Rückert, Tobias Busche, Daniel Wibberg, Jörn Kalinowski
Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany

Next-Generation and “Third Generation” sequencing technologies have led to a revolution with respect to the speed of generating knowledge on biological systems with reasonable effort. However, the application of these technologies on natural product discovery and analysis is only starting. The presentation will give an overview on recent technology developments (nanopore sequencing) and its application to generate (almost) gap-free genomes of natural product-producing microbes from prokaryotic[1] and eukaryotic[2] origin in a hybrid approach together with short read data. Not only single organisms can now be completely sequenced and their genomes mined for natural products, but also metagenome sequencing allows to have a look at communities and their production capacities. The applications presented will include sponge microbiomes[3] and other natural communities.
Sequencing of genomes is complemented by sequencing of transcriptomes, either for efficiently deducing the natural product synthesis pathways in higher organisms, namely plants, but also for deciphering transcriptional control of biosynthetic gene clusters.

[1]Senges CHR, Al-Dilaimi A, Marchbank DH, Wibberg D, Winkler A, Haltli B, Nowrousian M, Kalinowski J, Kerr RG, Bandow JE. (2018) The secreted metabolome of *Streptomyces chartreusis* and implications for bacterial chemistry. Proc Natl Acad
Sci U S A. 115(10):2490-2495.
[2]Schor R, Schotte C, Wibberg D, Kalinowski J, Cox RJ. (2018) Three previously unrecognised classes of biosynthetic enzymes revealed during the production of xenovulene A. Nat Commun. 9(1):1963.
[3]Mori T, Cahn JKB, Wilson MC, Meoded RA, Wiebach V, Martinez AFC, Helfrich JN, Albersmeier A, Wibberg D, Dätwyler S, Keren R, Lavy A, Rückert C, Ilan M, Kalinowski J, Matsunaga S, Takeyama H, Piel J. (2018) Single-bacterial genomics validates rich and varied specialized metabolism of uncultivated *Entotheonella* sponge symbionts. Proc Natl Acad Sci U S A. 115(8):1718-1723.

L 4

Towards a synthetic biology platform for natural product discovery

Charles M. Moore
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Basel, Switzerland

The maturation of DNA sequencing technologies coupled with the rapid emergence of synthetic biology as a discipline has turned (meta)genome mining into an attractive avenue to acquire novel chemical starting points to facilitate early lead discovery. Technical challenges persist; however, progress in this space has accelerated in recent years. Here we present our experiences from a multi-year effort to design and implement a natural product discovery pipeline based on principles of synthetic biology. This effort has permitted us to understand where bottlenecks reside and potentially how they could be addressed going forward.

Genetically taged small molecule libraries

M. Klika Škopić, V. Kunig, M. Potowski, A. Brunschweiger TU Dortmund University, Dortmund, Germany

The identification of compounds binding to a protein of interest is a crucial step for drug development. Genetic tagging of small molecules allows for efficient handling of large compound libraries (DNA-encoded libraries, DELs), and interrogating these efficiently by selection.[1] DELs consist of chemically synthesized or modified molecules covalently connected to individual DNA barcode strands. They are synthesized through combinatorial strategies. We have synthesized and validated a DNA-encoded 27.000-membered library based on functionalized scaffolds to establish the library synthesis process.[2] Heterocycles are essential structures in the chemical space of bioactive compounds. Currently, only few heterocycle-forming reactions are available for DEL synthesis. Transition metal catalysts, and acid organocatalysts enable access to diverse drug-like heterocycles, but interact or even react with purine bases eventually causing depurination of the DNA tag. To circumvent this impediment, we utilize a hexathymidine sequence “hexT” as an adapter oligonucleotide in the initial step of DEL synthesis (figure 1).[3-5] Testimony of the chemical stability of the hexT was the synthesis of hexT-pyrazole conjugates through a Au(I)-mediated annulation reaction in glacial acetic acid. The hexT- heterocycle conjugates were readily ligated to coding DNA sequences.

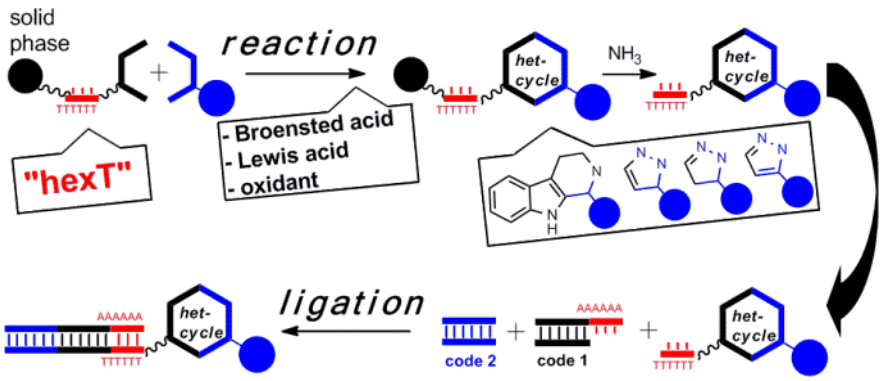

Figure 1: Access to DNA-encoded libraries based on the hexathymidine sequence “hexT”.

[1] H. Salamon, *et al.*, ACS Chem. Biol. 2016, 19, 296-307. [2] M. Klika Škopić, *et al.*, Med. Chem. Commun. 2016, 7, 1957-1965. [3] M. Klika Škopić, *et al.*, Chem. Sci., 2017, 8, 3356- 3361. [4] M. Klika Škopić, *et al.*, Org. Biomol. Chem., 2017, 15, 8648-8654. [5] A. Brunschweiger, *et al.*, 2015, EP15202448.5.

Integration of informatics and metabolic engineering for the discovery and analysis of Natural Products

Tilmann Weber
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Genome analyses of many microorganisms indicate that the genetic potential to synthesize secondary metabolites is far beyond the number of molecules observed in traditional chemical screenings. Thus, genome mining for natural products biosynthetic pathways has emerged as an important technology complementing the chemical approaches. This trend was supported by the public availability of powerful genome mining bioinformatics software that allow microbiologists or chemists to carry out such analyses.

In close collaboration with the group of M. Medema at Wageningen University, we are coordinating the development of the comprehensive genome mining platform antiSMASH (http://antismash.secondarymetabolites.org. With antiSMASH, users can easily mine microbial (and plant) genomic sequences for the presence of secondary metabolite biosynthetic gene clusters. To provide better analysis options of the data generated with antiSMASH, we have recently released version 2 of the antiSMASH database (http://antismash-db.secondarymetabolites.or), a user-friendly web-based application allowing to browse and query antiSMASH annotation of >6000 high-quality bacterial genomes.

These genome mining technologies build the foundations of our experimental work on actinomycetes, a group of organisms that historically have been – and still are – among the most important producers of natural products with anti-microbial activities. However, there are severe limitations concerning efficiency of mutagenesis protocols that often hamper systems metabolic engineering and Synthetic Biology approaches. We have developed a CRISPR/Cas9-based toolkit for streptomycetes and other actinomycetes that allows efficient mutagenesis and transcriptional repression.[3,] This toolkit was extended with our software CRISPy-web (http://crispy.secondarymetabolites.org, which enables users to design the sgRNAs required for the CRISPR/Cas9 experiments for any user-submitted microbial genome in a user-friendly web-interface.

Having such tools at hand, it is not only possible to identify the biosynthetic pathways, but also to engineer them directly. Based on the knowledge about the biosynthesis of the antibiotic kirromycin,[5–8] we were recently able to engineer a producer

strain, which upon feeding on the non-natural polyketide extender units allyl and propargyl-malonate, is able to synthesize allyl- and propargyl-kirromycin derivatives, the latter easily accessible for Click-chemistry modifications.

References

1. Blin, K. *et al.* AntiSMASH 4.0 - improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res.* 45, W36–W41 (2017).
2. Blin, K., Medema, M. H., Kottmann, R., Lee, S. Y. & Weber, T. The antiSMASH database, a comprehensive database of microbial secondary metabolite biosynthetic gene clusters. *Nucleic Acids Res.* 45, (2017).
3. Tong, Y., Robertsen, H. L., Blin, K., Weber, T. & Lee, S. Y. in *Methods in Molecular Biology* (eds. Krogh Jensen, M. & Keasling, J. D.) 1671, 163–184 (Humana Press, New York, NY, 2018).
4. Tong, Y., Charusanti, P., Zhang, L., Weber, T. & Lee, S. Y. CRISPR-Cas9 Based Engineering of Actinomycetal Genomes. *ACS Synth. Biol.* 4, (2015).
5. Musiol, E. M. *et al.* The AT 2 Domain of KirCI Loads Malonyl Extender Units to the ACPs of the Kirromycin PKS. 1343–1352 (2013). doi:10.1002/cbic.201300211
6. Ye, Z., Musiol, E. M., Weber, T. & Williams, G. J. Reprogramming acyl carrier protein interactions of an acyl-CoA promiscuous trans-acyltransferase. *Chem. Biol.* 21, (2014).
7. Robertsen, H. L. *et al.* Filling the Gaps in the Kirromycin Biosynthesis: Deciphering the Role of Genes Involved in Ethylmalonyl-CoA Supply and Tailoring Reactions. *Sci. Rep.* 8, 3230 (2018).
8. Musiol-Kroll, E. M. *et al.* Polyketide Bioderivatization Using the Promiscuous Acyltransferase KirCII. *ACS Synth. Biol.* 6, 421–427 (2017)

An Enzymatic Total Synthesis of Sesquiterpenes

Michael Dirkmann, Julia Nowack, Frank Schulz
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Ex vivo Biosynthesis flexibilizes Natural Product Analysis and Preparation

The enzymatic synthesis of terpenes was investigated using a cascade based on the mevalonic acid pathway. Suitable enzymes from all kingdoms of life were identified and combined to give rise to geosmin and patchoulol as representative compounds. The pathway was studied in three separate segments which were subsequently combined to a 10-step cascade plus added cofactor regeneration systems (Fig. 1).

