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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 Δgcn1* 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 $\Delta gcnl$ mutant in close proximity to wild type strains, and fully complemented by insertion of the $gcnl$ gene. This suggests that the $gcnl$ gene encodes the major $\beta$-1,6-glucanase activity of $E. festucae$. 
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TABLE OF CONTENTS

Abstract ...................................................................................................................... iii
Acknowledgements .................................................................................................. v
Table of contents ..................................................................................................... vi
Table of figures ......................................................................................................... xv
Table of tables .......................................................................................................... xviii
Table of abbreviations ............................................................................................. xix

CHAPTER 1: Introduction

1.1 FUNGAL LIFESTYLES ....................................................................................... 2
1.2 EPICHLÖÈ AND NEOTYPHODIUM ENDOPHYTES ......................................... 3
  1.2.1 Relationships between Epichloë and Neotyphodium endophytes and their hosts ............................................................ 3
  1.2.2 Endophyte secondary metabolites ............................................................ 5
  1.2.3 Endophyte growth and colonisation within their hosts ......................... 6
  1.2.4 Endophyte-host compatibility ................................................................. 8
  1.2.5 Endophyte nutrition within its host grass .............................................. 9
  1.2.6 Epichloë and Neotyphodium sp. endophytes as experimental systems ... 11
1.3 HYDROLYTIC ENZYMES ............................................................................... 12
1.4 SUBTILISIN-LIKE PROTEASES .................................................................... 14
  1.4.1 Family I of the fungal subtilisin-like proteases: pyrolysin-type proteases .... 15
  1.4.2 Family II of the fungal subtilisin-like proteases: proteinase K-type proteases ................................................................. 16
  1.4.3 Fungal subtilisin-like protease family III: kexin-type proteases .............. 18
  1.4.4 Distribution of subtilisin-like proteases in fungal genomes .................... 20
1.5 ROLE OF PROTEASES IN INTERACTIONS WITH THEIR HOSTS ............. 23
  1.5.1 Insect pathogenic fungi ........................................................................... 23
  1.5.2 Trichoderma species ............................................................................... 26
  1.5.3 Nematode pathogenic fungi ..................................................................... 27
    1.5.3.1 Arthrobotrys oligospora ..................................................................... 27
    1.5.3.2 Verticillium chlamydosporium ............................................................. 28
    1.5.3.3 Paecilomyces lilacinus ....................................................................... 29
  1.5.4 Candida albicans ...................................................................................... 29
  1.5.5 Dermatophytic fungi ................................................................................ 30
  1.5.6 Plant pathogenic fungi ............................................................................. 31
    1.5.6.1 Magnaporthe grisea ............................................................................ 31
    1.5.6.2 Botrytis cinerea ................................................................................ 32
    1.5.6.3 Sclerotinia sclerotiorum ..................................................................... 32
    1.5.6.4 Fusarium species .............................................................................. 33
    1.5.6.5 Verticillium dahliae ............................................................................ 33
    1.5.6.6 Stagonospora nodorum ..................................................................... 34
    1.5.6.7 Cochliobolus carbonum ..................................................................... 34
    1.5.6.8 Glomerella cingulata ........................................................................ 34
    1.5.6.9 Ophiostoma piliferum ........................................................................ 35
CHAPTER 2: Materials and methods

