Impact of HPLC profiling and secondary metabolomics on the discovery of new bioactive compounds from fungi

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Even after several decades of extensive exploitation, fungi remain to be an important source for novel lead compounds in the Life Science industries. Recent studies based on genome mining of filamentous Ascomycota have suggested the presence of dozens of PKS/NRPS genes, even in species that are not regarded as particularly creative metabolite producers. It will be a future challenge to elucidate the functions of such “silent” genes, and manipulate the secondary metabolism of these model organisms, employing molecular genetic tools and information provided by genomics. Nevertheless, new compounds have so far rarely resulted from genetic approaches, which are more useful to optimise selectivity and yields, once an interesting metabolite has been encountered. For discovery of novel leads, it still appears more worthwhile to more broadly explore the fungal biodiversity, using sophisticated methods. We will highlight the utility of secondary metabolomics in these processes.

In the past years, we have built a world-wide unprecedented, powerful HPLC-DAD-MS platform, allowing for efficient dereplication of natural products \textsuperscript{1}. This platform was designed for early-stage identification of known compounds in biologically active extracts, but it now plays a pivotal role in our approach for discovery of novel natural leads. This will be outlined, using some examples from our current work on xylariaceous Ascomycota and certain groups of Basidiomycota.

\textbf{a) From chemotaxonomy to novel compounds:} As model organisms, we have selected and extensively studied the Xylariaceae; over 7000 specimens were evaluated in the past decade in the course of chemotaxonomic studies. The inconspicuous xylariaceous stromata (fruiting bodies) had so far been widely neglected by natural product chemists, but they accumulate surprisingly large amounts of bioactive compounds. These compounds were still detectable even in ancient herbarium specimens collected more than 200 years ago. Over fifty novel natural products were obtained during “mycochemical” studies from eight representative species \textsuperscript{2}. Our metabolomic approach has revealed various rare and new species that produced additional unique compounds.

\textbf{b) A succession of secondary metabolite production} was observed in Xylariaceae\textsuperscript{3} by HPLC-DAD-MS monitoring during ontogeny and differentiation of the stromata in the natural environment throughout the vegetation zone. A total of ca. 150 (mostly unknown) compounds were observed in different developmental stages during the life cycle of a single species. A particularly intense metabolism was noted during colonisation of new substrates.

\textbf{c) Mycoalexins} Co-incubation of Xylariaceae in dual culture with mycoparasitic fungi, isolated from the natural habitats, resulted in the production of antibiotics that are not found under regular fermentation conditions. This phenomenon appears analogous to phytoalexin production by plants. However, “mycoalexins” are so far widely unknown to science. Cultures are now available for artificial production of stromata and scale-up of elicitation experiments, to obtain sufficient amounts of starting material for preparative work to elucidate the chemical background of the above observations.

\textbf{d) Utility of metabolomics in lead optimisation.} Once a lead compound has been found, it pays off to search natural analogues to establish structure activity relationships, providing important information for optimisation of biological effects. Even here, metabolomic techniques have great utility for characterisation of phylogenetically related strains, which often produce similar compounds. Furthermore, new congeners are usually detected during scale-up of fermentation and downstream processing. This will be demonstrated by our results on the elucidation of the secondary metabolome of the plant-parasitic basidiomycete, Armillaria gallica, and other species of this important genus. We will show that Armillaria species are not only the largest organism on Earth, but they are also highly creative secondary metabolite producers!

Literature
1) Bitzer J et al., Chimia 2007, 51, 332-338.