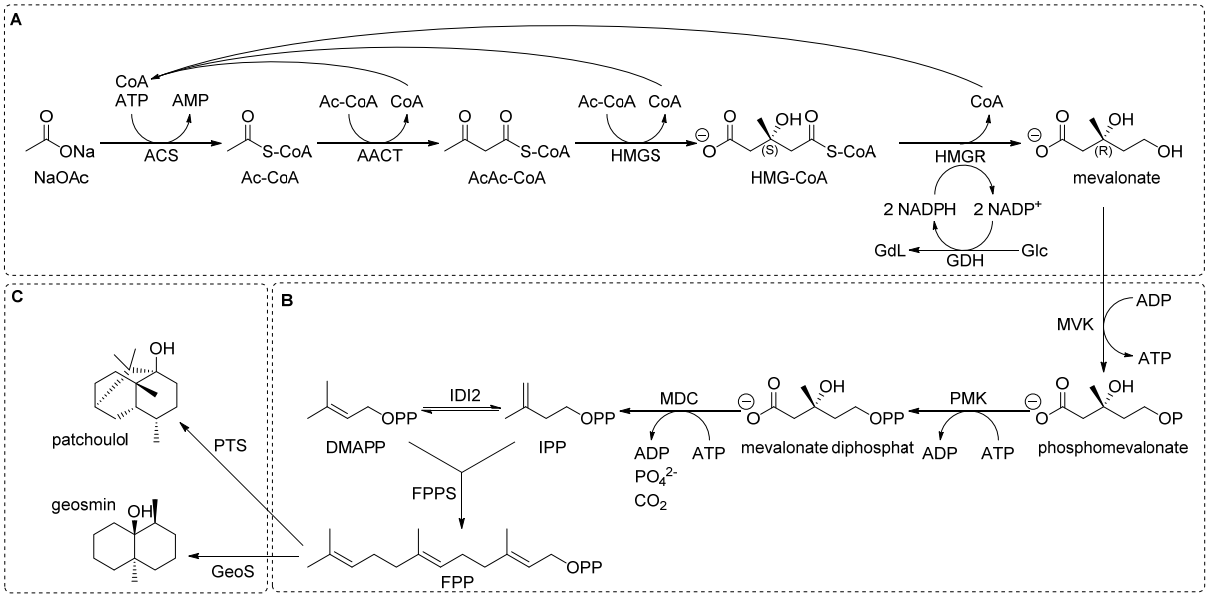

Figure 1: Synopsis of the synthesis of patchoulol and geosmin from acetic acid through an enzyme cascade.[1]

The synthesis starts from acetic acid as the smallest possible precursor and delivers farnesylpyrophophate in >40% yield. FPP itself is directly converted into the corresponding sesquiterpene with a yield of > 90%.

[1] Michael Dirkmann, Julia Nowack, Frank Schulz, **2018**, *in revision*

Metabolic engineering for monolignol production in *Escherichia coli*

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Abstract

Monolignols are the basic modules for the complex plant cell wall polymer lignin as well as for a plethora of secondary metabolites. Many plant secondary metabolites have beneficial characteristics like anti-inflammatory, antimicrobial and cancer-protective activities and can be precursors of mammalian enterolignans like enterodiol and enterlactone[2]. They are produced in very low amounts in the plants themselves and levels can hardly be elevated without disturbing the metabolisms of the plant. Thus, a heterologous production in industrially relevant microbial systems is desired.

The research presented here is part of the LignaSyn project and aims at the production of lignans in the host organism *E. coli*. The production of p-coumaryl alcohol was already established recently in our group and is currently further characterized and improved[2]. For the p-coumaryl alcohol synthetic pathway, we utilized the bacterial tyrosine ammonia lyase (TAL) and 4-coumarate-CoA ligase from *Petroselinum crispum* (4CL) as well as a cinnamoyl-CoA reductase (CCR) and cinnamoyl alcohol dehydrogenase (CAD) from *Zea mays*. Proceeding from this first established pathway the utilization of P450 monooxygenases and their corresponding reductases are required for the further production route towards coniferyl and sinapyl alcohol. Here, we present first data of our pathway from L-tyrosine to coniferyl alcohol in *E. coli*.

References:

1. Satake, H. *et al.* Recent advances in the metabolic engineering of lignin biosynthesis pathways for the production transgenic plant-based foods and supplements. *J. Agric. Food Chem.* **61**, 11721-11729 (2013).
2. Jansen, F. *et al.* Metabolic engineering for p-coumaryl alcohol production in *Escherichia coli* by introducing an artificial phenylpropanoid pathway *Biotechnol. Appl. Biochem.* **61**, 646-654 (2014).

Microbial production of plant polyphenols with *Corynebacterium glutamicum*

Lars Milke, Nicolai Kallscheuer, Jennifer Aschenbrenner, Jan Marienhagen
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The soil-bacterium *Corynebacterium glutamicum* is an important microorganism in industrial biotechnology for the production of bulk chemicals, especially amino acids (Vogt *et al*., 2014). However, until now, only a few *C. glutamicum* strains for the production of aromatic compounds are available, which can be mostly attributed to the presence of a complex (and partly unknown) catabolic network for a broad range of aromatic substances.

We were able to uncover the complete catabolic route for phenylpropanoids, which allows growth of *C. glutamicum* using these compounds as sole carbon and energy source. Phenylpropanoids are channeled into the central carbon metabolism by the coupled action of an identified peripheral, CoA-dependent, β-oxidative degradation pathway and the well-known β-ketoadipate pathway (Kallscheuer *et al*., 2016). By deleting four gene clusters comprising altogether 21 genes being essential for degradation of phenylpropanoids and other aromatic compounds, we obtained a *C. glutamicum* platform strain that was subsequently applied for the production of (2*S*)-flavanones and stilbenes (Milke *et al.,* 2018).

Heterologous expression of codon-optimized genes encoding a 4-coumarate: CoA-ligase (4CL), a chalcone synthase and a chalcone isomerase led to the production of 30 - 35 mg/L of corresponding (2*S*)-flavanones from supplemented phenylpropanoids in the constructed strain. The strain also produced stilbenes when genes coding for a 4CL and a stilbene synthase (STS) were heterologously expressed. Several cultivation parameters influencing growth and gene expression were optimized with respect to improved product titers. Under optimal conditions, stilbene concentrations ranging from 60 to 160 mg/L could be obtained from supplemented phenylpropanoids. A strain converting the phenylpropanoid *p*-coumaric acid into the stilbene resveratrol was further modified with a focus on the increased supply of the precursor metabolite L-tyrosine. By introduction of an additional gene coding for a tyrosine ammonia lyase combining aromatic amino acid metabolism with phenylpropanoid synthesis, the strain was able to produce 60 mg/L directly from glucose in defined medium simultaneous to biomass formation.

Beyond production of polyphenols, the strain will help to overcome current drawbacks of *C. glutamicum* resulting from degradation of aromatic compounds, offering

a huge potential for production of other high-value aromatic compounds with this industrially relevant organism.

Kallscheuer, N., Vogt, M., Kappelmann, J., Krumbach, K., Noack, S., Bott, M., & Marienhagen, J. (2016). *Applied microbiology and biotechnology*, 100(4), 1871-1881

Milke L., Aschenbrenner J., Marienhagen J., Kallscheuer N. (2018). Production of plant-derived polyphenols in microorganisms: current state and perspectives. *Appl. Environ. Microbiol.* 102: 1575-1585.

Vogt, M., Haas, S., Klaffl, S., Polen, T., Eggeling, L., van Ooyen, J., & Bott, M. (2014). *Metabolic engineering*, 22, 40-52.

K 3

P450 driven production of plant natural products

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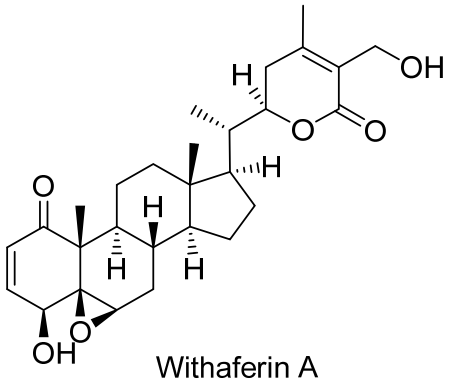
Recent work in our lab has resulted in the elucidation of the biosynthetic pathways of structurally complex diterpenoids from *Coleus forskohlii*, *Vitex agnus-castus*, *Eremophila lucida* and *Ginkgo biloba*, elucidation of the synthesis of vanillin in *Vanilla planifolia* and of the pathways for synthesis of cyanogenic glucosides in *Hordeum vulgare*, *Eucalyptus cladocalyx*, *Prunus amygdalus* and the ferns *Phlebodium aureum* and *Pteridium aquilinum.* The pathway discovery process has been guided by transcriptomics and proteomics and functional characterization of gene candidates using transient expression in tobacco and stable expression in yeast followed by LC -MS-NMR based structural identification of products obtained. MS bio-imaging technology has been optimized for plant cells to visualize the localization of the natural products at the cellular levels and has facilitated interpretation of the role of endogenous recycling of auto-toxic natural products. Using the styrene-maleic acid polymer based “cookie cutter” technology, the possibility to isolate membrane bound enzyme complexes (metabolons) catalyzing entire pathways has been demonstrated and the dynamic organization of the complexes demonstrated using fluorescence lifetime imaging microscopy. Using the approaches of synthetic biology for combinatorial biosynthesis, the functional modules identified have been assembled in new combinations to expand the natural product landscape. Successful transfer of entire pathways into chloroplasts, the power house of the photosynthetic cell, demonstrate the potential of this organelle as a direct production and storage site for different classes of phytochemicals pointing towards development of a light driven synthetic biology platform based on carbon dioxide from the atmosphere as the sole carbon source.

