Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

How does *Epichloë festucae* avoid the host defence response?

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

In

Genetics

at Massey University, Palmerston North
New Zealand

Nazanin Noorifar

2018

Abstract

Epichloë festucae is a filamentous fungus, which forms symbiotic associations with aerial tissues of *Lolium* and *Festuca* grass species. Chitin, a polymer of N-acetyl-D-glucosamine, is an important component of the fungal cell wall and a well-known pathogen associated molecular pattern (PAMP). Chitin promotes pathogen-triggered immunity (PTI) upon hydrolysis with plant chitinases and release of chitin oligomers. Therefore, to establish a stable and successful symbiosis, the endophyte needs to remain 'hidden' from the host immune system or actively suppress it. Confocal laser scanning microscopy (CLSM)-based analysis of leaf tissue infected with the *E. festucae* wild type strain and infiltrated with the chitin-specific molecular probe, WGA-Alexa Fluor-488, showed that only the septa of endophytic hyphae bound this probe while the entire cell wall was labelled in epiphyllous hyphae confirming previous observations that hyphal cell wall chitin is either masked or remodelled in endophytic hyphae. The aims of this project were (i) to test whether *E. festucae* LysM-containing proteins have a role in binding to or sequestering cell wall chitin oligomers and thereby preventing PAMP-triggered immunity and (ii) to analyse the composition of the cell wall of endophytic and epiphytic hyphae. An analysis of the *E. festucae* genome identified seven genes encoding proteins with LysM domains. Expression of two of these genes, *lymA* and *lymB*, increased *in planta* compared to in culture. Interestingly, both are divergently transcribed from chitinase encoding genes (*chiA* and *chiB* respectively), which also have increased expression *in planta*. Single gene deletion mutants of *lymA, lymB, chiA* and *chiB* as well as a double gene deletion Δ*lymA/B* were generated, and their plant interaction phenotype analysed. Plants infected with Δ*lymA*, Δ*lymB* or Δ*chiA* had the same plant-interaction phenotype as wild type whereas Δ*chiB* and Δ*lymA/B* mutants had defects in hyphal growth within the leaves. Analysis of hyphal cell wall structure using Chitin Binding Protein (CBP) and chitosan (CAP (Chitosan Affinity Protein) and OGA-488)-specific eGFP-based biosensors suggest that cell wall chitin is converted to chitosan in endophytic hyphae. This structural change is consistent with a lack of a defence response when *E. festucae* forms a mutualistic symbiotic association with *L. perenne*. Three *E. festucae* chitin deacetylase genes were identified (*cdaA*, *cdaB* and *cdaC*), and gene expression analysis showed *cdaA* expression is significantly increased *in planta* compare to in culture. Functional analysis of *cdaA* revealed that although plants infected with the Δ*cdaA* mutant had a similar whole plant interaction phenotype as wild type, they had an abnormal

cellular phenotype. Patches of chitin were exposed along the endophytic hyphae confirming this mutant was unable to convert chitin to chitosan. However, hyphae in these plants still labelled with the chitosan biosensor OGA-488 demonstrating that despite the deletion of the *cdaA*, the hyphal cell wall of endophytic hyphae still contain chitosan suggesting that another chitin deacetylase, possibly CdaB has a redundant function in *E. festucae*. Collectively these results show that *lymA*, *lymB* and *chiB* are required for establishment of the symbiosis between *E. festucae* and *L. perenne*. In addition, this study shows that chitin is converted to chitosan in the hyphal cell wall of endophytic hyphae during the infection and colonisation of the host. The *E. festucae* chitin deacetylase gene *cdaA* is also essential for proper hyphal growth *in planta* and the symbiotic interaction.

Acknowledgments

My deepest sense of gratitude to my main supervisor Professor Barry Scott for the patient guidance, encouragement and advice he has provided throughout the period of my doctoral study as his student. I have been extremely lucky to have a knowledgeable and experienced supervisor who cared so much about my work and who responded to my questions and queries so promptly. It was a rewarding experience to work under his supervision. I would like to thank my co-supervisor Dr Matthew Savoian for his technical training, constant support, availability and constructive suggestions which were determinant for the accomplishment of the work presented in this thesis.

A big thank you to our lab manager Arvina Ram, the first person who gave me technical training in the lab and whom throughout these years had always gave encouraging words and always stood with me and ready to help me specially during the hard time of writing. It was a wonderful time being with her and to work with her in the lab. She is like a sister to me far away from home.

A warm thank you to all Scottbase members, past and present, Alex, Kim, Daniel, Yonathan, Berit and Taryn for providing so pleasurable and friendly working atmosphere I am truly thankful for all the help, discussions and ideas whenever I needed them and a big thank you to Elli and Alyesha for helping me taking care of the plant room and their help whenever I needed them.

Thanks also to Dr Yvonne Becker, Dr Mathias Becker, Dr Carla Eaton and Dr David Winter for their valuable discussion and advice on microscopy and bioinformatic aspects.

I also want to thank present and past members of MMIC for providing such a high-quality level of help and guidance.

I would like to thank members of the microscopy centre at Fontera in Palmerston North for kindly helping me to analyse the plant samples in the second part of the project.

I would like to thank Professor Bruno Moerschbacher and Professor William Willats for providing chitin and chitosan biosensors which made the second part of this work possible.

I would also like to acknowledge the BioProtection Research Centre for funding this PhD project.

Thanks to all my friends back home in Iran and all here in New Zealand. I express my gratitude for their unconditional friendship, support and patience throughout these years.

A special thanks to my family, my grandmother, my parents, my sister, Nazila, my brother, Navid and my sister in law, Parisa, for their unconditional love, support, encouragement

and all the sacrifice you have made for me. Words cannot express how grateful I am to have you all in my life. Your prayers for me were what sustained me thus far. I consider myself the luckiest in the world to have such a lovely and caring family, standing beside me with their love and unconditional support.

And last but not the least my eternal gratitude goes to my husband, Naser, for his continued and unfailing love, support and understanding during my pursuit of PhD degree that made the completion of this thesis possible. He is always around at times when I thought that it is impossible to continue.

Chapter 1. Introduction…….....1

1.1. Epichloë species as ryegrass endophytes……....2

1.2. The endophyte life cycle……...2

1.3. *Epichloë festucae-Lolium perenne* as an experimental model system…….....3

1.4. Epichloë species grow within grass leaves by a novel mechanism……...4

1.4.1. Endophytic growth……4

1.4.2. Epiphytic growth……....6

1.5. Fungal cell wall structure……...8

1.5.1. The chitin component of the fungal cell wall……....9

1.5.2. The glucan component of the fungal cell wall……11

1.5.3. The glycoprotein component of the fungal cell wall……...11

1.6. Plant immunity…….....13

1.7. Chitin recognition and the host defence response……...13

1.8. Fungal effectors and LysM proteins……15

1.9. Chitosan……17

1.10. Fungal chitinases……...19

1.11. Aims and objectives of this project……20

Chapter 2. Materials and methods……...23

2.1. Sterile conditions……24

2.2. Molecular and biological material……24

2.3. Media and growth conditions……32

2.3.1. *Escherichia coli*……32

2.3.2. Luria-Bertani (LB) medium……32

2.3.3. SOC medium……32

2.3.4. *Epichloë festucae*……..32

2.3.5. Potato dextrose (PD) medium……...32

2.3.6. Regeneration medium (RG)……..33

2.3.7. Water agar medium (WA)……33

2.3.8. *Lolium perenne*……33

2.3.8.1. Seedling sterilization, plant inoculation and growth conditions……33

2.3.8.2. Plant growth condition……...33

2.3.8.3. Immunohistochemical determination of endophyte infection……34

2.4. DNA and RNA Isolation and Quantification……34

2.4.1. Plasmid isolation……...34
2.4.2. *E. festucae* crude genomic DNA and quantification……..34
2.4.3. *E. festucae* genomic DNA isolation and quantification…….35
2.4.4. *E. festucae* RNA isolation using TRIzol……...36
2.4.5. Reverse transcription……36
2.5. DNA Manipulation……..36
2.5.1. Restriction enzyme digestion……36
2.5.2. Ethanol precipitation of nucleic acid……37
2.5.3. Gel electrophoresis……...37
2.5.4. Gel purification…….....37
2.5.5. Southern blotting …….....37
2.5.6. DNA probe-labelling……38
2.5.7. Hybridisation and detection ……38
2.5.8. Colourimetric development of Southern blots……..39
2.6. Polymerase chain reaction (PCR)……..39
2.6.1. Standard PCR……...39
2.6.2. High fidelity PCR……39
2.6.3. DNA quantification……..39
2.6.4. Quantitative reverse transcription PCR (qRT-PCR) ……39
2.6.4.1. qRT-PCR…….....39
2.6.4.2. Generating qRT-PCR standard curves……...40
2.7. Cloning……40
2.7.1. Gibson assembly……...40
2.8. Transformation technique……41
2.8.1. Bacterial transformation……...41
2.8.1.1. Transformation of chemically competent *E. coli* cells……..41
2.8.1.2. Invitrogen clone checker system……41
2.8.3. Sequencing ……...41
2.8.4. Fungal transformation……...42
2.8.4.1. Preparation of fungal protoplast……42
2.8.4.2. Transformation of fungal protoplast……..42
2.9. Preparation of constructs……...43
2.9.1. *E. festucae lymA* gene replacement construct……43
2.9.2. *E. festucae lymB* gene replacement construct……43

2.9.3. *E. festucae chiA* gene replacement construct……43
2.9.4. *E. festucae chiB* gene replacement construct……44
2.9.5. *E. festucae lymA* gene localization construct……44
2.9.5.1. *lymA-eGFP* native promoter expression construct……44
2.9.5.2. *lymA-eGFP* over expression construct……...44
2.9.6. *E. festucae lymB* gene localization construct……45
2.9.6.1. *lymB-eGFP* native promoter expression construct……45
2.9.6.2. *lymB-eGFP* over expression construct……..45
2.9.7. *E. festucae cdaA* gene replacement construct……45
2.10. Microscopy……46
2.10.1. In culture phenotype……...46
2.10.2. *In planta*, cellular phenotype……..46
2.10.3. Endophyte cell wall composition……47
2.10.3.1. Biosensor detection of endophyte cell wall composition……47
2.10.3.1.1. Chitosan Affinity Protein (CAP) labelling *in planta*……47
2.10.3.1.2. Chitin Binding Protein (CBP) labelling *in planta*……47
2.10.3.1.3. CAP labelling in culture……47
2.10.3.1.4. WGA-AF488 and CBP labelling in culture……48
2.10.3.2. Oligogalactrunate (OGA-488) labelling……..48
2.10.3.2.2. OGA-488 labelling *in planta*……48
2.11. Bioinformatics……...48
2.11.1. Nucleotide sequence resources……...48
2.11.2. Multiple sequence alignment……..49
2.11.3. Protein structure prediction……49
2.12. A core gene set for mutualism and antagonism……..49
Chapter 3. Bioinformatic analysis of genes encoding LysM motifs and chitinases……51
3.1. Identifying genes encoding LysM proteins in the *Epichloë festucae* genome…52
3.2. Synteny analysis of *lymA* and *chiA* genes across the Clavicipitaceae……55
3.3. Synteny analysis of *lymB* and *chiB* genes across the Clavicipitaceae……69
Chapter 4. Functional analysis of genes encoding LysM and Glycoside hydrolase Family 18 (GH18) proteins……..81
4.1. Functional analysis of genes encoding LysM motif containing proteins……82

4.1.1. Generation of Δ*lymA* mutant…….....82
4.1.2. Δ*lymA* culture phenotype…….....84
4.1.3. Δ*lymA* symbiotic interaction phenotype……...86
4.1.4. Generation of Δ*lymB* mutant…….....89
4.1.5. Δ*lymB* culture phenotype…….....91
4.1.6. Δ*lymB* symbiotic interaction phenotype……...93
4.1.7. Generation of Δl*ymA/B* mutant……96
4.1.8. Δ*lymA/B* culture phenotype…….....98
4.1.9. Δ*lymA/B* symbiotic interaction phenotype……100
4.2. Functional analysis of genes encoding Glycoside hydrolase Family 18 (GH18) proteins……103
4.2.1. Generation of Δ*chiA* mutant……103
4.2.2. Δ*chiA* culture phenotype……105
4.2.3. Δ*chiA* symbiotic interaction phenotype……107
4.2.4. Generation of Δ*chiB* mutant……109
4.2.5. Δ*chiB* culture phenotype……112
4.2.6. Δ*chiB* symbiotic interaction phenotype……114
4.2.7. LymA cellular localization……117
4.2.8. LymB cellular localization……119
Chapter 5. Discussion……121
Chapter 6. Bioinformatic analysis of genes encoding chitin deacetylase enzymes……131
6.1. Identification of *E. festucae* Fl1 chitin deacetylase genes……132
Chapter 7. Functional analysis of *E. festucae* Fl1 chitin deacetylase (*cda*) genes……141
7.1. *E. festucae* Fl1 cell wall composition in endophytic and epiphyllous hyphae in infected *L. perenne* seedlings and pseudostem……...142
7.2. Cell wall composition in axenic culture……144
7.2.1. The cell walls of axenic cultures resemble those of epiphyllous hyphae…144
7.3. Fungal cell wall composition in endophytic and epiphyllous hyphae *in planta*.147
7.4. Chitosan presence in *E. festucae* cell wall in axenic culture……151
7.5. Functional analysis of the chitin deacetylase gene……153

7.5.1. Generation of Δ*cdaA* mutant……153
7.5.2. Δ*cdaA* culture phenotype……156
7.5.3. Δ*cdaA* symbiotic interaction phenotype……158
7.5.4. *cdaB* and *cdaC* gene expression level in Δ*cdaA* mutant strains *in planta*…163
Chapter 8. Discussion……165
Chapter 9. Conclusion and future directions……177
Chapter 10. Appendices……183
Chapter 11. References……255

List of figures

Figure 1.1. *E. festucae* sexual and asexual life cycle……3
Figure 1.2. *E. festucae* intercalary growth……5
Figure 1.3. Symbiosis of *E. festucae* within the host plant. ……7
Figure 1.4. Cell wall structure of fungal pathogens. ……9
Figure 1.5. The schematic of the zigzag model showing the quantitative output of the plant immune system. ……14
Figure 1.6. Fungal LysM proteins classification based on their domain structure……16
Figure 1.7. Distribution of chitin and chitosan on fungal infection structures……18

Figure 3.1. *E. festucae* Fl1 LysM predicted polypeptide structure……...52
Figure 3.2. Gene maps of *E. festucae* Fl1 *lymA-chiA* and *lymB-chiB* loci……53
Figure 3.3. *E. festucae* Fl1 ChiA and ChiB predicted polypeptide structure……54
Figure 3.4. Synteny analysis of genes at the *lymA-chiA* locus in *E. festucae* Fl1, other sexual *Epichloë* species and *Claviceps purpurea*……58
Figure 3.5. LysM protein predicted structure……...59
Figure 3.6. Multiple sequence alignment of *E. festucae* LymA protein and homologues from other *Epichloë* species and *Claviceps purpurea*……61
Figure 3.7. Chitinase protein predicted structure. ……63
Figure 3.8. Multiple sequence alignment of *E. festucae* ChiA protein and homologues from other *Epichloë* species and *Claviceps purpurea*. ……67
Figure 3.9. Synteny analysis of genes at the *lymB-chiB* locus in *E. festucae* Fl1 and other sexual *Epichloë* species. ……70
Figure 3.10. LysM protein predicted structure. ……...71
Figure 3.11. Multiple sequence alignment of *E. festucae* LymB protein and homologues from other *Epichloë* species. ……...73
Figure 3.12. Chitinase protein predicted structure. ……75
Figure 3.13. Multiple sequence alignment of the *E. festucae* Fl1 ChiB protein and homologues from other *Epichloë* species. ……79

Figure 4.1. Strategy for deletion of *E. festucae lymA* and confirmation by Southern analysis. ……82
Figure 4.2. Culture phenotype of wild-type and Δ*lymA* mutant strains……84

Figure 4.3. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*lymA* mutant strains. ……86
Figure 4.4. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*lymA* mutant strains. ……87
Figure 4.5. Strategy for deletion of *E. festucae lymB* and confirmation by Southern analysis. ……89
Figure 4.6. Culture phenotype of wild-type and Δ*lymB* mutant strains. ……...91
Figure 4.7. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*lymB* mutant strains.……93
Figure 4.8. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*lymB* mutant strains……94
Figure 4.9. Strategy for deletion of *E. festucae lymA/B* and confirmation by Southern analysis……97
Figure 4.10. Culture phenotype of wild-type and Δ*lymA/B* mutant strains…….98
Figure 4.11. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*lymA/B* mutant strains…….100
Figure 4.12. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*lymA/B* mutant strains……101
Figure 4.13. Strategy for deletion of *E. festucae chiA* and confirmation by Southern analysis……..103
Figure 4.14. Culture phenotype of wild-type and Δ*chiA* mutant strains……105
Figure 4.15. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*chiA* mutant strains…….107
Figure 4.16. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*chiA* mutant strains……108
Figure 4.17. Strategy for deletion of *E. festucae chiB* and confirmation by Southern analysis…….110
Figure 4.18. Culture phenotype of wild-type and Δ*chiB* mutant strains……112
Figure 4.19. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*chiB* mutant strains…….114
Figure 4.20. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*chiB* mutant strains……115
Figure 4.21. Localization of LymA::eGFP in *E. festucae* Δ*lymA*……..117
Figure 4.22. Localization of LymB::eGFP in *E. festucae* Δ*lymB*……...119

Figure 6.1. *E. festucae* chitin deacetylase predicted polypeptide structure……134
Figure 6.2. Multiple sequence alignment of the *E. festucae* chitin deacetylase proteins (CdaA, CdaB and CdaC) with homologues from *M. oryzae* (MGG12939, MGG09159, MGG14966, MGG05023, MGG08774, MGG03461, MGG01868, MGG08356, MGG04704, MGG04172), *N. crassa* (NCU10651, NCU09508) and *F. graminearum* (FGSC05847) ……139

Figure 7.1. *E. festucae* Fl1 endophytic and epiphyllous hyphae at site of inoculation of *L. perenne* seedlings……...142
Figure 7.2. Exit of *E. festucae* Fl1 endophytic hyphae from the *L. perenne* leaf by formation of an expressorium……142
Figure 7.3. *E. festucae* Fl1 grown in axenic culture labelled with WGA-AF488…….144
Figure 4.7. *E. festucae* Fl1 grown in axenic culture labelled with chitin binding protein (CBP)…….146
Figure 7.5. *E. festucae* Fl1 endophytic hyphae in *L. perenne* cell division zone at the base of leaf primordia…….147
Figure 7.6. *E. festucae* Fl1 endophytic hyphae in the *L. perenne* leaf sheath tissue labelled with CAP and OGA-488……147
Figure 7.7. *E. festucae* Fl1 epiphyllous hyphae in the *L. perenne* sheath tissue labelled with Chitosan Affinity Protein (CAP) and OGA-488……148
Figure 7.8. *E. festucae* Fl1 endophytic hyphae in the *L. perenne* leaf sheath tissue labelled with Chitin Binding Protein (CBP)……149
Figure 7.9. *E. festucae* Fl1 epiphyllous hyphae in *L. perenne* sheath tissue labelled with Chitin Binding Protein (CBP)……149
Figure 7.10. *E. festucae* Fl1 grown in axenic culture labelled with Chitosan Affinity Protein (CAP)…….....151
Figure 7.11. *E. festucae* E2368 grown in axenic culture labelled with Chitosan Affinity Protein (CAP)…….....152
Figure 7.12. Strategy for deletion of *E. festucae cdaA* and confirmation by Southern analysis) …….....154
Figure 7.13. Culture phenotype of wild-type and Δ*cdaA* mutant strains……156
Figure 7.14. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*cdaA* mutant strains…….....158

Figure 7.15. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*cdaA* mutant strains.…….....159
Figure 7.16. Cell wall changes in endophytic hyphae of sheath plant samples infected with Δ*cdaA* mutant strains. …….....160
Figure 7.17. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*cdaA* mutant strains.…….....161
Figure 7.18. Expression analysis of *cdaB*. Expression level of *cdaB* in wild type (Fl1) and Δ*cdaA* mutant strains. …….....163

List of tables

Table 2. 1. Strains used in this study…….24
Table 2. 2. Plasmids used in this study…….26
Table 2. 3. Primers used in this study……...27

Table 3.1. Fold differences of expression of *lysM* and *chitinase* genes *in planta* compared to in culture…….54
Table 3.2. Gene expression fold changes of *lysM* and *chitinase* genes in *E. festucae* genome between wild type and symbiotic mutants Δ*noxA*, Δ*proA* and Δ*sakA*……55
Table 3.3. BLASTn analysis of genes at the *lymA-chiA* locus in *E. festucae* Fl1 against other sexual Epichloë and related species…….57
Table 3.4. BLASTn analysis of genes at the *lymA-chiA* locus in *E. festucae* Fl1 against other sexual *Epichloë* and related species…….69

Table 6.1. tBLASTn analysis of *M. oryzae* chitin deacetylase protein sequences against the *E. festucae* Fl1 genome…….132
Table 6.2. tBLASTn analysis of *N. crassa* and *F. graminearum* chitin deacetylase protein sequences against the *E. festucae* Fl1 genome……133
Table 6.3. Fold differences of expression of *E. festucae chitin deacetylase* genes *in planta* compared to in culture……133
Table 6.4. Gene expression fold changes of *chitin deacetylase* genes in *E. festucae* genome between wild type and symbiotic mutants Δ*noxA*, Δ*proA* and Δ*sakA*…….133

Appendices

Appendix 1. Plasmid map of pRS426……183
Appendix 1. Plasmid map of pSF15.15……184
Appendix 3. Plasmid map of pSF17.1. ……185
Appendix 4. Plasmid map of pDB48. ……...186
Appendix 5. Plasmid map of pBH18. ……...187
Appendix 6. Plasmid map of pBH28. ……...188
Appendix 7. Plasmid map of pNN01. ……...189
Appendix 8. Plasmid map of pNN02. ……...190
Appendix 9. Plasmid map of pNN03. ……191
Appendix 10. Plasmid map of pNN04. ……192
Appendix 2. Plasmid map of pNN05. ……193
Appendix 3. Plasmid map of pNN11……...194
Appendix 4. Plasmid map of pNN12. ……195
Appendix 5. Plasmid map of pNN13. ……196
Appendix 6. Plasmid map of pNN14. ……197
Appendix 16. Multiple sequence alignment of the *E. festucae lymA* gene with homologues from other Epichloë species and *Claviceps purpurea*……198
Appendix 17. 7Multiple sequence alignment of the *E. festucae chiA* gene with homologues from other *Epichloë* species and *Claviceps purpurea*……212
Appendix 18. Multiple sequence alignment of the *E. festucae lymB* gene with homologues from other *Epichloë* species……...228
Appendix 19. Multiple sequence alignment of the *E. festucae chiB* gene with homologues from other *Epichloë* species……...237
Appendix 20. Standard curve for *cdaB* and *cdac* ……249
Appendix 8. Genes encoding chitinase (GH18) in *E. festucae* Fl1 genome……250
Appendix 9. Differences in expression of genes encoding chitinase proteins (GH18) in *E. festucae* Fl1 genome between wild type and symbiotic mutants……251
Appendix 10. Differences in expression of genes encoding chitinase proteins (GH18) in *E. festucae* Fl1 genome, *in planta* versus in culture……252

Abbreviations

aa Amino acid

Amp Ampicillin

AmpR Ampicillin resistant

ap appressorium

Avr Avirulence

BLAST Basic local alignment search tool

BLASTn Nucleotide database search using a nucleotide query

BLASTp Protein database search using a protein query

bp Base pair(s)

cda chitin deacetylase

cDNA Complementary DNA

chi chitinase

cf conidiophore

CFW Calcofluor white

CLSM Confocal Laser Scanning Microscopy

CWI Cell wall integrity

cu cuticle

DIC Differential interference contrast

DIG Digoxigenin

dir direction

DNA Deoxyribonucleic acid

dNTP Deoxynucleotide triphosphate

EDTA Ethylene diamine tetra-acetic acid

eGFP Enhanced green fluorescent protein

en endophytic hyphae

ep epiphyllous hyphae

ETI effector-triggered immunity

ETS effector-triggered susceptibility

g Gram

gDNA Genomic DNA

Gen Geneticin

GenR Geneticin resistant

GH18 glycoside hydrolase family 18

GFP Green fluorescent protein

GPI Glycosylphosphatidylinositol

gt germ tube

h Hour(s)

Hph Hygromycin

HR hypersensitive response

HygR Hygromycin resistant

IDC Impaired development of crippled growth

ih infection hyphae

kb Kilobase(s)

KO Knock-out

L Litre

LB Lysogeny broth

LysM lysin motif

M Molar

MAPK(K/K) Mitogen activated protein kinase (kinase/kinase)

MAMP Microbe associated molecular pattern

Mb millions of base pairs

mg Milligram

µg Microgram

min Minute(s)

µL Microlitre

mL Millilitre

µm Micrometre

µM Micromolar

mm Millimeter

mM Millimolar

Mo *Magnaporthe oryzae*

MOB monopolar spindle-one-binder

mRNA Messenger ribonucleic acid

Nc *Neurospora crassa*

NCBI National Centre for Biotechnology Information

*ntp*II Geneticin

Nox NADPH oxidase

PAMP Pathogen associated molecular pattern

PCR Polymerase chain reaction

PD Potato dextrose

PEG Polyethylene glycol

pro protoperithecia mutant

PTI PAMP-triggered immunity

PRR Pattern recognition receptors

RG Regeneration

RNA Ribonucleic acid

RNase Ribonuclease

RNA-seq Ribonucleic acid sequencing

rpm Revolutions per minute

RT Reverse transcriptase

RT-PCR Reverse transcriptase-polymerase chain reaction

SAK Stress-activated kinase

SAM Shoot apical meristem

SDS Sodium dodecyl sulfate

Sp spore

STRIPAK Striatin interacting phosphatase and kinase

sv substomatal vesicle

sym Symbiosis mutant

TBE Tris-boric acid-EDTA

tBLASTn Translated nucleotide database search using a protein query

T-DNA Transfer-deoxyribonucleic acid

TEM Transmission electron microscopy

TMD Transmembrane domain

Um *Ustilago maydis*

UV Ultraviolet

V Volts

v/v Volume/volume ratio

WT Wild-type

w/v Weight/volume ratio

YE Yeast extract

°C Degrees Celsius

Chapter 1. Introduction

1.1. Epichloë species as ryegrass endophytes

The term endophyte refers to the location of the organism, in the plant with "Endo" meaning within and "Phyte" the plant. So, at the basic level an endophyte is an organism, which lives inside a plant. In contrast, the term "epiphyte" refers to an organism, which lives outside the plant, usually on the surface of the leaves (Wilson, 1995). Epichloë species including *Epichloë festucae* and its asexual derivative *Epichloë festucae* var *lolii* (formerly *Neotyphodium lolii* (Leuchtmann et al., 2014)) belong to the family Clavicipitaceae (Ascomycota), and form symbiotic associations with grasses of the Festuca and Lolium genera. These species grow within the host apoplastic (intercellular) space of the plant host (Schardl *et al.*, 1994). The Epichloë-grass symbiosis is highly beneficial to both the endophyte and its host since *E. festucae* can be transmitted vertically through the seed and has access to nutrients from the host apoplast (Schardl, 1996) whereas the host obtains protection to biotic stress, such as insect and mammalian herbivory as a result of the fungus producing bio-protective molecules, and increased tolerance to abiotic stress such as drought. The presence of Epichloë increases host fitness and survival (Clay & Schardl, 2002).

1.2. The endophyte life cycle

During the vegetative state of the host, hyphae proliferate within the aerial tissues without any visible pathogenic symptoms, and when the host undergoes the reproductive stage the endophyte can be transmitted either vertically through seeds as part of its asexual life cycle or horizontally via proliferative external growth on the surface of the leaf to form a stroma where conidiospores are formed to initiate the sexual life cycle (Clay & Schardl, 2002). Stromata form on the flag leaf of the grass, which surrounds the developing inflorescence, thereby preventing emergence of the inflorescence to cause "choke" disease (Schardl *et al.*, 1997). The conidiospores provide spermatia of one mating type and are transferred to a stroma of the opposite mating type by female anthomyiid flies (Bultman *et al.*, 1998) (Figure 1.1).

In contrast, *Epichloë festucae* var *lolii* has lost the ability to enter the sexual cycle so the only mechanism for dissemination is vertical transmission through the seed (Chung *et al.*, 1997), following endophyte infection of the developing embryo. During reproductive

development of the plant, hyphae grow from the vegetative apex into the inflorescence primordium and floral apices, to infect the ovary and ovules. Immediately after fertilization, hyphae gain entry to the embryo sac. During early embryogenesis, hyphae can be found on the surface of the embryo. As the embryo matures, hyphae become widespread throughout the embryo and surrounding tissues including the plumule apex, embryo axis, the aleurone layer, and between the scutellum and the endosperm. During seed germination, hyphae colonize the developing shoot apex. Further colonization of leaf primordia, sheaths, and blades of leaves results in systemic infection of aerial tissues (May *et al.*, 2008; Philipson & Christey, 1986).

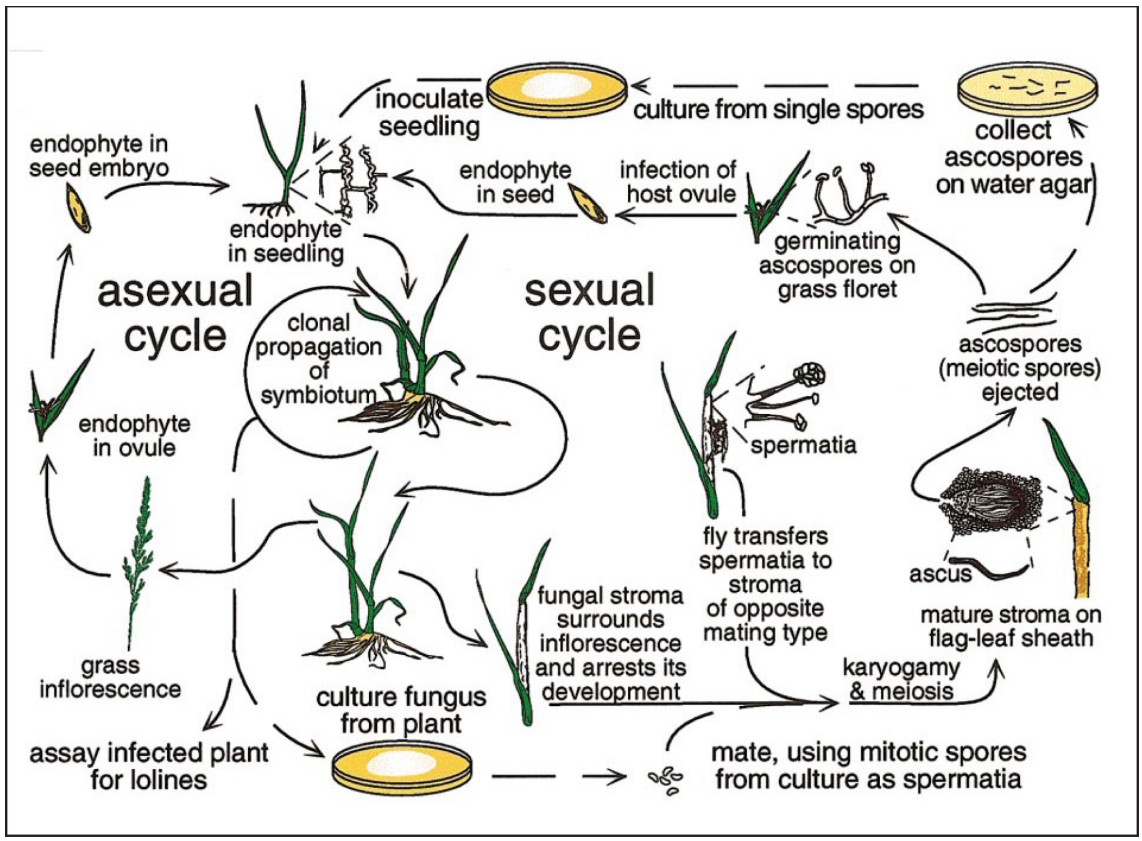


Figure 1.1. *E. festucae* sexual and asexual life cycle. Image adapted from (Schardl, 2001).

1.3. *Epichloë festucae-Lolium perenne* as an experimental model system

Perennial ryegrass (*Lolium perenne*) is a cool season grass, which is agriculturally important in New Zealand and many other parts of the world and is naturally found in association with the asexual endophyte *E. festucae* var *lolii*. This endophyte species is not suitable for genetic analysis due to a slow rate of growth in culture. In contrast, *E. festucae* (sexual species) grows faster and forms stable mutualistic symbiotic associations with

aerial tissues of cool seasons grasses such as Festuca and Lolium genera in nature and also under standard laboratory conditions (Christensen & Latch, 1991). Moreover, *E. festucae* is the most well studied species in terms of molecular genetics (Scott *et al.*, 2012). *E. festucae* is a haploid species with limited host range compared to *E. typhina* and the host association can be easily established in the laboratory, which makes it an ideal grass-endophyte model system to study this symbiosis ((Scott *et al.*, 2012; Schardl, 2010). Introduction of various constructs into the *E. festucae* genome to generate reporter tagged strains or targeted gene deletions is easily manageable in this species because it is quite amenable to both homologous and non-homologous recombination (Tanaka *et al.*, 2005; Young *et al.*, 2005). The genome sequence of *E. festucae* strains E2368 and E984 (Fl1) were sequenced using a combination of pyrosequencing and Sanger methods**,** yielding total genome assemblies of 34.7 Mb in E2368 and 34.9 Mb in Fl1 (Schardl *et al.*, 2013). The availability of the genome sequence of the *E. festucae* strains, their gene models and annotations at www.endophyte.uky.edu is another advantage of using these fungal strains as an experimental system. More recently, the genome sequence of strain Fl1 has been fully assembled to chromosome level using large contigs generated from PacBio sequencing combined with Hi-C analysis (Winter *et al.*, 2018).

1.4. Epichloë species grow within grass leaves by a novel mechanism

1.4.1. Endophytic growth

For establishment of the symbiotic interaction between *E. festucae* and its host, the endophyte needs to colonise the entire aerial tissues of the plant. While some pathogenic fungi have infection and feeding structures *E. festucae* relies entirely on the uptake of nutrients from either the apoplastic space or by way of specialised transport processes between the hypha and the attached plant cells (Christensen *et al.*, 2002; Hinton & Bacon, 1985). Host colonisation occurs by two very distinct fungal growth patterns. *E. festucae* initially forms a proliferative hyphal network among the cells in the zone below the SAM (Shoot apical meristem) (Christensen *et al.*, 2008). The hyphae colonise leaf primordia and axillary buds where they proliferate by tip growth in the cell division zones of the nascent leaf sheath and leaf blade. Following colonisation of these tissues, the hyphae become attached to the plant cell wall, where they switch from tip to intercalary growth in the leaf cell expansion zone of leaf sheath and blade. Once in this zone the number of hyphae does not increase, resulting in a restrictive mode of growth (Christensen *et al.*,

2008; Christensen *et al.*, 2002; Tan *et al.*, 2001; Scott *et al.*, 2012; Christensen & Voissey, 2007). Hyphae attach to the plant cell wall through an adhesive surface matrix of unknown origin. As the plant cells elongate in the leaf expansion zone, the attached hyphae are stretched, thereby triggering a switch from tip- to intercalary-growth resulting in strands of hyphae growing parallel to the leaf axis (Christensen *et al.*, 2008; Voissey, 2010). An assay was recently developed by Ariyawansa & Voisey to mechanically stretch hyphae *in planta* to enable a molecular analysis of the process of intercalary growth (Ariyawansa, 2015). These authors showed that application of a mechanical stretch resulted in the development of new cellular compartments that had a higher rate of nuclear division and septation compared to unstretched hyphae. This pattern of growth within the plant enables endophytic hyphae to grow at the same rate as plant leaf cells thereby preventing shearing of the attached hyphae. In the leaf expansion zone intercalary growth of attached hyphae are oriented in the direction of plant growth and thus follow the longitudinal axis of the leaf (Christensen *et al.*, 2008) (Figure 1.2 and 1.3).

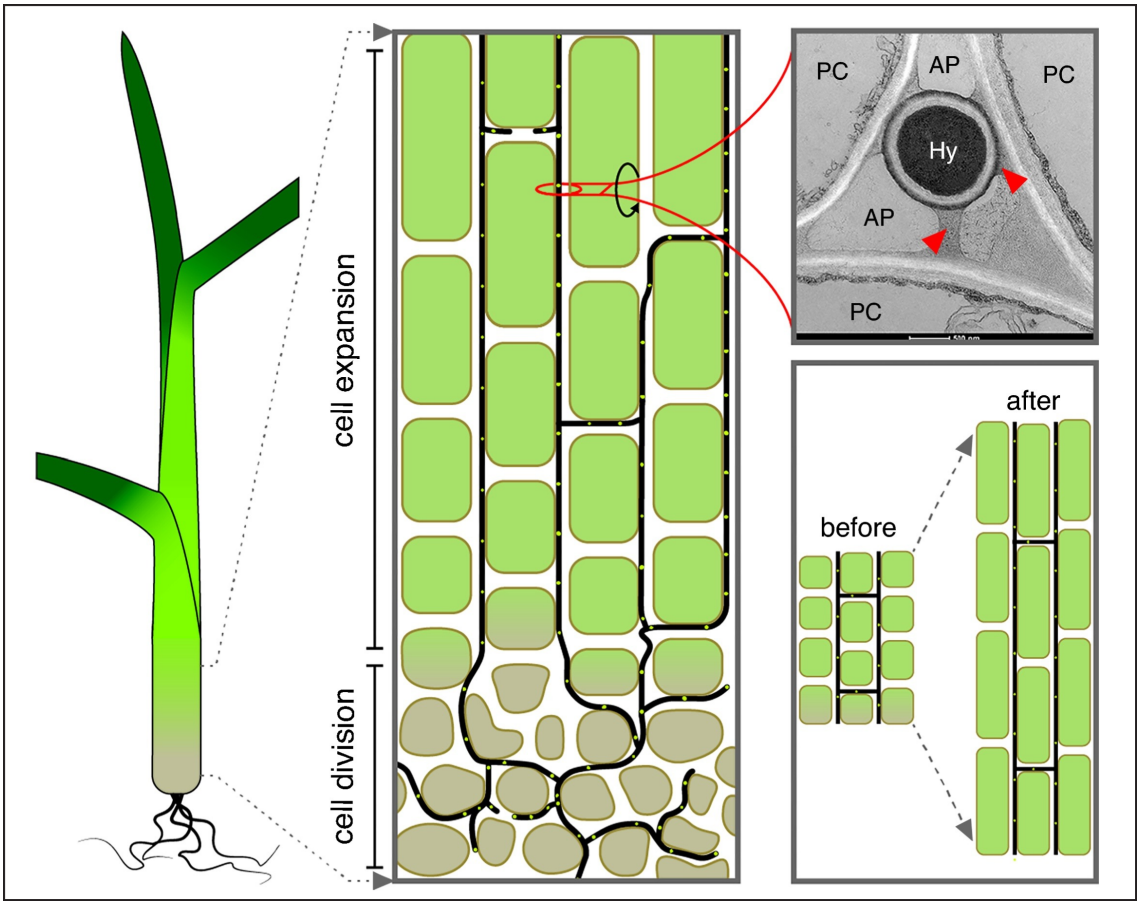


Figure 1.2. *E. festucae* intercalary growth. Schematic of *E. festucae* intercalary growth in cell division and cell expansion zone of pseudostem and blade tissues of *L. perenne*. Abbreviations:

PC, plant cell; AP, apoplast; Hy, hypha. Red arrowheads identify the extracellular matrix. Bar=500 nm. Image adopted from (Scott *et al.*, 2018).

1.4.2. Epiphytic growth

In addition to endophytic growth, *E. festucae* has been observed to grow epiphytically on leaves of *L. perenne* plants. It has been observed that the endophytic hyphae emerge from the plant expansion zone to form an epiphyllous hyphal net on the surface of the host grass leaves (Christensen *et al.*, 1997; Christensen *et al.*, 2002; Moy *et al.*, 2000). The endophytic and epiphytic hyphal network remains connected to one another, potentially allowing for transfer of nutrients or signal molecules between the two different growth forms. One hypothesis put forward as a potential benefit for the host from the epiphytic growth, is increased protection from fungal pathogens through 'niche exclusion' (Moy *et al.*, 2000). Recent work has indicated that endophytic hyphae emerge from the host plant by way of formation of an appressorium-like structure called an expressorium. Staining of these hyphae with WGA-AF488, a chitin specific stain, showed that after emergence of hyphae from the leaf there is extensive cell wall remodelling resulting in the presence of chitin throughout the cell wall within 2-3 cell divisions after breaking through the cuticle (Becker *et al.*, 2016). Epiphyllous hyphae were observed to be mostly attached to the leaf surface, growing along leaf surface depressions, but frequent bridging of furrows and loss of surface contact was also observed. Coil-like structures, identical to those observed on agar, frequent hyphal fusions, and the formation of conidiophores and conidia were also observed. Whether the conidia are able to germinate and penetrate the leaf surface has not been observed (Becker *et al.*, 2016; Scott B, 2012) (Figure 1.3).

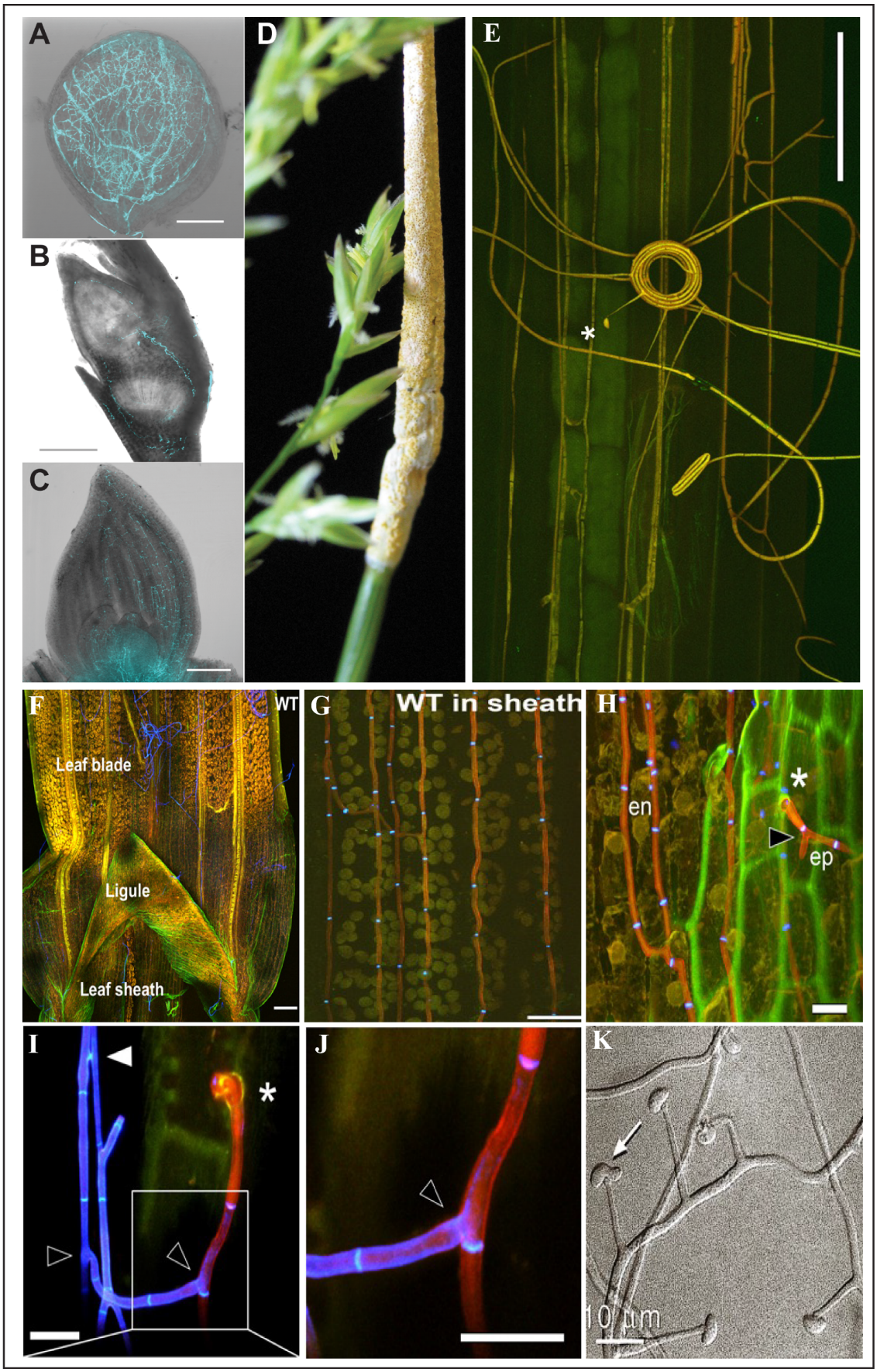


Figure 1.3. Symbiosis of *E. festucae* within the host plant. (A) Ovules. Bar=100 µm. (B) Embryos. Bar=200 µm. (C) Shoot apical meristem and surrounding new leaves. Bar=200 µm. (D)

Asymptomatic (on left) and stromata formation (on right) and "choked" inflorescence. Image adapted from (Schardl *et al.*, 2013). (E) *E. festucae* epiphyllous hyphae grow on *L. perenne* surface forming hyphal coil with conidia and conidium (white asterisk). Bar=25 μm. (F) *E. festucae* epiphyllous hyphal net on *L. perenne* leaf in blue pseudocolour. Bar=100 μm (G) Endophytic hyphae grow intercellular space of *L. perenne* in red pseudocolour. Chitin is visible in septa in blue pseudocolour. Bar=25 μm (H) Endophytic hyphae (en) and exiting point from plant tissue by the means of expressorium indicted with white asterisk. Epiphyllous hyphae (ep) branching soon after emergence on the surface of the leaf (black arrowhead). Bar=10 μm. (I) Hyphae emerging plant epidermal cells by forming the expressorium indicated with whit asterisk. Hyphal branching and fusion are indicated with black and white arrow heads respectively. Bar=10 μm. (J) Higher magnification of (I) showing the alteration of hyphal cell wall composition from red pseudocolour labelled with aniline blue to blue pseudocolour labelled with WGA-AF488. Bar=10 μm. Images adapted from (Becker *et al.*, 2016). (K) *E. festucae* hyphae grow in culture form a row of phialides with give rise to conidia. Germinated conidium is indicated with white arrow. Bar=10 μm. Image adapted from (Schardl, 2001).

1.5. Fungal cell wall structure

The cell wall is the outermost layer of the fungal cell and is the surface that is exposed to the external environment and is essential to maintain cellular structure and integrity of fungi. It is a dynamic structure which is essential for cell viability, morphogenesis and pathogenesis. The major components of fungal cell walls are polysaccharides: β-glucans (β-1,3-glucan and β-1,6-glucan), α-glucans (α-1,3-glucan and α-1,4-glucan), chitin and mannan (Latge, 2007; Perez & Ribas, 2004). Most fungal species have branched β-1,3-glucan, which has 3 to 4% interchain connections, and chitin, which is covalently attached through hydrogen bonds which comprises the inner cell wall (Latge, 2007; Fleet, 1991). These cell wall polysaccharides can also be assembled into fibrous microfibrils that form a basket-like scaffold around the cell. The β-1,3-glucan and chitin exoskeleton can protect the cell wall from the internal hydrostatic pressure exerted by the cytoplasm and membrane. β-1,3: β-1,6-glucan can be bound to other polysaccharides and proteins. However, these components vary considerably among fungal species. For example, the hyphae in *Candida albicans* contain shorter and less dense mannan fibrils than yeast cells or in dimorphic fungi such as *Histoplasma capsulatum* where the cell wall is covered with α-1,3-glucan during infection. By contrast *Cryptococcus neoformans* has a thick outer capsule largely made of glucuronoxylomannan and galactoxylomannan attached to α-

glucan. In *Aspergillus fumigatus* the cell wall structure is different in conidia and hyphae with a layer of α-glucan along with melanin and hydrophobic rodlets in the outer layer of conidia, while in hyphae it contains α-glucan, galactomannan and galactosaminoglycan (Erwig & Gow, 2016; Fujikawa *et al.*, 2009; Gow *et al.*, 2017; Hopke *et al.*, 2018) (Figure 1.4).

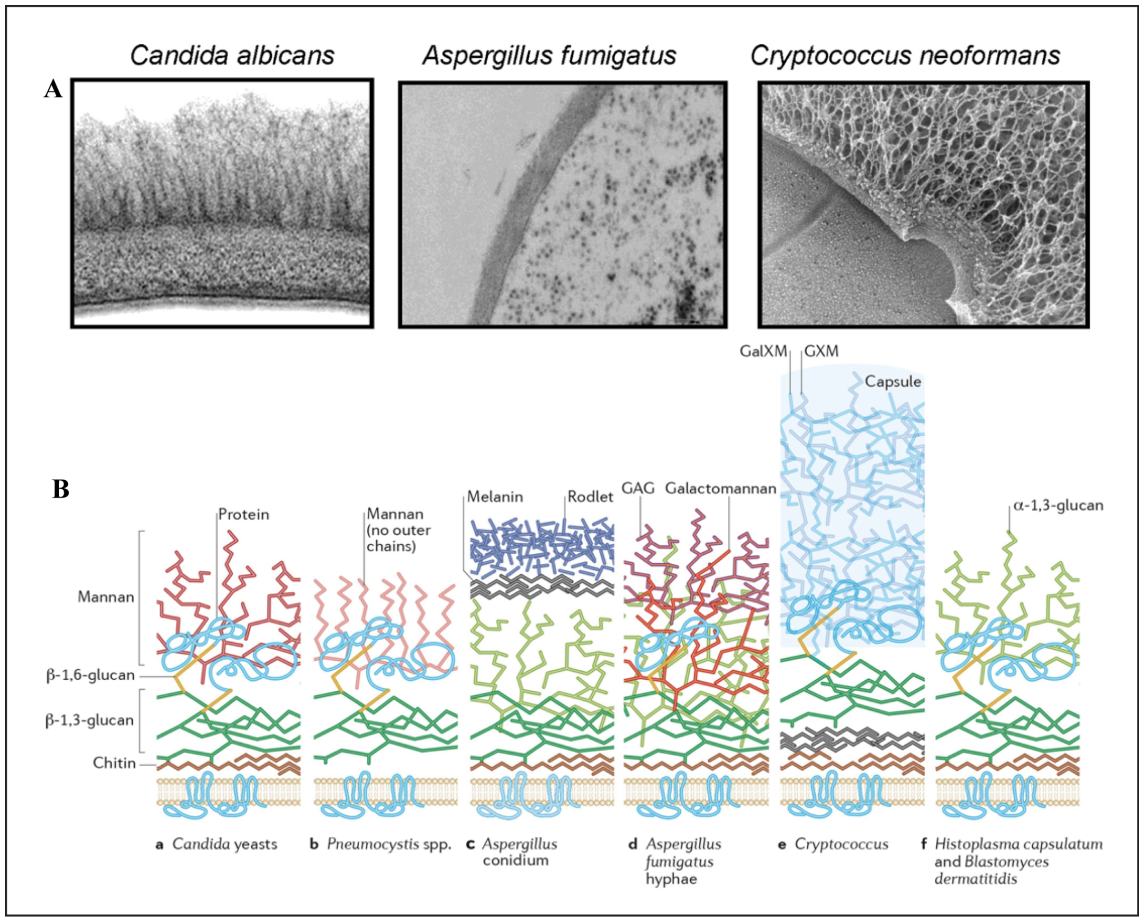


Figure 1.4. Cell wall structure of fungal pathogens. (A) Transmission electron microscopy images of cell walls showing mannoprotein fibrils in the outer walls of *C. albicans*, the fibril-free cell wall of an *A. fumigatus* hyphae, and the elaborate capsule of *C. neoformans*. (B) The schematic of major components of the fungal cell wall and their interconnections. Abbreviations: GAG, galactosaminoglycan; GalXM, galactoxylomannan; GXM, glucuronoxylomannan. Images adapted from (Gow *et al.*, 2017).

1.5.1. The chitin component of the fungal cell wall

Chitin is a linear homopolymer of β-1,4-linked N-acetyl-D-glucosamine (GlcNAc) monomers. Chitin is a structurally important component of the cell wall even though the amount present varies considerably from species to species. While only 1-2% of yeast cell wall the dry weight contains chitin, filamentous fungi such as *Aspergillus nidulans*

and *Neurospora crassa* contain 10-20% chitin. In filamentous fungi, chitin microfibrils are formed from inter-chain hydrogen bonding and produce a very flexible crystalline polymer that controls the overall integrity of the cell wall. Consequently, when chitin synthesis is disrupted, the wall becomes disordered and the fungal cell becomes and osmotically unstable (Bowman & Free, 2006; Latge, 2007; Latge, 2010). Chitin polysaccharide is synthesised by transmembrane enzymatic complexes at the plasma membrane which are targeted to the plasma membrane in an inactive form via secretory vesicles and then activated after insertion into the plasma membrane (Gow *et al.*, 2017). Chitin synthesis occurs at sites of active growth and for filamentous fungi this is at the hyphal apex or growing tip where the substrate, UDP-*N*-acetylglucosamine is abundant. Chitin synthase enzymes can be classified into two distinct families with each of these being further subdivided into three (I, II, III) and four (IV, V, VI, VII) classes depending on the primary amino acid sequence. Chitin synthases of classes III, V, VI and VII are filamentous fungal specific. Mutations in genes encoding chitin synthase genes results in two types of phenotypes, the first with reduced chitin content but normal chitin synthase activity *in vitro* and the second with enzyme activity disrupted but with regular cell wall chitin content. The number of *CHS* genes can vary from one to 20 among different fungal species. *A. fumigatus* and *C. neoformans* both contain eight *CHS* genes and all are predicted to be nonessential, although mutants of Δ*chs3* in *C. neoformans* are not able to grow at 37°C. In contrast *C. albicans* has four *CHS* genes with the class II *CHS1* essential for cell viability. *Aspergillus* mutants with single chitin synthase gene deletions are mostly viable, but some of the double mutants are lethal. Class V and VII of the chitin synthase enzymes contain myosin-motor like domains (MMD), which are often essential for growth, morphogenesis, virulence and stress tolerance in filamentous fungi. It is thought that the MMD is involved in actin-mediated cytoplasmic transport, as they bind to actin and influence apical localization and facilitate vesicle fusion with the plasma membrane. In *N. crassa* four specific chitin synthase enzymes have been identified encoded by *chs-1*, *chs-2*, *chs-3* and *chs-4* genes. Mutant strains of these genes showed different phenotypes. While disruption of *chs-2* resulted in strains with the same level of chitin and morphology as the wild type, deletion of *chs-1* resulted in reduction of chitin levels, slow growth and formation of swollen and bulbous hyphae. The *chs-4* gene is predicted to be functional in cell wall biosynthesis under environmental stress conditions and since the *chs3* gene of *N. crassa* is essential for survival it is suggested that the CHS3

enzyme may catalyse the majority of chitin synthesis under normal growth conditions (Gow *et al.*, 2017).

1.5.2. The glucan component of the fungal cell wall

The other major fungal cell wall polysaccharide is β-1,3-glucan which is synthesized by a plasma membrane-bound glucan synthase complex and uses UDP-glucose as its substrate. The linear β-1,3-glucan chain is extruded through the membrane into the cell wall and can act as a transglycosidase enzyme. Sixty five to ninety five percent of the fungal cell wall glucan is β-1,3-glucan and the other form of the glucan such as β-1,6 mixed β-1,3 and β-1,4, α-1,3 and α-1,4 linked glucans have been found in other fungal species cell walls (Bernard & Latge, 2001; Bowman & Free, 2006; Grun *et al.*, 2005). β-1,3-glucan synthases are essential for proper cell wall formation and normal development of fungi as this is the main structural constituent to which other cell wall components are covalently attached. While yeast cell walls contain β-1,3 and β-1,6-glucans, filamentous fungi such as *N. crassa* and *A. fumigatus* do not contain the latter (Borkovich *et al.*, 2004; Bowman & Free, 2006; Fontaine *et al.*, 2000). The branched glucans are cross-linked with each other, and to chitin and mannoproteins, to provide the cell wall with mechanical strength and integrity (Bowman & Free, 2006; Kollar *et al.*, 1997). Considering that β-glucan is important for fungal cell wall architecture, it is not surprising that disruption in its synthesis affects the fungal morphology and pathogenicity. Two functionally redundant genes encoding catalytic subunits of the glucan synthase complex, *FKS1* and *FKS2*, were identified for the first time in yeast. Single deletion of *FKS1* or *FKS2* resulted in a slow growth rate and cell wall defects whereas deletion of both genes was lethal. RNAi-mediated knockdown mutants of a β-1,3-glucan synthase in *C. graminicola* resulted in reduction of β-1,3-glucan levels in the cell wall and defects in appressorium differentiation. Fungal appressoria in these mutants were less adhesive to various surfaces, showed more elasticity, and were unable to withstand increases in appressorial turgor pressure. Deletion of a glucan synthase gene in *F. solani* similarly resulted in spore and hyphal lysis (Bowman & Free, 2006).

1.5.3. The glycoprotein component of the fungal cell wall

The fungal cell wall is comprised of a complex and interlinked network of polysaccharides and protein. Fungal cell walls of *S. cerevisiae* and *A. candidans* have between 30-50% of protein by dry weight. *N. crassa* has approximately 15% protein in

its cell wall. Most of the fungal cell wall proteins are glycoproteins, which go through the secretory pathway before transiting to the cell wall. Proteomics analyses conducted in various fungal species identified small cytosolic and mitochondrial proteins. In addition to these small proteins heat shock and glycolytic enzymes have been detected as being cross-linked and associated with the cell wall. The actual function of these non-traditional proteins is still unclear. Glycoproteins are traditional fungal cell wall proteins, which are exclusively modified with *N*-linked and *O*-linked oligosaccharides. The structures of the oligosaccharides, which are attached to these glycoproteins, are different amongst fungal species. The glycoproteins of *N. crassa* and *A. fumigatus* contain galactomannan structures, composed of both mannose and galactose residues. The main function of cell wall proteins is to maintain cell shape, mediate adhesion for cell migration and fusion, protect the cell against foreign substances, mediate the absorption of molecules, transmit intracellular signals from external stimuli, and synthesise and remodel cell wall components. In addition, the Glycosylphosphatidylinositol (GPI) anchor, a lipid and oligosaccharide-containing structure, is also added to some cell wall proteins that contain a C-terminal signal sequence. The GPI anchor directs and localises these proteins to the plasma membrane and cell wall. Approximately 20 proteins are involved in the GPI anchor production and attachment process, some defined as enzymes and other as auxiliary factors. Seven primary steps of the GPI anchor pathway have been identified, starting with anchor biosynthesis and finishing with the final attachment of the completed anchor structure to the recipient protein (Eisenhaber *et al.*, 2003; Kinoshita & Inoue, 2000). GPI anchor pathways have also been analysed in *S. cerevisiae* and *N. crassa*. In *S. cerevisiae* all but one of the genes are essential for fungal viability. The gene *GPI7* is the only gene involved in the GPI anchor pathway that is not required for fungal survival. Deletion of *GPI7* resulted in morphological defects such as alteration in cell wall remodelling and integrity. In *N. crassa* several genes involved in the GPI anchor pathway have also been identified. While deletion of some genes in this species are lethal, others were less severe resulting in different morphological phenotypes such as restricted hyphal growth and defects in the cell wall. As observed for GPI anchor proteins in *S. cerevisiae*, these proteins are also required for cell wall integrity in *N. crassa* (Bowman & Free, 2006; Bowman *et al.*, 2006).

1.6. Plant immunity

Microbial plant pathogens such as fungi have a very specific life strategy. They almost always occupy extracellular niches. However, the nutrients they need to grow are derived from host cells. Fungi can directly enter plant epidermal cells, or extend hyphae on top of, between, or through plant cells. Pathogenic and symbiotic fungi and oomycetes have a range of different structures for colonising and feeding on the host including appressoria, hyphopodia and haustoria. Plants do not have a mobile defence system such as the adaptive immune system found in mammals but do have an innate immune system that enables them to recognise specific molecules released by the pathogen and which trigger a defence response. Plants rely on cell autonomous events and innate immune system for defence. This system allows recognition of various pathogen-associated molecular patterns or microbe associated molecular patterns (PAMPs or MAMPs) such as proteins, carbohydrates, lipids and small molecules, including ATP (Dodds & Rathjen, 2010; Jones & Dangl, 2006).

1.7. Chitin recognition and the host defence response

Different fungal species deploy different strategies to colonise their host. Host recognition of fungi triggers a host immune response. Plants have evolved to recognise PAMPs by cell surface localised pattern recognition receptors (PRRs) in order to mount an immune reaction. Chitin as a component of the fungal cell wall acts as a PAMP following its hydrolysis and release of chitin oligomers by plant chitinases. PAMP recognition through PRRs, triggers the first line of defence reaction called PAMP-triggered immunity (PTI). Activation of PRRs results in a signalling response characterised by reactive oxygen accumulation, ion channel activation, defence related mitogen-activated protein kinase cascade activation, host transcriptional reprogramming and accumulation of antimicrobial compounds such as proteinases, chitinases and glucanases which can damage pathogen structure. Plant PRRs have been identified in *Arabidopsis thaliana* and *Oryza sativa* (rice). Chitin oligomers released from the fungal cell wall are detected and recognised by LysM-receptor like kinases (LysM-RLK) called CERK1/RLK1/LYK1, which bind chitin oligomers through three extracellular LysM domains. Rice has an additional receptor like protein (RLP) to CERK1 called chitin elicitor-binding protein (CEBiP), which is also required for chitin detection and immune

signalling. Chitin-induced homodimerization of CERK1 in both *A. thaliana* and *O. sativa* is essential for the activation of downstream signalling (Liu *et al.*, 2012; Lo Presti *et al.*, 2015; Sanchez-Vallet *et al.*, 2015). Fungi must avoid triggering the PTI to establish a compatible interaction and proliferate in their host. To overcome the PTI, the pathogen can secret effectors which can be toxic secondary metabolites, proteins that kill the host plant (in necrotrophic and hemibiotrophic pathogens), or proteins that shield the fungus or suppress the host immune response. Although secreted effectors can have a role in suppressing PTI they may be recognised by plant receptors and elicit another layer of immune response called effector triggered immunity (ETI). ETI can be activated following effector recognition by plant resistance proteins (R proteins) which are conserved intracellular receptors of the nucleotide-binding leucine-rich receptor (NB-LRR) class. PTI and ETI can cause similar responses, however, they differ in the strength of the response. ETI often triggers a localized cell death response, or hypersensitive response (HR). In the four-phased 'zigzag' model of the plant immune system put forward by (Jones & Dangl, 2006) ETI can also be suppressed by the pathogen. In this phase, due to natural selection, the pathogen can suppress ETI either by shedding or diversifying the recognized effector gene, or by acquiring additional effectors. Natural selection results in new R specificities so that ETI can be triggered again (Dodds & Rathjen, 2010) (Figure 1.5).

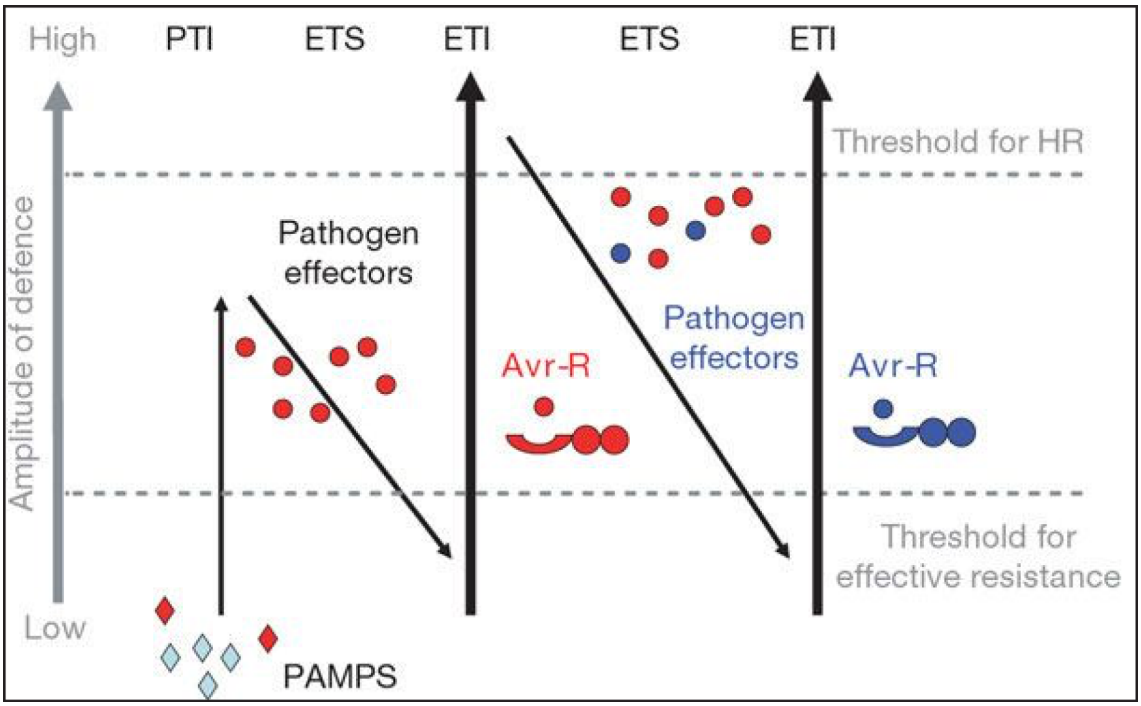


Figure 1.5. The schematic of the zigzag model showing the quantitative output of the plant immune system. In phase 1, PAMPs/MAMPs (red diamonds) are detected by PRRs to trigger PTI.

In phase 2, delivered effectors by successful pathogens interfere with PTI resulting in effector-triggered susceptibility (ETS). In phase 3, one pathogen effector (indicated in red) is recognized by an NB-LRR protein resulting in effector triggered immunity (ETI). Avr and R refer to pathogen avirulance and palnt resistance genes respectively. In phase 4, pathogen isolates which have lost the red effectors and perhaps gained new effectors (in blue) through horizontal gene flow will be selected to supress ETI. New plant NB-LRR alleles acquired through selection can help to recognize new effectors resulting again in ETI. Image adapted from Jones and Dangl, 2006.

1.8. Fungal effectors and LysM proteins

Fungal pathogens can overcome PTI induced by chitin via different mechanisms. *Cladosporium fulvum* secretes the chitin-binding effector protein, Avr4, which protects the fungal cell wall against plant chitinase activity (van Esse *et al.*, 2007). Functional homologues of Avr4 have been identified in several other fungal species, including *Mycosphaerella fijiensis* and *Dothistroma septosporum* (de Wit *et al.*, 2012). Recent studies have revealed that cell wall chitin is physically masked by other cell wall components. Rappleye *et al.* (2007), reported the presence of α-1,3-glucan, which masks a triggering of the host immune system, in the outermost layer of the cell wall of *Histoplasma capsulatum*, a pathogenic fungus of mammals. Macrophages recognize the infection by *H. capsulatum* in part through the interaction of β-glucan from the fungal cell wall and the receptor dectin-1, and thus α-1,3-glucan blocks recognition by covering the β-glucan (Rappleye *et al.*, 2007). Similarly, in *M. oryzae*, (Fujikawa *et al.*, 2009; Fujikawa *et al.*, 2012)) showed that α-1,3-glucan masks both chitin and β-1,3-glucan in the cell walls of *M. oryzae*. They also demonstrated that the presence of α-1,3-glucan results in increased tolerance to plant chitinase digestion. Thus, masking chitin by α-1,3-glucan is a major strategy by *M. oryzae* to evade attack by chitinases. Deletion of the α-1,3-glucan synthase reduced host virulence. *C. fulvum* secretes another chitin binding protein Ecp6 which sequesters chitin oligosaccharides that are released from the cell walls of invading hyphae to prevent elicitation of a host immune response (de Jonge *et al.*, 2010). Genomic and sequence analysis of 70 fungal species showed that secreted LysM containing proteins are widespread in the fungal kingdom and might have a role in sequestration of chitin oligosaccharides to dampen host defense (de Jonge & Thomma, 2009) (Figure 1.6). Genes encoding LysM motifs have been analysed in several other fungi including *Mycosphaerella graminicola, Trichoderma atriviridae, Colletotrichum*

higginsianum and *Penicillium expansum* (Bolton *et al.*, 2008; Marshall *et al.*, 2011; Gruber *et al.*, 2011; Mentlak *et al.*, 2012; Takahara *et al.*, 2016; Levin *et al.*, 2017; Seidl-Seiboth *et al.*, 2013; Kombrink & Thomma, 2013). Homologues of Ecp6 have been identified in other fungal pathogen species. For example, in the rice blast fungus *M. oryzae*, Slp1 accumulates at the plant-fungus interface at an early stage of infection, where it specifically binds chitin to supress chitin triggered immunity. Spl1 is also able to compete for chitin binding with chitin binding PRRs in rice. The Mg3LysM protein in *M. graminicola* is also involved in blocking elicitation of a chitin-induced plant defence response. Like Avr4 in *C. fulvum*, Mg3LysM provides protection of the hyphae against cell wall degradation by plant chitinases (Mentlak *et al.*, 2012; Marshall *et al.*, 2011; van Esse *et al.*, 2007).

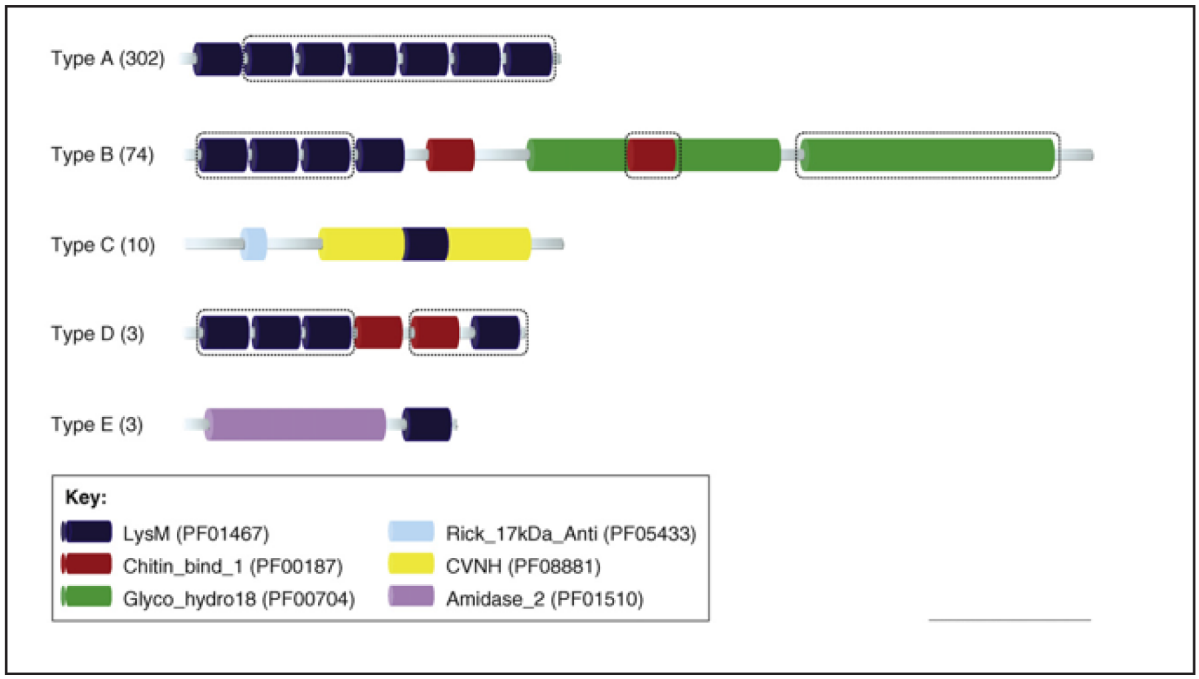


Figure 1.6. Fungal LysM proteins classification based on their domain structure. Fungal LysM proteins grouped into 5 types (A-E) according to their overall domain architecture. Numbers in brackets indicating the number of proteins that belong to each type. Image adapted from (de Jonge & Thomma, 2009).

Similar to chitin the glucan oligosaccharides released form fungal cell wall upon fungal attack can also act as PAMPs, such as the ß-1,6-1,3-heptaglucan derived from plant pathogen *Phytophthora sojae* (Aronson et al, 1967; Cheong et al, 1991). So, the fungi need to protect ß-glucan polymers from plant recognition and triggering the PTI. It has

been recently shown that the fungal lectin FGB1 from the root endophyte *Phytophthora indica* is secreted during the fungal colonization and specifically interact with ß-1,6 linked glucan and potentially alter cell wall composition to increase the resistance to the glucan- associated cell wall stressor Congo Red. Moreover, it has been shown that the FGB1 can have a similar function as chitin binding LysM effectors and suppress laminarin-triggered ROS production in different hosts and avoid ß-glucan trigger immunity (Wawra *et al.*, 2016).

In biotrophic fungi *Ustilago maydis*, the pathogen secrets effector proteins to colonise and cause disease in maize. A repetitive protein effector Rsp3 (repetitive secreted protein 3) has been functionally characterised and qRT-PCR analyses showed that *rsp3* is highly expressed during biotrophic phase. It has been shown that Rsp3 is responsible for suppressing the host defence response by shielding hyphae from antifungal maize proteins AFP1 and AFP2 (Ma *et al.*, 2018).

In tomato fungal pathogen *Fusarium oxysporum*, the pathogen deploys a specific strategy to overcome the deleterious effect of CBD (chitin binding domain) of plant chitinases using for degrading chitin component of the fungal cell wall. Jashni *et al.*, (2015) showed that both secreted metalloprotease FoMep1 and serine protease FoSep1 in *F. oxysporum* are responsible for cleavage in CBD of class I and IV tomato chitinase. Double deletion of these two CBD cleavage protease (Δ*FoMep1*/*Fosep1*) showed reduced virulence in tomato suggesting the importance of CBD chitinase in plant basal defence.

1.9.Chitosan

While chitin is important for pathogenic fungal growth and protection, it is also the intruder's 'Achilles heel' as even small amounts of released chitin oligomers can trigger a PTI defence response. Fungal pathogens have evolved different strategies to avoid a chitin-triggered immune response. Besides secreting effectors to bind chitin to protect them from chitinase hydrolysis or sequestering chitin oligomers to prevent them binding to plant receptors, fungal pathogens have another strategy; that of modifying the chitin into chitosan by deacetylation of the chitin in hyphal cell walls at the infection site. In contrast to chitin, chitosan is a poor activator of the plant immune response, and a poor substrate for host plant chitinases (Geoghegan *et al.*, 2017). Chitin deacetylase activity has been identified in some fungal species such as broad bean and wheat rust fungi *Uromyces faba*, *Puccinia graminis* and maize anthracnose fungus *Colletotricum*

graminicola. In these species only substromatal vesicles, infection hyphae and infection vesicles were labelled by a chitosan biosensor, showing that chitin is converted to chitosan at the stage when the pathogens penetrate the plant leaf tissues (El Gueddari *et al.*, 2002). Conversion of chitin to chitosan has also been observed in *M. oryzae*, but some studies showed that chitosan has a role in fungal morphology and appressorium development rather than serving as a stealth molecule (Fujikawa *et al.*, 2009; Fujikawa *et al.*, 2012; Geoghegan & Gurr, 2016; Geoghegan & Gurr, 2017) (Figure 1.7).