2.1 BIOLOGICAL MATERIAL ............................................................ 44
2.2 GROWTH OF BACTERIAL AND FUNGAL CULTURES .................... 47
  2.2.1 Bacterial cultures ............................................................... 47
  2.6.1 Fungal cultures ................................................................ 47
2.3 MEDIA ................................................................................. 47
  2.3.1 Aspergillus complete medium (ACM) .................................... 47
  2.3.2 Luria-Bertani medium (LB) ................................................ 47
  2.3.3 Potato dextrose medium (PD) ............................................ 48
  2.3.4 Pustulan or glucose media .................................................. 48
  2.3.5 Regeneration medium (RG) ................................................ 48
  2.3.6 SOC medium .................................................................. 48
  2.3.7 TOP agarose medium ........................................................ 48
  2.3.8 Water agar medium .......................................................... 48
  2.3.9 Media additions ................................................................. 49
2.4 BUFFERS AND SOLUTIONS ..................................................... 49
  2.4.1 Buffers ............................................................................. 49
    2.4.1.1 Byrd extraction buffer .................................................. 49
    2.4.1.2 20% PEG solution ...................................................... 49
    2.4.1.3 SM buffer ................................................................ 49
    2.4.1.4 20x SSPE buffer ....................................................... 49
    2.4.1.5 STE (100/10/1) buffer ............................................... 49
    2.4.1.6 STET buffer ............................................................... 49
    2.4.1.7 Taha lysis buffer ......................................................... 49
    2.4.1.8 TE (10/0.1) buffer ...................................................... 50
    2.4.1.9 TES buffer ................................................................. 50
    2.4.1.10 Tris acetate buffer ..................................................... 50
  2.4.2 Enzymes ........................................................................ 50
    2.4.2.1 DNase I .................................................................. 50
    2.4.2.2 Lysozyme ................................................................ 50
    2.4.2.3 Proteinase K ............................................................. 50
    2.4.2.4 RNase A (DNase free) ................................................. 50
  2.4.3 Commonly used stock solutions .......................................... 50
  2.4.4 Stains ............................................................................. 51
    2.4.4.1 Aniline Blue stain ....................................................... 51
    2.4.4.2 Congo Red stain ......................................................... 51
2.5 DNA ISOLATION AND PURIFICATION .................................. 51
2.5.1 Phenol-chloroform purification ........................................... 51
2.5.2 Precipitation of DNA with ethanol or isopropanol .................... 51
2.5.3 Gel purification ..................................................................... 51
  2.5.3.1 Freeze-thaw extraction .................................................. 51
  2.5.3.2 Extraction from agarose using the QiaQuick™ gel extraction kit
         (Qiagen) ........................................................................ 52
2.5.4 PCR product purification ...................................................... 52
2.5.5 Plasmid DNA isolation .......................................................... 52
  2.5.5.1 Rapid boil plasmid isolation ............................................ 52
  2.5.5.2 High Pure™ plasmid isolation kit (Roche) ......................... 53
  2.5.5.3 Quantum™ plasmid miniprep kit (Bio-Rad) ....................... 53
  2.5.5.4 Quantum™ plasmid midiprep kit (Bio-Rad) ....................... 53
2.5.6 Alkaline lysis purification of plasmids and cosmids .................... 54
  2.5.6.1 Alkaline lysis solutions .................................................. 54
    2.5.6.1.1 Alkaline lysis solution I .......................................... 54
    2.5.6.1.2 Alkaline lysis solution II ........................................ 54
    2.5.6.1.3 Alkaline lysis solution III ...................................... 54
  2.5.6.2 Alkaline lysis preparation of plasmid and cosmid DNA for
         sequencing ....................................................................... 54
  2.5.6.3 Large scale cosmid DNA isolation by alkaline lysis .............. 55
2.5.7 λ DNA isolation ................................................................. 55
  2.5.7.1 Plating λ phage ............................................................. 55
  2.5.7.2 Isolation of λ phage DNA ............................................... 56
2.5.8 Fungal and plant genomic DNA isolation .................................. 56
  2.5.8.1 Isolation of genomic DNA from fungal protoplasts ............... 56
  2.5.8.2 Isolation of fungal or plant genomic DNA using modified
         Taha method ...................................................................... 57
  2.5.8.3 Isolation of genomic DNA using the plant-fungal method .......... 57
  2.5.8.4 Isolation of fungal genomic DNA using modified Byrd method ... 58
2.6 DNA QUANTIFICATION .............................................................. 58
  2.6.1 Fluorometric quantitation with Hoescht dye .......................... 58
    2.6.1.1 Solutions for fluorometric quantitation ........................... 58
      2.6.1.1.1 Hoescht dye solution ............................................ 58
      2.6.1.1.2 10 x TNE buffer ................................................... 58
      2.6.1.1.3 Calf thymus DNA stock ....................................... 58
      2.6.1.1.4 Assay solution A (for low range assays) .................... 59
      2.6.1.1.5 Assay solution B (for high range assays) .................. 59
  2.6.1.2 Quantitation using the fluorometer .................................... 59
    2.6.1.2.1 Low concentration assays of DNA concentration ............ 59
    2.6.1.2.2 High concentration assays of DNA concentration .......... 59
  2.6.2 Quantitation by ethidium bromide staining ............................ 60
2.7 RESTRICTION ENDONUCLEASE DIGESTION OF DNA .................... 60
2.8 AGAROSE GEL ELECTROPHORESIS ........................................... 60
  2.8.1 Agarose gel electrophoresis solutions .................................. 60
    2.8.1.1 1 x TAE electrophoresis buffer .................................. 60
    2.8.1.2 1 x TBE electrophoresis buffer .................................. 60
    2.8.1.3 SDS loading dye ..................................................... 60
    2.8.1.4 Ethidium bromide staining solution ............................... 61
2.9 SOUTHERN BLOTTING .......................................................... 62

