Enhancement of the *Trichoderma reesei* expression system

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Abstract

Filamentous fungi are attractive as cell factories for recombinant protein production. *Trichoderma reesei*, an industrially-important filamentous fungus, has been used for the expression of recombinant gene products for over a decade and a variety of molecular genetics strategies has been applied to increase the yields of these products; yet no substantial improvements have been made and a significant increase in the yields remains a priority for economic production of non-fungal recombinant proteins. In this work, a new approach was developed to increase further recombinant protein production in *T. reesei*, featuring simultaneous use of multiple promoters for gene expression. Reliance on a single strong promoter, such as the one from the *cbh1* gene encoding the main cellobiohydrolase I (CBHI), for driving expression of the genes encoding proteins of interest may not utilise the full capacity of protein expression by the organism. Hence, the novel concept of a multiple promoter platform (MPP) was put into practice with the objective of improving the efficiency of recombinant (heterologous) protein production. Instead of using one type of promoter for gene expression, the strategy behind MPP was to increase overall transcription of the gene of interest, here *xynB* encoding a thermophilic bacterial xylanase enzyme (Xylanase B), by employing multiple different fungal gene promoters.

Enhancement of heterologous protein production was explored by combined proteomic, molecular genetic and glycoproteomic approaches. Major secreted proteins from *T. reesei*, including EGLII, CBHII and XYNII, were identified by 2-DE and MALDI-TOF analyses and the promoter sequences of the genes encoding these proteins were used to construct expression vectors for the production of the heterologous Xylanase B (XynB) from the thermophilic bacterium, *Dictyoglomus thermophilum*. Two generations of transformants carrying either a single promoter (SP) or multiple promoters (MP) controlling the expression of the *xynB* gene were examined. The best performing SP and MP recombinants were selected based on Xylanase B activity. The increase of activity in the best strain carrying the three different promoters to drive the expression of the *xynB* gene was 170 % compared to that of the best SP strain (transformation host for the MP strains). The copy number of the integrated genes and the mode of integration of the expression cassettes by homologous or non-homologous recombination were not significant factors in the production of Xylanase B, suggesting the presence of additional, as yet unidentified elements were involved in heterologous protein production. Examination of the glycosylation of Xylanase B fused to the linker region of EGLII revealed considerable

amounts of O-linked oligosaccharides attached to the protein. One of the sugars identified by LC-MS/MS was hexuronic acid, which presents a sugar not previously found in *Trichoderma* glycoproteins. Overall, production of Xylanase B was enhanced by using a combination of three promoters, *xyn2*, *cbh2* and *egl2* inducing *xynB* gene expression.

Declaration

The research presented in this thesis is original work conducted between March 2006 and October 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.

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Abbreviations

Abbreviations frequently used in the text are:

Amp	Ampicillin resistance
BSA	Bovine serum albumin
bp	Base pairs
cbh2	Gene coding CBHII enzyme
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleoside triphosphates
EDTA	Ethylenediamine-tetra-acetic acid
egl2	Gene encoding EGLII enzyme
ER	Endoplasmic reticulum
g	Specific gravity
IPTG	Isopropyl-β-D-thiogalactopyranoside
kb	Kilobase pairs
kDa	Kilodalton
lacZ	β-galactosidase gene
LB	Luria Broth
mRNA	Messenger ribonucleic acid
M	Moles per litre
min	Minute
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
RNA	Ribonucleic acid
rpm	Revolutions per minute
SAP	Shrimp alkaline phosphatase
SDS	Sodium dodecyl suphate
TBE	Tris-borate-EDTA
Tris	[2-amino-2-(hydroxymethyl)propane-1,3-diol, (tris)]
UV	Ultra violet
V	Volt
v/v	Volume per volume
v/w	Volume per weight
w/w	Weight per weight
X-Gal	5-bromo-4-chloro-3-indolyl β-D-galactopyranoside
xyn2	Gene encoding xylanase II enzyme