Developing a Plant-Based Witanolide Synthetic Biology Platform

Annika Stein, Stephan Rohrbach, Jakob Franke, Leibniz University Hannover, Centre of Biomolecular Drug Research (BMWZ), Hannover, Germany

Withanolides such as withaferin A are steroidal lactones found in numerous Solanaceous plants.[1] Over 400 representatives have been reported, many of which show potent antiproliferative activity *in vitro*. However, this enormous chemical complexity makes it difficult to obtain sufficient quantities of pure compounds for thorough drug testing.



Withaferin A

We will present how transient combinatorial gene expression in the wild tobacco plant *Nicotiana benthamiana*[2,3] can be used to develop a synthetic biology platform to identify biosynthetic genes and ultimately produce withanolides in a heterologous plant host. This approach will help to improve the toolkit available for the study of plant specialised metabolism as well as make withanolides as a promising class of anti-cancer drug candidates better accessible for medicinal research.

[1] L.-X. Chen, H. He, F. Qiu, *Nat. Prod. Rep.* **2011**, *28*, 705–740.

[2] J. Reed, M. J. Stephenson, K. Miettinen, B. Brouwer, A. Leveau, P. Brett, R. J. M. Goss, A. Goossens, M. A. O'Connell, A. Osbourn, *Metab. Eng.* **2017**, *42*, 185–193.

[3] T.-T. T. Dang, J. Franke, I. S. T. Carqueijeiro, C. Langley, V. Courdavault, S. E. O'Connor, *Nat. Chem. Biol.* **2018**, doi: 10.1038/s41589-018-0078-4.

L 10

Deciphering the effect of phytohormones and microRNAs in glandular trichome development and distribution

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Abstract
In contrast to the well-studied gene regulatory network of unicellular trichome in *A. thaliana*, the development process of glandular trichomes are almost unknown. First data indicate that the networks responsible for non-glandular formation differ from that of glandular trichome development. Since the biosynthesis of many organic compounds occurs in glandular trichomes they can be considered as biofactories. To use trichomes as a renewable resource for these compunds a detailed research is necessary to shed light on the complex network of glandular trichome formation.
The major objectives of this project are to identify the phytohormone-dependent variation of glandular trichome formation and to identify miRNAs and miRNA targets which contribute to the regulatory network responsible for the development and surface distribution of glandular trichomes. This will provide a detailed picture of the metabolic pathways involved in glands development and explores the cross talks between miRNA- and phytohormone-regulated trichome development.
Here we show that phytohormones can modulate the surface distribution of glands on *Cannabis* flowers. Furthermore, we were able to identify 38 miRNAs by a RNA-Seq approach of different *Cannabis* organs including trichomes. These miRNAs and the corresponding target sequences will be evaluated in a virus-induced gene silencing (VIGS) system that was optimized for the application in *Cannabis*.

Diversification of natural and non-natural products using engineered biosynthetic pathways and enzymes

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Abstract
Natural products often require further chemical modification, to improve their biological activities or physicochemical properties, for therapeutic and other applications. However, many of the most promising natural products, particularly the polyketides and nonribosomal peptides, are highly complex molecules which offer limited opportunity for semi-synthesis, and are invariably inaccessible through total synthesis on the scale required for drug development. Consequently, alternative biosynthetic engineering approaches are required, which can enable the rapid structural diversification and optimisation of promising natural product scaffolds. In this lecture our recent progress in biosynthetic engineering will be presented. In addition methods for using enzymes from biosynthetic pathways to create non-natural products will be described.

L 12

One enzyme, two products: Recombinant production of NRPS-like enzymes in fungal expression platforms

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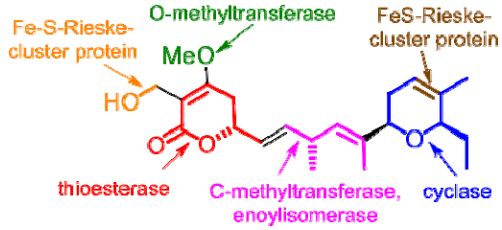
Background: Filamentous fungi are treasure chests for novel yet uncharacterised natural products with interesting biological activities of value for human exploitation. As biosynthesis genes of these secondary metabolites frequently remain silent under laboratory conditions, heterologous gene expression is one possibility to identify the corresponding metabolites. A recombinant expression system based on transcriptional elements from *Aspergillus terreus* has been established using *Aspergillus niger* and *Aspergillus oryzae* as expression platform strains. Furthermore, by using a self-cleaving viral 2A peptide, several genes can be simultaneously expressed from a polycistronic messenger using this system.
Results: Here, genes coding for so-called non-ribosomal peptide synthetase (NRPS) -like enzymes were investigated. These enzymes generally use α-keto acids from aromatic amino acids as substrates. Thereby, substrate condensation by a *C*-terminal thioesterase domain results in different interconnecting core structures such as furanones of quinones. Thus, by using the same substrate *p*-hydroxyphenylpyruvate, MelA from *A. terreus* produces aspulvinone E, whereas InvA5 from *Paxillus involutus* forms the involution precursor atromentin. Unexpectedly, product formation was dependent on the expression platform strain. While heterologous expression of InvA5 in *A. oryzae* resulted in atromentin, a novel previously undescribed metabolite called atrofuranic acid was produced in *A. niger*. Subsequent analyses revealed that atrofuranic acid does not derive from a modification of atromentin and is specifically produced in Aspergilli from section *Nigri*.
Conclusion: Despite a limited substrate spectrum, NRPS-like enzymes produce a wide range of different products. Several enzymes are embedded in biosynthesis gene clusters further broadening the diversity of metabolites produced. Even more, the host physiology may direct the structure of the resulting metabolite. Therefore, different expression platforms should be utilised when working on this class of enzymes.

In vitro studies on biosynthetic key enzymes and their applicability for chemoenzymatic synthesis

Frederick Lindner, Andreas Peil, Steffen Friedrich, Gesche Berkhan and Frank Hahn Department of Chemistry, Universität Bayreuth, Bayreuth/ Germany*

Natural products are ideal starting points for the development of novel drugs.1 Their structural complexity necessitates efficient strategies to prepare derivatives libraries of sufficient size for structure activity relationship (SAR) studies. For this, the combination of chemical-synthetic and biotechnological methodology is particularly promising.2,3 One such approach is chemoenzymatic synthesis using enzymes from secondary metabolism. By exploiting the advantages of enzyme catalysis, the efficiency of synthetic routes is markedly improved. Our research focuses on the discovery and characterisation of novel biocatalysts from the secondary metabolism, with a particular focus on polyketides. Enzymes with synthetically attractive activity are studied in detail and their applicability for preparative-organic purposes optimised.



Progress on the chemoenzymatic synthesis of the unusual reduced polyketide jerangolid A and the closely related ambruticins will be presented. An efficient routeto series of biosynthetic precursor derivatives has been developed and several key enzymes from the both biosynthetic pathway have recently been characterised.4-7

References:

1. D.J. Newman, G.M. Cragg, *J. Nat. Prod.* **2016**, *79*, 629.
2. A. Kirschning, F. Hahn, *Angew. Chem. Int. Ed.* **2012**, *51*, 4012.
3. S. Friedrich, F. Hahn, *Tetrahedron* **2015**, *71*, 1473.
4. G. Berkhan, F. Hahn, *Angew. Chem. Int. Ed.* **2014**, *53*, 14240.
5. G. Berkhan, C. Merten, C. Holec, F. Hahn, *Angew. Chem. Int. Ed.* **2016**, *55*, 13589.
6. K.-H. Sung, G. Berkhan, T. Hollmann, L. Wagner, W. Blankenfeldt, F. Hahn,*Angew. Chem. Int. Ed.* **2018**, *57*, 343.
7. S. Friedrich, F. Hemmerling, F. Lindner, A. Warnke, J. Wunderlich, G. Berkhan, F. Hahn, *Molecules* **2016**, *21*, 1443.

