Aspergillus nidulans as a platform for discovery and characterization of complex biosynthetic pathways

Filamentous fungi produce a wide range of bioactive compounds, classified as secondary metabolites, which have the potential to be used as pharmaceuticals, insecticides, fungicides and food additives. Secondary metabolites also include mycotoxins, which are produced by fungi that contaminate food and feed. Secondary metabolites therefore both have a positive and deleterious impact on the human health. The increase in available genome sequences of fungi has revealed that there is a large number of putative secondary metabolite biosynthetic gene clusters to be discovered and potentially exploited as pharmaceuticals. Access to this unexploited reservoir is hampered as many of the clusters are silent or barely expressed under laboratory conditions. Methods for activating these pathways are therefore essential for pathway discovery and elucidation.

Filamentous fungi and Aspergillus species in particular are used in industrial applications for the production of these bioactive compounds and other chemicals as well as for enzyme production. Especially Aspergillus niger and Aspergillus oryzae are used as industrial workhorses for the production of various enzymes. Many of the secreted proteins are glycosylated, indicating that glycosylation plays an important role in the secretory pathway. Thus, understanding the role and process of glycosylation will enable directed glycoengineering in Aspergilli to improve protein production and expand the repertoire of proteins, which can be produced by these fungi.

Aspergillus nidulans has been used as a model organism for a range of research disciplines and many genetic engineering tools are available for working in this organism. This PhD study therefore employed A. nidulans as a model system to address the following on two aspects: 1) Developing A. nidulans as a platform for pathway discovery of secondary metabolites and 2) Developing A. nidulans as a model system for protein production with human-like glycan structure.

The first part of this study resulted in the development of a method for the transfer and expression of intact biosynthetic gene clusters to A. nidulans to facilitate pathway and product discovery. As proof of concept the biosynthetic gene cluster for production of the polyketide geodin was identified and transferred from A. terreus to A. nidulans. The cluster was integrated in a well-characterized locus in A. nidulans. Reconstitution of the cluster resulted in the production of geodin. Expression of the enzymes in the pathway was validated by transcription analysis and the functions of specific genes were investigated by gene deletions. This proved that this method is a fast and easy way to transfer biosynthetic gene clusters regardless of size and characterize them. Furthermore, a different approach to activate silent clusters was demonstrated, as the heterologous expression of a putative transcription factor from A. niger in A. nidulans induced the synthesis of insect juvenile hormones in A. nidulans, which had previously not been reported as fungal metabolites.

The second part of the study focused on understanding the glycosylation pathway in A. nidulans and engineering a strain capable of producing precursors for the further modification of the glycan structure towards a more human-like pattern. Previous studies have shown that the deletion of the first mannosyltransferase in the ER (alg3) resulted in the accumulation of sizes from Man₃GlcNAc₂ to Man₇GlcNAc₂. This study shows that the remaining mannosyltransferases in the ER do not use the truncated structure generated from the alg3 deletion as a substrate, thus the remaining mannosyltransferase activity must take place in the Golgi. Furthermore, there is an indication that the Man₅GlcNAc₂ structure generated from the alg3 deletion is trimmed to Man₃GlcNAc₂ and extended to Man₇GlcNAc₂, which has a different structure that the first generated structure by the alg3 deletion. The work done during this study gives more insight into the glycosylation pathway of A. nidulans, which can be used as a basis for further engineering of the pathway to produce humanized glycans in Aspergilli.

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