Integrated Analytical Approaches Towards Fungal Natural Products Discovery, Pathway Elucidation and Synthetic Biology

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At Center for Microbial Biotechnology we have studied the chemistry of filamentous fungi such as *Aspergillus* and *Penicillium* for more than twenty years. Fungi possess an advanced secondary metabolism that is regulated and coordinated in a complex manner depending on environmental challenges. We have demonstrated that the profiles of secondary metabolites (SM) are species specific and that phenotypic based microbial taxonomy can therefore be used in microbial drug discovery (1). This involves the use of multivariate methods for analysis and clustering of mass spectrometric data allowing selection of talented strains for cultivation on various media using the OSMAC approach before testing in anticancer and antibiotic bioassays (2). Single compound identification is done by *dereplication*, where we use *state-of-the-art* high resolution mass spectrometry (<1 ppm mass accuracy and accurate isotope pattern) in combination with comprehensive compound databases (3). When likely unknown compounds have been identified an explorative solid-phase extraction approach (4) is applied for micro-scale fractionation on a set of different types of columns (RP, ion-exchange, size, NP), in order to clarify the chemical properties of the bioactive(s). This further aids selection of a fast optimal purification strategy towards the pure bioactive prior to NMR characterization. As part of our dereplication procedure we also use a novel algorithm (X-hitting) that use automated search of full UV-spectra to target both known and novel compounds having characteristic UV chromophore systems (1). More recently we have focused our effort towards characterization of the chemistry of full genome sequenced aspergilli (2). Bioinformatics analysis has demonstrated that for the majority of genes that putatively encode enzymes for SM production, the product is not known or detected, calling for sophisticated molecular biological to trigger and detect the production of SMs from cryptic gene clusters. This talk will highlight our recent efforts towards discovery and linking of novel SMs to their respective gene clusters.

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