Are peroxygenases the new P450s? Scope and current challenges of peroxygenases for selective oxyfunctionalisation chemistry

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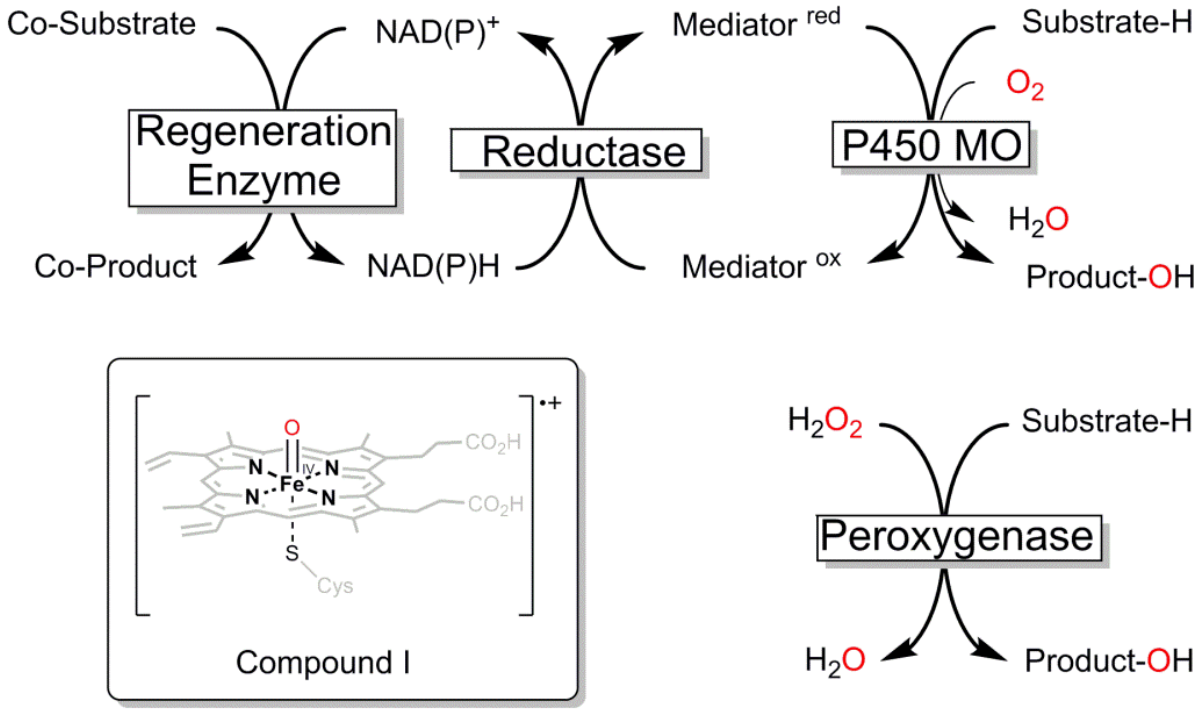
Peroxygenases are experiencing a renewed interest as catalysts for selective oxyfunctionalisation chemistry. Peroxygenases are promising alternatives to the well-known P450 monooxygenases due to the significantly simpler regeneration scheme.



Comparison of P450-monooxygenases and peroxygenases with respect to the regeneration of the catalytically Compound I.

New peroxygenases enable selective hydroxylation of non-activated C-H bonds in alkanes and aromatics, epoxidation and heteroatom oxygenation. Yet, to assess the full scope of this exciting enzyme class a range of challenges need to be met: (1) more enzyme (variants) with tailored properties need to be identified/evolved; (2) new *in situ* H_2O_2-generation systems are necessary to minimise oxidative enzyme inactivation and (3) peroxygenase-reactions in non-aqueous media need to be established.
These issues together with some promising solutions will be discussed.

Effectively Combining Biocatalysis and Photochemistry: Green and Mild *C*,*C*-Functionalizations

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Vanadium-dependent haloperoxidases (VHPOs) are a class of enzymes that catalyze the oxidative halogenation of organic substrates, with only minimal requirements: hydrogen peroxide as the oxidant together with a halide source and vanadate as the prosthetic group. Our group recently identified and heterologously produced a new VHPO from the cyanobacterium *Acaryochloris marina* (*Am*VHPO).[1] This novel and highly robust biocatalyst is capable of brominating a broad variety of (hetero-)aromatic compounds. In order to decrease over-bromination and oxidative degradation, it is desirable to produce hydrogen peroxide *in situ*. Inspired by previous examples using flavin mononucleotide (FMN) or Au-doped TiO_2 photochemical as redoxmediators to produce hydrogen peroxide[2] we optimized the system omitting any external electron donor from the reaction by simply using the buffer salt or even water itself as sacrificial electron source.[3] To our delight, the brominated products can be subjected to further downstream processes, like e.g. Pd catalyzed cross coupling reactions or photochemical transformations allowing for a one-pot formation of a new *C,C*-bond utilizing the halogen atom as a traceless directing group.

[1] C. J. Seel, A. Frank, M. Groll, T. Gulder, *ChemBioChem* **2016**, *17*, 2028-2032.
[2] a) F. Sabuzi, E. Churakova, P. Galloni, R. Wever, F. Hollmann, B. Floris, V. Conte, *Eur. J. Inorg. Chem.* **2015**, *2015*, 3519-3525.
b) D. I. Perez, M. M. Grau, I. W. C. E. Arends, F. , Hollmann, *Chem. Commun.* **2009**, 6848-6850.
c) W. Zhang, E. Fernández-Fueyo, Y. Ni, M. van Schie, J. Gacs, R. Renirie, R. Wever, F. G. Mutti, D. Rother, M. Alcalde, F. Hollmann, *Nat. Catal.* **2018**, *1*, 55-62.
[3] C. J. Seel, A. Králík, M. Hacker, A. Frank, B. König, T. Gulder, *ChemCatChem* **2018**, doi.org/10.1002/cctc.201800886; published online

Modular Enzyme Systems for Cofactor Regeneration

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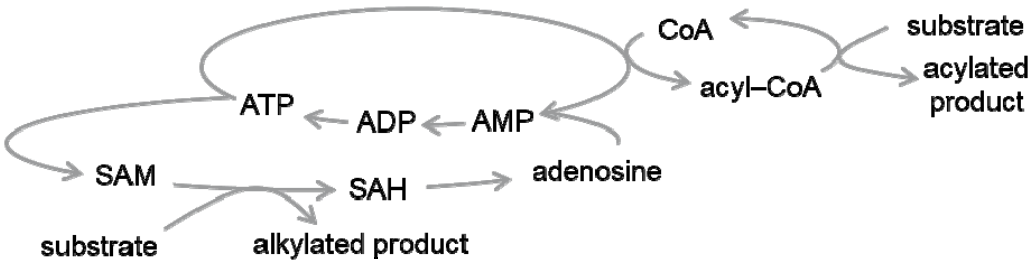
Development of biomimetic multistep cofactor regeneration cycles based on polyphosphate as energy source

Cofactor-dependent enzymes have great potential for chemical processes. However, their application is often hampered by the need for stoichiometric supply of the cofactor and inhibitory effects of cofactor-derived byproducts.[1] To counteract these issues, we implemented *in situ* cofactor regeneration systems based on natural principles.

Adenosine 5'-triphosphate (ATP) is involved in the biosynthesis of further organic cofactors such as (acyl-) coenzyme A (CoA) and *S*-adenosylmethionine (SAM). In these pathways, ATP is degraded to various products. In the *in vitro* systems presented, they are re-phosphorylated to ATP by using inexpensive and abundant inorganic polyphosphate as phosphate donor and energy source.

CoA-dependent acylation reactions rely on ATP to form an acyl-CoA intermediate yielding adenosine 5'-monophosphate as a byproduct. Following the acylation step, CoA is released and through ATP regeneration from AMP both cofactor cycles (ATP and CoA) are re-entered.[2]

Nature's methylating agent SAM is mainly used for chemo-, regio-, and stereoselective methylation (or alkylation) reactions. In a SAM supply system, SAM is generated *in situ* from ATP and L-methionine and the alkyl transfer is followed by hydrolytic cleavage of the SAM-derived byproduct. We extended the SAM supply system to a genuine SAM regeneration cycle by re-phosphorylating the hydrolysis product to ATP.[3]



[1] H. K. Chenault, E. S. Simon, G. M. Whitesides, *Biotechnol. Genet. Eng. Rev.* **1988**, *6*, 221–270.

[2] S. Mordhorst, A. Maurer, D. Popadić, J. Brech, Andexer, *ChemCatChem* **2017**, *9*, 4164–4168.

[3] S. Mordhorst, J. Siegrist, M. Müller, M. Richter, J. N. Andexer, *Angew. Chem. Int. Ed.* **2017**, *56*, 4037–4041

Enzymatic synthesis of cyclic dinucleotides

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The presence of deoxyribonucleic acids (DNA) in the cytosol of mammalian cells is a danger signal and triggers an innate immune response. Cytosolic DNA activates cyclic guanosine monophosphate (GMP) - adenosine monophosphate (AMP) synthase (cGAS), which catalyzes the synthesis of the cyclic GMP-AMP dinucleotide 2'3'-cGAMP [1, 2]. Recent studies have shown that 2'3'-cGAMP induces the production of interferons and appears to be promising for the use as a novel active ingredient to boost the innate immunity [3].

This study presents the synthesis of 2'3'-cGAMP and its derivatives using cGAS (Fig. 1).

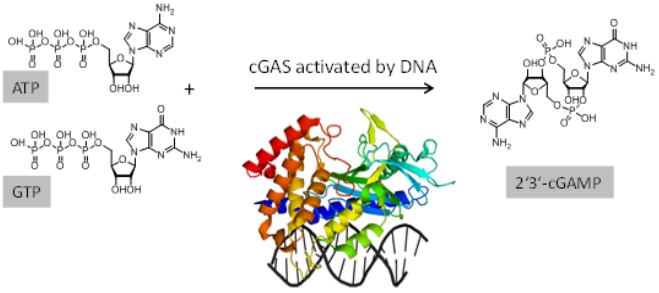

Figure 1: Synthesis of 2'3'-cGAMP using cGAS

The activity of cGAS to synthesize 2'3'-cGAMP from guanosine triphosphate (GTP) and adenosine triphosphate (ATP) was proven. Design of experiments was used to establish a multivariable regression model which facilitates the description of basic catalytic mechanisms and moreover allows the identification of reaction optima. The results offer compelling evidence for the existence of two major substrate inhibition mechanisms. The inhibition of the biocatalytic reaction appears to be the result of substrate inhibition caused by ATP concentrations above 0.5 mM and substrate competition for side reactions. Interestingly, cGAS not only catalyzes the formation of 2'3'-cGAMP but also the formation of cyclic di-AMP and cyclic di-GMP.