2.9.1 Southern blotting solutions ........................................... 62
  2.9.1.1 Solution 1 ................................................................. 62
  2.9.1.2 Solution 2 ................................................................. 62
  2.9.1.3 Solution 3 ................................................................. 62
  2.9.1.4 20 x SSC ................................................................. 62
  2.9.1.5 2 x SSC ................................................................. 62
  2.9.1.6 10 x Denhardt’s Solution ........................................ 62
  2.9.1.7 Library hybridisation solution ................................... 62
  2.9.1.8 Alkaline stripping solution ....................................... 62

2.9.2 Southern (capillary) blotting ......................................... 62

2.9.3 Radiolabelling of DNA probes ........................................ 63

2.9.4 Hybridisation of radio labelled DNA probes ..................... 64

2.9.5 Autoradiography .......................................................... 64

2.9.6 Stripping of Southern blots ............................................. 64

2.10 LIBRARY SCREENING ...................................................... 65

2.11 DNA SEQUENCING .......................................................... 65

2.12 DNA LIGATION .............................................................. 66
  2.12.1 CAP treatment of vector DNA ...................................... 66
  2.12.2 DNA ligation .............................................................. 67
  2.12.3 Shot gun cloning of λ and cosmid DNA fragments ............ 67

2.13 VECTOR CONSTRUCTION .................................................. 68
  2.13.1 Construction of vectors to give heterologous *prt1* or *prt2* expression ........................................ 68
    2.13.1.1 Construction of the phFunGus vector ..................... 68
    2.13.1.2 Construction of vectors to give heterologous *prt1* expression ........................................ 68
    2.13.2 Construction of vectors to give heterologous *prt2* expression ........................................ 70
    2.13.3 Construction of the *gcnl* gene replacement vector ...... 73

2.14 BACTERIAL TRANSFORMATION .......................................... 73
  2.14.1 Preparation of electro-competent *E. coli* cells .......... 73
  2.14.2 Transformation of DNA by electroporation .................... 75
  2.14.3 Screening of transformants ....................................... 75
    2.14.3.1 Blue-white selection .......................................... 75
    2.14.3.2 Clone Checker™ analysis (Invitrogen) .................... 75
    2.14.3.3 Colony PCR ..................................................... 76

