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Discovery and exploitation of the transcriptional regulatory system of pectinases in *Aspergillus niger*

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Summary

Pectin is a plant cell wall polysaccharide made of mainly D-galacturonic acid (GA) subunits. The potency of *Aspergillus niger* to naturally secrete high amounts of pectinases to degrade pectin has been utilized for the industrial production of pectinases. Industrially produced pectinases by *A. niger* are subsequently used mainly in the food industry, and for the hydrolysis of plant biomass to produce renewable energy. In this thesis the transcriptional activator GaaR, required for the expression of the genes encoding several pectinases, the (putative) GA transporters and the GA catabolic pathway enzymes was identified (Chapter 3). The $\Delta gaaR$ strain could not grow on GA or PGA, but showed residual growth on pectin, indicating that deletion of *gaaR* does not affect the utilization of monomeric carbon sources other than GA which are present in pectin. Transcriptome analysis of the $\Delta gaaR$ strain on pectin confirmed that the $\Delta gaaR$ strain still expresses several enzymes which mainly act on the side chains of rhamnogalacturonan I. Metabolic analysis of the GA catabolic pathway deletion mutants resulted in the identification of 2-keto-3-deoxy-L-galactonate accumulating in the $\Delta gaaC$ mutant. Gene expression analysis showed that accumulation of 2-keto-3-deoxy-L-galactonate in the $\Delta gaaC$ mutant resulted in hyperinduction of GA-responsive genes, indicating that 2-keto-3-deoxy-L-galactonate is the physiological inducer of the GA-responsive genes (Chapter 5).

Genome sequencing of several mutants that showed constitutive expression of the genes encoding pectinases resulted in the identification of the repressor protein GaaX that likely inhibits the transcriptional activity of GaaR via protein-protein interaction under non-inducing conditions (Chapter 4). One mutant with constitutive expression of the genes encoding pectinases contained the W361R mutation in GaaR, indicating that W361 is important for the proper interaction of GaaX and GaaR under non-inducing conditions (Chapter 7).

Overexpression of *gaaR* via the strong constitutive *A. nidulans* *gpdA* promoter brought about constitutive expression of the genes encoding pectinases, providing further evidence that GaaR is active as long as it is not inhibited by GaaX. This suggests that in the presence of the inducer, the inducer binds to GaaX resulting in a GaaX-unbound and active form of GaaR. Deletion of the main repressor involved in carbon catabolite repression, *creA*, enhanced the constitutive expression pectinases in the *OEgaaR* strain (Chapter 6).

Constitutive expression of the genes encoding plant biomass degrading enzymes like pectinases would benefit especially the use of cheap and renewable plant biomass as a feedstock for fungal fermentation. This thesis also contains a chapter reviewing the approaches to modulate the transcriptional regulation in order to increase or constitutively express plant biomass degrading enzymes in filamentous fungi (Chapter 2). These approaches include accumulation of the inducer, deletion of the carbon source specific repressor, constitutive activation and overexpression of the transcriptional activator and elimination of carbon catabolite repression. All of these approaches have been implemented successfully in this thesis to increase and/or constitutively express pectinases in *A. niger*.