Further experiments focus on the systematic analysis and characterization of cGAS and its application for new reactions. The development of libraries of such CDN analogs is thought to be a useful tool in future enabling to get a more detailed insight into the meaning of CDNs as second messenger.

1. Hall J, et al. (2017): Prot Sci 26 (12):2367-2380. doi: 10.1002/pro.3304
2. Ablasser A, et al. (2013): Nature 498 (7454):380-384. doi: 10.1038/nature12306
3. Corrales L, et al. (2015): Cell Rep 11 (7):1018-1030. doi: 10.1172/JCI86892

P 1

Identification and heterologous production of the mycobacteria associated cofactor mycofactocin using a metabolomics and systems biology approach

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Faculty of Pharmacy and Biology, Friedrich-Schiller-Universität Jena, Jena, Germany

The discovery of novel enzyme cofactors and their corresponding enzymes could reveal unusual biochemistry and promise new biotech applications as well as antibiotic targets. A known characteristic of biosynthetic gene clusters (BCG) yielding cofactors as a final product is their genomic co-occurrence with genes that encode the corresponding cofactor-utilizing enzymes. This comparative genomics approach was employed for proposing the existence of "mycofactocin" as a novel redox cofactor [1]. Its BGC is widespread across various phyla and particularly abundant in Actinobacteria, e.g. in the clinically important Mycobacteriaceae family. The biosynthetic pathway has been partially decoded *in vitro* using heterologously expressed enzymes [2-4]. However, further steps are still uncharacterized and the final structure and activity of the cofactor is still unknown. Our research is focused on alternative research approaches, in which the whole BGC is expressed in heterologous hosts. Comparative metabolomics is employed to identify candidate molecules *in vivo*. Furthermore, the putative precursor VY* has been chemically synthetized and a redox activity has been determined. Taken together, these two approaches should lead to the elucidation of the structure and function of mycofactocin.

1. Haft, D. H. BMC Genomics, 2011, 12, 21
2. Bruender, N. A. & Bandarian V. Biochemistry, 2016, 55, 2813-2816
3. Khaliullin, B. *et al.* FEBS Lett, 2016, 590, 2538-2548
4. Bruender, N. A. & Bandarian, V. J Biol Chem, 2017, 292, 4371-4381

P 2

Fermentative production of L-tryptophan derivatives

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Amino acids are the most important organic compounds because of their fundamental function in protein biosynthesis. There is an increasing interest of amino acid production by chemical industries. Especially the production of aromatic amino acids as L-phenylalanine, L-tyrosine and L-tryptophan gain in importance since they can not be synthesized by animals and humans and have to be assimilated by food (Leuchtenberger et al., 2005). Bacteria can produce these amino acids via the Shikimate pathway through diverse intermediates. One derivative of tryptophan is the cyclic and aromatic compound indole, which serves an in important signal molecule in bacteria (Li & Lee, 2010). L-Tryptophan can be hydroxylated and 5-hydroxytryptophan, for example, has an important impact in medicine, where it improves the sleep state (Bongaerts et al., 2001).
Here we study the production of tryptophan derivatives by metabolically engineered *Corynebacterium glutamicum*, a bacterium suited for production of aromatic compounds (Lee & Wendisch, 2017). To enable β-elimination of L-tryptophan to yield indole (Yoshida et al., 2009), the gene for pyridoxal 5-phosphate dependent tryptophanase (*TnaA*) from various microorganisms was expressed in a *C. glutamicum* strain. Furthermore, a *C. glutamicum* strain was engineered to produce hydroxylated aromatic compounds by heterologous expression of a phenylalanine-4-hydroxylase gene (*phhA*) from *Chromobacterium violaceum.* Hydroxylation of L-tryptophan required introduction of two amino acid exchanges.

Bongaerts, J., Krämer, M., Müller, U., Raeven, L., & Wubbolts, M. (2001) *Metabolic engineering*, *3*(4), 289-300.
Lee JH, Wendisch VF. (2017) *J Biotechnol*. 257:211-221.
Leuchtenberger, W., Huthmacher, K., & Drauz, K. (2005) *Applied microbiology and biotechnology*, *69*(1), 1-8.
Lee, J. H., & Lee, J. (2010). *FEMS microbiology reviews*, *34*(4), 426-444.
Yoshida, Y., Sasaki, T., Ito, S., Tamura, H., Kunimatsu, K., & Kato, H. (2009). *Microbiology*, *155*(3), 968-978.

P 3

Fast production of patchoulol with metabolically engineered *Corynebacterium glutamicum*

*Nadja A. Henke [1], Carina Prell [1], Julian Wichmann [2], Thomas Baier [2], Jonas Frohwitter [1], Kyle J. Lauersen [2], Joe M. Risse [3], Petra Peters-Wendisch [1], Olaf Kruse [2] and Volker F. Wendisch [1],**

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Abstract: Patchoulol is a sesquiterpene alcohol found in patchouli oil and represents an important product of the perfumery industry. The central enzyme for its biosynthesis – the patchoulol synthase *PcPS* from the plant *Pogostemon cablin* – was used to produce patchoulol in a metabolically engineered *C. glutamicum* strain. *C. glutamicum* is a prominent host for biotechnological production of value-added products[a] such as various amino acids, vitamins and isoprenoid compounds. Due to favourable characteristics, like its robustness and fast growth, *C. glutamicum* has previously been engineered for the production of the sesquiterpenoid valencene[b] and the high-value carotenoid astaxanthin[c,d]. In this study, *C. glutamicum* strain ATCC 13032 was metabolically engineered for patchoulol production by pursuing different strategies: Prevention of carotenoid accumulation by genomic deletions, an increased precursor supply by heterologous overexpression of the FPP synthase (*ispA*) from *E. coli* and the overexpression of limiting enzymes from the MEP-pathway contributed to an FPP overproducing strain. Overexpression of a codon optimized heterologous patchoulol synthase gene enabled production of the fragrance compound patchoulol from glucose. It was shown, that upscaling to liter-scale fermenters improved both titer and volumetric productivities 60 mg L^{-1} and 18 mg L^{-1} d^{-1}, respectively[e]. Patchoulol production was also demonstrated from the non-food competitive carbon sources arabinose and xylose underlying the robustness and flexibility of *C. glutamicum* as a platform system in white biotechnology. The space time yield here presented exceeds that of production hosts described in literature and makes the recombinant *C. glutamicum* strain an attractive candidate for patchoulol production in industrial scale.

P 4

CRISPR-Cas9 mediated reduction of the nicotine content of tetraploid smoking tobacco (*Nicotiana tabacum*)

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Abstract

Tobacco alkaloids, especially nicotine as the most abundant alkaloid, represent a large group of secondary metabolites with a great research interest. Some approaches were already done to reduce the nicotine level of tobacco plants according to health benefits or to investigate the function of these enzymes in nicotine biosynthesis. Additionally, tobacco represents a model plant for heterologous production of proteins and metabolites. With lower nicotine content, the plant represents a suitable platform organism for biotechnological use, since alkaloids do not act as interfering factors anymore. The reduction of the expression level of several genes involved in alkaloid biosynthesis resulted in developmental abnormalities or changes of the content of other alkaloids. The final oxidation step of alkaloid biosynthesis is catalysed by proteins of the berberine bridge enzyme-like (BBL) family, localised in the vacuole, encoded by six different genes. Lower expression levels of three of the six related genes resulted in lowered nicotine level without any side effects. Thus, the *BBL* genes represent promising targets for knock-out studies concerning a low nicotine yield.

Here we describe the CRISPR-Cas9 mediated knock-out of the six known *BBL* genes targeted with just one sgRNA. Since *N. tabacum* is a tetraploid plant, 24 gene copies have to be knocked out at the same time. Regenerated plants from transformed tissue were grown up to F_3 generation. Nicotine content of these plants was analyzed, sequences of the six *BBL* genes have been checked for successful knock-out and it was tested if the plants do not carry the transformation cassette anymore. It was possible to identify plants with nicotine contents between 3 % - 8 % compared to the wild type. Finally we obtained one plant without the transformation cassette and with knock-out of all six *BBL* genes.