2.15 FUNGAL PROTOPLAST PREPARATION AND CHEF ELECTROPHORESIS 76
  2.15.1 Protoplasting solutions ............................................ 76
    2.15.1.1 OM buffer ...................................................... 76
    2.15.1.2 Glucanex ....................................................... 76
    2.15.1.3 ST buffer ...................................................... 77
    2.15.1.4 STC buffer ..................................................... 77
    2.15.1.5 40% PEG buffer ............................................... 77
    2.15.1.6 GMB buffer .................................................... 77
    2.15.1.7 LMP in GMB ................................................... 77
    2.15.1.8 SE buffer ..................................................... 77
2.15.1.9 10 x ET buffer with SLS ................................................................. 77
2.15.1.10 1 x ET buffer .......................................................... 77
2.15.2 Protoplast preparation .......................................................... 77
2.15.3 Preparation of protoplast plugs for CHEF ...................................... 78
2.15.4 CHEF electrophoresis .......................................................... 78
2.16 FUNGAL TRANSFORMATION ....................................................... 79
2.16.1 Transformation of fungal protoplasts ........................................... 79
2.16.2 Screening of fungal transformants ............................................. 80
   2.16.2.1 Screening using alkaline lysis of fungal hyphae ....................... 80
2.16.2.2 Screening using the plant Extract-N-Amp™ PCR kit (Sigma) ....... 80
2.17 PCR ................................................................. 80
2.17.1 PCR reagents .......................................................... 80
   2.17.1.1 Oligonucleotide primers ................................................. 80
   2.17.1.2 dNTPs ......................................................... 83
2.17.2 Standard PCR .......................................................... 83
2.17.3 Gradient PCR .......................................................... 84
2.17.4 PCR using Expand™ Long Template (Roche) ................................ 84
2.17.5 PCR using Expand™ High Fidelity (Roche) .................................. 84
2.17.6 Inverse PCR .......................................................... 85
2.17.7 TripleMaster® PCR .................................................. 85
2.17.8 RT-PCR .......................................................... 85
2.18 RNA ISOLATION AND PURIFICATION ........................................... 85
2.18.1 Purification of total RNA using Trizol ....................................... 86
2.18.2 Purification of polyA RNA from total RNA .................................... 86
2.18.3 RNA quantitation by measuring absorbance and A_{260}/A_{280} nm .... 86
2.18.4 DNase I treatment of RNA .................................................. 87
2.18.5 cDNA synthesis ....................................................... 87
2.19 PLANT-ENDOPHYTE SYMBIOTA GROWTH AND MAINTENANCE ......... 88
2.19.1 Plant maintenance .......................................................... 88
2.19.2 Inoculation of grass seedlings with endophyte hyphae ................. 88
   2.19.2.1 Surface sterilisation of grass seeds .................................... 88
   2.19.2.2 Inoculation of grass seedlings with endophytes ................. 88
   2.19.2.3 Root training of inoculated seedlings ............................... 88
2.19.3 Detection of infected seedlings after endophyte inoculation .......... 89
   2.19.3.1 Aniline blue staining .................................................. 89
   2.19.3.2 Immunodetection by immunoblotting ................................. 89
   2.19.3.2.1 Immunoblotting blocking solution .................................. 89
   2.19.3.2.2 Immunoblotting Tris buffer ........................................ 89
   2.19.3.2.3 Fast Red chromogen .................................................. 89
   2.19.3.2.4 Immunoblot detection of endophyte in grass tissues ........... 89
2.20 MICROSCOPY AND PHOTOGRAPHY .................................................... 90
2.21 BIOINFORMATICS ................................................................. 91
CHAPTER 3: Gene family

3.1 E. FESTUCAE AND N. LOLII PROTEINASE K FAMILY GENES (SUBFAMILIES 1 AND 2) ............................................................. 94
  3.1.1 The prt1 and prt5 genes ............................................................. 94
  3.1.2 The prt2 gene ........................................................................ 101
  3.1.3 The prt3 gene ........................................................................ 108
    3.1.3.1 Isolation of the N. lolii Lp19 and E. festucae Fll prt3 genes ... 108
    3.1.3.2 The N. lolii Lp19 and Lp5 prt3 genes encode non-functional proteins 112
  3.1.4 Phylogenetic analysis of E. festucae Fll and N. lolii Lp19 prt1, prt2, 
     prt3 and prt5 genes .................................................................... 115
  3.2 E. FESTUCAE FL1 PROTEINASE K FAMILY GENE (SUBFAMILY 3) .... 117
  3.2.1 The prt4 gene ........................................................................ 117
  3.2.2 Phylogenetic analysis of proteinase K subfamily 3 genes .......... 122
  3.3 THE E. FESTUCAE KEX2 GENE ..................................................... 123
  3.4 E. FESTUCAE FL1 CONTAINS OTHER GENES ENCODING 
     SUBTILISIN-LIKE PROTEASES .................................................... 128
  3.5 CHROMOSOMAL LOCALISATION OF THE PRT AND KEX2 GENES.... 132
  3.6 EXPRESSION OF THE PRT AND KEX2 GENES IN CULTURE AND IN PLANTA ................................................................. 134

CHAPTER 4: Functional analysis of prt1 and prt2

4.1 CONSTRUCTION OF VECTORS TO GIVE ALTERED EXPRESSION 
     OF THE PRT1 OR PRT2 GENES ..................................................... 140
4.2 TRANSFORMATION OF E. FESTUCAE FL1 WITH THE ALTERED 
     EXPRESSION VECTORS ................................................................ 141
4.3 ANALYSIS OF TRANSFORMANT PRT1 OR PRT2 EXPRESSION ...... 143
  4.3.1 Expression of the transformant prt1 or prt2 genes in culture ...... 143
  4.3.2 Expression of the transformant prt1 or prt2 genes in planta ...... 150
4.4 PHENOTYPE OF TRANSFORMANTS DURING GROWTH IN 
     CULTURE AND IN PLANTA .......................................................... 153

