Project Julia Bandow

Polyether ionophore

Background and preliminary work: In the Center for System-based Antibiotic Research (CESAR), headed by Bandow, we investigate the biosynthetic capabilities of *Streptomyces* species. We developed a workflow, which combines genomics, bioinformatics, untargeted data-dependent tandem mass spectrometry, and molecular networking [Senges et al., 2018]. We analyzed the secreted metabolome of *S. chartreusis* NRRL 3882 and detected more than 1000 distinct metabolites in organic extracts of culture supernatants, only ten of which at the time were identified by searching public databases and approx. 40 more based on literature, analytical standards, NMR analysis, and manual annotation of mass and fragment spectra. Structures and functions of the vast majority of metabolites are unknown. Despite their fame as microbial chemists, the biosynthetic potential of *Actinomycetes* might still be underestimated. Our data suggest that individual biosynthetic gene clusters are able to convert substrates into a number of secondary metabolites [Sagurna et al., 2023].

To test our hypothesis within the Research Training Group MiCon, we singled out the biosynthetic gene cluster, which produces the polyether ionophore calcimycin. Calcimycin mediates iron and manganese release from *Bacillus subtilis* cells and triggers siderophore synthesis and thus can be considered a sophisticated tool aiding in iron acquisition [Raatschen et al., 2013]. In the above-mentioned study, calcimycin was detected in culture supernatants of *S. chartreusis* NRRL 3882 alongside three analogs of approximately equal abundance, two of which were identified as cezomycin and de-methyl-calcimycin, previously described in the literature as precursors of calcimycin (Wu *et al*., 2011 – PMID: 21173184). We elucidated the structure of the fourth compound by NMR analysis and it proofed to be a previously unknown derivative [Senges et al., 2018]. By supplementing different proteinogenic amino acids to a chemically defined medium, we detected further analogs [Arend and Bandow, 2021]. We found the semi-synthetic analog 4-Br-A23187 has a different ion transport profile. It acts as copper ionophore [Senges et al., 2022]. What might be the biological properties of the uncharacterized natural analogs?

Work planned: The goal of the MiCon PhD project will be to further investigate the polyether biosynthetic machinery and its products, to deepen our understanding of the origins and benefits of chemical diversity. The workplan will comprise the verification of the structure of additional new derivatives by purification and subsequent NMR analysis (collaboration with Metzler-Nolte). Further, selected genes in the biosynthetic gene cluster will be investigated with regard to their contribution to chemical diversity using single-gene knockout mutants and quantitative LC-MS/MS. Finally, using antimicrobial activity assays and element analysis, the biological properties of analogs will be investigated.

Selected references:

Raatschen N, Wenzel M, Leichert LIO, Düchting P, Krämer U, Bandow JE. 2013. Extracting iron and manganese from bacteria with ionophores – a mechanism against competitors characterized by increased potency in environments low in micronutrients. Proteomics 13:1358-1770.

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 USA. 10:2490-2595

Sagurna L, Heinrich S, Kaufmann LS, Rückert C, Busche T, Wolf A, Eickhoff J, Klebl B, Kalinowski J, Bandow JE. 2023. Characterization of the antibacterial activity of quinone-

based compounds originating from the alnumycin biosynthetic gene cluster of a *Streptomyces* isolate. Antibiotics 12:1116.

Arend KI, Bandow JE. 2021. Influence of amino acid feeding on production of calcimycin and analogs in *Streptomyces chartreusis*. Int J Environ Res Public Health 18:8740.

Senges CHR, Warmuth HL, Vázquez-Hernández M, Uzun HD, Sagurna L, Dietze P, Schmidt C, Mücher B, Herlitze S, Krämer U, Ott I, Günther Pomorski T, Bandow JE. 2022. Effects of 4-Br-A23187 on *Bacillus subtilis* cells and unilamellar vesicles reveal it to be a potent copper ionophore. Proteomics e2200061.