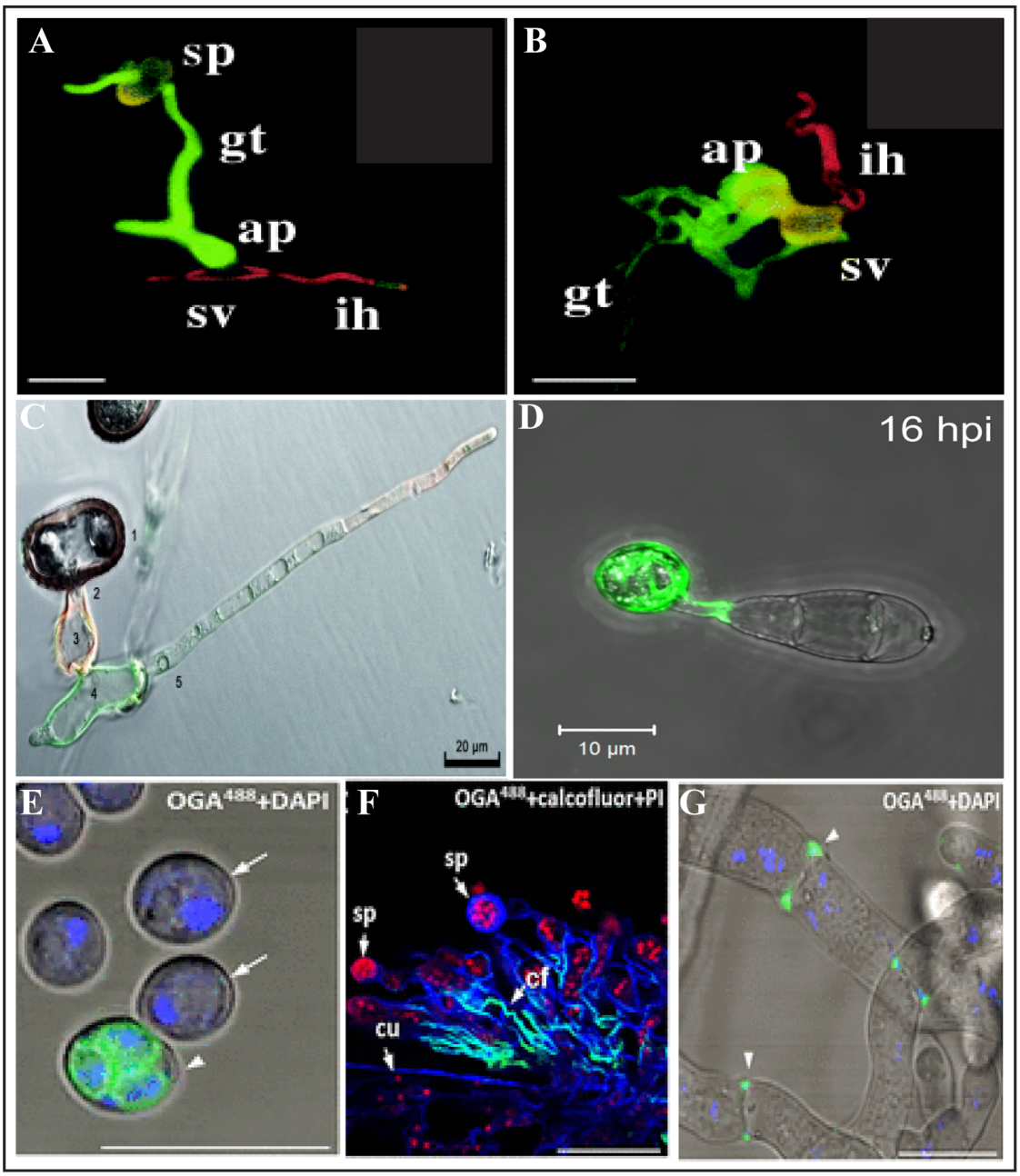


Figure 1.7. Distribution of chitin and chitosan on fungal infection structures. (A) and (B) Staining of a *P. graminis* f.sp. *tritici* and *U. faba* germinated spore (sp) respectively with FITC-WGA

(green fluorescence and Texas-Red labelled antibody (red fluorescence). Germ tube (gt) and appressorium (ap) formed on the surface strongly labelled with FITC-WGA indicating the chitin exposure. Substomatal vesicle (sv) and infection hyphae (ih) formed in intercellular space strongly labelled with chitosan antibody. Bars= 30 µm. Images adapted from (El Gueddari *et al.*, 2002). (C) Double staining of the *P. graminis* f.sp. *tritici* spore (1) with WGA-Texas-Red. Chitin was labelled in germ tube (2), appressorium (3) and tip of the infection hyphae in red florescence with WGA-Texas-Red. Substomatal vesicle (4) and infection hyphae (5) are stained with CAP in green fluorescence indicating the presence of chitosan. Bar=20 µm. Image adapted from (Nampally *et al.*, 2012). (D) Germ tube and appressorium of *M. oryzae* were labelled with anti-chitosan probe OGA-488, indicating the presence of chitosan in these structures. Bar=10 µm. Image adapted from (Geoghegan & Gurr, 2016). (E-G) OGA-488 labelling of different fungal species. (E) Chitosan labelling (green) of a tetrad of four *S. cervisiae* spores. Ascus wall and diploid cells indicated with white arrowhead and arrows respectively are not labelled. Bar=10 µm. (F) OGA-488, Calcofluor and propidium iodide (PI) labelling of the pathogenic fungus *Entomophthora scizhophorae* outgrowing on house fly *Musca domestica*. chitosan labelling (green) is visible only in conidiophore (cf) structure but not spores (sp) or cuticle of the fly (cu). Chitin and nuclei are stained by Calcofluor and PI in blue and red respectively. Bar=10 µm. (G) chitosan labelling is restricted to septa (white arrowhead) between cells in N. *crassa* hyphae. Bar=10 µm. Images adapted from (Mravec *et al.*, 2014).

1.10. Fungal chitinases

The fungal cell wall is a highly dynamic structure that undergoes various changes during different physiological stages such as spore germination, hyphal branching and septum formation. The presence of glycoproteins within the cell wall structure is the source of this dynamic nature. Sequence and homology analysis revealed that cell wall associated proteins can be grouped into two categories, wall-associated enzymes (WAEs) and structural proteins. The WAEs include chitinases, glucanases and peptidases involved in enzymatic remodelling of the cell wall. Structural proteins are relatively small proteins incorporated into the fungal cell wall structure, but they do not have any functional domains or enzymatic activity. These proteins may play roles in cell migration, adhesion, fusion and mating, or serve as scaffolding proteins to which other cell wall components are attached (Adams, 2004; Bowman & Free, 2006). A number of chitinases and glucanases have been purified from *S. cerevisiae* and *A. fumigatus* and shown to have endo- or exo- glycolytic activity. The yeast and filamentous fungal genomes contain

several genes encoding chitinases and glucanases with overlapping hydrolysis activity important for cell wall remodelling. Chitinases are classified as endochitinases or exochitinases depending on the site of chitin cleavage. Endochitinases cleave chitin randomly and release chitotetraose, chitotriose, and di-acetylchitobiose oligomers (Sahai & Manocha, 1993). In contrast, exochitinases only release the dimer di-acetylchitobiose from the nonreducing end of the chitin polymer (Sahai & Manocha, 1993). In *S. cerevisiae* two genes, *CTS1* and *CTS2,* encode endochitinases that are involved in cell/bud separation and spore wall assembly, respectively (Adams, 2004). A chitinase was shown to be one of the major secreted proteins in cultures of a *Neotyphodium. spp* (Li et al., 2004). This enzyme has also been detected in the apoplastic fluid of *Poa ampla* infected with *Neotyphodium. spp*, suggesting that it might have a role in fungal morphology such as hyphal branching and conidia development, germination in culture, or autolysis of older mycelia and re-assimilation of nutrients for continued growth in the apoplast of infected plants (Li *et al.*, 2004).

1.11. Aims and objectives of this project

Chitin, the main component of the cell wall, acts as a PAMP to trigger the plant immune response. Several studies have shown that phytopathogenic fungal genomes contain many genes encoding LysM proteins, and some of these have been revealed to have a role in sequestering chitin oligomers released by plant chitinases to suppress the host defence response. In addition to LysM effector secretion, pathogens can deploy a complementary strategy to avoid activation of the plant immune response by remodeling the fungal cell wall through chitin deacetylation, resulting in the conversion of chitin into chitosan. This molecule is a poor substrate for chitinases and elicits a relatively weak PTI reaction. Chitin deacetylase activity has been identified in several different fungal pathogens and chitosan is present in the cell walls of infection structures, findings that suggest chitosan can act as a stealth molecule during host infection. Given that *E. festucae* must suppress its plant host defense reaction to establish a stable symbiotic interaction, the aim of this project is to understand how *E. festucae* is able to suppress host defense to maintain its mutualistic symbiosis with the following objectives:

1. Identify candidate genes encoding LysM proteins in the *E. festucae* genome using bioinformatic approaches.

2. Determine the role of LysM proteins in the symbiotic interaction by performing targeted gene deletion of a selection of genes identified in objective 1.
3. Analyze the fungal cell wall structure in seedlings and mature plants infected with *E. festucae* Fl1 to understand how cell wall structure, especially chitin, change during the initial stages of host infection, during endophytic growth and epiphyllous growth using different biosensors and microscopy approaches.
4. Identify candidate genes encoding chitin deacetylases in the *E. festucae* genome using bioinformatic approaches.
5. Determine the role of chitosan in the symbiotic interaction by performing targeted deletion of a gene encoding a chitin deacetylase upregulated *in planta*.

Chapter 2. Materials and Methods

2.1. Sterile conditions

Unless otherwise specified all media were prepared using purified Milli-Q water supplied by the Barnstead NANOpure ultrapure water purification system (Thermo Scientific™ Barnstea™) and sterilized by autoclaving for 20 min at 121°C. All the experiments involving culturing bacteria or fungal cells were performed under sterile conditions within a UV sterilized laminar flow hood.

2.2. Molecular and biological material

Organisms (Table 2.1), plasmids (Table 2.2) and primers (Table 2.3) that were used are listed as follows:

Table 2. 3. Strains used in this study.

Strain	Relevant characteristics	Reference
Fungal strains		
Epichloë festucae		
PN2278 wild type	Fl1	(Young *et al.*, 2005)
PN3096 (Δ*lymA*#7)	Fl1/Δ*lymA*::P*trpC-hph*;Hyg^R	This study
PN3097 (Δ*lymA*#27)	Fl1/Δ*lymA*::P*trpC-hph*;Hyg^R	This study
PN3098 (Δ*lymA*#30)	Fl1/Δ*lymA*::P*trpC-hph*;Hyg^R	This study
PN3099 (Δ*lymA*#32)	Fl1/Δ*lymA*::P*trpC-hph*;Hyg^R	This study
PN3100 (Δ*chiA*#5)	Fl1/Δ*chiA*::P*trpC-hph*;Hyg^R	This study
PN3101 (Δ*chiA*#22)	Fl1/Δ*chiA*::P*trpC-hph*;Hyg^R	This study
PN3102 (Δ*chiA*#39)	Fl1/Δ*chiA*::P*trpC-hph*;Hyg^R	This study
PN3125 (Δ*lymB*#41)	Fl1/Δ*lymB*::P*trpC-npt*II-T*trpC*;Gen^R	This study
PN3126 (Δ*lymB*#52)	Fl1/Δ*lymB*::P*trpC-npt*II-T*trpC*;Gen^R	This study
PN3127 (Δ*lymB*#116)	Fl1/Δ*lymB*::P*trpC-npt*II-T*trpC*;Gen^R	This study
PN3158 (Δ*chiB*#36)	Fl1/Δ*chiB*::P*trpC-npt*II-T*trpC*;Gen^R	This study
PN3159 (Δ*chiB*#68)	Fl1/Δ*chiB*::P*trpC-npt*II-T*trpC*;Gen^R	This study
PN3160 (Δ*lymA/B*#12)	Δ*lymA*/Δ*lymB*::P*trpC-npt*II-T*trpC*;Gen^R, Hyg^R	This study
PN3161 (Δ*lymA/B*#29)	Δ*lymA*/Δ*lymB*::P*trpC-npt*II-T*trpC*;Gen^R, Hyg^R	This study
PN3162 (Δ*lymA/B*#42)	Δ*lymA*/Δ*lymB*::P*trpC-npt*II-T*trpC*;Gen^R, Hyg^R	This study
PN3200 (Δ*cdaA*#28)	Fl1/Δ*cda-A*::P*trpC-hph*-T*trpC*;Hyg^R	This study

PN3201 (Δ*cdaA*#41)	Fl1/Δ*cda-A*::P*trpC-hph*-T*trpC*;Hyg^R	This study
PN3202 (Δ*cdaA*#49)	Fl1/Δ*cda-A*::P*trpC-hph*-T*trpC*;Hyg^R	This study
PN 3236(Δ*lymA*#7/*lymA-e*GFP#14)	Δ*lymA/teflymA*::e*GFP*-T*trpC*-P*trpC-hph*-T*tub*;Gen^R, Hyg^R	This study
PN 3237(Δ*lymB*#41/*lymB-e*GFP#1)	Δ*lymB/teflymB*::e*GFP*-T*trpC*-P*trpC-npt*II-T*tub*;Hyg^R, Gen^R	This study
Bacterial strains		
E. coli		
DH5α	F$^-$Φ80lac ZΔM15 Δ(*lac* ZYA-*arg* F) U169 *rec*A1 *end*A1 *hsd*R17 (rk$^-$, mk$^+$) *phoAsup* E44λ*thi*$^-$1 *gyr*A96*rel*A1	ThermoFisher Scientific
NN01	DH5α/pNN01	This study
NN02	DH5α/pNN02	This study
NN03	DH5α/pNN03	This study
NN04	DH5α/pNN04	This study
NN05	DH5α/pNN05	This study
NN11	DH5α/pNN11	This study
NN12	DH5α/pNN12	This study
NN13	DH5α/pNN13	This study
NN14	DH5α/pNN14	This study

Table 2. 4. Plasmids used in this study.

Biological material	Relevant characteristics	Reference
Plasmids		
pRS426	ori(f1)-lacZ-T7 promoter-MCS (*Kpn*I-*Sac*I)-T3 promoter lacIori pMB1)-ampR-ori (2 micron), URA3, Amp^R	(Winston *et al.*, 1995)
pSF15.15	P*trpC*-hph;Amp^R,Hyg^R	S. Foster
pSF17.1	P*trpC*-ntpII-T*trpC*; Amp^R,Gen^R	S. Foster
pDB48	P*trpC*-hph-T*trpC*; Amp^R,Hyg^R	D. Berry
pBH18	P*tef*-m*cherry*-T*trpC*-P*trpC-hph*-T*tub*;Amp^R,Hyg^R	B. Hassing
pBH28	P*gpd*-e*GFP*-T*trpC*-P*trpC-ntp*II-T*tub*;Amp^R,Gen^R	B. Hassing
pNN01	pRS426 containing 5'*lymA*-PtrpC-*hph*-3'*lymA*;Amp^R ,Hyg^R	This study
pNN02	pRS426 containing 5'*chiA*-PtrpC-*hph*-3'*chiA*;Amp^R ,Hyg^R	This study
pNN03	pRS426 containing 5'*lymB*-P*trpC*- *npt*II-T*trpC* -3'*lymB*; Amp^R ,Gen^R	This study
pNN04	pRS426 containing 5'*chiB*-PtrpC- *npt*II-T*trpC* -3'*chiB*; Amp^R ,Gen^R	This study
pNN05	pRS426 containing 5'*cdaA*-P*trpC*- *hph*-T*trpC* -3'*cdaA*; Amp^R ,Hyg^R	This study
pNN11	pNN12 containing *PlymA*:*e*GFP-*TtrpC*;Amp^R,Gen^R	This study
pNN12	pBH28 containing *PlymA*:*e*GFP-*TtrpC*;Amp^R,Gen^R	This study
pNN13	pBH18 containing P*tef*::*lymB*:*e*GFP-*TtrpC*;Amp^R,Hyg^R	This study
pNN14	pBH18 containing P*tef*::*lymB*:*e*GFP-*TtrpC*;Amp^R,Hyg^R	This study

Table 2. 3. Primers used in this study.

Name	Primer sequence (5'-3')	Purpose
NN10	ACGCCAGGGTTTTCCCAGTCACGACG TTAACTGCCTGGAGAACCAACTCAC	pNN01 generation
NN11	CACTCGTCCGAGGGCAAAGGAATAGC TTGGTGGAGCAAAAGCCAG	pNN01 generation
NN12	GCTCCTTCAATATCAGTTCCAAGCTC AAGCAGAATGACTCGTGCG	pNN01 generation
NN13	ATAACAATTTCACACAGGAAACAGCG AATTCCCAAGACGCAAACCTGTTCG	pNN01 generation
NN22	GTCGTACGATGTGGCATTGC	Amplify within *lymA*
NN25	ACGACGCCAGGTTAGAACAG	Amplify within *lymA*
NN26	GAGCAGCAAGTGTTCAACGG	Amplify across *lymA* deletion construct left border
NN29	ATACGAGGTCGCCAACATCC	Amplify across *lymA* deletion construct left border
NN31	ACAGCATACCCCGACCAAAG	Amplify across *lymA* deletion construct right border
NN32	TGTAGAAACCATCGGCGCAG	Amplify across *lymA* deletion construct right border
NN42	ACGCCAGGGTTTTCCCAGTCACGACG TTAACAGGTCTGCGCAACTCTTCTC	pNN03 generation
NN43-2	CAAAAAGTGCTCCTTCAATATCTAGA CTCTGTGCTTGCCAGC	pNN03 generation
NN44-2	CATTCCTACTAAGATGGGTAGGCGGA AGTCGAAGCATTGTG	pNN03 generation
NN45	ATAACAATTTCACACAGGAAACAGCA CTAGTGCCGAAACATCACCAAGACG	pNN03 generation
NN50	AGCATAAGTCGCCGTCTTGG	Amplify within *lymB*
NN51	GCGGGATTACGCAGTGTCTC	Amplify within *lymB*
NN52	ATCCACACGGCGATGAAGAG	Amplify across *lymB* deletion construct right border
NN53	TGCTTGACAAACGCACCAAG	Amplify across *lymB* deletion construct right border
NN54	ACCGCTTCCTCGTGCTTTAC	Amplify across *lymB* deletion construct left border
NN55	TCATTTCCCCGTGAGAGTGC	Amplify across *lymB* deletion construct left border

NN18	ACGCCAGGGTTTTCCCAGTCACGACG TTAACTGTCCATTGCAGTCTCGTCC	pNN02 generation
NN19	GCTCCTTCAATATCAGTTCCAAGCTTC GATCTGCCGGGAATGATG	pNN02 generation
NN20	CACTCGTCCGAGGGCAAAGGAATAGT CGTCTTGCCATCCTTGGAG	pNN02 generation
NN21	ATAACAATTTCACACAGGAAACAGCG AATTCAGTTACATCTATCTGTGC	pNN02 generation
NN34	TGAAAGCCCGACCGTAGAAC	Amplify within *chiA*
NN35	TCCTTGTCCTGAGTCGTTGC	Amplify within *chiA*
NN29	ATACGAGGTCGCCAACATCC	Amplify across *chiA* deletion construct left border
NN37	TCCAAACCGGCATATCTCGG	Amplify across *chiA* deletion construct left border
NN32	TGTAGAAACCATCGGCGCAG	Amplify across *chiA* deletion construct right border
NN36	CTTGAAAACTGTGCCCCAGC	Amplify across *chiA* deletion construct right border
NN56	ACGCCAGGGTTTTCCCAGTCACGACG TTAACATCGTCTAATGAGCAGAAGA	pNN04 generation
NN57	CAAAAAGTGCTCCTTCAATATCTCTG CGACTCTAACGAAGCC	pNN04 generation
NN58	CATTCCTACTAAGATGGGTAGTCCTTT GCCTCTTACGGCTG	pNN04 generation
NN59	ATAACAATTTCACACAGGAAACAGCA CTAGTTGTCAGCTCCGCGATATGTC	pNN04 generation
NN60	TCCGACGATCAGTATATGGC	Amplify within *chiB*
NN61	CGAGACTTCTCTCATCTAGT	Amplify within *chiB*
NN62	CAGTTGCCTATCTGGATGAT	Amplify across *chiB* deletion construct left border
NN53	TGCTTGACAAACGCACCAAG	Amplify across *chiB* deletion construct left border
NN54	ACCGCTTCCTCGTGCTTTAC	Amplify across *chiB* deletion construct right border
NN63	CTATAGACCCTCGCTCATCA	Amplify across *chiB* deletion construct right border

NN117	GTGACACTATAGAACTCGACTTGACC CTGCTTCCTCTCCGC	pNN11 generation
NN118	CATAGATCCACCTCCACCAGAACCTC CACCTCCCTCTCCAATACAGACAGC	pNN11 generation
NN119	GGAGGTGGAGGTTCTGGTGGAGGTGG ATCTATGGTGAGCAAGGGCGAGGA	pNN11 generation
NN121	GTCGAGTTCTATAGTGTCACC	pNN11 generation
NN112	CCATACATCACCGTCAAACCTCATGT CCGCTCTTTTCATAAC	pNN12 generation
NN118	CATAGATCCACCTCCACCAGAACCTC CACCTCCCTCTCCAATACAGACAGC	pNN12 generation
NN119	GGAGGTGGAGGTTCTGGTGGAGGTGG ATCTATGGTGAGCAAGGGCGAGGA	pNN12 generation
NN121	GTCGAGTTCTATAGTGTCACC	pNN12 generation
NN120	GTGACACTATAGAACTCGACGGTAGC AAACGGTGGTCAAAG	pNN12 generation
BH77	GAGGTTTGACGGTGATGTATG	pNN12 generation
NN122	GTGACACTATAGAACTCGACAATAAT ACCATCATCTGTGGA	pNN13 generation
NN123	CATAGATCCACCTCCACCAGAACCTC CACCTCCAATCTCGTCCTTTGCAC	pNN13 generation
NN119	GGAGGTGGAGGTTCTGGTGGAGGTGG ATCTATGGTGAGCAAGGGCGAGGA	pNN13 generation
BH115	CAAGCTGTTTGATGATTTCAGTTACTT GTACAGCTCGTCCATG	pNN13 generation
BH76	CTGAAATCATCAAACAGCTTG	pNN13 generation
NN121	GTCGAGTTCTATAGTGTCACC	pNN13 generation
NN104	CATACATCACCGTCAAACCTCATGCT TCGACTTCCGCTTCT	pNN14 generation
NN123	CATAGATCCACCTCCACCAGAACCTC CACCTCCAATCTCGTCCTTTGCAC	pNN14 generation
BH76	CTGAAATCATCAAACAGCTTG	pNN14 generation
NN121	GTCGAGTTCTATAGTGTCACC	pNN14 generation
BH76	CTGAAATCATCAAACAGCTTG	pNN14 generation
BH77	GAGGTTTGACGGTGATGTATG	pNN14 generation

NN122	GTGACACTATAGAACTCGACAATAAT ACCATCATCTGTGGA	pNN13 generation
NN123	CATAGATCCACCTCCACCAGAACCTC CACCTCCAATCTCGTCCTTTGCAC	pNN13 generation
NN119	GGAGGTGGAGGTTCTGGTGGAGGTGG ATCTATGGTGAGCAAGGGCGAGGA	pNN13 generation
BH115	CAAGCTGTTTGATGATTTCAGTTACTT GTACAGCTCGTCCATG	pNN13 generation
BH76	CTGAAATCATCAAACAGCTTG	pNN13 generation
NN121	GTCGAGTTCTATAGTGTCACC	pNN13 generation
NN104	CATACATCACCGTCAAACCTCATGCT TCGACTTCCGCTTCT	pNN14 generation
NN123	CATAGATCCACCTCCACCAGAACCTC CACCTCCAATCTCGTCCTTTGCAC	pNN14 generation
BH76	CTGAAATCATCAAACAGCTTG	pNN14 generation
NN121	GTCGAGTTCTATAGTGTCACC	pNN14 generation
BH76	CTGAAATCATCAAACAGCTTG	pNN14 generation
BH77	GAGGTTTGACGGTGATGTATG	pNN14 generation
NN119	GGAGGTGGAGGTTCTGGTGGAGGTGG ATCTATGGTGAGCAAGGGCGAGGA	pNN14 generation
BH115	CAAGCTGTTTGATGATTTCAGTTACTT GTACAGCTCGTCCATG	pNN14 generation
NN92	ACGCCAGGGTTTTCCCAGTCACGACC CCGGGACAAGGAACACGAACGGAAG	pNN05 generation
NN93	CCTTCAATATCAGTTCCAAGCTCATG CTAGAGTCGTCGTCCA	pNN05 generation
NN94	GCGCCCACTCCACATCTCCACTCGAA GGAGTTCGGCTCTTGAACA	pNN05 generation
NN95	ATAACAATTTCACACAGGAAACAGCG TTAACAAGCCACAGACCAAGTACGG	pNN05 generation
NN96	CGGATTAGGAAGCATGTCGT	Amplify within *cdaA*
NN97	AATCGCTGATGTCCTTGGTC	Amplify within *cdaA*
NN98	CATGCAGTCATGGAGTCTGG	Amplify across *cdaA* deletion construct right border
NN99	ATTTGTGTACGCCCGACAGT	Amplify across *cdaA* deletion construct right border

NN100	AGTTGAGACAAATGGTGTTC	Amplify across *cdaA* deletion construct left border
NN101	CCCAAGACATTCGACAAGTA	Amplify across *cdaA* deletion construct left border
hph-F	AGCTTGGAACTGATATTGAAGG	Amplification of P*trp*C–*hph* cassette
hph-R	CTATTCCTTTGCCCTCGGACG	Amplification of P*trp*C–*hph* cassette
hph-F	AGCTTGGAACTGATATTGAAGG	Amplification of P*trp*C–*hph*-T*trp*C cassette
pDB33_7	CAGGTCGAGTGGAGATGTGG	Amplification of P*trp*C–*hph*-T*trp*C cassette
*ntp*II-F	GATATTGAAGGAGCACTTTTTG	Amplification of P*trp*C–*ntp*II-T*trp*C cassette
*ntp*II-R	CTACCCATCTTAGTAGGAATG	Amplification of P*trp*C–*ntp*II-T*trp*C cassette
pRS426-F	GTAACGCCAGGGTTTTCCCAGTCACGAC	Amplify vector backbone for Gibson assembly
pRS426-R	GCGGATAACAATTTCACACAGGAAACAGC	Amplify vector backbone for Gibson assembly
NN135	ACATTCTGAGCATGGTGCAT	*cdaB* expression level (qRT-PCR)
NN136	CGACTTTGTACCCCTGCTGT	*cdaB* expression level (qRT-PCR)
NN137	TGATCCAGCAGTCCAAGGAT	*cdaC* expression level (qRT-PCR)
NN138	TGTAAACCGTCTGCTCATGG	*cdaC* expression level (qRT-PCR)

2.3. Media and growth conditions

2.3.1. *Escherichia coli*

E. coli strains were cultured overnight (approx. 18 hours) at 37°C either on Luria-Bertani (LB) (2.3.2) agar plates or in sterilized test tubes containing 2-4 ml of broth medium with shaking at 200 RPM. Filter sterilized antibiotic was added to the media where appropriate to a final concentration of 100 μg/ml for ampicillin post autoclaving. *E. coli* strains were grown by streaking on to LB plates or transferring 1 μl of an overnight culture into liquid culture. Strains were stored at 4°C for short term storage or in 50% (v/v) glycerol at -80°C for long term storage.

2.3.2. Lysogeny Broth (LB) medium

LB liquid medium (Miller 1972) contained 5 g/L tryptone, 10 g/L yeast extract and 5 g/L NaCl, with pH adjusted to 7-7.5 with 5 M NaOH. 15 g/L agar was added for solid media.

2.3.3. SOC medium

SOC medium (Dower et al., 1988) contained 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4.7H_2O$, 20 mM glucose, 20 g/L tryptone and 5 g/L yeast extract.

2.3.4. *Epichloë festucae*

E. festucae strains were cultured at 22°C for 5 days in 50 ml of PD (2.3.5) broth medium shaking at 200 RPM or on PD agar plates for 5-7 days. *E. festucae* protoplasts were regenerated on 1.5% (w/v) RG (2.3.6) agar plates for 10-14 days. When selection was required the plates were overlaid with an additional layer of 0.8% RG agar containing antibiotic. Strains were stored on plates at 4°C for short-term storage or on PD agar slants under mineral oil or 30% (v/v) glycerol at -80°C for long-term storage.

2.3.5. Potato dextrose (PD) medium

PD broth medium contained 24 g/L PD. The agar (1.5%) was added to obtain solid medium. Where selection was required, antibiotics were added to the molten medium post autoclaving to final concentrations of 150 μg/ml of hygromycin (*hph*) or 250 μg/ml of geneticin (*npt*II).

2.3.6. Regeneration medium (RG)

RG medium contained 24 g/L PD, 0.8 M sucrose. The pH was adjusted to 6.5. Agar was added at a final concentration of 1.5% and 0.8% for base medium and the soft overlays before autoclaving respectively.

2.3.7. Water agar medium (WA)

WA medium contained Milli-Q water (H_2O) as well as 3% (w/v) agar which was added before autoclaving. When a culture phenotype analysis was performed, a glass slide was positioned on 3% WA base medium and overlaid with 5-10 ml of 1.5% WA. The strains were sub-cultured at the edge of the slides and then were taken for microscopic analysis two weeks post sub-culturing.

2.3.8. *Lolium perenne*

2.3.8.1. Seedling sterilization, plant inoculation and growth conditions

L. perenne seedlings were surface sterilized by soaking in 50% (v/v) H_2SO_4 for 30 min with shaking to have a consistent incubation, rinsed 3 times in sterile milli-Q water, followed by soaking in 50% (v/v) chlorine bleach for 30 min and finally 3 times rinsing in sterile milli-Q water. They were air-dried on sterile filter papers using sterile conditions in a laminar flow cabinet and stored in sterile petri dishes at 4°C. Ten to twelve seeds were placed and germinated on 3% (w/v) water agar set in petri dishes. The plates were kept 7 days at 22°C for germination. A 2-3 mm longitudinal slit of the germinated seedlings between the mesocotyl and coleoptile was cut using a scalpel under a dissecting microscope and a small piece of 7-day old freshly grown mycelia on PD agar (the same age as the germinated seedlings) was inoculated into the cut seedlings. The seedlings were then incubated at 22°C for 7 days in the dark and 7 days in the light to complete the infection. The seedlings were then planted in root trainers filled with potting mix free from fungicide (provided by AgResearch) and grown under standard conditions in the plantroom facility.

2.3.8.2. Plant growth condition

Seedlings were grown in root trainers (in groups of 56) in an environmentally controlled growth room at 22°C with a photoperiod of 16 h of light (~100 $\mu E/m^2$ per sec). Plants were watered every 2 days during the first week post planting and then twice a week, or as required.

2.3.8.3. Immunohistochemical determination of endophyte infection

A single tiller of each plant was cut at the base using a scalpel. Dead leaves were removed, and the exposed end of the tiller pressed into a nitrocellulose membrane (NCM). The membrane was immersed in fresh blocking solution containing 20 mM Tris, 50 mM NaCl, 0.5% (w/v) non-fat milk powder at room temperature for at least 2 hours. The blocking solution was decanted off and membrane sheets were incubated in 5 ml of fresh blocking solution with 5 µl of primary antibody (polyclonal rabbit antibody raised against homogenised mycelium of *Epichloë festucae* var. *lolii* (Christensen *et al.*, 1993) and shaken overnight in 4°C . The solution was decanted off the following day and replaced with 5 ml blocking solution and 2.5 µl of secondary antibody (goat anti-rabbit antibody with an alkaline phosphatase conjugate, Sigma), then shaken at room temperature for 2 hours. The result was visualized using developing solution containing SIGMAFAST™ Fast Red TR/Naphthol AS-MX tablets, as per the manufacturer's instructions, after decanting off the solution and washing the sheets three times with fresh blocking solution. The membrane was developed after 15 min shaking at room temperature.

2.4. DNA and RNA Isolation and Quantification

2.4.1. Plasmid isolation

E. coli strains were grown overnight in 2-4 ml of LB selective media containing 100 µg/ml of ampicillin. Plasmid DNA was extracted using a High Pure Plasmid Isolation Kit (Roche) according to the manufacturer's instructions.

2.4.2. *E. festucae* crude genomic DNA and quantification

E. festucae fungal mycelia were grown on solid media for 7 days. One cm^2 mycelial blocks from the outer edge of the colony were removed and chopped using sterile tooth picks and transferred to 1.7 ml Eppendorf tubes containing 150 µl of lysis buffer (100 mM Tris-HCl, 100 mM EDTA and 1% SDS adjusted to a pH of 8.0). Samples were incubated at 70°C for 30 min then 150 µl of 5 M potassium acetate was added, inverted 6-8 times and incubated on ice for 10 min. The tubes were centrifuged for 20 min at 13,000 RPM and 200 µl aliquots of the supernatant were transferred to new tubes with extra care to avoid dislodging the pellet. Ice cold isopropanol (140 µl) was added to precipitate the DNA. Samples were either centrifuged for 15 min or kept at -20°C

overnight and then centrifuged. The supernatant was discarded, and the pellet was washed using 180 µl of 70% ethanol by centrifuging for 5 min. The supernatant was removed by pipetting and resuspended in 50 µl of sterile H_2O and heated at 70 for 10 min, centrifuged for 10 min and transferred into new tubes before using as the template for PCR reaction or kept at 4°C.

2.4.3. *E. festucae* genomic DNA isolation and quantification

Fungal mycelia were obtained from the strains cultured in PD broth media with shaking for 5 days. The mycelia were collected from the liquid media and filtered through sterile nappy liners and washed three times with sterile H_2O. The mycelia were lyophilized for at least 24 hours. Freeze-dried mycelia (15-20 µg) was ground in liquid nitrogen using a mortar and pestle and transferred to 2 ml Eppendorf tubes containing 800 µl extraction buffer (150 mM EDTA, 50 mM Tris-HCl, 1% SDS adjusted pH to 8.0) and proteinase K at a final concentration of 2 mg/ml. The samples were incubated at 37°C for 20 min and then centrifuged for 10 min. The aqueous phase was carefully transferred to new microtubes and 400 µl of phenol and 400 µl of chloroform were added, mixed and centrifuged again for 10 min. The phenol/chloroform treatment step was repeated 2 more times retaining the upper aqueous phase. The final extraction step was done by adding 800 µl of chloroform and centrifuging for 10 min. The DNA was precipitated by adding 800 µl of ice cold isopropanol to the aqueous phase and centrifuging again for 10 min. The DNA pellet was washed with 1 ml of 70% ethanol, centrifuged for 10 min, air dried for 10 min and resuspended in 100 µl of sterile Milli-Q H_2O.

To avoid RNase contamination, all the work spaces and utensils were cleaned and treated with RNase AWAY® (Sigma-Aldrich), only RNase free pipettes with filter tips and RNase free consumables were used.

2.4.4. *E. festucae* RNA isolation using TRIzol

Samples for RNA extraction were harvested from infected plants. The plants were harvested, dead leaves and tissues were removed, and the plant pseudostems sectioned into approximately 10 mm segments and frozen in liquid nitrogen immediately. Plant samples were ground in liquid nitrogen using a mortar and pestle, 1.6 µl of the TRIzol was added in a way to cover the ground samples and make a paste. The samples were kept at room temperature for 2-3 min to be thawed and transferred to 2 ml microtubes and centrifuged at 4°C/ 12000 x g for 5 min. The supernatant (1.5 µl) was transferred to new 1.7 ml microtubes, 300 µl of chloroform was added, kept at room temperature for 2-3 min and centrifuged for 15 min. The aqueous phase (800µl) was pipetted into new tubes followed by addition of 400 µl of isopropanol and 400 µl of high salt solution (0.8 M sodium citrate, 1.2 M NaCl in DEPC H_2O), incubated at room temperature for 10 min and centrifuged for 10 min. The supernatant was discarded, and the RNA pellet was washed with 1 ml of 70% ethanol (in DEPC H_2O), centrifuged for 5 min and the supernatant discarded and the RNA pellet retained. The pellet was air dried for 5 min and resuspended in 50 µl of 1× RNA secure solution and incubated at 60°C for 10 min to inactivate RNases and kept at -80°C for storage.

2.4.5. Reverse transcription

cDNA was synthesised from 0.5-1 µg of RNA using the QuantiTect® Reverse Transcription Kit (Qiagen), as per the manufacturer's instructions.

2.5. DNA Manipulation

2.5.1. Restriction enzyme digestion

Restriction enzyme digestion of DNA was performed using restriction enzymes purchased from New England Biolabs. The digestion was set up using 10 units of the restriction enzyme per 1 µg of DNA, 5 µl of 10× appropriate buffer adjusting the total volume of the reaction in 50 µl with sterile Milli-Q H_2O and incubated at a suitable temperature for 30-60 min. However, for Southern blot analysis, 1-1.5 µg of DNA was

digested using 20 units of restriction enzyme and the reaction mixture incubated for approximately 4 hours. If necessary, an additional 10 units of enzyme was added and the mixture incubated overnight at an appropriate temperature. When double digestions were performed a suitable buffer for both enzymes was used.

2.5.2. Ethanol precipitation of nucleic acid

DNA was precipitated by adding 2.5 volumes of absolute ethanol, 0.1 volume of 3 M sodium acetate to the DNA solution and mixing well by inversion. Samples were kept at -20°C from 1 hour to overnight before centrifuging for 10 min at 13,000 RPM. The DNA pellet was washed with 1 ml of 70% ethanol then centrifuged for 5 min at 13,000 RPM. The pellet was air dried for 10 min at room temperature before resuspending in sterile Milli-Q water.

2.5.3. Gel electrophoresis

Agarose gels were prepared from molten 0.8% to 2% (v/w) agarose for separation of large and small DNA fragments respectively. Agarose was melted in 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA) by boiling. The gel was set and immersed in 1× TBE buffer and the DNA samples mixed with 6× loading dye (20% sucrose (w/v), 5 mM EDTA, 1% (w/v) SDS and 0.2% (w/v) bromophenol blue) in a ratio of 5:1. Samples were loaded and run at 100-120 V using standard electrophoresis equipment. The gel was stained in ethidium bromide solution (1 µg /mL ethidium bromide) for 15-20 min, de-stained in Milli-Q water for 10 min, visualized and photographed using a UV Transilluminator Gel Documentation System (Bio-Rad).

2.5.4. Gel purification

DNA fragments were column purified from PCR reaction mixtures using the Wizard® SV Gel and PCR Clean-UP System (Promega), as per the manufacturer's instructions. PCR fragments were gel purified using the same method if there were additional bands present in the reaction mixture.

2.5.5. Southern blotting

To confirm deletion of genes, high quality gDNA (1-1.5 µg) was digested using the appropriate restriction enzyme overnight. The reaction volume was reduced by ethanol

precipitation, then the sample mixed (5:1) with 6× loading dye and loaded into the wells of a pre-set 0.8% agarose gel. The gel was run overnight at 30V. The following day the gel was stained with ethidium bromide for 15 min, destained in water for 5 min, visualized and photographed under UV light with a ruler for DNA fragment size estimation. The gel was depurinated in solution I (0.25 M HCl) for 15 min, denatured in solution II (0.5 M NaOH, 0.5 M NaCl) for 30-45 min, neutralized in solution III (0.5 M Tris, pH 7.4, 2 M NaCl) for 30-60 min and then washed in 2× SSC (0.3 M NaCl, 30 mM sodium citrate) for 2 min. The blotting apparatus was assembled, the gel was placed on it and the DNA transferred to a positively charged nylon membrane (Roche) overnight. The blotting apparatus was assembled as follows: a double layer of "wick" of 320 x 156 mm 3MM Whatman sheet soaked in 20× SSC (3 M NaCl, 0.3 M sodium citrate), the ends of the sheets submerged in wells of assembly trays filled by 20× SSC. The gel was placed on top of it, covered with positively charged nylon membrane cut the same size as the gel. To prevent the capillary action and liquid bypassing the membrane, the gel edges were protected by plastic cling film cut slightly smaller than the gel with a square hole in it. Two wick layer sheets with the exact size of the gel were soaked in 2× SSC placed on top of the gel followed by two identical dry sheets. A pile of paper towels was placed on top of all this and weighted down evenly using a filled 300 ml Schott bottle, left overnight. The following day the blot was disassembled, the membrane was washed in 2× SSC for 2 min and air dried on paper towel, the DNA was cross linked to the nylon membrane by UV irradiation for 2 min using a CEX-800 UV cross-linker (120 mJ/cm2, 254 nm; Ultralum, Inc.).

2.5.6. DNA probe-labelling

DNA probes were labelled using the DIG High Prime DNA Labelling and Detection Starter Kit (Roche), as per the manufacturer's instructions. One µg of DNA was labelled at 37°C for 18-20 hours, and the reaction stopped by adding 2 µl of 200 mM EDTA buffer (pH 8). Probe labelling resulted in 20 µl of 100 ng/µl probe solutions.

2.5.7. Hybridisation and detection

The membrane was pre-hybridised and hybridised for 2 hours and overnight respectively using the DIG High Prime DNA Labelling and Detection Starter Kit (Roche), as per the manufacturer's instructions. The incubation was conducted in PYREX^{TM} hybridization

tubes (Fisher Scientific) at 48-50°C (optimal hybridization temperature calculated) with slow agitation.

2.5.8. Colourimetric development of Southern blots

The hybridized DNA and membrane were developed and detected using NBT/BCIP colour detection (DIG-labelled probes), following the manufacturer's instruction.

2.6. Polymerase chain reaction (PCR)

2.6.1. Standard PCR

Standard PCR reactions were performed in a total reaction mixture of 25 or 50 µl containing *Taq* DNA polymerase (Roche®) or One*Taq*® DNA polymerase (New England Biolabs, Inc.) with reaction conditions as per the manufacturer's instructions. The PCR reactions were performed using a thermocycler (Mastercycler™ Nexus Thermal Cycler, Eppendorf™).

2.6.2. High fidelity PCR

When high fidelity PCR products were required for downstream applications high fidelity polymerase enzymes such as Phusion® DNA polymerase (ThermoFisher Scientific) or Q5® DNA polymerase (New England Biolabs, Inc.) were used with reaction conditions as per the manufacturer's instructions.

2.6.3. DNA quantification

The DNA concentration of the plasmids and PCR products were quantified using a NanoPhotometer™ (Implen) as per the manufacturer's instructions. The concentration of the fungal genomic DNA was determined using a DNA Quant 200 Fluorometer (Hoefer), as per the manufacturer's instructions.

2.6.4. Quantitative reverse transcription PCR (qRT-PCR)

2.6.4.1. qRT-PCR

qRT-PCR was performed using SsoFast™ EvaGreen® supermix (Bio-Rad). The master mix was prepared under clean conditions using filter tips containing 1 µl cDNA template, 10 µM forward and reverse primers and 5 µl SsoFast Evagreen 2x supermix in a reaction volume of 10 µl. The reaction was performed in 96-well plates with two technical

replicates in all experiments and with the LightCycler® 480 Instrument II (Roche). The reaction was run with an initial denaturation step at 95ºC for 30 s, followed by 40 cycles of denaturation at 95ºC for 5 s and annealing/ extension at 60ºC for 10 s. A melting curve was captured after 40 cycles at 65/98ºC with ramp of 0.4ºC /s. Data was analysed by obtaining and comparing the relative amount of cDNA between target and reference genes by the $2^{(\Delta cp)}$ method (Livak & Schmittgen, 2001).

2.6.4.2. Generating qRT-PCR standard curves

A standard curve was generated using cDNA standards amplified with the respective primer pairs using High Fidelity DNA polymerase (2.6.2). The amplicon was resolved by electrophoresis on 1.5% (w/v) agarose gel, excised and then purified using Wizard® SV Gel and PCR Clean-UP System (Promega) (2.5.4). Serial dilutions were made from concentrations of 10 aM to 10 pM and used to generate the standard curve for absolute quantification.

2.7. Cloning

2.7.1. Gibson assembly

The Gibson assembly (GA) master mix (2x concentration) was prepared on ice in a final volume of 250 µl using 100 µl of 5× isothermal buffer (25% (w/v) PEG-8000, 500 mM Tris-HCl (from 1 M pH 7.5 stock solution), 50 mM $MgCl_2$, 50 mM 1,4-dithiothreitol, 5 mM NAD and 1 mM dNTPs), 2 µl T5 exonuclease (10 U/µl; New England Biolabs, Inc.), 6.25 µl Phusion DNA polymerase (2 U/µl; ThermoFisher Scientific), 50 µl *Taq* ligase (40 U/µl; New England Biolabs, Inc.) and 91.75 µl of Milli-Q water. The GA master mix was then divided into PCR tubes (25 x 10 µl) and stored at -20°C. The fragments used for Gibson assembly reaction were designed to be joined based on homologous recombination with at least 15 bp long overlaps with T_m >50°C, with no additional sequence beyond those overlaps. All the fragments were generated by PCR amplification using high fidelity DNA polymerase enzymes, purified and combined to a final volume of 10 µl containing 50-100 ng of vector with 3-fold excess of each insert fragment (molar concentration). The reaction was performed on ice and 10 µl of GA2× was added, incubated at 50°C for 1 hour in a thermocycler. The resulting mixture was then transferred into chemically competent *E. coli* cells (Gibson *et al.*, 2009).

2.8. Transformation techniques

2.8.1. Bacterial transformation

2.8.1.1. Transformation of chemically competent *E. coli* cells

One hundred µl of chemically competent *E. coli* cells were thawed on ice, 2-10 µl of plasmid solution or Gibson assembly reaction was added and mixed gently with pipetting, cells incubated on ice for 20 min, heat shocked at 42°C for 60 s, and then placed back on ice again for 2 min. Three hundred µl of SOC media was added and incubated at 37°C with shaking at 200 RPM. Aliquots (200 µl) were spread on solid selective LB medium containing 100 µg/ml ampicillin using a sterile glass spreader and incubated at 37°C overnight.

2.8.1.2. Invitrogen clone checker system

Putative *E. coli* transformants derived from *E. coli* transformation were picked using 20 µl sterile pipette tips transformed into 6 µl liquid LB medium, mixed well by pipetting and 3 µl of this mixture transferred into 8 µl of the lysis solution (0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, 0.5% Triton X-100, pH 8) from CloneChecker™ System (ThermoFisher) and incubated at 99°C for 30 s. After cooling to room temperature 2 µl of the restriction enzyme (RE) mixture (1 unit of RE and 1 µl of the corresponding 10× buffer) was added and the mixture incubated at an appropriate temperature for 30 min. DNA fragments of digested plasmid were separated by gel electrophoresis. *E. coli* colonies containing the correct plasmid were inoculated into 2-4 ml liquid LB medium for isolation of plasmid DNA and subsequent experiments.

2.8.3. Sequencing

DNA sequencing of plasmids and PCR products were conducted by the Massey Genome Service located in Palmerston North using a 3730 DNA Analyser (applied Biosystems) with BigDye™ Terminator Version 3.1 chemistry (Applied Biosystems).

2.8.4. Fungal transformation

2.8.4.1. Preparation of fungal protoplast

E. festucae mycelia were obtained from fungal liquid cultures incubated at 22°C for 4-5 days shaking at 200 RPM. The mycelia were harvested, washed 3 times with sterile water followed by a final rinse with OM buffer (1.2 M $MgSO_4$, 10 mM $NaHPO_4$, pH to 5.8

using NaH_2PO_4). Lysing enzyme (40ml) (10 ml per 1 mg of mycelia wet weight) from *Trichoderma harzianum* (10 mg/ml stock in OM buffer; Sigma-Aldrich) was filer sterilized and added to the mycelia and incubated overnight at 22°C with shaking at 80 RPM. Samples were checked under the microscope to confirm formation of protoplasts, then filtered through sterile nappy liners and transferred into a sterile bottle. Filtered protoplasts were divided into 15 ml Falcon tubes (5 ml each) and overlaid carefully with 2 ml of ST buffer (0.6 M sorbitol, 100 mM Tris, pH 8.0) using 1 ml tips to form a clear layer. Tubes were spun for 5 min at 4300 RPM at 4°C so protoplasts formed a white layer between the ST buffer and lysis enzyme. Protoplasts were transferred carefully to new tubes using 1 ml filter tips following by addition of 5 ml of STC buffer (1 M sorbitol, 50 mM $CaCl_2$ or $CaCl_2.2H_2O$, 1 M Tris, pH 8.0) and mixed gently. Samples were spun for 5 min at 4300 RPM at 4°C. The washing step with STC buffer was repeated 3 times and samples were pooled after each washing step into a single tube. Protoplasts were diluted in 500 µl of STC buffer and a sample taken for estimation of number using a haemocytometer. The stock solution was diluted to 1.25×10^8 protoplast/ml in STC buffer and divided into 1.5 ml microtubes (80 µl each tubes) and 20 µl of 40% (v/v) polyethylene glycol was added to each tube and stored at -80°C until required (Yelton *et al.*, 1985).

2.8.4.2. Transformation of fungal protoplast

One hundred µl of protoplast aliquots were thawed on ice, and 5 µl of 5 mg/ml filter-sterilized heparin and 2 µl of 50 mM filter-sterilized spermidine was added to each tube and mixed by gentle agitation. Two to five µg of sample DNA solution (maximum volume of 30 µl), positive control DNA or water as the negative control (protoplast viability) was added to each tube. Tubes were incubated on ice for 30 min and 900 µl of 40% (v/v) polyethylene glycol was then added to each tube. Fifty to hundred µl of transformed protoplasts, 100-300 µl positive control transformed protoplasts and negative control (protoplasts only) were mixed into 3.5 ml pre-warmed (50°C) molten 0.8% RG agar medium, spread onto pre-set 1.5% RG solid plates and incubated at 22°C overnight. The following day all plates, except the protoplast viability check plates, were overlaid with 5 ml of molten pre-warmed (50°C) 0.8% RG medium containing the appropriate quantity of antibiotic. Plates were stored for 14 days at 22°C or until colonies began emerging through the overlay. The colonies were transferred to solid PD medium containing an appropriate antibiotic. Cultures were sub-cultured three times to achieve nuclear purification (Young *et al.*, 2005).

2.9. Preparation of constructs

2.9.1. *E. festucae lymA* gene replacement construct

For generation of the *lymA* replacement construct, pNN01, 3' and 5' *lymA* flanks and a hygromycin cassette were amplified from wild type *E. festucae* gDNA and pSF15.15 (appendix.2) respectively using Q5 high fidelity DNA polymerase and the primer pairs NN10/11, NN12/13, and *hph*-F/R respectively. All these flanks (1357 bp *lymA* 5'flank; 1416 bp *lymA* 3' flank; 1394 bp hygromycin cassette) were purified, recombined and assembled in the pRS426 (appendix.1) vector to generate pNN01 plasmid using Gibson assembly (appendix.7). DNA sequencing was performed to check for an error-free construct for subsequent experiments.

2.9.2. *E. festucae lymB* gene replacement construct

For generation of the *lymB* replacement construct, pNN03, 3' and 5' of *lymB* flanks and a geneticin cassette were amplified from wild type *E. festucae* gDNA and pSF17.1 (appendix.3) respectively using Q5 high fidelity DNA polymerase and the primer pairs NN42/43-2, NN44-2/45, and *npt*II-F/R, respectively. All of these flanking regions (1288 bp *lymB* 5'flank /1367 bp *lymB* 3' flank/1741 bp geneticin cassette) were purified, recombined and assembled in pRS426 (appendix.1) vector to generate pNN03 plasmid using Gibson assembly (appendix.9). DNA sequencing was performed to check for an error-free construct for subsequent experiments.

2.9.3. *E. festucae chiA* gene replacement construct

For generation of the *chiA* replacement construct, pNN02, 3' and 5' of *chiA* flanks and a hygromycin cassette were amplified from wild type *E. festucae* gDNA and pSF15.15 (appendix.2) respectively using Q5 high fidelity DNA polymerase and the primer pairs NN18/19, NN20/21, and *hph*-F/R, respectively. These flanks (1131 bp *chiA* 5'flank /1429 bp *chiA* 3' flank/1394 bp hygromycin cassette) were purified, recombined and assembled in pRS426 (appendix.1) vector to generate pNN02 plasmid using Gibson assembly (appendix.8). DNA sequencing was performed to check for an error-free construct for subsequent experiments.

2.9.4. *E. festucae chiB* gene replacement construct

For generation of the *chiB* replacement construct, pNN04, 3' and 5' of *chiB* flanks and a geneticin cassette were amplified from wild type *E. festucae* gDNA and pSF17.1 (appendix.3) respectively using Q5 high fidelity DNA polymerase and the primer pairs NN56/57, NN58/59, and *npt*II-F/R respectively. All these flanks (1257 bp *chiB* 5'flank /1303 bp *chiB* 3' flank/1741 bp geneticin cassette) were purified, recombined and assembled in pRS426 (appendix.1) vector to generate pNN04 plasmid using Gibson assembly (appendix.10). DNA sequencing was performed to check for an error-free construct for subsequent experiments.

2.9.5. *E. festucae lymA* gene localization construct

2.9.5.1. *lymA-eGFP* native promoter expression construct

For generation of the *lymA-eGFP* native promoter expression construct (pNN11) (appendix.12) a 3567 bp PCR purified fragment of the wild type copy of *lymA* containing native promoter (999 bp) and a 5560 bp PCR purified fragment containing $(GGGGS)_2$-eGFP and a geneticin cassette amplified from *E. festucae* wild type gDNA and pNN12 (appendix.13) using Q5 high fidelity DNA polymerase and the primer pairs NN117/NN118 and NN119/NN121 respectively. These two fragments were combined together using Gibson assembly. DNA sequencing was performed to check an error-free construct for subsequent experiments.

2.9.5.2. *lymA-eGFP* over expression construct

For generation of the *lymA-eGFP* over expression promoter construct (pNN12) (appendix.13) a 2623 bp PCR purified fragment of wild type copy of *lymA*, a 5560 bp PCR purified fragment containing $(GGGGS)_2$-eGFP and geneticin cassette and an 817 bp containing *tef* promoter amplified from *E. festucae* wild type g DNA, pBH28 (appendix.6) and pBH18 (appendix.5) using Q5 high fidelity DNA polymerase and the primer pairs NN112/NN118, NN119/NN121 and NN120/BH77 respectively. These three fragments were combined together using Gibson assembly. DNA sequencing was performed to check for an error-free construct for subsequent experiments.

2.9.6. *E. festucae lymB* gene localization construct

2.9.6.1. *lymB-eGFP* native promoter expression construct

For generation of the *lymB-eGFP* native promoter expression construct (pNN113) (appendix.14) a 3239 bp PCR purified fragment of wild type copy of *lymB* containing native promoter (999 bp), a 771 bp PCR purified fragment containing $(GGGGS)_2$-eGFP

and a 5073 bp PCR purified fragment containing hygromycin cassette amplified from *E. festucae* wild type gDNA, pBH28 (appendix.6) and pBH18 (appendix.5) using Q5 high fidelity DNA polymerase and the primer pairs NN122/NN123, NN119/BH115 and BH76/NN121 respectively. These three fragments were combined together using Gibson assembly. DNA sequencing was performed to check for an error-free construct for subsequent experiments.

2.9.6.2. *lymB-eGFP* over expression construct

For generation of the *lymB-eGFP* over expression promoter construct (pNN14) (appendix.15) a 2240 bp PCR purified fragment of wild type copy of *lymB*, a 5870 bp PCR purified fragment containing Geneticin cassette and *tef* promoter and a 771 bp containing $(GGGGS)_2$-eGFP amplified from *E. festucae* wild type g DNA, pBH18 (appendix.5) and pBH28 (appendix.6) using Q5 high fidelity DNA polymerase and the primer pairs NN104/NN123, BH76/BH77 and NN119/BH115 respectively. These three fragments were combined together using Gibson assembly. DNA sequencing was performed to check for an error-free construct for subsequent experiments.

2.9.7. *E. festucae cdaA* gene replacement construct

For generation of the *cdaA* replacement construct, pNN05 (appendix.11), 3' and 5' of *cdaA* flanks and hygromycin cassette were amplified from wild type *E. festucae* gDNA and pDB48 (appendix.4) respectively using Q5 high fidelity DNA polymerase and the primer pairs NN92/93, NN94/95, and *hph*-F, pDB33_7 respectively. All these flanks (1069 bp *cdaA* 5'flank / 1059 bp *cdaA* 3' flank/ 2181 bp hygromycin cassette) purified, recombined and assembled in pRS426 (appendix.1) vector to generate pNN05 plasmid using Gibson assembly. DNA sequencing was performed to check for an error-free construct for subsequent experiments.

2.10. Microscopy

2.10.1. In culture phenotype

For analysis of the culture phenotype cultures were grown on WA. Sterile microscopy slides were placed on pre-set 1.5% WA, overlaid with 5 ml of 1.5% WA. Mycelial plugs were placed near the edge of the slide and kept at 22°C for 7-10 days. A square block of the media containing growth fungal mycelia was extracted and placed on the new slides,

stained with 4-5 µl of Calcofluor white (3 mg/ml) (Fluorescent Brighter 28; Sigma) and covered with a cover slip. Samples were imaged using an Epi-flourescence microscope (Olympus IX83) with a 40x oil immersion objective, NA = 0.075, outfitted with differential interference contrast (DIC) optics and U-MWU2 filtercubes for imaging Calcoflour white. Images were captured with a Retiga 6000M (QImaging) camera using a Bin2x2.

Samples prepared to analyse protein localization were imaged using a confocal laser scanning microscopy (CLSM) using a Leica SP5 DM6000B confocal microscope (488 nm argon laser, 40x oil immersion objective, NA = 1.3) (Leica Microsystems). Images were produced with ImageJ software (NIH). To collapse multiple optical sections into a single image, selected planes were displayed as maximum intensity projections.

2.10.2. *In planta*, cellular phenotype

The cellular phenotype of hyphae *in planta* was determined by analysing leaves using CLSM. Grass pseudostems were cut to 3-5 cm sections, dead leaves and tissues were removed and these sections again cut in half to get longitudinal sections. Sheath samples were transferred to 2 ml microtubes, incubated in 95% ethanol at 4°C overnight. Samples were transferred into new tubes containing 10% w/v KOH at room temperature for 3 h or overnight at 4°C. Samples were carefully washed three times with 1 ml PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) and incubated in staining solution containing (0.02% aniline blue, 10 ng/ml WGA-AF488, 0.02% Tween 20 and 0.002% (w/v) propidium iodide in PBS (pH 7.4)) for 30 min including 10 min staining at room temperature and a 20 min vacuum infiltration step. Hyphal growth and fungal cellular phenotypes were documented by CLSM using a Leica SP5 DM6000B confocal microscope (488 nm argon and 561 nm DPSS laser, 40x oil immersion objective, NA = 1.3) (Leica Microsystems). Images were produced with ImageJ software (NIH). To collapse multiple optical sections into a single image, selected planes were displayed as maximum intensity projections.

2.10.3. Endophyte cell wall composition

2.10.3.1. Biosensor detection of endophyte cell wall composition

2.10.3.1.1. Chitosan Affinity Protein (CAP) labelling *in planta*

Fungal cell wall chitosan distribution was analysed in plant sheath samples using an engineered biosensor. This probe (Chitosan Affinity Protein (CAP)) was formed by

fusing a chitosan affinity protein with GFP, (Nampally *et al.*, 2012). Fixed plant samples in 95% ethanol were incubated in 10% KOH at room temperature for 3 hours or at 4°C for overnight, samples then washed 3 times carefully in 1 ml PBS buffer and incubated in staining solution containing 0.1 mg/ml of CAP (30 mg/ml stock solution in TEA buffer (20 mM triethanolamine (pH 8)), 400 mM NaCl, 10% (v/v) glycerol, 0.02% Tween 20 and 0.002% (w/v) propidium iodide in PBS (pH 7.4). Samples were vacuum infiltrated for 30 min and stored at 4°C overnight before analysing using CLSM. Fungal cell wall composition and chitosan analysis was determined as described above (2.10.2). Images were produced with ImageJ software using the maximum intensity method.

2.10.3.1.2. Chitin Binding Protein (CBP) labelling *in planta*

Presence of chitin in in fungal cell wall was also analysed using the same staining and analysing procedure as described in 2.10.3.1.1, using an engineered Chitin Binding Protein (CBP) which was formed by fusion of a chitin binding domain from a *Bacillus lichenformis* chitinase to GFP (Fuenzalida et al, 2014).

2.10.3.1.3. CAP labelling in culture

Chitosan distribution in hyphal cell wall grown in axenic culture were also analysed. Conidia were isolated from *E. festucae* Fl1 or *E. festucae* E2368 strains grown on PD agar for 6 days. Fifty µl of conidia suspension was spread on sterile microscopy slides placed on pre-set 1.5% WA solid media (2.10.1). A block of media containing mycelia developed form single colonies was washed 3 times carefully in 1 ml PBS buffer and incubated in staining solution containing 0.1 mg/ml of CAP, 0.02% Tween 20 and 0.002% (w/v) propidium iodide in PBS (pH 7.4). Analyses of the samples were performed as described above (2.10.3.1.1).

2.10.3.1.4. WGA-AF488 and CBP labelling in culture

For analysis the fungal cell wall composition and chitin distribution of hyphal cell wall grown on the axenic culture, a block of mycelia was grown on WA (2.10.1) for 6 days and then stained with WGA-AF488 or CBP independently. Samples were washed 3 times carefully in 1 ml PBS buffer and incubated in staining solution containing 0.1 mg/ml of CBP or WGA-AF488, 0.02% Tween 20 in PBS (pH 7.4). Samples were analysed using the same microscopy settings described as above (2.10.3.1.1).

2.10.3.2. Oligogalactrunate (OGA-488) labelling

2.10.3.2.1. OGA-488 labelling *in planta*

Endophyte cell wall composition was also analysed using OGA-488 (oligogalacturonate-Alexa Fluor 488) (Mravec *et al.*, 2014). The same staining procedure was applied (2.10.2) and plant sheath samples were stained, and vacuum infiltrated for 15 min in staining solution containing 0.1% (v/v) OGA-488 (1 mg/ml stock solution in 0.1 M sodium acetate buffer pH 4.9), 0.02% Tween 20 and 0.002% (w/v) propidium iodide in PBS (pH 7.4). Fungal cell wall chitosan labelling was determined and imaged by CLSM using the same microscopy setting as described in 2.10.3.1.1.

2.10.3.2.2. OGA-488 labelling *in planta*

Hyphal cell wall composition was also analysed using OGA-488 using a different staining procedure (Geoghegan & Gurr, 2016). Plant sheath samples fixed in 95% ethanol were washed briefly with 25 mM MES buffer (pH 5.6). Samples then were stained and vacuum infiltrated for 15 min in staining solution containing 0.1% (v/v) OGA-488 (1 mg/ml stock solution in 0.1 M sodium acetate buffer pH 4.9), 0.02% Tween 20 and 0.002% (w/v) propidium iodide in 25 mM MES buffer (pH 5.6). Fungal cell wall chitosan labelling was determined and imaged by CLSM using a Zeiss LSM800 confocal microscope (488 nm argon and 561 nm DPSS laser, ×63 oil immersion objective). Images were produced with ImageJ software using the maximum intensity method.

2.11. Bioinformatics

2.11.1. Nucleotide sequence resources

E. festucae genes encoding LysM motif proteins and chitin deacetylases were obtained from http://Epichloë.massey.ac.nz/pfam_annotations in *E. festucae* (E2368) genome and the domain structures of the encoded products annotated using Pfam. *E. festucae* (Fl1) as well as other species gene and protein sequences were obtained from the Kentucky genome databases (http://www.endophyte.uky.edu) as curated by C. L. Schardl at the University of Kentucky (Schardl *et al.*, 2013). The proposed gene models were validated based on HMM-based gene structure prediction by FGENESH (http://www.softberry.com). The other *Epichloë* and Clavicipitacae genome sequences used in this study and utilised in the synteny analysis are also available on the Clavicipitaceae genome database (http://www.endophyte.uky.edu/) provided and

maintained by Prof. Chris Schardl, University of Kentucky. The *Magnaporthe oryzea* chitin deacetylase gene sequences (Geoghegan & Gurr, 2016) used in this study to perform tBLASTn against the Fl1 genome were obtained through FungiDB, Fungal and Oomycete Genomics Resources at http://fungidb.org/fungidb.

2.11.2. Multiple sequence alignment

Alignment of nucleotide and amino acid sequences were performed with ClustalW (Larkin *et al.*, 2007) as provided with MacVector 14.5 (MacVector, Inc.) and using default parameters. The DNA and amino acid sequence alignments utilised for synteny analyses were performed using the MAFFT (v7.017) algorithm (Katoh *et al.*, 2002; Katoh & Standley, 2013).

2.11.3. Protein structure prediction

SignalP (v. 4.1) (Petersen *et al*., 2011, Nielsen *et al*., 1997) was used for detection of the N terminal signal peptide and TMHMM (v. 2.0c) (Krogh *et al*., 2001, Sonnhammer *et al*., 1998) was used fordetection of transmembrane domains. Domain analysis was also performed using InterProScan (v. 71.0) (Quevillon *et al*., 2005, Zdobnov & Apweiler, 2001), PredGPI (Pierleoni *et al*, 2008) was used to check for GPI-lipid anchor modification sites.

2.12. A core gene set for mutualism and antagonism

High-throughput mRNA sequencing was performed on three biological replicates for each of the wild-type, Δ*noxA,* Δ*proA* and Δ*sakA* plant associations to identify key genes significantly up or down regulated compared to Fl1 which may be essential for symbiosis (Eaton *et al.,* 2015). Differences between the three mutant data sets and their associated wild-type samples were then compared to identify a core set of *E. festucae* genes that show common differential expression patterns in all three mutants. Only those genes that exhibited differential expression in the same direction (all upregulated or all downregulated) were included. This analysis identified a core gene set of 182 genes, based on statistically significant differences (Corrected $P < 0.05$) and a two-fold change cutoff. Of the 182 genes in the core gene set, 143 were upregulated and 39 downregulated in the antagonistic mutants relative to the wild type (Eaton *et al.,* 2015).

Chapter 3. Bioinformatic analysis of genes encoding LysM motifs and chitinases

3.1. Identifying genes encoding LysM proteins in the *Epichloë festucae* genome

Genes encoding LysM proteins were identified by Pfam annotation analysis through http://Epichloë.massey.ac.nz/pfam_annotations. Seven genes encoding proteins with LysM domains were identified in the *E. festucae* (E2368) genome. All seven genes were also found in the *E. festucae* Fl1 genome at http://www.endophyte.uky.edu. The deduced domain structure of the proteins encoded by these genes showed that there were various numbers of LysM domains from one in EfM3.010700 and EfM3.061280, two in EfM3.029340 and EfM3.062860, three in EfM3.078100 and four in EfM3.000820 and EfM3.035020. All, except EfM3.010700 and EfM3.061280 had predicted N terminal signal peptides suggesting they are candidates for secretion into the apoplastic space (Figure 3.1).

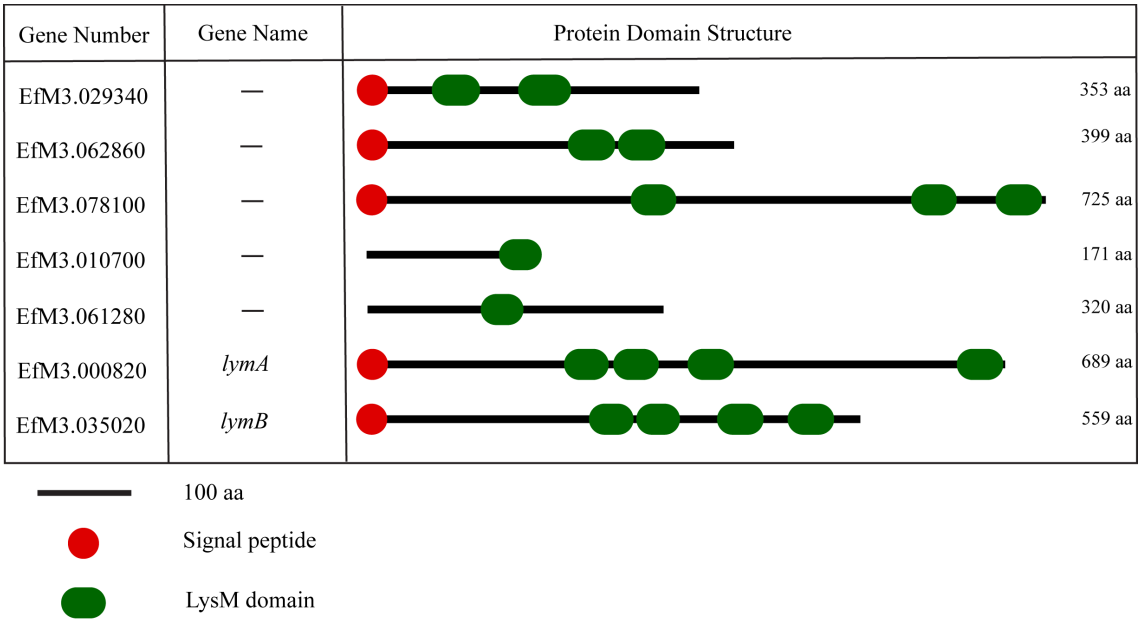


Figure 3.1. *E. festucae* Fl1 LysM predicted polypeptide structure. Protein domain structure of *E. festucae* LysM proteins showing the predicted signal peptide and LysM domains. Bar=100 amino acids.

To gain further insight into which genes might have a crucial role in the symbiotic interaction gene expression data was analysed. Analysis of RNA seq data of *E. festucae* gene expression *in planta* compared to in culture showed that five out of the seven genes were significantly increased *in planta* compared to in culture (Table 3.1). Further analysis of RNA seq data generated for three symbiotic mutants, *noxA*, *proA* and *sakA in planta* compared to wild-type showed that two genes (EfM3.000820 and EfM3.035020) were significantly downregulated in the *noxA* and *proA* data set. They were also

downregulated in the *sakA* mutant, but the change was not significant. These two genes were designated as *lymA* and *lymB* respectively (2.12) (Table 3.2). Checking the genome organization of *lymA* and *lymB* at http://www.endophyte.uky.edu revealed that both are divergently transcribed from genes encoding chitinases in the *E. festucae* Fl1 genome (EfM3.000810 and EfM3.035030). These two chitinase genes were named as *chiA* and *chiB* based on their linkage with *lymA* and *lymB* on the genome (Figure 3.2). Like *lymA* and *lymB* the expression of both chitinase genes were significantly increased *in planta* compared to in culture (Table 3.1). Additional analysis of the RNA seq data generated for *noxA*, *proA* and *sakA* symbiotic mutants *in planta* compared to wild-type showed that *chiA* was significantly downregulated in all three symbiotic mutants whereas *chiB* was significantly downregulated in just the *noxA* and *proA* mutants. While *chiB* was also downregulated in the *sakA* data set the change was not significant (Table 3.2). Analysis of the domain structure of the proteins encoded by *chiA* and *chiB* using InterPro scan at https://www.ebi.ac.uk/interpro revealed that both proteins are members of the glycoside hydrolase family 18 (GH18) with a secretion signal, chitin binding and chitinase insertion domains (Figure 3.3).

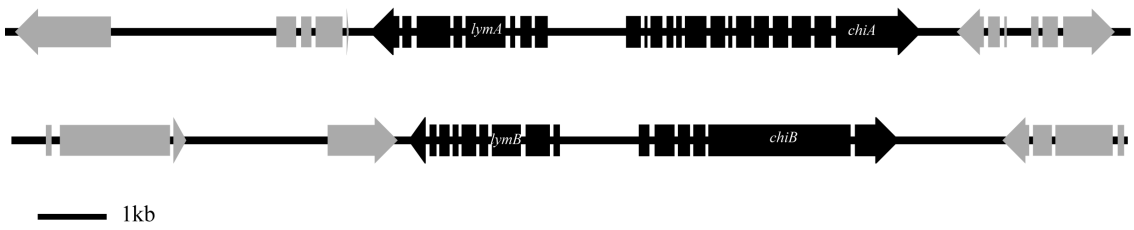


Figure 3.2. Gene maps of *E. festucae* Fl1 *lymA*-*chiA* and *lymB*-*chiB* loci. Fl1 *lymA* and *lymB* contain 8 and 9 exons respectively and are divergently transcribed from *chiA* and *chiB*, which contain 14 and 6 exons. Bar=1000 bp.

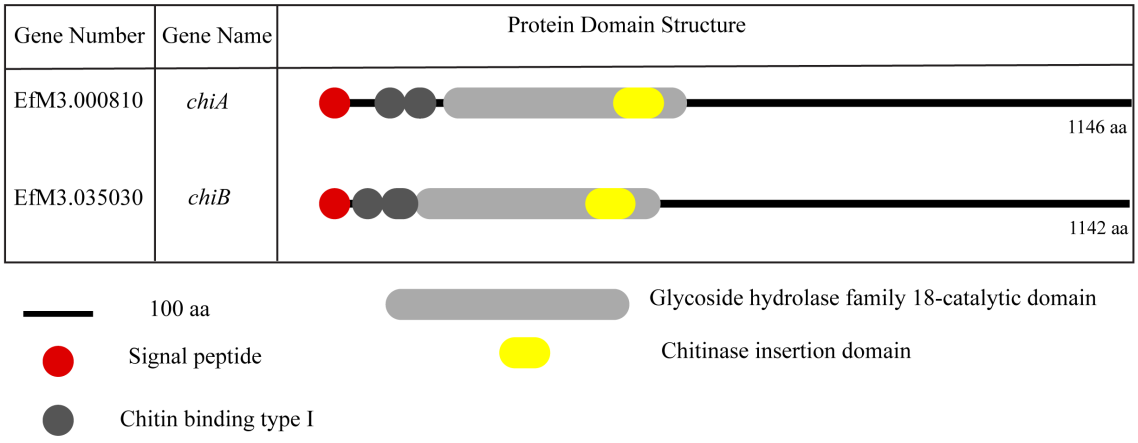


Figure 3.3. *E. festucae* Fl1 ChiA and ChiB predicted polypeptide structure. Domain structure of Fl1 ChiA and ChiB showing the predicted signal peptide, chitin binding type I, Glycoside hydrolase family 18 catalytic domain and chitinase insertion domains. Bar=100 amino acids.

Table 3.1. Fold differences of expression of *lysM* and *chitinase* genes *in planta* compared to in culture

Gene Model	Gene Name	Culture_RPKM [a]	*In Planta*_RPKM [a]	Ratio	Direction	Fold Diff
EfM3.029340		280.462	8260.020	29.451	+	29.451
EfM3.062860		8.889	6.863	0.772	-	1.295
EfM3.010700		26.509	55.115	2.079	+	2.079
EfM3.078100		12.264	11.955	0.975	-	1.026
EfM3.061280		0.000	0.000	1.000	+	1.000
EfM3.000820	*lymA*	8.590	310.551	36.151	+	36.151
EfM3.035020	*lymB*	4.497	5.669	1.261	+	1.261
EfM3.000810	*chiA*	4.373	143.571	32.834	+	32.834
EfM3.035030	*chiB*	0.948	2.485	2.623	+	2.623

[a] Data obtained from T. Chujo (in preparation). Direction indicates the level of expression change. Minus and plus indicate the direction of change (down regulated and up regulated respectively). RPKM: Reads Per Kilobase of transcript per Million mapped reads.

Table 3.2. Gene expression fold changes of *lysM* and *chitinase* genes in *E. festucae* genome between wild type and symbiotic mutants Δ*noxA*, Δ*proA* and Δ*sakA*.

Gene Model	Gene Name	*noxA* dir [b]	*noxA* Fold [a]	*proA* dir [b]	*proA* dir [b]	*sakA* dir [b]	*sakA* Fold [a]
EfM3.029340		+	1.52	+	2.40	+	1.16
EfM3.062860		-	1.12	+	1.07	+	2.52
EfM3.010700		+	1.16	+	1.8	+	2.57
EfM3.078100		-	1.06	+	1.05	+	1.08
EfM3.061280			NA	-	1.38		NA
EfM3.000820	*lymA*	-	2.72	-	2.21	-	1.01
EfM3.035020	*lymB*	-	NA	-	5.53	-	3.17
EfM3.000810	*chiA*	-	3.98	-	3.82	-	2.88
EfM3.035030	*chiB*	-	10.80	-	3.69	-	1.59

[a] Fold changes in red and green are significant whereas those in black are not significant.

[b] Direction indicates the level of expression change. Minus and plus indicate the direction of change (down regulated and up regulated respectively).

Data obtained from Eaton *et al*, 2015.

Analysing the exon and intron structure of *lymA*, *lymB*, *chiA* and *chiB* using RNA seq data at http://www.endophyte.uky.edu revealed that the cDNAs of these genes encode proteins of 689, 559, 1146, 1142 amino acids with predicted unmodified molecular weights of 73.38, 60.44, 125.15 and 124.08 kD respectively (Figure 3.1 and 3.3).

3.2. Synteny analysis of *lymA* and *chiA* genes across the Clavicipitaceae

To check for the presence of *lymA* and *chiA* homologues in other Epichloë species genomes within the Clavicipitaceae a BLASTn search was conducted using the nucleotide sequences of *lymA*, *chiA* and two other genes located in the same contig as the *lymA* (nitrogen metabolite repression and muramidase) and two genes located in the same contig as the *chiA* (FCF and replication factor C) from the Fl1 genome as query. The first and second lowest E-value of Fl1 against the other Epichloë species within Clavicipitaceae family showed that *lymA* was not *lymB* homologues and these two genes were not duplicated (Table 3.3). BLASTn analysis revealed the presence of homologous genes of the *lymA*, *chiA* and the other four linked genes in different Epichloë and Claviceps species (Table 3.3). These sequences were then used to check the gene structure

and synteny in other Epichloë species, which revealed that these genes share conserved synteny in some of the species in Clavicipitaecae (Figure 3.4). A nucleotide sequence alignment done to check the gene structure in these species also revealed that there are gene deletion in genes homologous to *lymA* in *E. amarillans* E57, E4668, *E. brachylyetri*, *E. elymi*, *E. typhina* and *C. purpurea* (Figures 3.4 and Appendix 16). However, checking the protein structure encoded by all these *lymA* genes in Epichloë and Claviceps species showed that despite the gene deletions they all appear to be functional, as the virtual translation of these genes shows that they can be translated into proteins. Domain analysis of the encoded products which had the same gene organization as Fl1 with genes encoding LysM motifs divergently transcribed from genes encoding chitinase genes showed that LysM proteins in these species also carried different numbers of LysM domains which varied from one in *E. brachyelytri* and *E. elymi* and five in *E. sylvatica.* Only LysM proteins encoded with *E. festucae* Fl1*, E. festucae* E2368*, E. baconii, E. sylvatica, E. typhina* and *C. purpurea* contained N terminal signal peptides (Figure 3.5). The proteins without an N terminal signal peptide and predicted not to be secreted were excluded from the amino acid sequence alignment and the alignment was conducted only on the LysM proteins including N terminal signal peptide and the results showed that the LysM motifs are highly conserved among these species (Figure 3.6).