CHAPTER 5: Functional analysis of gcn1

5.1 ENDOPHYTE GENES ENCODING β-1,6-GLUCANASES ..................... 160
5.2 REPLACEMENT OF THE E. FESTUCAE FL1 GCNI GENE ............... 161
  5.2.1 Transformation of E. festucae Fll with a gcnl::hph construct ....... 161
  5.2.2 Phenotype of the Δgcn1 strains during growth in culture .......... 167
5.3 COMPLEMENTATION OF THE ΔGCNI STRAIN ................................ 170
5.4 GROWTH OF ΔGCNI STRAINS IN PLANTA .................................... 174

CHAPTER 6: Discussion

6.1 E. FESTUCAE CONTAINS A GENE FAMILY OF SUBTILISIN-LIKE 
     PROTEASES .............................................................................. 178
6.1.1 Proteinase K-type subtilisin-like proteases .................................................. 178
  6.1.1.1 The prt5-prtl locus ................................................................................. 178
  6.1.1.2 The prt2-gcn1 locus .............................................................................. 179
  6.1.1.3 The prt3 locus ....................................................................................... 181
  6.1.1.4 The prt4 gene ..................................................................................... 184
6.1.2 Kex2 is a member of the kexin family of subtilisin-like proteases .............. 184
6.1.3 Regulation of expression of genes encoding subtilisin-like proteases ....... 184
6.1.4 Genomic distribution of subtilisin-like proteases in filamentous fungi .... 191

6.2 HETEROLOGOUS EXPRESSION OF PRT1 AND PRT2 IN 
  EPICHLÓE FESTUCAE FL1 .................................................................................. 195
6.3 FUNCTION OF THE E. FESTUCAE FL1 GCN1 GENE .................................. 200

APPENDIX

Appendix A1: Restriction maps ................................................................................. 209
  Appendix A1.1: Vectors for general use ............................................................... 210
    A1.1.1 pFungUS ................................................................................................. 210
    A1.1.2 pAN7-1 .................................................................................................. 210
    A1.1.3 phGFP2 ................................................................................................. 211
    A1.1.4 pF199 .................................................................................................... 211
    A1.1.5 pPN1688 ............................................................................................... 212
    A1.1.6 pUC118 .................................................................................................. 212
    A1.1.7 pXZ56 .................................................................................................... 213
    A1.1.8 pGEM-T Easy ....................................................................................... 213
  Appendix A1.2: prtl vectors .................................................................................. 214
    A1.2.1 pMM2 .................................................................................................. 214
    A1.2.2 pMM3 .................................................................................................. 214
    A1.2.3 pMM4 .................................................................................................. 215
    A1.2.4 pMM51 ................................................................................................. 215
  Appendix A1.3: prt2 vectors .................................................................................. 216
    A1.3.1 pMM7 .................................................................................................. 216
    A1.3.2 pMM44 ................................................................................................. 216
  Appendix A1.4: kex2 vectors ................................................................................ 217
    A1.4.1 pMM65 ................................................................................................. 217
  Appendix A1.5: Other genomic sequences .......................................................... 217
    A1.5.1 Ite cluster 1 from E. festucae FL1 ......................................................... 217
    A1.5.2 The E. festucae FL1 tub2 gene ............................................................. 218
    A1.5.3 The A. nidulans gpDA gene ................................................................. 218
  Appendix A2: Comparison of E. festucae and N. lolii sequences ................. 219
    Appendix A2.1: Comparison of the E. festucae FL1 and N. lolii Lp19 prtl and prtl
                     sequences ....................................................................................... 220
Appendix A2.2: Comparison of the *E. festucae* Fll and *N. lolii* Lp19 *prt2* and *gon1* sequences..............................................................226
Appendix A2.3: Comparison of the endophyte sequences homologous to *prt3*...232

Appendix A3: Analysis of Orf4 .....................................................................235

Appendix A4: Analysis of Cyc1 .....................................................................237

