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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

beneficial. This beneficial effect can be explained by the extensive size of the surviving sub-population in the high heterogeneous population. Evolutionary experiments were carried out in the presence of the antifungal agent, fluconazole. The ancestral strains were cultivated in parallel cultures in 96 well plates. 10^5 cells were serial transferred into fresh medium in the adaptation experiment using constant level of fluconazole. In contrary, 10^7 cells were serial transferred in the adaptation experiment using gradually increasing level of fluconazole.

After 100 generations, there was no difference in the evolutionary adaptation rate of the different heterogeneous strains, which suggests a heterogeneity-independent adaptation. The high heterogeneity provides advantage when the population faces a higher selective pressure: the survival subpopulation is greater which provides increased chance of accumulation of beneficial mutations. The bistable system remained the same after the evolutionary experiment; therefore, the acquired resistance of HH strain is presumably caused by adaptive beneficial mutations.

We suggest that these beneficial mutations interact with the synthetic construct. The mean expression did not change, but the coefficient of variation increased after 100 generations. For the first time, our results provide experimental evidence that phenotypic heterogeneity of an isogenic population can contribute to adaptive advantage under high level of stress. In sharp contrast, under low level of stress this enormous advantage vanished.

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The role of ATAC histone acetyltransferase complex on steroidogenic gene expression

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The *Sf-1* (steroidogenic factor 1) plays an important role in steroidogenic gene expression and also in the adrenogonadal development. *Sf-1* and similarly its *Drosophila* orthologue the *ftz-f1* transcription factor belongs to the nuclear hormone receptor family. The transcriptional activity of *Sf-1/ftz-f1* is controlled by posttranslational modifications. Phosphorylation at Ser203 and acetylation by GCN5 and p300 enhance *Sf-1* function.

The *Sf-1* shows tissue specific expression (adrenal cortex, testis, ovary, hypophysis, ventromedial hipothalamus, skin and spleen) and its mutation, absence or in some cases, overexpression can lead to tumor formation.

Recently we have reported that the lack-of-function mutations of the GCN5 histone acetyltransferase (HAT)-containing ATAC complex influence steroid biosynthesis. In contrast, the lack of the other GCN5-containing HAT complex, SAGA has only mild effect on steroid biosynthesis. The mechanism by which ATAC affects steroid synthesis, however, remains to be discerned. The two most probable scenarios could be that ATAC influences the transcription of genes involved in steroid hormone biosynthesis directly by histone acetylation at their promoters, or that it acetylates FTZ-F1/SF1 and by this regulates the transcription of steroid converting gene indirectly.

We demonstrated that *Halloween* gene expression is detectable and modified by protein acetylation in S2 insect cells. We found that the stability of FTZ-F1 was increased after treatment of TSA (histone deacetylase inhibitor). Furthermore, we established that the overexpression of *ftz-f1* significantly increases the expression of *Halloween* genes in the *Drosophila* S2 embryonic cell line.

We performed chromatin immunoprecipitation experiments to answer whether histone acetylation has a role in steroid hormone biosynthesis. We found that H4K5 acetylation can be observed at the regulatory regions of *disembodied (dib)* and *shade (shd) Halloween* genes, while we did not detect H3K9 acetylation at any regions of these genes. In contrast to that, H3K9 acetylation can be observed at the initiator region of the mammalian *Cyp11a1* gene, while H4K5 acetylation can be detected at its promoter and initiator regions.

Based on our findings we conclude that the ATAC HAT complex plays a role in *Drosophila* steroid hormone biosynthesis through histone acetylation. To provide further proofs to this conclusion we continue our studies with the aim to detect the presence of the ATAC complex at the regulatory regions of the *Cyp11a1* gene.

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