Table 3.3. BLASTn analysis of genes at the *lymA-chiA* locus in *E. festucae* Fl1 against other sexual Epichloë and related species.

subject genome		*Nitrogen metabolite repression*			*Muramidase*			*lymA*			*chiA*			*FCF1*			*Replication factor C*		
Species	**strain**	**a**	**b**	**contig**	**a**	**b**	**contig**	**a**	**b**	**contig**	**a**	**b**	**contig**	**a**	**b**	**contig**	**a**	**b**	**contig**
Epichloë amarillans	ATCC 200744	0	2.00E-04	140	0	2	140	0	0.32	140	0	0.53	140	0	0.022	140	0	0.15	140
Epichloë amarillans	E4668	0	2.00E-04	6	0	2.1	6	0	0.34	6	0	0.56	6	0	0.024	6	0	0.16	6
Epichloë baconii	ATCC 200745	0	7.00E-04	122	0	0.51	122	0	0.32	122	0	0.53	122	0	0.35	122	0	0.15	122
Epichloë brachyelytri	E4804	0	8.00E-04	30	0	0.59	30	0	0.37	30	0	2.4	30	0	0.4	30	0	0.17	30
Epichloë bromicola	AL0426/2	0	2E.00-04	363	0	0.44	363	8.00E-54	0.27	363	1.00-E72	0.45	363	0	0.3	363	0	0.13	363
Epichloë bromicola	AL0434	0	0.003	1160	1.00E-154	0.51	1160	1.00E-53	0.32	1160	1.00E-78	0.53	1160	0	0.35	1160	0	0.15	1160
Epichloë bromicola	ATCC 200750	0	1.00E-04	165	1.00E-154	0.37	6067	1.00E-21	0.23	6067	2.00E-70	0.38	13614	0	0.25	9644	0	0.11	9027
Epichloë elymi	ATCC 201551	0	1.00E-04	195	1.00E-180	0.38	195	0	0.24	195	1.00E-121	0.39	195	0	0.066	195	0	0.11	195
Epichloë festucae	E2368	0	2.00E-04	28	0	0.46	28	0	0.29	28	0	0.48	28	0	0.02	28	0	0.13	28
Epichloë festucae	Fl1	0	2.00E-04	96	0	0.46	96	0	0.29	96	0	0.48	96	0	0.02	96	0	0.14	96
Epichloë glyceria	ATCC 200747	0	0.001	285	0	2.6	285	0	0.41	285	9.00E-22	2.7	285	0	0.45	285	0	0.19	285
Epichloë sylvatica	GR 10156	0	8.00E-04	19	0	2.2	19	0	0.34	19	0	0.14	19	0	0.024	19	0	0.16	19
Epichloë typhina	ATCC 200736	0	1.00E-05	298	0	2.2	314	0	0.34	314	0	0.15	314	0	0.095	314	0	0.16	314
Metarhizium acridum	CQMa 102	8.00E-84	1.00E-05	111	1.00E-174	2	566	0.32	0.32	1260	0.53	0.53	340	1.00E-25	0.022	111	1.00E-123	0.009	111
Metarhizium robertsii	ARSEF 23	2.00E-62	0.003	794	0	0.52	586	4.00E-84	1.3	586	2.1	2.1	858	3.00E-23	0.09	794	1.00E-121	0.01	794
Claviceps fusiformis	PRL 1980	0	2.00E-04	2274	3.00E-09	2.7	88	0.11	0.43	130	9.00E-22	0.18	88	1.00E-112	0.12	307	0	0.2	307
Claviceps paspali	RRC 1481	0	1.00E-04	107	0.097	0.38	728	0.061	0.95	30	0.1	0.1	1085	1.00E-108	0.017	107	0	5.00E-04	107
Claviceps purpurea	20.1	0	0.002	114	3.00E-05	0.11	789	1.00E-43	1.1	789	1.00E-66	0.02	789	1.00E- 110	0.074	114	1.00E-148	0.13	114
Atkinsonella texensis	B6155	E-162	1.00E-04	380	2E.00-46	0.095	78	7.00E-51	0.24	78	0.025	0.02	676	5.00E-98	0.017	380	1.00E-149	0.43	380
Atkinsonella texensis	B6156	E-162	1.00E-04	166	2E.00-46	0.095	815	7.00E-51	0.24	815	0.025	0.02	526	5.00E-98	0.016	166	1.00E-149	0.43	166
Periglandula ipomoeae	IasaF13	E-158	0.003	331	0	1.9	52	0.29	0.29	100	1.00E-107	0.12	52	1.00E-115	0.32	331	1.00E-165	0.54	787

[a]Lowest E-value. [b]Second lowest E-value.

Genes located in the same contig as *lymA* and *chiA* are highlighted in green whereas those in different contigs are highlighted in yellow. The second strongest match against the Fl1 genome was used as a minimum-similarity reference to identify putative homologs in the other species.

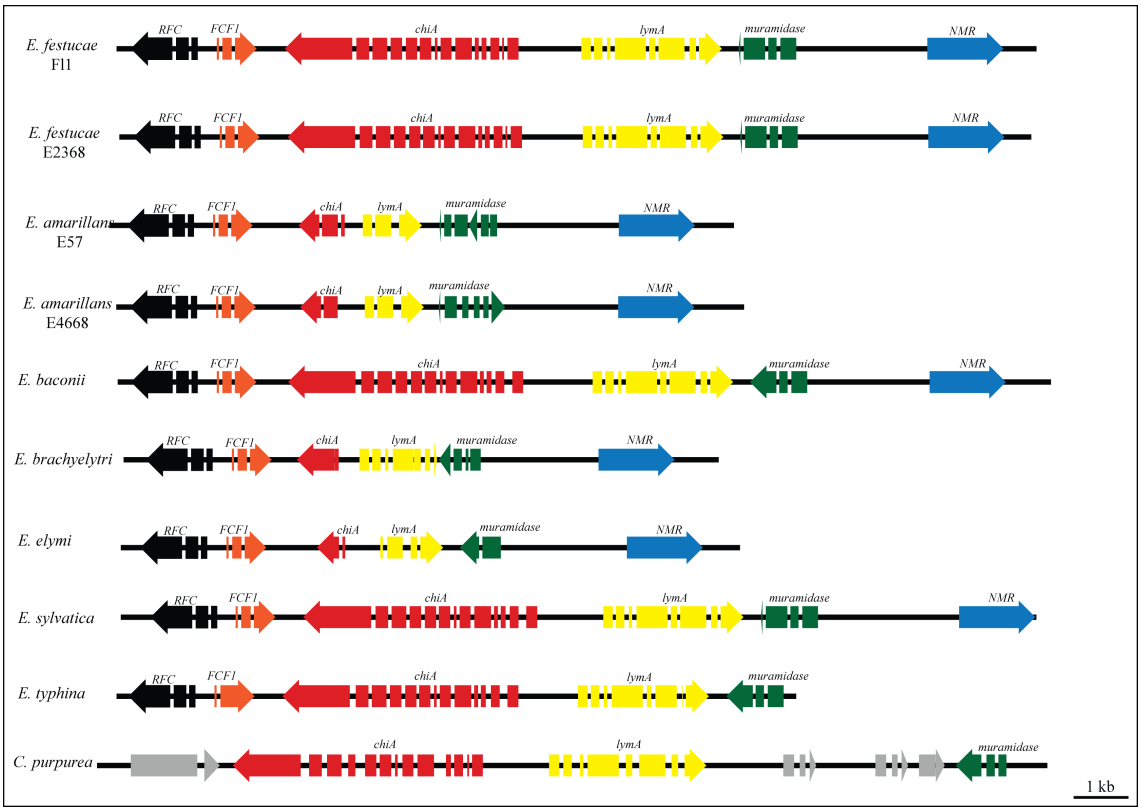


Figure 3.4. Synteny analysis of genes at the *lymA-chiA* locus in *E. festucae* Fl1, other sexual *Epichloë* species and *Claviceps purpurea*. Homologous genes share a single colour across species. Arrows represent the direction of transcription. Sequence data was sourced from http://www.endophyte.uky.edu. Bar= 1 kb.

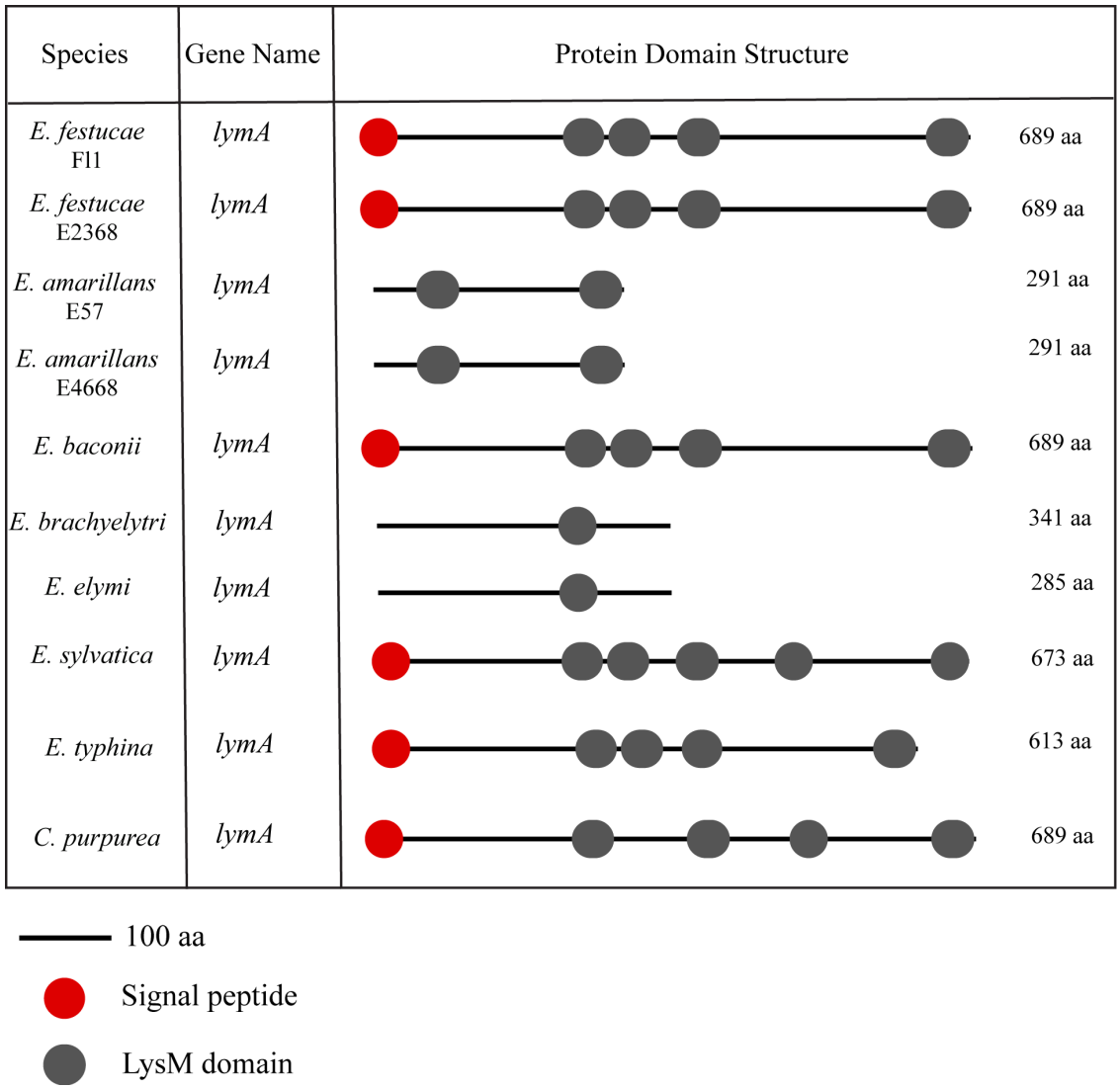
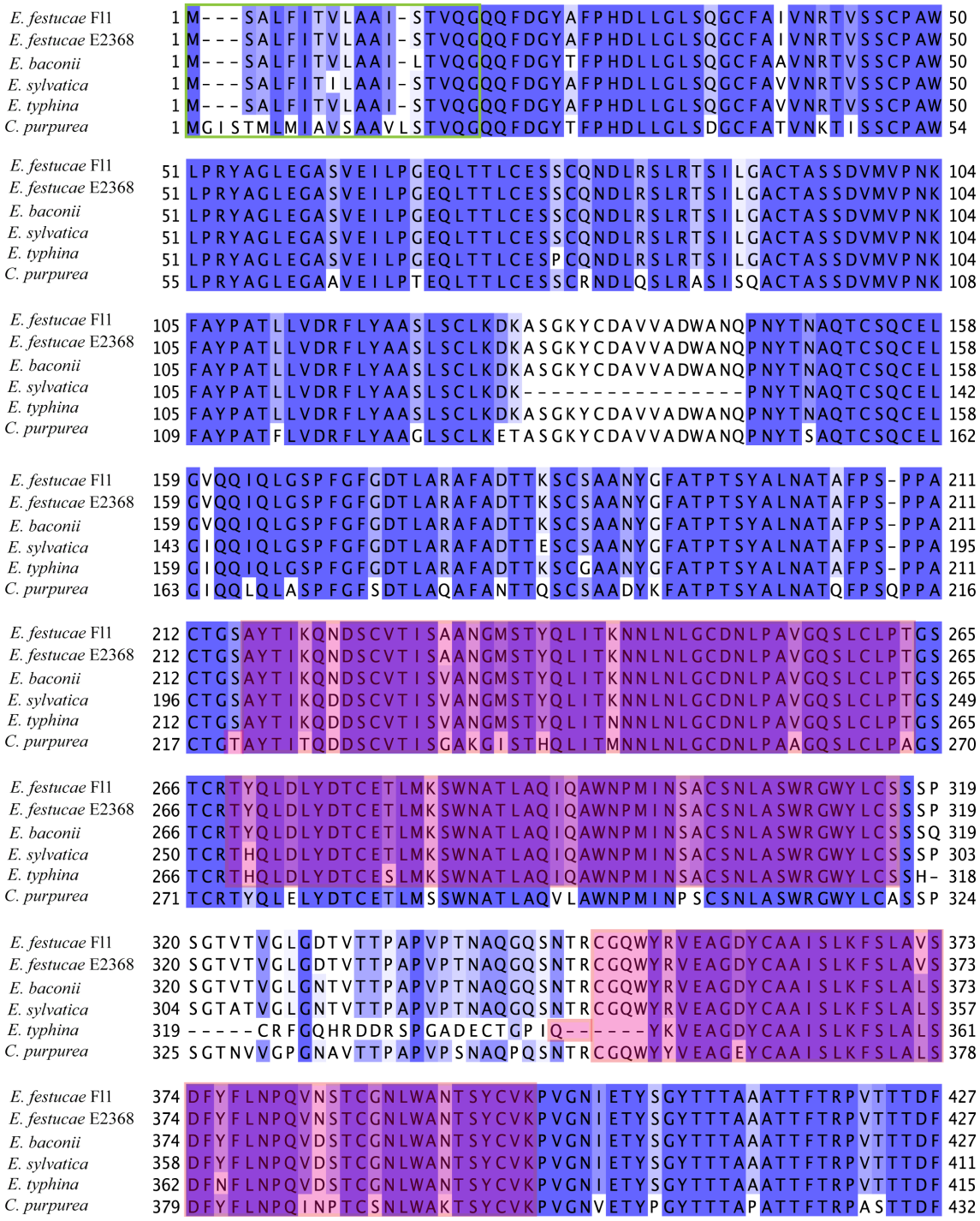


Figure 3.5. LysM protein predicted structure. *E. festucae* LymA protein and homologues from other *Epichloë* species and *Claviceps purpurea* showing the predicted signal peptide and LysM domains. Bar=100 amino acids.


E. festucae Fl1 1 M---SALFITVLAAI-STVQGQQFDGYAFPHDLLGLSQGCFAIVNRTVSSCPAW 50
E. festucae E2368 1 M---SALFITVLAAI-STVQGQQFDGYAFPHDLLGLSQGCFAIVNRTVSSCPAW 50
E. baconii 1 M---SALFITVLAAI-LTVQGQQFDGYTFPHDLLGLSQGCFAAVNRTVSSCPAW 50
E. sylvatica 1 M---SALFITILAAI-STVQGQQFDGYAFPHDLLGLSQGCFAVVNRTVSSCPAW 50
E. typhina 1 M---SALFITVLAAI-STVQGQQFDGYAFPHDLLGLSQGCFAVVNRTVSSCPAW 50
C. purpurea 1 MGISTMLMIAVSAAVLSTVQGQQFDGYTFPHDLLGLSDGCFATVNKTISSCPAW 54
E. festucae Fl1 51 LPRYAGLEGASVEILPGEQLTTLCESSCQNDLRSLRTSILGACTASSDVMVPNK 104
E. festucae E2368 51 LPRYAGLEGASVEILPGEQLTTLCESSCQNDLRSLRTSILGACTASSDVMVPNK 104
E. baconii 51 LPRYAGLEGASVEILPGEQLTTLCESSCQNDLRSLRTSILGACTASSDVMVPNK 104
E. sylvatica 51 LPRYAGLEGASVEILPGEQLTTLCESSCQNDLRSLRTSILGACTASSDVMVPNK 104
E. typhina 51 LPRYAGLEGASVEILPGEQLTTLCESPCQNDLRSLRTSILGACTASSDVMVPNK 104
C. purpurea 55 LPRYAGLEGAAVEILPTEQLTTLCESSCRNDLQSLRASISQACTASSDVMVPNK 108
E. festucae Fl1 105 FAYPATLLVDRFLYAASLSCLKDKASGKYCDAVVADWANQPNYTNAQTCSQCEL 158
E. festucae E2368 105 FAYPATLLVDRFLYAASLSCLKDKASGKYCDAVVADWANQPNYTNAQTCSQCEL 158
E. baconii 105 FAYPATLLVDRFLYAASLSCLKDKASGKYCDAVVADWANQPNYTNAQTCSQCEL 158
E. sylvatica 105 FAYPATLLVDRFLYAASLSCLKDK----------PNYTNAQTCSQCEL 142
E. typhina 105 FAYPATLLVDRFLYAASLSCLKDKASGKYCDAVVADWANQPNYTNAQTCSQCEL 158
C. purpurea 109 FAYPATFLVDRFLYAAGLSCLKETASGKYCDAVVADWANQPNYTSAQTCSQCEL 162
E. festucae Fl1 159 GVQQIQLGSPFGFGDTLARAFADTTKSCSAANYGFATPTSYALNATAFPS-PPA 211
E. festucae E2368 159 GVQQIQLGSPFGFGDTLARAFADTTKSCSAANYGFATPTSYALNATAFPS-PPA 211
E. baconii 159 GVQQIQLGSPFGFGDTLARAFADTTKSCSAANYGFATPTSYALNATAFPS-PPA 211
E. sylvatica 143 GIQQIQLGSPFGFGDTLARAFADTTESCSAANYGFATPTSYALNATAFPS-PPA 195
E. typhina 159 GIQQIQLGSPFGFGDTLARAFADTTKSCGAANYGFATPTSYALNATAFPS-PPA 211
C. purpurea 163 GIQQLQLASPFGFSDTLAQAFANTTQSCSAADYKFATPTSYALNATQFPSQPPA 216
E. festucae Fl1 212 CTGSAYTIKQNDSCVTISAANGMSTYQLITKNNLNLGCDNLPAVGQSLCLPTGS 265
E. festucae E2368 212 CTGSAYTIKQNDSCVTISAANGMSTYQLITKNNLNLGCDNLPAVGQSLCLPTGS 265
E. baconii 212 CTGSAYTIKQNDSCVTISVANGMSTYQLITKNNLNLGCDNLPAVGQSLCLPTGS 265
E. sylvatica 196 CTGSAYTIKQDDSCVTISVANGMSTYQLITKNNLNLGCDNLPAVGQSLCLPTGS 249
E. typhina 212 CTGSAYTIKQDDSCVTISVANGMSTYQLITNNNLNLGCDNLPAVGQSLCLPTGS 265
C. purpurea 217 CTGTAYTITQDDSCVTISGAKGISTHQLITMNNLNLGCDNLPAAGQSLCLPAGS 270
E. festucae Fl1 266 TCRTYQLDLYDTCETLMKSWNATLAQIQAWNPMINSACSNLASWRGWYLCSSSP 319
E. festucae E2368 266 TCRTYQLDLYDTCETLMKSWNATLAQIQAWNPMINSACSNLASWRGWYLCSSSP 319
E. baconii 266 TCRTYQLDLYDTCETLMKSWNATLAQIQAWNPMINSACSNLASWRGWYLCSSSQ 319
E. sylvatica 250 TCRTHQLDLYDTCETLMKSWNATLAQIQAWNPMINSACSNLASWRGWYLCSSSP 303
E. typhina 266 TCRTHQLDLYDTCESLMKSWNATLAQIQAWNPMINSACSNLASWRGWYLCSSH- 318
C. purpurea 271 TCRTYQLELYDTCETLMSSWNATLAQVLAWNPMINPSCSNLASWRGWYLCASSP 324
E. festucae Fl1 320 SGTVTVGLGDTVTTPAPVPTNAQGQSNTRCGQWYRVEAGDYCAAISLKFSLAVS 373
E. festucae E2368 320 SGTVTVGLGDTVTTPAPVPTNAQGQSNTRCGQWYRVEAGDYCAAISLKFSLAVS 373
E. baconii 320 SGTVTVGLGNTVTTPAPVPTNAQGQSNTRCGQWYRVEAGDYCAAISLKFSLALS 373
E. sylvatica 304 SGTATVGLGNTVTTPAPVPTNAQGQSNTRCGQWYRVEAGDYCAAISLKFSLALS 357
E. typhina 319 -----CRFGQHRDDRSPGADECTGPIQ-------YKVEAGDYCAAISLKFSLALS 361
C. purpurea 325 SGTNVVGPGNAVTTPAPVPSNAQPQSNTRCGQWYYVEAGEYCAAISLKFSLALS 378
E. festucae Fl1 374 DFYFLNPQVNSTCGNLWANTSYCVKPVGNIETYSGYTTTAAATTFTRPVTTTDF 427
E. festucae E2368 374 DFYFLNPQVNSTCGNLWANTSYCVKPVGNIETYSGYTTTAAATTFTRPVTTTDF 427
E. baconii 374 DFYFLNPQVDSTCGNLWANTSYCVKPVGNIETYSGYTTTAAATTFTRPVTTTDF 427
E. sylvatica 358 DFYFLNPQVDSTCGNLWANTSYCVKPVGNIETYSGYTTTAAATTFTRPVTTTDF 411
E. typhina 362 DFNFLNPQVDSTCGNLWANTSYCVKPVGNIETYSGYTTTAAATTFTRPVTTTDF 415
C. purpurea 379 DFYFLNPQINPTCSNLWAKTSYCVKPVGNVETYPGYTTTAPATTFTRPASTTDF 432

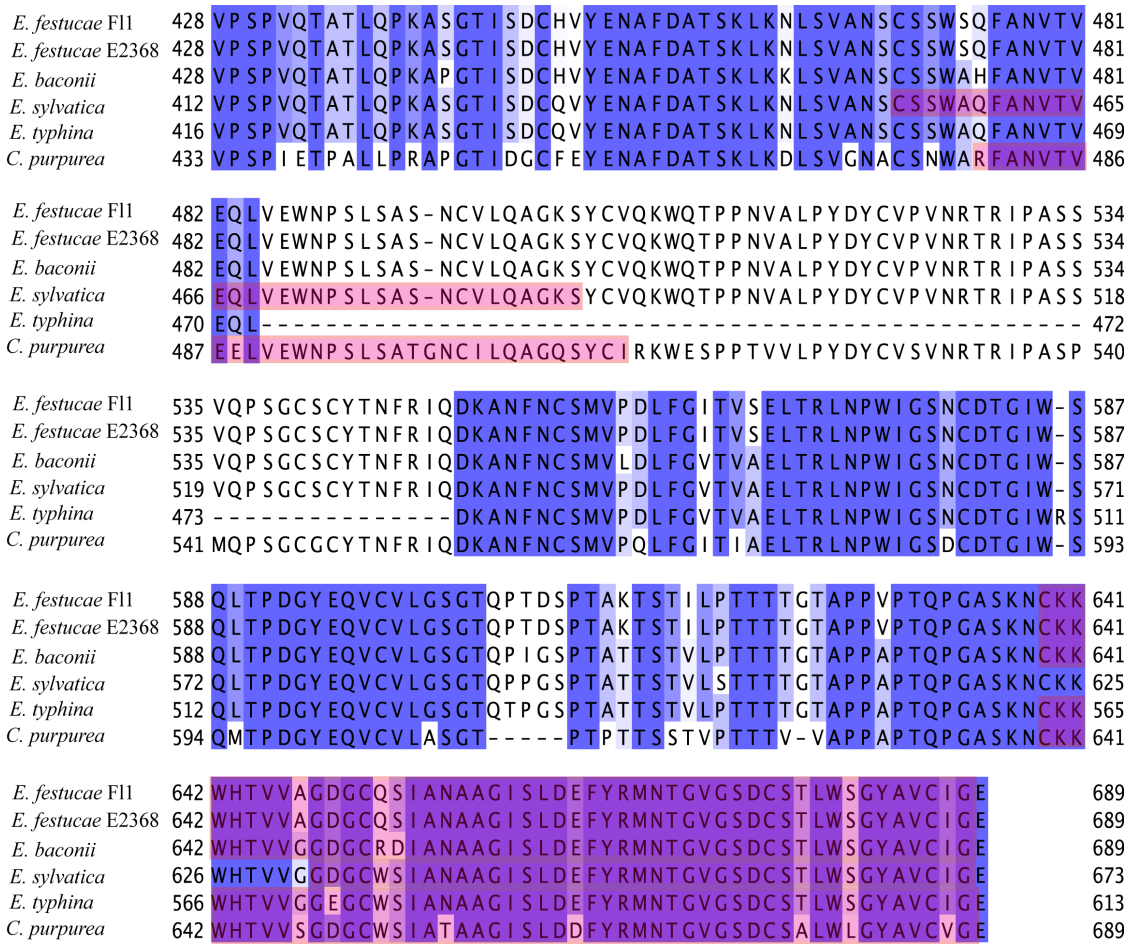


Figure 3.6. Multiple sequence alignment of *E. festucae* LymA protein and homologues from other *Epichloë* species and *Claviceps purpurea*. *E. festucae* (Fl1) EfM3.000820, E. *festucae* (E2368), *E. baconii, E. sylvatica, E. typhina,* and *C. purpurea.* Intensity of colouring indicates the level of conservation. N terminal signal peptides are bordered in green. LysM motifs are indicated in pink.

Gene structure analysis and nucleotide sequence alignment of the *chiA* genes divergently transcribed with *lymA* genes in Epichloë and Claviceps species showed that although there was conservation of synteny among these species, there were gene deletions in *E. amarillans* E57 and E4668, *E brachylyetri*, *E. elymi*, *E baconii*, *E. sylvatica*, *E. typhina* and *C. purpurea* (Figure 3.4 and Appendix 17). However, checking the protein structure encoded by these *chiA* genes in Epichloë and Claviceps species showed that despite the gene deletions they all appear to be functional. The domain structure of the proteins encoded by *chiA* genes revealed that some of these proteins are part of glycoside hydrolase family 18 (GH18), containing chitin binding, chitinase insertion and glycoside hydrolase family 18 catalytic domains including *E. festucae* Fl1, E2368, *E. baconii*, *E. sylvatica*, *E. typhina* and *C. purpurea* with N terminal signal peptide prediction in all of these species. Protein domain analysis of the genes divergently transcribed with *lymA* in *E. amarillans* E57, E4668, *E. brachylyetri* and *E. elymi* using InterPro scan at https://www.ebi.ac.uk/interpro showed that there was not any specific protein domain predicted for these proteins, and just the protein encoded with *E. amarillans* E57 included an N terminal signal peptide, although tBLASTn analysis of these protein sequences conducted at https://www.ncbi.nlm.nih.gov/ showed that they are chitinase proteins (Fig 3.7). The proteins without any specific predicted domains were excluded from further analysis and amino acid sequence alignment was conducted on GH18 chitinase proteins and the results showed that they are conserved in chitin binding, chitinase insertion and glycoside hydrolase family 18 catalytic domains (Figure 3.8).

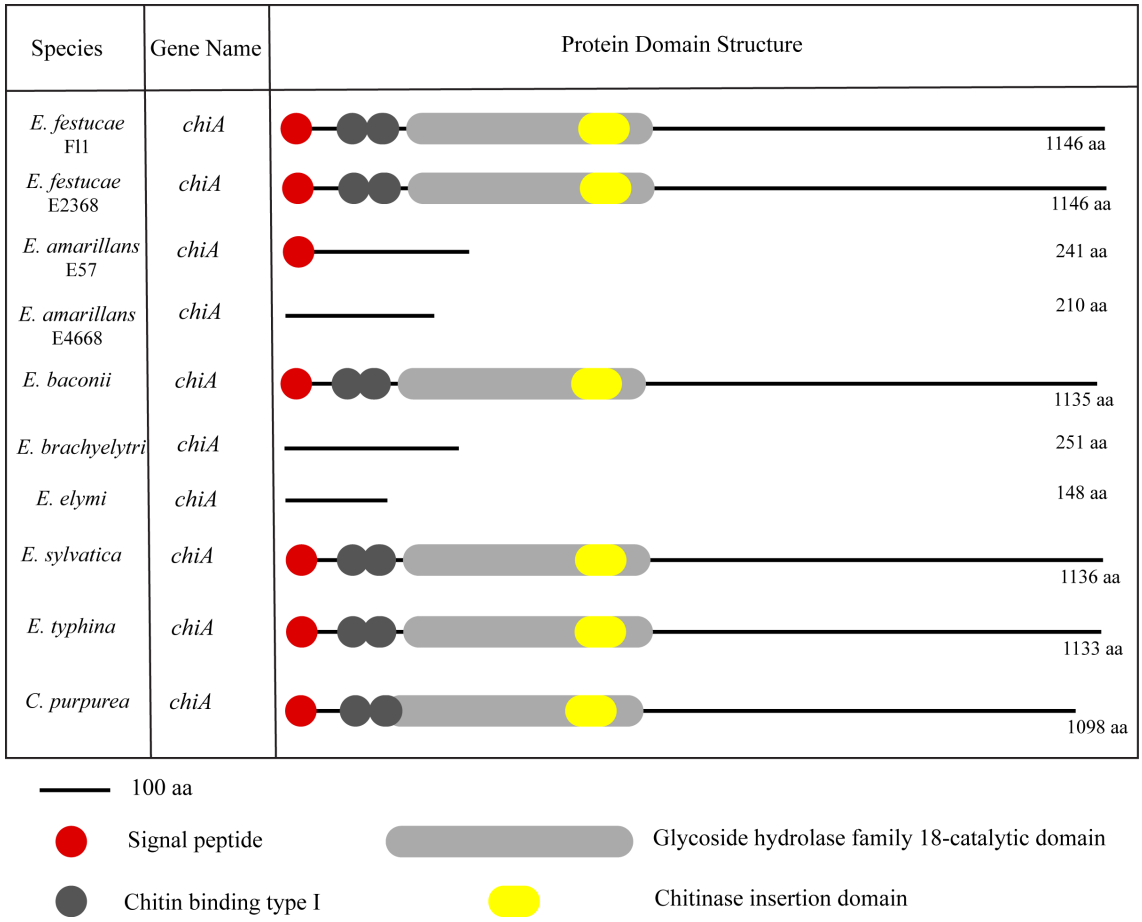
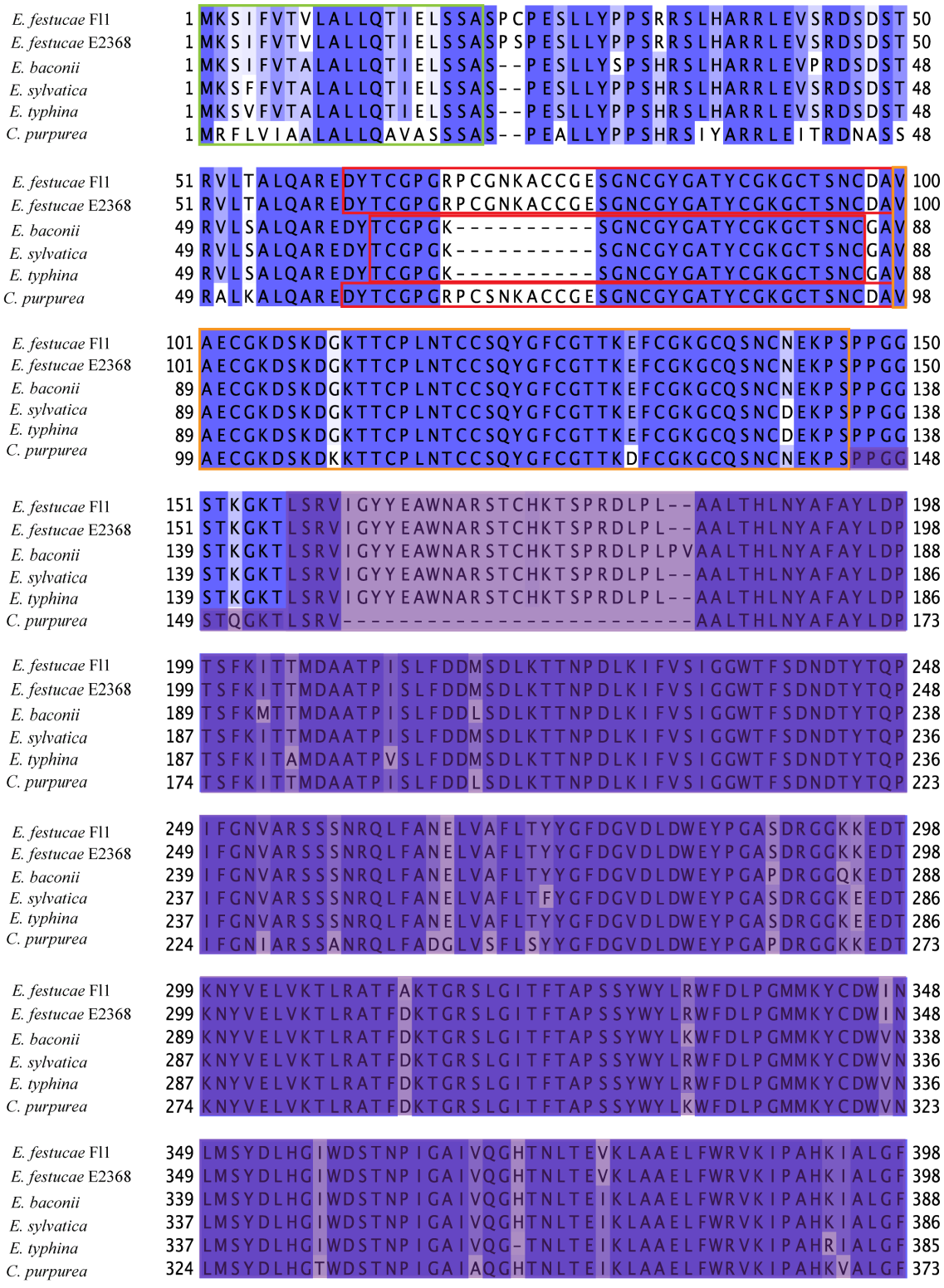
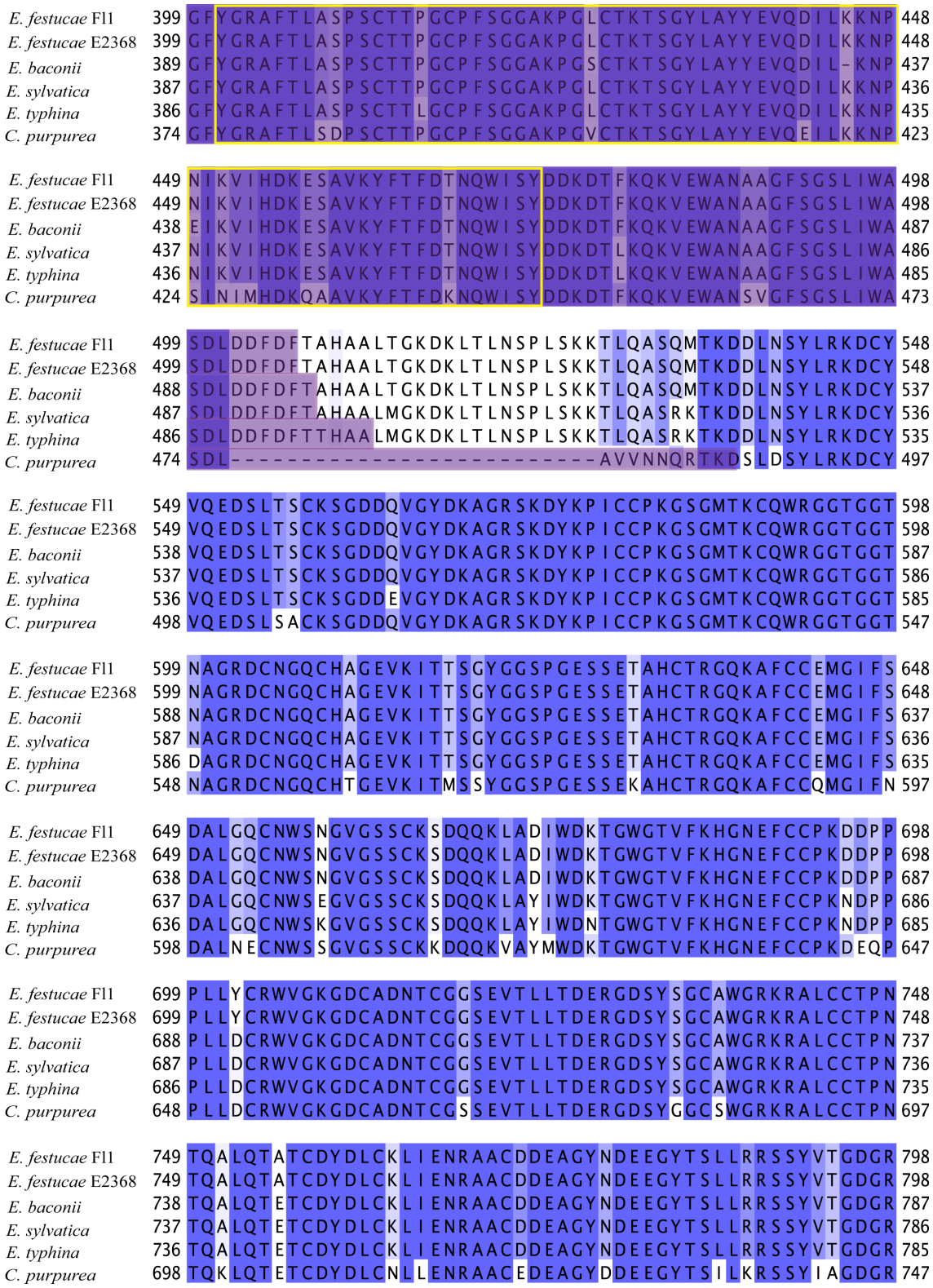


Figure 3.7. Chitinase protein predicted structure. *E. festucae* ChiA protein and homologues from other *Epichloë* species and *Claviceps purpurea* showing the predicted signal peptide, chitin binding type I, Glycoside hydrolase family 18 catalytic domain and chitinase insertion domains. Bar=100 amino acids.


E. festucae Fl1 1 MKSIFVTVLALLQTIELSSASPCPESLLYPPSRRSLHARRLEVSRDSDST 50
E. festucae E2368 1 MKSIFVTVLALLQTIELSSASPSPESLLYPPSRRSLHARRLEVSRDSDST 50
E. baconii 1 MKSIFVTALALLQTIELSSAS--PESLLYSPSHRSLHARRLEVPRDSDST 48
E. sylvatica 1 MKSFFVTALALLQTIELSSAS--PESLLYPPSHRSLHARRLEVSRDSDST 48
E. typhina 1 MKSVFVTALALLQTIELSSAS--PESLLYPPSHRSLHARRLEVSRDSDST 48
C. purpurea 1 MRFLVIAALALLQAVASSSAS--PEALLYPPSHRSIYARRLEITRDNASS 48
E. festucae Fl1 51 RVLTALQAREDYTCGPGRPCGNKACCGESGNCGYGATYCGKGCTSNCDAV 100
E. festucae E2368 51 RVLTALQAREDYTCGPGRPCGNKACCGESGNCGYGATYCGKGCTSNCDAV 100
E. baconii 49 RVLSALQAREDYTCGPGK----------SGNCGYGATYCGKGCTSNCGAV 88
E. sylvatica 49 RVLSALQAREDYTCGPGK----------SGNCGYGATYCGKGCTSNCGAV 88
E. typhina 49 RVLSALQAREDYTCGPGK----------SGNCGYGATYCGKGCTSNCGAV 88
C. purpurea 49 RALKALQAREDYTCGPGRPCSNKACCGESGNCGYGATYCGKGCTSNCDAV 98
E. festucae Fl1 101 AECGKDSKDGKTTCPLNTCCSQYGFCGTTKEFCGKGCQSNCNEKPSPPGG 150
E. festucae E2368 101 AECGKDSKDGKTTCPLNTCCSQYGFCGTTKEFCGKGCQSNCNEKPSPPGG 150
E. baconii 89 AECGKDSKDGKTTCPLNTCCSQYGFCGTTKEFCGKGCQSNCNEKPSPPGG 138
E. sylvatica 89 AECGKDSKDGKTTCPLNTCCSQYGFCGTTKEFCGKGCQSNCDEKPSPPGG 138
E. typhina 89 AECGKDSKDGKTTCPLNTCCSQYGFCGTTKEFCGKGCQSNCDEKPSPPGG 138
C. purpurea 99 AECGKDSKDKKTTCPLNTCCSQYGFCGTTKDFCGKGCQSNCNEKPSPPGG 148
E. festucae Fl1 151 STKGKTLSRVIGYYEAWNARSTCHKTSPRDLPL--AALTHLNYAFAYLDP 198
E. festucae E2368 151 STKGKTLSRVIGYYEAWNARSTCHKTSPRDLPL--AALTHLNYAFAYLDP 198
E. baconii 139 STKGKTLSRVIGYYEAWNARSTCHKTSPRDLPLPVAALTHLNYAFAYLDP 188
E. sylvatica 139 STKGKTLSRVIGYYEAWNARSTCHKTSPRDLPL--AALTHLNYAFAYLDP 186
E. typhina 139 STKGKTLSRVIGYYEAWNARSTCHKTSPRDLPL--AALTHLNYAFAYLDP 186
C. purpurea 149 STQGKTLSRV----------AALTHLNYAFAYLDP 173
E. festucae Fl1 199 TSFKITTMDAATPISLFDDMSDLKTTNPDLKIFVSIGGWTFSDNDTYTQP 248
E. festucae E2368 199 TSFKITTMDAATPISLFDDMSDLKTTNPDLKIFVSIGGWTFSDNDTYTQP 248
E. baconii 189 TSFKMTTMDAATPISLFDDLSDLKTTNPDLKIFVSIGGWTFSDNDTYTQP 238
E. sylvatica 187 TSFKITTMDAATPISLFDDMSDLKTTNPDLKIFVSIGGWTFSDNDTYTQP 236
E. typhina 187 TSFKITAMDAATPVSLFDDMSDLKTTNPDLKIFVSIGGWTFSDNDTYTQP 236
C. purpurea 174 TSFKITTMDAATPISLFDDLSDLKTTNPDLKIFVSIGGWTFSDNDTYTQP 223
E. festucae Fl1 249 IFGNVARSSSNRQLFANELVAFLTYYGFDGVDLDWEYPGASDRGGKKEDT 298
E. festucae E2368 249 IFGNVARSSSNRQLFANELVAFLTYYGFDGVDLDWEYPGASDRGGKKEDT 298
E. baconii 239 IFGNVARSSSNRQLFANELVAFLTYYGFDGVDLDWEYPGAPDRGGQKEDT 288
E. sylvatica 237 IFGNVARSSSNRQLFANELVAFLTFYGFDGVDLDWEYPGASDRGGKEEDT 286
E. typhina 237 IFGNVARSSSNRQLFANELVAFLTYYGFDGVDLDWEYPGASDRGGKEEDT 286
C. purpurea 224 IFGNIARSSANRQLFADGLVSFLSYYGFDGVDLDWEYPGAPDRGGKKEDT 273
E. festucae Fl1 299 KNYVELVKTLRATFAKTGRSLGITFTAPSSYWYLRWFDLPGMMKYCDWIN 348
E. festucae E2368 299 KNYVELVKTLRATFDKTGRSLGITFTAPSSYWYLRWFDLPGMMKYCDWIN 348
E. baconii 289 KNYVELVKTLRATFDKTGRSLGITFTAPSSYWYLKWFDLPGMMKYCDWVN 338
E. sylvatica 287 KNYVELVKTLRATFDKTGRSLGITFTAPSSYWYLRWFDLPGMMKYCDWVN 336
E. typhina 287 KNYVELVKTLRATFDKTGRSLGITFTAPSSYWYLRWFDLPGMMKYCDWVN 336
C. purpurea 274 KNYVELVKTLRATFDKTGRSLGITFTAPSSYWYLKWFDLPGMMKYCDWVN 323
E. festucae Fl1 349 LMSYDLHGIWDSTNPIGAIVQGHTNLTEVKLAAELFWRVKIPAHKIALGF 398
E. festucae E2368 349 LMSYDLHGIWDSTNPIGAIVQGHTNLTEVKLAAELFWRVKIPAHKIALGF 398
E. baconii 339 LMSYDLHGIWDSTNPIGAIVQGHTNLTEIKLAAELFWRVKIPAHKIALGF 388
E. sylvatica 337 LMSYDLHGIWDSTNPIGAIVQGHTNLTEIKLAAELFWRVKIPAHKIALGF 386
E. typhina 337 LMSYDLHGIWDSTNPIGAIVQG-TNLTEIKLAAELFWRVKIPAHRIALGF 385
C. purpurea 324 LMSYDLHGTWDSTNPIGAIAQGHTNLTEIKLAAELFWRVKIPAHKVALGF 373


E. festucae Fl1 399 GFYGRAFTLASPSCTTPGCPFSGGAKPGLCTKTSGYLAYYEVQDILKKNP 448
E. festucae E2368 399 GFYGRAFTLASPSCTTPGCPFSGGAKPGLCTKTSGYLAYYEVQDILKKNP 448
E. baconii 389 GFYGRAFTLASPSCTTPGCPFSGGAKPGSCTKTSGYLAYYEVQDIL-KNP 437
E. sylvatica 387 GFYGRAFTLASPSCTTPGCPFSGGAKPGLCTKTSGYLAYYEVQDILKKNP 436
E. typhina 386 GFYGRAFTLASPSCTTLGCPFSGGAKPGLCTKTSGYLAYYEVQDILKKNP 435
C. purpurea 374 GFYGRAFTLSDPSCTTPGCPFSGGAKPGVCTKTSGYLAYYEVQEILKKNP 423
E. festucae Fl1 449 NIKVIHDKESAVKYFTFDTNQWISYDDKDTFKQKVEWANAAGFSGSLIWA 498
E. festucae E2368 449 NIKVIHDKESAVKYFTFDTNQWISYDDKDTFKQKVEWANAAGFSGSLIWA 498
E. baconii 438 EIKVIHDKESAVKYFTFDTNQWISYDDKDTFKQKVEWANAAGFSGSLIWA 487
E. sylvatica 437 NIKVIHDKESAVKYFTFDTNQWISYDDKDTLKQKVEWANAAGFSGSLIWA 486
E. typhina 436 NIKVIHDKESAVKYFTFDTNQWISYDDKDTLKQKVEWANAAGFSGSLIWA 485
C. purpurea 424 SINIMHDKQAAVKYFTFDKNQWISYDDKDTFKQKVEWANSVGFSGSLIWA 473
E. festucae Fl1 499 SDLDDFDFTAHAALTGKDKLTLNSPLSKKTLQASQMTKDDLNSYLRKDCY 548
E. festucae E2368 499 SDLDDFDFTAHAALTGKDKLTLNSPLSKKTLQASQMTKDDLNSYLRKDCY 548
E. baconii 488 SDLDDFDFTAHAALTGKDKLTLNSPLSKKTLQASQMTKDDLNSYLRKDCY 537
E. sylvatica 487 SDLDDFDFTAHAALMGKDKLTLNSPLSKKTLQASRKTKDDLNSYLRKDCY 536
E. typhina 486 SDLDDFDFTTHAALMGKDKLTLNSPLSKKTLQASRKTKDDLNSYLRKDCY 535
C. purpurea 474 SDL----------AVVNNQRTKDSLDSYLRKDCY 497
E. festucae Fl1 549 VQEDSLTSCKSGDDQVGYDKAGRSKDYKPICCPKGSGMTKCQWRGGTGGT 598
E. festucae E2368 549 VQEDSLTSCKSGDDQVGYDKAGRSKDYKPICCPKGSGMTKCQWRGGTGGT 598
E. baconii 538 VQEDSLTSCKSGDDQVGYDKAGRSKDYKPICCPKGSGMTKCQWRGGTGGT 587
E. sylvatica 537 VQEDSLTSCKSGDDQVGYDKAGRSKDYKPICCPKGSGMTKCQWRGGTGGT 586
E. typhina 536 VQEDSLTSCKSGDDEVGYDKAGRSKDYKPICCPKGSGMTKCQWRGGTGGT 585
C. purpurea 498 VQEDSLSACKSGDDQVGYDKAGRSKDYKPICCPKGSGMTKCQWRGGTGGT 547
E. festucae Fl1 599 NAGRDCNGQCHAGEVKITTSGYGGSPGESSETAHCTRGQKAFCCEMGIFS 648
E. festucae E2368 599 NAGRDCNGQCHAGEVKITTSGYGGSPGESSETAHCTRGQKAFCCEMGIFS 648
E. baconii 588 NAGRDCNGQCHAGEVKITTSGYGGSPGESSETAHCTRGQKAFCCEMGIFS 637
E. sylvatica 587 NAGRDCNGQCHAGEVKITTSGYGGSPGESSETAHCTRGQKAFCCEMGIFS 636
E. typhina 586 DAGRDCNGQCHAGEVKITTSGYGGSPGESSETAHCTRGQKAFCCEMGIFS 635
C. purpurea 548 NAGRDCNGQCHTGEVKITMSSYGGSPGESSEKAHCTRGQKAFCCQMGIFN 597
E. festucae Fl1 649 DALGQCNWSNGVGSSCKSDQQKLADIWDKTGWGTVFKHGNEFCCPKDDPP 698
E. festucae E2368 649 DALGQCNWSNGVGSSCKSDQQKLADIWDKTGWGTVFKHGNEFCCPKDDPP 698
E. baconii 638 DALGQCNWSNGVGSSCKSDQQKLADIWDKTGWGTVFKHGNEFCCPKDDPP 687
E. sylvatica 637 DALGQCNWSEGVGSSCKSDQQKLAYIWDKTGWGTVFKHGNEFCCPKNDPP 686
E. typhina 636 DALGQCNWSKGVGSSCKSDQQKLAYIWDNTGWGTVFKHGNEFCCPKNDPP 685
C. purpurea 598 DALNECNWSSGVGSSCKKDQQKVAYMWDKTGWGTVFKHGNEFCCPKDEQP 647
E. festucae Fl1 699 PLLYCRWVGKGDCADNTCGGSEVTLLTDERGDSYSGCAWGRKRALCCTPN 748
E. festucae E2368 699 PLLYCRWVGKGDCADNTCGGSEVTLLTDERGDSYSGCAWGRKRALCCTPN 748
E. baconii 688 PLLDCRWVGKGDCADNTCGGSEVTLLTDERGDSYSGCAWGRKRALCCTPN 737
E. sylvatica 687 PLLDCRWVGKGDCADNTCGGSEVTLLTDERGDSYSGCAWGRKRALCCTPN 736
E. typhina 686 PLLDCRWVGKGDCADNTCGGSEVTLLTDERGDSYSGCAWGRKRALCCTPN 735
C. purpurea 648 PLLDCRWVGKGDCADNTCGSSEVTLLTDERGDSYGGCSWGRKRALCCTPN 697
E. festucae Fl1 749 TQALQTATCDYDLCKLIENRAACDDEAGYNDEEGYTSLLRRSSYVTGDGR 798
E. festucae E2368 749 TQALQTATCDYDLCKLIENRAACDDEAGYNDEEGYTSLLRRSSYVTGDGR 798
E. baconii 738 TQALQTETCDYDLCKLIENRAACDDEAGYNDEEGYTSLLRRSSYVTGDGR 787
E. sylvatica 737 TQALQTETCDYDLCKLIENRAACDDEAGYNDEEGYTSLLRRSSYVTGDGR 786
E. typhina 736 TQALQTETCDYDLCKLIENRAACDDEAGYNDEEGYTSLLRRSSYVTGDGR 785
C. purpurea 698 TQKLQTETCDYDLCNLLENRAACEDEAGYDDEEGYTSILKRSSYIAGDGR 747

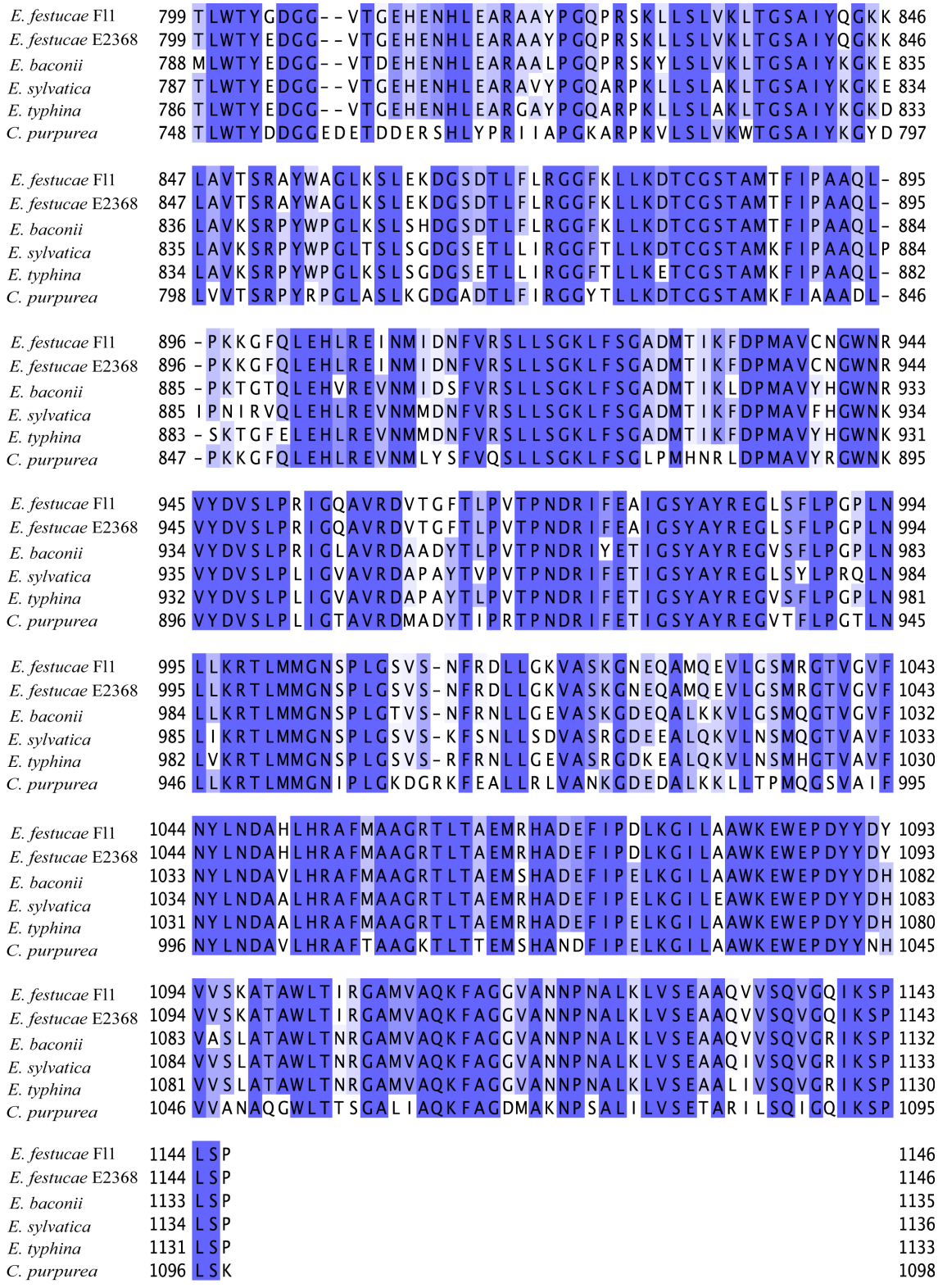

E. festucae Fl1 799 TLWTYGDGG--VTGEHENHLEARAAYPGQPRSKLLSLVKLTGSAIYQGKK 846
E. festucae E2368 799 TLWTYEDGG--VTGEHENHLEARAAYPGQPRSKLLSLVKLTGSAIYQGKK 846
E. baconii 788 MLWTYEDGG--VTDEHENHLEARAALPGQPRSKYLSLVKLTGSAIYKGKE 835
E. sylvatica 787 TLWTYEDGG--VTGEHENHLEARAVYPGQARPKLLSLAKLTGSAIYKGKE 834
E. typhina 786 TLWTYEDGG--VTGEHENHLEARGAYPGQARPKLLSLAKLTGSAIYKGKD 833
C. purpurea 748 TLWTYDDGGEDETDDERSHLYPRIIAPGKARPKVLSLVKWTGSAIYKGYD 797
E. festucae Fl1 847 LAVTSRAYWAGLKSLEKDGSDTLFLRGGFKLLKDTCGSTAMTFIPAAQL- 895
E. festucae E2368 847 LAVTSRAYWAGLKSLEKDGSDTLFLRGGFKLLKDTCGSTAMTFIPAAQL- 895
E. baconii 836 LAVKSRPYWPGLKSLSHDGSDTLFLRGGFKLLKDTCGSTAMTFIPAAQL- 884
E. sylvatica 835 LAVKSRPYWPGLTSLSGDGSETLLIRGGFTLLKDTCGSTAMKFIPAAQLP 884
E. typhina 834 LAVKSRPYWPGLKSLSGDGSETLLIRGGFTLLKETCGSTAMKFIPAAQL- 882
C. purpurea 798 LVVTSRPYRPGLASLKGDGADTLFIRGGYTLLKDTCGSTAMKFIAAADL- 846
E. festucae Fl1 896 -PKKGFQLEHLREINMIDNFVRSLLSGKLFSGADMTIKFDPMAVCNGWNR 944
E. festucae E2368 896 -PKKGFQLEHLREINMIDNFVRSLLSGKLFSGADMTIKFDPMAVCNGWNR 944
E. baconii 885 -PKTGTQLEHVREVNMIDSFVRSLLSGKLFSGADMTIKLDPMAVYHGWNR 933
E. sylvatica 885 IPNIRVQLEHLREVNMMDNFVRSLLSGKLFSGADMTIKFDPMAVFHGWNK 934
E. typhina 883 -SKTGFELEHLREVNMMDNFVRSLLSGKLFSGADMTIKFDPMAVYHGWNK 931
C. purpurea 847 -PKKGFQLEHLREVNMLYSFVQSLLSGKLFSGLPMHNRLDPMAVYRGWNK 895
E. festucae Fl1 945 VYDVSLPRIGQAVRDVTGFTLPVTPNDRIFEAIGSYAYREGLSFLPGPLN 994
E. festucae E2368 945 VYDVSLPRIGQAVRDVTGFTLPVTPNDRIFEAIGSYAYREGLSFLPGPLN 994
E. baconii 934 VYDVSLPRIGLAVRDAADYTLPVTPNDRIYETIGSYAYREGVSFLPGPLN 983
E. sylvatica 935 VYDVSLPLIGVAVRDAPAYTVPVTPNDRIFETIGSYAYREGLSYLPRQLN 984
E. typhina 932 VYDVSLPLIGVAVRDAPAYTLPVTPNDRIFETIGSYAYREGVSFLPGPLN 981
C. purpurea 896 VYDVSLPLIGTAVRDMADYTIPRTPNDRIFETIGSYAYREGVTFLPGTLN 945
E. festucae Fl1 995 LLKRTLMMGNSPLGSVS-NFRDLLGKVASKGNEQAMQEVLGSMRGTVGVF 1043
E. festucae E2368 995 LLKRTLMMGNSPLGSVS-NFRDLLGKVASKGNEQAMQEVLGSMRGTVGVF 1043
E. baconii 984 LLKRTLMMGNSPLGTVS-NFRNLLGEVASKGDEQALKKVLGSMQGTVGVF 1032
E. sylvatica 985 LIKRTLMMGNSPLGSVS-KFSNLLSDVASRGDEEALQKVLNSMQGTVAVF 1033
E. typhina 982 LVKRTLMMGNSPLGSVS-RFRNLLGEVASRGDKEALQKVLNSMHGTVAVF 1030
C. purpurea 946 LLKRTLMMGNIPLGKDGRKFEALLRLVANKGDEDALKKLLTPMQGSVAIF 995
E. festucae Fl1 1044 NYLNDAHLHRAFMAAGRTLTAEMRHADEFIPDLKGILAAWKEWEPDYYDY 1093
E. festucae E2368 1044 NYLNDAHLHRAFMAAGRTLTAEMRHADEFIPDLKGILAAWKEWEPDYYDY 1093
E. baconii 1033 NYLNDAVLHRAFMAAGRTLTAEMSHADEFIPELKGILAAWKEWEPDYYDH 1082
E. sylvatica 1034 NYLNDAALHRAFMAAGRTLTAEMRHADEFIPELKGILEAWKEWEPDYYDH 1083
E. typhina 1031 NYLNDAALHRAFMAAGRTLTAEMRHADEFIPELKGILAAWKEWEPDYYDH 1080
C. purpurea 996 NYLNDAVLHRAFTAAGKTLTTEMSHANDFIPELKGILAAWKEWEPDYYNH 1045
E. festucae Fl1 1094 VVSKATAWLTIRGAMVAQKFAGGVANNPNALKLVSEAAQVVSQVGQIKSP 1143
E. festucae E2368 1094 VVSKATAWLTIRGAMVAQKFAGGVANNPNALKLVSEAAQVVSQVGQIKSP 1143
E. baconii 1083 VASLATAWLTNRGAMVAQKFAGGVANNPNALKLVSEAAQVVSQVGRIKSP 1132
E. sylvatica 1084 VVSLATAWLTNRGAMVAQKFAGGVANNPNALKLVSEAAQIVSQVGRIKSP 1133
E. typhina 1081 VVSLATAWLTNRGAMVAQKFAGGVANNPNALKLVSEAALIVSQVGRIKSP 1130
C. purpurea 1046 VVANAQGWLTTSGALIAQKFAGDMAKNPSALILVSETARILSQIGQIKSP 1095
E. festucae Fl1 1144 LSP 1146
E. festucae E2368 1144 LSP 1146
E. baconii 1133 LSP 1135
E. sylvatica 1134 LSP 1136
E. typhina 1131 LSP 1133
C. purpurea 1096 LSK 1098

Figure 3.8. Multiple sequence alignment of *E. festucae* ChiA protein and homologues from other *Epichloë* species and *Claviceps purpurea*. *E. festucae* (Fl1) EfM3.000820, E. *festucae* (E2368), *E. baconii, E. sylvatica, E. typhina,* and *C. purpurea.* Intensity of colouring indicates the level of conservation. N terminal signal peptides are bordered in green. Chitin binding domains are bordered in red and orange. Glycoside hydrolase family 18 catalytic domain is indicated in pink and chitinase insertion domain inside it, is bordered in yellow. Intensity of colouring indicates the level of conservation.

3.3. Synteny analysis of *lymB* and *chiB* genes across the Clavicipitaceae

To check the presence of *lymB* and *chiB* homologues in other Epichloë species of Clavicipitaceae a BLASTn search was conducted using the nucleotide sequences of *lymB*, *chiB* and two other genes located in the same contig as the *lymB* (phoD like phosphatase and hypothetical protein) and two genes with linkage to the *chiB* (F-box containing protein and DUF 1665) from the Fl1 genome as query. The first and second lowest E-value of Fl1 against the other fungi within the Clavicipitaceae family showed that *lymB* and *lymA* appear not to be paralogues from a gene duplication event (Table 3.4). BLASTn analysis revealed the presence of homologues of *lymB*, *chiB* and the other four linked genes in different Epichloë species only (Table 3.4). These sequences were then used for checking the gene structure and synteny in other Epichloë species, which revealed that these genes share conserved synteny in some of the species in the Clavicipitaceae (Figure 3.9). However, there are gene deletions in the homologues to *lymB* in *E. bromicola* E 5761, AL 04334, E 502 and *E. glyceria* (Figures 3.9 and Appendix 18). Checking the protein structure encoded by these *lymB* genes showed that despite the deletions the virtual translation of these genes shows that they can be translated into proteins that appear to be functional. Domain analysis of the encoded products which had the same gene organization as Fl1 with genes encoding LysM motifs divergently transcribed from genes encoding chitinase proteins showed that most of the LysM proteins in these species also carried four LysM domains. LysM proteins encoded by *E. bromicola* E502 and *E. glyceria* harboured 3 LysM domains and the protein in *E. glyceria* did not include any N terminal signal peptide (Figure 3.10). The protein without an N terminal signal peptide and predicted not to be secreted was excluded from the amino acid sequence alignment and the alignment was conducted only on the LysM proteins with an N terminal signal peptide and the results showed that the LysM motifs are conserved among these species (Figure 3.11).

Table 3.4. BLASTn analysis of genes at the *lymA-chiA* locus in *E. festucae* Fl1 against other sexual *Epichloë* and related species.

subjest genome		*phoD-like phosphatase*			*Hypothetical protein*			*lymB*			*chiB*			*F-box containing protein*			*DUF 1665*		
Species	**strain**	**a**	**b**	**contig**	**a**	**b**	**contig**	**a**	**b**	**contig**	**a**	**b**	**contig**	**a**	**b**	**contig**	**a**	**b**	**contig**
Epichloë amarillans	ATCC 200744	0	3.9	2	0	0.49	2	0	4	2	1.8	1.8	278	2.00E-11	0.85	457	0	0.45	2
Epichloë amarillans	E4668	0	1.1	4	0	0.52	4	0	4.3	4	2	2	601	3.00E-13	0.92	266	0	0.49	4
Epichloë baconii	ATCC 200745	0	0.99	154	0	0.49	154	0	4	154	0	0.002	154	0	3.00E-90	1395	0	0.12	250
Epichloë brachyelytri	E4804	0	1.1	203	0	0.57	200	0	1.2	200	0	6.00E-07	200	1.00E-117	0.99	39	0	0.13	39
Epichloë bromicola	AL0426/2	0	0.85	52	0	0.42	52	0	0.88	52	0	0.002	52	8.00E-10	0.74	1824	0	0.099	52
Epichloë bromicola	AL0434	0	0.99	698	0	0.49	698	0	1	698	0	1.00E-10	698	0.85	0.85	692	0	0.12	698
Epichloë bromicola	ATCC 200750	0	0.72	3003	0	0.36	119	0	0.75	119	0	0	119	0.62	0.62	1984	0	0.084	138
Epichloë elymi	ATCC 201551	0	0.74	150	0	0.37	150	0	0.76	150	0	2.00E-08	150	4.00E-08	7.00E-04	257	0	0.086	150
Epichloë festucae	E2368	0	3.6	251	0	0.45	233	0	0.24	233	0	5.00E-07	233	0	8.00E-04	233	0	0.42	233
Epichloë festucae	Fl1	0	0.23	364	0	0.45	364	0	0.94	364	0	5.00E-07	364	0	8.00E-04	365	0	0.42	365
Epichloë glyceria	ATCC 200747	0	1.3	162	0.01	0.16	264	4.00E-16	1.3	230	1.00E-14	0.039	230	9.00E-17	1.1	751	0	0.15	162
Epichloë sylvatica	GR 10156	0	0.27	240	0	0.034	357	3.00E-84	1.1	357	3.00E-05	2	509	6.00E-05	0.92	751	0	0.12	1179
Epichloë typhina	ATCC 200736	0	0.27	271	0	0.53	113	0.28	1.1	72	2	2	301	0.001	3.7	327	0	0.13	551
Metarhizium acridum	CQMa 102	7.00E-45	0.99	1265	0.49	1.9	248	0.065	0.26	669	0.46	1.8	978	0.22	0.22	871	1.00E-13	0.12	1256
Metarhizium robertsii	ARSEF 23	1.00E-46	0.26	642	0.032	0.5	28	1	4.1	130	5.00E-04	0.12	249	0.014	0.22	547	1.00E-16	0.47	642
Claviceps fusiformis	PRL 1980	0	0.34	54	0.17	2.7	171	1.4	1.4	847	2.5	2.5	245	1.2	4.6	17	1.00E-48	0.01	54
Claviceps paspali	RRC 1481	0	0.012	626	1.5	1.5	380	0.2	0.78	307	0.089	1.4	947	0.65	2.6	691	2.00E-30	0.35	626
Claviceps purpurea	20.1	0	0.21	229	0.41	0.41	924	3.4	3.4	1234	0.39	1.6	789	0.18	2.9	238	2.00E-49	0.38	229
Atkinsonella texensis	B6155	0	0.19	18	0.092	0.092	533	0.19	0.19	1154	0.087	0.35	960	1.00E-145	0.64	729	2.00E-82	0.086	18
Atkinsonella texensis	B6156	0	0.19	64	0.092	0.092	742	0.19	0.76	285	0.087	0.34	158	1.00E-145	0.16	778	6.00E-80	0.085	64
Periglandula ipomoeae	IasaF13	0	0.92	37	0.45	0.45	604	0.95	0.95	745	0.43	0.43	144	1.00E-18	0.051	35	1.00E-103	0.11	37

[a]Lowest E-value. [b]Second lowest E-value.

Genes located in the same contig as *lymB* and *chiB* are highlighted in green whereas those in different contigs are highlighted in yellow.

The second strongest match against the Fl1 genome was used as a minimum-similarity reference to identify putative homologs in the other species.

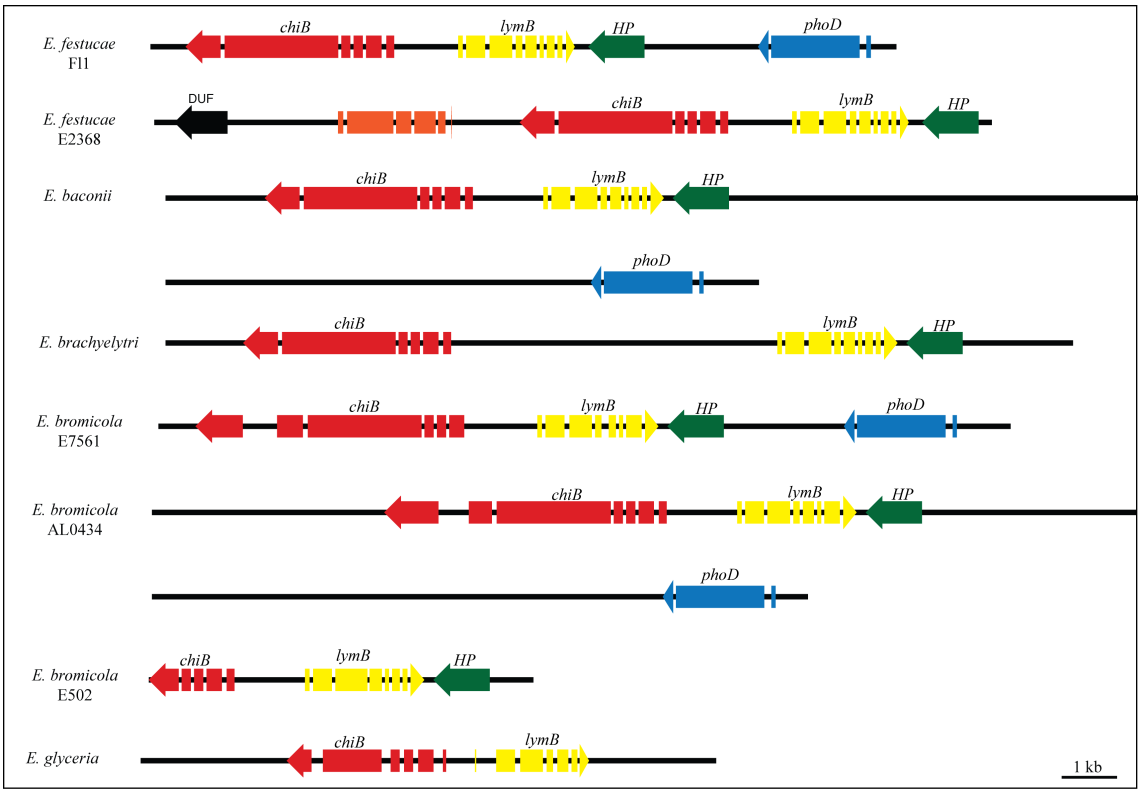


Figure 3.9. Synteny analysis of genes at the *lymB-chiB* locus in *E. festucae* Fl1 and other sexual *Epichloë* species. Homologous genes share a single colour across species. Arrows represent the direction of transcription. Sequence data was sourced from http://www.endophyte.uky.edu. Bar= 1 kb.