Appendix A5: Analysis of Ptn1 .....................................................................239
  Appendix A5.1 Alignment of the *E. festucae* Fll Ptn1 with phosphoinositide 3-
  phosphatase sequences.............................................................................240
  Appendix A5.2 Phylogenetic relationship of Ptn1 to fungal PTEN-like phosphatases.........................................................................................241

Appendix A6: Analysis of Gao1 .....................................................................243
  Appendix A6.1 Alignment of Gaol with GaoA from *Fusarium* sp..............244
  Appendix A6.2 Phylogenetic analysis of the *E. festucae* Fll Gaol protein
  with D-galactose oxidases.........................................................................245

Appendix A7: Design of degenerate primers
  Appendix A7.1 Design of degenerate PCR primers used to amplify the vacuolar
  protease encoding gene *prt4*....................................................................248
  Appendix A7.2 Design of degenerate primers for *prt* isolation.................249-250

Appendix A8: Analysis of Orf2 .....................................................................251

Appendix A9: Analysis of Orf3 .....................................................................253

Appendix A10: Analysis of Nc25 ..................................................................255

Appendix A11: MEME analysis for *prt* promoters........................................257
  Appendix A11.1 MEME analysis of the *E. festucae* Fll *prt* promoters........258
  Appendix A11.2 MEME motifs....................................................................258

Appendix A12: Raw data for assessing transgene copy number ......................263
  Appendix A12.1: Raw data for copy number analysis in pMM32
  transformants.........................................................................................264
  Appendix A12.2: Raw data for copy number analysis in pMM33
  transformants.........................................................................................265
  Appendix A12.3: Raw data for copy number analysis in pMM26
  transformants.........................................................................................266
  Appendix A12.4: Raw data for copy number analysis in pMM27
  transformants.........................................................................................267

Appendix A13: Sequences used in phylogenetic analysis...............................269
  Appendix A13.1 Nucleotide sequences used in rRNA phylogenetic analysis....270
Appendix A13.2 Polypeptide sequences used in Prt1, Prt2, Prt3 and Prt5 phylogenetic analysis ................................................................. 271
Appendix A13.3 Polypeptide sequences used in Prt4 phylogenetic analysis ...... 272
Appendix A13.4 Polypeptide sequences used in Kex2 phylogenetic analysis .... 273
Appendix A13.5 Polypeptide sequences used in Gcn1 phylogenetic analysis .... 274
Appendix A13.6 Polypeptide sequences used in Cyc1 phylogenetic analysis .... 275
Appendix A13.7 Polypeptide sequences used in Ptn1 phylogenetic analysis ...... 276
Appendix A13.8 Polypeptide sequences used in Gaol phylogenetic analysis .... 277

Appendix A14: Intron conservation .......................................................... 279
Appendix A14.1 Conservation of intron position in prt genes ...................... 280
Appendix A14.2 Conservation of intron position in Fl1 prt4 ....................... 281
Appendix A14.3 Intron conservation in kexin-encoding genes ..................... 282

Appendix A15: Growth of E. typhina PN2311 in planta .................................. 283
Appendix A16: Gene features ...................................................................... 285

Appendix A17: SignalP 3.0 analysis .............................................................. 287