P 5

Phlorobenzophenone from metabolically engineered *Escherichia coli*

Anuwatchakij Klamrak, Natsajee Nualkaew

Faculty of Pharmaceutical Sciences: Khon Kaen University, Khon Kaen, Thailand

Background: phlorobenzophenone (also known as 2,4,6-trihydroxybenzophenone) is a major product obtained from the catalytic activity of benzophenone synthase (BPS) by using one molecule of benzoyl CoA and three molecules of malonyl CoA as the substrates. This compound plays a crucial role as the key precursor in the biosynthesis of bioactive prenylated benzophenones and xanthones found in medicinal plants belong to Clusiaceae. **Aims:** *E. coli* cells carrying the genes encoded benzoate CoA ligase (Bad A) from *Rhodopseudomonas palustris* and benzophenone synthase (BPS) from *Garcinia mangostana* was previously engineered. Herein, we investigate the ability to produce phlorobenzophenone from both intracellular (cell pellets) and extracellular (medium) fractions. **Methods:** The production of phlorobenzophenone was carried out by culturing the strains carrying plasmid pETDuet/BadA/BPS in LB medium supplemented 3 mM $MgCl_2$. The IPTG- induced cultures were cultivated at 18 ^{0}C, 250 rpm for 5 hr. Sodium benzoate (5 mM) was added into the cultures to initiate the biosynthesis of target compound and further cultivated for 18 hr. The cell pellets and medium were collected from the cultures. The extracellular phlorobenzophenone was isolated from the medium by partition with ethyl acetate and re-dissolved with methanol prior to analysis. Intracellular phlorobenzophenone was extracted with EtOAc from the cell pellets treated with lysis buffer. The enzymatic reactions of recombinant BPS (wild type) and its mutant (T133L) were used for preparation of standard in HPLC analysis. **Results:** Phlorobenzophenone could be detected as the major product from both intracellular and extracellular fractions. Higher production of this compound found in extracellular fraction, indicated its secretion into the cultured medium. The peaks corresponded to the tetraketide lactone and triketide lactone, the derailments products of BPS could also be detected from both fractions as the minor products. **Conclusion:** The engineered strain capable of producing phlorobenzophenone was established. This suggested our engineered strains could possibly be used for production of prenylated benzophenones by means of metabolic engineering and synthetic biology.

De-novo Design of a Biosynthetic Gene Cluster for the Highly Bioactive RiPP Wewakazole

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Lyngbya majuscula is marine cyanobacterium which synthesizes different natural compounds: PKs, NRPs, hybrid PK-NRPs and also ribosomal peptides (RiPPs) such as the wewakazoles (Nogle *et al.*, 2003). Wewakazole A (Fig. 1) and wewakazole B are cyclic peptides and present a potent cytotoxic activity against the human H460 lung cancer (10 mM and 1 mM, respectively) and the human MCF7 breast cancer (0.58 mM) cell lines.
The natural producers and chemical synthesis produce only a minor amount of these compounds. Heterologous expression is also not an option since the biosynthetic gene cluster is completely unknown. These facts lead to a scarcity of the compound that prevents its further development as a lead structure.

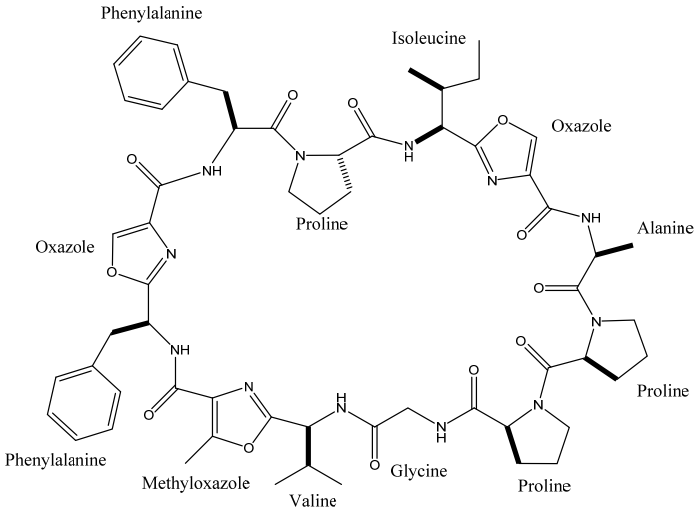

Figure 1. Wewakazole A structure.

Here we present the bioinformatic studies and *de-novo* design of a wewakazole biosynthetic gene cluster in *Escherichia coli* DH10B using the accumulated knowledge in biosynthesis of similar pathways and, especially, **Synthetic Biology** tools.

References

Nogle LM, Marquez BL, Gerwick WH. (2003) Wewakazole, a novel cyclic dodecapeptide from a Papua New Guinea *Lyngbya majuscula*. Org Lett. 2003 5:3-6.

P 7

De novo triterpene biosynthesis in *E. coli*

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Abstract

Terpenes and terpenoids represent one of the largest and most diverse groups of natural products. These secondary metabolites are commonly found in plants where they are involved in stress responses to biotic and abiotic factors, as well as in pollinator attraction or other plant-environment communication processes, making them key components for plant survival and health. Besides their biological roles, terpenes and terpenoids are used as medicines, nutritional additives, flavours, fragrances or biofuels. However, their concentration in plants is rather low what makes their extraction an inefficient and costly process and led to the rising interest in biotechnological ways towards more efficient production of these chemically complex compounds.

While many di- or sesquiterpenes have already been successfully produced in microorganisms, *de novo* biosynthesis of triterpenes still remains a challenge. In plants they are produced via cyclization of (*S*)-2,3-oxidosqualene catalysed by oxidosqualene cyclases, which are hard to express in soluble form in microorganisms.

We constructed an *E. coli* based platform for screening of potentially interesting plant oxidosqualene cyclases and *de novo* triterpene biosynthesis. Using easily manipulated *E. coli* cells, three plasmids with a total of up to 12 bacterial, fungal and plant genes were combined in a modular manner to mimic plant triterpene pathways. This *E. coli* based platform allowed us not only to identify several oxidosqualene cyclases active in *E. coli*, but also to produce their respective triterpene products lupeol, β-amyrin and cycloartenol.

P 8

Wiring *Escherichia coli* for the biosynthesis of Coenzyme F_{420}

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Organic cofactors have a decisive role supporting many enzymes to catalyse biochemical reactions. While many are ubiquitous, others are involved in specialized metabolism and might have an unearthed potential for biotechnology. Coenzyme F_{420} is an exotic cofactor whose structural peculiarities endows it with low redox potential and, consequently, with the ability to aid challenging redox reactions, e.g. biosynthesis of antibiotics and transformation of xenobiotics. However, the appreciation of F_{420}-dependent enzymes is hindered due to the cofactor being commercially unavailable and to the current biological source, *Mycobacterium smegmatis*, being a human pathogen. Aiming to reconstitute and evaluate the biosynthesis of coenzyme F_{420} in a more tractable microorganism, we report the production of F_{420} key precursor (F_O) in *Escherichia coli*. We identified a biosynthetic gene cluster for the cofactor F_{420} in the genome of the Gram-negative proteobacterium *Paraburkholderia rhizoxinica* HKI454. The sequence encoding F_O synthase was heterologously expressed in *E. coli* either in its native form or as a hexa-histidine fusion protein. HRMS/MS and fluorimetric evidences allowed the unambiguous detection of intra- and extracellular F_O in both cases. Finally, we also show the activity and biochemical attributes of F_{420-0} glutamyl-ligase (CofE) from *P. rhizoxinica*. In this sense, we were able to enzymatically remove and reconstitute the characteristic polyglutamylated tail of F_{420} *in vitro* using CofE and could determine the number of L-glutamate residues preferably attached by this enzyme. Our results support the use of *E. coli* may be a safe, suitable source of the F_O towards the complete biosynthesis the cofactor F_{420}. Moreover, this is the first report on the biosynthetic gene cluster for F_{420} from *P. rhizoxinica*. The functionality of both F_O synthase and F_{420-0} glutamyl-ligase encourages the investigation of F_{420}-dependent processes in this microorganism.

Engineering pseudochelin production in *Myxococcus xanthus*

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Myxobacteria utilize the catechol natural products myxochelin A and B in order to maintain their iron homeostasis.[1] Recently, the production of these siderophores was reported from the marine bacterium *Pseudoalteromonas piscicida* S2040, along with a new myxochelin derivative named pseudochelin A.[2] The latter features a characteristic imidazoline moiety, which was proposed to originate from an intramolecular condensation reaction of the β-aminoethyl amide group in myxochelin B. To identify the enzyme catalyzing this conversion, we compared the myxochelin regulons of two myxobacterial strains, which solely produce myxochelin A and B, with *P. piscicida* S2040. This approach revealed a gene exclusive to the myxochelin regulon in *P. piscicida* S2040, coding for an enzyme of the amidohydrolase superfamily. To prove that this enzyme is indeed responsible for the postulated conversion, the reaction was reconstituted *in vitro* using a hexahistidyl-tagged recombinant protein made in *Escherichia coli* and myxochelin B as substrate.[3] To test the production of pseudochelin A under *in vivo* conditions, the amidohydrolase gene was cloned into the myxobacterial plasmid pZJY156 and placed under the control of a copper-inducible promoter. The resulting vector was introduced into the myxobacterium *Myxococcus xanthus* DSM16526, a native producer of myxochelin A and B. Following the induction with copper, the myxobacterial expression strain was found to synthesize small quantities of pseudochelin A. Replacement of the copper-inducible promoter with the constitutive *pilA* promoter led to increased production levels in *M. xanthus*, which facilitated the isolation and subsequent structural verification of the heterologously produced compound.[3]

References

[1] B. Kunze, N. Bedorf, W. Kohl, G. Höfle, H. Reichenbach, *J. Antibiot.* **1989**, *42*, 14-17

[2] E. C. Sonnenschein, *et al.*, *Tetrahedron* **2017**, *73*, 2633-2637

[3] J. Korp, L. Winand, A. Sester, M. Nett, *Appl. Environ. Microbiol.* **2018**, in press

P 10

Production of N-methylglutamate by recombinant *Pseudomonas putida*

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N-methylated amino acids are present in diverse biological molecules in bacteria, archaea and eukaryotes. There is an increasing interest in this new molecular class of alkylated amino acids by the pharmaceutical and chemical industries. Since these modified amino acids have desired functions such as higher proteolytic stability, enhanced membrane-permeability and longer peptide half-lives, they can be used for the design of new peptide-based drugs, the so-called peptidomimetics.
N-methylated amino acids can be synthesized chemically e.g. by reductive amination, reductive ring opening of 5-oxazolidinones or using methylating reagents.[1] Here, we describe metabolic engineering *Pseudomonas putida* KT2440, which has been used for sustainable production of a broad spectrum of chemicals (e.g. terpenoid or aromatic compounds), for the fermentative production of *N*-methylglutamate. The *P. putida* GRAS strain KT2440, which has the naturally ability to grow with the alternative feedstock glycerol as sole carbon and energy source, was engineered for conversion of glutamate to *N*-methylglutamate using heterologous enzymes when monomethylamine was added to the medium. About 3.9 g L^{-1} *N*-methylglutamate accumulated within 48 h in shake flask cultures with glycerol minimal medium. Upscaling of the process in a fed-batch cultivation resulted in an enhanced *N*-methylglutamate titer of 17.9 g L^{-1}.

