PRODUCTION OF HYDROPHOBINS IN THE TWO EXPRESSION SYSTEMS BASED ON *TRICHODERMA*

Victor Lobanov[a], Feng Cai[a,b], Agnes Przylucka[a], Lizaveta Sidarenka[a], Qirong Shen[b], Irina Druzhinina[a]

[a]E166 - Institute of Chemical, Environmental and Biological Engineering, Research Area Biochemical Technology, Microbiology Group, TU Wien, Vienna, Austria
[b]Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing, China

INTRODUCTION

Genetically modified organisms have grown to play an integral role in modern day industry, visible from the substantial amount of research currently pursued on the topic. Strain modification is a process compromising of screening for ideal traits and transforming designed gene vectors into the chosen host organism. Such vectors may be optimised depending on the host and secretion product. Due to their versatility and high secretory capacity, filamentous fungi are common in the production of proteins for industrial applications. Certain species, e.g. *Trichoderma reesei* QM 6a, are regarded as model organisms. Extensive research and optimisation of fermentations using *T. reesei* allow industrial bioreactors to secrete products in excess of 100 gL^{-1} (1), part of a global multibillion euro market (2). *Trichoderma* are ubiquitous worldwide, finding niches in saprotrophic as well as mycotrophic settings, enabled by a highly complex cocktail of enzymes and auxiliary proteins. One class of small molecular weight proteins, hydrophobins (HFB), is particularly interesting. These amphipathic proteins are known for their acute surface activity and spontaneous layer formation. The tertiary structure is marked by a conserved α-helix barrel with peripheral β-sheets defining largely the variability across hydrophobins (3).

Hydrophobins offer potential solutions and improvements to numerous industrial needs, largely based around the exploitation of their amphipathic and sheet-forming properties as agents to functionalise material surfaces (4). HFBs may be loosely distinguished into two classes differentiated by their layer formation (robust or transient), thus ideal for diverse material coatings (5). Furthermore, their application in water significantly decreases surface tension (6), thereby increasing wettability. HFBs are safe for ingestion and trigger no immune response (7-9), rendering them relevant for biomedical as well as food industries (10, 11). Ironically, the very properties of hydrophobins so desired for industrial use, e.g. the surface activity, mire potential production upscaling. Assuming improvement in the purification of hydrophobins from the bioreactor medium, the potential for strain optimisation remains nonetheless open. In light of this, the current study presents a case comparison of the recently discovered *T. guizhouense* NJAU 4742 (12) as a potential replacement of the traditional cell factory *T. reesei* for overexpression of *Trichoderma* class II hydrophobins, namely HFB4 and HFB7.

METHODS

As a platform for comparison between the two species, several gene constructs for heterologous and homologous overexpression were designed and transformed into both organisms. Initially, the hydrophobin encoding genes* $hfb4_{vir}$ and likewise $hfb7_{vir}$, both originating from *T. virens* Gv 29-8, were overexpressed in *T. reesei* QM 6a using a pUC19 plasmid containing a *cdna1* promoter, *cbh1* terminator, and 6His cassette. Concurrently, the homologous expression of $hfb4_{gui}$ in *T. guizhouense*

using a *cdna1* promoter and native terminator were investigated. The *cdna1*P*hfb4*$_{vir}$6His*cbh1*T construct, as well as its *hfb7*$_{vir}$ counterpart, were transformed into *T. guizhouense*. Furthermore, *hfb4*$_{gui}$ was transformed into *T. reesei* QM 6a first with the original *cdna1*P*hfb4*$_{gui}$*hfb4*$_{gui}$T construct and with a *cdna1*P*hfb4*$_{gui}$6His*cbh1*T construct.

RESULTS AND DISCUSSION

By manipulating both strain and gene overexpression construct, the initial comparison of *T. reesei* and *T. guizhouense,* whereby the overexpression mutants from *T. guizhouense* outproduced their *T. reesei* counterparts, may be expanded in a meaningful way. Shake flask fermentations provide an initial summary as to which organism and construct combination allows greater hydrophobin secretion. Regardless, this will necessitate further bioreactor fermentations as well as extensive optimisation of the fermentation set-up to study how the cell factories may permit further upscaling of hydrophobin production.

CONCLUSION

Given the demand for an effective production mechanism for large-scale hydrophobin production, it is imperative that not only well-known organisms be tested, but also those more recently discovered. In line with this, the current study has focused on the potential of *T. guizhouense* as a viable candidate for this task. This was explored by comparing overexpression mutants from the newcomer candidate against the traditionally used *T. reesei* using various hydrophobin overexpression cassettes.

* To differentiate orthologous hydrophobin genes, the first three letters of the respective species name is appended to the gene; i.e. *hfb4*$_{vir}$, *hfb4*$_{gui.}$

REFERENCES

1. Druzhinina I, Kubicek C. Chapter Two-Familiar Stranger: Ecological Genomics of the Model Saprotroph and Industrial Enzyme Producer Trichoderma reesei Breaks the Stereotypes. Advances in applied microbiology. 2016;95:69-147.

2. Nevalainen KM, Te'o VS, Bergquist PL. Heterologous protein expression in filamentous fungi. Trends Biotechnol. 2005;23(9):468-74.

3. Wosten HA, Scholtmeijer K. Applications of hydrophobins: current state and perspectives. Appl Microbiol Biotechnol. 2015;99(4):1587-97.

4. Espino-Rammer L, Ribitsch D, Przylucka A, Marold A, Greimel KJ, Acero EH, et al. Two novel class II hydrophobins from Trichoderma spp. stimulate enzymatic hydrolysis of poly (ethylene terephthalate) when expressed as fusion proteins. Applied and environmental microbiology. 2013;79(14):4230-8.

5. Linder MB, Szilvay GR, Nakari-Setälä T, Penttilä ME. Hydrophobins: the protein-amphiphiles of filamentous fungi. FEMS Microbiology Reviews. 2005;29(5):877-96.

6. Wosten HA, de Vocht ML. Hydrophobins, the fungal coat unravelled. Biochim Biophys Acta. 2000;1469(2):79-86.

7. Giles SS, Dagenais TR, Botts MR, Keller NP, Hull CM. Elucidating the pathogenesis of spores from the human fungal pathogen Cryptococcus neoformans. Infection and immunity. 2009;77(8):3491-500.

8. Hakanpää J, Paananen A, Askolin S, Nakari-Setälä T, Parkkinen T, Penttilä M, et al. Atomic resolution structure of the HFBII hydrophobin, a self-assembling amphiphile. Journal of Biological Chemistry. 2004;279(1):534-9.

9. Hohl TM, Van Epps HL, Rivera A, Morgan LA, Chen PL, Feldmesser M, et al. Aspergillus fumigatus triggers inflammatory responses by stage-specific β-glucan display. PLoS Pathog. 2005;1(3):e30.

10. Fischer G, Dott W. Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. Archives of Microbiology. 2003;179(2):75-82.

11. Bimbo LM, Makila E, Raula J, Laaksonen T, Laaksonen P, Strommer K, et al. Functional hydrophobin-coating of thermally hydrocarbonized porous silicon microparticles. Biomaterials. 2011;32(34):9089-99.

12. Li Q-R, Tan P, Jiang Y-L, Hyde KD, Mckenzie EH, Bahkali AH, et al. A novel Trichoderma species isolated from soil in Guizhou, T. guizhouense. Mycological progress. 2013;12(2):167-72.