IMPACT OF INDUCER MOLECULES ON DNA ACCESSIBILITY IN CELLULASE AND XYLANASE EXPRESSION IN *TRICHODERMA REESEI*

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INTRODUCTION

Trichoderma reesei is a filamentous ascomycete, which exerts a saprotrophic lifestyle [1]. It gains its nutrients by degradation of plant cell wall material. For this purpose, it secretes various cellulases and hemicellulases to break down complex polysaccharides. The most naturally abundant plant-based biomass is cellulose, followed by hemicellulose (*e.g.* xylan) as second most abundant. Synergistic action of *T. reesei's* secreted enzyme cocktail leads to a degradation of the plant biomass.

These enzymes are industrially used [2]. Especially cellulases are highly secreted in the fungal strain Rut-C30, which is the ancestor of industrial strains. Their expression is regulated by the interplay of transcription factors (TFs) in response to different carbon sources or specific inducers.

AIM OF STUDY

In *Trichoderma*, the transactivator Xyr1 (encoded by *xyr1*) [3], the repressor Cre1 (encoded by *cre1*) [4], and gene-specific transcription factors regulate the expression of two major cellulases (encoded by *cbh1* and *cbh2*) and xylanases (encoded by *xyn1* and *xyn2*). Inducer substances such as sophorose (a transglycosylation product of cellobiose) and D-xylose achieve an induction of gene expression of these enzymes. On D-glucose, Cre1 mediates carbon catabolite repression (CCR), which leads to a down-regulation of expression of *xyr1* and of both cellulase and xylanase-encoding genes.

Transcription factors do not regulate gene expression exclusively, the chromatin packaging and other DNA-protein interactions add an additional layer in gene regulation. Therefore, the chromatin status as well as differences in protein-DNA interactions on TF binding motifs were investigated by chromatin accessibility real-time PCR (CHART-PCR) and *in vivo* footprinting in the wild-type strain and in the CCR-released industrial ancestor strain Rut-C30.

RESULTS AND DISCUSSION

For the cellulase-encoding genes (*cbh1* and *cbh2*) no remarkable changes in chromatin on repressing and inducing conditions in both strains were observed, whereas changes were detected for the xylanase-encoding genes (*xyn1* and *xyn2*). Together with *in vivo* footprinting analyses differences in protein-DNA interactions were detected, particularly for *xyn2,* depending on the inducer applied in the wild-type strain. Using sophorose, the protein-DNA interactions on the functional TF motifs of *xyn2* were similar in the wild-type strain and Rut-C30.

CONCLUSION

Both methods, the CHART-PCR and the *in vivo* footprinting, contribute to a deeper understanding of an important aspect in gene regulation. Besides that, the chromatin status of cellulase and xylanase-encoding genes react differently to applied inducers. It can be therefore concluded that

additional regulatory mechanisms are involved that shape the final promoter architecture in response to different inducers.

REFERENCES

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