1 M. L. Di Gioia, A. Leggio, F. Malagrinò, E. Romio, C. Siciliano and A. Liguori, Mini-Rev. Med. Chem., 2016, 16, 683–690.

P 11

A genome streamlining-based bacterial chassis generation

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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.

P 12

Chemical fingerprinting of single glandular trichomes of Cannabis sativa by Coherent anti-Stokes Raman scattering (CARS) microscopy

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Cannabis possesses a rich spectrum of phytochemicals i.e. cannabinoids, terpenes and phenolic compounds of industrial and medicinal interests. Most of these high-value plant products are synthesised and stored in glandular trichomes, small protrusions of epidermal origin on the surface of leaves and flower buds. While the biosynthesis of the most abundant cannabinoids is well known regarding its biochemical pathway, localisation of the occurring processes lacks suitable *in vivo* methods. Here we show that Coherent anti-Stokes Raman scattering, a microspectroscopic technique, in combination with transmission and fluorescence enables the label-free imaging of cannabinoids and its precursors inside drug- and fibre-type hemp on single-trichome level, even in 3D. These results provide a deeper insight in trafficking and localisation of cannabinoids in trichomes and serve as a base for more in depth and especially spatially resolved analysis of natural biochemical factories of bioactive metabolites. Moreover, this method offers an easy and fast control of biotechnological processes, harvesting times and high throughput screenings for an optimised natural compound production.

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The Chemical Treasure of Microbial Communication Associated with Fungus Growing Termites

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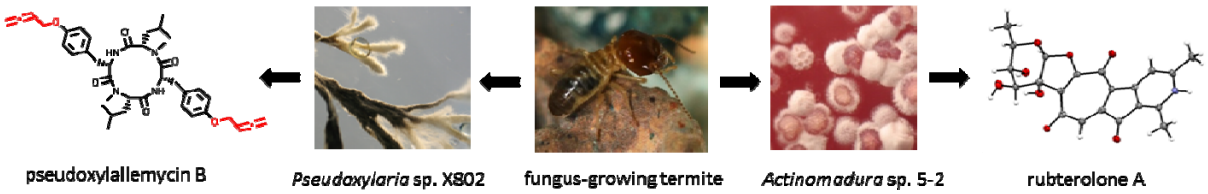

The rapid development of OMICs technologies has enabled a renaissance of natural product research. In particular, the detailed chemical analysis of complex symbiotic systems has attracted substantial attention amongst natural product chemists. As an immediate result, many different microbial derived metabolites with diverse structural features were identified and found to be important regulators of symbiotic interactions and essential for microbial communication, thus making them promising pharmaceutical drug candidates.

Here, I will present our recent results of the chemical analysis of symbiotic microbes associated with fungus-growing termites. First, I will report on the discovery of new highly substituted tropolone alkaloids from an Actinomycete (*Actinomadura* sp. 5-2), isolated from the gut of the fungus-growing termite *Macrotermes natalensis*. Rubterolones were identified using fungus-bacteria challenge assays, a HRMS-based dereplication strategy, and were characterized by NMR and HRMS analyses and X-ray crystallography. In a second example, I will highlight the discovery of cyclic tetrapeptides named pseudoxylallemycins from fungus *Pseudoxylaria* sp. X802, an antagonist of the cultivar of *M. natalensis*. Derivatives B–D were particularly intriguing as they possess a rare and chemically accessible allene moiety amenable for synthetic modifications, showed antimicrobial activity against Gram-negative human -pathogenic *Pseudomonas aeruginosa*. Both studies highlight that microbes present a prolific source of secondary metabolites and that we can use the chemical and genomic information to increase compound diversity.

P 14

Development of an Analytical Method for Metabolic Profiling of Monolignol and Lignan-Producing *Escherichia coli*

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Lignans are plant secondary metabolites that exhibit biological activity such as anticancer, antiinflammatory and antioxidant[1]. They find application as leading compounds for cancer treatment drugs as well as dietary supplements for the protection against lifestyle-related diseases. To circumvent high cost and environmental disruption linked to the isolation of lignans from natural plant sources, biosynthesis of lignans in microorganisms via novel multi-enzyme cascades is desired. However, bottlenecks as the occurrence of byproducts are probable. These can be detected by means of metabolic profiling, thereby identifying targets for optimization of lignan production[2]. Metabolic profiling requires the precise, reliable and reproducible quantification of the metabolites of interest, which has not yet been reported for the pathway from glucose or tyrosine to the lignan matairesinol.
Here, the development of a sample preparation method and a protocol for RP-HPLC-MS analysis for the quantification of metabolites involved in the synthesis of lignans, starting with the precursor tyrosine, is presented. The optimized HPLC protocol was used to analyse the exometabolome of a *p*-coumaryl alcohol producing *E. coli* strain, starting from tyrosine. The extraction efficiency with ethyl acetate was evaluated via spike-in with analytical standards of the intermediates of the monolignol pathway.

References

[1] Satake et al. (2015) Essences in metabolic engineering of lignan biosynthesis. Metabolites 5:270–290.
[2] Juminaga et al. (2012) Modular engineering of L-tyrosine production in *Escherichia coli*. Applied and Environmental Microbiology 78:89–98.

P 15

Free-Flow Electrophoresis for the Continuous Purification and Characterisation of Enzymatic Reaction Products

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Free-flow electrophoresis (FFE) is a method that allows the continuous separation and parallel fractionation of charged analytes in a liquid matrix. A major difference to more common electrophoretic techniques such as capillary electrophoresi (CE) and gel electrophoresis is the continuous nature of the injection and fractionation process. The continuity of the workflow makes FFE a versatile tool to be coupled to different devices up- and downstream like other flow-through reactors, CE or mass spectrometry (MS).

Here, we present a way to use microfluidic FFE (µFFE) to purify and monitor enzymatic reaction products coming from a flow-through reactor which contains immobilised enzymes. The idea is to continuously harvest the enzymatic products while cycling unused educts back into the system and getting rid of unwanted side products at the same time. The analytes being electrophoretically separated in the device are monitored at-line via electrospray ionisation mass spectrometry (ESI-MS). The coupling of µFFE to MS offers several advantages over commonly used analytical methods which are typically based on optical techniques. Proteins and DNA for example can be labelled with fluorescent dyes. Labelling, however, is not an option when the analytes ought to keep their original state. With MS no modifications of the analytes are required. Specificity and sensitivity are quite high and only little sample is consumed in the process.

Monitoring Cell-Free Miniaturized Enzymatic Production Units – On-Line and At-Line Analytics

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Background

Natural product research is of high interest for the development of new drugs. Most reactions rely on enzymes though, making their availability a limiting factor for research and development. One strategy to circumvent this limitation is miniaturisation of the whole process including reaction vessels, separation and analysis. Such miniature laboratories are frequently referred to as “lab on a chip” (LOC). A LOC design allows to perform multiple reactions (also in parallel) requiring only minimal amounts of sample and enzyme.

Aim

In our work in the Leibniz research cluster “Bio/synthetic multifunctional micro production units – novel ways of compound development” we aim to develop a LOC that allows for cell-free enzyme-catalyzed production of polyketides. This LOC shall comprise all necessary reaction steps: sequential or parallel reactors with on-line (or at-line) analytics and subsequent microfluidic separation of the reaction products. One major challenge is the destruction-free on-line analysis of the small volumes.

Results

We utilize specific enzymes to produce typical polyketide educts such as Malonyl-CoA or Acetyl-CoA from comparatively cheap starting materials. The enzymes are immobilized on magnetic beads so they can be reversibly transferred into the LOC reactors. We follow the enzymatic reactions of free as well as immobilized enzymes with several different techniques such as e.g. capillary electrophoresis, coherent Anti-Stokes-Raman spectroscopy or multi-photon spectroscopy in order to determine and quantify all reaction educts and products. We intend to expand this method on complex enzyme cascades to analyse entire polyketide syntheses.

