Highlights of Analytical Sciences in Switzerland

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Genome Mining-guided and MALDI Imaging-assisted Discovery of New Antibiotics

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Despite rapid technological progress, the discovery of new antibiotics has stalled. Meanwhile, long-term prescription and misuse of antibiotics has resulted in increased resistances culminating in the current antibiotic resistance crisis. Most therapeutically relevant antibiotic classes are based on natural products discovered from bacteria. Traditionally, 'talented' natural product producers such as soil Actinomycetes were used as sources for antibiotics discovery. However, the focus on taxonomically related bacteria from similar habitats increasingly results in the re-isolation of known compounds.
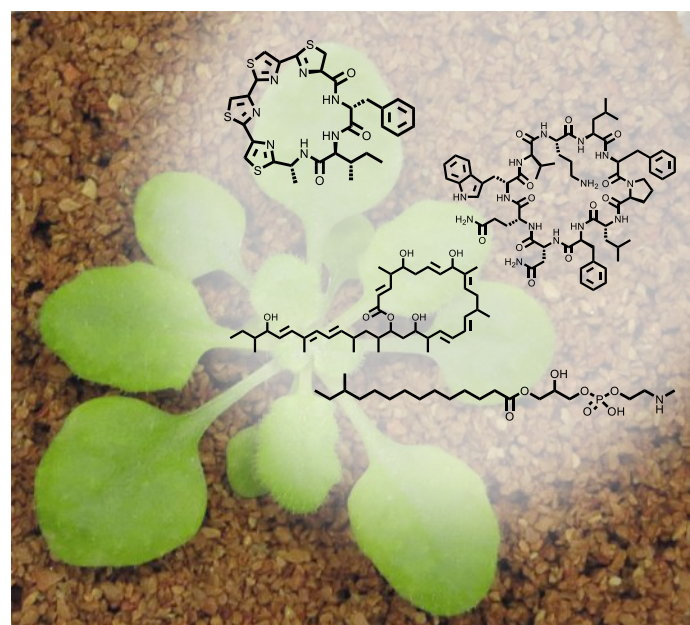
A powerful discovery strategy is based on the computational prediction of biosynthetic products from microbial genome sequences. Natural products are generated by enzymes encoded in clustered sets of genes called biosynthetic gene clusters (BGC). Knowledge of their function allows prediction of natural product structures for some compound classes. Advanced bioinformatic tools are used to identify BGCs in bacterial genomes and compare these to known biosynthetic pathways, thus providing insights into the biosynthetic potential for novel natural products in a bacterium.

These *in silico* dereplication techniques can be applied to bacteria from as-yet chemically poorly explored taxa and ecosystems. We studied bacteria associated with leaves of the model plant *Arabidopsis thaliana* as bacteria colonizing this nutrient-poor habitat might employ antimicrobial warfare against competitors. A large collection of leaf bacteria were subjected to genomic analysis in combination with thousands of binary interaction assays, which revealed a large number of novel BGCs and antagonistic interactions. For bacteria showing potential in both analyses, Matrix Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI IMS) was used to scan the spatial distribution of ions around colonies grown on sensitive strains. It allows metabolic changes to be detected, such as an increased secretion of metabolites when confronting the producer strain with a competing organism. Moreover, it suggests which ions belong to an antibiotic by comparing the shape of the inhibition zone around the colony with the distribution of the detected compounds. Compounds of interest were purified guided by the *in silico*, analytical, and activity data, followed by full structure elucidation. This resulted in the identification of two novel antibiotics from a single producer.

This combinatorial approach of interaction data, genome mining and MALDI IMS streamlines the identification of new talented producers and simplifies the HPLC purification and elucidation of novel molecules.

Reference
E. J. N. Helfrich, C. M. Vogel, R. Ueoka, M. Schäfer, F. Ryffel, D. B. Müller, S. Probst, M. Kreuzer, J. Piel, J. A. Vorholt, *Nature Microbiol.* **2018**, *3.8,* 909.

Arabidopsis thaliana overlaid by bioactive compounds from the isolated *Brevibacillus* sp. Leaf182.
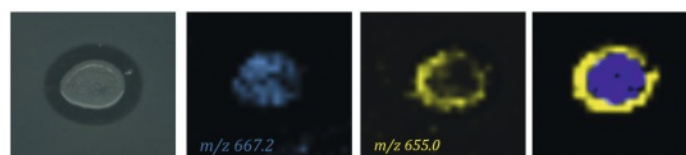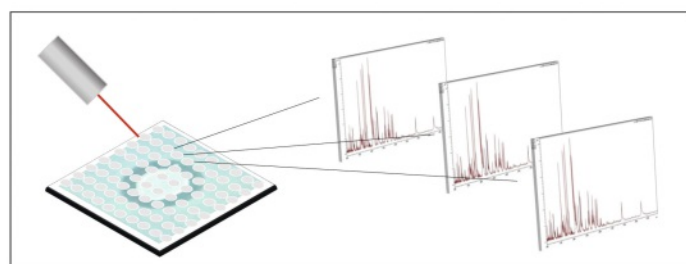
MALDI IMS of a bacterial colony forming an inhibition zone on a lawn of competing bacteria. The intensities of color-coded masses correspond to two isolated secondary metabolites, the antibiotics macrobrevin (*m/z* 667.2) and marthiapeptide A (*m/z* 655.0).

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