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DISSERTATION SUMMARIES

Microbial production, purification and structural elucidation as well as biological activity of ophiobolins

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Ophiobolins are sesterterpene-type secondary metabolites produced by filamentous fungi belonging to the genera *Bipolaris*, *Cochliobolus*, *Drechslera* and *Aspergillus*. Until now 28 ophiobolin analogues have been described and assigned into 15 subgroups based on their characteristic structure in the carbon skeleton. The best known member of this family of compounds is ophiobolin A which has several biological activities such as antimicrobial, cytotoxic, nematocid or calmodulin antagonist.

In our work, initially an isocratic HPLC method was developed and optimized for the detection of the different ophiobolin compounds. The chromatographic parameters of the analysis were determined and the method was validated.

After that the ophiobolin A production abilities of numerous *Bipolaris* and *Cochliobolus* isolates representing 23 different species are characterized with the optimized HPLC method. Six of the tested isolates produced remarkable amounts of ophiobolin A (>1 mg/g [dry weight]). The ophiobolin secretion kinetics of the examined *Ascomycetes* were determined during the whole cultivation procedure. The strains aggregated into the following four groups based on their production abilities: I. the ophiobolin A production showed one maximum level in the range of 5-8 days; II. strains showed also one maximum level at days 5-7, however in case of these microorganisms strong decreasing tendencies were observed after the maximum production level; III. the production had two maximum level during the cultivation period at 3-5 days and 9-10 days; IV. strains did not show any ophiobolin A production under the applied cultivation conditions.

With the selected isolates the fermentation were carried out at larger scales to gain higher amount ophiobolin compounds for the further purifications. For this purposes a preparative HPLC method was also developed, which was combined with a foregoing and cost effective Solid Phase Extraction pre-cleanup procedures. Using our new preparative method we have successfully cleaned up three potential ophiobolin analogues from a *B. oryzae* strain. One of them was identified as ophiobolin A using the available analytical standard compounds (Sigma). The purities of our batch was determined by HPLC, and proved to be 95% for ophioblin A, and above 94% for the two other compounds. The mass spectrometric examinations indicated that the isolated secondary metabolites have ophiobolin-like fragmentation patterns, and the recorded *m/z* values suggested their structures, however the identification of their identity requires the applications of further structure-determination methods.

During the investigations of biological activities of the purified compounds, agar diffusion and *in vitro* antagonism tests were used. Their antimicrobial activity were determined against a number of microorganisms and in some cases it showed remarkable antimicrobial activity.

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Phenotypic heterogeneity provides evolutionary advantage under high level of stress

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Isogenic individuals within a population generally show a certain level of phenotypic variability. This can be explained by the different nature of promoters which fluctuate between active and inactive status. If the switching time is long enough, then at a given time point a certain gene is active in specific cells, while this certain gene is inactive in other cells of the population, resulting a phenotypically heterogeneous population. The role of this phenomenon in evolutionary processes is highly debated and needs to be explained.

To shed light on the possible role of heterogeneity in evolution, two isogenic strains with significantly different heterogeneity of gene expression of a GFP fused efflux pump (Pdr5p) were established, by transforming two synthetic genomic constructs into the same yeast (Δ*pdr5*, *Saccharomyces cerevisiae*) background. The high heterogeneous (HH) strain and the low heterogeneous (LH) strain have similar mean expression level of *PDR5-GFP*, while the coefficient of variation is different. Pdr5p is a good candidate to examine gene expression heterogeneity, since it provides resistance against a well-known antifungal agent, fluconazole.

Under low level of stress, the high heterogeneity of a population provides no advantage, however under high level of stress it can be