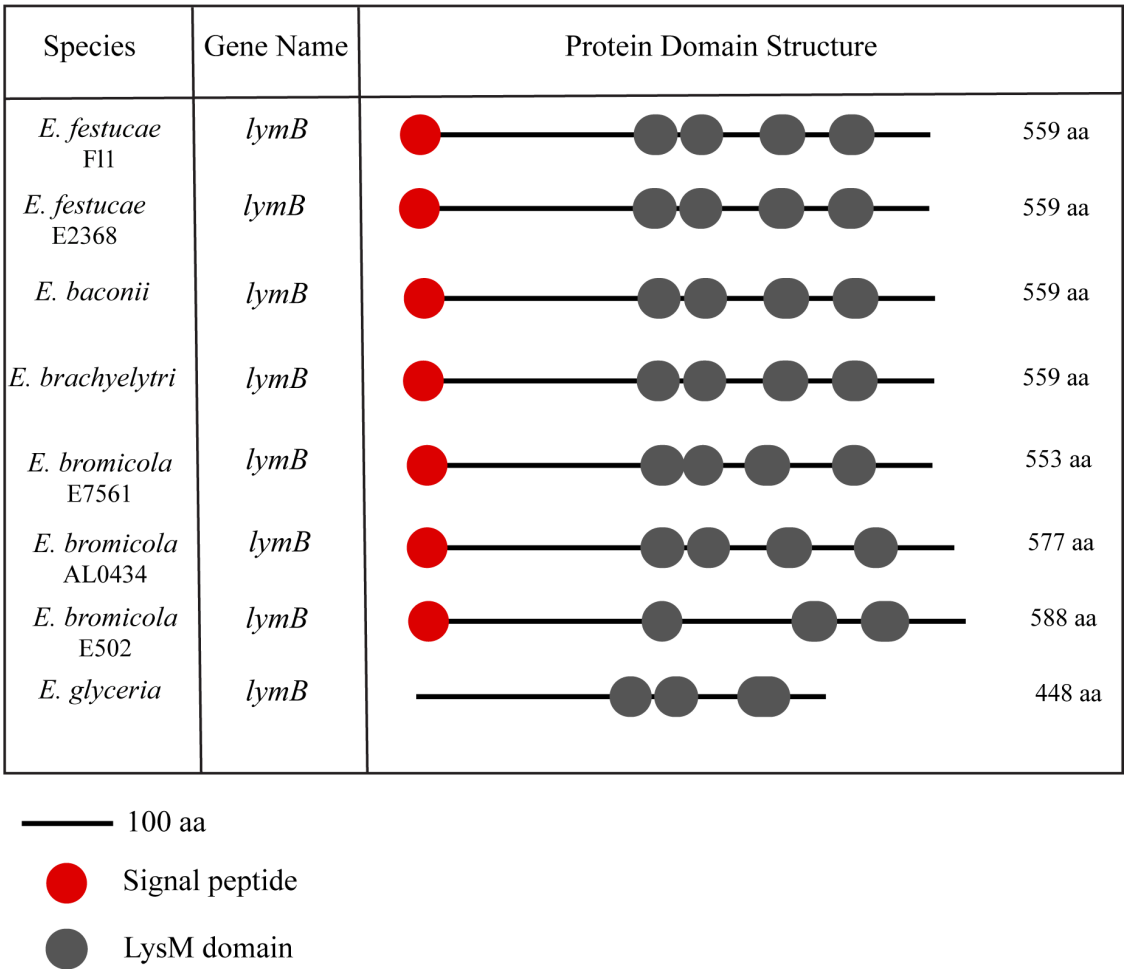
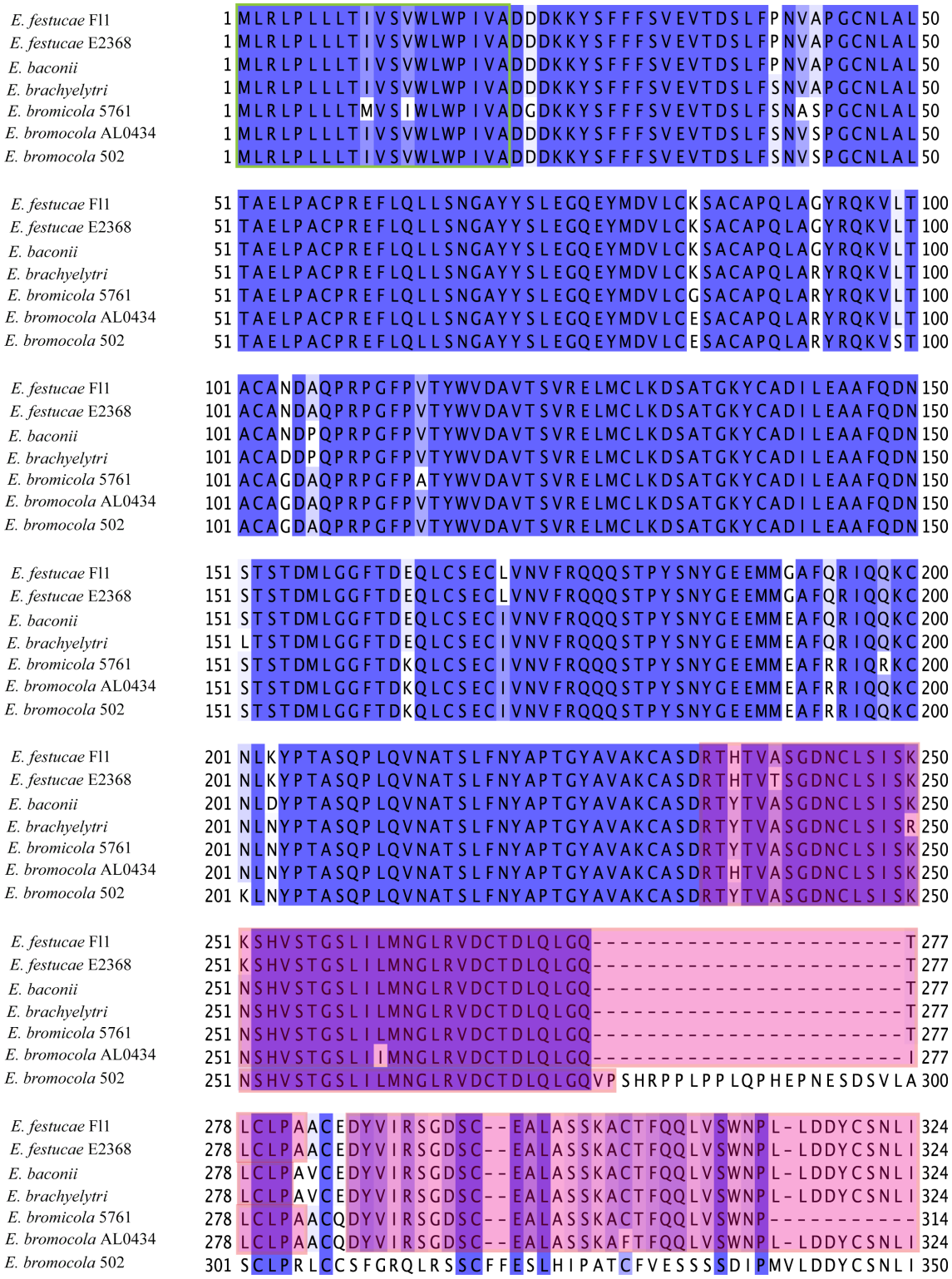


Figure 3.10. LysM protein predicted structure. *E. festucae* LymB protein and homologues from other *Epichloë* species showing the predicted signal peptide and LysM domains. Bar=100 amino acids.


E. festucae Fl1 1 MLRLPLLLTIVSVWLWPIVADDDKKYSFFFSVEVTDSLFPNVAPGCNLAL 50
E. festucae E2368 1 MLRLPLLLTIVSVWLWPIVADDDKKYSFFFSVEVTDSLFPNVAPGCNLAL 50
E. baconii 1 MLRLPLLLTIVSVWLWPIVADDDKKYSFFFSVEVTDSLFPNVAPGCNLAL 50
E. brachyelytri 1 MLRLPLLLTIVSVWLWPIVADDDKKYSFFFSVEVTDSLFSNVAPGCNLAL 50
E. bromicola 5761 1 MLRLPLLLTMVSIWLWPIVADGDKKYSFFFSVEVTDSLFSNASPGCNLAL 50
E. bromocola AL0434 1 MLRLPLLLTIVSVWLWPIVADDDKKYSFFFSVEVTDSLFSNVSPGCNLAL 50
E. bromocola 502 1 MLRLPLLLTIVSVWLWPIVADDDKKYSFFFSVEVTDSLFSNVSPGCNLAL 50
E. festucae Fl1 51 TAELPACPREFLQLLSNGAYYSLEGQEYMDVLCKSACAPQLAGYRQKVLT 100
E. festucae E2368 51 TAELPACPREFLQLLSNGAYYSLEGQEYMDVLCKSACAPQLAGYRQKVLT 100
E. baconii 51 TAELPACPREFLQLLSNGAYYSLEGQEYMDVLCKSACAPQLAGYRQKVLT 100
E. brachyelytri 51 TAELPACPREFLQLLSNGAYYSLEGQEYMDVLCKSACAPQLARYRQKVLT 100
E. bromicola 5761 51 TAELPACPREFLQLLSNGAYYSLEGQEYMDVLCGSACAPQLARYRQKVLT 100
E. bromocola AL0434 51 TAELPACPREFLQLLSNGAYYSLEGQEYMDVLCESACAPQLARYRQKVLT 100
E. bromocola 502 51 TAELPACPREFLQLLSNGAYYSLEGQEYMDVLCESACAPQLARYRQKVST 100
E. festucae Fl1 101 ACANDAQPRPGFPVTYWVDAVTSVRELMCLKDSATGKYCADILEAAFQDN 150
E. festucae E2368 101 ACANDAQPRPGFPVTYWVDAVTSVRELMCLKDSATGKYCADILEAAFQDN 150
E. baconii 101 ACANDPQPRPGFPVTYWVDAVTSVRELMCLKDSATGKYCADILEAAFQDN 150
E. brachyelytri 101 ACADDPQPRPGFPVTYWVDAVTSVRELMCLKDSATGKYCADILEAAFQDN 150
E. bromicola 5761 101 ACAGDAQPRPGFPATYWVDAVTSVRELMCLKDSATGKYCADILEAAFQDN 150
E. bromocola AL0434 101 ACAGDAQPRPGFPVTYWVDAVTSVRELMCLKDSATGKYCADILEAAFQDN 150
E. bromocola 502 101 ACAGDAQPRPGFPVTYWVDAVTSVRELMCLKDSATGKYCADILEAAFQDN 150
E. festucae Fl1 151 STSTDMLGGFTDEQLCSECLVNVFRQQQSTPYSNYGEEMMGAFQRIQQKC 200
E. festucae E2368 151 STSTDMLGGFTDEQLCSECLVNVFRQQQSTPYSNYGEEMMGAFQRIQQKC 200
E. baconii 151 STSTDMLGGFTDEQLCSECIVNVFRQQQSTPYSNYGEEMMEAFQRIQQKC 200
E. brachyelytri 151 LTSTDMLGGFTDEQLCSECIVNVFRQQQSTPYSNYGEEMMEAFQRIQQKC 200
E. bromicola 5761 151 STSTDMLGGFTDKQLCSECIVNVFRQQQSTPYSNYGEEMMEAFRRIQRKC 200
E. bromocola AL0434 151 STSTDMLGGFTDKQLCSECIVNVFRQQQSTPYSNYGEEMMEAFRRIQQKC 200
E. bromocola 502 151 STSTDMLGGFTDKQLCSECIVNVFRQQQSTPYSNYGEEMMEAFRRIQQKC 200
E. festucae Fl1 201 NLKYPTASQPLQVNATSLFNYAPTGYAVAKCASDRTHTVASGDNCLSISK 250
E. festucae E2368 201 NLKYPTASQPLQVNATSLFNYAPTGYAVAKCASDRTHTVTSGDNCLSISK 250
E. baconii 201 NLDYPTASQPLQVNATSLFNYAPTGYAVAKCASDRTYTVASGDNCLSISK 250
E. brachyelytri 201 NLNYPTASQPLQVNATSLFNYAPTGYAVAKCASDRTYTVASGDNCLSISR 250
E. bromicola 5761 201 NLNYPTASQPLQVNATSLFNYAPTGYAVAKCASDRTYTVASGDNCLSISK 250
E. bromocola AL0434 201 NLNYPTASQPLQVNATSLFNYAPTGYAVAKCASDRTHTVASGDNCLSISK 250
E. bromocola 502 201 KLNYPTASQPLQVNATSLFNYAPTGYAVAKCASDRTYTVASGDNCLSISK 250
E. festucae Fl1 251 KSHVSTGSLILMNGLRVDCTDLQLGQ----------T 277
E. festucae E2368 251 KSHVSTGSLILMNGLRVDCTDLQLGQ----------T 277
E. baconii 251 NSHVSTGSLILMNGLRVDCTDLQLGQ----------T 277
E. brachyelytri 251 NSHVSTGSLILMNGLRVDCTDLQLGQ----------T 277
E. bromicola 5761 251 NSHVSTGSLILMNGLRVDCTDLQLGQ----------T 277
E. bromocola AL0434 251 NSHVSTGSLIIMNGLRVDCTDLQLGQ----------I 277
E. bromocola 502 251 NSHVSTGSLILMNGLRVDCTDLQLGQVPSHRPPLPPLQPHEPNESDSVLA 300
E. festucae Fl1 278 LCLPAACEDYVIRSGDSC--EALASSKACTFQQLVSWNPL-LDDYCSNLI 324
E. festucae E2368 278 LCLPAACEDYVIRSGDSC--EALASSKACTFQQLVSWNPL-LDDYCSNLI 324
E. baconii 278 LCLPAVCEDYVIRSGDSC--EALASSKACTFQQLVSWNPL-LDDYCSNLI 324
E. brachyelytri 278 LCLPAVCEDYVIRSGDSC--EALASSKACTFQQLVSWNPL-LDDYCSNLI 324
E. bromicola 5761 278 LCLPAACQDYVIRSGDSC--EALASSKACTFQQLVSWNP----------314
E. bromocola AL0434 278 LCLPAACQDYVIRSGDSC--EALASSKAFTFQQLVSWNPL-LDDYCSNLI 324
E. bromocola 502 301 SCLPRLCCSFGRQLRSSCFFESLHIPATCFVESSSSDIPMVLDDYCSNLI 350

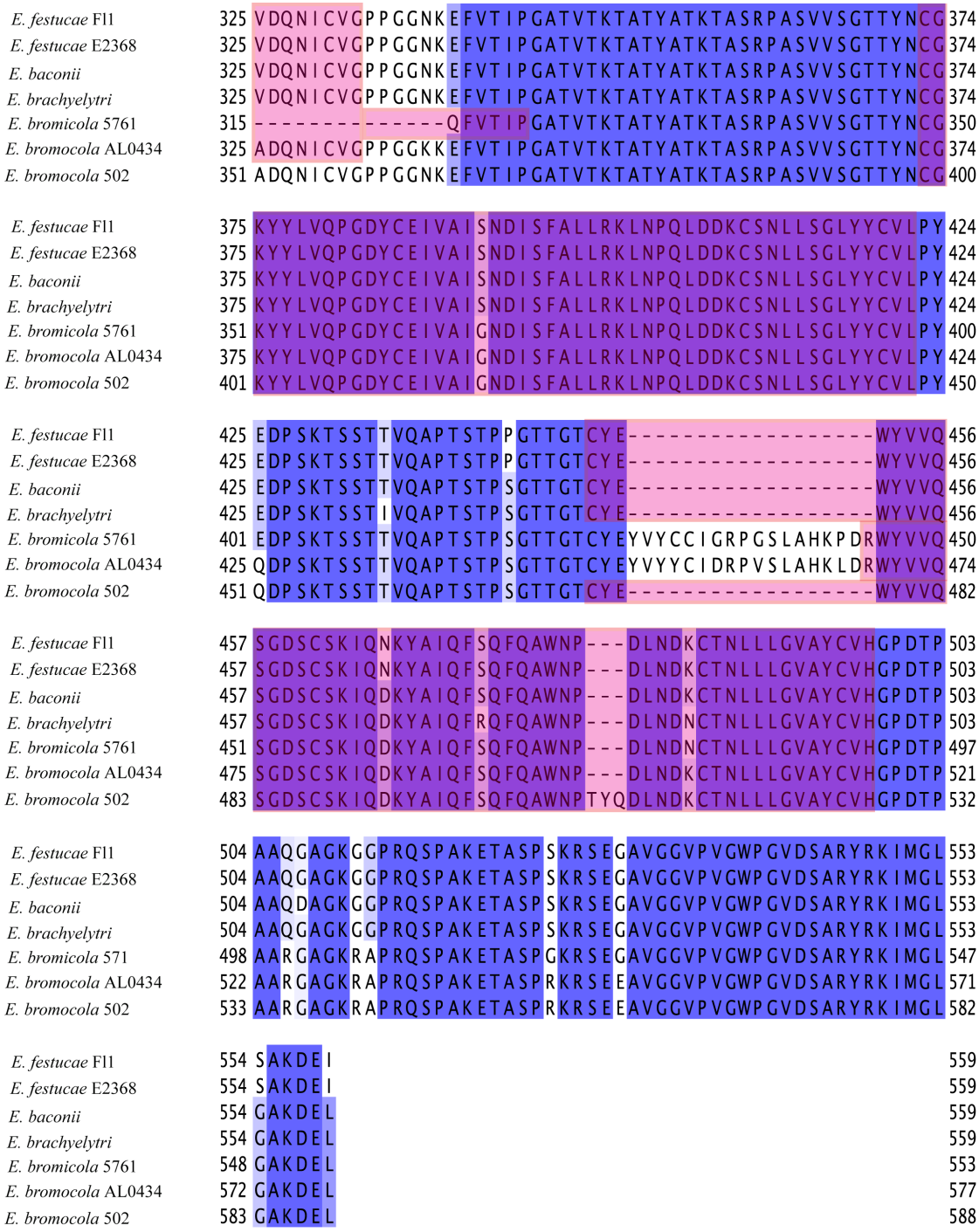


Figure 3.11. Multiple sequence alignment of *E. festucae* LymB protein and homologues from other *Epichloë* species. *E. festucae* (Fl1) EfM3.035020, E. *festucae* (E2368), *E. baconii, E. brachyelytri, E. bromicola* 5761, AL0434 and 502. Intensity of colouring indicates the level of conservation. N terminal signal peptides are bordered in green. LysM motifs are indicated in pink.

Gene structure analysis of the *chiB* genes divergently transcribed with *lymB* genes in Epichloë species showed that although there was conservation of synteny among these species, there were gene deletions in *E. bromicola* E5761, AL0434, E502 and *E. glyceria* (Figure 3.9 and appendix 19). The domain structure of the proteins encoded by *chiB* genes revealed that these proteins are part of glycoside hydrolase family 18 (GH18) with at least one chitin binding domain in all the species and two in *E. festucae* Fl1, *E. festucae* E2368 and *E. baconii*, chitinase insertion and glycoside hydrolase family 18 catalytic domains. All had an N terminal signal peptide prediction with the exception of *E. bromicola* E5761 and *E. bromicola* E502. *E. bromicola* E502 in addition lacks a stop codon which could be due to the gene misannotation (Appendix 19), so these two proteins were excluded from further amino acid sequence alignment (Figure 3.12). The alignment was conducted only on the chitinase proteins that had an N terminal signal peptide and the results showed these proteins have conserved domains for chitin binding, chitinase insertion and glycoside hydrolase. Checking the protein structure encoded by these *chiB* genes showed that despite the gene deletions and insertions they appear to be functional, an exception being *E. bromicola* E502, which is predicted to lack a stop codon (Figure 3.13).

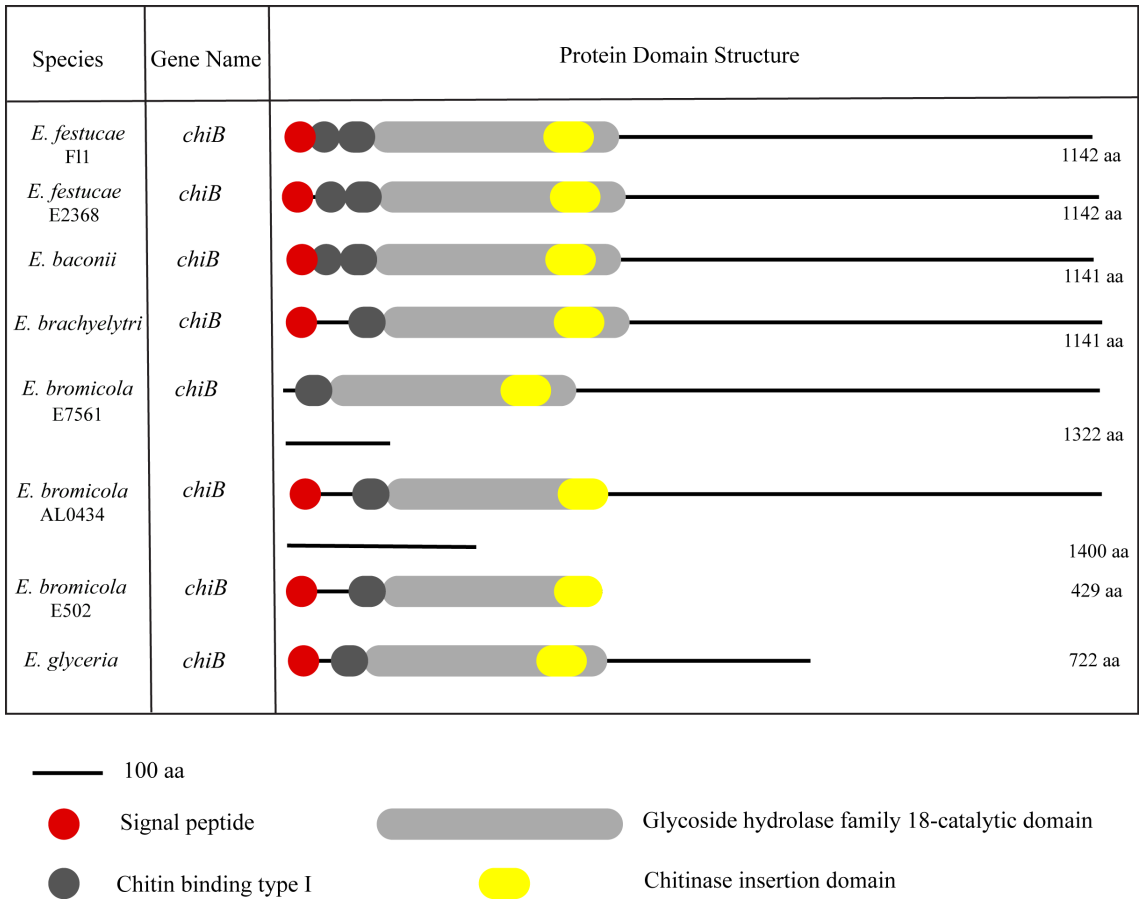
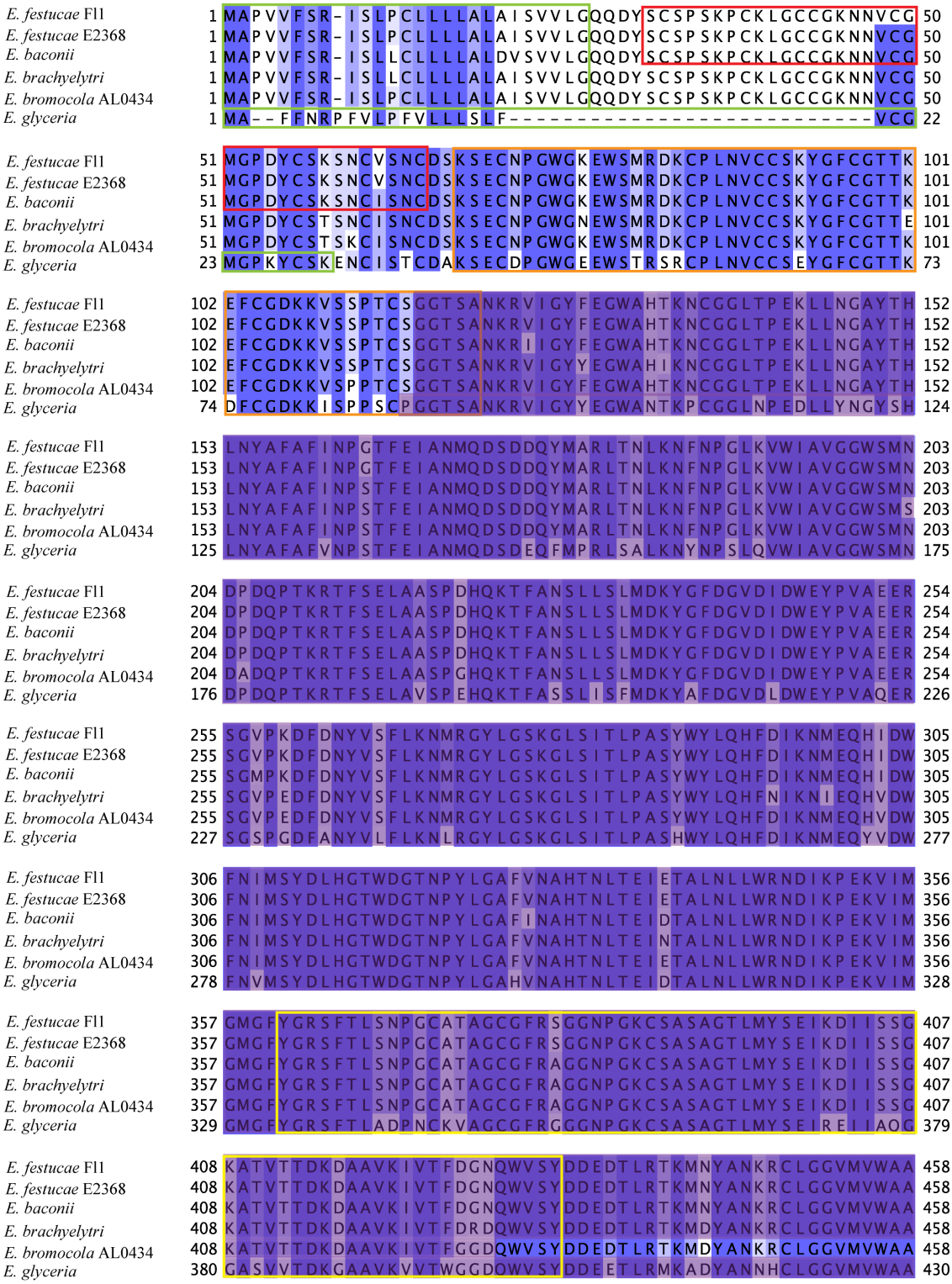
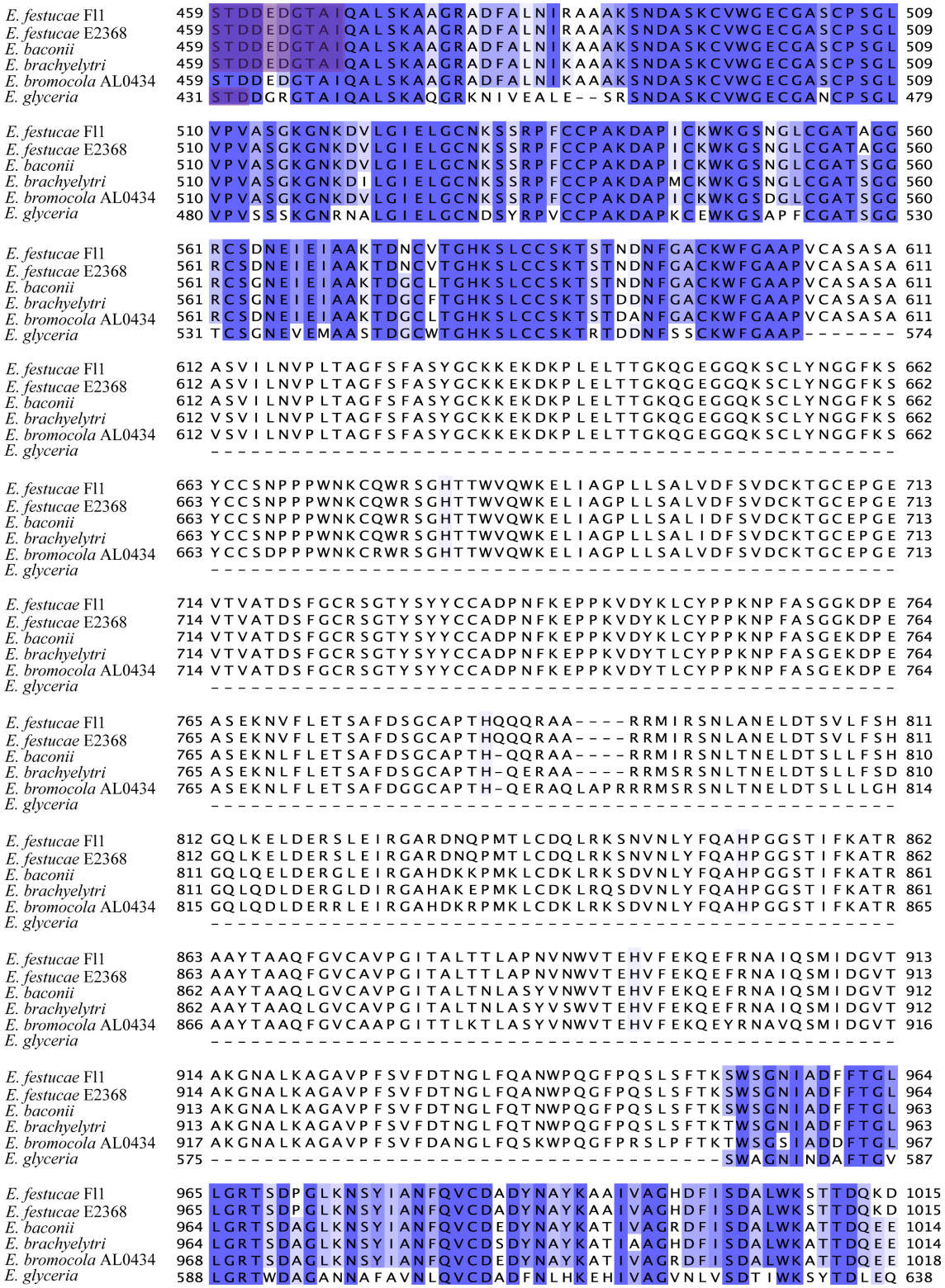


Figure 3.12. Chitinase protein predicted structure. *E. festucae* ChiB protein and homologues from other *Epichloë* species showing the predicted signal peptide, chitin binding type I, Glycoside hydrolase family 18 catalytic domain and chitinase insertion domains. Bar=100 amino acids.


E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromocola AL0434
E. glyceria


E. festucae Fl1 459 STDDEDGTAIQALSKAAGRADFALNIRAAAKSNDASKCVWGECGASCPSGL 509
E. festucae E2368 459 STDDEDGTAIQALSKAAGRADFALNIRAAAKSNDASKCVWGECGASCPSGL 509
E. baconii 459 STDDEDGTAIQALSKAAGRADFALNIKAAAKSNDASKCVWGECGASCPSGL 509
E. brachyelytri 459 STDDEDGTAIQALSKAAGRADFALNIKAAAKSNDASKCVWGECGASCPSGL 509
E. bromocola AL0434 459 STDDEDGTAIQALSKAAGRADFALNIKAAAKSNDASKCVWGECGASCPSGL 509
E. glyceria 431 STDDGRGTAIQALSKAQGRKNIVEALE--SRSNDASKCVWGECGANCPSGL 479
E. festucae Fl1 510 VPVASGKGNKDVLGIELGCNKSSRPFCCPAKDAPICKWKGSNGLCGATAGG 560
E. festucae E2368 510 VPVASGKGNKDVLGIELGCNKSSRPFCCPAKDAPICKWKGSNGLCGATAGG 560
E. baconii 510 VPVASGKGNKDVLGIELGCNKSSRPFCCPAKDAPICKWKGSNGLCGATSGG 560
E. brachyelytri 510 VPVASGKGNKDILGIELGCNKSSRPFCCPAKDAPMCKWKGSNGLCGATSGG 560
E. bromocola AL0434 510 VPVASGKGNKDVLGIELGCNKSSRPFCCPAKDAPICKWKGSDGLCGATSGG 560
E. glyceria 480 VPVSSSKGNRNALGIELGCNDSYRPVCCPAKDAPKCEWKGSAPFCGATSGG 530
E. festucae Fl1 561 RCSDNEIEIAAKTDNCVTGHKSLCCSKTSTNDNFGACKWFGAAPVCASASA 611
E. festucae E2368 561 RCSDNEIEIAAKTDNCVTGHKSLCCSKTSTNDNFGACKWFGAAPVCASASA 611
E. baconii 561 RCSGNEIEIAAKTDGCLTGHKSLCCSKTSTNDNFGACKWFGAAPVCASASA 611
E. brachyelytri 561 RCSGNEIEIAAKTDGCFTGHKSLCCSKTSTDDNFGACKWFGAAPVCASASA 611
E. bromocola AL0434 561 RCSDNEIEIAAKTDGCLTGHKSLCCSKTSTDANFGACKWFGAAPVCASASA 611
E. glyceria 531 TCSGNEVEMAASTDGCWTGHKSLCCSKTRTDDNFSSCKWFGAAP------- 574
E. festucae Fl1 612 ASVILNVPLTAGFSFASYGCKKEKDKPLELTTGKQGEGGQKSCLYNGGFKS 662
E. festucae E2368 612 ASVILNVPLTAGFSFASYGCKKEKDKPLELTTGKQGEGGQKSCLYNGGFKS 662
E. baconii 612 ASVILNVPLTAGFSFASYGCKKEKDKPLELTTGKQGEGGQKSCLYNGGFKS 662
E. brachyelytri 612 VSVILNVPLTAGFSFASYGCKKEKDKPLELTTGKQGEGGQKSCLYNGGFKS 662
E. bromocola AL0434 612 VSVILNVPLTAGFSFASYGCKKEKDKPLELTTGKQGEGGQKSCLYNGGFKS 662
E. glyceria ----------
E. festucae Fl1 663 YCCSNPPPWNKCQWRSGHTTWVQWKELIAGPLLSALVDFSVDCKTGCEPGE 713
E. festucae E2368 663 YCCSNPPPWNKCQWRSGHTTWVQWKELIAGPLLSALVDFSVDCKTGCEPGE 713
E. baconii 663 YCCSNPPPWNKCQWRSGHTTWVQWKELIAGPLLSALIDFSVDCKTGCEPGE 713
E. brachyelytri 663 YCCSNPPPWNKCQWRSGHTTWVQWKELIAGPLLSALIDFSVDCKTGCEPGE 713
E. bromocola AL0434 663 YCCSDPPPWNKCRWRSGHTTWVQWKELIAGPLLSALVDFSVDCKTGCEPGE 713
E. glyceria ----------
E. festucae Fl1 714 VTVATDSFGCRSGTYSYYCCADPNFKEPPKVDYKLCYPPKNPFASGGKDPE 764
E. festucae E2368 714 VTVATDSFGCRSGTYSYYCCADPNFKEPPKVDYKLCYPPKNPFASGGKDPE 764
E. baconii 714 VTVATDSFGCRSGTYSYYCCADPNFKEPPKVDYKLCYPPKNPFASGEKDPE 764
E. brachyelytri 714 VTVATDSFGCRSGTYSYYCCADPNFKEPPKVDYTLCYPPKNPFASGEKDPE 764
E. bromocola AL0434 714 VTVATDSFGCRSGTYSYYCCADPNFKEPPKVDYTLCYPPKNPFASGEKDPE 764
E. glyceria ----------
E. festucae Fl1 765 ASEKNVFLETSAFDSGCAPTHQQQRAA----RRMIRSNLANELDTSVLFSH 811
E. festucae E2368 765 ASEKNVFLETSAFDSGCAPTHQQQRAA----RRMIRSNLANELDTSVLFSH 811
E. baconii 765 ASEKNLFLETSAFDSGCAPTH-QQRAA----RRMIRSNLTNELDTSLLFSH 810
E. brachyelytri 765 ASEKNLFLETSAFDSGCAPTH-QERAA----RRMSRSNLTNELDTSLLFSD 810
E. bromocola AL0434 765 ASEKNLFLETSAFDGGCAPTH-QERAQLAPRRRMSRSNLTNELDTSLLLGH 814
E. glyceria ----------
E. festucae Fl1 812 GQLKELDERSLEIRGARDNQPMTLCDQLRKSNVNLYFQAHPGGSTIFKATR 862
E. festucae E2368 812 GQLKELDERSLEIRGARDNQPMTLCDQLRKSNVNLYFQAHPGGSTIFKATR 862
E. baconii 811 GQLQELDERGLEIRGAHDKKPMKLCDKLRKSDVNLYFQAHPGGSTIFKATR 861
E. brachyelytri 811 GQLQDLDERGLDIRGAHAKEPMKLCDKLRQSDVNLYFQAHPGGSTIFKATR 861
E. bromocola AL0434 815 GQLQDLDERRLEIRGAHDKRPMKLCDKLRKSDVNLYFQAHPGGSTIFKATR 865
E. glyceria ----------
E. festucae Fl1 863 AAYTAAQFGVCAVPGITALTTLAPNVNWVTEHVFEKQEFRNAIQSMIDGVT 913
E. festucae E2368 863 AAYTAAQFGVCAVPGITALTTLAPNVNWVTEHVFEKQEFRNAIQSMIDGVT 913
E. baconii 862 AAYTAAQLGVCAVPGITALTNLASYVNWVTEHVFEKQEFRNAIQSMIDGVT 912
E. brachyelytri 862 AAYTAAQLGVCAVPGITALTNLASYVSWVTEHVFEKQEFRNAIQSMIDGVT 912
E. bromocola AL0434 866 AAYTAAQFGVCAAPGITTLKTLASYVNWVTEHVFEKQEYRNAVQSMIDGVT 916
E. glyceria ----------
E. festucae Fl1 914 AKGNALKAGAVPFSVFDTNGLFQANWPQGFPQSLSFTKSWSGNIADFFTGL 964
E. festucae E2368 914 AKGNALKAGAVPFSVFDTNGLFQANWPQGFPQSLSFTKSWSGNIADFFTGL 964
E. baconii 913 AKGNALKAGAVPFSVFDTNGLFQTNWPQGFPQSLSFTKSWSGNIADFFTGL 963
E. brachyelytri 913 AKGNALKAGAVPFSVFDTNGLFQTNWPQGFPQSLSFTKTWSGNIADFFTGL 963
E. bromocola AL0434 917 AKGNALKAGAVPFSVFDANGLFQSKWPQGFPRSLPFTKTWSGSIADDFTGL 967
E. glyceria 575 ----------SWAGNINDAFTGV 587
E. festucae Fl1 965 LGRTSDPGLKNSYIANFQVCDADYNAYKAAIVAGHDFISDALWKSTTDQKD 1015
E. festucae E2368 965 LGRTSDPGLKNSYIANFQVCDADYNAYKAAIVAGHDFISDALWKSTTDQKD 1015
E. baconii 964 LGRTSDAGLKNSYIANFQVCDEDYNAYKATIVAGRDFISDALWKATTDQEE 1014
E. brachyelytri 964 LGRTSDAGLKNSYIANFQVCDSDYNAYKATIAAGHDFISDALWKATTDQEE 1014
E. bromocola AL0434 968 LGRTSDAGLKNSYIANFQVCDEDYNAYKATIVAGRDFISDALWKATTDQEE 1018
E. glyceria 588 LGRTWDAGANNAFAVNLQVCDADFNLHKEHIVAGVNLVSDTIWKSYTDLEQ 638

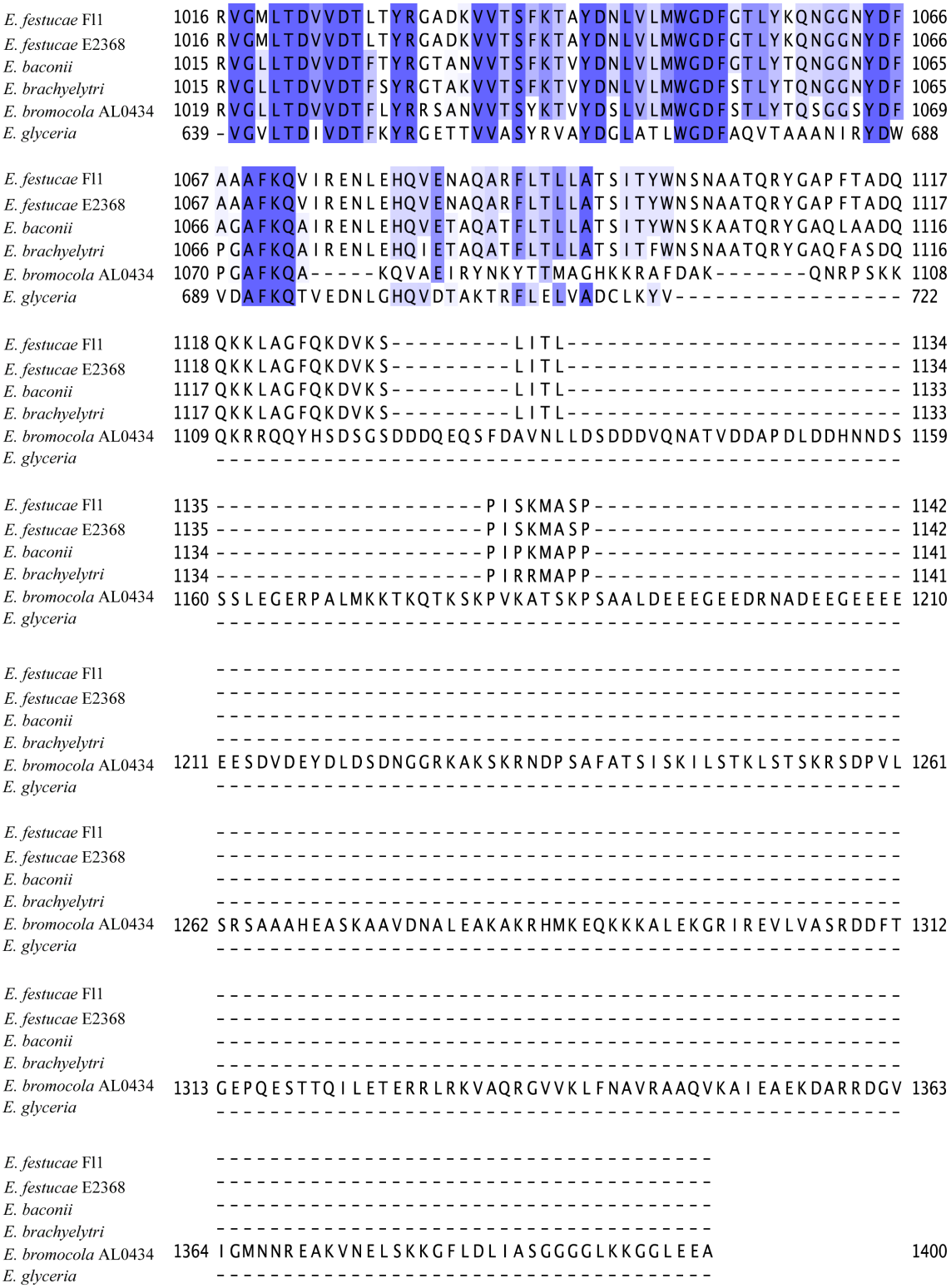

E. festucae Fl1 1016 RVGMLTDVVDTLTYRGADKVVTSFKTAYDNLVLMWGDFGTLYKQNGGNYDF 1066
E. festucae E2368 1016 RVGMLTDVVDTLTYRGADKVVTSFKTAYDNLVLMWGDFGTLYKQNGGNYDF 1066
E. baconii 1015 RVGLLTDVVDTFTYRGTANVVTSFKTVYDNLVLMWGDFGTLYTQNGGNYDF 1065
E. brachyelytri 1015 RVGLLTDVVDTFSYRGTAKVVTSFKTVYDNLVLMWGDFSTLYTQNGGNYDF 1065
E. bromocola AL0434 1019 RVGLLTDVVDTFLYRRSANVVTSYKTVYDSLVLMWGDFSTLYTQSGGSYDF 1069
E. glyceria 639 -VGVLTDIVDTFKYRGETTVVASYRVAYDGLATLWGDFAQVTAAANIRYDW 688
E. festucae Fl1 1067 AAAFKQVIRENLEHQVENAQARFLTLLATSITYWNSNAATQRYGAPFTADQ 1117
E. festucae E2368 1067 AAAFKQVIRENLEHQVENAQARFLTLLATSITYWNSNAATQRYGAPFTADQ 1117
E. baconii 1066 AGAFKQAIRENLEHQVETAQATFLTLLATSITYWNSKAATQRYGAQLAADQ 1116
E. brachyelytri 1066 PGAFKQAIRENLEHQIETAQATFLTLLATSITFWNSNAATQRYGAQFASDQ 1116
E. bromocola AL0434 1070 PGAFKQA-----KQVAEIRYNKYTTMAGHKKRAFDAK-------QNRPSKK 1108
E. glyceria 689 VDAFKQTVEDNLGHQVDTAKTRFLELVADCLKYV---------- 722
E. festucae Fl1 1118 QKKLAGFQKDVKS---------LITL---------- 1134
E. festucae E2368 1118 QKKLAGFQKDVKS---------LITL---------- 1134
E. baconii 1117 QKKLAGFQKDVKS---------LITL---------- 1133
E. brachyelytri 1117 QKKLAGFQKDVKS---------LITL---------- 1133
E. bromocola AL0434 1109 QKRRQQYHSDSGSDDDQEQSFDAVNLLDSDDDVQNATVDDAPDLDDHNNDS 1159
E. glyceria
E. festucae Fl1 1135 ----------PISKMASP---------- 1142
E. festucae E2368 1135 ----------PISKMASP---------- 1142
E. baconii 1134 ----------PIPKMAPP---------- 1141
E. brachyelytri 1134 ----------PIRRMAPP---------- 1141
E. bromocola AL0434 1160 SSLEGERPALMKKTKQTKSKPVKATSKPSAALDEEEGEEDRNADEEGEEEE 1210
E. glyceria
E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromocola AL0434 1211 EESDVDEYDLDSDNGGRKAKSKRNDPSAFATSISKILSTKLSTSKRSDPVL 1261
E. glyceria
E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromocola AL0434 1262 SRSAAAHEASKAAVDNALEAKAKRHMKEQKKKALEKGRIREVLVASRDDFT 1312
E. glyceria
E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromocola AL0434 1313 GEPQESTTQILETERRLRKVAQRGVVKLFNAVRAAQVKAIEAEKDARRDGV 1363
E. glyceria
E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromocola AL0434 1364 IGMNNREAKVNELSKKGFLDLIASGGGGLKKGGLEEA 1400
E. glyceria

Figure 3.13. Multiple sequence alignment of the *E. festucae* Fl1 ChiB protein and homologues from other *Epichloë* species. *E. festucae* (Fl1) EfM3. 035030, E. *festucae* (E2368), *E. baconii, E. brachyelytri, E. bromicola* AL0434, *E. glyceria.* Intensity of colouring indicates the level of conservation. N terminal signal peptides are bordered in green. Chitin binding domains are bordered in red and orange. Glycoside hydrolase family 18 catalytic domain is indicated in pink and chitinase insertion domain inside it, is bordered in yellow. Intensity of colouring indicates the level of conservation.

Chapter 4. Functional analysis of genes encoding LysM and Glycoside hydrolase Family 18 (GH18) proteins.

To investigate the role of *E. festucae* genes encoding LysM motifs and the associated, divergently transcribed, chitinase genes, a reverse genetics approach was conducted. The genes encoding LymA, LymB, ChiA and ChiB were deleted towards determining their protein product function in hyphal growth, fungal development and effect on the host, *L. perenne.*

4.1. Functional analysis of genes encoding LysM motif containing proteins

4.1.1. Generation of Δ*lymA* mutant

To test whether *lymA* has a role in suppressing host defence in the symbiotic interaction of *E. festucae* with *L. perenne*, a deletion mutant of *lymA* was generated by targeted replacement of *lymA* with a hygromycin B phosphotransferase (*hph*) cassette (Figure 4.1 A). Protoplasts of *E. festucae* Fl1 (2.8.4.1) were transformed with an *Hpa*I/*Eco*RI digested linear fragment of pNN01 (Appendix 7) and screened for growth on PD agar medium (2.8.4.2) containing hygromycin. One hundred and two Hyg^R colonies were picked and sub-cultured three times to confirm transformant stability and to ensure they were homokaryons (Young *et al.*, 2005). Crude genomic DNA was isolated from each transformant (2.4.2) and screened by PCR (2.6.1) using primers NN22 and NN25, to confirm the absence of the wild-type fragment. Thirteen putative Δ*lymA* candidates were identified from this screen. Further PCR screening using primer sets that amplify the left (NN31/NN32) and right (NN29/NN26) flanks of the *hph* cassette confirmed that 10 were Δ*lymA* candidates. High quality genomic DNA (2.4.3) was isolated from four of these transformants, digested with either *Sma*I or *Nde*I, separated by gel electrophoresis and Southern blots of the gels prepared. Probing of these blots with a DIG-labelled linear fragment of pNN01 confirmed that all four candidates were 'clean' single copy replacement mutants (Figure 4.1 B). The presence of the additional 12.6-kb band in the *Nde*I digests of transformants #7 and #32 is likely due to incomplete digestion with this enzyme given that there were no extra bands in the *Sma*I digest.

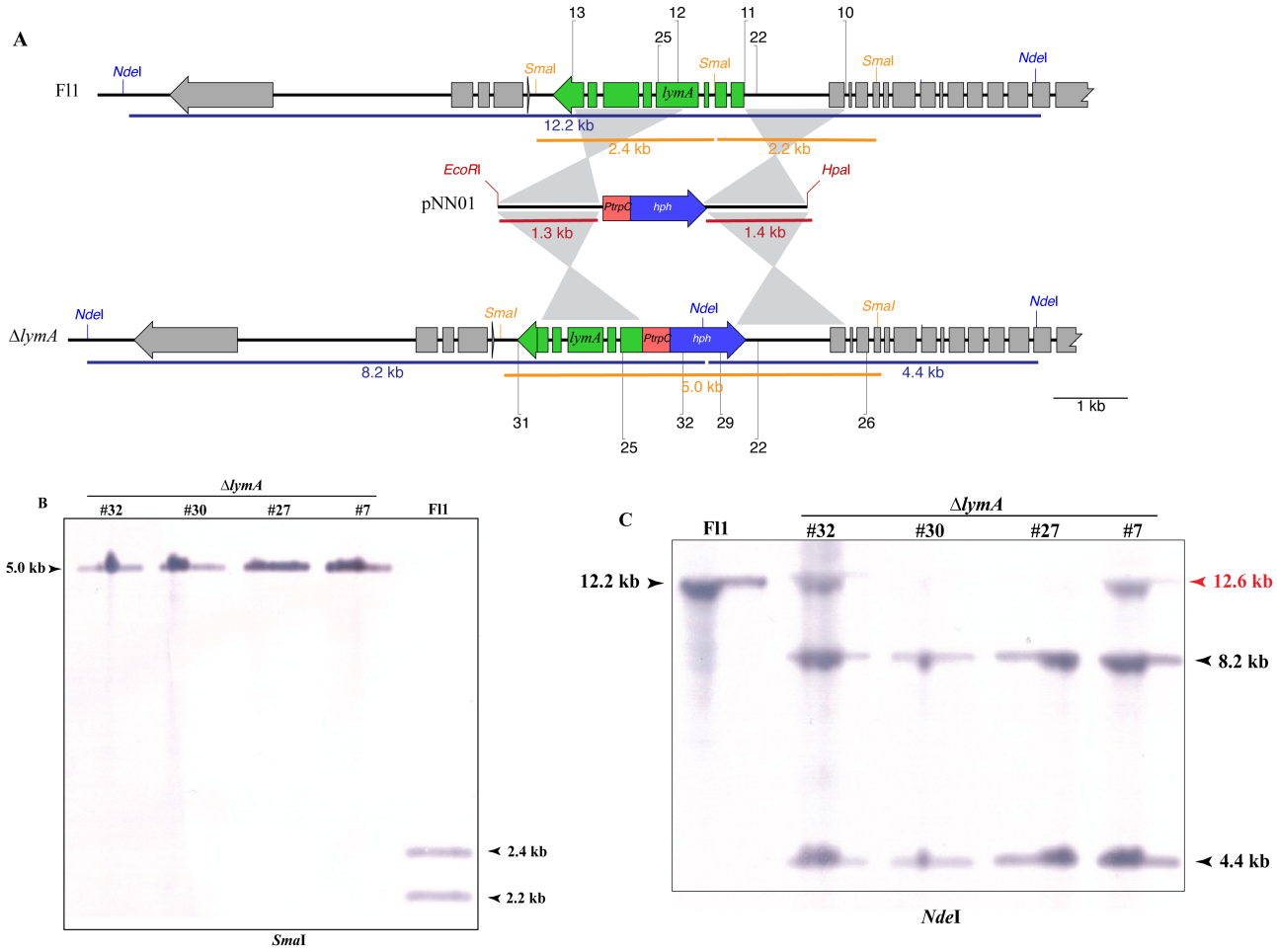


Figure 4.1. Strategy for deletion of *E. festucae lymA* and confirmation by Southern analysis. (A) Physical map of *lymA* wild-type genomic locus, linear insert of *lymA* replacement construct, pNN01, showing restriction enzyme sites for *Nde*I and *Sma*I. Grey shading indicates regions of recombination. Numbers indicate the PCR primers used for Gibson assembly and knock–out screening. (B) and (C) NBT/BCIP stained Southern blot of *Sma*I (B) and *Nde*I (C) digests (1 µg) of *E. festucae* wild-type (Fl1) Δ*lymA*#32 (PN3099), Δ*lymA*#30 (PN3098), Δ*lymA*#27 (PN3097) and Δ*lymA*#7 (PN3096) strains probed with digoxigenin (DIG)-11-dUTP labelled linear insert of pNN01 amplified with primers NN10/NN13.

4.1.2. Δ*lymA* culture phenotype

Cultures of *E. festucae* wild type and two independent Δ*lymA* mutant strains, Δ*lymA*#7 and Δ*lymA*#27, were grown on PD agar or WA to determine if there were any differences in colony growth or fungal morphology. Radial growth and morphology of Δ*lymA* was indistinguishable from the wild-type strain (Figure 4.2 A). Epi-fluorescence light microscopy analysis of cultures grown on water agar and stained with Calcofluor white (CFW) (2.10.1), which binds to chitin in the cell wall and septa (Ram & Klis, 2006), revealed that Δ*lymA* forms hyphal bundles, undergoes hyphal cell-cell fusion and forms coils from which conidiophores arise, all with a morphology and frequency similar to wild-type (Figure 4.2 B-E).

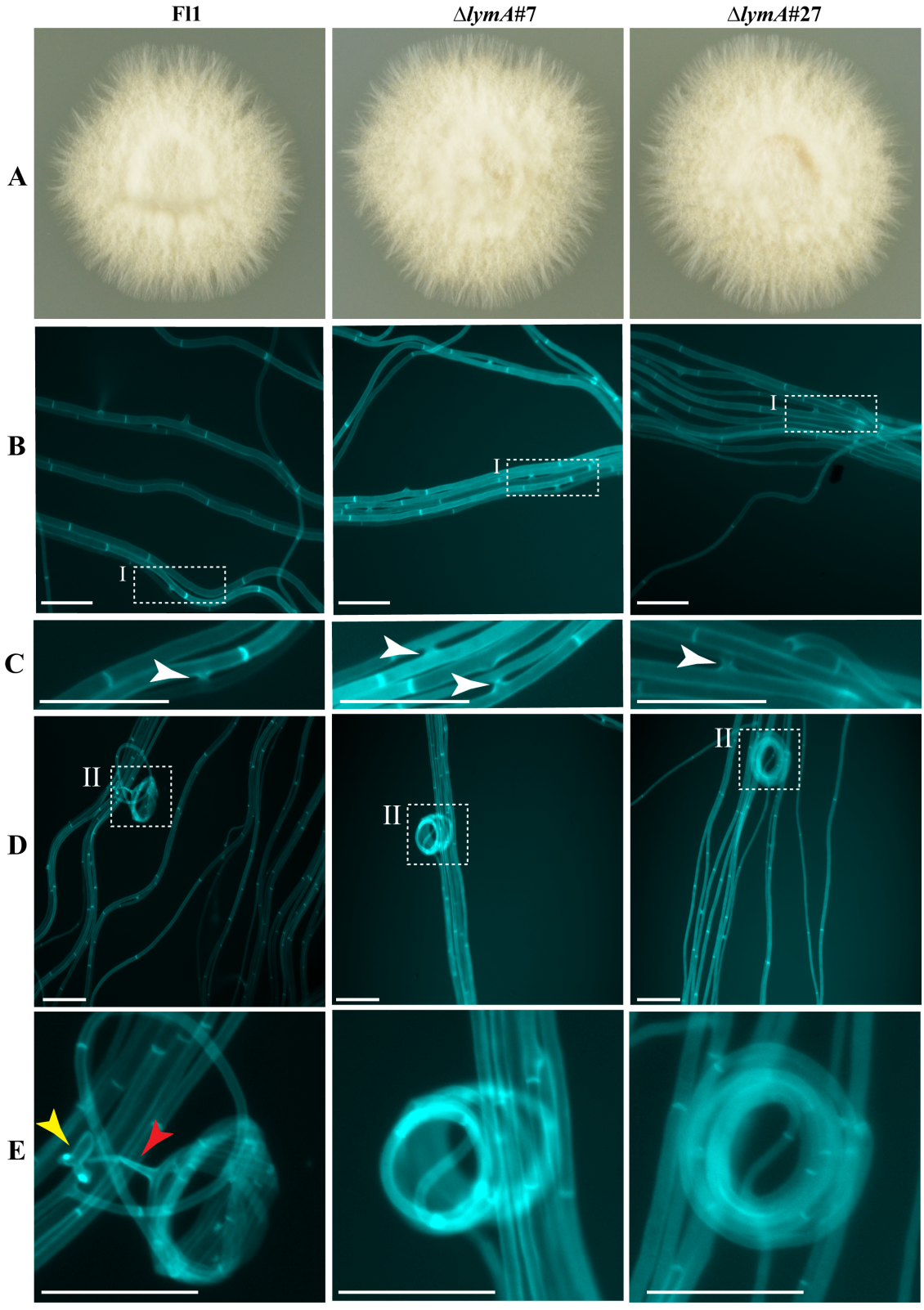


Figure 4.2. Culture phenotype of wild-type and Δ*lymA* mutant strains. (A) Colony morphology of wild type (Fl1) and Δ*lymA* strains grown on 2.4% PD agar at 22°C for 7 days. (B) and (D) Fluorescent images captured by Epi-fluorescence light microscopy of hyphae grown for 7 days on water agar and stained with Calcofluor white. Bar=20 µm. (C) and (E) Higher magnification

of (I) and (II) indicating fusion points and coiled structures respectively. White, Red and yellow arrowheads identify hyphal fusion points, conidiophore and conidia respectively. Bar=20 μm.

4.1.3. Δ*lymA* symbiotic interaction phenotype

To test whether *lymA* is required for the symbiotic interaction of *E. festucae* with its host, *L. perenne*, 7-day old seedlings were inoculated with wild type and two independent mutant strains, Δ*lymA*#7 and Δ*lymA*#27, and grown under controlled environment conditions for 8-12 weeks (2.3.8.2). After this period of growth, single tillers were immunoblotted to identify which plants were infected (2.3.8.3). Although statistical analysis of the average tiller length and number of infected plants showed a significant difference for plants infected with Δ*lymA*#27 and just tiller length of plants infected with Δ*lymA*#7. For all other associations there were no significant differences in these parameters. These variations are sometimes observed in similarly maintained WT plants so are likely to reflect the variation between biological plants. In summary, this analysis revealed that the phenotype of plants infected with the Δ*lymA* mutants was largely similar to those infected with WT with similar root, stem and leaf morphologies (Figure 4.3).

To determine if any phenotypic differences were occurring at the cellular level, plant sheath samples were fixed with ethanol and stained with aniline blue, wheat germ agglutinin-conjugated with Alexa Fluor 488 (WGA-AF488) and propidium iodide (2.10.2), which stain fungal β-glucan, chitin and DNA respectively, and examined by confocal laser scanning microscopy (CLSM). In plants infected with wild type, fungi grew predominantly as single elongated hyphae, parallel to the leaf longitudinal axis with occasional lateral branching and fusion. Importantly, only the septa stained with WGA-AF488 suggesting as previously observed (Becker *et al.*, 2015; Eaton *et al.*, 2015), that chitin is absent from the cell walls of endophytic hyphae. The morphology and growth pattern of Δ*lymA* mutants *in planta* was indistinguishable from that of WT (Figure 4.4).

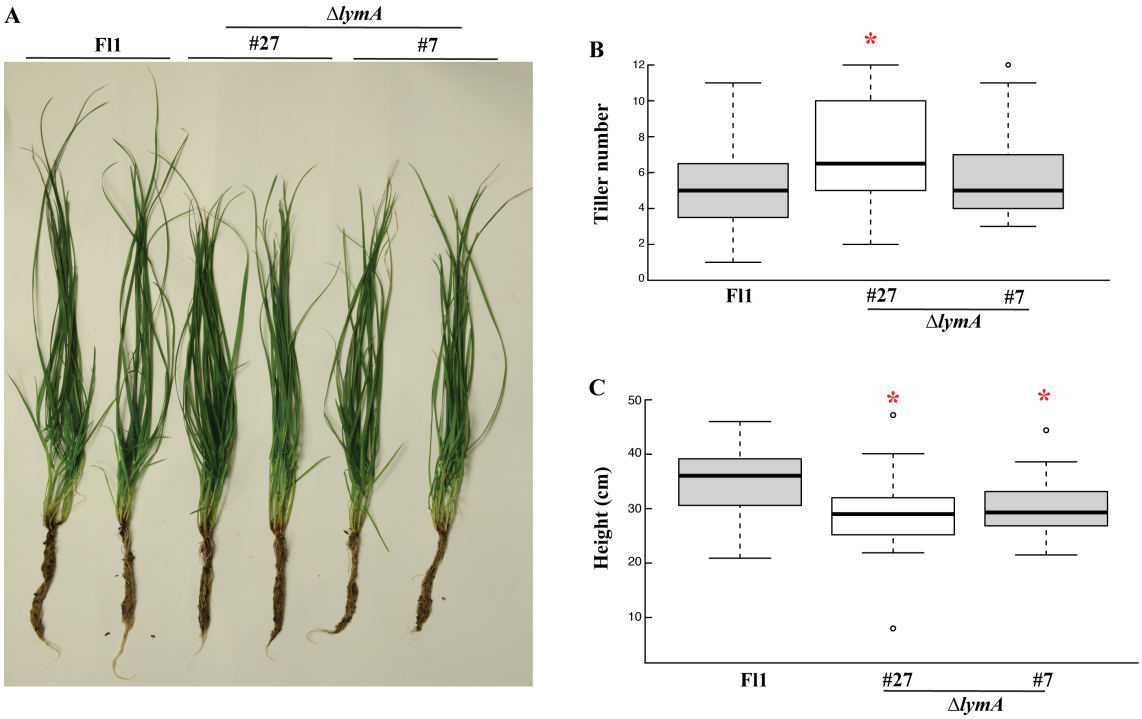


Figure 4.3. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*lymA* mutant strains. (A) Whole plant interaction phenotype of *L. perenne* plants infected with *E. festucae* strains 10 weeks after inoculation. (B) and (C) Box plots of tiller number and tiller height of *L. perenne* plants infected with *E. festucae* strains. **P* <0.05 determined by Student's *t*-test (two-tailed, n≥10). Outliers are indicated by circles. Significant difference is indicated by red asterisk.

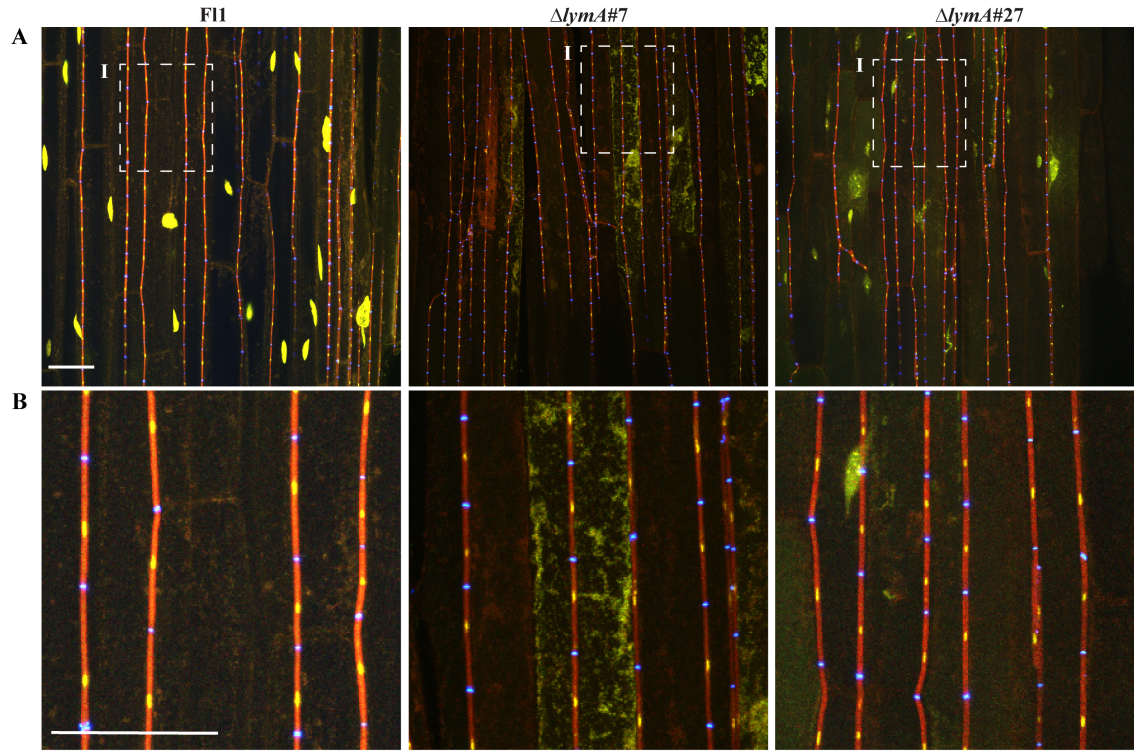


Figure 4.4. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*lymA* mutant strains. (A) Confocal depth series images of longitudinal sections of *L. perenne* leaf sheath tissue infected with wild type (Fl1) and Δ*lymA* strains 10 weeks post inoculation, showing hyphae stained with aniline blue (red pseudocolour), chitin in cell wall septa with WGA-AF488 (blue pseudocolour), and fungal and plant nuclei with propidium iodide (yellow pseudocolour). Bar=50 µm. (B) Higher magnification of (I) showing hyphal compartments. Images were generated by maximum intensity projection of a 10× 1.2 µm confocal z-stack. Bar=50 µm. Scale bars are applicable for images in the same panel. The analyses were conducted at least in three biological replicates.

4.1.4. Generation of Δ*lymB* mutant

To examine the function of *lymB* in suppressing host defence in the symbiotic interaction of *E. festucae* with *L. perenne*, a deletion mutant of *lymB* was generated by targeted replacement of *lymB* with a neomycin phosphotransferase (*ntp*II) cassette (Figure 4-5 A). Protoplasts of *E. festucae* Fl1 (2.8.4.1) were transformed with an *Hpa*I/*Spe*I digested linear fragment of pNN03 (Appendix 9) and screened for growth on PD agar medium (2.8.4.2) containing geneticin. Two hundred Gen^R colonies were picked and sub-cultured three times to confirm transformant stability and to ensure they were homokaryons. Crude genomic DNA was isolated from each transformant (2.4.2) and 68 colonies were screened by PCR (2.6.1) using primers NN50 and NN51, to confirm the absence of a wild-type fragment. Four putative Δ*lymB* candidates were identified from this screen. Further PCR screening using primer sets that amplify the left (NN52/NN53) and right (NN54/NN55) flanks of the *nptII* cassette confirmed that all 4 were Δ*lymB* candidates. High quality genomic DNA (2.4.3) was isolated from all four transformants, digested with either *Kpn*I or *Nde*I, the digests separated by gel electrophoresis, and Southern blots of the gels prepared. Probing of these blots with a DIG-labelled linear fragment of pNN03 confirmed that all four candidates were 'clean' replacement mutants. All strains including WT showed hybridization to a non-specific band of 4.5 kb. The presence of the additional 9 kb band in the *Nde*I digests of transformant #122 is likely due to tandem copies integrated into genome, given a single band of the same size as the other strains was present in the *Kpn*I digest. The presence of the 4 kb and 7 kb bands in *Kpn*I digestion could be due to non-specific hybridization as well. A 2 kb band in the *Kpn*I digestion including WT could be a result of digestion in *chiB* and probed incompletely.

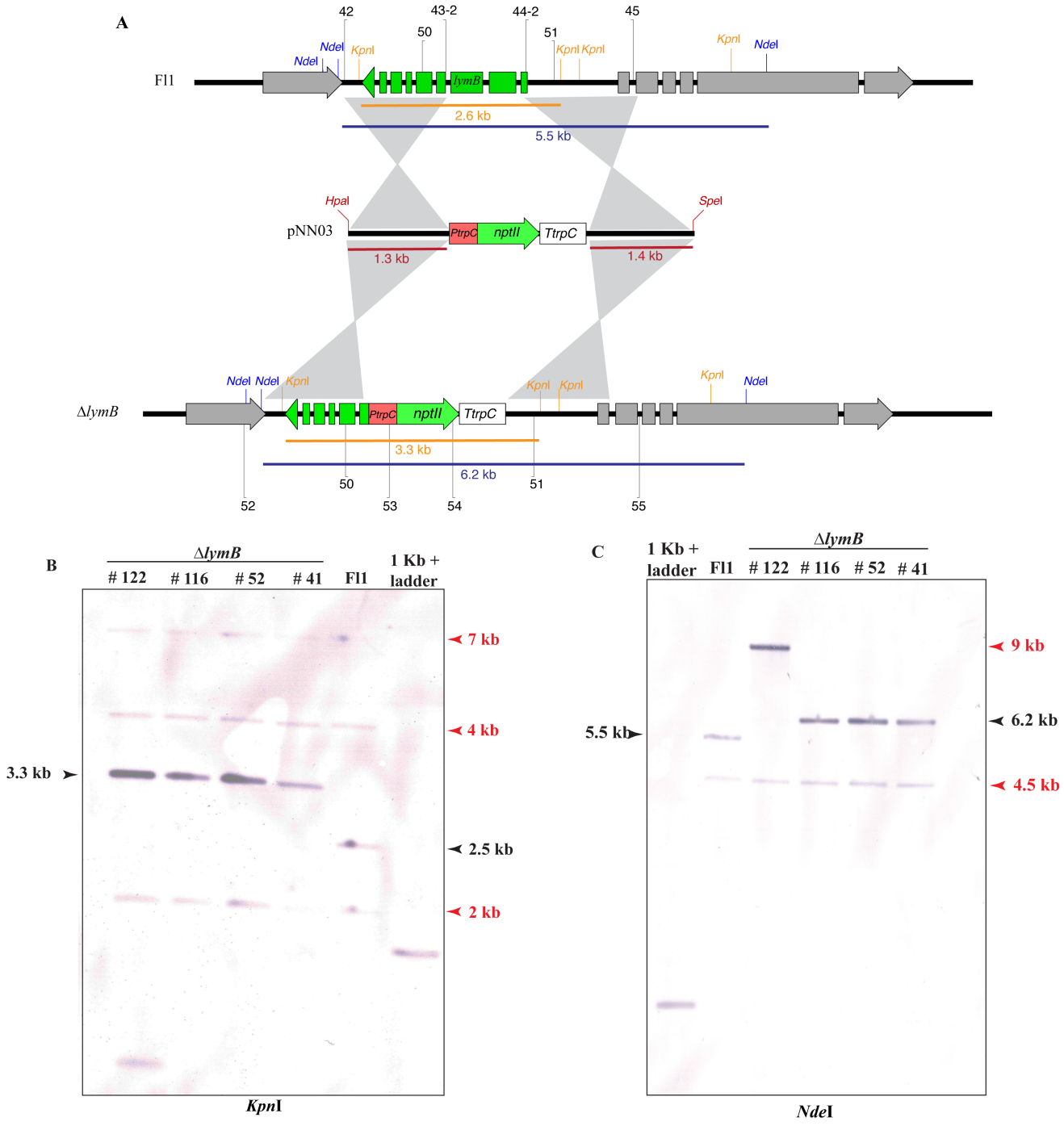


Figure 4.5. Strategy for deletion of *E. festucae lymB* and confirmation by Southern analysis. (A) Physical map of *lymB* wild-type genomic locus, linear insert of *lymB* replacement construct, pNN03, showing restriction enzyme sites for *Nde*I and *Kpn*I. Grey shading indicates regions of recombination. Numbers indicate the PCR primers used for Gibson assembly and knock–out screening. (B) and (C) NBT/BCIP stained Southern blot of *Kpn*I (B) and *Nde*I (C) digests (1 µg) of *E. festucae* wild-type (Fl1) Δ*lymB*#41 (PN3125), Δ*lymB*#52 (PN3126), Δ*lymB*#116 (PN3127)

and Δ*lymB*#122 strains probed with digoxigenin (DIG)-11-dUTP labelled linear insert of pNN03 amplified with primers NN42/NN45.

4.1.5. Δ*lymB* culture phenotype

In axenic culture radial growth and morphology of Δ*lymB* mutant strains (#41 and #52) was indistinguishable from the wild-type strain (Figure 4.6 A). Epi-fluorescence light microscopy analysis of cultures grown on WA and stained with CFW (2.10.1), showed that Δ*lymB* forms hyphal bundles, undergoes hyphal cell-cell fusion and forms coils from which conidiophores arise, with a morphology and frequency similar to wild-type (Figure 4.6 B-E).

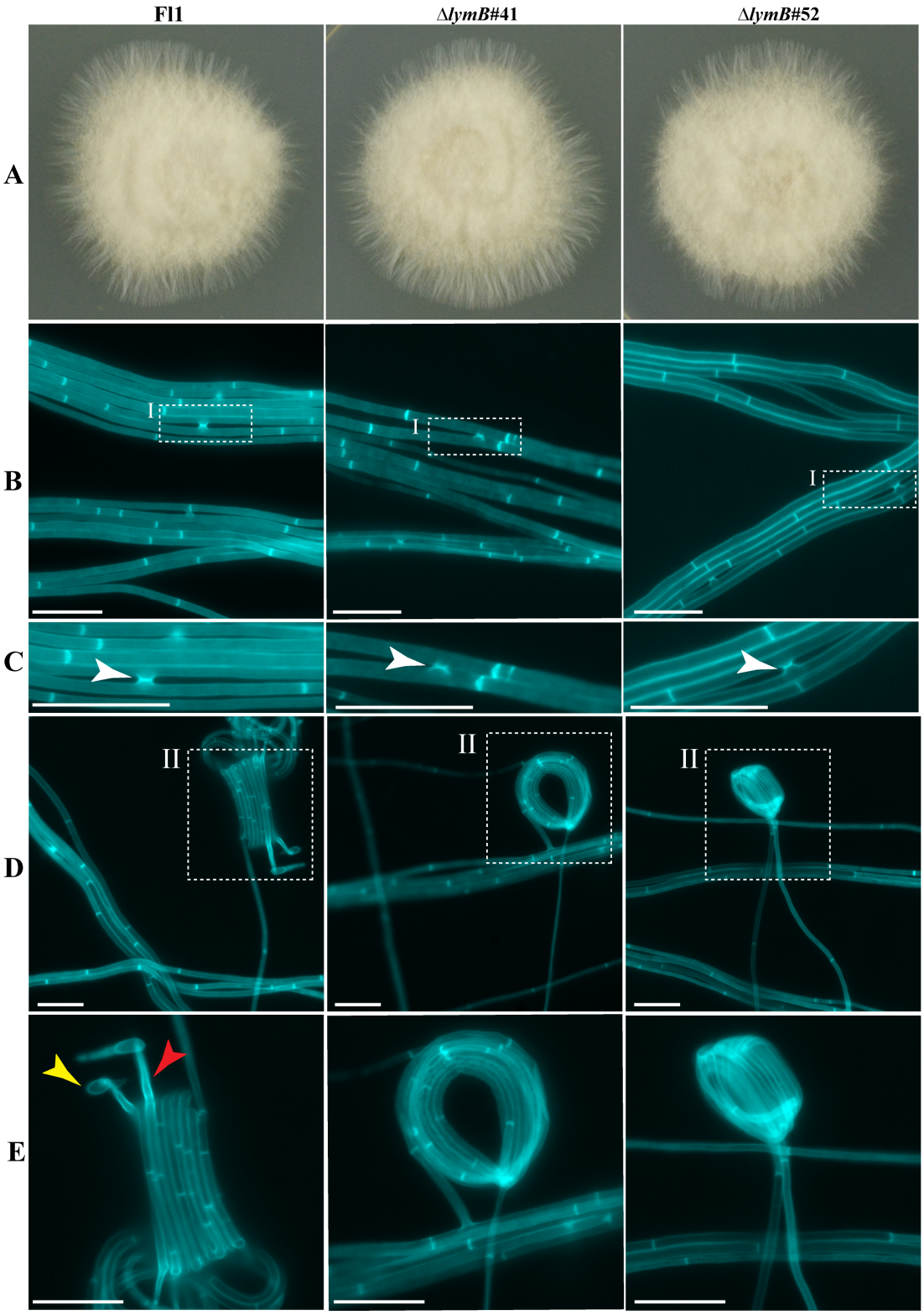


Figure 4.6. Culture phenotype of wild-type and Δ*lymB* mutant strains. (A) Colony morphology of wild type (Fl1) and Δ*lymB* strains grown on 2.4% PD agar at 22°C for 7 days. (B) and (D) Fluorescent images captured by Epi-fluorescence light microscopy of hyphae grown for 7 days on water agar and stained with Calcofluor white. Bar=20 µm. (C) and (E) Higher magnification

of (I) and (II) indicating fusion points and coiled structures respectively. White, red and yellow arrowheads identify hyphal fusion points, conidiophores and conidia respectively. Bar=20 μm.

4.1.6. Δ*lymB* symbiotic interaction phenotype

To test whether the *lymB* is required for the symbiotic interaction of *E. festucae* with its host, *L. perenne*, 7-day old seedlings were inoculated with WT and two independent mutant strains, Δ*lymB*#41 and Δ*lymB*#52, and grown under controlled environment conditions (2.8.3.2). Single tillers were immunoblotted 8-12 weeks post inoculation to identify which plants were infected (2.3.8.3). The phenotype of plants infected with the Δ*lymB* mutants was similar to those infected with WT, and examination of root, stem and leaf morphologies did not reveal significant differences (Figure 4.7 A). There was a significant change in tiller length and number in plants infected with Δ*lymB*#41 compared to WT. These variations are sometimes observed in similarly maintained WT plants and likely reflect the variation between biological plants. Therefore, plant phenotype analysis revealed that there were no significant differences in tiller number or tiller length between Δ*lym*B and WT (Figure 4.7 B & C).

To check if there were any differences in phenotype at the cellular level, plant sheath samples were fixed in ethanol, stained with aniline blue, WGA-AF488 and propidium iodide (2.10.2) to label fungal ß-glucan, chitin, DNA, respectively and examined by CLSM (Figure 4.8). As with WT, Δ*lymB* mutants formed hyphae that were linear and grew parallel to the longitudinal axis of the infected leaf. This was occasionally interrupted by lateral branching. In all cases only the septa stained with WGA-AF488. This is consistent with chitin being absent from the cell walls of endophytic hyphae.

