

***Trichoderma reesei* proteasome and genome-wide  
effects of the expression of mutant  
cellobiohydrolase I**

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## Abstract

*Trichoderma reesei* has a naturally high capacity for protein secretion and is currently employed for industrial production of a range of enzymes and recombinant gene products for a variety of biotechnological applications. A major limitation for the use of *T. reesei* as a universal production host is that industrial-scale production of heterologous proteins often results in lower yields than those achieved from native proteins. One reason for the low secretion yields of heterologous proteins is their improper folding and consequent elimination from the cell by the protein quality control mechanisms mediated by the unfolded protein response and the ER-associated degradation. Proteasome plays an important role in protein quality control by degradation the misfolded or aberrant proteins. In the current study three different mutant versions of the main secreted protein, cellobiohydrolase I (CBHI) tagged with the fluorescent protein Venus, were produced in *T. reesei* and their effects on physiology and gene expression were explored. The transcriptional response of the fungal hyphae was determined by CustomArray™ 12K slides at three different time points. Potential interaction between the mutant CBHIs and the fungal proteasome was studied by fluorescence and the immunoelectron microscopy.

A new rapid purification method for the fungal proteasome was developed during this study followed by separation of the proteasome subunit proteins by 2DE. Several proteasome interacting proteins (PIPs) were also identified. The purified 26S proteasome was visualised by transmission electron microscopy. The three mutant CBHI strains differed in terms of protein production and CBHI enzyme activity, although there were similarities between them showing a "pulsing" phenomenon both in protein secretion and transcription of the CBHI mRNA. Interestingly only one of the mutant CBHI strains could secrete the Venus-tagged fusion protein into the culture medium.

The genome wide transcriptional study showed that two mutations in the *cbh1* core gene did not cause UPR or ERAD activation, even though physiological signs of the stress were evident. Four and five mutations in the *cbh1* core gene lead to expression changes in genes related to UPR and ERAD pathways and the physiological indications of stress were also seen under the light microscope. A new finding was up-regulation of a group of genes

involved in ribosome structure and synthesis in all mutant CBHI strains. In previous studies, secretion stress has been applied to fungal hyphae by drugs such as dithiothreitol (DTT) or tunicamycin, which seem to result in a different feedback to the protein translation machinery.

Fluorescence and immunoelectron microscopy studies supported the microarray results indicating that four mutations in the *cbh1* core gene lead to the interaction of the mutant CBHI with the 20S proteasome and at least partial retention of the mutant CBHI protein in the fungal hyphae.

## **Declaration**

The research presented in this thesis is original work conducted between April 2005 and April 2009 by the author. This material has not been submitted as part of the requirement for any other degree or course to any other institution. To the best of my knowledge it contains no material previously published or written by any other person except where due reference is made in the text.

Liisa Kaarina Kautto

## Attachments to this thesis

Grinyer, J., Kautto, L., Traini, M., Willows, R.D., Teo, J., Bergquist, P. and Nevalainen, H. 2007. Proteome mapping of the *Trichoderma reesei* 20S proteasome. *Current Genetics*. 51:79-88.

Kautto, L., Grinyer, J., Birch, D., Kapur, A., Baker, M., Traini, M., Bergquist, P. and Nevalainen, H. 2009. Rapid purification method of the 26S proteasome from filamentous fungi *Trichoderma reesei*. *Protein Expression and Purification*. 67:156-163.

DVD - Supplementary material for the microarray data  
- Supplementary material for the fluorescence microscopy studies  
- Thesis in PDF-format

## Abbreviations

Abbreviations frequently used in the text are:

bp	Base pairs
CBHI	Cellobiohydrolase I
CLSM	Confocal laser scanning microscopy
DEPC	Diethylpyrocarbonate
DIC	Differential interference contrast
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
2D	Two dimensional
EDTA	Ethylene diamine-tetra-acetic acid
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum associated degradation
FBS	Fetal bovine serum
g	Specific gravity
h	Hour
kb	Kilobase
kDa	Kilodalton
LB	Luria broth
MW	Molecular weight
M	Moles per litre
min	Minute
ORF	Open reading frame
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
RNA	Ribonucleic acid
RT	Room temperature
s	Second
SDS	Sodium dodecyl sulphate
TEM	Transmission electron microscopy
Tris	Tris(hydroxymethyl)aminomethane
UPR	Unfolded protein response
V	Voltage
v/v	Volume per volume
w/v	Weight per volume

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