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

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Stellingen

behorende bij het proefschrift

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

1. Loss of constitutive expression of pectinase genes in the *A. niger* $\Delta gaaX$ mutant when *gaaR* is deleted indicates that GaaX functions upstream of GaaR as a signal (presence of GA) sensor (Chapter 4).
2. The transcription level of pectinase genes is positively correlated with the nuclear concentration of GaaX-unbound GaaR (This thesis).
3. Based on previous observations on QutR (the repressor of quinate-utilization in *A. nidulans*), and the proposed common evolutionary origin of QutR and GaaX, overexpression of *gaaX* is expected to result in super-repression of pectinase genes under inducing conditions (This thesis; Lamb *et al.*, *Microbiology* 1996).
4. N- or C-terminal eGFP-tagging of GaaR affects its proper functioning or proper nuclear localization under non-inducing conditions, respectively. Therefore, the natural subcellular distribution of GaaR should be studied by methods other than fluorescent tagging (Chapter 6; Chapter 7).
5. Increased expression of the genes encoding plant biomass degrading enzymes in the $\Delta creA\Delta creB$ double deletion mutant compared to the $\Delta creA$ single deletion mutant strongly prompts further study of the role of CreB and its possible targets other than CreA, involved in carbon catabolite repression in filamentous fungi (Ichinose *et al.*, *Applied Microbiology and Biotechnology* 2014; Ichinose *et al.*, *Journal of Bioscience and Bioengineering* 2017).
6. In terms of research and application, pectinases represent a crucial but underrated class of enzymes for the full exploitation of plant biomass to produce valuable chemicals (Edwards and Doran-Peterson, *Applied Microbiology and Biotechnology* 2012; Latarullo *et al.*, *Frontiers in Plant Science* 2016).
7. Lack of knowledge on functional domains and species-specificity of the activation mechanisms and gene regulons of transcription factors, even if highly similar in sequence, make the design of fungal strains with increased production of plant biomass degrading enzymes based on these transcription factors unpredictable

(Makita *et al.*, *Bioscience Biotechnology and Biochemistry* 2009; Suzuki *et al.*, *Applied Microbiology and Biotechnology* 2015; Lv *et al.*, *Biofuels* 2015; Tamayo *et al.*, *Fungal Genetics and Biology* 2008; Calero-Nieto *et al.*, *Molecular Plant-Microbe Interactions* 2007; Chapter 2).

8. More research should be performed on characterizing the functional domains of fungal transcription factors to successfully design and use synthetic transcription factors, engineered to combine the DNA-binding and effector modules of different transcription factors, as a powerful strategy to produce plant biomass degrading enzymes (Gao *et al.*, *Biotechnology Journal* 2017; Chapter 2).
9. Utilization of CRISPR-Cas9 based gene editing techniques allows more efficient functional and localization studies of transcription factors in comparison to homologous recombination based techniques, as it alleviates the use of a selection marker in the gene replacement cassette (Chapter 7).
10. To have real fun in performing PhD research, a biologist needs to have a basic understanding of bioinformatics.

Ebru Alazi, 2018