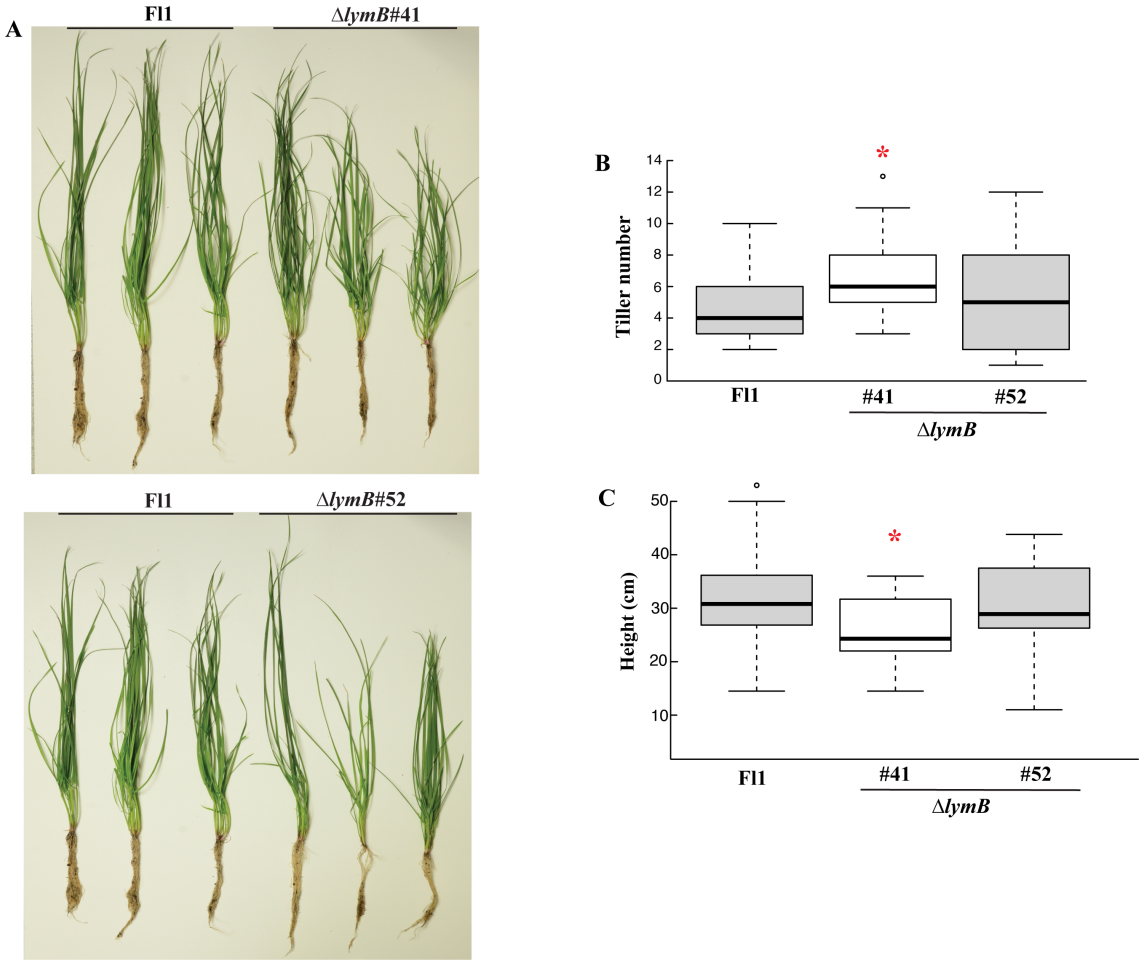


Figure 4.7. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*lymB* mutant strains. (A) Whole plant interaction phenotype of *L. perenne* plants infected with *E. festucae* strains 10 weeks after inoculation. (B) and (C) Box plots of tiller number and tiller height of *L. perenne* plants infected with *E. festucae* strains. **P* <0.05 determined by Student's *t*-test (two-tailed, n≥10). Outliers are indicated by circles. Significant difference is indicated by red asterisk.

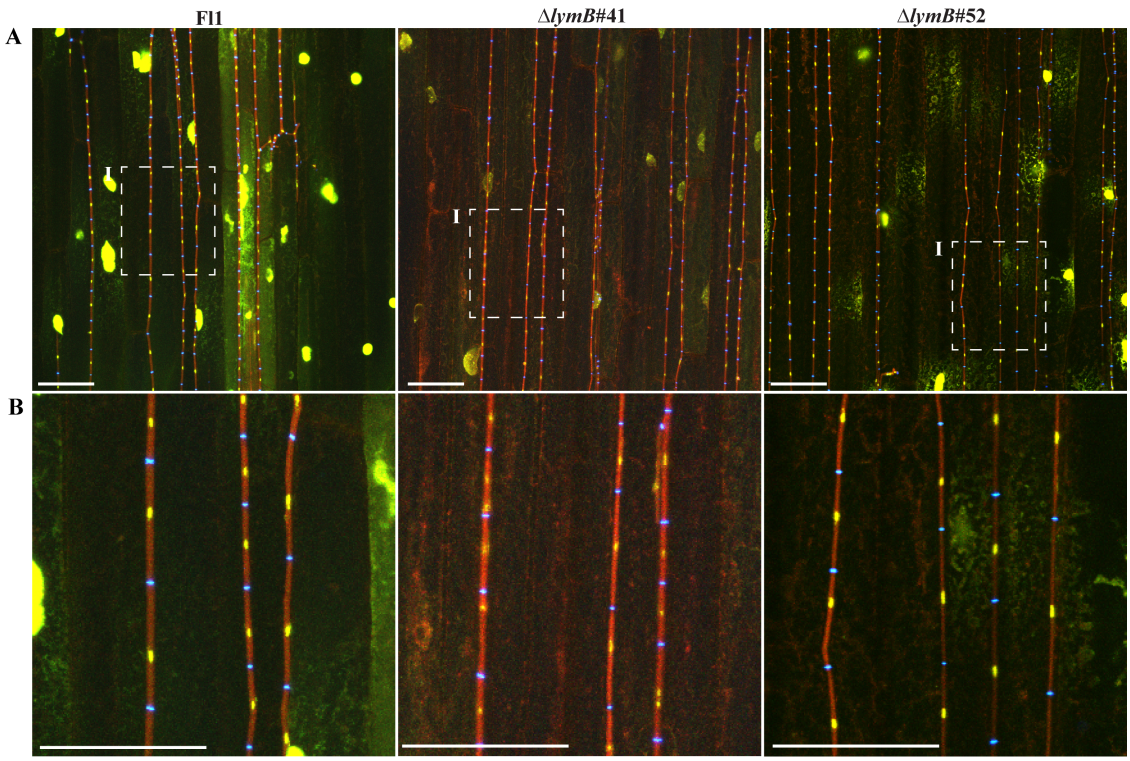
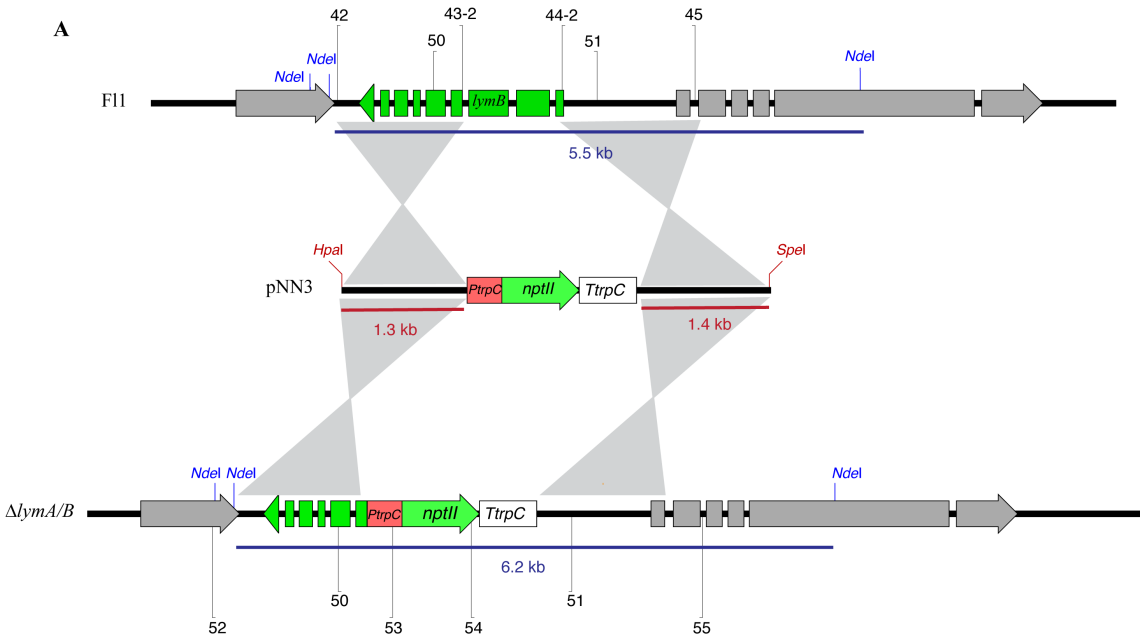


Figure 4.8. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*lymB* mutant strains. (A) Confocal depth series images of longitudinal sections of *L. perenne* leaf sheath tissue infected with wild type (Fl1) and Δ*lymB* strains 10 weeks post inoculation, showing hyphae stained with aniline blue (red pseudocolour), chitin in cell wall septa with WGA-AF488 (blue pseudocolour), and fungal and plant nuclei with propidium iodide (yellow pseudocolour). Bar=50 µm. (B) Higher magnification of (I) showing hyphal compartments. Images were generated by maximum intensity projection of a 10× 1.2 µm confocal z-stack. Bar=50 µm. The analyses were conducted at least in three biological replicates.

4.1.7. Generation of Δl*ymA/B* mutant

To determine whether *lymA* and *lymB* are functionally redundant and together have a role in suppressing host defence in the symbiotic interaction of *E. festucae* with *L. perenne*, a double deletion mutant was engineered. This was done stepwise through the targeted replacement of *lymB* with a neomycin phosphotransferase (*npt*II) cassette (Figure 4.9 A). Protoplasts of *E. festucae* Δ*lymA*#7 (2.8.4.1) were transformed with an *Hpa*I/*Spe*I digested linear fragment of pNN03 (Appendix 9) and screened for growth on PD agar medium (2.8.4.2) containing geneticin. One hundred and twelve Gen^R colonies were picked and sub-cultured three times to confirm transformant stability and to ensure they were homokaryons. Crude genomic DNA was isolated from each transformant (2.4.2) and 48 colonies screened by PCR (2.6.1) using primers NN50 and NN51, for absence of a wild-type fragment. Five putative Δ*lymA/B* candidates were identified from this screen. Further PCR screening using primer sets that amplify the left (NN52/NN53) and right (NN54/NN55) flanks of the *npt*II cassette confirmed that all 5 were Δ*lymA/B* candidates. High quality genomic DNA (2.4.3) was isolated from all five transformants, digested with *Nde*I, the digests separated by gel electrophoresis, and Southern blots of the gels prepared. Probing of these blots with a DIG-labelled linear fragment of pNN03 confirmed that four of the five candidates (#52, #42, #29 and #12) were 'clean' single copy replacement mutants (Figure 4.9 B).


A
42
43-2
44-2
45
50
51
NdeI
NdeI
NdeI
Fl1
lymB
5.5 kb
HpaI
SpeI
pNN3
PtrpC
nptII
TtrpC
1.3 kb
1.4 kb
NdeI NdeI
NdeI
ΔlymA/B
PtrpC
nptII
TtrpC
6.2 kb
50
51
52
53
54
55

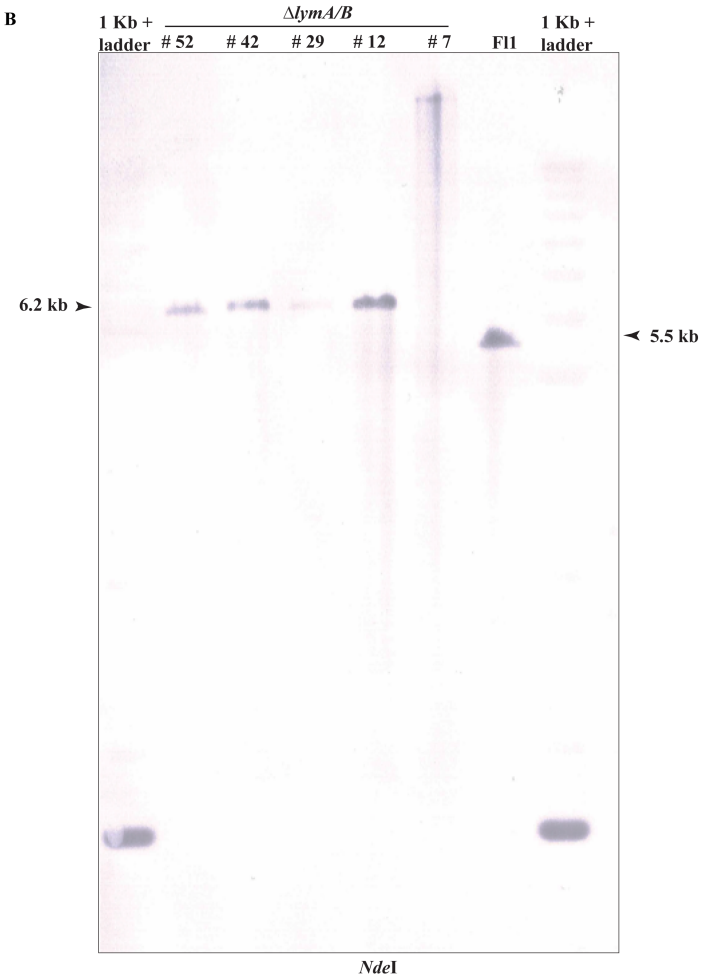

B
1 Kb + ladder
ΔlymA/B
52
42
29
12
7
Fl1
1 Kb + ladder
6.2 kb
5.5 kb
NdeI

Figure 4.9. Strategy for deletion of *E. festucae lymA/B* and confirmation by Southern analysis. (A) Physical map of *lymB* wild-type genomic locus, linear insert of *lymB* replacement construct, pNN03, showing restriction enzyme sites for *Nde*I. Grey shading indicates regions of recombination. Numbers indicate the PCR primers used for Gibson assembly and knock–out screening. (B) NBT/BCIP stained Southern blot of *Nde*I (B) digests (1 µg) of *E. festucae* wild-type (Fl1) Δ*lymA/B*#12 (PN3160), Δ*lymA/B*#29 (PN3161), Δ*lymA/B*#42 (PN3162), strains probed with digoxigenin (DIG)-11-dUTP labelled linear insert of pNN03 amplified with primers NN42/NN45.

4.1.8. Δ*lymA/B* culture phenotype

Radial growth and morphology of *E. festucae* wild type and three independent Δ*lymA/B* mutant strains, Δ*lymA/B*#12, Δ*lymA/B*#29 and Δ*lymA/B*#42, Δ*lymA/B* were checked in axenic culture. None of these appeared different than the wild-type strain (Figure 4.10 A). Epi-fluorescence light microscopy analysis of cultures grown on WA and stained with CFW (2.10.1), showed that Δ*lymA/B* forms hyphal bundles, undergoes hyphal cell-cell fusion and forms coils from which conidiophores arise, with a morphology and frequency similar to wild-type (Figure 4.10 B-E).

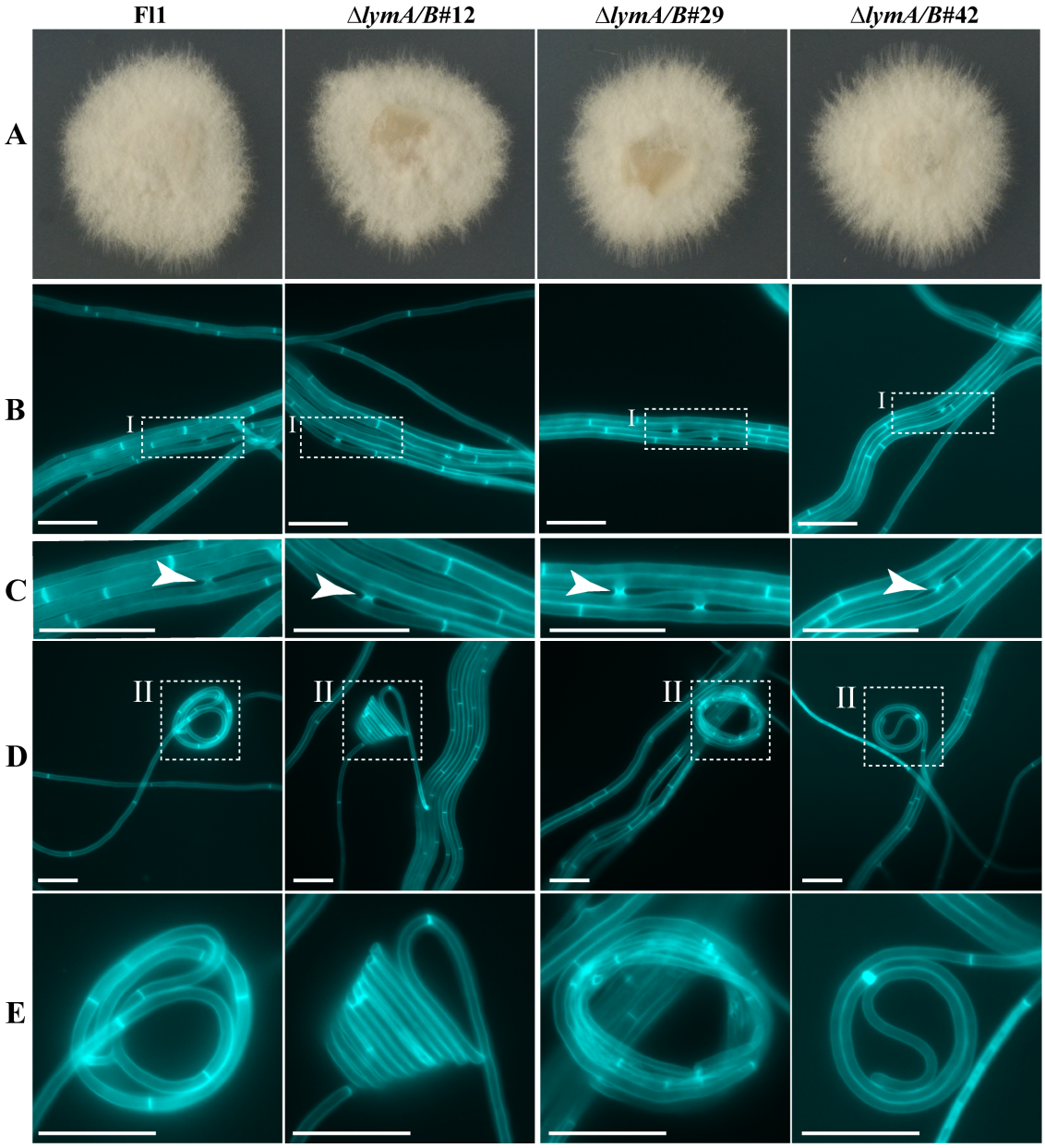


Figure 4.10. Culture phenotype of wild-type and Δ*lymA/B* mutant strains. (A) Colony morphology of wild type (Fl1) and Δ*lymA/B* strains grown on 2.4% PD agar at 22°C for 7 days. (B) and (D) Fluorescent images captured by Epi-fluorescence light microscopy of hyphae grown for 7 days on water agar and stained with Calcofluor white. Bar=20 µm. (C) and (E) Higher magnification of (I) and (II) indicating hyphal fusion points and coiled structures respectively. White arrowheads identify fusion points. Bar=20 µm.

4.1.9. Δ*lymA/B* symbiotic interaction phenotype

To test whether the *lymA* and *lymB* both are required for the symbiotic interaction of *E. festucae* with its host, 7-day old seedlings of *L. perenne*, were inoculated with WT and three independent mutant strains, Δ*lymA/B*#12, Δ*lymA/B*#29 and Δ*lymA/B*#42 and grown under controlled environment conditions (2.8.3.2). After 8-12 weeks post-inoculation, single tillers were immunoblotted to confirm the infection (2.3.8.3). Statistical analysis of the whole plant phenotype following infection with the Δ*lymA/B* mutants and WT showed a decrease in average tiller height for plants infected with the Δ*lymA/B* mutant strains, indicating a mild stunting plant phenotype (Figure 4.11).

To check the cellular phenotype, plant sheath samples were fixed with ethanol and stained with aniline blue, WGA-AF488 and propidium iodide (2.10.2) to reveal the distribution of 1,3 ß glucan, chitin and nuclei, respectively prior to examination by CLSM. The morphology and growth pattern of the Δ*lymA/B* mutants *in planta* was significantly different from that of WT. Mutant hyphae had a break down in the symbiosis with abnormal hyphal growth in intercellular spaces. Hyphae were swollen and branched forming highly convoluted structures with smaller hyphal compartments. Strikingly, unlike WT hyphae, the mutants displayed patches of WGA-AF488 along their cell walls as well as the septa. This observation raises the possibility that loss of *lymA* and *lymB* alters the composition or structure of the fungal cell wall *in planta* (Figure 4.12).

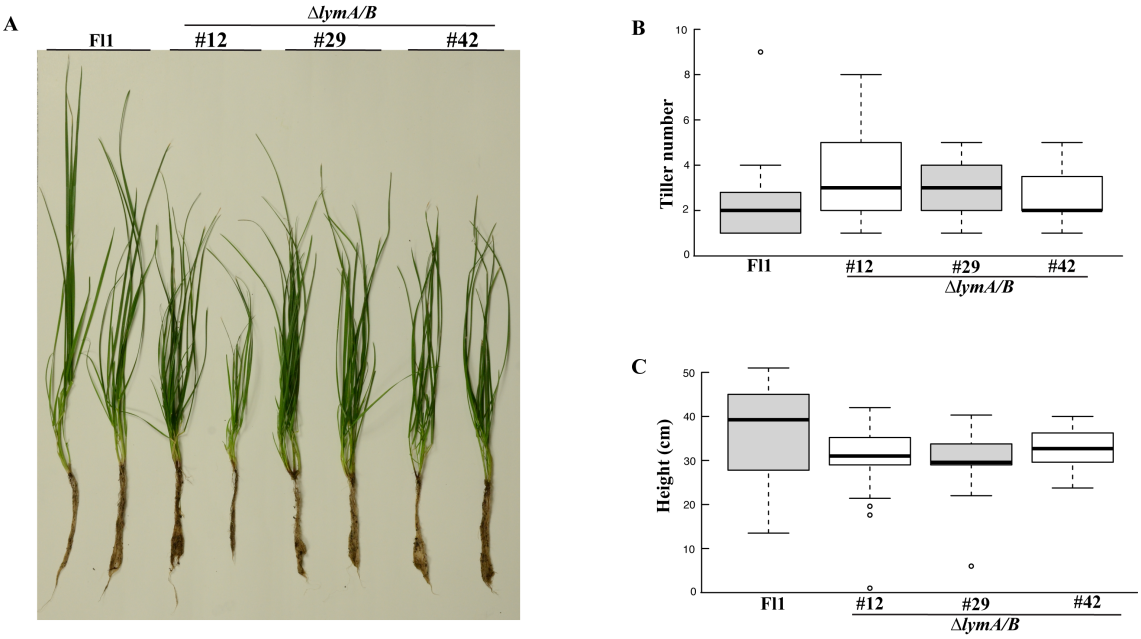


Figure 4.11. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*lymA/B* mutant strains. (A) Whole plant interaction phenotype of *L. perenne* plants infected with *E. festucae* strains 10 weeks after inoculation. (B) and (C) Box plots of tiller number and height of *L. perenne* plants infected with *E. festucae* strains. Outliers are indicated by circles.

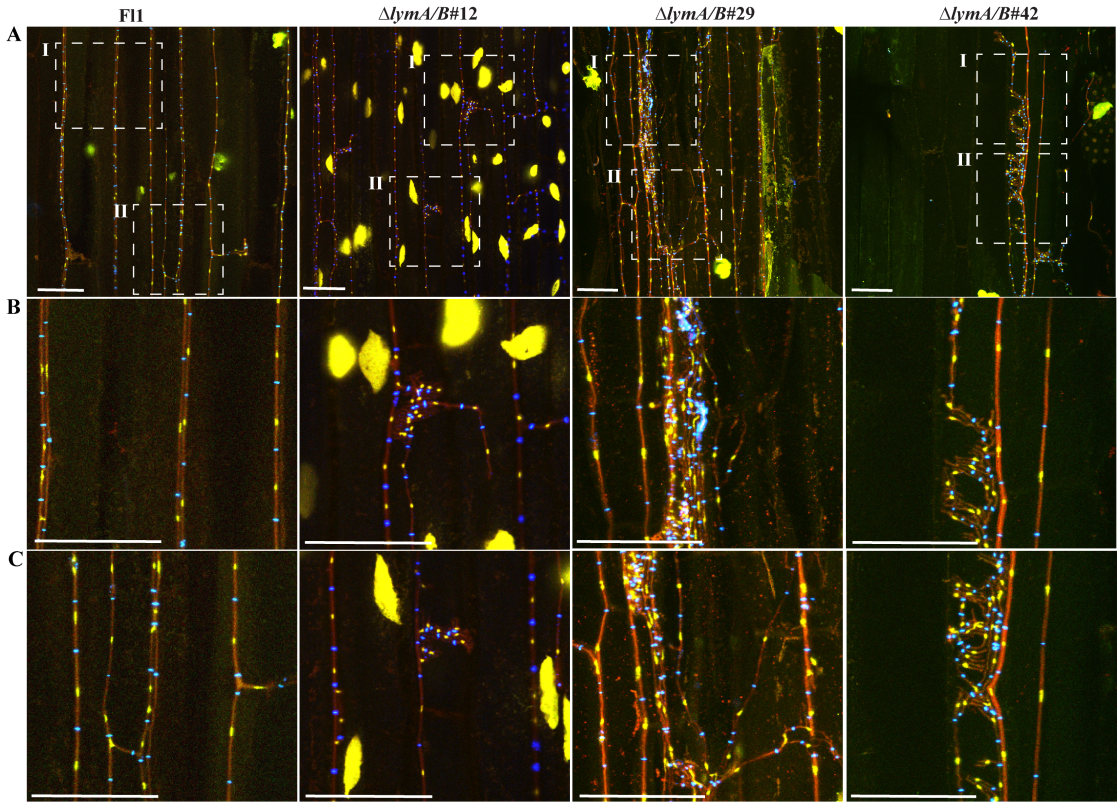


Figure 4.12. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*lymA/B* mutant strains. (A) Confocal depth series images of longitudinal sections of *L. perenne* leaf sheath tissue infected with wild type (Fl1) and Δ*lymA/B* strains 10 weeks post inoculation, showing hyphae stained with aniline blue (red pseudocolour), chitin in cell wall septa with WGA-AF488 (blue pseudocolour), and fungal and plant nuclei with propidium iodide (yellow pseudocolour). Bar=50 µm. (B) and (C) Higher magnification of (I) and (II) showing normal hyphal compartments in the wild type, abnormal growth pattern of fungal hyphae and increasing of hyphal branching in Δ*lymA/B* mutant strains respectively. Images were generated by maximum intensity projection of a 10× 1.2 µm confocal z-stack. Bar=50 µm. The analyses were conducted at least in three biological replicates.

4.2. Functional analysis of genes encoding Glycoside hydrolase Family 18 (GH18) proteins.

4.2.1. Generation of Δ*chiA* mutant

To examine the possible function of *chiA* gene for establishment and maintenance of the symbiotic interaction of *E. festucae* with *L. perenne*, *chiA* gene deletion was conducted by targeted replacement of *chiA* with a hygromycin B phosphotransferase (*hph*) cassette (Figure 4.13 A). Protoplasts of *E. festucae* Fl1 (2.8.4.1) were transformed with an *Hpa*I/*Eco*RI digested linear fragment of pNN02 (Appendix 8) and screened for growth on PD agar medium (2.8.4.2) containing hygromycin. One hundred and twenty Hyg^R colonies were picked and transformant stability confirmed by three times subculturing (2.4.2) and screened by PCR (2.6.1) using primers NN34 and NN35, for absence of a wild-type fragment. Four putative Δ*chiA* candidates were identified from this screen. Further PCR screening using primer sets that amplify the left (NN36/NN32) and right flanks (NN29/NN37) of the *hph* cassette confirmed that these 4 transformants were Δ*chiA* candidates. High quality genomic DNA (2.4.3) was isolated from these four transformants, digested with *Eco*RI, the digests separated by gel electrophoresis, and Southern blots of the gels prepared. Probing of these blots with a DIG-labelled linear fragment of pNN02 confirmed that three of the four candidates (#5, #22 and #39) were 'clean' single copy replacement mutants (Figure 4.13 B).

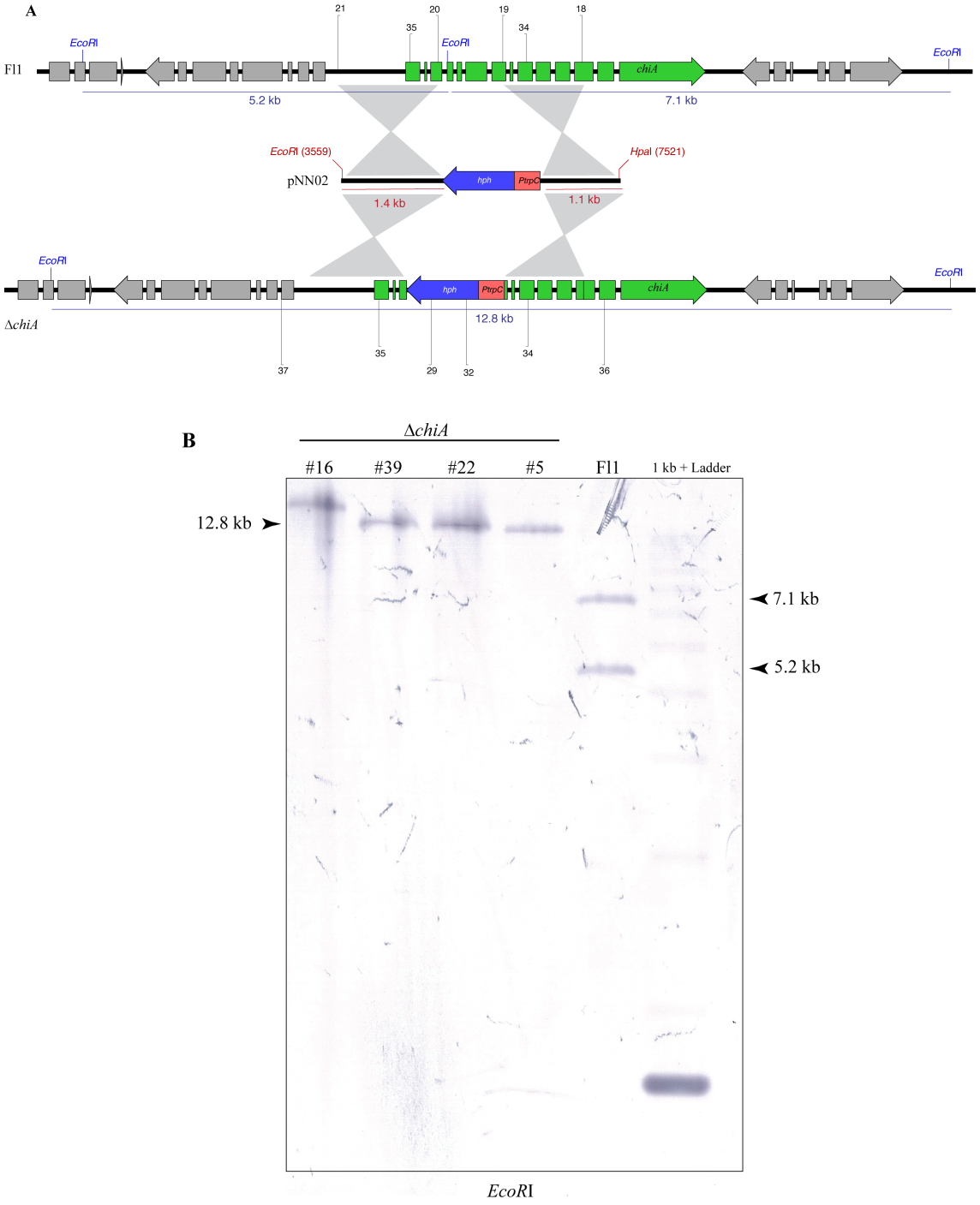


Figure 4.13. Strategy for deletion of *E. festucae chiA* and confirmation by Southern analysis. (A) Physical map of *chiA* wild-type genomic locus, linear insert of *chiA* replacement construct, pNN02, showing restriction enzyme sites for *Eco*RI. Grey shading indicates regions of recombination. Numbers indicate the PCR primers used for Gibson assembly and knock–out screening. (B) NBT/BCIP stained Southern blot of *EcoR*I digest (1 µg) of *E. festucae* wild-type

(Fl1) Δ*chiA*#5 (PN3100), Δ*chiA*#22 (PN3101), Δ*chiA*#39 (PN3102) and Δ*chiA*#16 strains probed with digoxigenin (DIG)-11-dUTP labelled linear insert of pNN02 amplified with primers NN18/NN21.

4.2.2. Δ*chiA* culture phenotype

The colony morphology phenotypes of Δ*chiA*#5 and Δ*chiA*#39 mutant strains grown on axenic culture were the same as WT and no distinct differences in either radial growth or shape were observed (Figure 4.14 A). Epi-fluorescence light microscopy analysis of cultures grown on WA and stained with CFW (2.10.1), showed that Δ*chiA* forms hyphal bundles, undergoes hyphal cell-cell fusion and forms coils from which conidiophores arise, with a morphology and frequency similar to wild-type (Figure 4.14 B-E).

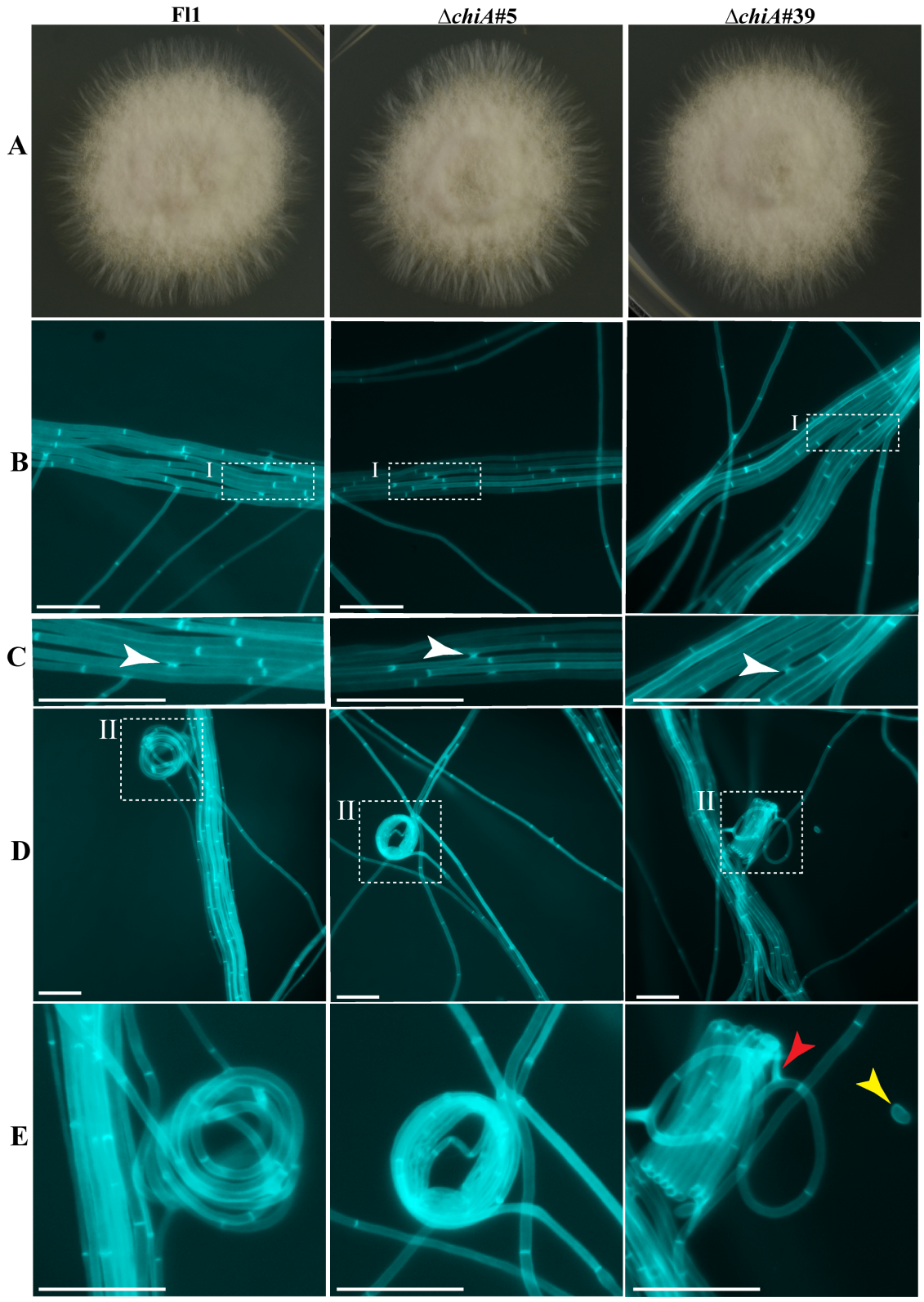


Figure 4.14. Culture phenotype of wild-type and Δ*chiA* mutant strains. (A) Colony morphology of wild type (Fl1) and Δ*chiA* strains grown on 2.4% PD agar at 22°C for 7 days. (B) and (D) Fluorescent images captured by Epi-fluorescence light microscopy of hyphae grown for 7 days on water agar and stained with Calcofluor white. Bar=20 µm. (C) and (E) Higher magnification

of (I) and (II) indicating fusion points and coiled structures respectively. White, red and yellow arrowheads identify hyphal fusion points, conidiophores and conidia respectively. Bar=20 μm.

4.2.3. Δ*chiA* symbiotic interaction phenotype

To test whether the *chiA* gene is required for the symbiotic interaction of *E. festucae* with its host, *L. perenne*, 7-day old seedlings were inoculated with WT and two independent mutant strains, Δ*chiA*#5 and Δ*chiA*#39, and grown under controlled environment conditions for 8-12 weeks (2.8.3.2). After this period of growth, single tillers were immunoblotted to identify which plants were infected (2.3.8.3). Statistical analysis of the average number and length of tillers in infected plants with Δ*chiA* mutant strains, showed a significant difference in the average number and length of tillers in plants infected with Δ*chiA*#39. This differs from plants infected by Δ*chiA*#5 which only differ from WT in the number of tillers (Figure 4.15 B & C) as these variations are sometimes observed in similarly maintained WT plants, so this analysis showed that the phenotype of plants infected with the Δ*chiA* mutants was similar to those infected with WT with similar root, stem and leaf morphologies (Figure 4.15 A).

To check the cellular phenotype, plant sheath samples stained with aniline blue, WGA-AF488 and propidium iodide (2.10.2), were examined by CLSM. The morphology and growth pattern of the Δ*chiA* mutants *in planta* was indistinguishable from that of WT. Consistently the hyphae grow predominantly parallel to the leaf longitudinal axis, a pattern interrupted by occasional lateral branching and fusion. Similarly, WGA-AF488 staining only labelled the septa in each. This suggested that the cell walls in Δ*chiA* like WT lack chitin (Figure 4.16 B).

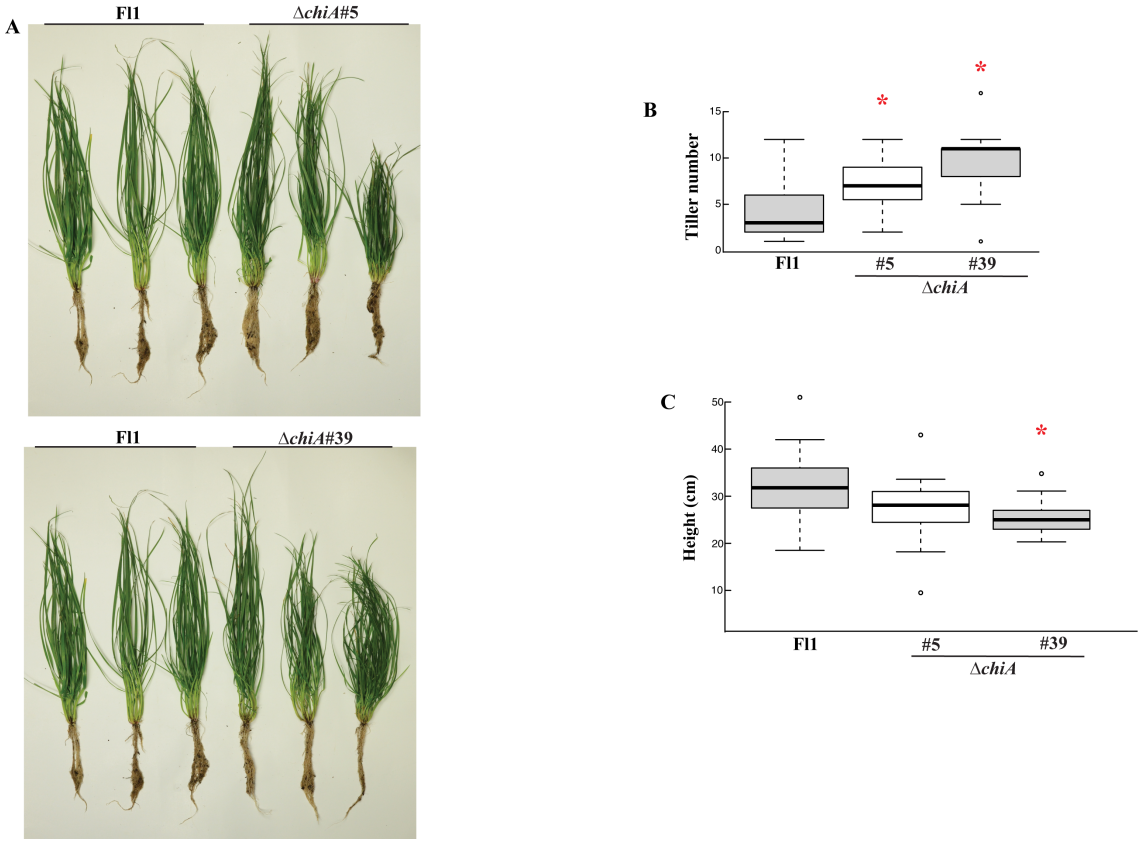


Figure 4.15. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*chiA* mutant strains. (A) Whole plant interaction phenotype of *L. perenne* plants infected with *E. festucae* strains 10 weeks after inoculation. (B) and (C) Box plots of tiller number and tiller height of *L. perenne* plants infected with *E. festucae* strains. **P* <0.05 determined by Student's *t*-test (two-tailed, n≥10). Outliers are indicated by circles. Significant difference is indicated by red asterisk.

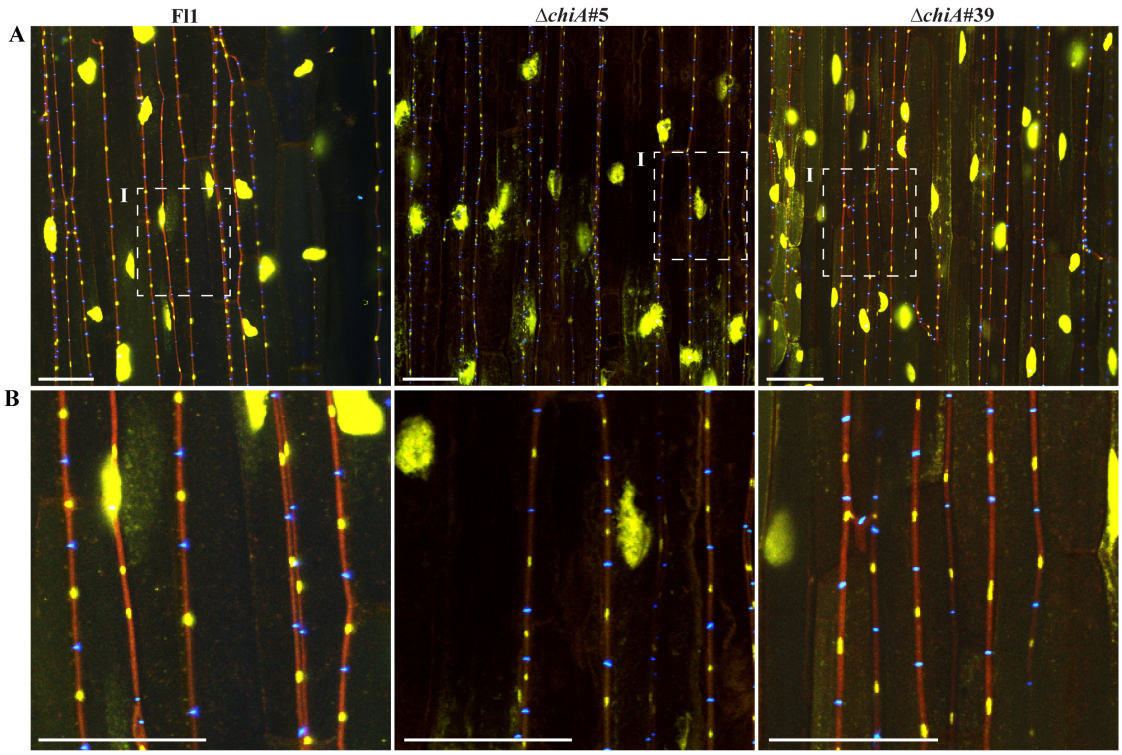


Figure 4.16. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*chiA* mutant strains. (A) Confocal depth series images of longitudinal sections of *L. perenne* leaf sheath tissue infected with wild type (Fl1) and Δ*chiA* strains 10 weeks post inoculation, showing hyphae stained with aniline blue (red pseudocolour), chitin in cell wall septa with WGA-AF488 (blue pseudocolour), and fungal and plant nuclei with propidium iodide (yellow pseudocolour). Bar=50 µm. (B) Higher magnification of (I) showing hyphal compartments. Images were generated by maximum intensity projection of 10× 1.2 µm confocal z-stack. Bar=50 µm. The analyses were conducted at least in three biological replicates.

4.2.4. Generation of Δ*chiB* mutant

To test the possible role of *chiB* for establishment and maintenance of the symbiotic interaction of *E. festucae* with *L. perenne*, a deletion mutant of *chiB* was generated by targeted replacement of *chiB* with a neomycin phosphotransferase (*npt*II) cassette (Figure 4.17 A). Protoplasts of *E. festucae* Fl1 (2.8.4.1) were transformed with an *Hpa*I/*Spe*I digested linear fragment of pNN04 (Appendix 10) and screened for growth on PD medium (2.8.4.2) containing geneticin. 198 Gen^R colonies were picked and three times sub-cultured to confirm transformant stability and to ensure they were homokaryons. Crude genomic DNA was isolated from each transformant (2.4.2) and 48 colonies were screened by PCR (2.6.1) using primers NN60 and NN61, to confirm the absence of a wild

type fragment. Three putative Δ*chiB* candidates were identified from this screen. Further PCR screening using primer sets that amplify the left (NN62/NN53) and right (NN54/NN63) flanks of the *npt*II cassette confirmed that all 3 were Δ*chiB* candidates. High quality genomic DNA (2.4.3) was isolated from all three transformants, digested with either *EcoR*V or *Pvu*I, the digests separated by gel electrophoresis, and Southern blots of the gels prepared. Probing of these blots with DIG-labelled linear fragment of pNN04 confirmed that two of three candidates were 'clean' single copy replacement mutants. (Figure 4.17 B). The presence of the additional 2.1 kb and 2.8 kb bands in the *Pvu*I digests of transformants #36 is likely due to incomplete digestion with this enzyme, given a single band of the correct size was present in the *EcoR*V digest.

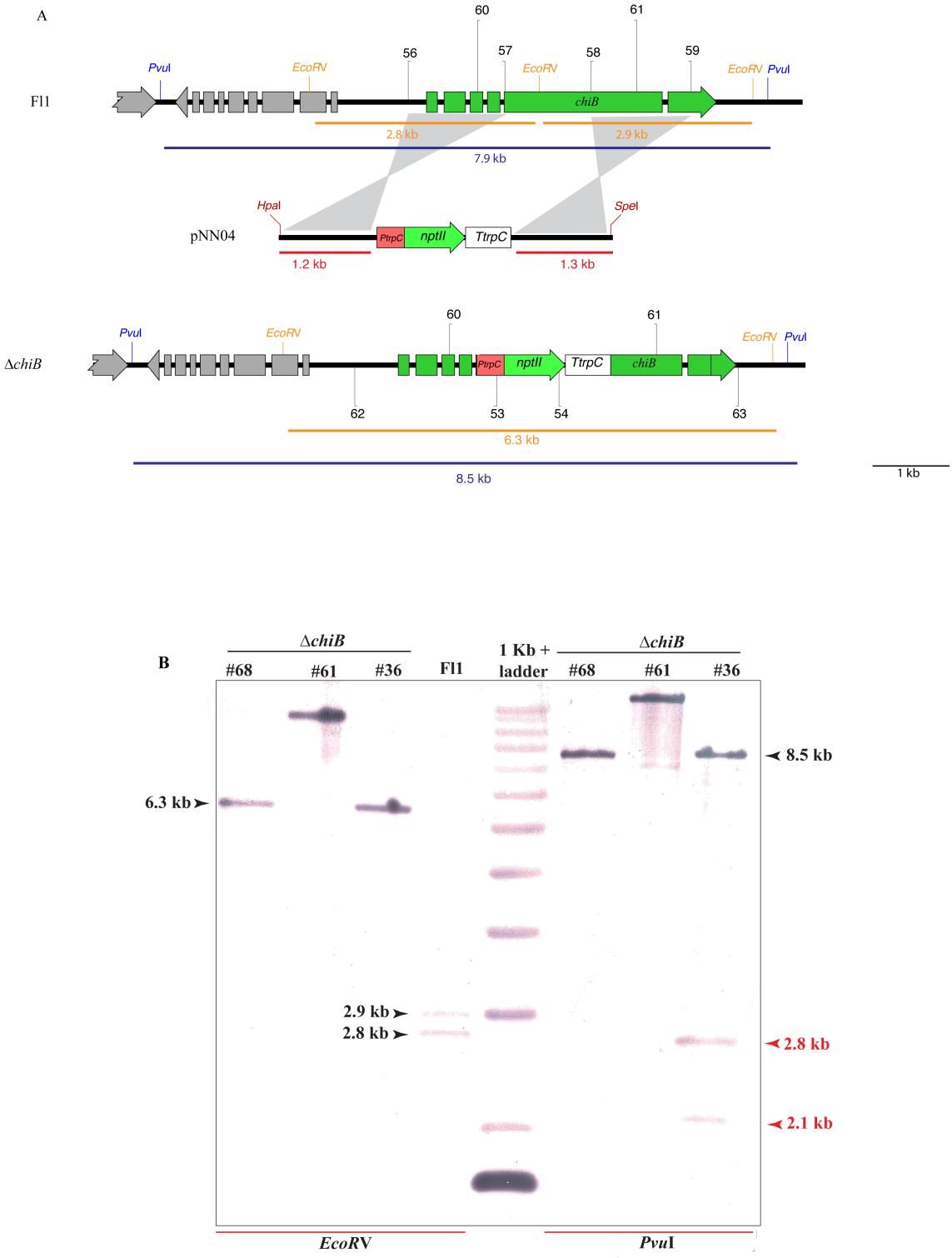


Figure 4.17. Strategy for deletion of *E. festucae chiB* and confirmation by Southern analysis. (A) Physical map of *chiB* wild-type genomic locus, linear insert of *chiB* replacement construct, pNN04, showing restriction enzyme sites for *Eco*RV and *Pvu*I. Grey shading indicates regions of recombination. Numbers indicate the PCR primers used for Gibson assembly and knock–out screening. (B) NBT/BCIP stained Southern blot of *Eco*RV and *Pvu*I *(*B) digests (1 µg) of *E.*

festucae wild-type (Fl1), Δ*chiB*#36 (PN3158), Δ*chiB*#61 and Δ*chiB*#68 (PN3159) strains probed with digoxigenin (DIG)-11-dUTP labelled linear insert of pNN04 amplified with primers NN56/NN59.

4.2.5. Δ*chiB* culture phenotype

Cultures of *E. festucae* wild type and two independent Δ*chiB* mutant strains, Δ*chiB*#36 and Δ*chiB*#68, were indistinguishable from WT on PD agar with the same radial growth and morphology (Figure 4.18 A). Epi-fluorescence light microscopy analysis of cultures grown on water agar and stained with CFW (2.10.1), showed that Δ*chiB* mutant strains behave normally; they regularly form bundles, undergo hyphal cell-cell fusion and form coils from which conidiophores arise, all of which appear morphologically similar to wild-type (Figure 4.18 B-E).

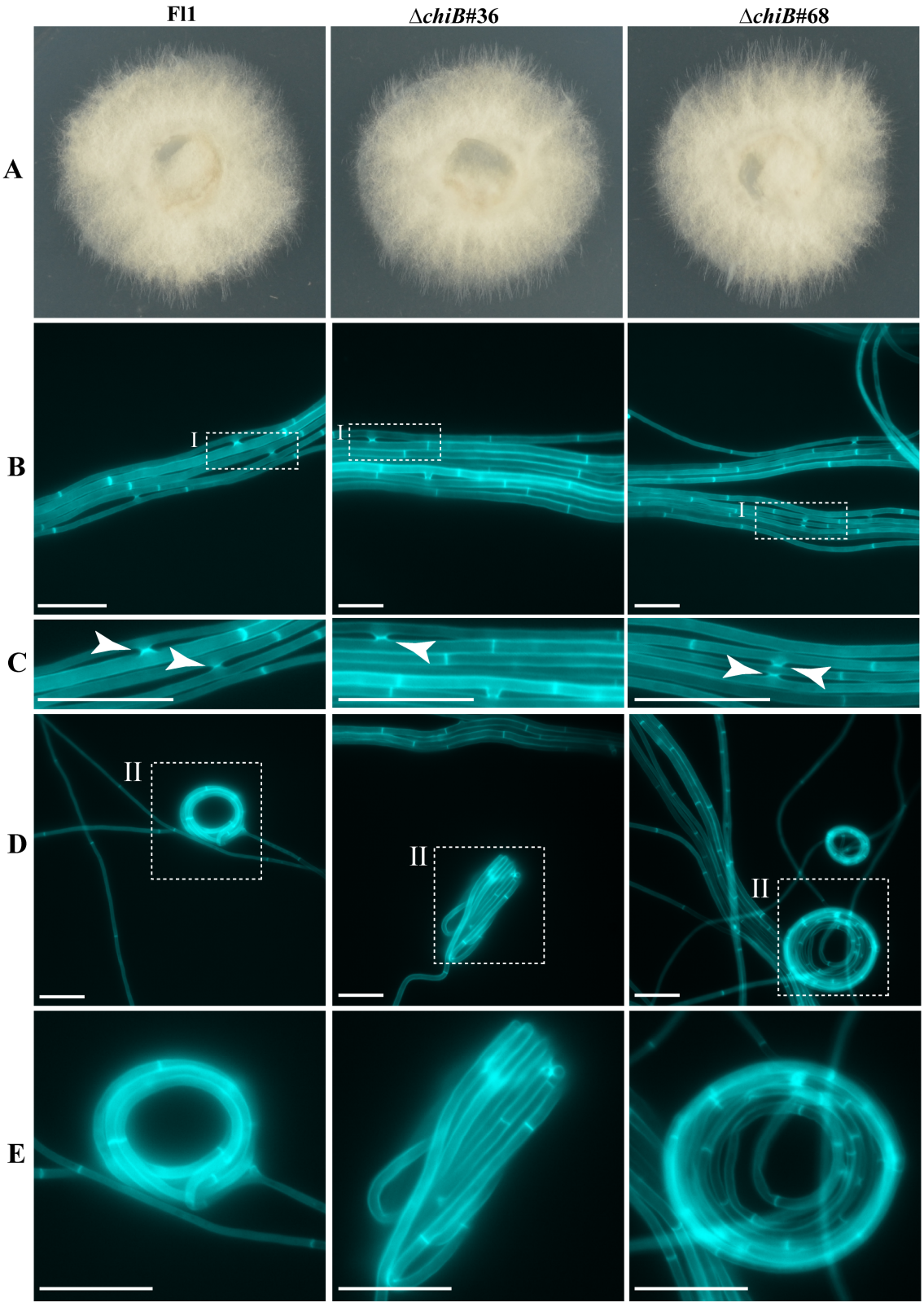


Figure 4.18. Culture phenotype of wild-type and Δ*chiB* mutant strains. (A) Colony morphology of wild type (Fl1) and Δ*chiB* strains grown on 2.4% PD agar at 22°C for 7 days. (B) and (D) Fluorescent images captured by Epi-fluorescence light microscopy of hyphae grown for 7 days on water agar and stained with Calcofluor white. Bar=20 µm. (C) and (E) Higher magnification

of (I) and (II) indicating hyphal fusion points and coiled structures respectively. White arrowheads indicate fusion points. Bar=20 μm.

4.2.6. Δ*chiB* symbiotic interaction phenotype

To test whether the *chiB* is required for the symbiotic interaction of *E. festucae* with its host, *L. perenne*, 7-day old seedlings were inoculated with WT and two independent mutant strains, Δ*chiB*#36 and Δ*chiB*#68, and grown under controlled environment conditions for 8-12 weeks (2.3.8.2). After this time, single tillers were immunoblotted to identify infected plants (2.3.8.3). Although the phenotype of plants infected with the Δ*chiB* mutants looked similar to those infected with WT with similar root, stem and leaf morphologies (Figure 4.19 A), statistical analysis showed a decrease in tiller height of plants infected with Δ*chiB* mutants with significant changes in Δ*chiB*#68. There were not any significant changes in tiller number of infected plants with Δ*chiB* mutants compared to the WT. These results showed a mild stunting plant phenotype in Δ*chiB*. (Figure 4.19 B & C).

To check if there were any differences in phenotype at the cellular level, sheath samples were fixed with ethanol and stained with aniline blue, WGA-AF488 and propidium iodide (2.10.2) and examined with CLSM. In plants infected with the WT, hyphae grow predominantly as single elongated hyphae, parallel to the leaf longitudinal axis with occasional lateral branching and fusion. Only the septa stained with WGA-AF488 suggesting that chitin is absent from the cell walls of endophytic hyphae. The morphology and growth pattern of the Δ*chiB* mutants *in planta* was significantly different from that of WT. Hyphae were overgrown, exhibiting aberrant positioning and density in the intercellular spaces and sometimes appeared “swollen” or wider than normal. Moreover, mutant hyphae displayed increased branches and formed highly convoluted structures. In these instances, hyphal size appeared to be reduced (Figure 4.20).

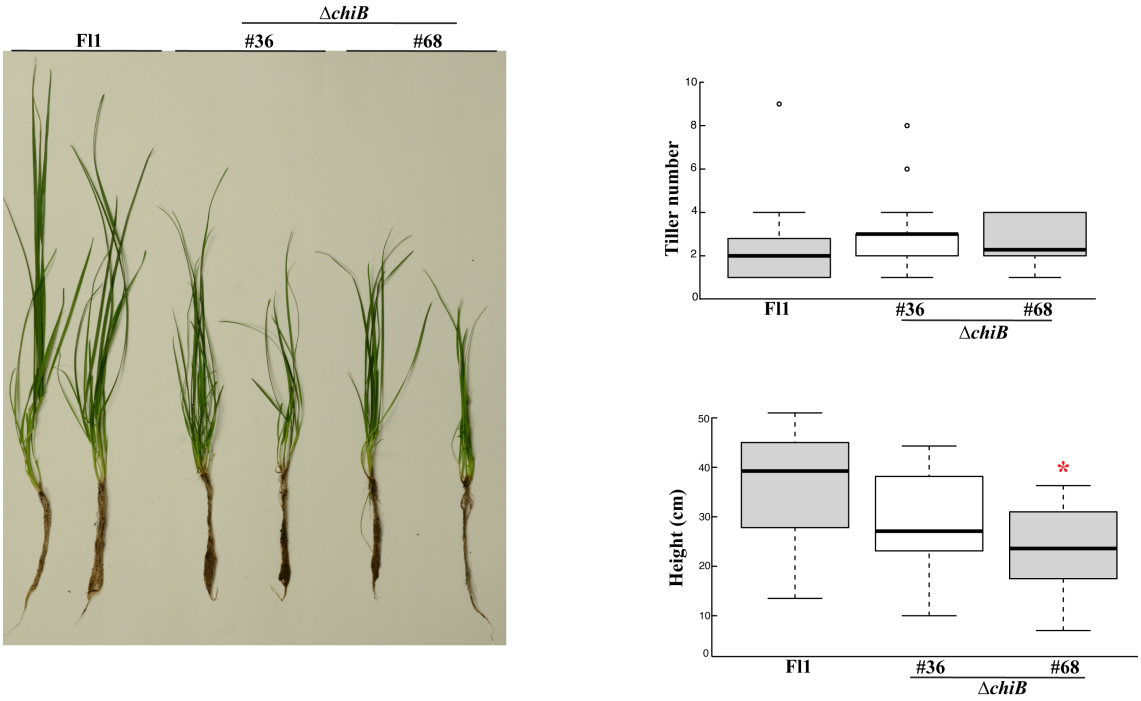


Figure 4.19. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*chiB* mutant strains. (A) Whole plant interaction phenotype of *L. perenne* plants infected with *E. festucae* strains 10 weeks after inoculation. (B) and (C) Box plots of tiller number and tiller height of *L. perenne* plants infected with *E. festucae* strains. **P* <0.05 determined by Student's *t*-test (two-tailed, n≥10). Outliers are indicated by circles. Significant difference is indicated by red asterisk.

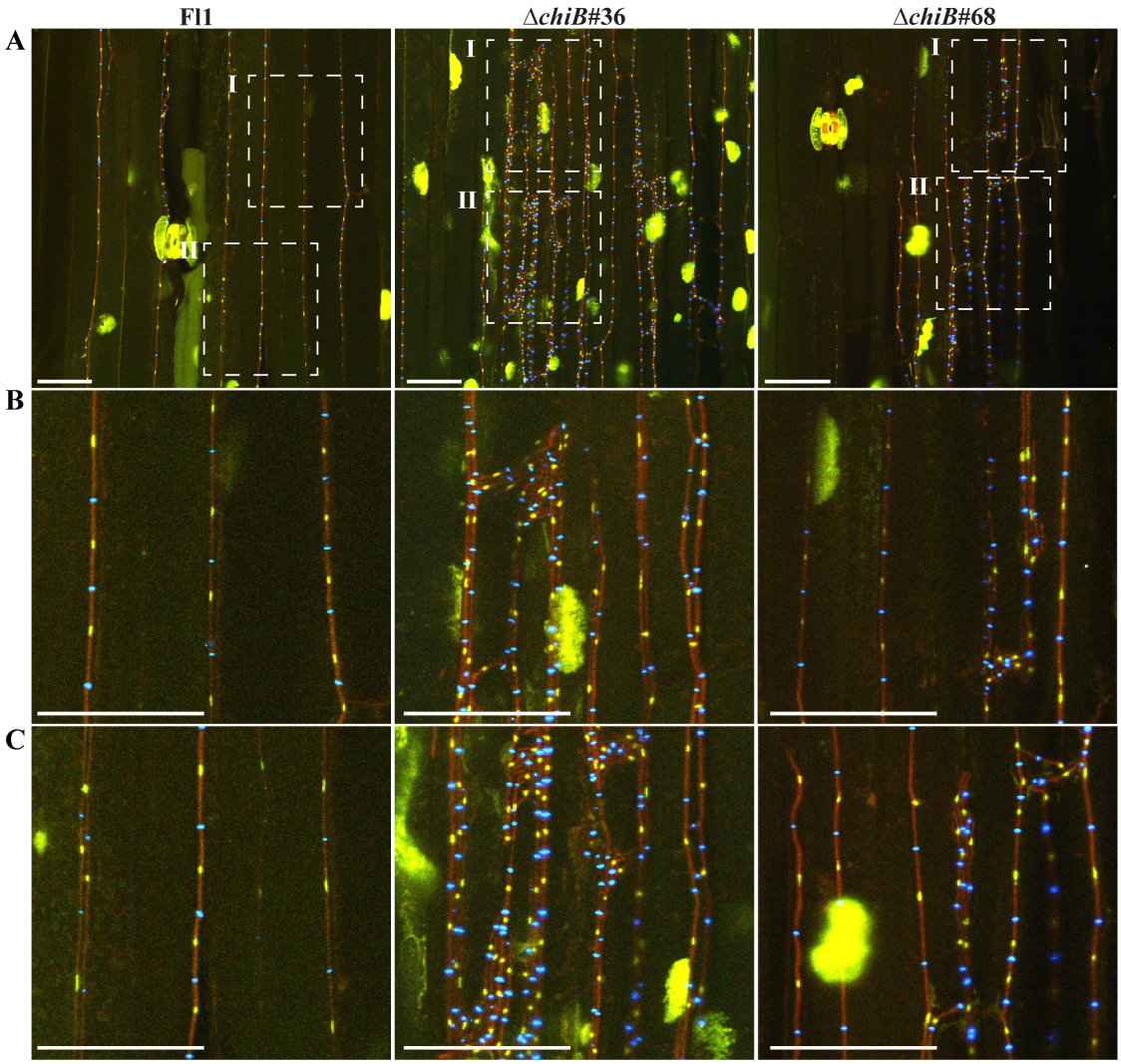


Figure 4.20. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*chiB* mutant strains. Confocal depth series images of longitudinal sections of *L. perenne* leaf sheath tissue infected with wild type (Fl1) and Δ*chiB* strains 10 weeks post inoculation, showing hyphae stained with aniline blue (red pseudocolour), chitin in cell wall septa with WGA-AF488 (blue pseudocolour), and fungal and plant nuclei with propidium iodide (yellow pseudocolour). Bar=50 µm. (B) and (C) Higher magnification of (I) and (II) showing normal hyphal compartment in wild type, abnormal growth pattern of fungal hyphae and increasing of hyphal branching in Δ*chiB* mutant strains respectively. Images were generated by maximum intensity projection of a 10× 1.2 µm confocal z-stack. Bar=50 µm. The analyses were conducted at least in three biological replicates.

4.2.7. LymA cellular localization

To check the LymA protein cellular localization *lymA-eGFP* constructs were made under the control of their native and *tef* over expression promoter (2.9.5) and transformed into Δ*lymA* protoplasts (2.8.4.1). Twenty *lymA* Gen^R transformants were picked and cultured on PD agar media containing geneticin as the selectable marker (2.8.4.2) and checked for fluorescent signals and cellular localisation using Epi-fluorescence light microscopy (2.10.1). Given that the fluorescent signal under the native promoters was weak the overexpression strains were analysed. Microscopic analyses showed fluorescent signals in the fungal cell walls and septa with stronger signals and localisation in hyphal septa (Figure 4.21).

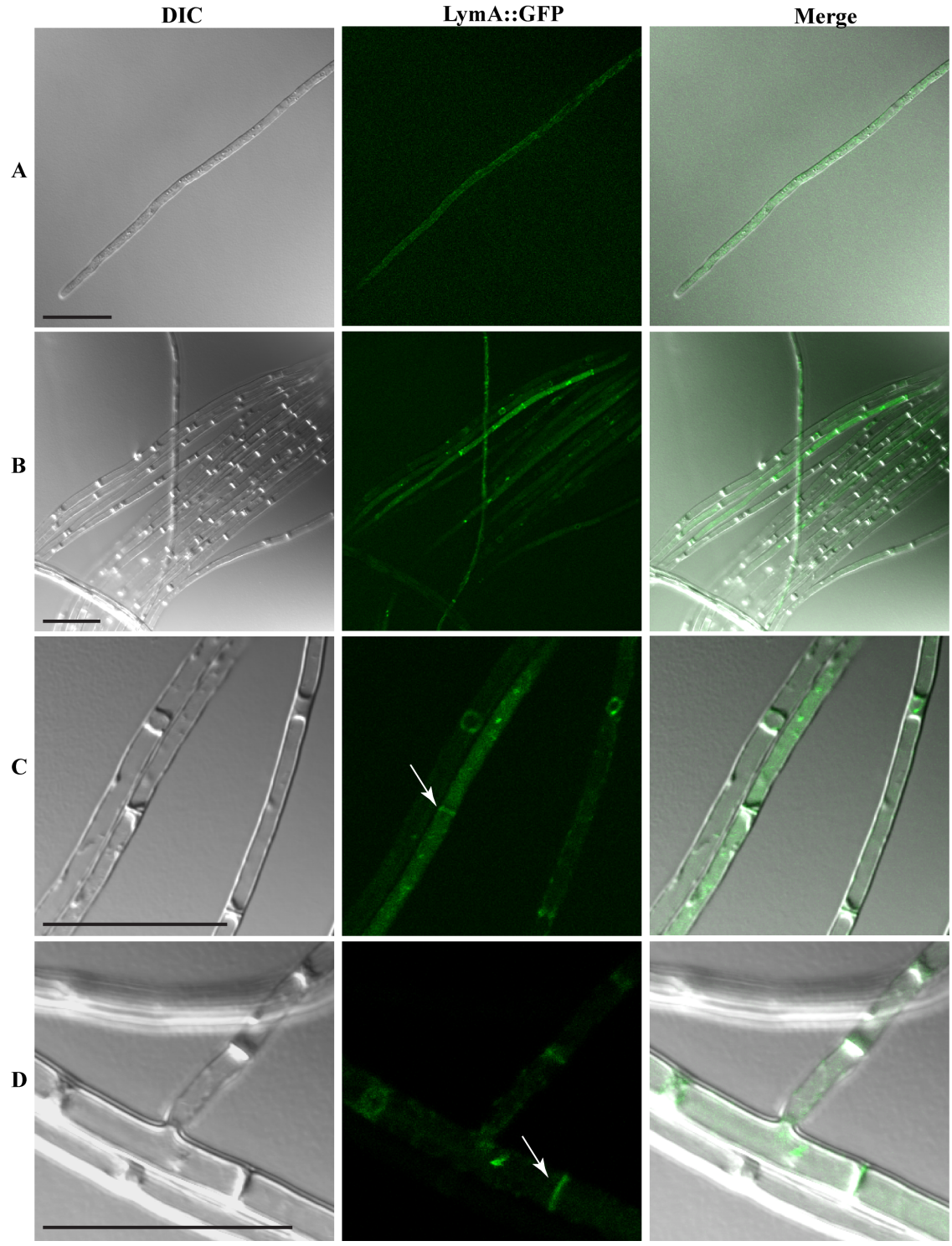


Figure 4.21. Localization of LymA::eGFP in *E. festucae* Δ*lymA*. Confocal laser scanning and DIC microscopy of LymA::eGFP (green pseudocolour) cellular localization in axenic culture of PN 3236 (Δ*lymA*#7/pNN12) at A, young hyphae at hyphal tip, B, mid region and centre of the colony, C and D higher magnification of the protein localization in fungal cell wall and septa. The PtefA promoter was used to express LymA::eGFP. White arrow heads indicate localization point at hyphal septa. Bar = 20 μm.

4.2.8. LymB cellular localization

lymB-eGFP constructs were made under the control of their native and *tef* over expression promoter (2.9.6) to check the LymB protein cellular localization and transformed into Δ*lymB* protoplasts (2.8.4.1). Twenty *lymB* Hyg^R transformants were picked and cultured on PD agar media containing hygromycin as the selectable marker (2.8.4.2) and checked for fluorescent signals and cellular localization using Epi-fluorescence light microscopy (2.10.1). Due to the weak fluorescent signal observed using the native promoter, the overexpression strains were analysed. Microscopy analyses showed fluorescent signals in fungal cell walls and septa with stronger signals and localization in hyphal septa (Figure 4.22).

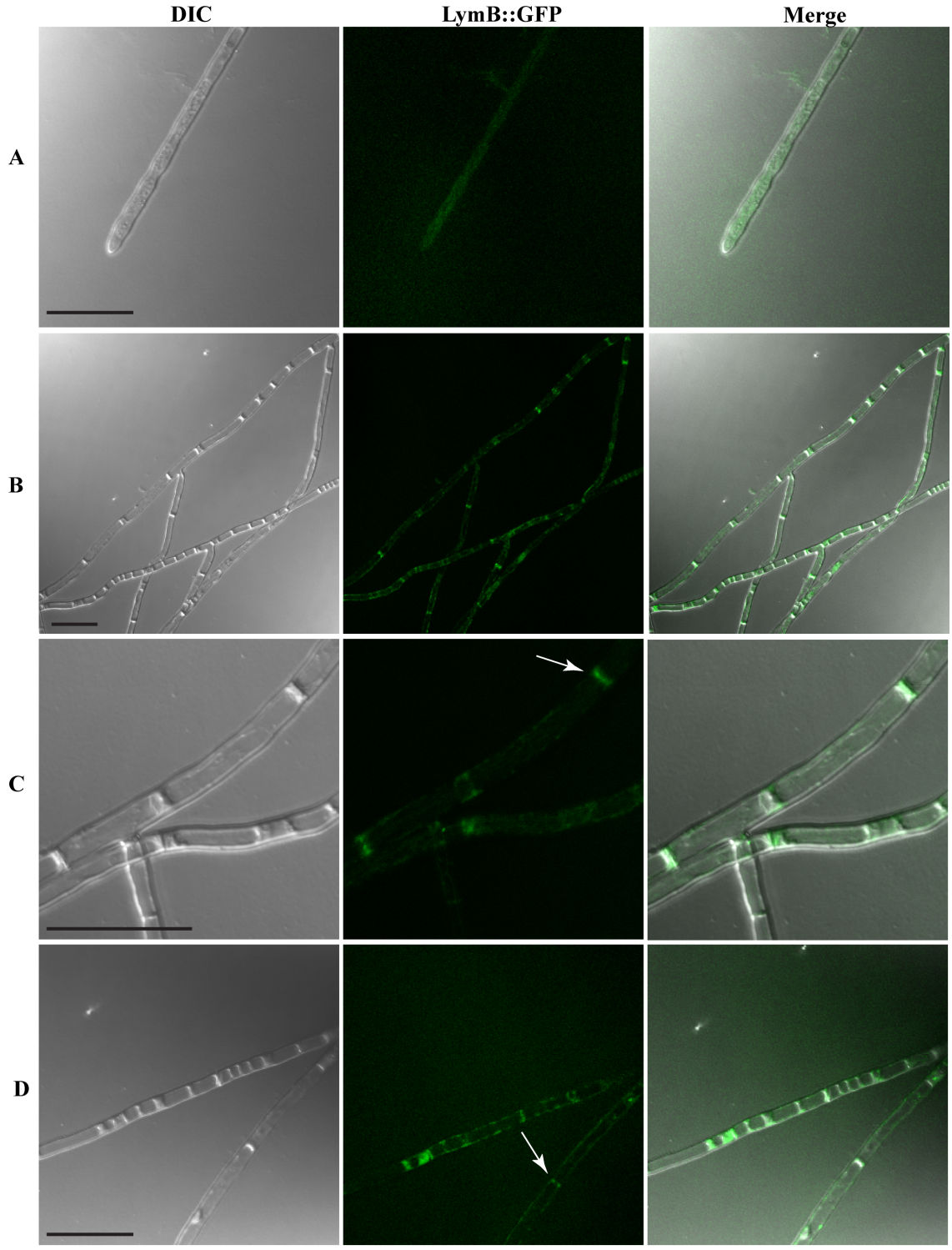


Figure 4.22. Localization of LymB::eGFP in *E. festucae* Δ*lymB*. Confocal laser scanning and DIC microscopy of LymAB::eGFP (green pseudocolour) cellular localization in axenic culture of PN 3237 (Δ*lymA*#741/pNN14) at A, young hyphae at hyphal tip, B, mid region and centre of the colony, C and D higher magnification of the protein localization in fungal cell wall and septa. The PtefA promoter was used to express LymB::eGFP. White arrow heads indicate localization point at hyphal septa. Bar = 20 μm.

Chapter 5. Discussion

LysM containing proteins have been identified in many different fungi from mammalian and plant pathogens to saprophytes. They have a role not only in sequestration of chitin oligomers released from fungal cell walls during host invasion to prevent the triggering of host immunity defence, but also in fungal development (de Jonge & Thomma, 2009; Levin *et al.*, 2017; Seidl-Seiboth *et al.*, 2013; Takahara *et al.*, 2016). The current study shows that two *E. festucae* Fl1 *lysM* genes, *lymA* and *lymB*, which are divergently transcribed from two chitinase genes (*chiA* and *chiB*), are required for the symbiotic interaction between *E. festucae* and its grass host *L. perenne.* Functional analysis of these genes by gene deletion showed that these two *lysM* genes, *lymA*, *lymB* and one of the chitinase genes, *chiB*, are required for fungal restrictive growth in the plant host *L. perenne*. Both LymA and LymB proteins are localised to the hyphal cell wall and septa, where chitin is an abundant oligosaccharide.

