An investigation into the *hex1* gene and gene promoter for the enhancement of protein production in *Trichoderma reesei*

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Abbreviations

Abbreviations frequently used in the text are:

bp	Base pairs
BSA	Bovine serum albumin
CLSM	Confocal laser scanning microscopy
DAPI	4'-6-Diamidino-2-phenylindole
DEPC	Diethylpyrocarbonate
DIC	Differential interference contrast
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediamine-tetra-acetic acid
EM	Electron microscopy
ER	Endoplasmic reticulum
g	Specific gravity
h	Hour
IPTG	Isopropyl-beta-D-thiogalactopyranoside
kb	Kilobase pairs
kDa	Kilodalton
LB	Luria Broth
M	Moles per litre
min	Minute
MOPS	3-(N-morpholino) propanesulfonic acid
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
ss	Signal sequence
TBE	Tris-borate-EDTA buffer
TBS	Tris-buffered saline
TEM	Transmission electron microscopy
Tris	[2-amino-2-(hydroxymethyl) propane-1,3-diol, (tris)]
tsp	Transcription start point
V	Volt
v/v	Volume per volume
w/v	Weight per volume
X-Gal	5-bromo-4-chloro-3-inodyl-beta-D-galactopyranoside
xyn1	Gene encoding xylanase I enzyme

Attachments to this thesis

Curach, N. C., Te'o, V. S. J., Gibbs, M. D., Bergquist, P. L. Nevalainen, K. M. H. (2004). Isolation, characterisation and expression of the *hex1* gene from *Trichoderma reesei*. Gene, **331**: 133-140.

DVD - Supplementary Material to Figures.

CD – Thesis in PDF format.

Declaration

The research presented in this thesis is original work conducted between January 2001 and January 2005 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.

Natalie Claire Curach

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Abstract

For *Trichoderma reesei* to be developed as an efficient producer of a large variety of proteins, the expression system requires diversification. In particular, the choice of promoters available needs to be broadened to include promoters which are active in conditions other than those conducive to induction of cellulase expression. Using proteomics, the HEX1 protein was identified as an abundant protein of the cell envelope of *T. reesei* when grown on a range of carbon sources, suggesting that a strong constitutive promoter drives the expression of this physiologically important protein. This thesis is an exploration into the *hex1* gene promoter and the role of *hex1* in the maintenance of mycelium integrity in *T. reesei* with consideration for the application of this gene in the further development of filamentous fungi as protein expression systems.

The single copy *hex1* gene and flanking regions were isolated from *T. reesei* and another biotechnologically important fungus, *Ophiostoma floccosum*. The fluorescent reporter protein DsRed1-E5 was expressed under the *T. reesei hex1* promoter and promoter activity was monitored by fluorescence CLSM and RNA analysis. During the rapid growth phase of a culture, the *hex1* promoter was active in a range of carbon sources and three transcript types with alternative *tsp* and splicing sites were discovered for the *hex1* gene. The distribution of fluorescence throughout the mycelium suggested spatial regulation of the *hex1* promoter as well as temporal regulation. The promoter was continually active in the absence of a functional *hex1* gene product suggesting that the *hex1* promoter is regulated in part, by negative feedback from the endogenous gene product. Interruption of the *hex1* gene produced hyphae that leaked excessive volumes of cytoplasm when physically damaged which may be advantageous for the

externalisation of selected protein products. The results indicate that the regulation of the *hex1* gene promoter is complex and that the *hex1* gene is integral to the maintenance of the integrity of the fungal mycelium.