BIBLIOGRAPHY

Bibliography ................................................................................................. 290
| Figure 1.1 | Life cycles of *Epichloë* and *Neotyphodium* species within their grass hosts | 4 |
| Figure 1.2 | Exo- and endohydrolytic cleavage of molecules | 13 |
| Figure 1.3 | Hydrolytic reaction catalysed by subtilisin-like proteases | 14 |
| Figure 1.4 | Phylogenetic relationships of fungal species | 22 |
| Figure 1.5 | Reaction catalysed by endo-β-1,6-glucanases | 37 |
| Figure 2.1 | Construction of the phFunGus vector | 69 |
| Figure 2.2 | Construction of vectors directing heterologous expression of *prt1* | 71 |
| Figure 2.3 | Construction of vectors directing heterologous expression of *prt2* | 72 |
| Figure 2.4 | Construction of the *gcn1*:kph replacement vector pMM54 | 74 |
| Figure 3.1 | Southern analysis of *N. lolii* Lp19 and *E. festucae* Fl1 *prt1* | 95 |
| Figure 3.2 | Structure of the *N. lolii* Lp19 *prt5* and *prt1* genes | 96 |
| Figure 3.3 | Structure of the *E. festucae* Fl1 *prt5* and *prt1* genes | 96 |
| Figure 3.4 | Southern analysis of *E. festucae* Fl1 *prt5* | 97 |
| Figure 3.5 | Gene structure of the *prt5* and *prt1* genes | 98 |
| Figure 3.6 | Potential binding sites for fungal global transcription regulators in *E. festucae* Fl1 *prt5* and *prt1* | 100 |
| Figure 3.7 | MEME analysis of repeated sequence elements found in the *prt* promoters | 102 |
| Figure 3.8 | Southern analysis of *prt2* | 103 |
| Figure 3.9 | Structure of the *N. lolii* Lp19 *prt2* locus | 105 |
| Figure 3.10 | Structure of the *E. festucae* Fl1 *prt2* locus | 105 |
| Figure 3.11 | Gene structure of the *E. festucae* Fl1 *prt2, gcn1, cyc1* and *pml1* genes | 107 |
| Figure 3.12 | Potential binding sites for fungal global transcription regulators in *E. festucae* Fl1 *prt2* | 109 |
| Figure 3.13 | Sequence of the At1 homologue from *N. lolii* Lp19 | 110 |
| Figure 3.14 | Southern analysis of *E. festucae* Fl1 *prt3* | 111 |
| Figure 3.15 | Structure of the *E. festucae* Fl1 *prt3* genomic region | 112 |
| Figure 3.16 | Gene structure of the *E. festucae* Fl1 *prt3* and *gao1* genes | 112 |
| Figure 3.17 | Potential binding sites for fungal global transcription regulators in *E. festucae* Fl1 *prt3* locus | 114 |
| Figure 3.18 | Phylogenetic relationships of *Prt1, Prt2, Prt3* and *Prt5* | 116 |
| Figure 3.19 | Strategy for identifying a vacuolar protease homologue | 117 |
| Figure 3.20 | Sequence of the *prt4* degenerate PCR product | 118 |
| Figure 3.21 | Southern analysis of the *E. festucae* Fl1 *prt4* | 119 |
| Figure 3.22 | Structure of the *E. festucae* Fl1 *prt4* gene | 120 |
| Figure 3.23 | Gene structure of the *E. festucae* Fl1 *prt4* gene | 120 |
| Figure 3.24 | Potential binding sites for fungal global transcription regulators in *E. festucae* Fl1 *prt4* locus | 121 |
| Figure 3.25 | Phylogenetic relationship of *E. festucae* Fl1 *Prt4* to fungal vacuolar proteases | 122 |
Figure 3.26 Southern analysis of *E. festucae* Fl1 *kex2* ........................................... 123
Figure 3.27 Structure of the *E. festucae* Fl1 *kex2* gene ........................................... 124
Figure 3.28 Gene structure of *E. festucae* Fl1 orf2, orf3, Ne25 and *kex2* genes .......... 124
Figure 3.29 Potential binding sites for fungal global transcription regulators in *E. festucae* Fl1 *kex2* locus ................................................................. 126
Figure 3.30 Phylogenetic relationship of the *E. festucae* Fl1 Kex2 protein with fungal kexins ................................................................. 127
Figure 3.31 Degenerate PCR amplification of subtilisin-like protease-encoding sequences from *E. festucae* Fl1 ..................................................... 128
Figure 3.32 The *E. festucae* Fl1 prt6 gene ................................................................. 129
Figure 3.33 The *E. festucae* Fl1 *prt7* gene ................................................................. 130
Figure 3.34 The *E. festucae* Fl1 *prt8* gene ................................................................. 131
Figure 3.35 Chromosomal location of the *prt* genes .................................................... 133
Figure 3.36 Chromosomal location of the *kex2* gene .................................................... 133
Figure 3.37 Equalisation between in culture and *in planta* tub2 expression ................. 135
Figure 3.38 Comparison of hydrolytic enzyme gene expression in culture and *in planta* ................................................................. 136
Figure 3.39 Comparison of *prt* gene regulation in different grass-endophyte symbiota ... 137