P 17

Potential use of *Pueraria candollei* var. *mirifica* single cell suspension for large scale isoflavonoid production

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Scalable isoflavonoid production from suspension cell culture of *Pueraria candollei* var. *mirifica*

Isoflavonoids are naturally occurring polyphenolic compounds that have various biochemical and pharmacological properties. The tuberous roots of *Pueraria candollei* var. *mirifica* is a storehouse of isoflavonoids and are widely used in therapeutics and as dietary supplements. The present work was carried out with the aim of producing reliable sources of isoflavonoids from single *Pueraria* cells and at optimizing a growth medium with increased biomass and isoflavonoid content. The medium optimized for prolonged maintenance of callus was MS medium supplemented with 200 mg/L KH_2PO_4, 1 mg/L thiamine HCl, 100 mg/L of myo-inositol with 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) with routine subculture after every 30 days for the highest growth. The amount of different isoflavonoids were quantified using HPLC using standard reference. The highest amounts of puerarin and daidzein were quantified as 0.63 and 1.80 mg/g of dry weight in the examined suspension cell culture of single clones, whereas daidzein (0.50 mg/g) and genistein (0.03 mg/g) were present in the suspension cell culture obtained from a mixture of cells. The suspension of cell cultures from the same origin gave similar amounts of isoflavonoids, but cells from different sources varied in the amount of isoflavonoids. The suspension cell culture raised from single cells ensured low variation in the amount of isoflavonoids and are being scaled up using 1L/2L flasks and batch to batch consistency in the amount of isoflavonoids are routinely checked using HPTLC.

P 18

De-novo transcriptomic analysis revealed cannabinoid-like compound in *Radula marginata*

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The liverwort Radula marginata belongs to the bryophyte division of land plants and is a prospective alternate source of cannabinoid-like compound. However, lack of genetic information for such a distinguished pathway lead us to study *R. marginata* at transcriptomic level. Therefore, transcriptome of *R. marginata* was captured, deep sequenced, *de novo* assembled and annotated leading to the identification and validation of key gene for this distinct pathway. BLAST (Basic local alignment search tool) was used to infer the homology of all the unigenes from de-novo assembled transcriptome against NCBI (National Centre for Biotechnology Information) non-redundant (nr) protein database. Gene ontology analysis revealed 4820 gene functions from three categories of biological process (BP) molecular function (MF) and cellular components (CC). InterProScan (IPR) was used for the functional classification of unigenes and found 3,487 protein families, 3,529 domains, 515 sites and 108 repeats. As a result of complete annotation process 11,421 candidate genes were identified that encoded 1,554 enzymes in 145 biosynthesis pathways. Interestingly, we have identified all the upstream genes of the central precursor of cannabinoid biosynthesis, cannabigerolic acid (CBGA), including its two first intermediates. The large-scale transcriptomic resource generated in this study would further serve as a reference transcriptome to explore the *Radulaceae* family.

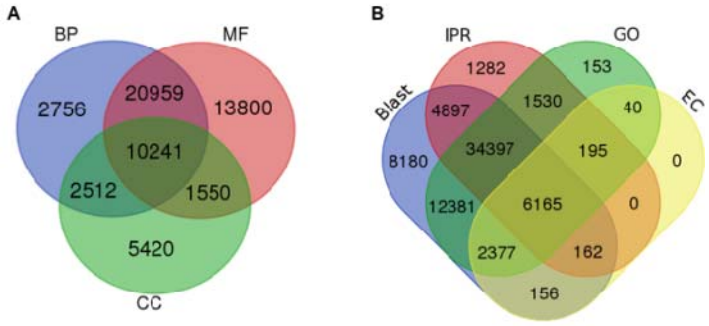

Figure: (A) Gene ontology functional annotation for biological process (BP), Molecular function (MF), and Cellular component (CC). **(B)** Overall annotation results from BLAST, InterProScan (IPR), Gene ontology (GO), and KEGG pathway databases.

These findings were recently published in the following referenced article:
Hussain T, Plunkett B, Ejaz M, Espley R V. Identification of Putative Precursor Genes for the Biosynthesis of Cannabinoid-Like Compound in Radula marginata. 2018; 9: 1–17

P 20

Regeneration of *Maytenus heterophylla* by a stem cutting technique and genome mining for cross-species maytansine biosynthesis

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Abstract

Background: The ecological role of maytansine, an important antineoplastic and antimicrobial compound with high cytotoxicity, particularly as a chemical defense compound, has remained elusive since its discovery in the 1970s in Celastraceae plants such as *Maytenus* and *Putterlickia* species. We recently provided proof-of-concept of ecospecific function of maytansine in Celastraceous plants [1,2]. However, the concept of cross-species maytansine biosynthesis is still open to further elucidation. Herein we evaluate the sharing steps in maytansine production between *Maytenus heterophylla* and its endophytes, as well as develop a sustainable protocol to regenerate the plant for further investigations. **Methods:** To achieve these goals, the plant materials from different places in South Africa were divided into two groups to perform parallel experiments (genome mining and plant regeneration). Firstly, the plant samples were separated into three subgroups according to their tissues, such as roots, leave and twigs. All these groups were surface sterilized before subjected to isolation of the endophytes. After that, all plant materials and isolated endophytes were screened for the present of three key genes in maytansine production i.e., AHBA synthase, KS and halogenase. Secondly, the stems of *M. heterophylla* were cut at the axillary bud in 45 degree angle and putted into a liquid fertilizer to initiate the root. **Results:** Ten endophytic communities and fourteen endophytic bacteria were isolated. On the one hand, only one endophytic community contained the halogenase gene, whereas three endophytic bacteria showed AHBA synthase gene and two of those endophytes also carried KS and halogenase genes. On the other hand, only the twig of *M. heterophylla* from Barrydale on the left side presented all genes that could produce maytansine. The other samples were carried one, two or none of those genes. After combining the results, it is possible that sharing biosynthesis coevolved in *M. heterophylla* from Seweweeks Poort and its endophytes. Finally, the roots were generated after cutting 8 weeks and we were successful in transferring the plants into the soil.

Ref.

Kusari et al. *J. Nat. Prod.*, **2014**, 77, 2577-2584

Kusari et al. *RSC Adv.*, **2016**, 6, 10011-10016

P 22

Catalytic activity of cannabinoid synthases in organic phase

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Cannabinoids are terpenophenolic compounds, which are produced and accumulated in glandular trichomes of Cannabis sativa. Nowadays, they have become promising therapeutic agents for treating cancer, inflammations, appetite disorders, and neurological diseases. The increasing demand for pharmaceutical grade cannabinoids as well as complicated law status of Cannabis made scientists to look for new biotechnology-based strategies of their synthesis.
In this study we examined catalytic activity of the exudates from Cannabis glandular trichomes in organic phase. We applied microsuction technique to collect the material from Cannabis glandular trichomes and subjected it for biotransformation studies in hexane. We also performed proteomic studies to identify enzymatic component of the exudates.
We identified cannabinoid synthases in the samples prepared from extracted exudates. The accumulation of the enzymes increased substantially from 5th to 7th week of the flowering stage. After incubating hexane-diluted trichome exudates with cannabigerolic acid we observed increased concentration of major cannabinoids.
Our results indicate that apart from metabolites Cannabis glandular trichomes secrete active enzymes, which accumulate over the flowering period. The enzymes remained their catalytic activity in organic phase. Our results also support the concept of extracellular biosynthesis of cannabinoids in the exudates of glandular trichomes. Further studies are needed to elucidate the exact mechanism which allows enzymatic activity in organic phase.

P 23

Salix amplexicaulis Bory – a novel source of bioactive compounds

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Willow bark (Salix spp., Salicaceae) is a traditional herbal remedy used to treat pain, fever and inflammation. Leaves are usually treated as waste material after bark collection and are mainly not studied. *Salix amplexicaulis* Bory is a species naturally occurring and widely spread on the Balkan Peninsula, yet there is lack of data about its chemical and medicinal properties. Therefore, the aim of this study was to chemically characterize and investigate, for the first time, the antioxidant capacity of ethanolic bark and leaf extracts of *S. amplexicaulis*. Chemical composition was determined by HPLC, while the antioxidant activity was assessed by the ability of extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals (OH). HPLC analysis revealed the presence of chlorogenic, *p*-hydroxybenzoic and *p*-coumaric acids, flavonoids epicatechin, rutin, quercetin, and naringenin and salycilic glycoside salicin.The most abundant compounds in both bark and leaf extracts were salicin, rutin and quercetin. DPPH scavenging capacity of bark (2.12 ± 0.12 μg/mL) extract was stronger than that of leaf (7.05 ± 0.15 μg/mL), while the OH radical inhibition of leaf extract (31.38 ± 0.14 μg/mL) was stronger than that of bark (51.69 ± 0.31 μg/mL). The obtained results suggest that bark and leaves of *S. amplexicaulis*, a species of willow not in commercial use, have great potential as sources of bioactive compounds.

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BIOZENTRUM

The *Biocenter* (www.biozentrum.nrw) was implemented at TU Dortmund University in 2016 following the appointments of Prof. Lütz and Prof. Nett. Having its home at the Department of Biochemical and Chemical Engineering, the *Biocenter* integrates expertises from both life sciences and engineering to promote excellence in interdisciplinary natural product research. The center is commitment to biotechnology, natural product biosynthesis and metabolic engineering.

The overall aim is to establish a center of excellence, providing long-term support to outstanding scientists as well as institutional and departmental program support in research and teaching. The specific goal is to enhance the innovative ability of TU Dortmund University and our partners with a highly sophisticated research program, latest technology in bioengineering as well as sharing ideas and competences in international grant applications as strategic partner.

We are prepared for accepting the challenges of the future in a dramatically changing world of synthetic biology, systems biotechnology, and metabolic engineering. We believe that future tasks of natural product biotechnology can only be solved in an interdisciplinary environment, and this is exactly what we have started here in Dortmund.

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