E. festucae strain Fl1 has seven genes encoding proteins with a variable number of LysM motifs without catalytic modules and are therefore classified as type-A fungal LysM proteins (de Jonge & Thomma, 2009). Five of these Fl1 proteins were shown to have an N-terminal signal peptide suggesting they are secreted into the apoplast and therefore possibly have a role in suppressing the host defence response by overcoming PAMP triggered immunity. A recent proteomics analysis of apoplastic fluids demonstrated that EfM3.029340 is one of the most abundant fungal proteins in apoplastic fluid. However, peptides derived from LymA and LymB were not detected in this analysis (Green et al, unpublished data). A possible reason for this result is their lack of abundance or localisation to some other cellular region. Genes encoding LysM motifs have been analysed in several other fungi including *Cladosporium fulvum, Mycosphaerella graminicola, Trichoderma atriviridae, Magnaporthe oryzae, Colletotrichum higginsianum* and *Penicillium expansum* (Bolton *et al.*, 2008; Marshall *et al.*, 2011; Gruber *et al.*, 2011; Mentlak *et al.*, 2012; Takahara *et al.*, 2016; Levin *et al.*, 2017). The best characterised of these proteins is the extracellular protein Ecp6 from *C. fulvum* which has three LysM motifs. The 3-D structure of this protein revealed that just two of the three LysM domains (LysM1 and LysM3) bind chitin oligosaccharides. Binding studies revealed that these LysM motifs bind chitin oligomers with ultra-high affinity which is sufficient to outcompete host immune receptors for chitin binding and prevent chitin triggered immunity (Sanchez-Vallet *et al.*, 2015). The third LysM motif (LysM2) also binds to chitin but at a lower affinity suggesting that it has the potential to perturb chitin

triggered immunity by disruption of dimerization of plant receptors, which is essential for initiation of the host immune response (Sanchez-Vallet *et al.*, 2015; Kombrink & Thomma, 2013; Liu *et al.*, 2012). The LysM proteins identified in *M. graminicola* (Marshall *et al.*, 2011) and *M. oryzae* (Mentlak *et al.*, 2012) are proposed to have similar functions as Ecp6 in sequestering chitin oligosaccharide released from invading hyphae, thereby preventing elicitation of host immunity.

Although proteins corresponding to LymA and LymB were absent in the *E. festucae* Fl1 apoplastic fluid analysis, the significant upregulation of *lymA in planta* compared to in culture suggests that at least *lymA* has an important role in the symbiosis (Chujo and Scott unpublished). Consistent with this result is a decrease in expression of these genes in three symbiotic mutants (significantly in *noxA* and *proA* and not significant in *sakA* mutant strains) that dramatically affect the interaction of *E. festucae* with *L. perenne,* resulting in severe stunting, hypertillering and premature host senescence. Unlike the wild type, hyphae do not align parallel to the leaf axis and have an abnormal growth pattern in these three symbiotic mutants (Tanaka *et al.*, 2006; Tanaka *et al.*, 2013; Eaton *et al.*, 2015). In addition to these *lysM* genes, three other *E. festucae* lysM genes were more highly expressed *in planta* compared to in culture but only EfM3.029340, which is predicted to have an N-terminal signal peptide was identified in the proteome analysis (Green et al, unpublished data). Although currently unexamined in *E. festucae*, analysis of genes encoding LysM proteins in other fungi shows that changes in expression occur during different developmental stages. Other studies have shown that the expression profile of fungal effectors may be affected by the cell type or organ being invaded (Lo Presti *et al.*, 2015; Okmen & Doehlemann, 2014). In *M. graminicola Mg3LysM* and *Mg1LysM* LysM effectors were transcriptionally activated during the symptomless phase of leaf infection (Marshall *et al.*, 2011). In *Penicillium expansum* the expression level of four *lysM* gene effectors, *PeLysM1*, *PeLysM2*, *PeLysM3* and *PeLysM4* increased during host infection (Levin *et al.*, 2017). In *C. higginsianum*, *ChELP1* and *ChELP2* are the most highly expressed of all *lysM* genes described in this species and have roles during early pathogenesis and the biotrophic stage (Takahara *et al.*, 2016).

Analysing the chromosomal organisation of the *E. festucae lysM* genes revealed that *lymA* and *lymB* are both divergently transcribed from two chitinase genes *chiA* and *chiB* and are therefore likely to be coregulated. Chitinase proteins encoded by *chiA* and *chiB* both belong to chitinase hydrolase family 18 with predicted chitin binding, chitinase hydrolase

family 18 catalytic and chitinase insertion domains. Both proteins also contain a putative N-terminal signal peptide and are predicted to be secreted into the apoplast. *chiA* and *chiB* follow the same transcriptional pattern as *lymA* and *lymB*; both are up-regulated *in planta* compared to in culture, and are down-regulated in the Δ*noxA,* Δ*proA* and Δ*sakA* symbiotic mutants suggesting that these two chitinase proteins are working at the same stage of fungal development as the LymA and LymB proteins. A similar regulation pattern was observed in *T. atriviridae* with the *lysM* gene *tal6* which encodes a protein with 7 lysM motifs and a subgroup C chitinase gene *tac6. tac6* is strongly expressed during hyphal network formation and involved in self-cell wall remodelling during fungal development and growth. Deletion of the *tac6* gene resulted in faster growth than the wild type. *tac6 and tal6* share a common upstream region and are therefore likely to be also coregulated. Spore germination assays conducted on *T. atrivirdae*, *T. reesei*, *N. crassa* and *A. niger* using different TAL6 protein concentrations showed that TAL6 has a complete inhibitory effect on spore germination of *T. atrivirdae* and *T. reesei* at 1.8 μM and 0.18 μM of protein concentrations respectively and does not have any effect on spore germination of *N. crassa* and *A. niger*, showing TAL6 has a strong inhibitory effect on spore germination of *Trichoderma.* spp and not on the other fungi. In the same study it was shown that the truncated version of the TAL6 protein (TAL6-4) with only four C terminal LysM motifs, which are highly similar to each other, does not have the same inhibitory effect on either *Trichoderma*. spp or other fungi. So *tal6* is involved in self signalling processes during fungal growth rather than in fungal-plant interaction and with *tac6* these two genes appear to have a role in fine-tuning the regulation of fungal growth and hyphal development (Gruber *et al.*, 2011; Seidl-Seiboth *et al.*, 2013). Although *tal6* and *tac6* are divergently transcribed from each other in *T. atriviridae,* homologues of these genes in *E. festucae* Fl1 genome are at two different loci with corresponding gene models of EfM3.078100 and EfM3.062780 respectively. Interestingly the homologue of tal6, EfM3.078100, is divergently transcribed with a chitinase gene (EfM.078090) but they do not share the same expression profile either in the three mutant strains (Δ*noxA*, Δ*proA*, Δ*sakA*) or *in planta* compared to in culture, suggesting that these two genes are probably involved in different pathways from each other. The chitinase gene EfM3.062870 identified as the homologue of *tac6* is also divergently transcribed from a *lysM* gene EfM3.062860 with a similar expression pattern in the three symbiotic mutant strains (Δ*noxA*, Δ*proA*, Δ*sakA*), and both are downregulated *in planta* compared to in culture, suggesting that they are co-expressed and may have a functional role in fungal

growth as in *T. atrivirdae.* Functional analysis of these genes in *E. festucae* Fl1 has not been checked yet.

One possible joint role for LymA/ChiA and LymB/ChiB proteins is in hyphal intercalary growth which is identified as a unique form of *E. festucae* hyphal growth *in planta* enabling hyphal growth to be tightly synchronised with development of the host, a situation which is not required in plant pathogens. *E. festucae* grows between undifferentiated plant cells in the true stem of the grass by hyphal tip growth and branching (Christensen and Voissey, 2007). As grass leaf primordia develop they differentiate into two limited cell division zones corresponding to the, leaf sheath and blade intercalary division zones (Christensen *et al.*, 2008; Scott *et al*, 2010). First the leaf cell division zone is colonised with highly branching hyphae attached to it, then colonisation of the intercellular space of expanding and differentiating tissues occurs where hyphae become attached to the plant cell wall. But in contrast to the cell division zone the number of the hyphae do not increase and they are restricted in growth (Christensen *et al.*, 2002; Tan *et al.*, 2001). Hyphae attach to the plant cell wall with an adhesive matrix and when the plant cells elongate the hyphae are stretched triggering a switch from tip to intercalary growth (Christensen *et al.*, 2008) Voissey *et al*, 2010). For hyphae to be stretched and undergo intercalary growth the cell wall will need to be constantly remodelled. Chitinases may play a key role in remodelling the cell wall thereby necessitating the need for fungal LysM effectors to sequester the chitin oligomers that are released. Co-expression of the *lysM* and *chitinase* genes would enable these two functions to be coordinated.

Homologues of *lymA* and *lymB*, and their associated co-regulated *chiA* and *chiB genes*, have been identified in other *Epichloë* and *Claviceps* species. An analysis of the chromosomal organisation of these genes in other *Epichloë* and *Claviceps* species revealed that there is conserved synteny of both *lymA*/*chiA* and *lymB*/*chiB* loci and the two genes positioned on either side of them. However, many of these homologues have undergone gene deletions, including *E. amarillans* E57 and E4668, *E. brachylyetri*, *E. elymi*, *E. typhina*, *E. baconii* and *E. sylvatica* in the *lymA*/*chiA* genes and in *E. bromicola* E5761, AL04334, E502 and *E. glyceria* in *lymB*/*chiB* genes. Interestingly, despite these deletions, the translation of the ORFs still generate what appear to be functional proteins, although in some cases signal peptides are lost and the number of LysM motifs is reduced compared to the number found in *E. festucae* Fl1. Protein sequence alignment of LysM

and chitinase proteins in different species also showed conservation in their protein domains among these species suggesting that they are essential for plant-symbiotic interactions.

Single and double deletion of *lymA* and *lymB* did not have any effect on fungal morphology and growth when these mutants were grown on PD agar media, which perhaps is not surprising given that both genes are preferentially expressed *in planta* compared to in culture. As discussed before, EfM3.062860 is divergently transcribed with a chitinase gene homologous to *tac6* in *T. atriviridae* and EfM3.078100 is homologous to tal6 in *T. atrivirdae*, and divergently transcribed with another chitinase gene. Both *lysM* genes encode proteins that contain N-terminal signal peptides with two and three LysM motifs respectively. Given they are preferentially expressed in culture it is likely they may have a role in fungal growth and development in culture. Deletion of *PeLysM3*, one of four *lysM* genes in *P. expansum*, resulted in a mutant with reduced radial growth, a lower percentage of germination and shorter germ tubes, suggesting that *PeLysM3* has a role like TAL6 from *T. atrivirdae* in self signalling processes during fungal growth rather than a role in fungal-plant interaction (Levin *et al.*, 2017; Seidl-Seiboth *et al.*, 2013). Although the silencing of *ChELP1* and *ChELP2* in *C. higginsianum* did not affect mycelial growth *in vitro*, the mutants did show abnormal appressorium function and morphology, suggesting that silencing these *lysM* genes affects the ability of the pathogen to penetrate Arabidopsis resulting in less disease symptoms (Takahara *et al.*, 2016).

Single deletions of *E. festucae chiA* and *chiB* also did not have any effect on fungal morphology or growth in culture, a result consistent with the pattern of gene expression. Both *chiA* and *chiB* have higher expression *in planta* compared to in culture suggesting they have a role in the plant-interaction. The *E. festucae* Fl1 genome contains 13 chitinase genes belonging to glycoside hydrolase family 18 and five of these genes have higher expression in culture compared to *in planta*. (Appendix 18 and 20). Surprisingly only two, EfM3.062870 and EfM3.064090, out of the five chitinases contain N-terminal signal peptides and are therefore predicted to be secreted, suggesting that they may have a role in fungal development in culture. Chitinases from filamentous fungi are required for various cellular processes such as cell wall modification, degradation, hyphal branching, tip growth and spore germination (Adams, 2004). *T. atriviridae* strains with *tac6* deleted grew faster than wild type suggesting a role for this protein in self-cell wall remodelling

during fungal development and growth (Seidl-Seiboth *et al.*, 2013). As described before it is interesting that the homologue of *tac6* has been identified in Fl1 as EfM3.062870, which has a higher expression in culture compared to *in planta* and therefore may have a role in fungal growth in culture.

Although there was no plant interaction phenotype for the Δ*lymA* and Δ*lymB* single gene mutants there was a dramatic cellular phenotype for the Δ*lymA/B* double mutant indicative of some functional redundancy between these two genes. A similar result was observed in *P. expansum* where deletion of a single *lysM* gene did not have any plant phenotype and did not show any changes in virulence, possibly because of gene redundancy (Levin *et al.*, 2017). Gene redundancy has also been described in *C. higginsianum ChELP1* and *ChELP2* which encode extracellular LysM proteins, where RNAi silencing of these two *lysM* genes, but not the individual genes, resulted in appressorium malfunction and reduced host penetration (Takahara *et al.*, 2016).

In wild type *E. festucae*, hyphae grow parallel to the leaf longitudinal axis with occasional lateral branching and fusion. In the Δ*lymA/B* double mutant there appears to be a switch from restrictive intercalary growth to proliferative tip growth characterised by increased branching and formation of convoluted bundles of hyphae in leaf tissues. These phenotypes suggest that *lymA* and *lymB* together are important in controlling intercalary growth as this pattern of growth requires that the fungal cell wall needs be attached to the host cell wall, resulting in stretching and growth parallel to the leaf axis. The breakdown in the symbiosis in this mutant may be due to an inability of the fungal cell wall to attach and thereby be stretched. Ariyawansa and Voisey have recently shown that this pattern of growth requires stretching as application of mechanical stretch to hyphae *ex planta* results in the development of new cellular compartments with a higher rate of nuclear division and septation compared to unstretched hyphae (Ariyawansa 2015). An intercalary pattern of growth enables the endophytic hyphae to synchronise growth with that of the plant and therefore avoid shearing. In an important recent study, the small Rho GTPase, Cdac42 has been shown to play a key role in controlling intercalary growth (Kayano *et al.*, 2018). Cdc42 is proposed to be responsible for controlling vesicle transportation to the hyphal tip by regulating reorganisation of the cytoskeleton and therefore may have a key role in re-directing vesicle movement to sites of intercalary growth. Plants infected with *cdc42* mutants showed hyphal breakage in the lower part of

the leaf tissue and impaired colonisation of the upper part of the mature leaf blades. Interestingly, plants infected with this mutant did not have any whole plant interaction phenotype, as found here for the *ΔlymA/B* double mutant (Kayano *et al.*, 2018; Scott *et al.*, 2018). A role for LysM motif proteins in intercalary growth is a potentially a new function for this family of proteins. Most studies to date on plant pathogenic fungi highlight the importance of at least some members of this family in controlling plant virulence. Ecp6 is an extracellular LysM effector protein secreted by the fungal tomato leaf mould pathogen, *C. fulvum*, which has a role in pathogen virulence through sequestering chitin oligomers derived from the cell wall of the pathogen thereby interfering with the host chitin detection system and perturbing chitin-induced immunity (Bolton *et al.*, 2008; de Jonge & Thomma, 2009; de Jonge *et al.*, 2010; Kombrink & Thomma, 2013). Additional *lysM* genes have been characterised in other fungi as well. In the wheat blotch fungus, *M. graminicola,* and the rice blast pathogen *M. oryzae*, the LysM effectors Mg3LysM and Slp1 have been shown to bind chitin oligomers, a feature shared with Ecp6 (Marshall *et al.*, 2011; Mentlak *et al.*, 2012). A role in virulence has also been reported for ChELP2 extracellular LysM protein from *C. higginsianum* (Takahara *et al.*, 2016). However, as discussed above, LysM effectors in *M. graminicola* and *C. higginsianum* have dual functions being required for pathogen virulence and fungal growth and development (Marshall *et al.*, 2011; Takahara *et al.*, 2016). Taken together these results demonstrate that LysM proteins have key roles in both suppressing host defence response and fungal development.

Unlike the *chiA* single deletion mutant which did not show any whole plant or cellular interaction phenotype, the *chiB* deletion mutant did have a cellular phenotype; instead of restrictive growth hyphae showed a proliferative pattern of tip growth similar to the cellular phenotype that has been observed in the *lymA* and *lymB* double deletion mutant. Hyphae are highly branched, and form convoluted structures with smaller hyphal compartments as a result of switching to proliferative growth. Alteration from hyphal restrictive intercalary growth to proliferative growth can change the symbiotic interaction from mutualistic to antagonistic however our results show that disruption in restrictive growth does not lead to an antagonistic interaction as has been observed for deletion of genes involved in production of reactive oxygen species, *noxA*, *racA* and *noxR*; or components of the cell wall integrity (CWI) signalling pathway, *mpkA* and *mkkA;* or genes encoding membrane associated proteins *symB* and *symC* in *E. festucae* Fl1, where

plants become stunted and undergo premature senescence (Takemoto *et al.,* 2006; Tanaka *et al.,* 2006; Tanaka *et al.,* 2008; Green et al*., 2017;* Becker et al*.,* 2015). Both *chiA* and *chiB* expression is higher *in planta* compared to in culture suggesting they have a role in the symbiotic interaction *in planta*. Although *chiA* has higher expression than *chiB*, deletion of the *chiA* does not have any effect on the symbiotic interaction. One explanation for not having any phenotype in Δ*chiA* mutant strains could be that these two chitinase genes are involved in different pathways and clearly these results show that *chiB* is required for maintenance of hyphal restrictive growth *in planta* and establishment of the symbiosis. However double deletion of *chiA* and *chiB* together has not been conducted to check if there is any redundancy between these genes and this can be considered as a future experiment to do.

To gain further insight into the function of the proteins encoded by *lymA* and *lymB*, eGFP fusion proteins of LymA and LymB proteins were generated and their localisation checked in axenic culture. Both LymA and LymB proteins localised in fungal cell wall and septa. This result is consistent with their proposed role of binding to chitin oligomers and the fact that they both contain secretion signals. It would be also interesting to examine their localisation *in planta* or the colocalization of LymA/ChiA and LymB/ChiB together to find out more about their function in symbiosis. Other studies done on LysM effectors in other pathogens indicate that LysM proteins are localised on the surface of the biotrophic primary hyphae in *C. higginsianum* (Takahara *et al.*, 2016) or at the tip and later around the invasive hyphae in *M. oryzae* (Mentlak *et al.*, 2012), supporting their role in sequestering the chitin oligomers and suppressing the chitin-triggered immunity response in their host.

In conclusion I have shown that two *E. festucae* Fl1 *lysM* genes and one *chitinase* gene, *lymA*, *lymB* and *chiB*, are necessary for hyphal growth and regulation *in planta*. I have established for the first time that deletion of *lymA* and *lymB* together and *chiB* resulted in breakdown of the symbiotic interaction suggesting they are required for hyphal intercalary growth, establishment and maintenance of symbiosis. These results suggest that LysM and chitinase proteins in *E. festucae* Fl1 are involved in different pathways and may have different contributions toward plant-fungal symbiosis. Chitin binding and chitinase enzyme assays to check the affinity of these LysM proteins for various polysaccharides such as chitin, chitosan, xylan and cellulose and their ability to protect the fungal hyphae against chitinases hydrolases can be future experiments to be done to

confirm their role in suppressing the plant host defence. Analysing their localisation *in planta* also could be another investigation to find out the actual role of these proteins during development, colonisation and growth inside the host.

Chapter 6. Bioinformatic analysis of genes encoding chitin deacetylase enzymes

6.1. Identification of *E. festucae* Fl1 chitin deacetylase genes

To determine if the *E. festucae* genome contains any chitin deacetylase genes, a tBLASTn search was conducted using *Magnaporthe oryzae* chitin deacetylase (CDA) protein (MGG_12939, 14966, 09159, 04172, 08774, 01868, 08356, 05023, 04704 and 03461) sequences as the query sequences at http://www.endophyte.uky.edu. The first and second hits identified three chitin deacetylase genes EfM3.042050, EfM3.035615 and EfM3.030650 sharing identities from 24% to 56% with *M. oryzae* sequences (Table 6.1). A similar analysis was done against *Neurospora crassa* and *Fusarium graminearum* chitin deacetylase protein sequences. The first and second hits obtained from tBLASTn analysis confirmed EfM3.042050 and EfM3.035615 respectively in the *E. festucae* Fl1 genome (Table 6.2). Identified chitin deacetylase gene models, EfM3.035615, EfM3.030650 and EfM3.042050, were designated as *cdaA*, *cdaB* and *cdaC* respectively. The deduced domain structure of the proteins encoded by these genes using InterPro scan https://www.ebi.ac.uk/interpro showed that they all contain polysaccharide deacetylase domains but only *cdaA* contains an N terminal signal peptide which is predicted to be secreted into the apoplastic space (Figure 6.1). Analysis of RNA-seq data of *E. festucae* gene expression *in planta* compared to in culture was conducted to find out the possible role of these genes in the symbiotic interaction between *E. festucae* and the host plant. The RNA seq data analysis showed that *cdaA* and *cdaB* were upregulated *in planta* compare to in culture while *cdaC* was downregulated (Table 6.3). Comparison of RNA-seq data generated *in planta* vs. in culture for three symbiotic mutants, *noxA*, *proA* and *sakA* showed that *cdaA* was downregulated in *noxA*, *proA* and *sakA* compared to WT. However, these changes were only significant in the *sakA* mutant. *cdaB* was upregulated in the three mutant data sets but likewise was only significant in the *sakA* data set. *cdaC* did not have any changes in any of three mutant data sets (2.12) (Table 6.4).

Table 6.1. tBLASTn analysis of *M. oryzae* chitin deacetylase protein sequences against the *E. festucae* Fl1 genome.

Gene number (*M. oryzae*)	Gene name (*M. oryzae*)	E-value	%Identity	score	Gene number (*E. festucae*)	Gene name (*E. fectucae*)
MGG_12939	CP1	1.00E-64[a]	44	217	EfM3.042050	*cdaC*
MGG_12939	CP1	2.00E-60[b]	56	230	EfM3.035615	*cdaA*
MGG_14966	CDA1	6.00E-53[a]	45	205	EfM3.042050	*cdaC*
MGG_14966	CDA1	2.00E-39[b]	34	122	EfM3.035615	*cdaA*
MGG_09159	CDA2	3.00E-69[a]	46	273	EfM3.042050	*cdaC*
MGG_09159	CDA2	7.00E-54[b]	51	209	EfM3.035615	*cdaA*
MGG_04172	CDA3	3.00E-14[a]	26	78	EfM3.042050	*cdaC*
MGG_04172	CDA3	1.00E-06[b]	24	52	EfM3.035615	*cdaA*
MGG_08774	CDA4	2.00E-49[a]	42	193	EfM3.042050	*cdaC*
MGG_08774	CDA4	2.00E-33[b]	35	140	EfM3.035615	*cdaA*
MGG_01868	CDA5	5.00E-33[a]	34	140	EfM3.042050	*cdaC*
MGG_01868	CDA5	2.00E-18[b]	30	92	EfM3.035615	*cdaA*
MGG_08356	CDA6	7.00E-33[a]	37	139	EfM3.042050	*cdaC*
MGG_08356	CDA6	3.00E-21[b]	35	100	EfM3.035615	*cdaA*
MGG_05023	CDA7	5.00E-46[a]	40	182	EfM3.042050	*cdaC*
MGG_05023	CDA7	1.00E-31[b]	37	134	EfM3.035615	*cdaA*
MGG_04704	CDA8	1.00E-61[a]	52	233	EfM3.030650	*cdaB*
MGG_04704	CDA8	9.00E-09[b]	27	58	EfM3.042050	*cdaC*
MGG_03461	CDA9	7.00E-37[a]	39	150	EfM3.042050	*cdaC*
MGG_03461	CDA9	2.00E-20[b]	28	78	EfM3.035615	*cdaA*

[a]Lowest E-value. [b]Second lowest E-value.

M. oryzae data obtained from Geoghegan and Gurr (2016).

Table 6.2. tBLASTn analysis of *N. crassa* and *F. graminearum* chitin deacetylase protein sequences against the *E. festucae* Fl1 genome.

Species	Gene name	E-value	Gene number (*E. festucae*)	Gene name (*E. festucae*)
N. crassa	NCU10651[a]	3.00E-70	EfM3.042050	*cdaC*
N. crassa	NCU10651[b]	2.00E-60	EfM3.035615	*cdaA*
N. crassa	NCU09508[a]	3.00E-46	EfM3.042050	*cdaC*
N. crassa	NCU09508[b]	2.00E-30	EfM3.035615	*cdaA*
F. graminearum	FGSC05847[a]	4.00E-79	EfM3.042050	*cdaC*
F. graminearum	FGSC05847[b]	2.00E-60	EfM3.035615	*cdaA*

[a]Lowest E-value. [b]Second lowest E-value.
Data obtained from http://fungidb.org/fungidb/ and https://www.ncbi.nlm.nih.gov.

Table 6.3. Fold differences of expression of *E. festucae chitin deacetylase* genes *in planta* compared to in culture.

Gene Model	Gene Name	Culture RPMK[a]	*InPlanta* RPMK[a]	Ratio	Direction	FoldDiff
EfM3.035615	*cdaA*	3.93	28.75	7.31	+	7.31
EfM3.030650	*cdaB*	11.77	14.03	1.19	+	1.19
EfM3.042050	*cdaC*	NA	NA	NA	NA	NA

[a] Data obtained from T. Chujo (in preparation). Direction indicates the level of expression change. Minus and plus indicate the direction of change (down regulated and up regulated respectively). RPKM: Reads Per Kilobase of transcript per Million mapped reads.

Table 6.4. Gene expression fold changes of *chitin deacetylase* genes in *E. festucae* genome between wild type and symbiotic mutants Δ*noxA*, Δ*proA* and Δ*sakA*.

Gene Model	Gene Name	*noxA* dir[b]	*noxA* Fold[a]	*proA* dir[b]	*proA* Fold[a]	*sakA* dir[b]	*sakA* Fold[a]
EfM3.035615	*cdaA*	-	1.34	-	1.56	-	6.49
EfM3.030650	*cdaB*	+	1.14	+	1.13	+	11.84
EfM3.042050	*cdaC*	NA	NA	NA	NA	NA	NA

[a] Fold changes in red and green are significant whereas those in black are not significant.
[b] Direction indicates the level of expression change. Minus and plus indicate the direction of change (down regulated and up regulated respectively). Data obtained from Eaton *et al*, 2015.

Analysing the exon and intron structure of *cdaA*, *cdaB*, *cdaC* at http://www.endophyte.uky.edu revealed that the cDNAs of these genes encode proteins of 259, 268, and 213 amino acids with predicted unmodified molecular weights of 29.77, 30.8, and 23.86 kD respectively (Figure 6.1).

To gain further insight into these proteins, an amino acid sequence alignment was conducted on the Fl1 chitin deacetylase proteins and their homologues from *M. oryzae*, *N. crassa* and *F. graminearum*. This analysis indicated that residues implicated in both enzymatic activity and zinc (Zn) binding are conserved among these proteins (Figure 6.2).

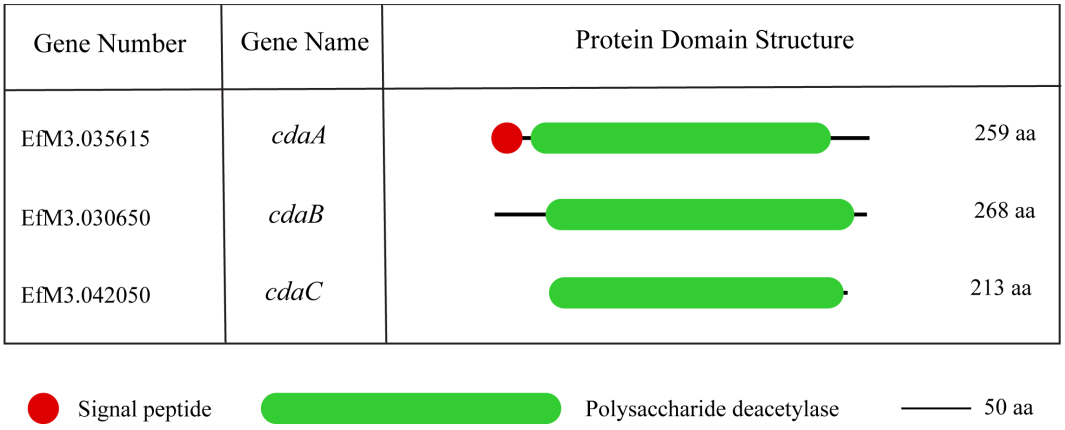
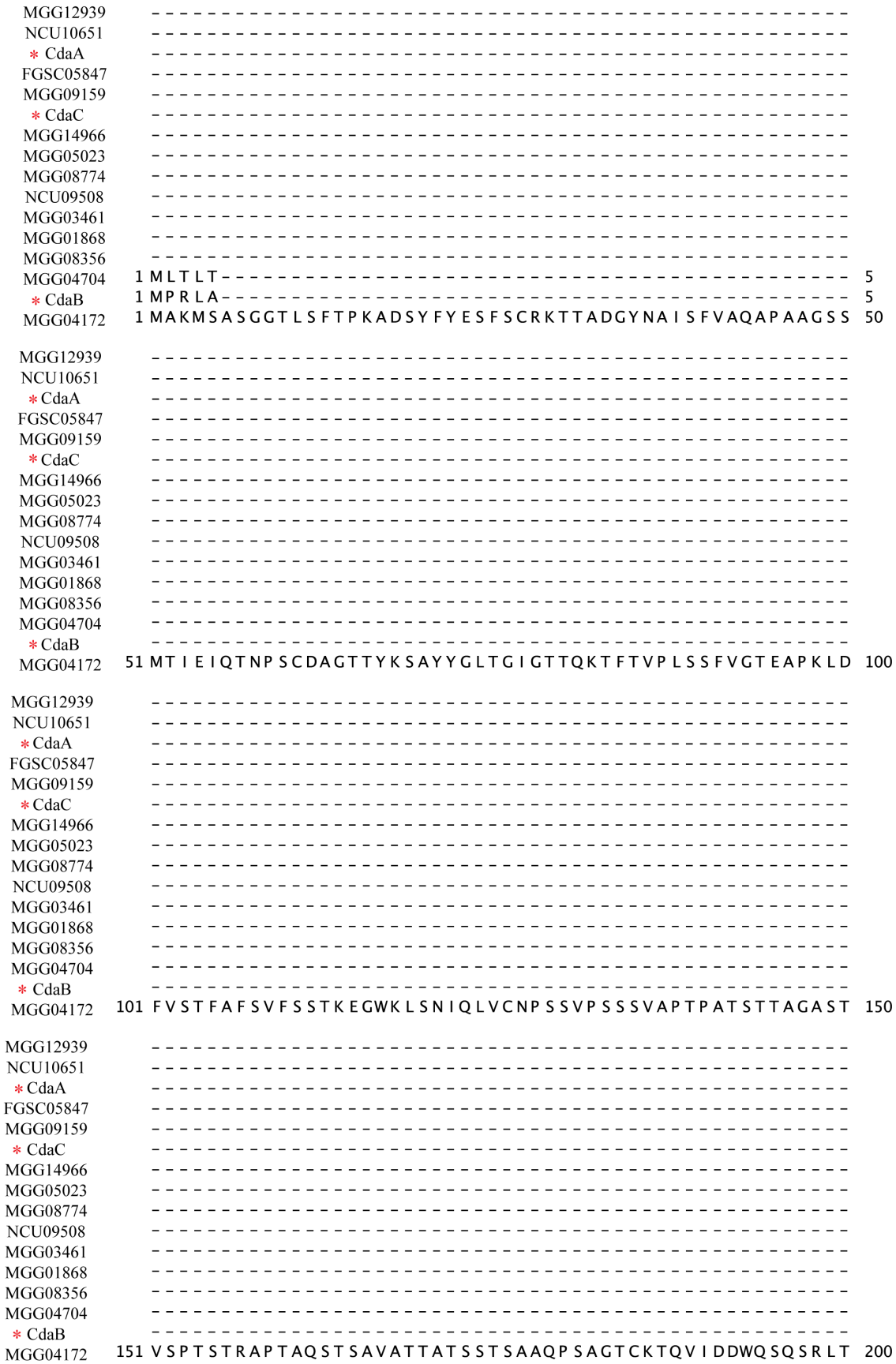
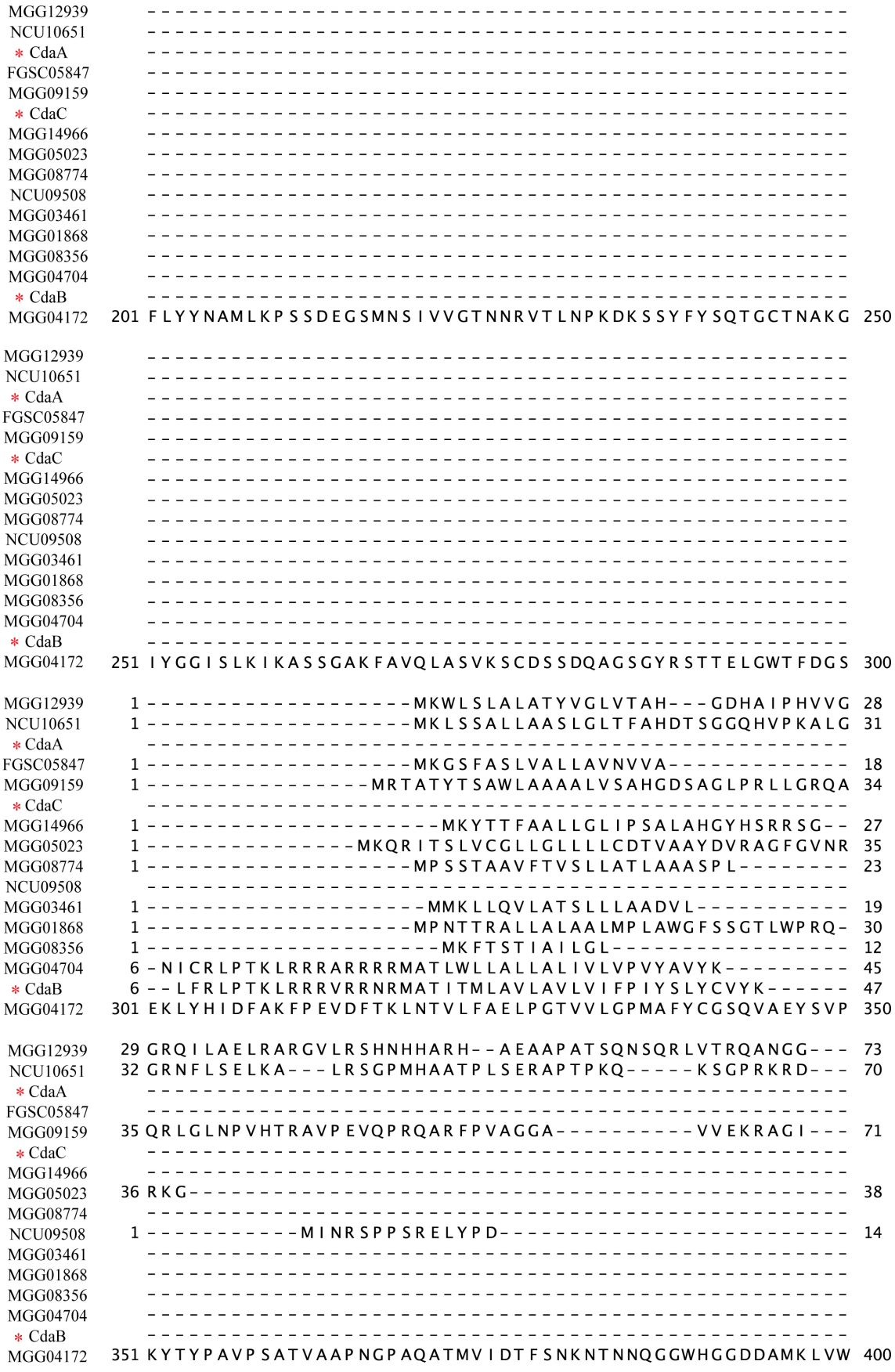
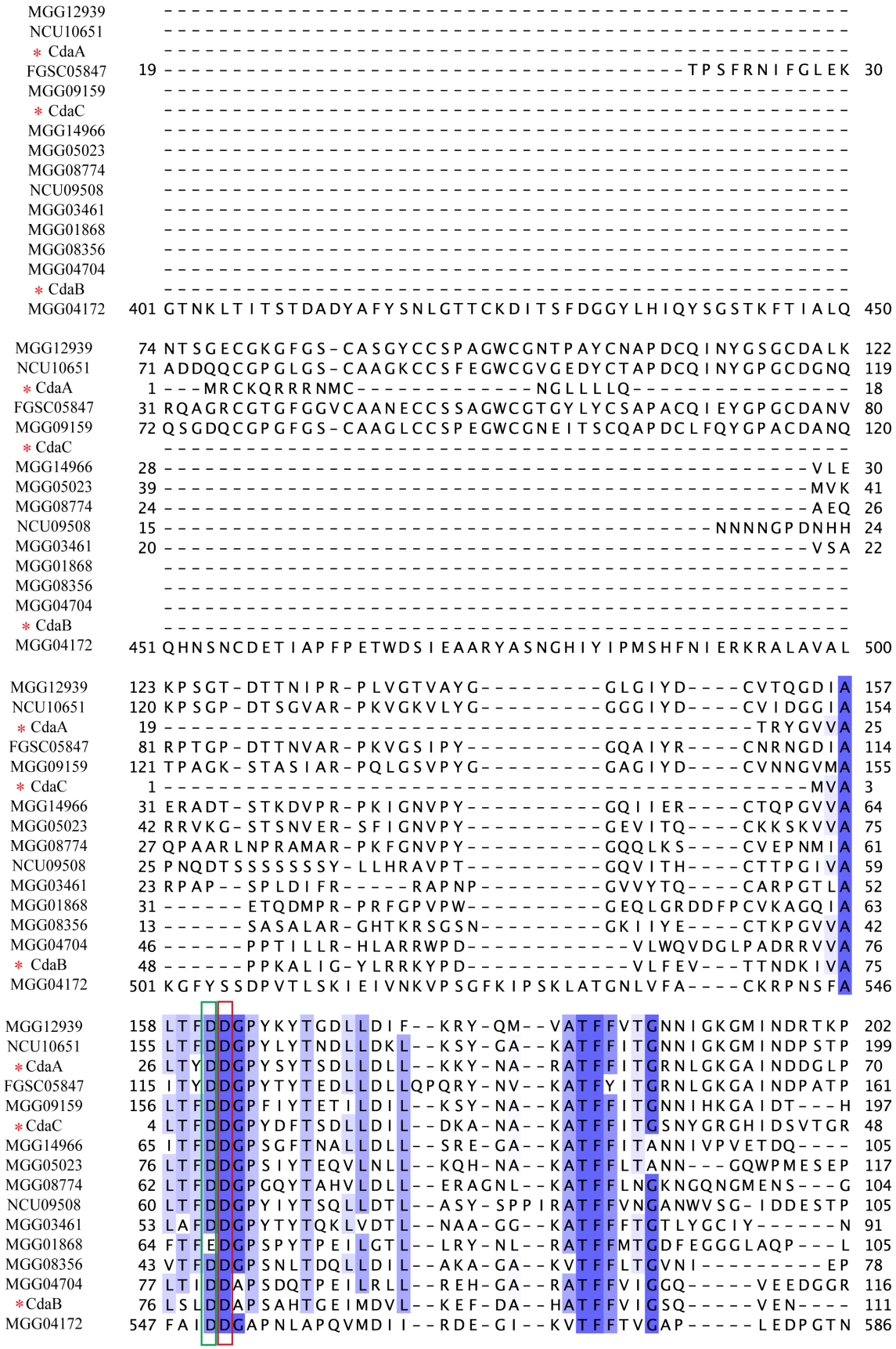


Figure 6.1. *E. festucae* chitin deacetylase predicted polypeptide structure. Protein domain structure of *E. festucae* chitin deacetylase proteins showing the predicted signal peptide and polysaccharide deacetylase domains. Bar=50 amino acids.


MGG12939
NCU10651
* CdaA
FGSC05847
MGG09159
* CdaC
MGG14966
MGG05023
MGG08774
NCU09508
MGG03461
MGG01868
MGG08356
MGG04704
* CdaB
MGG04172
1 M L T L T 5
1 M P R L A 5
1 M A K M S A S G G T L S F T P K A D S Y F Y E S F S C R K T T A D G Y N A I S F V A Q A P A A G S S 50
51 M T I E I Q T N P S C D A G T T Y K S A Y Y G L T G I G T T Q K T F T V P L S S F V G T E A P K L D 100
101 F V S T F A F S V F S S T K E G W K L S N I Q L V C N P S S V P S S S V A P T P A T S T T A G A S T 150
151 V S P T S T R A P T A Q S T S A V A T T A T S S T S A A Q P S A G T C K T Q V I D D W Q S Q S R L T 200


MGG12939
NCU10651
* CdaA
FGSC05847
MGG09159
* CdaC
MGG14966
MGG05023
MGG08774
NCU09508
MGG03461
MGG01868
MGG08356
MGG04704
* CdaB
MGG04172 201 F L Y Y N A M L K P S S D E G S M N S I V V G T N N R V T L N P K D K S S Y F Y S Q T G C T N A K G 250
MGG12939
NCU10651
* CdaA
FGSC05847
MGG09159
* CdaC
MGG14966
MGG05023
MGG08774
NCU09508
MGG03461
MGG01868
MGG08356
MGG04704
* CdaB
MGG04172 251 I Y G G I S L K I K A S S G A K F A V Q L A S V K S C D S S D Q A G S G Y R S T T E L G W T F D G S 300
MGG12939 1 M K W L S L A L A T Y V G L V T A H G D H A I P H V V G 28
NCU10651 1 M K L S S A L L A A S L G L T F A H D T S G G Q H V P K A L G 31
* CdaA
FGSC05847 1 M K G S F A S L V A L L A V N V V A 18
MGG09159 1 M R T A T Y T S A W L A A A A L V S A H G D S A G L P R L L G R Q A 34
* CdaC
MGG14966 1 M K Y T T F A A L L G L I P S A L A H G Y H S R R S G 27
MGG05023 1 M K Q R I T S L V C G L L G L L L L C D T V A A Y D V R A G F G V N R 35
MGG08774 1 M P S S T A A V F T V S L L A T L A A A S P L 23
NCU09508
MGG03461 1 M M K L L Q V L A T S L L L A A D V L 19
MGG01868 1 M P N T T R A L L A L A A L M P L A W G F S S G T L W P R Q 30
MGG08356 1 M K F T S T I A I L G L 12
MGG04704 6 N I C R L P T K L R R R A R R R R M A T L W L L A L L A L I V L V P V Y A V Y K 45
* CdaB 6 L F R L P T K L R R R V R R N R M A T I T M L A V L A V L V I F P I Y S L Y C V Y K 47
MGG04172 301 E K L Y H I D F A K F P E V D F T K L N T V L F A E L P G T V V L G P M A F Y C G S Q V A E Y S V P 350
MGG12939 29 G R Q I L A E L R A R G V L R S H N H H A R H A E A A P A T S Q N S Q R L V T R Q A N G G 73
NCU10651 32 G R N F L S E L K A L R S G P M H A A T P L S E R A P T P K Q K S G P R K R D 70
* CdaA
FGSC05847
MGG09159 35 Q R L G L N P V H T R A V P E V Q P R Q A R F P V A G G A V V E K R A G I 71
* CdaC
MGG14966
MGG05023 36 R K G 38
MGG08774
NCU09508 1 M I N R S P P S R E L Y P D 14
MGG03461
MGG01868
MGG08356
MGG04704
* CdaB
MGG04172 351 K Y T Y P A V P S A T V A A P N G P A Q A T M V I D T F S N K N T N N Q G G W H G G D D A M K L V W 400


MGG12939
NCU10651
* CdaA
FGSC05847
MGG09159
* CdaC
MGG14966
MGG05023
MGG08774
NCU09508
MGG03461
MGG01868
MGG08356
MGG04704
* CdaB
MGG04172

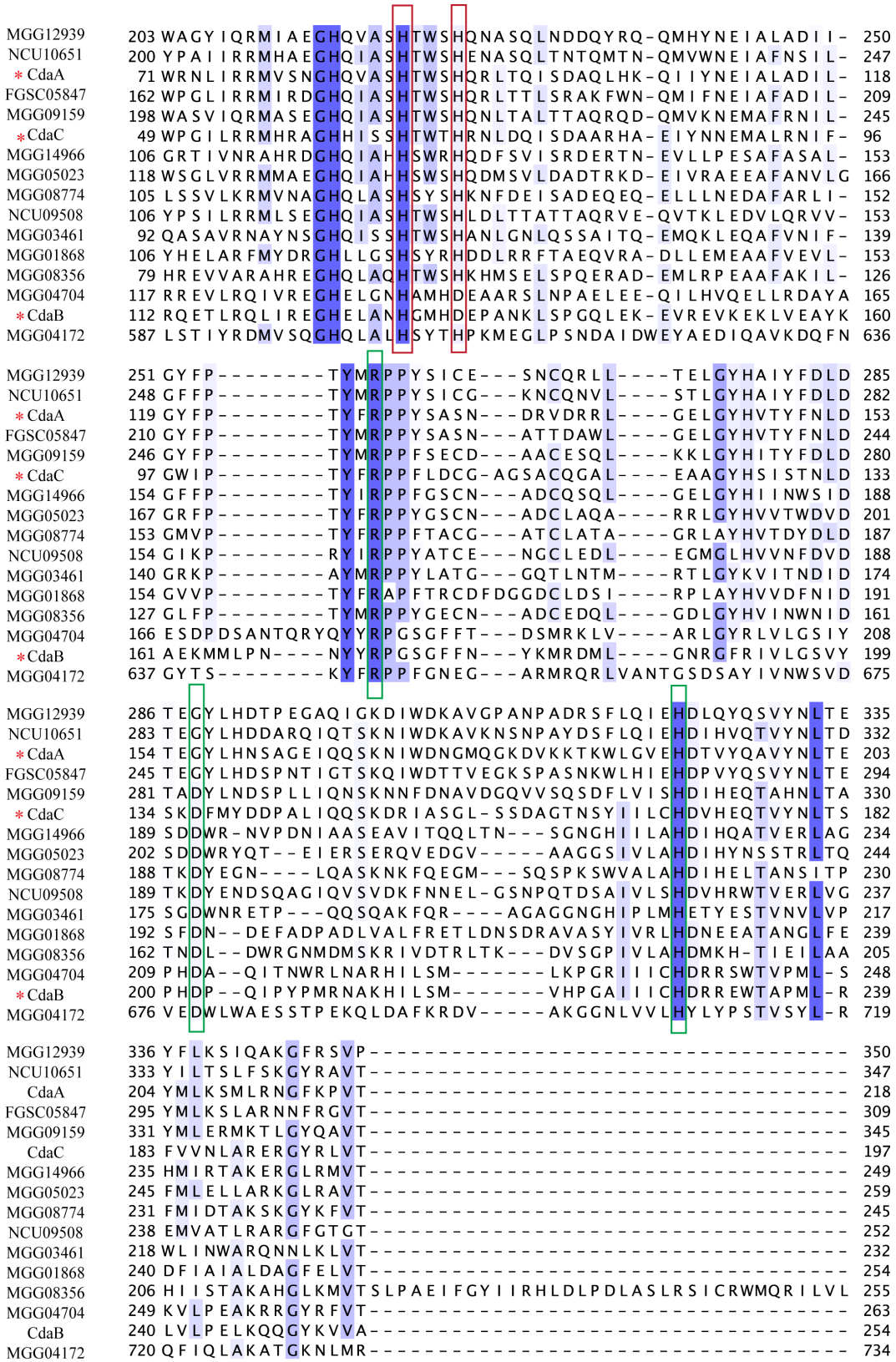

MGG12939 203 WAGYIQRMIAEGHQVASHTWSHQNASQLNDDQYRQ-QMHYNEIALADII- 250
NCU10651 200 YPAIIRRMHAEGHQIASHTWSHENASQLTNTQMTN-QMVWNEIAFNSIL- 247
*CdaA 71 WRNLIRRMVSNGHQVASHTWSHQRLTQISDAQLHK-QIIYNEIALADIL- 118
FGSC05847 162 WPGLIRRMIRDGHQIASHTWSHQRLTTLSRAKFWN-QMIFNEIAFADIL- 209
MGG09159 198 WASVIQRMASEGHQIASHTWSHQNLTALTTAQRQD-QMVKNEMAFRNIL- 245
*CdaC 49 WPGILRRMHRAGHHISSHTWTHRNLDQISDAARHA-EIYNNEMALRNIF- 96
MGG14966 106 GRTIVNRAHRDGHQIAHHSWRHQDFSVISRDERTN-EVLLPESAFASAL- 153
MGG05023 118 WSGLVRRMMAEGHQIAHHSWSHQDMSVLDADTRKD-EIVRAEEAFANVLG 166
MGG08774 105 LSSVLKRMVNAGHQLASHSYSHKNFDEISADEQEQ-ELLLNEDAFARLI- 152
NCU09508 106 YPSILRRMLSEGHQIASHTWSHLDLTTATTAQRVE-QVTKLEDVLQRVV- 153
MGG03461 92 QASAVRNAYNSGHQISSHTWSHANLGNLQSSAITQ-EMQKLEQAFVNIF- 139
MGG01868 106 YHELARFMYDRGHLLGSHSYRHDDLRRFTAEQVRA-DLLEMEAAFVEVL- 153
MGG08356 79 HREVVARAHREGHQLAQHTWSHKHMSELSPQERAD-EMLRPEAAFAKIL- 126
MGG04704 117 RREVLRQIVREGHELGNHAMHDEAARSLNPAELEE-QILHVQELLRDAYA 165
*CdaB 112 RQETLRQLIREGHELANHGMHDEPANKLSPGQLEK-EVREVKEKLVEAYK 160
MGG04172 587 LSTIYRDMVSQGHQLALHSYTHPKMEGLPSNDAIDWEYAEDIQAVKDQFN 636
MGG12939 251 GYFP--------TYMRPPYSICE---SNCQRLL----TELGYHAIYFDLD 285
NCU10651 248 GFFP--------TYMRPPYSICG---KNCQNVL----STLGYHAIYFDLD 282
*CdaA 119 GYFP--------TYFRPPYSASN---DRVDRRL----GELGYHVTYFNLD 153
FGSC05847 210 GYFP--------TYMRPPYSASN---ATTDAWL----GELGYHVTYFNLD 244
MGG09159 246 GYFP--------TYMRPPFSECD---AACESQL----KKLGYHITYFDLD 280
*CdaC 97 GWIP--------TYFRPPFLDCG-AGSACQGAL----EAAGYHSISTNLD 133
MGG14966 154 GFFP--------TYIRPPFGSCN---ADCQSQL----GELGYHIINWSID 188
MGG05023 167 GRFP--------TYFRPPYGSCN---ADCLAQA----RRLGYHVVTWDVD 201
MGG08774 153 GMVP--------TYFRPPFTACG---ATCLATA----GRLAYHVTDYDLD 187
NCU09508 154 GIKP--------RYIRPPYATCE---NGCLEDL----EGMGLHVVNFDVD 188
MGG03461 140 GRKP--------AYMRPPYLATG---GQTLNTM----RTLGYKVITNDID 174
MGG01868 154 GVVP--------TYFRAPFTRCDFDGGDCLDSI----RPLAYHVVDFNID 191
MGG08356 127 GLFP--------TYMRPPYGECN---ADCEDQL----GDLGYHVINWNID 161
MGG04704 166 ESDPDSANTQRYQYYRPGSGFFT---DSMRKLV----ARLGYRLVLGSIY 208
*CdaB 161 AEKMMLPN----NYYRPGSGFFN---YKMRDML----GNRGFRIVLGSVY 199
MGG04172 637 GYTS--------KYFRPPFGNEG---ARMRQRLVANTGSDSAYIVNWSVD 675
MGG12939 286 TEGYLHDTPEGAQIGKDIWDKAVGPANPADRSFLQIEHDLQYQSVYNLTE 335
NCU10651 283 TEGYLHDDARQIQTSKNIWDKAVKNSNPAYDSFLQIEHDIHVQTVYNLTD 332
*CdaA 154 TEGYLHNSAGEIQQSKNIWDNGMQGKDVKKTKWLGVEHDTVYQAVYNLTE 203
FGSC05847 245 TEGYLHDSPNTIGTSKQIWDTTVEGKSPASNKWLHIEHDPVYQSVYNLTE 294
MGG09159 281 TADYLNDSPLLIQNSKNNFDNAVDGQVVSQSDFLVISHDIHEQTAHNLTA 330
*CdaC 134 SKDFMYDDPALIQQSKDRIASGL-SSDAGTNSYIILCHDVHEQTVYNLTS 182
MGG14966 189 SDDWR-NVPDNIAASEAVITQQLTN---SGNGHIILAHDIHQATVERLAG 234
MGG05023 202 SDDWRYQT--EIERSERQVEDGV-----AAGGSIVLAHDIHYNSSTRLTQ 244
MGG08774 188 TKDYEGN----LQASKNKFQEGM---SQSPKSWVALAHDIHELTANSITP 230
NCU09508 189 TKDYENDSQAGIQVSVDKFNNEL-GSNPQTDSAIVLSHDVHRWTVERLVG 237
MGG03461 175 SGDWNRETP---QQSQAKFQR----AGAGGNGHIPLMHETYESTVNVLVP 217
MGG01868 192 SFDN--DEFADPADLVALFRETLDNSDRAVASYIVRLHDNEEATANGLFE 239
MGG08356 162 TNDL--DWRGNMDMSKRIVDTRLTK---DVSGPIVLAHDMKH-TIEILAA 205
MGG04704 209 PHDA--QITNWRLNARHILSM-------LKPGRIIICHDRRSWTVPML-S 248
*CdaB 200 PHDP--QIPYPMRNAKHILSM-------VHPGAIIICHDRREWTAPML-R 239
MGG04172 676 VEDWLWAESSTPEKQLDAFKRDV-----AKGGNLVVLHYLYPSTVSYL-R 719
MGG12939 336 YFLKSIQAKGFRSVP---------- 350
NCU10651 333 YILTSLFSKGYRAVT---------- 347
CdaA 204 YMLKSMLRNGFKPVT---------- 218
FGSC05847 295 YMLKSLARNNFRGVT---------- 309
MGG09159 331 YMLERMKTLGYQAVT---------- 345
CdaC 183 FVVNLARERGYRLVT---------- 197
MGG14966 235 HMIRTAKERGLRMVT---------- 249
MGG05023 245 FMLELLARKGLRAVT---------- 259
MGG08774 231 FMIDTAKSKGYKFVT---------- 245
NCU09508 238 EMVATLRARGFGTGT---------- 252
MGG03461 218 WLINWARQNNLKLVT---------- 232
MGG01868 240 DFIAIALDAGFELVT---------- 254
MGG08356 206 HIISTAKAHGLKMVTSLPAEIFGYIIRHLDLPDLASLRSICRWMQRILVL 255
MGG04704 249 KVLPEAKRRGYRFVT---------- 263
CdaB 240 LVLPELKQQGYKVVA---------- 254
MGG04172 720 QFIQLAKATGKNLMR---------- 734

Figure 6.2. Multiple sequence alignment of the *E. festucae* chitin deacetylase proteins (CdaA, CdaB and CdaC) with homologues from *M. oryzae* (MGG12939, MGG09159, MGG14966, MGG05023, MGG08774, MGG03461, MGG01868, MGG08356, MGG04704, MGG04172), *N. crassa* (NCU10651, NCU09508) and *F. graminearum* (FGSC05847). Intensity of colouring indicates the level of conservation with darker shades being more highly conserved. Predicted conserved active residues and zinc binding sites are bordered in green and red respectively. *E festucae* Fl1 chitin deacetylase proteins are indicted with red asterisk.

Chapter 7. Functional analysis of *E. festucae* Fl1 chitin deacetylase (*cda*) genes

To investigate the role of an *E. festucae* chitin deacetylase gene a reverse genetics approach was conducted to delete *cdaA*, to determine its function in fungal cell wall composition, hyphal growth, fungal development and effect on the grass host *L. perenne.*

7.1. *E. festucae* Fl1 cell wall composition in endophytic and epiphyllous hyphae in infected *L. perenne* seedlings and pseudostem.

To investigate the hyphal cell wall structure in endophytic and epiphyllous hyphae in the early stage of infection, *L. perenne* seedlings were inoculated with *E. festucae* Fl1 and stained two weeks post inoculation with the fluorescent dyes WGA-AF488 and aniline blue (2.10.2). Hyphae at the inoculation site were checked using CLSM microscopy. These analyses showed that the cell wall and septa in epiphyllous hyphae labelled with WGA-AF488 while only the septa in endophytic hyphae labelled with WGA-AF488 and endophytic fungal cell wall stained with aniline blue (Figure 7.1). Fungal cell wall composition was also examined in infected *L. perenne* sheath samples inoculated with Fl1. 10-week-old plant sheath samples were stained with aniline blue and WGA-AF488 and checked using CLSM (2.10.2). Microscopy results showed the same staining as seedlings with labelling of the septa in endophytic hyphae, and septa and cell wall in epiphyllous hyphae with WGA-AF488. Strikingly, hyphae exhibited a combination of both staining patterns near points of hyphal emergence and expressorium formation. At these locations a transition was observed from WGA-AF488 only labelling septa to it assuming a patchy and progressively more homogeneous distribution on the hyphal cell walls outside of the plant (Figure 7.2).

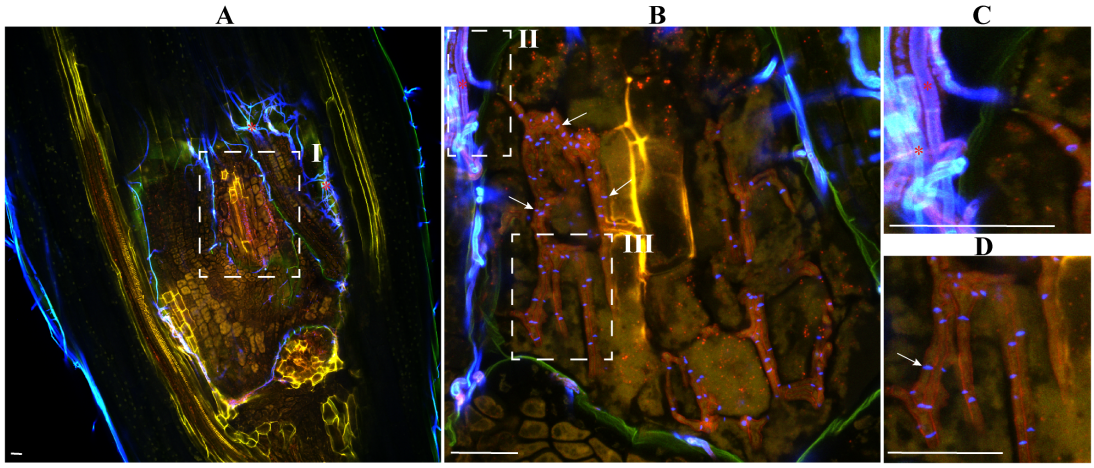


Figure 7.1. *E. festucae* Fl1 endophytic and epiphyllous hyphae at site of inoculation of *L. perenne* seedlings. (A) *L. perenne* seedling infected with *E. festucae* wild type (Fl1) two weeks post inoculation showing epiphyllous hyphae (red asterisk) stained with WGA-AF488 (blue pseudocolour). (I) endophytic hyphae colonising the seedling meristem zone stained with aniline blue (red pseudocolour) and chitin in cell wall septa with WGA-AF488 (blue pseudocolour). (B) Higher magnification of (I) showing plant meristem colonisation. (C) and (D) Higher magnification of (II) and (III) indicating epiphyllous (red asterisk) and endophytic hyphae respectively. White arrows identify fungal septa. Images were generated by maximum intensity projection of confocal z-stacks. Bar=20 µm.

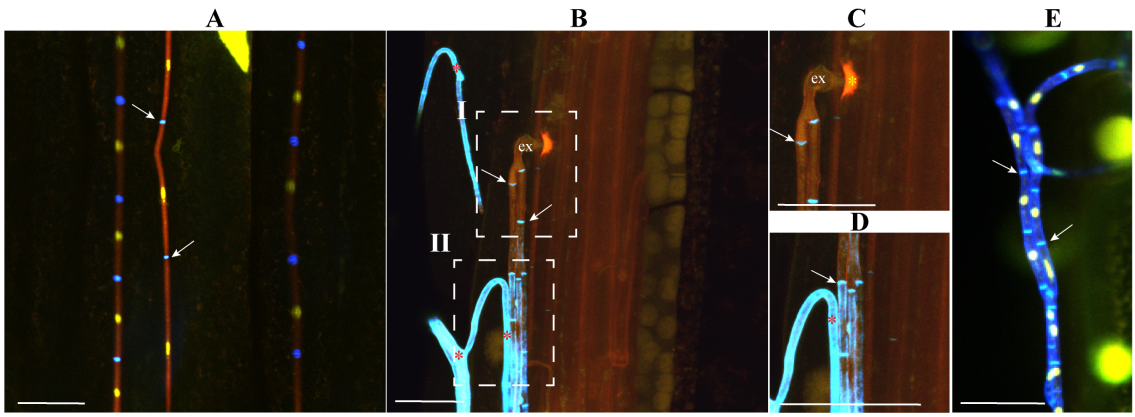


Figure 7.2. Exit of *E. festucae* Fl1 endophytic hyphae from the *L. perenne* leaf by formation of an expressorium. (A) *E. festucae* Fl1 endophytic hyphae stained with aniline blue (red pseudocolour), chitin in cell wall septa with WGA-AF488 (blue pseudocolour), and fungal and plant nuclei with propidium iodide (yellow pseudocolour). (B) *E. festucae* Fl1 expressorium (ex) and emergence point (yellow asterisk). The epiphyllous hyphae (red asterisk) stained with WGA-AF488 (blue pseudocolour). (C) and (D) Higher magnification of (I) and (II) showing the expressorium (ex) and epiphyllous hyphae (red asterisk). (E) *E. festucae* Fl1 epiphyllous hyphae, chitin in cell wall and fungal septa stained with WGA-AF488 (blue pseudocolour), plant and

fungal nuclei stained with propidium iodide (yellow pseudocolour). White arrows identify fungal septa. Images were generated by maximum intensity projection of confocal z-stacks. Bar=20 μm.

7.2. Cell wall composition in axenic culture

7.2.1. The cell walls of axenic cultures resemble those of epiphyllous hyphae

The E. festucae Fl1 strain was cultured on WA media to examine fungal cell wall composition. This was performed by staining with WGA-AF488 which binds chitin, or using a chitin specific probe, Chitin Binding Protein (CBP) (Fuenzalida *et al.*, 2014) followed by imaging with CLSM (2.10.3.1.4). Microscopy analysis revealed that the hyphal cell wall and septa of axenic cultures labelled with both chitin binding probes. This labelling pattern was similar to that observed when staining the epiphyllous hyphae (Figures 7.3 and 7.4).

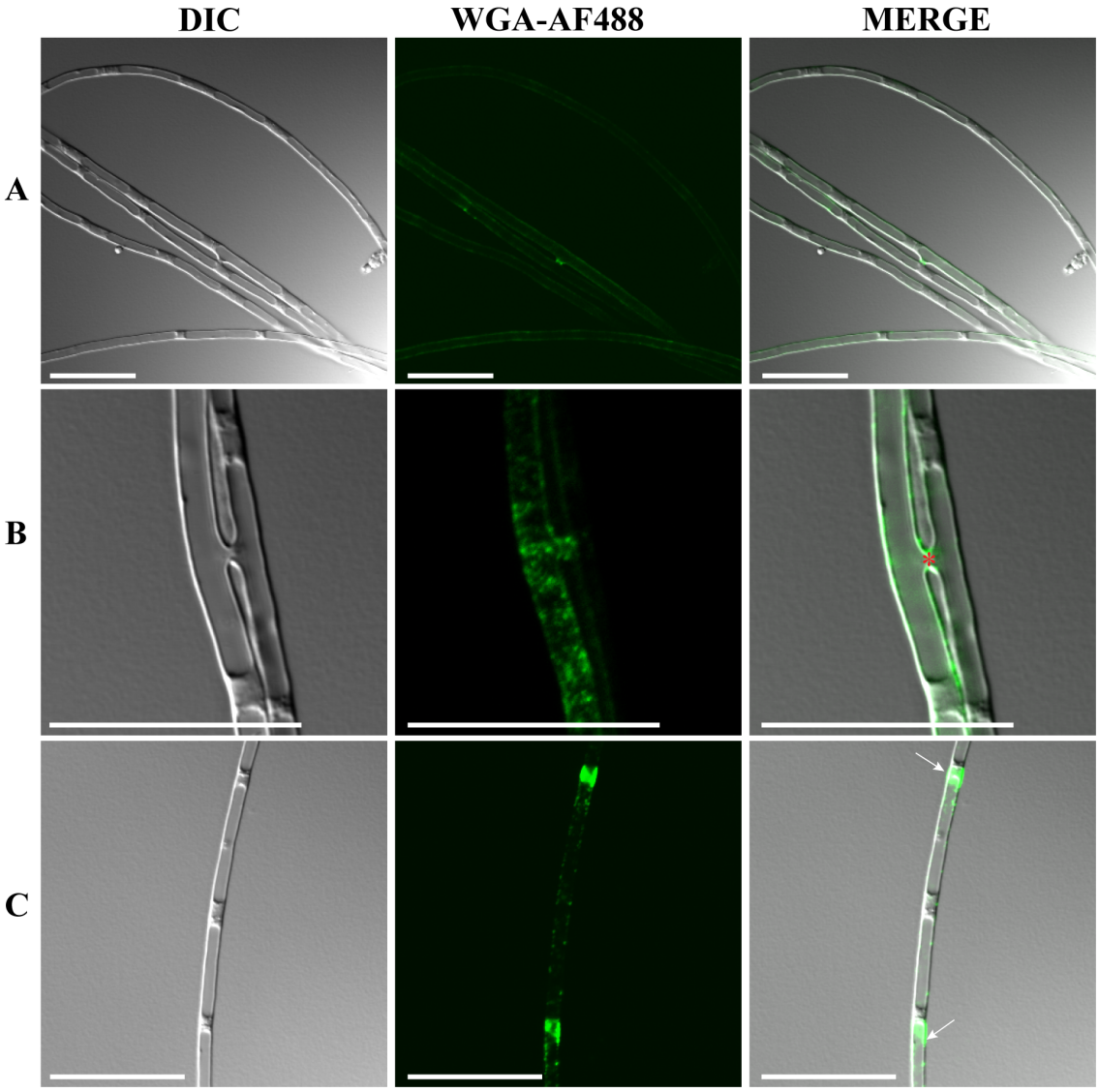


Figure 7.3. *E. festucae* Fl1 grown in axenic culture labelled with WGA-AF488. (A), (B) and (C) showing fungal cell wall, hyphal fusion point (red asterisk) and fungal septa (white arrows) in green pseudocolour respectively. Images were generated by maximum intensity projection of confocal z-stacks. Bar=20 μm.

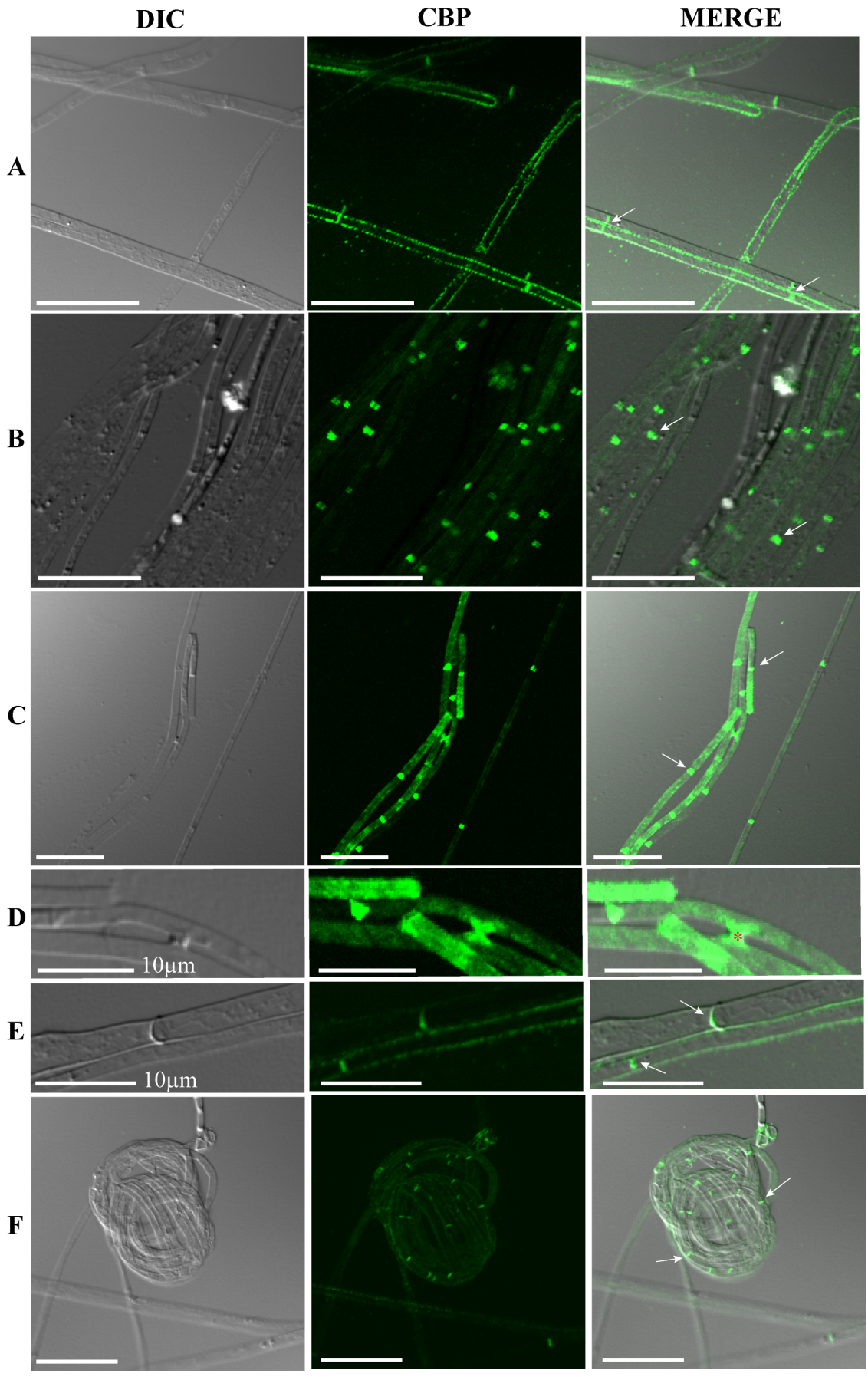

DIC
CBP
MERGE
A
B
C
D
E
F
10µm
10µm

Figure 4.7. *E. festucae* Fl1 grown in axenic culture labelled with chitin binding protein (CBP). (A), (B) and (C) showing fungal cell wall and septa stained with CBP (green pseudocolour). (D), (E) and (F) indicating chitin labelling in hyphal cell wall, fusion point (red asterisk) and coiled structure respectively. White arrows identify fungal septa. Images were generated by maximum intensity projection of confocal z-stacks. Bar=20 μm.

7.3. Fungal cell wall composition in endophytic and epiphyllous hyphae *in planta*

To further investigate the fungal cell wall composition *in planta,* plant samples infected with Fl1 were stained with Chitosan Affinity Protein (CAP), which selectively recognizes chitosan but not chitin (Nampally *et al.*, 2012) (2.10.3.1.1). Investigation of the plant cell division zone and leaf primordia by CSLM revealed that CAP exclusively labelled cell walls in the division zone. No CAP signal could be detected at septa (Figure 7.5). This indicates that hyphal cell walls are composed of chitosan and not chitin in this infected tissue.

Next, the leaf sheath tissues were examined. The plant sheath samples infected with *E. festucae* Fl1 were independently stained with two chitosan specific probes, CAP and OGA-488 (Mravec *et al.*, 2014) as well as propidium iodide (PI) and studied by CLSM (2.10.3.1.1, 2.10.3.2.1 and 2.10.3.2.2). Microscopy results revealed that the hyphal cell wall in endophytic hyphae was labelled along its full visible length with each of these two chitosan specific probes. Accordingly, no labelling was observed either in septa or at the hyphal cell wall of epiphyllous hyphae (Figures 7.6 and 7.7).

Additional experiments were conducted by staining the plant sheath samples infected with *E. festucae* Fl1 with CBP and PI before examining with CLSM (2.10.3.1.2). As expected, only fungal septa were labelled with CBP and there was not any labelling of the fungal cell wall in endophytic hyphae, while the entire cell wall was labelled in epiphyllous hyphae. In some fungal septa only, partial staining was observed irrespective of hyphal position within or outside of the leaf. This could be due to the incomplete infiltration of the probe within the tissue (Figures 7.8 and 7.9).

Taken together, these molecule-specific labelling indicate that chitin is a component of hyphal septa. Chitin is only detectable in cell walls of epiphyllous hyphae and this is replaced by chitosan when *E. festucae* colonises its plant host to become an endophyte.

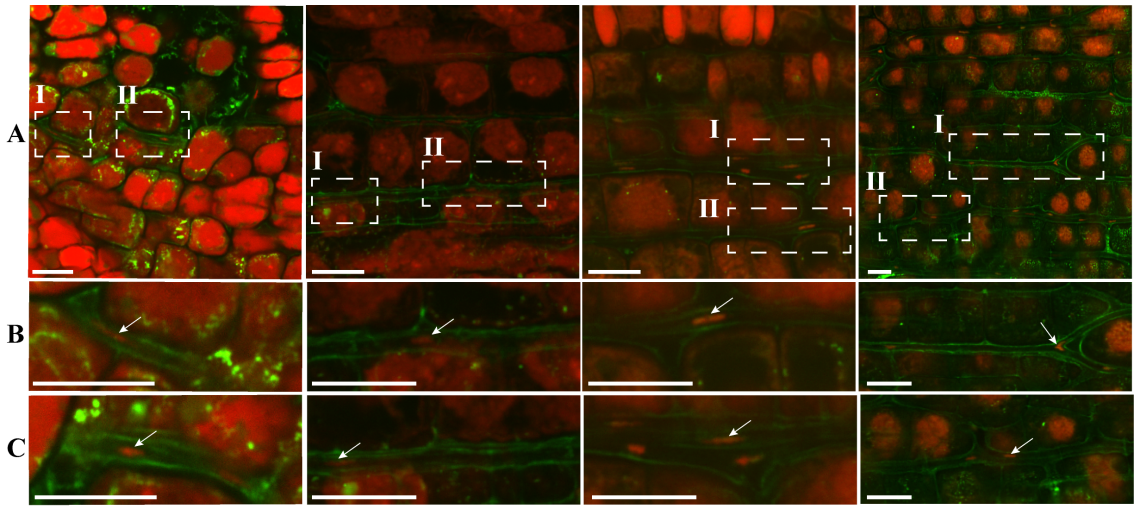


Figure 7.5. *E. festucae* Fl1 endophytic hyphae in *L. perenne* cell division zone at the base of leaf primordia. (A) Fungal cell wall labelled with Chitosan Affinity Protein (CAP) (green pseudocolour) and fungal nuclei stained with propidium iodide (red pseudocolour). (B) and (C) Higher magnification of (I) and (II) indicating hyphal cell wall in plant intercellular space. White arrows identify fungal nuclei. Images were generated by maximum intensity projection of confocal z-stacks. Bar=10 µm.