Figure 4.1 Constructs for altered expression of *prt1* and *prt2* .................................... 140
Figure 4.2 Strategy for assessing the number of intact transgene copies in transformant genomes ........................................................................ 142
Figure 4.3 Southern blot analysis of pMM32 transformants ........................................... 144
Figure 4.4 Southern blot analysis of pMM33 transformants ........................................... 145
Figure 4.5 Southern blot analysis of pMM26 transformants ........................................... 146
Figure 4.6 Southern blot analysis of pMM27 transformant ............................................ 147
Figure 4.7 Expression of the *E. festucae* Fl1 *prt1* and *prt2* wild type genes and transgenes in culture ................................................................. 149
Figure 4.8 Expression of the wild type and transgene copies of *prt1* *in planta* .......... 151
Figure 4.9 Expression of the wild type and transgene copies of *prt2* *in planta* .......... 152
Figure 4.10 Growth of pMM32 transformants *in planta* ............................................ 154
Figure 4.11 Growth of pMM33 transformants *in planta* ............................................ 155
Figure 4.12 Growth of pMM26 transformants *in planta* ............................................ 156
Figure 4.13 Growth of pMM27 transformants *in planta* ............................................ 157

Figure 5.1 Comparison of the *E. festucae* Fl1 and *N. lolii* Lp19 *prt2-gcn1* intergenic region ........................................................................ 160
Figure 5.2 Alignment of endophyte β-1,6-glucanases ..................................................... 161
Figure 5.3 Phylogenetic analysis of fungal β-1,6-glucanases ........................................... 162
Figure 5.4 The gcn1 deletion construct ....................................................................... 163
Figure 5.5 PCR analysis of selected gcn1::hph transformants ........................................ 164
Figure 5.6 Southern analysis of selected gcn1::hph transformants ................................... 166
Figure 5.7 Growth of Δgcn1 strains on media containing glucose ................................ 168
Figure 5.8 Growth of Δgcn1 strains on media containing pustulan, a β-1,6-glucan polymer ........................................................................ 169
Figure 5.9  Genetic complementation of the gcnl deletion by co-transformation with pMM44 and pII99............................171
Figure 5.10 Growth screening of Δgcnl strains complemented with pMM44..........172
Figure 5.11 Phenotype of Δgcnl hyphae during growth in planta..........................175
### TABLE OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Degradation of macromolecules by hydrolytic enzymes</td>
<td>13</td>
</tr>
<tr>
<td>Table 1.2</td>
<td>Distribution of subtilisin-like protease encoding genes in fungal genomes</td>
<td>21</td>
</tr>
<tr>
<td>Table 2.1</td>
<td>Biological material</td>
<td>44</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Supplements added to media</td>
<td>49</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Stock solutions</td>
<td>50</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Primers used in this study</td>
<td>81</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Size of fragments homologous to <em>N. lolii</em> Lp19 and <em>E. festucae</em> Fl1 <em>prt1</em></td>
<td>95</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Size of fragments homologous to <em>E. festucae</em> Fl1 <em>prt5</em></td>
<td>97</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Fragments homologous to <em>N. lolii</em> Lp19 <em>prt2</em></td>
<td>104</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Fragments homologous to <em>E. festucae</em> Fl1 <em>prt3</em></td>
<td>111</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>Fragments homologous to <em>E. festucae</em> Fl1 <em>prt4</em></td>
<td>119</td>
</tr>
<tr>
<td>Table 3.6</td>
<td>Fragments homologous to <em>E. festucae</em> Fl1 <em>kex2</em></td>
<td>124</td>
</tr>
<tr>
<td>Table 3.7</td>
<td>Characterised products from degenerate PCR with the MM149- MM150 primers</td>
<td>128</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Transformation frequency for different plasmid constructs</td>
<td>141</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Intact copies of pMM32</td>
<td>144</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Intact copies of pMM33</td>
<td>145</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Intact copies of pMM26</td>
<td>146</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Intact copies of pMM27</td>
<td>147</td>
</tr>
<tr>
<td>Table 6.1</td>
<td>Regulation of fungal subtilisin-like proteases</td>
<td>185</td>
</tr>
</tbody>
</table>
# TABLE OF ABBREVIATIONS

<table>
<thead>
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<th>ABBREVIATION</th>
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<td>reverse transcriptase polymerase chain reaction</td>
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</tr>
</tbody>
</table>