FUNCTIONAL ANALYSIS OF GENES ENCODING HYDROLYTIC ENZYMES IN THE INTERACTION OF *EPICHLOË FESTUCAE* WITH PERENNIAL RYEGRASS

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ABSTRACT

Hydrolytic enzymes degrade macromolecules into smaller components. These enzymes are important in fungal nutrition and have been implicated in the pathogenicity and virulence of pathogenic fungi towards their hosts. However, it is unknown if hydrolytic enzymes play important roles in mutualistic symbioses. In this study, the function of two different classes of hydrolytic enzymes was examined in the mutualistic symbiosis between the fungal endophyte *Epichloë festucae* and perennial ryegrass (*Lolium perenne* cv. Nui).

Nine members of a gene family encoding subtilisin-like proteases were identified in *E. festucae*. The *prt2, prt3* and *prt5* genes encode putative extracellular proteins belonging to the proteinase K subfamily 1, and *prt1* and *prt6* encode putative extracellular proteins belonging proteinase K subfamily 2. The *prt7* and *prt8* genes encoded pyrolysin-like enzymes from subfamilies 1 and 2. The *prt4* gene encodes a putative vacuolar protease, while the *kex2* gene encodes a putative proprotein convertase. Expression analysis showed that the *prt1, prt3, prt5, prt4* and *kex2* genes, but not the *prt2* gene, were expressed in culture. The *prt1* and *prt3* genes appeared to be up-regulated *in planta* compared to culture.

The function of *prt1* and *prt2* in the symbiotum between *E. festucae* and perennial ryegrass was characterised by expressing these genes under the control of the *Aspergillus nidulans gpdA* or the *E. festucae* Fl1 *ltmM* promoters. No major differences in hyphal or plant morphology were observed between symbioses containing wild type *E. festucae* or endophyte strains containing the *prt1* or *prt2* transgenes.

The *gcn1* gene, which encodes a β-1,6-glucanase, was identified immediately downstream of the *prt2* gene. The function of the *gcn1* gene was characterised by gene replacement and testing the phenotype during growth in culture and *in planta*. *E. festucae* Δ*gcn1* strains grew normally on glucose-containing media. On media containing the β-1,6-glucan pustulan, Δ*gcn1* strains did not form aerial hyphae or hydrolyse pustulan, which the wild type strain did. This phenotype was partially

complemented by growth of the Δ*gcn1* mutant in close proximity to wild type strains, and fully complemented by insertion of the *gcn1* gene. This suggests that the *gcn1* gene encodes the major β-1,6-glucanase activity of *E. festucae*.

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ABBREVIATION	IN FULL
ACM	*Aspergillus* complete medium
BcAPs	*Botrytis cinerea* Aspartic Proteases
bp	base pair
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CAP	calf alkaline phosphatase
CDK	cyclin-dependent kinase
cDNA	complementary cDNA
CHEF	contour-clamped homogeneous electric field
CTAB	hexadecyltrimethylammonium bromide
CTD	carboxy-terminal domain
cv.	cultivar
dCTP	deoxycytosine
DEPC	Dierucoyl phosphatidylcholine
DMAT	dimethylallyltryptophan
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleotide
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
ER	Endoplasmic Reticulum
EST	Expressed Sequence Tag
FAD	Flavin-adenine dinucleotide
GPI	Glycophosphoinositidol
GUS	β-glucuronidase
HMP	hydroxymethylpyrimidine
HR	Hypersensitive Response
IEF	isoelectric focusing
IP	imaging plate
IWF	Intercellular Wash Fluid
kb	kilobase
LB	Luria-Bertani
LMP	low melting point
MEME	Multiple EM for Motif Elicitation
mRNA	messenger RNA
NJ	Neighbour joining
NRPS	Non-Ribosomal Peptide Synthetase
PA	Protease-associated
PCD	Programmed Cell Death
PCR	polymerase chain reaction
PD	Potato dextrose
PDA	potato dextrose agar
PDB	Potato dextrose broth
PEG	polyethylene glycol
PFU	plaque forming units
PIP3	phosphatidylinositol 3,4,5-triphosphate
Pir	Protein with Internal Repeat
PTEN	phosphatase and tensin
PTP	protein tyrosine phosphatase
RG	regeneration medium
RNA	ribonucleic acid
RNAi	Ribonucleic Acid Interference
RNase	ribonuclease
RO	reverse osmosis
RT-PCR	reverse transcriptase polymerase chain reaction
SAPs	Secreted Aspartic Proteases
SDS	sodium dodecyl sulfate
SLS	sodium lauryl sarcosine
TGN	Trans Golgi Network
UTR	untranslated region
X-Gal	5-bromo-4-chloro-3-idolyl beta-D-galactoside