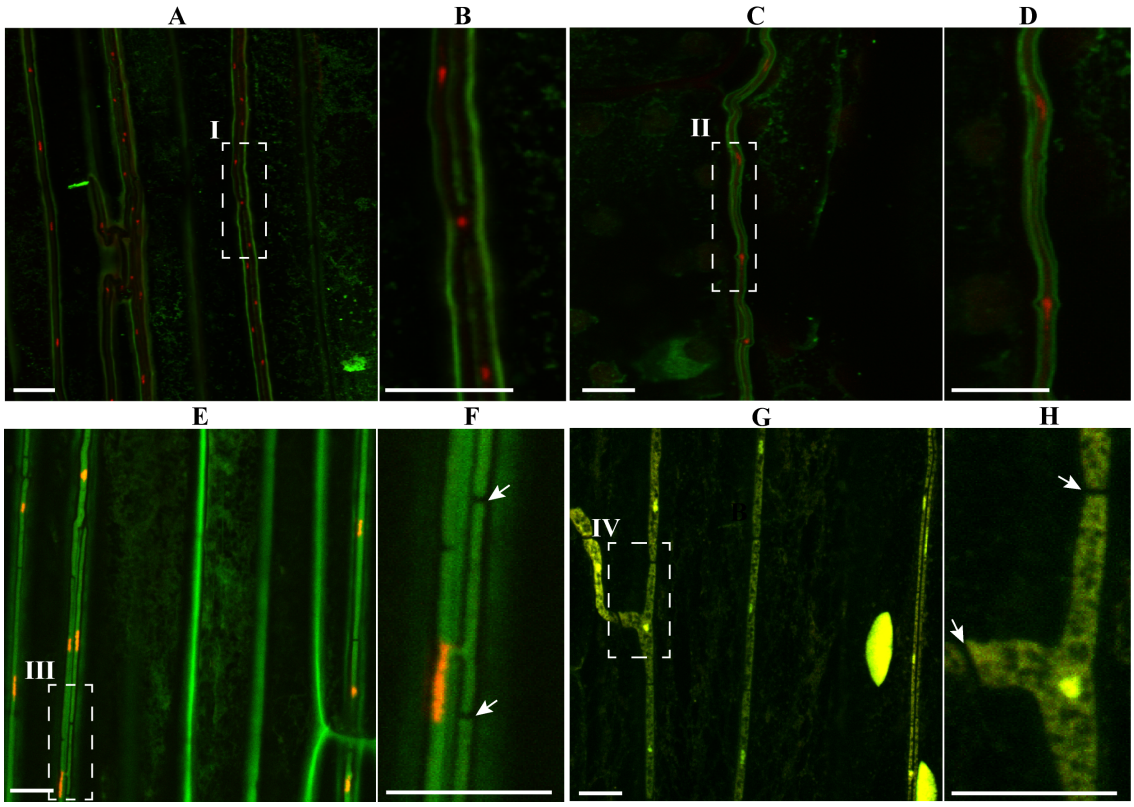


Figure 7.6. *E. festucae* Fl1 endophytic hyphae in the *L. perenne* leaf sheath tissue labelled with CAP and OGA-488. (A) and (C) Fl1 endophytic hyphae labelled with Chitosan Affinity Protein (CAP) in green pseudocolour. Fungal nuclei stained with propidium iodide in red pseudocolour.

(B) and (D) Higher magnification of (I) and (II) respectively showing the fungal cell wall labelled with CAP. (E) and (G) Fl1 endophytic hyphae labelled with OGA-488 in green pseudocolour. (F) and (H) Higher magnification of III and IV respectively indicating the fungal cell wall labelled with OGA-488. Plant and fungal nuclei stained with propidium iodide in red and yellow pseudocolour. White arrows identify unlabelled fungal septa position. (A-F) Samples were washed carefully in PBS buffer and stained in PBS staining solution. (G-H) Samples washed and stained in MES buffer. Images were generated by maximum intensity projection of confocal z-stacks. Bar=10 µm.

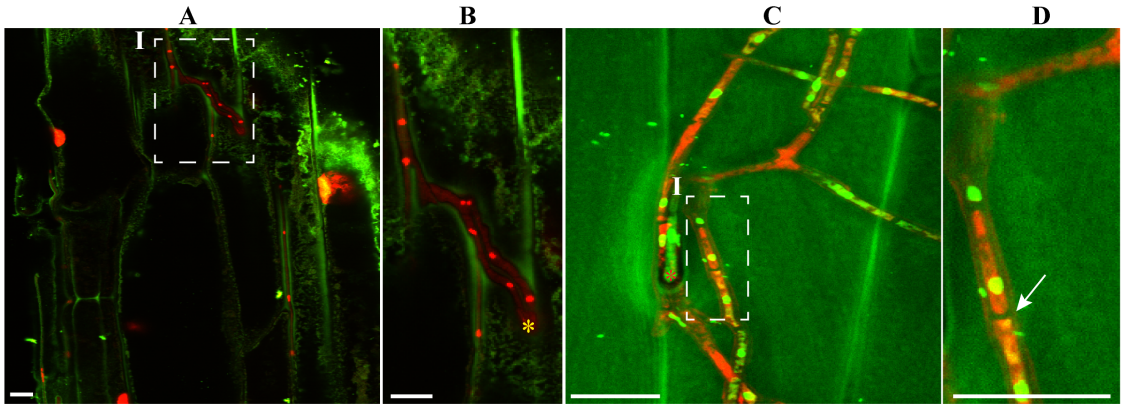


Figure 7.7. *E. festucae* Fl1 epiphyllous hyphae in the *L. perenne* sheath tissue labelled with Chitosan Affinity Protein (CAP) and OGA-488. (A) Fl1 epiphyllous hypha is visible in red (has not been labelled with CAP). Fungal nuclei stained with propidium iodide in red pseudocolour (red dots). (B) Higher magnification of (I) indicating the epiphyllous hyphae and expressorium emergence point (yellow asterisk). (C) Unlabelled Fl1 epiphyllous hyphae are visible in red. Fungal nuclei stained with propidium iodide in yellow pseudocolour (yellow dots). Expressorium emergence point is indicated with red asterisk (D) Higher magnification of (I) indicating the epiphyllous hyphae and emerging point (red asterisk). White arrows identify unlabelled fungal septa position. Images were generated by maximum intensity projection of confocal z-stacks. Bar=10 µm.

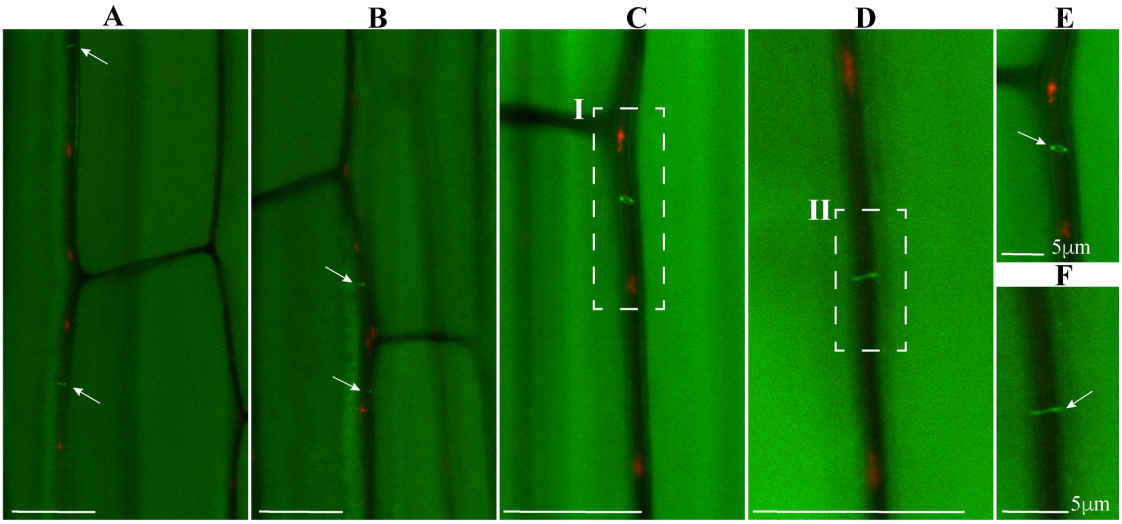


Figure 7.8. *E. festucae* Fl1 endophytic hyphae in the *L. perenne* leaf sheath tissue labelled with Chitin Binding Protein (CBP). (A), (B), (C) and (D) Fungal septa in endophytic hyphae labelled with CBP in green pseudo colour. Fungal nuclei stained with propidium iodide in red pseudocolour (red dots). (E) and (F) Higher magnification of (I) and (II) respectively indicating fungal septa. White arrows identify fungal septa. Images were generated by maximum intensity projection of confocal z-stacks. Bar=20 µm otherwise stated.

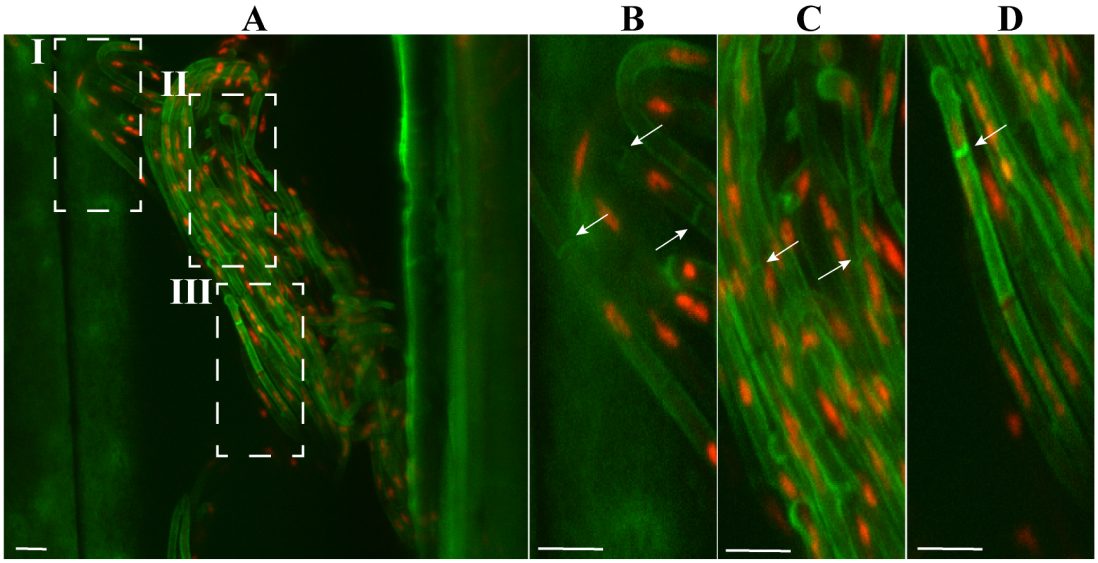


Figure 7.9. *E. festucae* Fl1 epiphyllous hyphae in *L. perenne* sheath tissue labelled with Chitin Binding Protein (CBP). (A) fungal cell wall and septa of epiphyllous hyphae labelled with CBP in green pseudocolour. Fungal nuclei stained with propidium iodide in red pseudocolour (red dots). (B), (C) and (D) Higher magnification of (I), (II) and (III) showing epiphyllous hyphal cell wall and septa. White arrows identify fungal septa. Images were generated by maximum intensity projection of confocal z-stacks. Bar=5 µm.

7.4. Chitosan presence in *E. festucae* cell wall in axenic culture

To check the fungal cell wall composition of *E. festucae* Fl1 in culture, conidia were placed and cultured on WA media, and after six days the hyphae were stained with CAP and PI and checked using CLSM (2.10.3.1.3). Microscopy results showed a strong and specific labelling of the phialides with CAP confirming the presence of chitosan in these structures and absence of the deacetylated chitin (chitosan) in other parts of the hyphae (Figure 7.10).

In order to confirm these results, the experiment was repeated with *E. festucae* strain E2368 which produce more conidia and has a higher frequency of phialide production. *E. festucae* E2368 conidia were grown on WA media and labelled with CAP and PI (2.10.3.1.3). Again, CAP specifically labelled just the phialides and not the other cells that comprised the mycelial network, indicating the presence of chitosan in the cell wall of phialides of *E. festucae* E2368 hyphae as well (Figure 7.11).

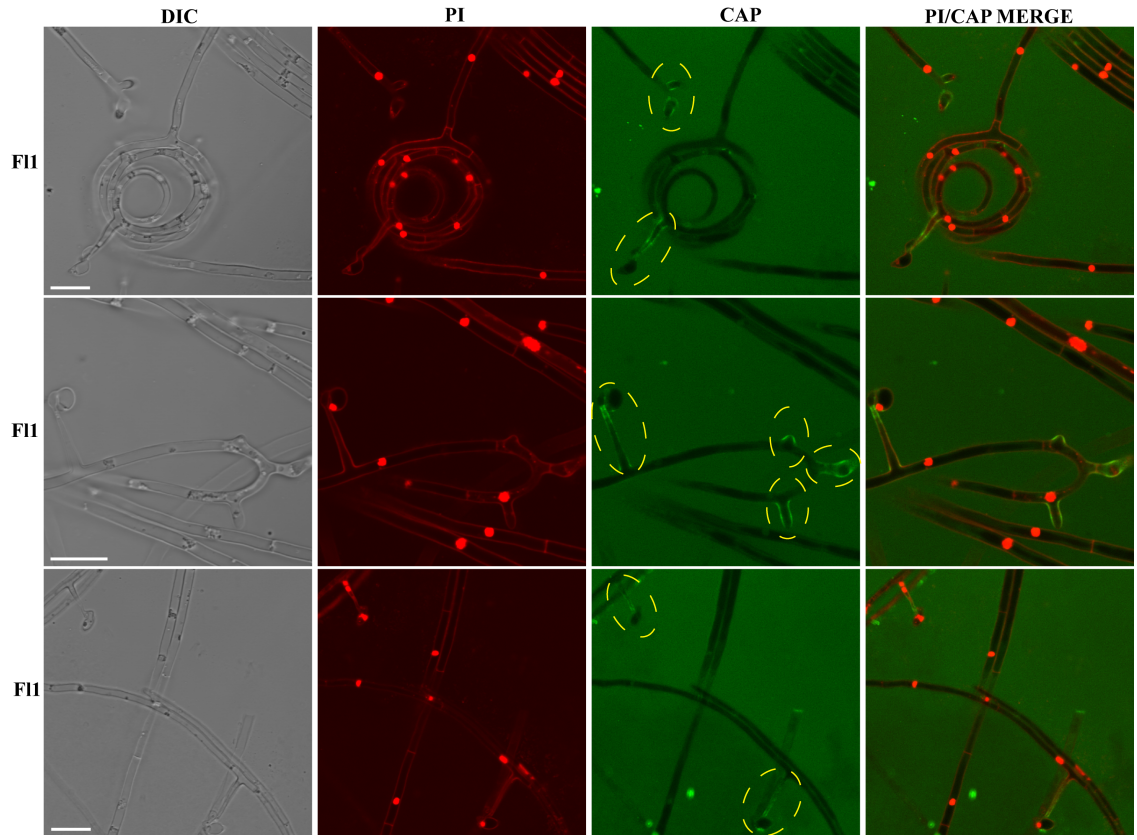


Figure 7.10. *E. festucae* Fl1 grown in axenic culture labelled with Chitosan Affinity Protein (CAP). Fungal nuclei labelled with propidium iodide (PI) in red pseudocolour (red dots). Only fungal phialides labelled with CAP in green pseudocolour. Yellow circles identify fungal phialides. Images were generated by maximum intensity projection of confocal z-stacks. Bar=10 µm.

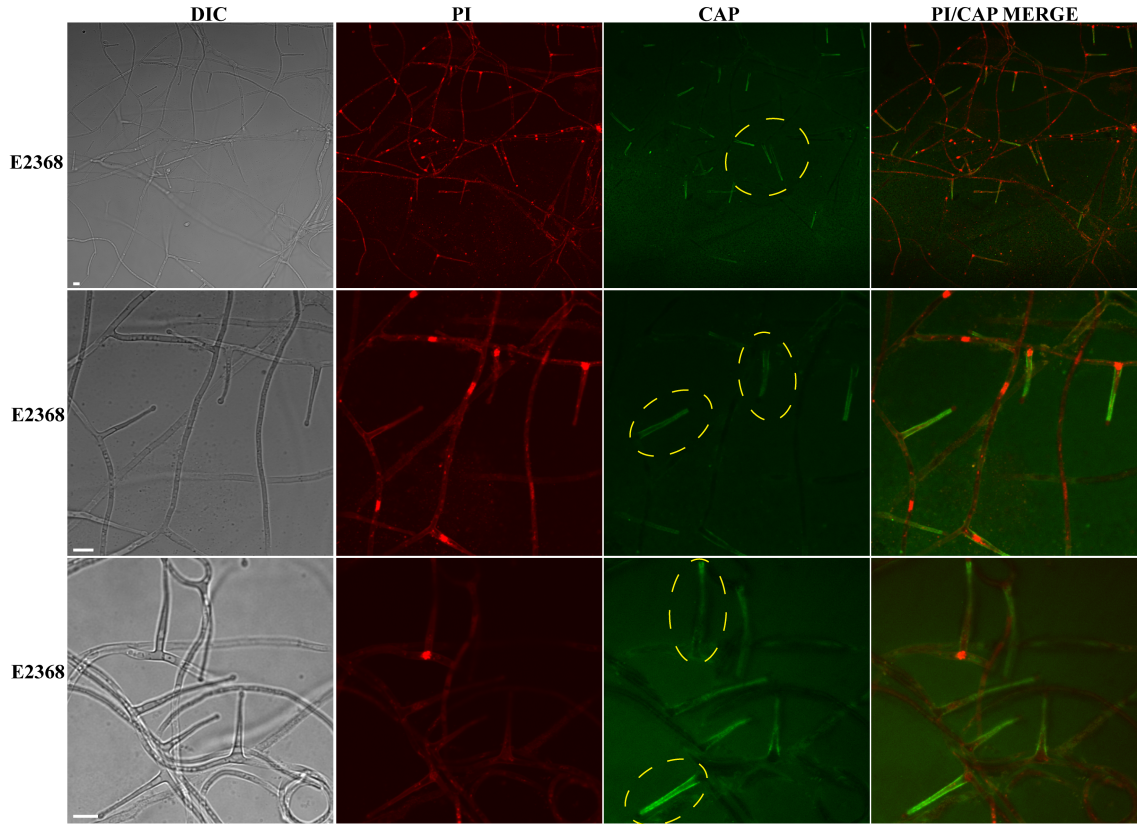


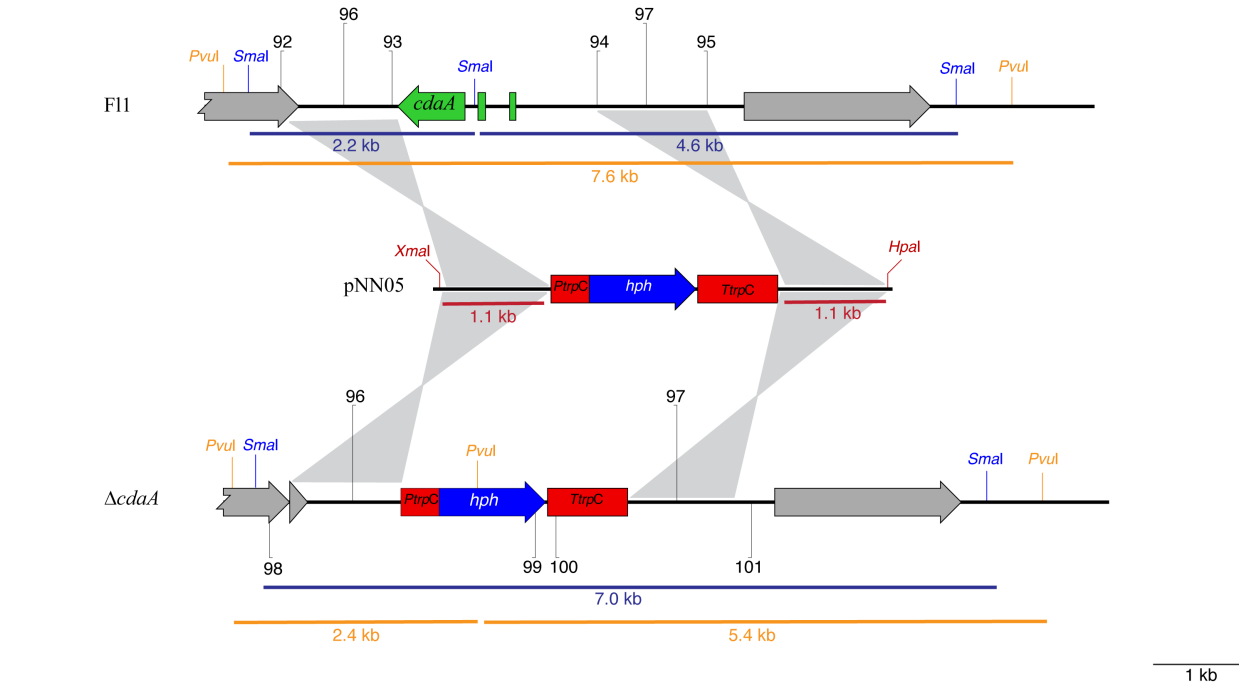
Figure 7.11. *E. festucae* E2368 grown in axenic culture labelled with Chitosan Affinity Protein (CAP). Fungal nuclei labelled with propidium iodide (PI) in red pseudocolour (red dots). Only fungal phialides labelled with CAP in green pseudocolour. Yellow circles identify fungal phialides. Images were generated by maximum intensity projection of confocal z-stacks. Bar=5 µm.

7.5. Functional analysis of the chitin deacetylase gene

7.5.1. Generation of Δ*cdaA* mutant

To test whether *cdaA* has a role in hyphal cell wall chitin modification and the subsequent suppression of a host defence in the symbiotic interaction of *E. festucae* with *L. perenne*, a deletion mutant of *cdaA* was generated by targeted replacement of *cdaA* with a hygromycin B phosphotransferase (*hph*) cassette (Figure 7.12 A). Protoplasts of *E. festucae* Fl1 (2.8.4.1) were transformed with an *Xma*I/*Hpa*I digested linear fragment of pNN05 (Appendix 10) and screened for growth on PD agar medium (2.8.4.2) containing hygromycin. One hundred and fifty-six Hyg^R colonies were picked and sub-cultured three times to confirm transformant stability and to ensure they were homokaryons (Young *et al.*, 2005). Crude genomic DNA was isolated from each transformant (2.4.2) and screened by PCR (2.6.1) for absence of a wild-type fragment using primers NN96 and NN97. Three

putative Δ*cdaA* candidates were identified from this screen. Further PCR screening using primer sets that amplify the left (NN98/NN99) and right (NN100/NN101) flanks of the *hph* cassette confirmed that all three were Δ*cdaA* candidates. High quality genomic DNA (2.4.3) was isolated from all of these transformants and digested with either *Sma*I or *Pvu*I before gel separation and subsequent Southern blotting. Probing of these blots with a DIG-labelled linear fragment of pNN05 confirmed that all three candidates were 'clean' single copy replacement mutants (Figure 7.12 B).



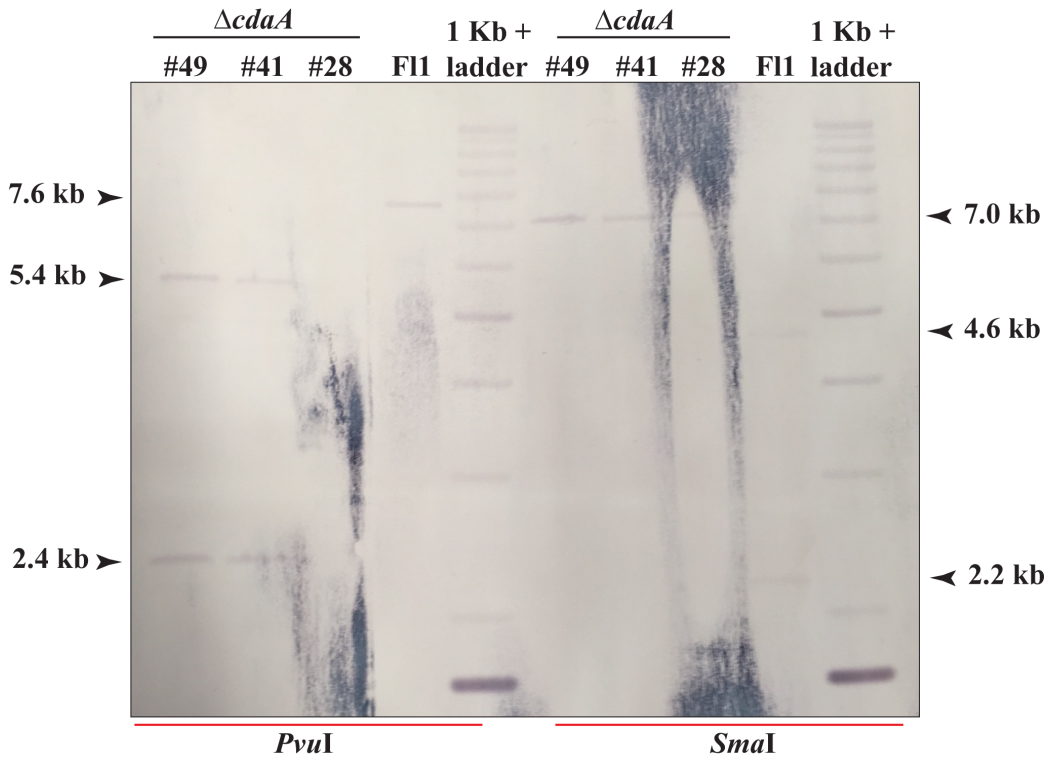


Figure 7.12. Strategy for deletion of *E. festucae cdaA* and confirmation by Southern analysis. (A) Physical map of the *cdaA* wild-type genomic locus, linear insert of the *cdaA* replacement construct, pNN05, showing restriction enzyme sites for *Xma*I and *Hpa*I. Grey shading indicates regions of recombination. Numbers indicate the PCR primers used for Gibson assembly and

knockout screening. (B) and (C) NBT/BCIP stained Southern blot of *Sma*I (B) and *Pvu*I (C) digests (1 µg) of *E. festucae* wild-type (Fl1) Δ*cdaA*#28 (PN3200), Δ*cdaA*#41 (PN3201), and Δ*cdaA*#49 (PN3203), strains probed with digoxigenin (DIG)-11-dUTP labelled linear insert of pNN5 amplified with primers NN92/NN95.

7.5.2. Δ*cdaA* culture phenotype

Cultures of *E. festucae* wild-type and three independent Δ*cdaA* mutant strains, Δ*cdaA*#28 Δ*cdaA*#41 and Δ*cdaA*#49, were grown on PD agar or WA to check if there were any differences in colony growth or fungal morphology (2.10.1). Radial growth and morphology of Δ*cdaA* was indistinguishable from the wild-type strain (Figure 7.13A). Epi-fluorescence light microscopy analysis of cultures grown on WA and stained with CFW showed that Δ*cdaA* forms hyphal bundles, undergoes hyphal cell-cell fusion and forms coils from which conidiophores arise, each with a morphology and frequency similar to wild-type (Figure 7.13 B-E).

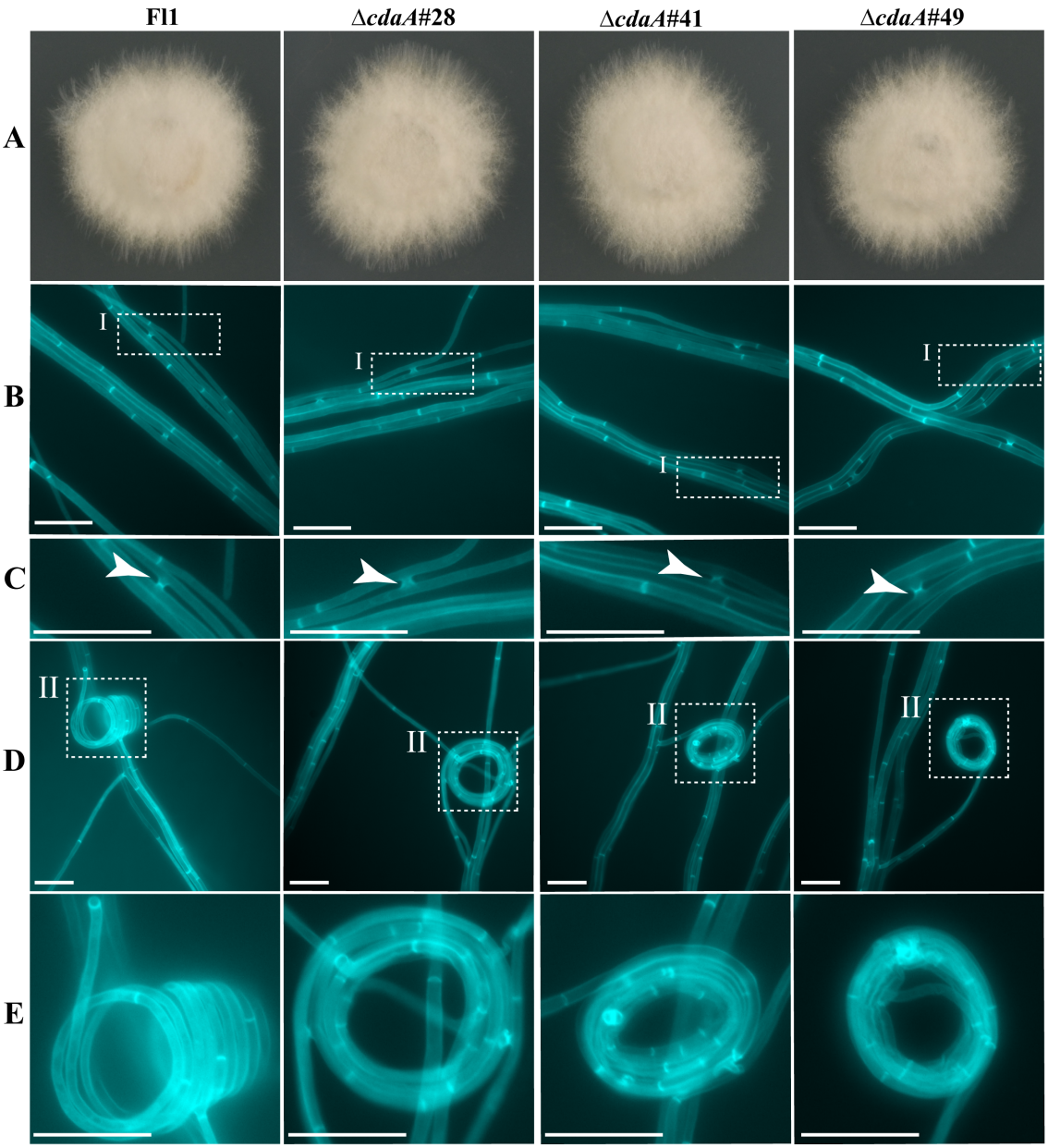


Figure 7.13. Culture phenotype of wild-type and Δ*cdaA* mutant strains. (A) Colony morphology of wild type (Fl1) and Δ*cdaA* strains grown on 2.4% PD agar at 22°C for 7 days. (B) and (D) Fluorescent images captured by widefield microscopy of hyphae grown for 7 days on water agar and stained with Calcofluor white. Bar=20 µm. (C) and (E) Higher magnification of (I) and (II) indicating fusion points and coiled structures respectively. White arrowheads identify hyphal fusion points. Bar=20 µm.

7.5.4. Δ*cdaA* symbiotic interaction phenotype

To determine whether *cdaA* is required for the symbiotic interaction of *E. festucae* with its host, 7-day old seedlings of *L. perenne*, were inoculated with WT and three independent mutant strains, Δ*cdaA*#28, Δ*cdaA*#41 and Δ*cdaA*#49 and grown under controlled environment conditions (2.8.3.2). eight to twelve weeks after inoculation, single tillers were immunoblotted to check for fungal infection of the plants (2.3.8.3). Analysis of the whole plant phenotype in plants infected with the Δ*cdaA* mutants and WT showed a decrease in the average height of the tillers from plants infected with the Δ*cdaA* mutant strains which was only significant for plants infected with *cdaA*#49. Overall the plants infected with the mutants showed a mild stunting phenotype. There was no significant difference in number of the tillers between plants infected with WT and the Δ*cdaA* mutant strains observed (Figure 7.14).

To check the cellular phenotype, plant sheath samples were fixed with ethanol and stained with aniline blue, WGA-AF488 and PI (2.10.2) and examined by CLSM. The morphology and growth pattern of the Δ*cdaA* mutants *in planta* was significantly different from that of WT. Plants infected with mutant strains showed a breakdown in the symbiosis with abnormal hyphal growth in intercellular spaces. Swollen and branched hyphal structures were present. These commonly formed dense and convoluted structures. Interestingly, the inter-septal distances also appeared altered in the mutants and were qualitatively smaller than in the WT (Figure 7.15). The presence of WGA-AF488 labeled patches in endophytic hyphae in samples infected with Δ*cdaA* mutant strains suggested that the cell wall structure and composition was affected by deletion of the *cdaA* gene with increased presence of chitin. (Figure 7.16).

To confirm that deleting the *cdaA* affected fungal cell wall composition, plant sheath samples were fixed with ethanol and stained with OGA488 and PI before being examined by CLSM (2.10.3.2.1). Endophytic hyphae in both plants infected with WT and Δ*cdaA* mutant strains, labelled with CAP (Figure 7.17A-B). However not all cell walls were robustly stained with this probe (Figure 7.17 C-D). This may indicate an absence of chitosan or reflect infiltration difficulties for deeper positioned hyphae.

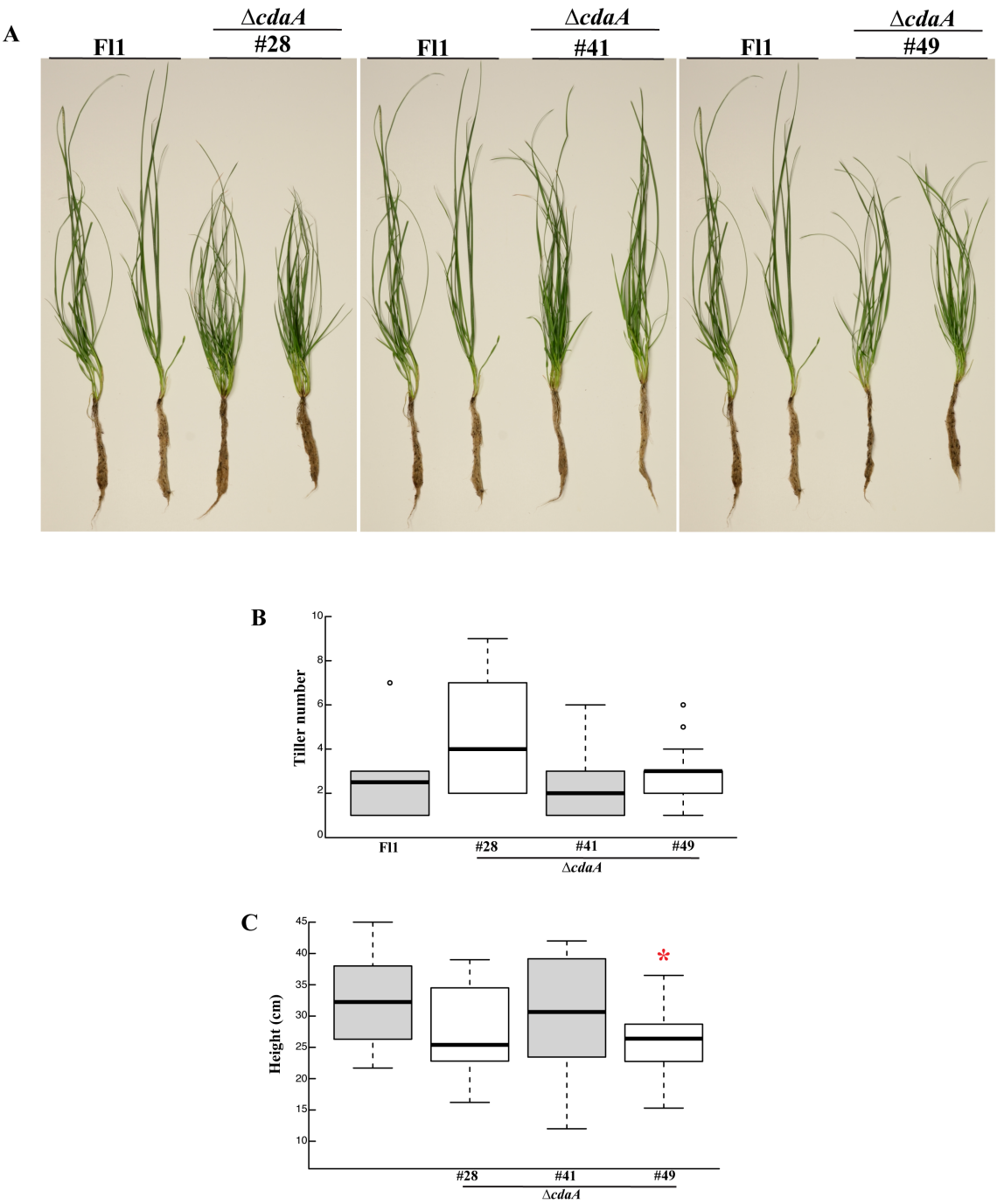


Figure 7.14. Plant phenotype of *Lolium perenne* infected with *E. festucae* wild-type and Δ*cdaA* mutant strains. (A) Whole plant interaction phenotype of *L. perenne* plants infected with *E. festucae* strains 10 weeks after inoculation. (B) and (C) Box plots of tiller number and tiller height of *L. perenne* plants infected with *E. festucae* strains. **P* <0.05 determined by Student's *t*-test (two-tailed, n≥10). Outliers are indicated by circles. Significant difference is indicated by red asterisk.

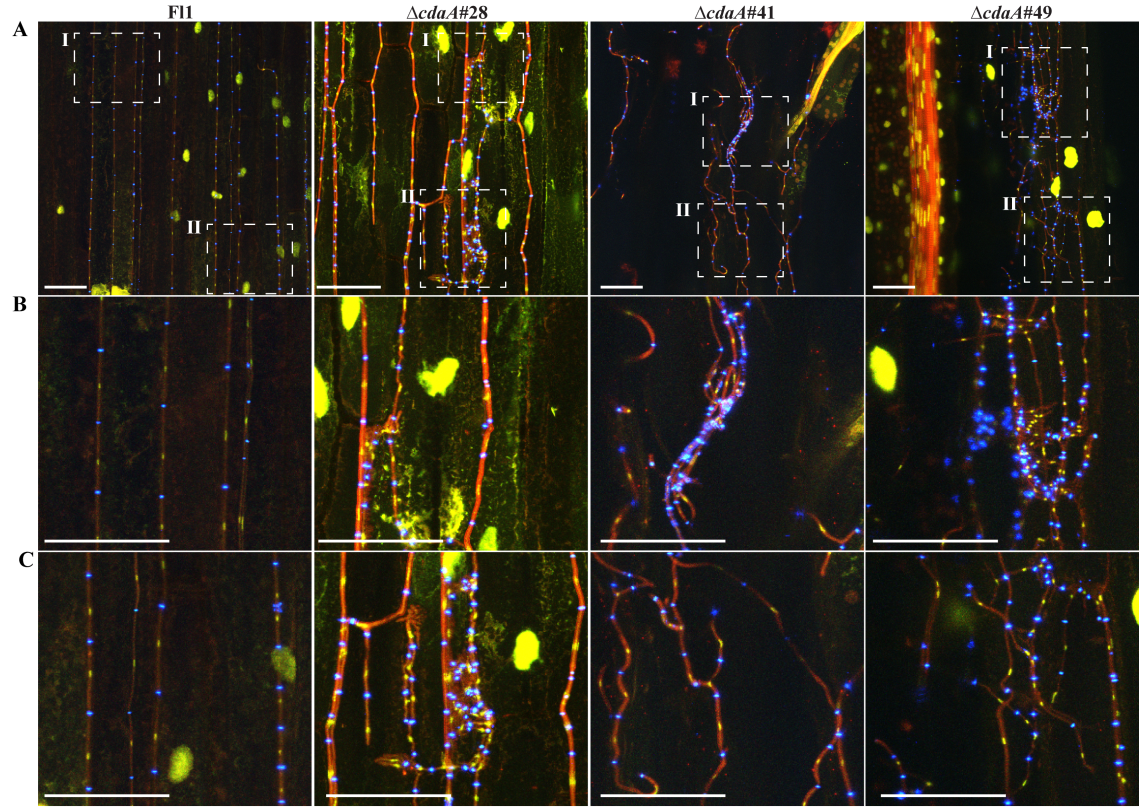


Figure 7.15. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*cdaA* mutant strains. (A) Confocal depth series images of longitudinal sections of *L. perenne* leaf sheath tissue infected with wild-type (Fl1) and Δ*cdaA* strains 10 weeks post inoculation, showing hyphae stained with aniline blue (red pseudocolour), chitin in cell wall septa with WGA-AF488 (blue pseudocolour), and fungal and plant nuclei with propidium iodide (yellow pseudocolour). Bar=50 µm. (B) and (C) Higher magnification of (I) and (II) showing normal hyphal compartment in wild type, abnormal growth pattern of fungal hyphae and increasing of hyphal branching in Δ*cdaA* mutant strains respectively. Images were generated by maximum intensity projection of a 10× 1.2 µm confocal z-stack. Bar=50 µm. The analyses were conducted at least in three biological replicants.

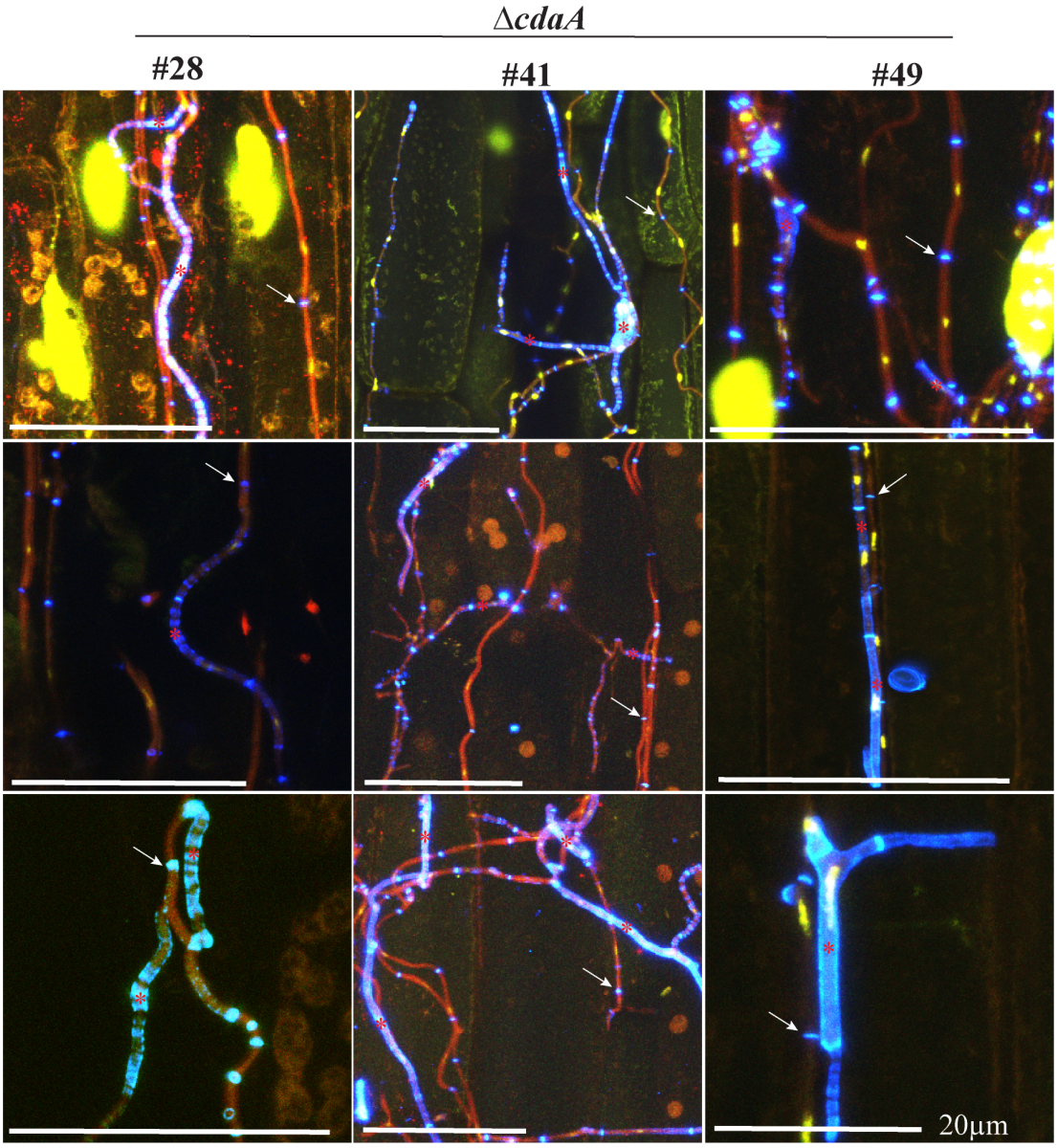


Figure 7.16. Cell wall changes in endophytic hyphae of sheath plant samples infected with Δ*cdaA* mutant strains. Large patches of chitin stained with WGA-Alexafluor-488 shows in blue pseudocolour suggesting a change in fungal cell wall composition. White arrows identify fungal septa labelled with WGA-AF488. Images were generated by maximum intensity projection of confocal z-stacks. Bar=50 µm.

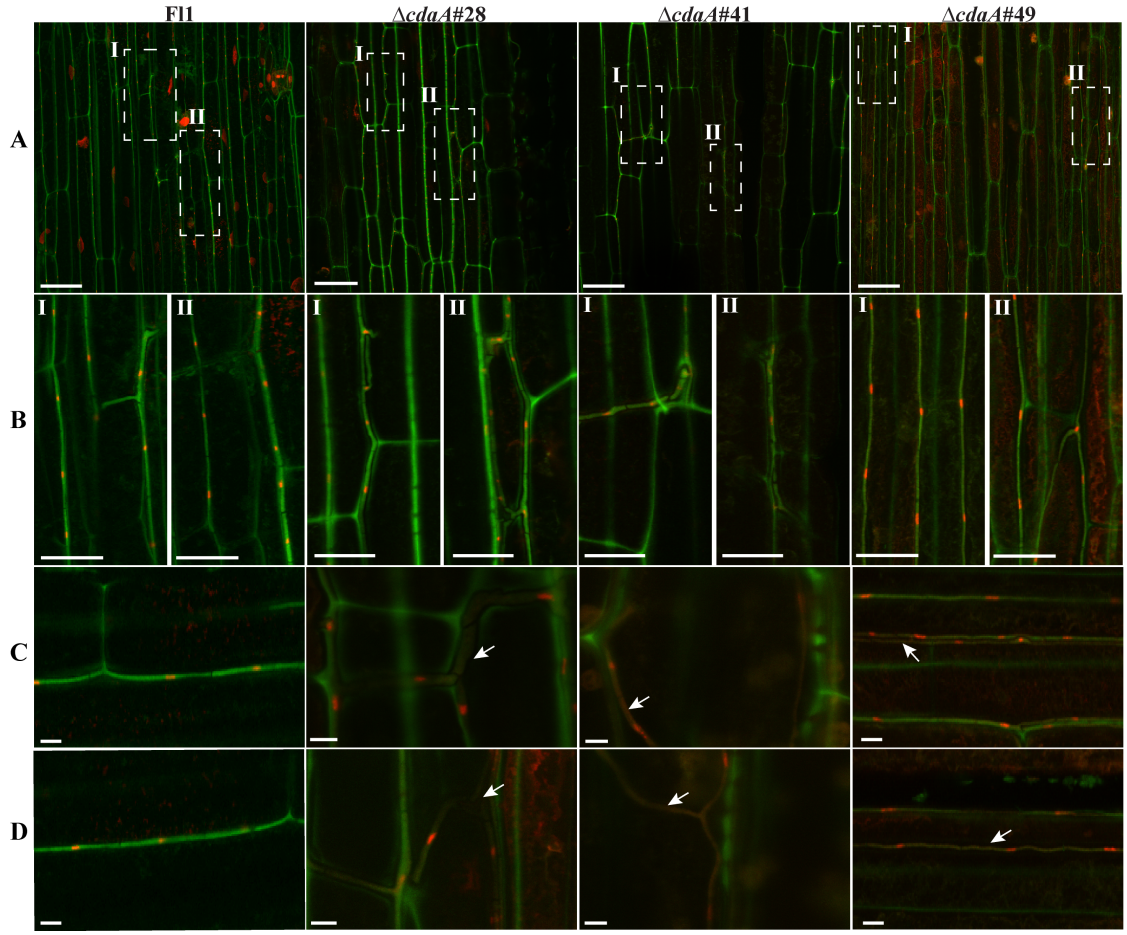


Figure 7.17. *In planta* cellular phenotype of *L. perenne* infected with *E. festucae* wild-type and Δ*cdaA* mutant strains. (A) Confocal depth series images of longitudinal sections of *L. perenne* leaf sheath tissue infected with wild type (Fl1) and Δ*cdaA* strains 10 weeks post inoculation, showing hyphae stained with OGA-488 (green pseudocolour), and fungal and plant nuclei with propidium iodide (red pseudocolour) Bar=50 µm. (B) Higher magnification of (I) and (II) indicating the labelled hyphae. Bar= 20 µm (C) and (D) Unlabelled hyphae in plants infected with Δ*cdaA* strains compared to those infected with WT. White arrows identify unlabelled hyphae. Bar=5 µm. Images were generated by maximum intensity projection of a 10× 1.2 µm confocal z-stacks in (A) and (B). Images were generated by maximum intensity projection of a 2× 1.2 µm confocal z-stacks in (C) and (D).

7.5.4. *cdaB* and *cdaC* gene expression level in Δ*cdaA* mutant strains *in planta*

To investigate if deletion of the *cdaA* gene had any effect on the expression levels of the other *E. festucae* Fl1 *cda* genes, *cdaB* and *cdaC*, RNA was extracted from pseudostem plant samples infected with WT and Δ*cdaA* mutant strains and used to generate cDNA (2.4.4 and 2.4.5). One µl of the cDNA was used as the template for a 40-cycle RT-qPCR reaction with primer pairs designed and targeted to *cdaB* and *cdaC* exons (2.6.4.1). RT-qPCR data were normalised against the expression of the 40S ribosomal protein S22 (*S22*) and elongation factor-2 (*EF-2*). Analysis by RT-qPCR revealed that the expression level of *cdaB* in the Δ*cdaA* mutant strains did not change significantly when compared to WT. However, *cdaB* expression level in Δ*cdaA*#41 mutant strains was significantly different compared to WT (Figure 7.18). On the other hand, the expression level of *cdaC* was below the detection level in both Δ*cdaA* mutants and WT.

Standard curves were generated for *cdaB* and *cdaC* genes using primer pairs NN135, NN136 and NN137, NN138 respectively (2.6.4.2). The primer efficiencies were found to be within the range of 1.9 to 2 (Appendix 19).

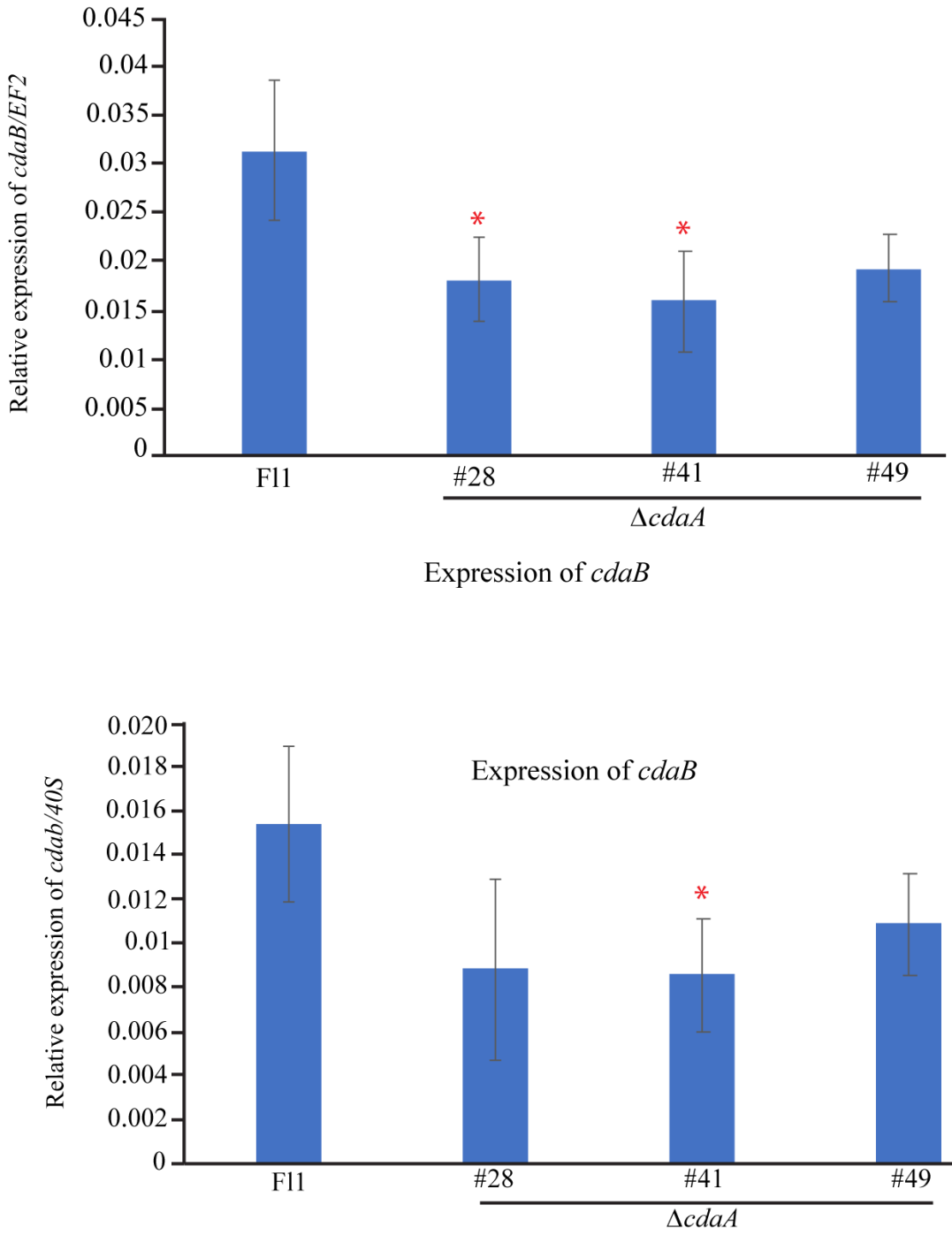


Figure 7.18. Expression analysis of *cdaB*. Expression level of *cdaB* in wild type (Fl1) and Δ*cdaA* mutant strains. RT-qPCR data were normalised against the expression of the elongation factor-2 (*EF-2*) and 40S ribosomal protein S22 (*S22*). Error bars represent ±SEM (n=3). *P<0.05 (two-tailed-ttest).

Chapter 8. Discussion

For successful infection and plant tissue colonisation, the invading fungi need to be undetectable to avoid the host immune response. Besides secreting fungal effectors to sequester chitin oligomers, chitin can be modified into chitosan thereby remodelling the fungal cell wall of the endophyte to initiate and establish symbiosis. This study shows that chitin is abundant in the cell walls of *E. festucae* hyphae growing in axenic culture but is restricted to septa of endophytic hyphae. Instead the cell walls of endophytic hyphae contain chitosan. Exit of endophytic hyphae from the host leaf to form an epiphyllous hyphal net on the surface of the leaf results in restoration of chitin in the cell wall. Chitin deacetylases are responsible for the conversion of chitin to chitosan. Functional analysis of one of the chitin deacetylase genes in the *E. festucae* Fl1 genome, *cdaA*, showed that this gene is required for establishing a symbiotic interaction between *E. festucae* and its plant host and is essential for remodelling the cell wall of endophytic hyphae.

Lectin binding, fluorescence labelling and imaging techniques revealed that there is a dramatic change in cell wall composition between endophytic and epiphyllous hyphae in *L. perenne* seedlings infected with *E. festucae* Fl1. Endophytic hyphae undergo proliferative tip growth in the leaf cell division zones but when they become attached to cells in the leaf expansion zone they switch to intercalary growth, a pattern of growth maintained in mature leaves. The chitin binding lectin WGA-AF488 (Robin *et al.*, 1986) labelled the entire fungal cell wall and septa in epiphyllous hyphae while only the septa in endophytic hyphae were labelled. In contrast the hyphal cell walls of endophytic hyphae are fully demarcated with aniline blue, which binds β-glucan. This suggests that chitin is either masked or modified in endophytic hyphae in the early stage of the infection in young seedlings. Endophytic hyphae in both the young rapidly growing and mature tissues of the leaf maintain this pattern of cell wall labelling. Besides endophytic growth, *E. festucae* also can grow epiphytically on aerial plant tissues by emerging from epidermal cells in the lower part of the leaf expansion zone through the formation of a unique expressorium structure (Christensen *et al.*, 1997; Tanaka *et al.*, 2006; Eaton *et al.*, 2010; Becker *et al.*, 2016; Christensen & Voissey, 2007; Scott *et al.*, 2012). Fluorophore labelling of hyphae immediately below the leaf cuticle of mature leaves showed the same fluorescence distribution as infected seedlings. Cell wall remodelling commences soon after the hyphae emerge on the surface of the leaf; usually after two to four cell divisions. At this stage the entire cell wall and septa of epiphyllous hyphae are labelled with WGA-AF488 whereas only the septa of endophytic hyphae label. These results confirm the

earlier observations made by (Becker *et al.*, 2016) that there is major remodelling of the hyphal cell wall of *E. festucae* upon exiting the leaf.

Further evidence of cell wall remodelling was observed through the labelling of hyphae in leaves of basal tissue and developing leaves with chitosan specific probes Chitosan Affinity Protein (CAP); which comprises a fusion of the functionally inactive *Bacillus* chitosanase to GFP, (Nampally *et al.*, 2012), and OGA-488, an oligogalacturonide coupled to AF488 (Mravec *et al.*, 2014). These engineered probes indicate that the cell walls of endophytic hyphae have an abundance of chitosan instead of chitin. Consistent with this observation is an absence of chitosan in the cell walls of epiphytic hyphae. Remodelling of chitin in the cell wall of endophytic and epiphyllous hyphae was also confirmed by staining infected leaves with a chitin specific probe, Chitin Binding Protein (CBP) developed by (Fuenzalida *et al.*, 2014) from the chitin binding domain of a *Bacillus lichenformis* chitinase fused to GFP. Using this probe, the entire cell wall and septa of epiphyllous hyphae on the plant surface labelled with CBP, while only septa in endophytic hyphae labelled with this biosensor. This confirms that chitin is abundant in the cell wall of epiphyllous hyphae but is only present in the septa of endophytic hyphae. Variable accessibility of the fungal cell wall oligosaccharides and deacetylation of chitin into chitosan has been observed in other fungal pathogens during infection processes. An analysis of fungal surfaces in broad bean and wheat rust fungi *Uromyces faba* and *Puccinia graminis* as well as maize anthracnose fungus, *Colletotricum graminicola*, indicated that chitin was readily accessible on the cell wall surface of germ tubes and appressoria, which are normally formed on the leaf surface, as these structures stained with FITC-WGA, a chitin biosensor, but not with Texas red labelled polyclonal antibodies to chitosan. In contrast, other tissues and structures such as substromatal vesicles and infection hyphae in rust fungi and infection vesicles of the maize anthracnose fungus were only labelled by the chitosan biosensor. These findings are consistent with the conversion of chitin to chitosan when the pathogens penetrate the plant leaf tissues (El Gueddari *et al.*, 2002). The hyphal cell wall polysaccharide composition in rice blast fungus *M. oryzae* has been analysed in great detail using fluorophore-labelled monoclonal antibodies specific to α-1,3-glucan, β-1,3-glucan, and fluorophore-labelled lectins to chitin (WGA-AF350) and mannan (FITC conjugated Concanavalin A), and the fluorescent dye EosinY to detect chitosan. The results revealed that chitin, chitosan and β-1,3-glucan are detectable on germ tubes and appressoria whereas only α-1,3-glucan and chitosan are labelled in infectious hyphae showing that α-1,3-glucan masks chitin and β-1,3-glucan to protect the fungal cell wall from access by cell wall degrading

enzymes produced by the plant. Deletion of the single gene *MoAGS*1 (*ags*1 strain) encoding α-1,3-glucan, resulted in a reduction in plant pathogenicity; rice leaves inoculated with *ags*1 did not form any lesions, whereas inoculation with wild type or *ags*1+*MoAGS*1 induced lesions, suggesting that α-1,3-glucan is indispensable for live host plant infection by *M. oryzae* (Fujikawa *et al.*, 2009; Fujikawa *et al.*, 2012). However, analysis of the *E. festucae* Fl1 genome revealed that it does not contain any α-1,3-glucan synthase gene (Becker *et al.*, 2015) so masking chitin and β-1,3-glucan does not appear to be a mechanism employed by *E. festucae* to avoid a host defence response. In contrast, deacetylation of chitin to chitosan could be an important strategy used by *E. festucae* to avoid a host defence response, as observed in some other plant pathogens.

Hyphal cell wall labelling with different chitin and chitosan biosensors in axenic culture indicated that fungal cell wall composition is different during vegetative growth compared to *in planta* and has the same composition as epiphytic hyphae. The hyphal cell wall and septa labelled with CBP and WGA-AF488 suggesting that the cell wall contains chitin and not chitosan. However, phialides, which are produced when hyphae differentiate during asexual development, strongly labelled with CAP showing that the cell walls of these structures are predominantly chitosan rather than chitin. The conversion of chitin to chitosan in these structures would suggest that a chitin deacetylase (*cda*) gene is specifically expressed or activated during phialide development. In nature these form in the mycelial mat that forms stromata around the host inflorescence when *E. festucae* initiates the sexual life cycle. The formation of chitosan and the involvement of chitin deacetylases was first described in the *S. cerevisiae* ascospore cell wall. Deletion of the two redundant chitin deacetylase genes *cda1* and *cda2* resulted in complete loss of chitosan resulting in disorganisation of this structure and an increase in cell wall permeability. As a result, the ascospore cell wall became abnormally susceptible to stress conditions and chemicals. While ascospore germination was unaffected, further development was impaired, suggesting that chitin deacetylases have a role in the later rather than the earlier stages of ascospore development. Mutant strains of the single chitin deacetylase gene found in *Ashbya gossypii* were defective in sporulation suggesting that this gene is required for fungal development. It has been shown that chitosan is necessary for cell wall integrity in *Cryptococcus neoformans as deletion of three out of the four chitin deacetylase genes in this* Basidiomycete fungus resulted in *defects in chitosan synthesis, reduced pathogenicity and leakage of melanin from the cell wall (Christodoulidou et al., 1996; Christodoulidou et al., 1999; Mishra et al., 1997; Suda et*

al., 2009; Lickfeld & Schmitz, 2012; Baker et al., 2007; Baker et al., 2011; Banks et al., 2005). *In situ* double staining of germinating urediniospores of the wheat stem rust fungus *P. graminis f sp*. *tritici* with the chitin-specific lectin WGA coupled to Texas Red and the chitosan specific CAP showed that chitin could be detected in germ tubes and appressoria as well as at the tip of infection hyphae. Conversely, chitosan was only detected on the substomatal vesicles and weakly on the infection hyphae. These distributions are indicative of cell wall remodelling and conversion of chitin to chitosan. There likely function is to prevent degradation of the cell wall and consequent activation of a plant immune response (Nampally *et al.*, 2012). In some recent work done on other fungi it has been shown that chitin to chitosan conversion is important for septa formation and conidiation in the filamentous fungi *N. crassa* and the house fly pathogenic fungus *Entomophthora schizophorae* respectively (Mravec *et al.*, 2014). Experiments conducted on the rice blast fungus *M. oryzae* showed that chitosan is a component of the fungal cell wall during vegetative growth. Immune detection using monoclonal and polyclonal anti-chitosan antibodies showed that chitosan was localised to the germ tube and appressorium. Using a chitosan specific probe OGA-488 to probe appressorium development in *M. oryzae* revealed that the entire germ tube and appressorium were labelled with the chitosan probe. A double deletion mutant of two chitin deacetylase genes (*CBP* and *CDA1*) (out of three genes), identified by transcriptome analysis to have a highest expression level during appressorium formation, resulted in both delayed and defective appressorium (Geoghegan & Gurr, 2016). However, the single deletion mutants had only a mild phenotype suggesting that these two genes are functionally redundant. In another study (Geoghegan & Gurr, 2017) found that *M. oryzae* fungal cell walls contain chitosan during fungal vegetative growth and two chitin deacetylase genes (*CDA1* and *CDA4*) are responsible for chitin deacetylation in mature hyphae and colony margins, respectively.

Recent work has shown that conversion of chitin to chitosan is important for a number of developmental processes including formation of septa in *N. crassa* and conidiophore in the pathogenic fungus *Entomophthora schizophorae*.

Chitin deacetylase genes are found in all fungal genomes (Ruiz-Herrera & Ortiz-Castellanos, 2010) but the number is quite variable. *E. festucae* strain Fl1 is no exception containing three genes encoding chitin deacetylases with the distinct signature of a polysaccharide/chitin deacetylase domain. Of the three, only CdaA contains an N-terminal signal peptide suggesting that this enzyme is secreted into the apoplast where it could modify the cell wall. Conversion of chitin to chitosan may be a mechanism for *E.*

festucae to avoid elicitation of a host defence response allowing establishment of a symbiotic interaction. Chitin deacetylase genes and their corresponding enzymes have been identified in other fungal pathogens. In the rice blast fungus *M. oryzae* ten chitin deacetylase genes have been identified containing the chitin deacetylase domain. With one exception they all encode proteins with an N-terminal signal peptide (MGG_04704). Four of these proteins also contain a chitin binding domain and three of them contain transmembrane domains (Geoghegan & Gurr, 2016). A tBLASTn analysis using the *M. oryzae* protein sequence as a query against the *E. festucae* Fl1 genome revealed that *cdaC*, has the greatest homology to 9 of the 10 *M. oryzae* chitin deacetylase genes, whereas *cdaA* and *cdaB* were the second and third strongest hits. The same result was obtained for a BLAST analysis against the *N. crassa* and *F. graminearum* genomes. Although gene and protein structure analysis of *cdaB* in http://www.endophyte.uky.edu/ and https://www.ebi.ac.uk/interpro based on M3 gene models showed that the product of this gene does not have an N-terminal signal peptide, chitin binding or transmembrane domain, an alternative gene model (FGENESH) suggests that *cdaB* may have a different gene structure with the encoded predicted protein containing an N-terminal signal peptide and a transmembrane domain. These features raise the possibility that *cdaB* may have a role in chitin deacetylation of the fungal cell wall. Chitin deacetylase activity has been identified in other fungal pathogens as well. In the fungal pathogen *Colletotrichum lindematinaum* chitin deacetylase activity is dependent on a zinc-binding Asp-His-His triad and key active sites including four catalytic base residues an Asp, a His which interacts with an Arg, and an Asp respectively (Blair *et al.*, 2006; Hekmat *et al.*, 2003). An alignment of protein sequences of CdaA, CdaB and CdaC from *E. festucae* Fl1 and *M. oryzae*, *N. crassa* and *F. graminearum* chitin deacetylase proteins showed that they all share conserved active site residues and zinc binding sites and are therefore likely to have chitin deacetylase activity like those chitin deacetylase protein from *M. oryzae* and *C. lindematianum* (Blair *et al.*, 2006; Hekmat *et al.*, 2003; Geoghegan & Gurr, 2016).
Although amino acid sequence alignment showed that *cdaC* is the most similar gene to nine of the ten *M. oryzae* chitin deacetylase genes, expression levels of this gene both in culture and *in planta* are very low. In contrast *cdaA* and *cdaB* are expressed both in culture and *in planta* with the former showing a significant increase *in planta* compared to in culture suggesting these genes are more likely to have active roles in chitin deacetylation. Based on these transcriptome analyses *cdaA* is the strongest candidate for having a role in fungal cell wall remodelling during the symbiotic interaction. While RNA seq data

analysis shows a decrease in the expression level of *cdaA* in three symbiotic mutants, the only significant change is for the *sakA* mutant. In contrast, expression of *cdaB* increases in the three symbiotic mutants, but again the change is only significant for the Δ*sakA* mutant. *cdaC* does not show any changes in these three symbiotic mutants (Eaton *et al.*, 2015). Taken together *cdaA* was identified as the best candidate to have a role in the symbiotic interaction between *E. festucae* Fl1 and its host plant. In the rust fungus *U. faba* chitin deacetylase activity was observed to change at different developmental stages. Radiometric and gel electrophoretic analysis of crude extracts and extracellular washing fluids showed that the enzyme activity was increased during formation of intercellular infection structures, substomatal vesicles, infection hyphae and haustorial mother cells, a result consistent with the demonstration that chitin is converted to chitosan during the fungal infection (Deising & Siegrist, 1995). Four potential polysaccharide deacetylation genes, *CDA1*, *CDA2*, *CDA3* and *FPD1* have been identified in the opportunistic human fungal pathogen *C. neoformans*. EosinY staining of the fungal cell wall showed that it readily binds to fungal cell walls of the wild type whereas the triple gene deletion mutant (*CDA1*/*CDA2*/*CDA3*) or the quadruple gene deletion mutant (*CDA1*/*CDA2*/*CDA3*/*FPD1*) lost the ability to bind this chitosan stain. Further deletion analysis showed that a single WT copy of *CDA1*, *CDA2* or *CDA3* is sufficient to get chitosan labelling similar to wild type. The ability of the fungus to grow at the same temperature as the host is important for virulence in *C. neoformans*. To rule out any contribution of temperature, northern blot analysis was conducted to check expression of the chitin deacetylase genes at different temperatures. *CDA1*, *CDA2* and *CDA3* were expressed at different temperature conditions while FDP1 was expressed only at 25°C. However, it has been revealed that the triple *CDA1*/*CDA2*/*CDA3* or quadruple chitin deacetylase gene deletion mutants did not affect the fungal growth at elevated temperatures and chitosan is not vital for fungal growth (Baker *et al.*, 2007). In the plant fungal pathogen *M. oryzae*, chitin deacetylase genes have different gene expression levels during appressorium development. A RT-qPCR was performed on total RNA extracted form rice leaves inoculated with WT conidia, at early and later stages of plant inoculation. Relative quantification of expression for all ten *M. oryzae* chitin deacetylase genes revealed that *CDA3*, *CDA2* and *CBP1* are highly expressed at the early stages of the infection and during appressorium formation, while *CDA1* and *CDA6* are the most highly expressed genes during the later stages of the leaf infection (Geoghegan & Gurr, 2016).

Deletion of just *cdaA* does not have any effect on fungal morphology in axenic culture which is not surprising as this gene is preferentially expressed *in planta* compared to in culture. Chitosan specific labelling in culture with CAP showed that only phialide structures labelled with the chitosan specific probe which could be the result of specific expression of either the *cdcA* or *cdaB* gene. The presence of chitosan rather than chitin in these structures may allow for rapid expansion of the stalk as it matures to produce conidia. Surprisingly, deletion of *cdaA* in *E. festucae* had no effect on phialide formation or conidiation. One explanation for this result could be due to redundancy with *cdaB,* which is also expressed in culture. Functional redundancy between chitin deacetylase genes has been reported in other fungi. In *C. neoformans* chitosan is not vital for fungal growth and retention of just one of the four genes found in this species is sufficient to get chitosan labelling in culture. However, it has been also found that in *C. neoformans* chitosan is necessary for proper separation of mother and daughter cells as deletion of three of four chitin deacetylase genes or deletion of all four genes resulted in abnormal bud separation. Experiments done on different media to check how chitosan affects cell wall integrity during specific stress conditions showed that chitosan is essential for cell wall integrity as well. These combined results indicate that there is functional redundancy between the different chitin deacetylase genes in *C. neoformans* (Baker *et al.*, 2007). In a recent study in *M. oryzae* it was demonstrated that the chitin deacetylase gene *cbp1* encodes a product with CDA activity that has an important role in appressorium formation. It has also been found that upregulation of all other seven homologous chitin deacetylase genes may compensate for the Δ*cbp1* mutant. Deletion of another chitin deacetylase in *M. oryzae cbl1* did not have any effect on colony growth and morphology during appressorium differentiation, however the double mutant strain, Δ*cbp1/cdl1* showed a decrease in conidiation, conidial adhesion and appressorium formation, suggesting that these two genes act synergistically and redundantly. Comparing the chitosan labelling with OGA-488 in wild type and the Δ*cbp1*mutant strain showed that accumulation of chitosan in the tip of germ tubes plays an important role during appressorium formation as very little fluorescence signal was detected in the tip of the Δ*cbp1* mutant strains compared to wild type strains (Kuroki *et al.*, 2017). As discussed previously an independent research study conducted on the role of chitosan in *M. oryzae* growth showed that only 3% of conidia in Δ*cbp1* mutant strains developed appressoria compared to 80% of wild type conidia. Appressorium development was both delayed and defective in Δ*cbp1*with abnormal elongation and failing to hook at the end distal from the spores.

Single and double deletion of the other chitin deacetylase genes *cda2* and *cda3* did not lead to any detectable appressorium malfunction. However, double deletion of *cbp1* and *cda2* resulted in a more severe phenotype compared to that observed for the single deletion, suggesting that *cbp1* and *cda2* exhibit partial redundancy. In this study a unique role for chitosan was found through its requirement in germling adhesion; while the conidial germination of wild type and triple deletion strains of *cbp1*/*cda2*/*cda3* are the same, germling adhesion of the triple mutant strains was significantly lower compared to wild type. The addition of chitosan back into the system restored adhesion, confirming this novel function (Geoghegan & Gurr, 2016). An additional explanation for the lack of vegetative culture phenotype in the *E. festucae cdaA* mutant strain is functional compensation by *cdaB*. Functional redundancy has been observed in *M. oryzae*, where *cda1*, *cda4* and to lesser extent *cda5* are responsible for chitin deacetylation in vegetative hyphae in culture. This was shown by generating targeted gene deletions of *cda1* and *cda4*/*cda5* and labelling the hyphae with the chitosan specific probe OGA-488. This showed that chitosan was almost absent in hyphae grown on CM and on solid surface in Δ*cda1* and ΔΔ*cda4*/*cda5* respectively, suggesting that *cda1* and *cda4* have developmentally distinct roles. *cda1* and *cda4* are responsible for chitin deacetylation in mature hyphae and in hyphae at colony margins, respectively, and no changes were observed in the conidiation of mutant strains. It has also been demonstrated that *cda1*, *cda4* and *cda5* do not have an effect on optimal fungal vegetative growth under stress conditions. However, the cell wall of Δ*cda1* is more resistant to the a mixture of chitinases, glucanases and proteolytic enzymes showing an alteration of the fungal cell wall (Geoghegan & Gurr, 2017). Confirmation of *cdaA* and *cdaB* redundancy in *E. festucae* could be examined by using a double deletion analysis. The generation of such a mutant would be a future experimental challenge. Given that only phialide structures were labelled with CAP in *E. festucae*, the localisation of chitin deacetylase proteins in culture could also be examined. This would provide useful insight into their specific roles in fungal growth and development. Accordingly, transcriptome analysis of chitin deacetylase gene expression levels in culture would also serve as a strong indicator for which genes responsible for chitosan production in culture and specifically in phialide structures.

Although deletion of *cdaA* did not result in a whole plant interaction phenotype, there was a breakdown in symbiosis with a dramatic cellular phenotype. This confirms the importance of the balance between chitin and chitosan for maintaining a healthy

symbiotic interaction between Epichloë and its host plant. Unlike wild type, which undergoes restrictive intercalary growth *in planta* and grows parallel to the leaf axis with chitin restricted to septa in endophytic hyphae, the *cdaA* mutant has an abnormal growth pattern with an increase in hyphal branching, resulting in more convoluted structures and hyphal compartments that were different in length to the wild type. Strikingly, in the *cdaA* mutant chitin is accessible not only in fungal septa but also in the hyphal cell wall of endophytic hyphae where it was observed as patches along the fungal cell wall, suggesting that there is a disruption in chitin deacetylation activity and synthesis of chitosan. An increase in chitin accessibility has been reported before in other symbiotic mutants, including Δ*symB* and Δ*symC*, the STRIPAK complex mutant Δ*mobC* and both Δ*mpkA* and Δ*mkkA* (Becker *et al.,* 2015; Green *et al.,* 2017; Green *et al.,* 2016). However, the patches of chitin seen in the Δ*cdaA* mutant are the most extensive of all the symbiotic mutants examined to date. In *symB*, *symC*, *mobC*, *mpkA* and *mkkA* mutant strains, disruption of restrictive intercalary growth resulted in a severe plant phenotype characterised by stunted plants while there was no obvious whole plant interaction phenotype detected in the Δ*cdaA* mutant strains. However, a crucial difference between these mutants and the Δ*cdaA* mutant is their inability to undergo cell-cell fusion. While defective in the ability to convert chitin to chitosan the Δ*cdaA* mutant is still able to undergo cell-cell fusion. Also, there may be some functional redundancy with the other *cda* genes. Transcriptome analysis of *cdaB* and *cdaC* in plants infected with Δ*cdaA* mutant strains revealed that while *cdaC* is not expressed, consistent with the results obtained from RNA seq data analysis, *cdaB* still expresses which could result in some deacetylation of the chitin and suppression of the phenotype. Like the results observed in *C. neoformans* where deletion of just one *CDA* gene was insufficient to see defects in fungal pathogenicity a double deletion Δ*cdaA*/*cdaB* mutant may need to be generated to see a more severe phenotype.

In *M. oryzae* pathogenicity was unaffected in single Δ*cbp1*, Δ*cda2* and Δ*cda3* mutant strains and in double Δ*cda2*/*cda3* mutant strains and plants inoculated with these mutant strains had the same lesion number as wild type. Although these mutants were defective in appressorium development on artificial surfaces, appressorium development was restored in single and triple mutant strains when they were inoculated into plants as they were able to germinate and penetrate rice sheaths similar to wild type (Geoghegan & Gurr, 2016; Kamakura *et al.*, 2002). It has been also shown that while in the triple mutant strains *cda2/cbp1/cda3, chitosan was undetectable using OGA-488, appressorium*

development was restored in the presence of plant surface chemicals on leaves, suggesting that they can trigger appressorium development and therefore replace the need for chitosan (Geoghegan & Gurr, 2016). In another study conducted on three other chitin deacetylase genes in *M. oryzae*, deletion of *cda1*, *cda4* or *cda5* did not affect fungal virulence. Therefore, neither *cda1*, *cda4* or *cda5* are required for pathogenicity in *M. oryzae* (Geoghegan & Gurr, 2017).

E. festucae Fl1 has a highly beneficial mutualistic symbiosis with its host plant so chitosan may have a role as a stealth molecule to avoid chitin triggering an immune response and protecting the fungal cell wall from chitinases and proteases. These lytic enzymes lead to host immune response activation. Rather, chitosan is only a poor substrate for chitinase and it cannot act as a PAMP to trigger the host immune response. The labelling of both β-glucan and chitosan during fungal endophytic growth and their lack of labelling in epiphytic growth, suggests that fungal hyphae are a complex matrix of different components which entirely remodel during the symbiotic interaction. Other possible roles for chitosan in *E. festucae* Fl1 symbiosis interaction with plants could be to generate a positive charge on the outside of the cell wall to promote adhesion to the plant cell walls or alternatively to give the fungal cell wall a more flexible structure compared to chitin during intercalary growth. Therefore, deletion of the *cdaA* gene could lead to loss of attachment or alter the composition of the cell wall, thereby affecting intercalary growth in the mutant strains. The deactivation of chitin conversion to chitosan when the hyphae switch to epiphytic growth could have a role in expressorium formation. This was not checked in *E. festucae* Fl1 in this study, but it has been reported in symbiotic interaction mutants in genes involved in production of reactive oxygen species, *noxA*, *noxB* and *noxR* (Becker *et al.*, 2016).

In conclusion, in this study it has been shown for the first time that the chitin deacetylase gene, *cdaA*, in *E. festucae* Fl1 is required for proper hyphal growth and development of endophytic hyphae during the symbiotic interaction between this endophyte and its host plant. It has also been shown that deletion of *cdaA* results in breakdown of the symbiotic interaction suggesting it is necessary for hyphal adhesion during the intercalary growth necessary for maintenance and establishment of the symbiosis. These results suggest that chitosan can have a dual function and act more than a stealth molecule to facilitate the infection and plant colonisation as it is also important for fungal development and morphogenesis as well. As *E. festucae* Fl1 contains three chitin deacetylases further analysis of their activity and role at different fungal developmental stages will be

important in understanding their specific role for growth in axenic culture and for growth in symbiotic association with the grass host.

Chapter 9. Conclusion and future directions

The fungal cell wall is the first fungal surface, which makes connections with the environment and the host. It is a dynamic structure and plays crucial roles not only in cell viability and shape but also in maintaining cellular integrity. Fungal cell wall composition and structure is altered during fungal growth and morphogenesis as part of cell differentiation processes but also in response to the environmental conditions encountered growing within and external to a host. Chitin is one of the core components of the fungal cell wall and is well known as an elicitor of the plant immune response. For fungi to establish a stable infection or symbiosis it is that chitin in the cell wall is protected from enzymatic degradation, and release of chitin oligosaccharides that will be detected by plant receptors. While almost all the phytopathogenic fungi encounter a common plant defence system, different fungi utilise different strategies to overcome this. Secretion of LysM effector proteins to sequester chitin oligosaccharides to supress chitin-induced PTI is a well-characterised mechanism used by *C. fulvum* and other fungi to suppress host defence (de Jonge *et al.*, 2010). Alterations to the cell wall structure and composition such as deacetylation of chitin to chitosan is another mechanism used by fungi to bypass chitin triggered immunity, as chitosan is a poor chitinase substrate and a weak PTI activator.

The aim of this study was to obtain a better understanding of the molecular basis of the strategies employed by *E. festucae* to establish and maintain a symbiotic interaction with its host plant *L. perenne*. In this study seven genes encoding proteins containing LysM motifs were identified in the *E. festucae* genome. Subsequent bioinformatic approaches narrowed down the candidates to two genes, *lymA* and *lymB*, as the most likely candidates to be involved in the symbiotic interaction. This analysis was based on an analysis of RNA seq data generated for three symbiotic mutants, Δ*noxA*, Δ*proA* and Δ*sakA* and also on gene expression differences between *in planta* versus in culture. Gene structure analysis led to the identification of two chitinase genes, *chiA* and *chiB*, which are divergently transcribed from *lymA* and *lymB* respectively, suggesting that the expression of these *lysM* and *chitinase* genes is coregulated. Data obtained from RNA seq analysis and gene expression level of *chiA* and *chiB* showed the same pattern as *lymA* and *lymB,* supporting the hypothesis that *lymA* and *chiA* as well as *lymB* and *chiB* are co-expressed, and have a role in symbiosis.

Bioinformatic analysis showed that there is conserved synteny shared in both *lymA*/*chiA* and *lymB*/*chiB* loci and the two genes positioned on either side of them among other

Epichloë and Claviceps species. Although many of these gene homologues have undergone some deletions the virtual translation of these ORFs generated potentially functional proteins suggesting that these genes could be important for symbiosis establishment in other Epichloë or Claviceps species.

Consequently, reverse genetic approaches were conducted to test *lymA* and *lymB* function in the symbiotic interaction. The culture and symbiotic interaction phenotype of single deletion mutant strains of Δ*lymA* and Δ*lymB* were indistinguishable from wild type, suggesting that there might be functional redundancy between these two genes. Therefore, a double deletion of *lymA*/*lymB* was generated to check this hypothesis. Both *lymA* and *lymB* together are indispensable in culture while deletion of both genes resulted in a mild stunting host phenotype. However, plants infected with Δ*lymA/lymB* double mutant strains showed disruption in fungal intercalary growth with hyphae appearing to grow by tip growth rather than intercalary growth, which led to hyper branching and the production of convoluted hyphal structures, a pattern of growth that led to breakdown of the symbiosis. Observing this interesting cellular phenotype suggested that LysM proteins can be involved in different pathways in symbiosis regulation. To switch from tip growth to intercalary growth in expanding and differentiating tissues, hyphae need to attach to the plant cell wall and stretch following plant cell elongation. To activate intercalary growth, hyphae will need to be remodelled, so chitinase may play a role in remodelling the cell wall and lysM proteins might be required to sequester chitin oligomers during this cell wall remodelling process to prevent activation host defence response.

Fungal morphology of Δ*chiA* and Δ*chiB* mutant strains in axenic culture was indistinguishable from wild type, a result consistent with the gene expression patterns. While single deletion of *chiA* did not result in a plant interaction phenotype, single deletion of *chiB* interestingly caused a dramatic cellular phenotype with disruption in hyphal intercalary growth and formation of highly branched hyphae and convoluted structures that contained smaller hyphal compartments. Unlike the wild type where hyphae grow parallel to the leaf longitudinal axis with occasional lateral branching and fusion, in the Δ*chiB* mutant the hyphae appear to switch from restrictive intercalary growth to proliferative growth indicating that *chiB* is essential for maintenance of hyphal restrictive growth and establishment of the symbiosis. However single deletion of *chiA*

did not affect culture growth or the symbiosis interaction suggesting that there might be functional redundancy between *chiA* and other chitinase genes such as *chiB*.
LymA and LymB were both found to localise to fungal cell walls and septa, a result consistent with their proposed role to bind to chitin oligomers. Based on the key results found in this study it would be useful to generate double deletion mutants of *lymA*/*chiA* and *lymB*/*chiB* to test the impact of loss of these coordinately regulated genes on the symbiotic interaction. It would also be helpful to know if ChiA and ChiB localise to the same subcellular regions as their corresponding partner LymA and LymB products. Knowledge of their localisation will help build up a picture as to where and how these proteins function in the cell, particularly during the interaction with the host. The results obtained in this thesis suggest they may have a key role in intercalary growth but more evidence is needed to support this hypothesis.

The presence of LysM motifs in LymA and LymB suggests that these proteins have glycan-binding activity, so the affinity of LymA and LymB for insoluble polysaccharide such as chitin, chitosan, xylan and cellulose could be examined to confirm their ability for binding to chitin oligomers to sequester them. Subsequently their ability to protect the fungal cell wall against hydrolysis by plant chitinases could also be checked, although it is not expected that LymA and LymB have this protective role which has not been observed for Ecp6 in *C. fulvum* (de Jonge *et al.*, 2010). In addition, it will be important to generate a double deletion mutant of *chiA* and *chiB* to check if there is any functional redundancy between these two genes.

The second part of this study looked at the remodelling of the cell wall in endophytic and epiphytic hyphae during different stages of the infection and the role of genes encoding chitin deacetylases in establishment of the symbiotic interaction between *E. festucae* and its host plant. Fluorescence labelling and microscopy approaches revealed that there is a dramatic change in cell wall composition between endophytic and epiphytic hyphae during early and late stages of the infection of seedlings and mature plants respectively. While only septa in endophytic hyphae labelled with the chitin binding lectin WGA-AF488, the entire cell wall and septa of epiphytic hyphae labelled with WGA-AF488, suggesting that chitin in endophytic hyphae is either masked or remodelled. This microscopy observation was consistent with the results obtained by (Becker *et al.*, 2016)) that there is major hyphal cell wall alteration in *E. festucae* hyphae upon exiting the leaf.

Subsequent lectin binding and imaging techniques were conducted to check fungal cell wall composition using chitosan specific probes CAP and OGA-488. Using these chitosan specific probes indicated that cell walls of endophytic hyphae contain chitosan instead of chitin. In contrast, chitosan is absent in epiphyllous hyphal nets on the plant surface. Remodelling of the fungal cell wall and presence of chitin in cell wall and septa in epiphyllous hyphae and only in septa in endophytic hyphae was confirmed by using a chitin specific probe, CBP, which was consistent with the results obtained from WGA-AF488. *E. festucae* hyphae grown in axenic culture stained with WGA-AF488 and CBP revealing that the fungal cell wall in culture has the same composition as epiphytic hyphae. Interestingly, CAP specifically labelled phialide structures suggesting that the cell wall of these structures contain chitosan. Why the cell wall is modified during conidiophore development is an interesting question for further study.

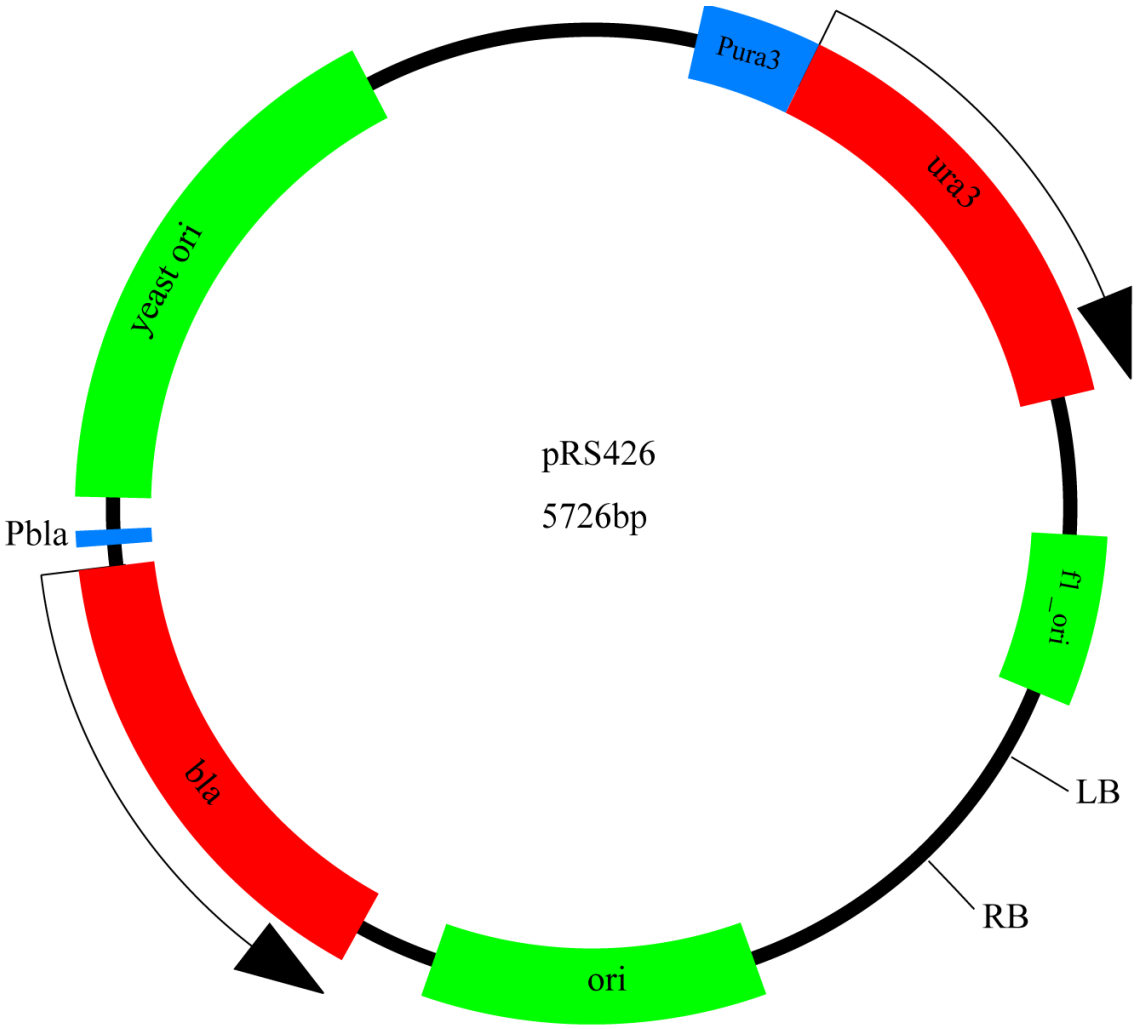
Bioinformatic analysis identified three chitin deacetylase genes based on their homology to *M. oryzae* chitin deacetylase genes. Subsequent bioinformatic characterisation identified *cdaA* as the potential candidate responsible for deacetylation of chitin into chitosan in the *E. festucae* hyphal cell wall, based on gene expression levels *in planta* compared to in culture and complementary RNA seq data analysis performed in three symbiotic mutants, *noxA*, *proA* and *sakA* compared to wild type. Single gene deletion of *cdaA* was conducted to find out its function in chitin cell wall remodelling to chitosan. While the culture and whole plant phenotype of *cdaA* mutant strains was not different from wild type, there was a break down in symbiosis observed as a dramatic cellular phenotype. Restrictive intercalary growth was clearly disrupted as hyphal growth was proliferative, with highly branched hyphae and convoluted structures containing smaller hyphal compartment. Strikingly, chitin accessibility in the *cdaA* mutant strains was different from the wild type as shown by labelling with WGA-AF488 as a chitin binding lectin. Interestingly, in addition to fungal septa in endophytic hyphae, chitin was observed as patches along the fungal cell wall showing disruption in chitin deacetylation activity.

Based on the results obtained in this study, it appears that chitosan can serve a dual function during the symbiotic interaction. As chitosan is a poor substrate for plant chitinases, and therefore a weak activator of PTI, it can function as a stealth molecule to avoid chitin triggering immune response. Chitosan may also play a key role in providing flexibility to the fungal cell wall during intercalary growth or generate a positive charge

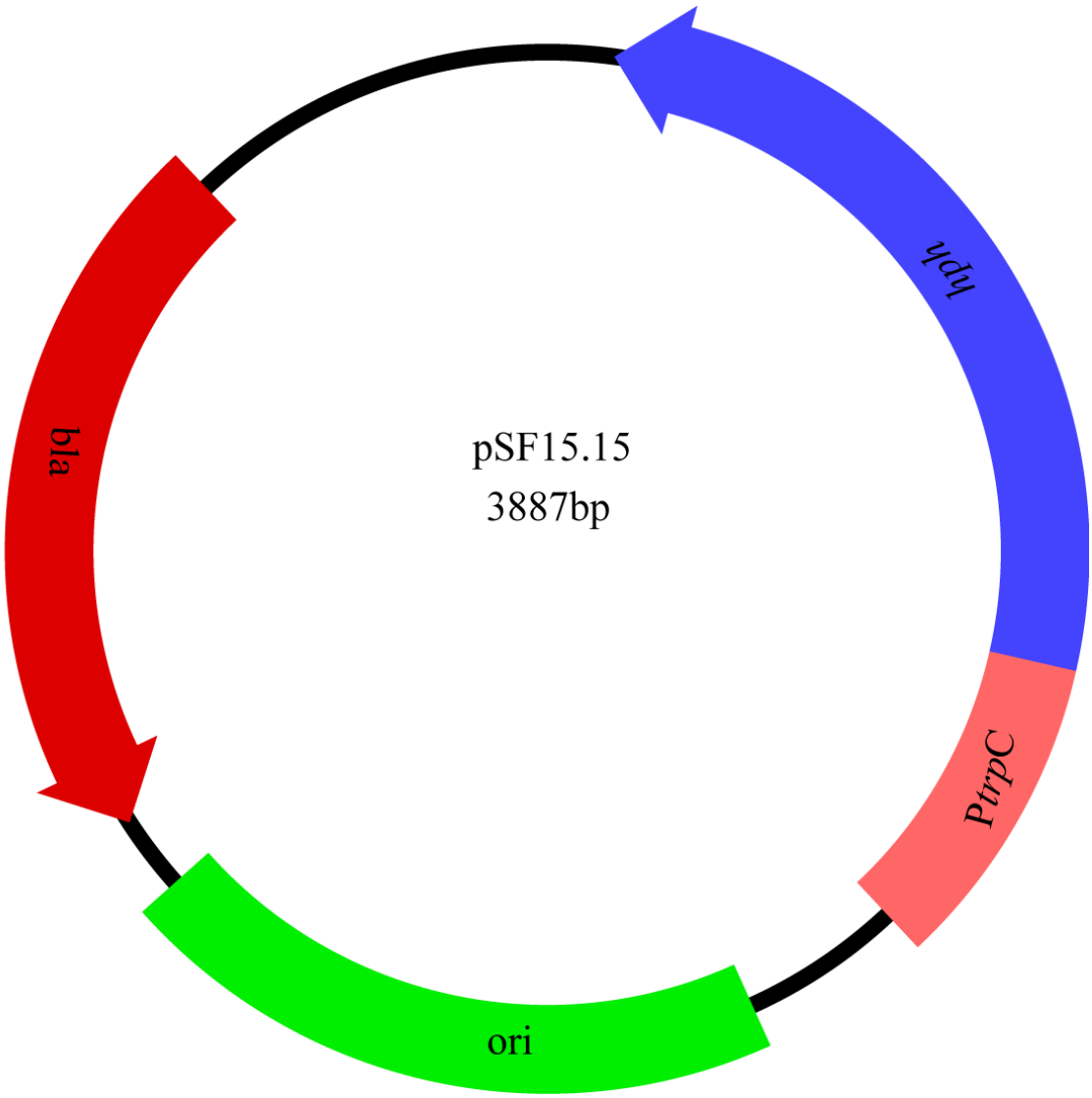
on the outside of the cell wall to facilitate fungal cell wall adhesion to the plant cell wall. Transcriptome analysis of *cdaB* and *cdaC* in plants infected with Δ*cdaA* mutant strains showed that while *cdaC* is very weakly expressed, *cdaB* is still expressed suggesting it may have a redundant function with *cdaA* and result in some deacetylation activity and suppression of the phenotype. Based on transcriptome analysis double deletion of *cdaA* and *cdaB* would be an interesting experiment to conduct and might result in complete abolishment of chitin deacetylation and consequently disrupt the symbiotic interaction. Given that single deletion of *cdaA* did not affect fungal morphology in culture and *E. festucae* was still able to produce phialide structures, localisation of chitin deacetylase proteins in culture as well as transcriptome analysis could be performed to gain a better understanding of the exact role of CdaA during fungal vegetative growth and phialide formation.

Chapter 10. Appendices

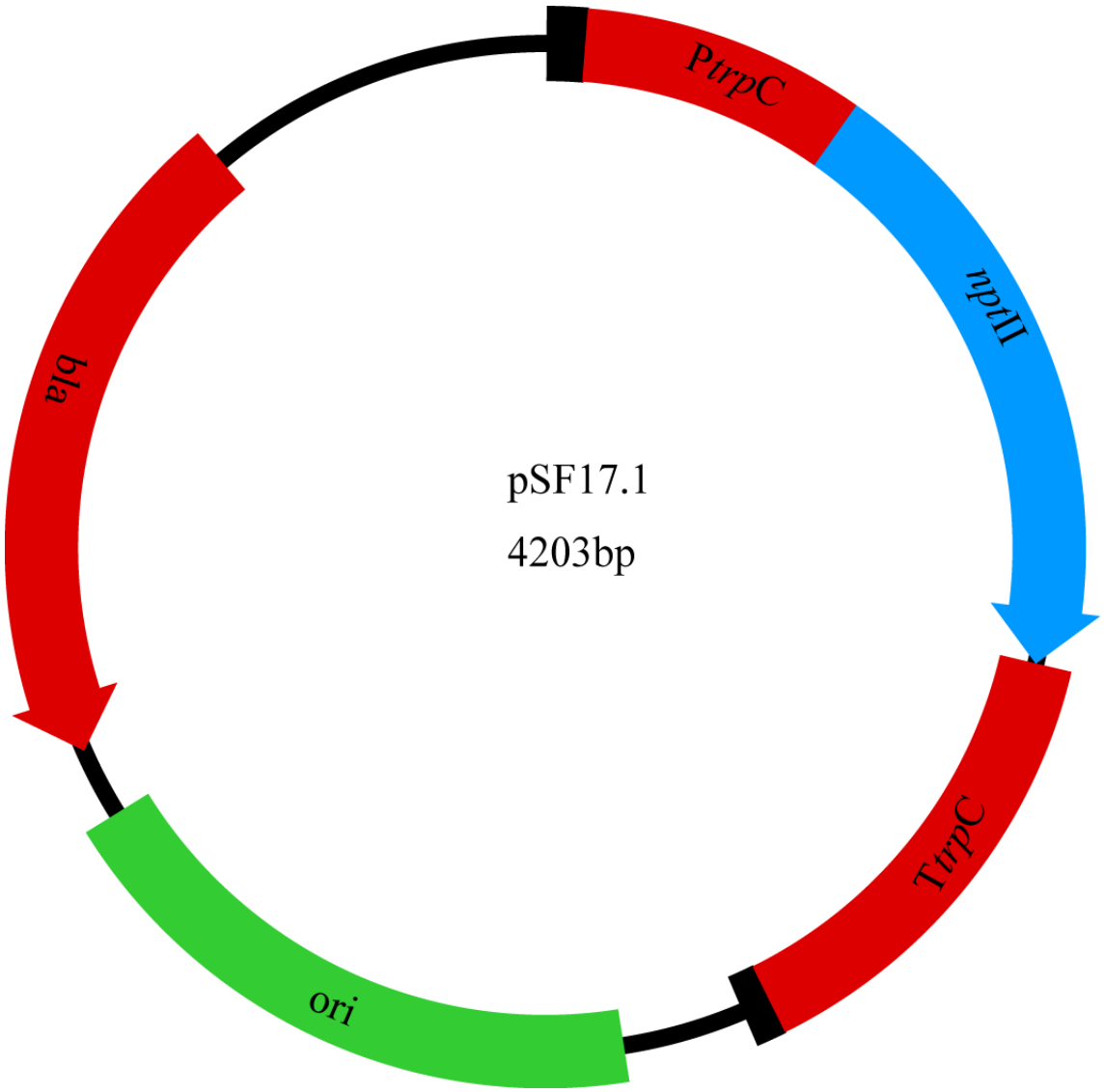
Appendix 1. Plasmid map of pRS426.



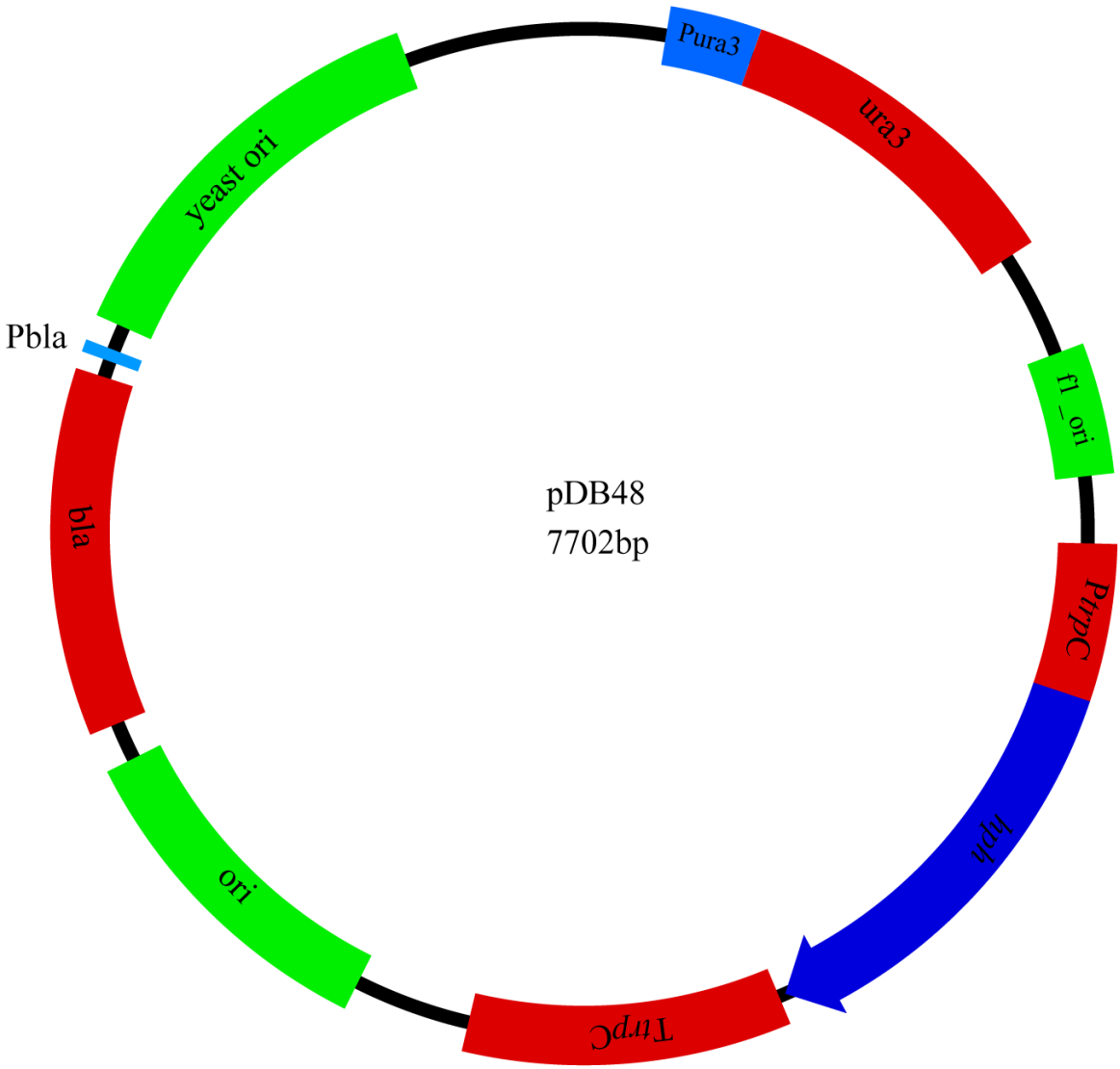
Appendix 11. Plasmid map of pSF15.15.



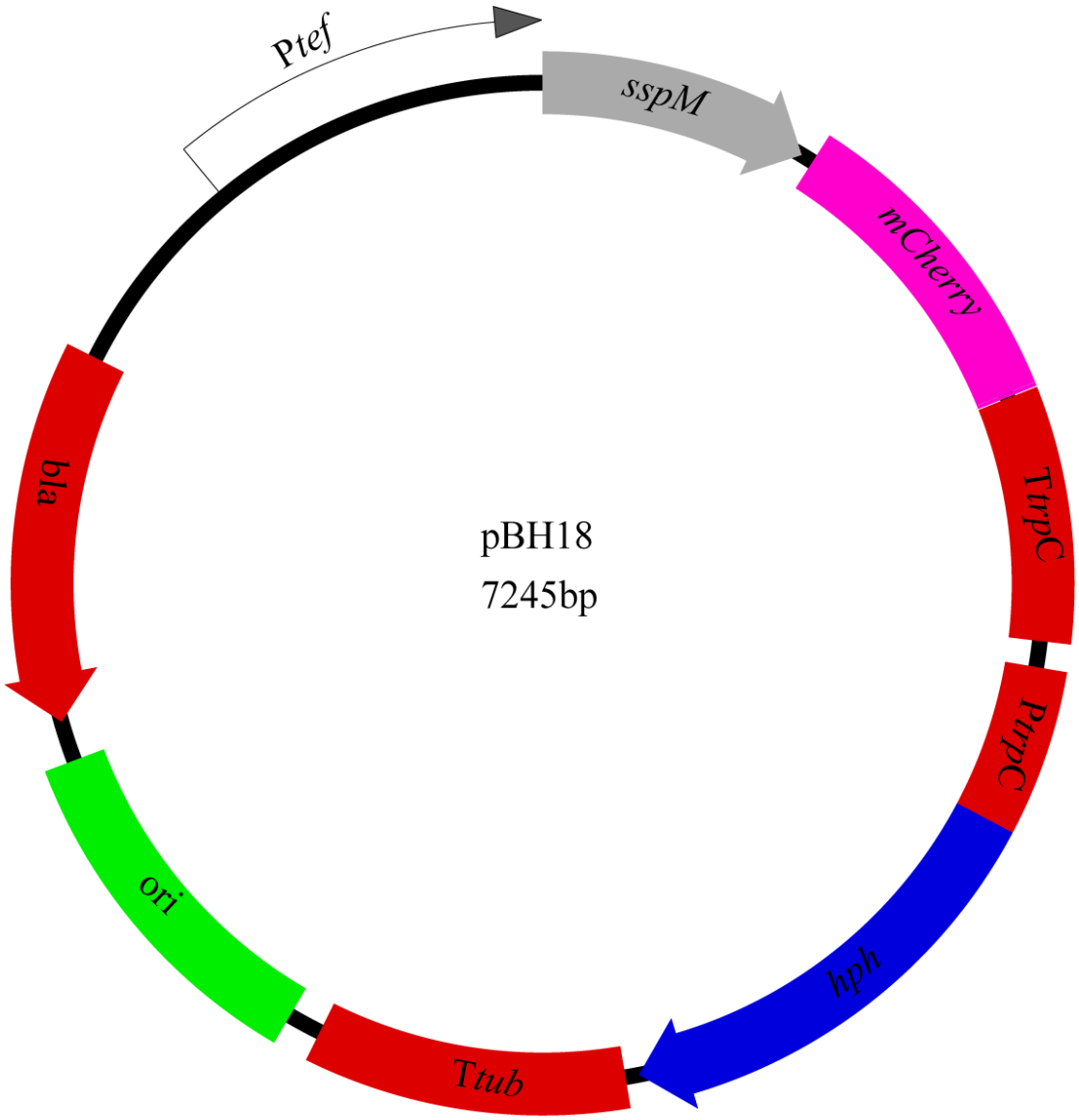
Appendix 3. Plasmid map of pSF17.1.



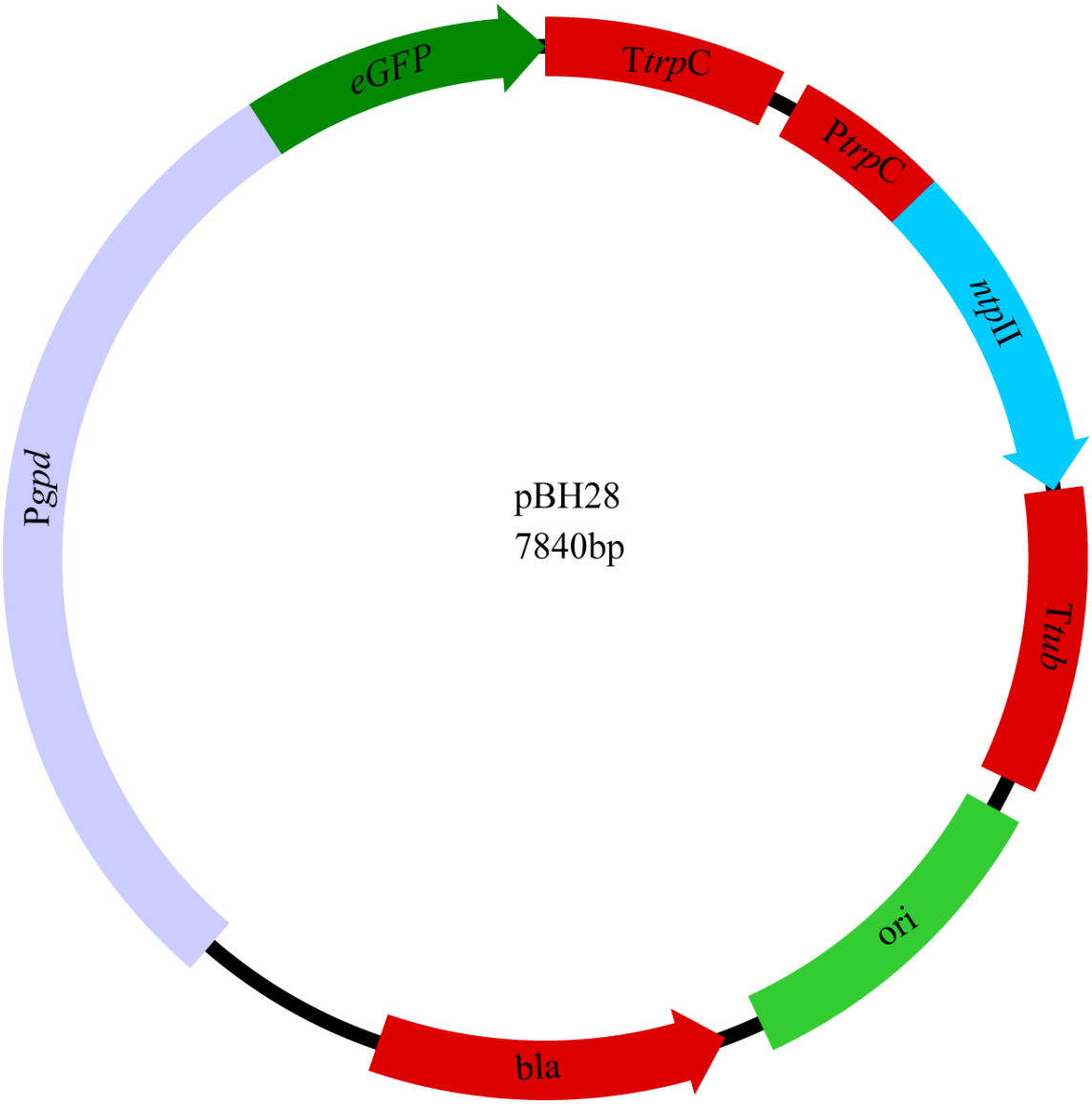
Appendix 4. Plasmid map of pDB48.



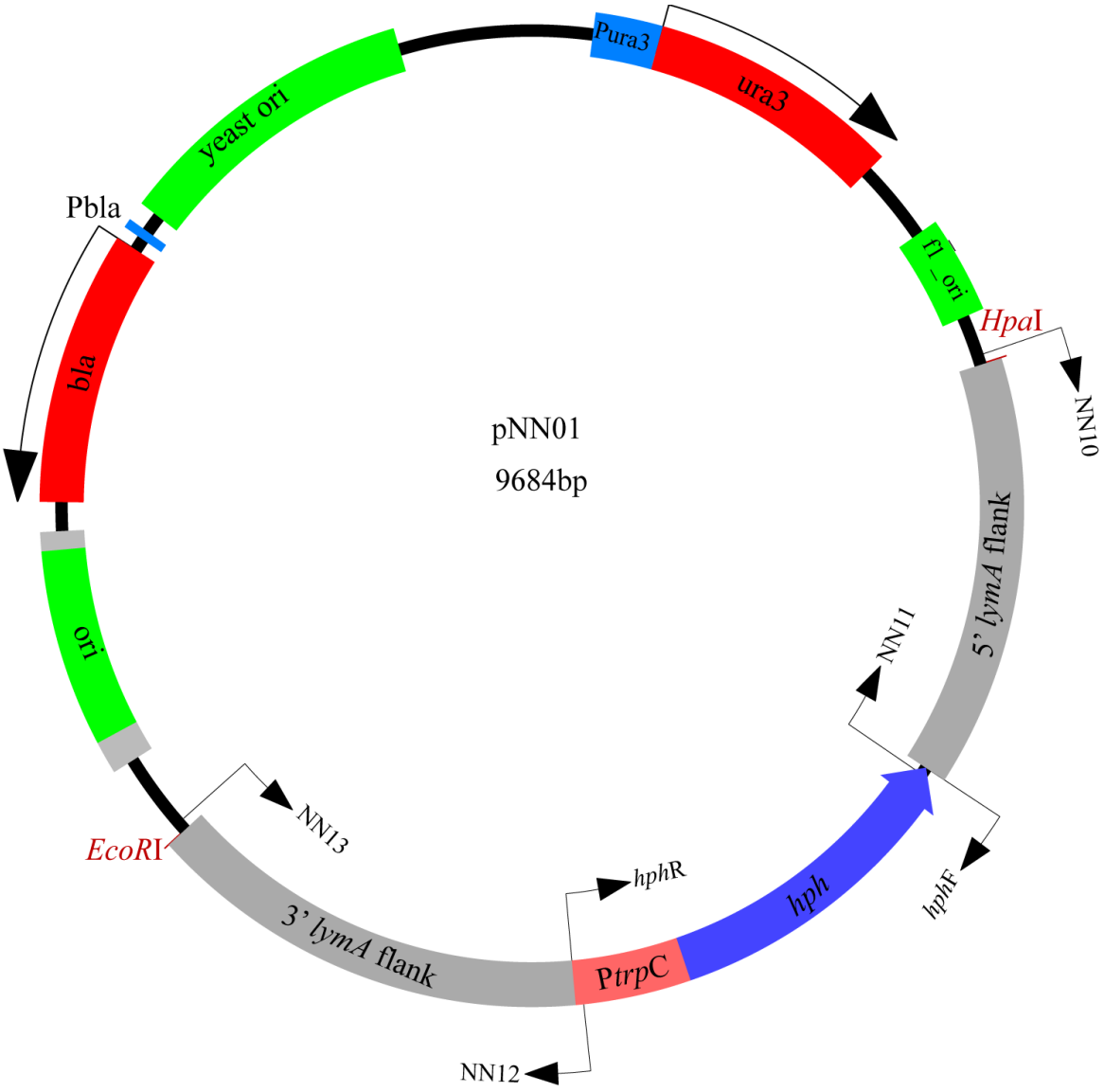
Appendix 5. Plasmid map of pBH18.



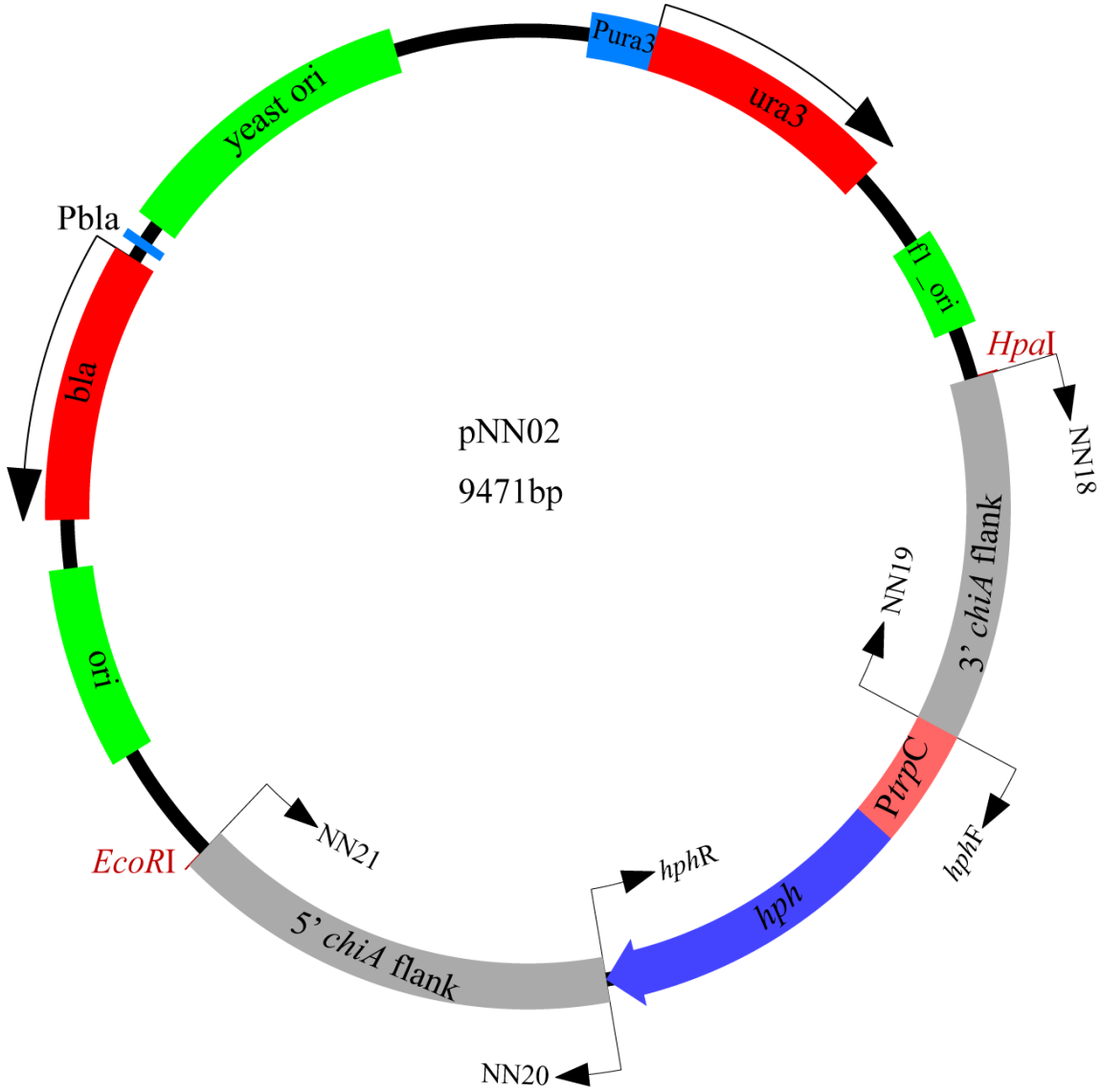
Appendix 6. Plasmid map of pBH28.



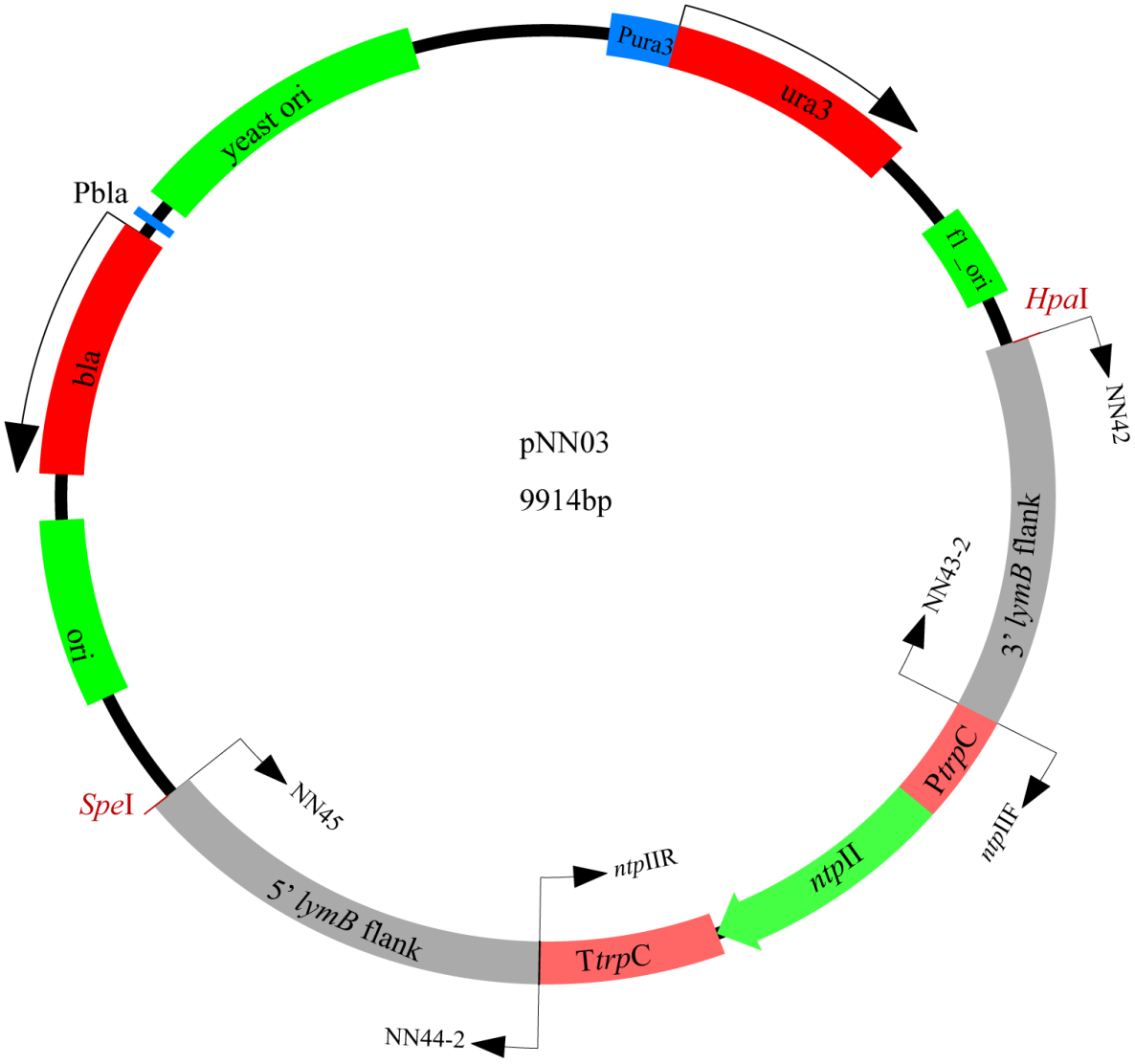
Appendix 7. Plasmid map of pNN01.



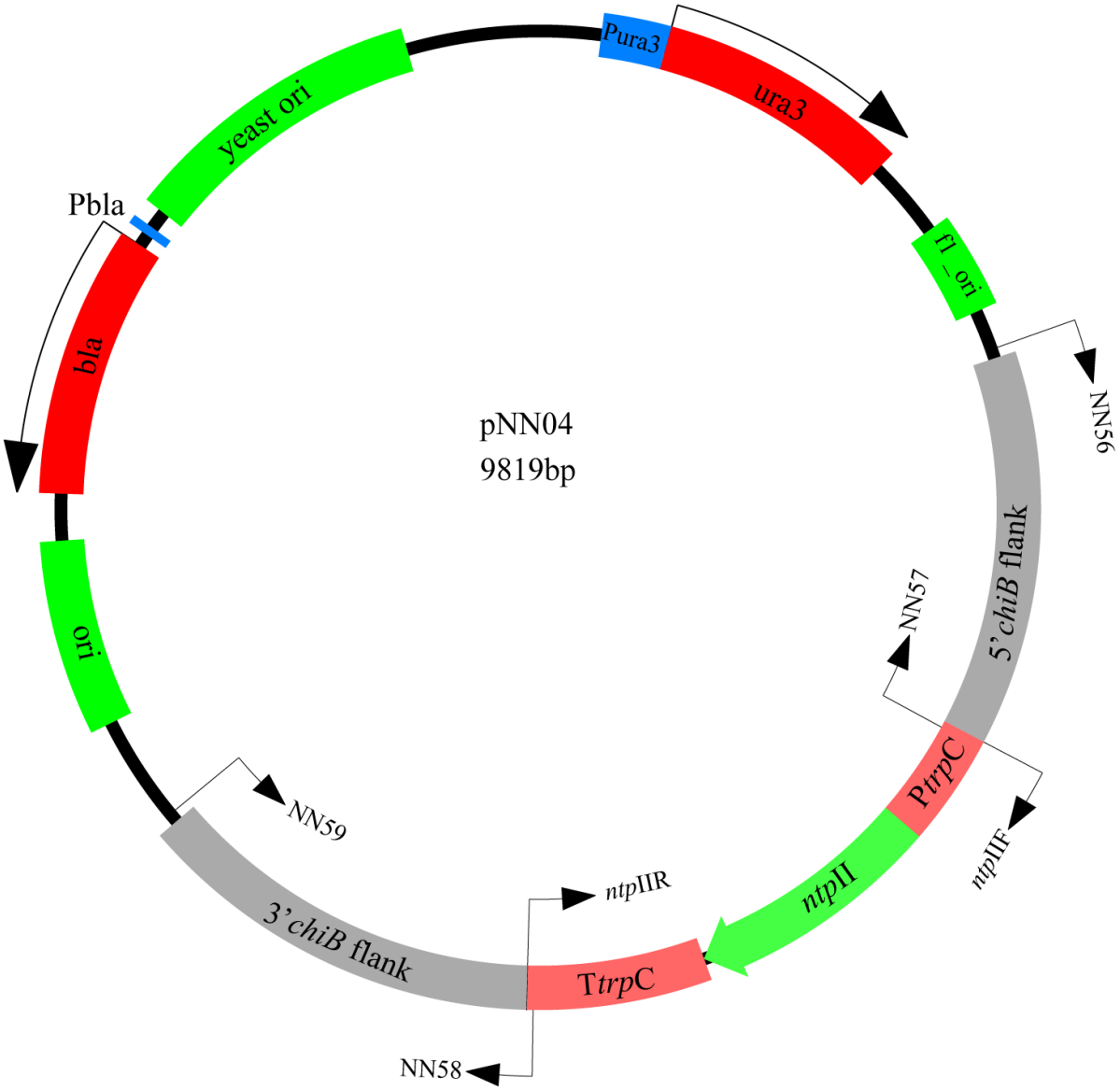
Appendix 8. Plasmid map of pNN02.



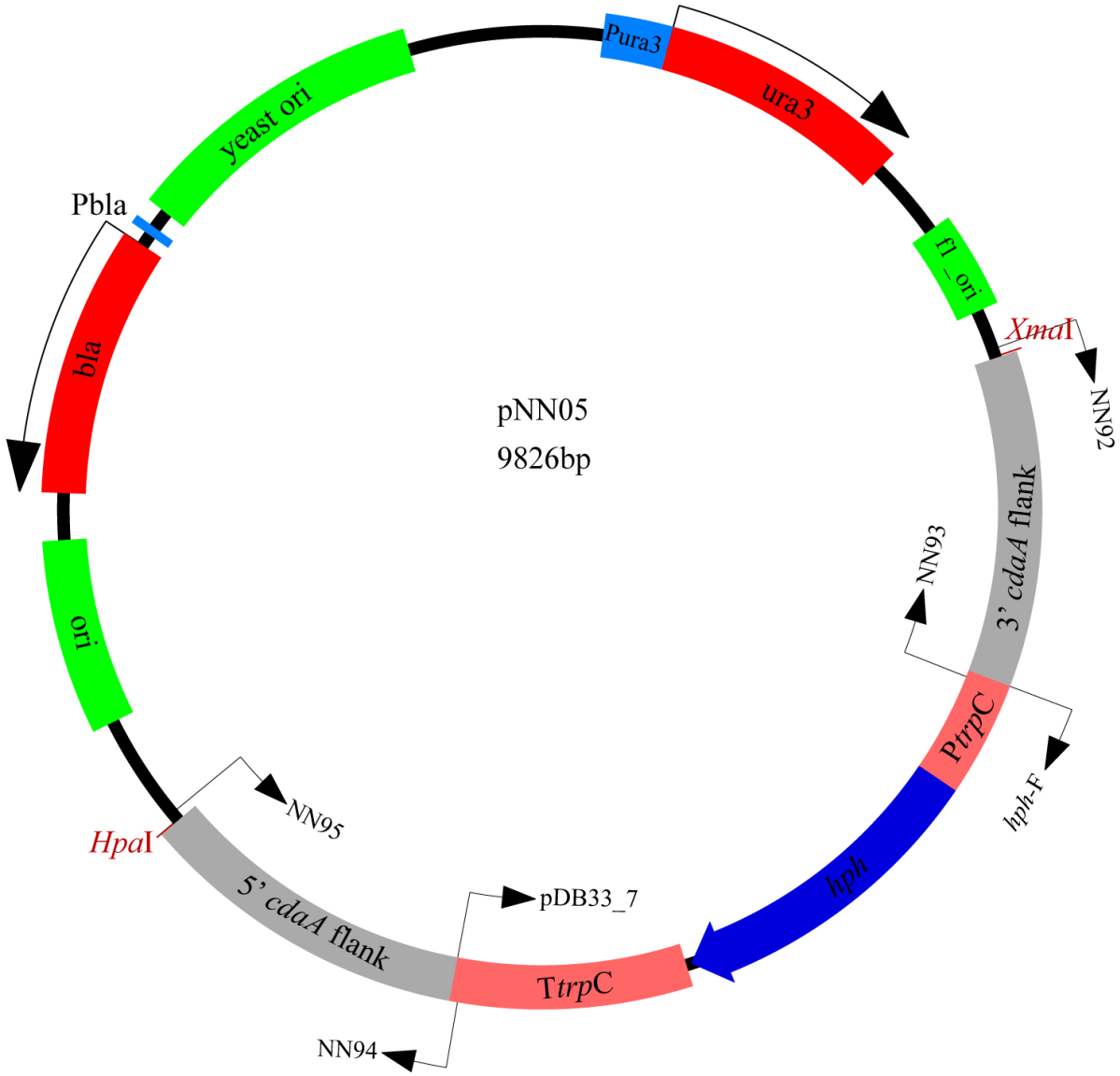
Appendix 9. Plasmid map of pNN03.



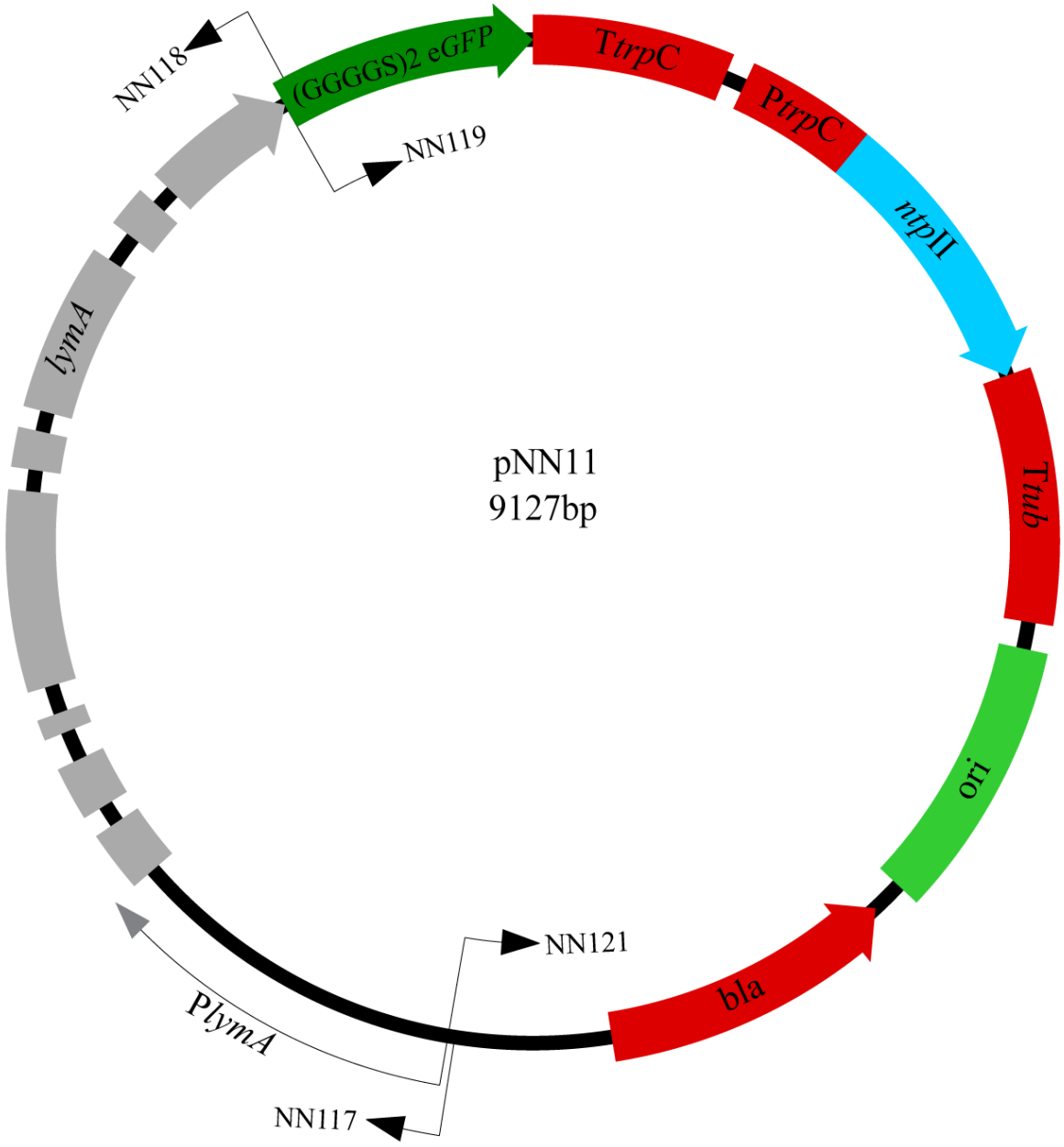
Appendix 10. Plasmid map of pNN04.



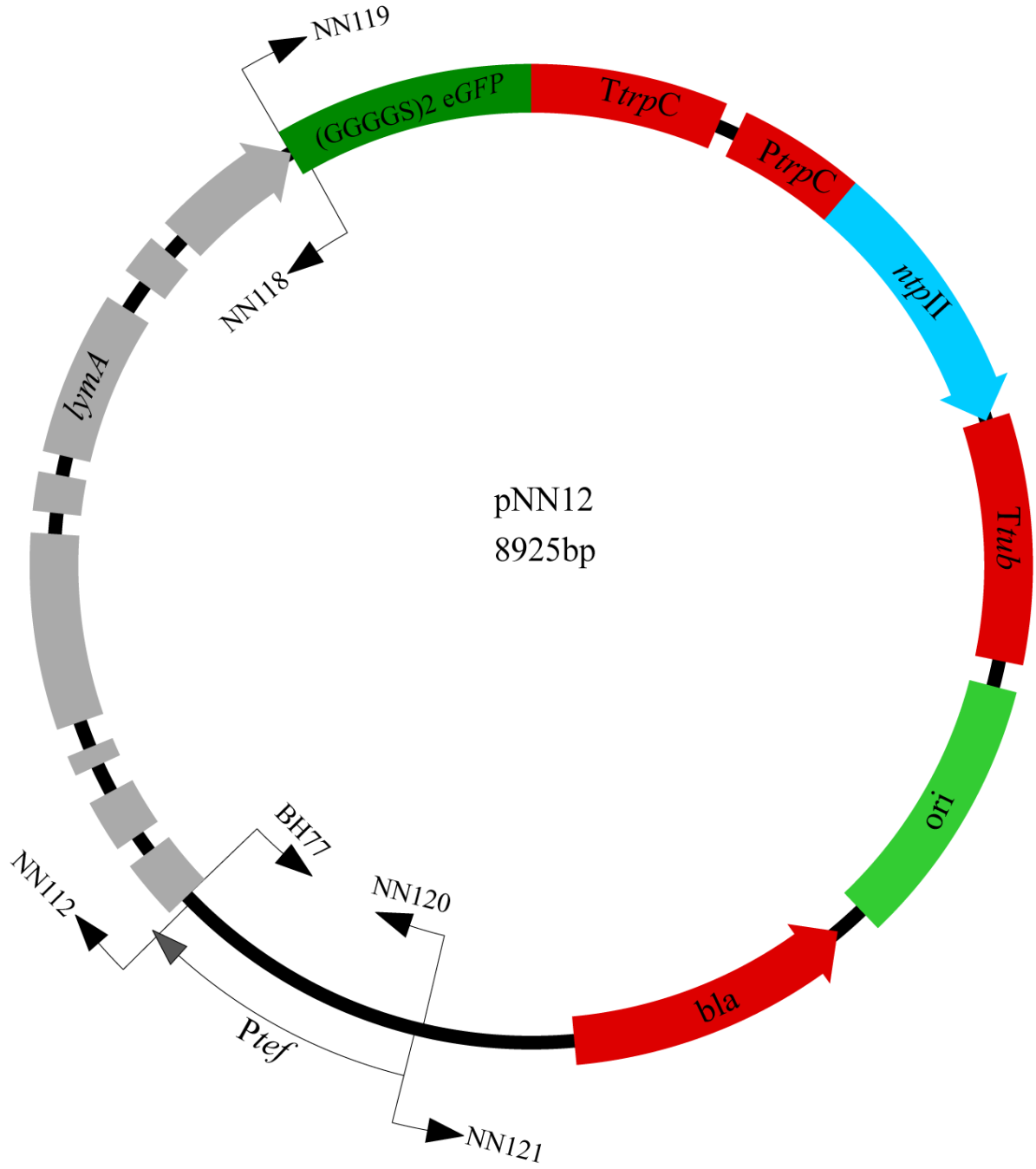
Appendix 12. Plasmid map of pNN05.



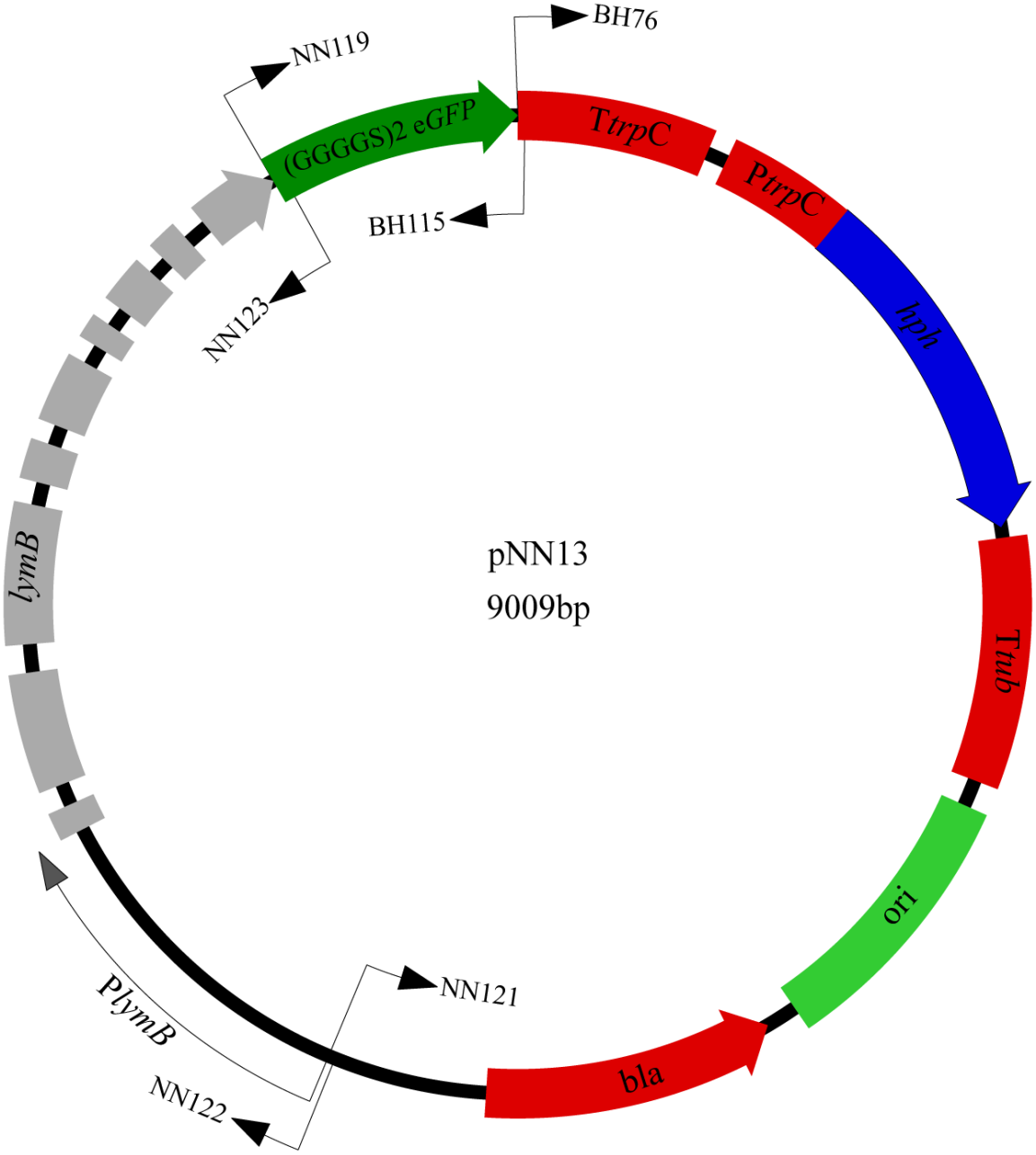
Appendix 13. Plasmid map of pNN11.



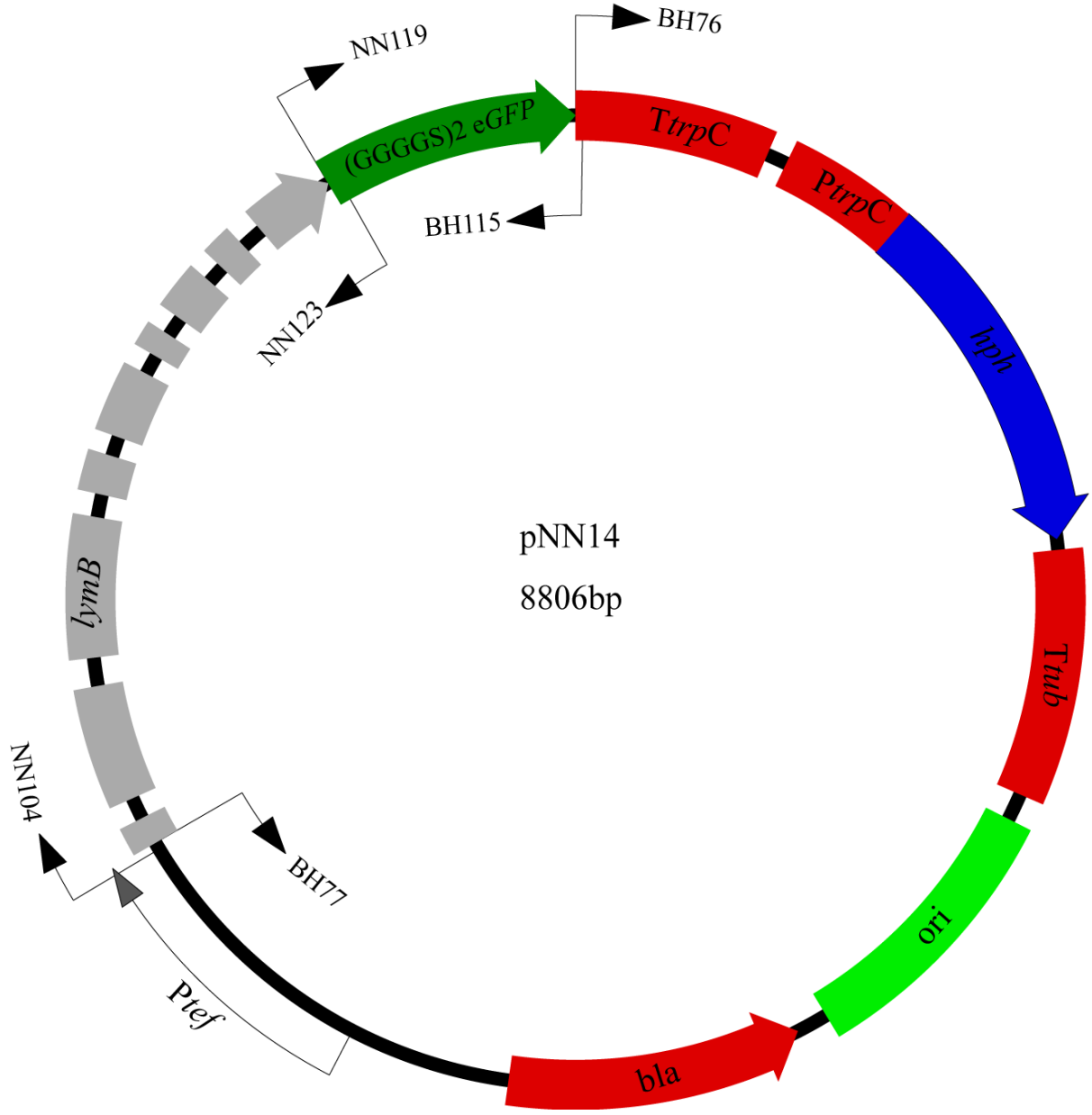
Appendix 14. Plasmid map of pNN12.



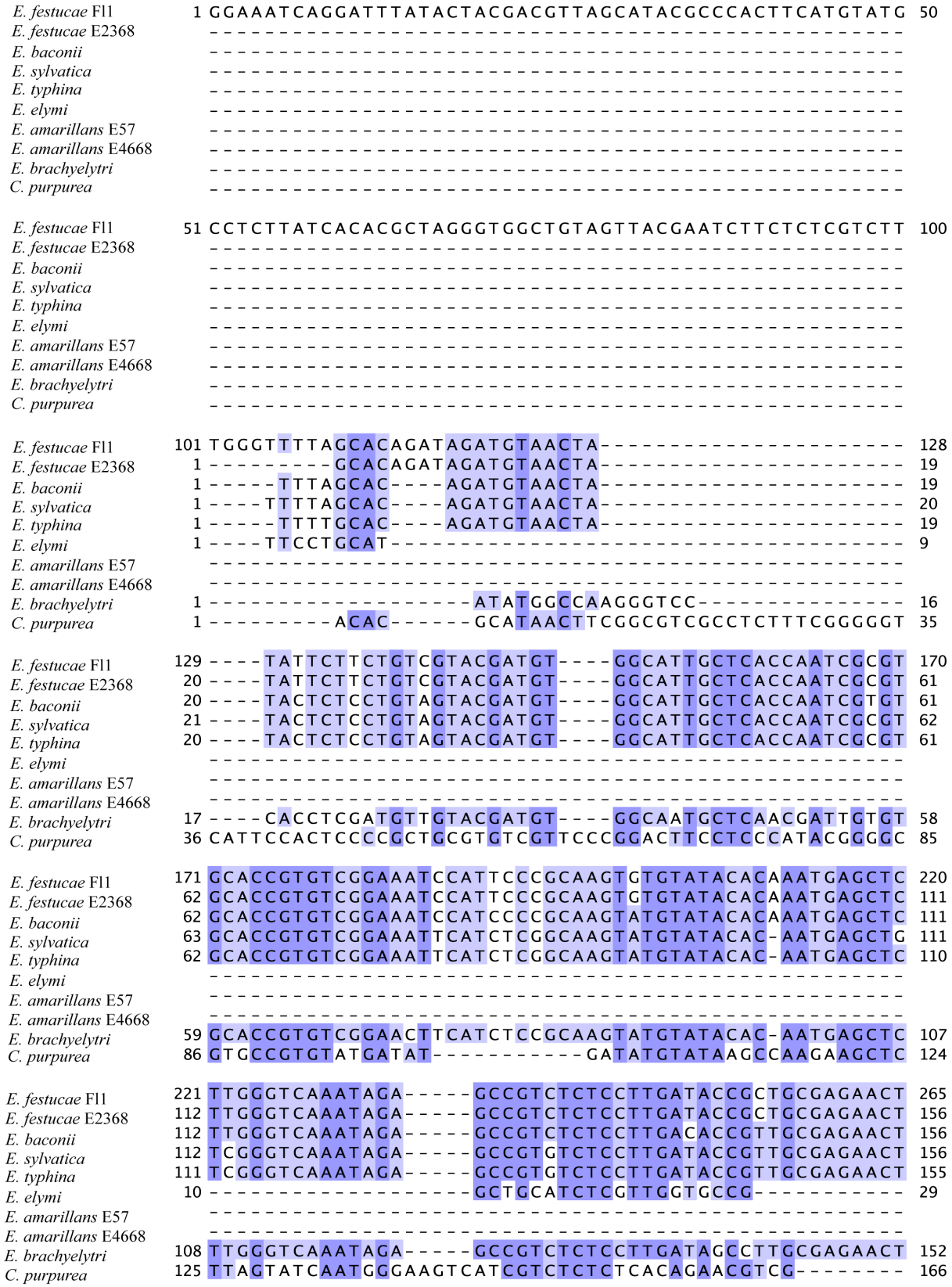
Appendix 15. Plasmid map of pNN13.

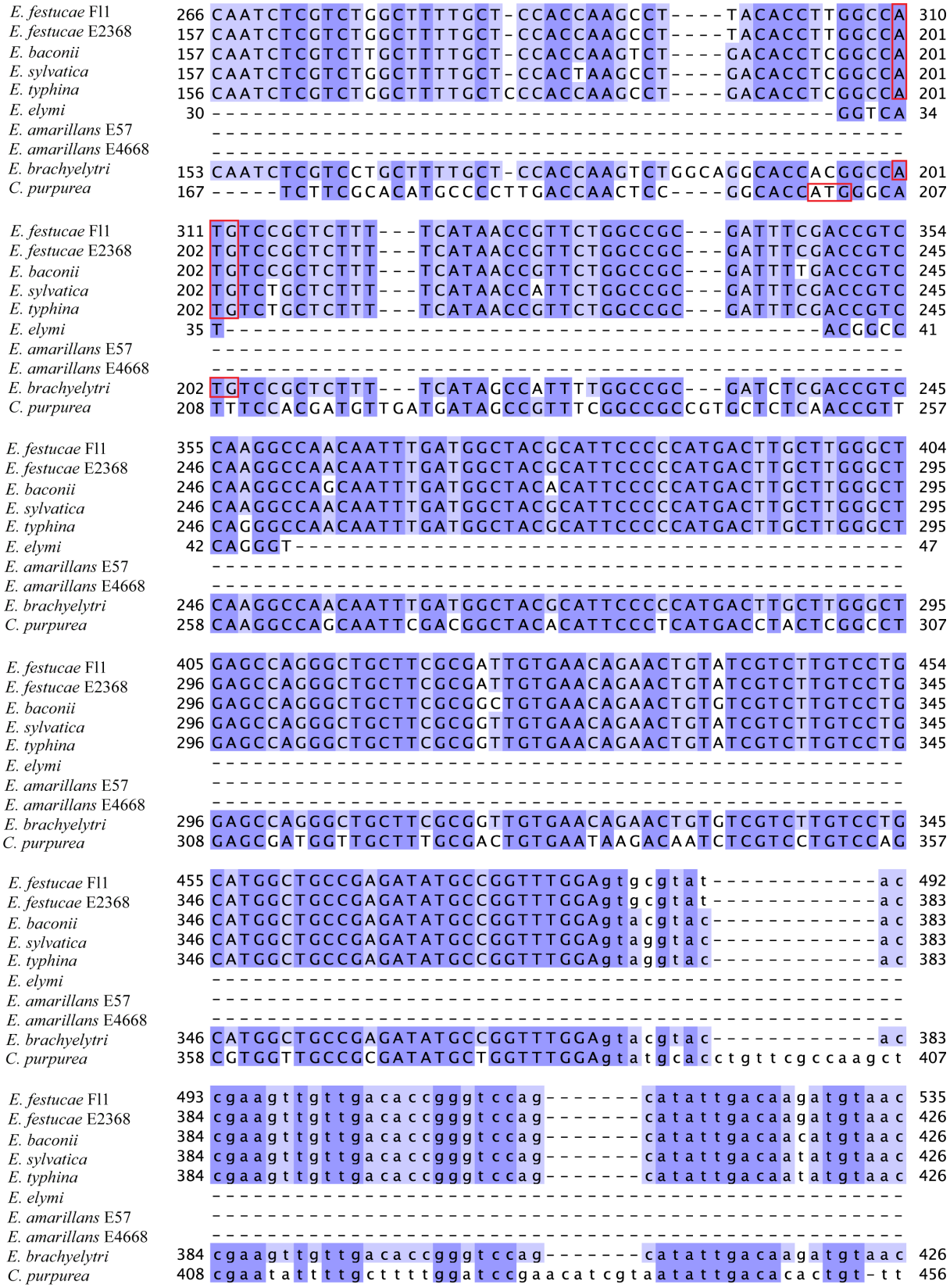


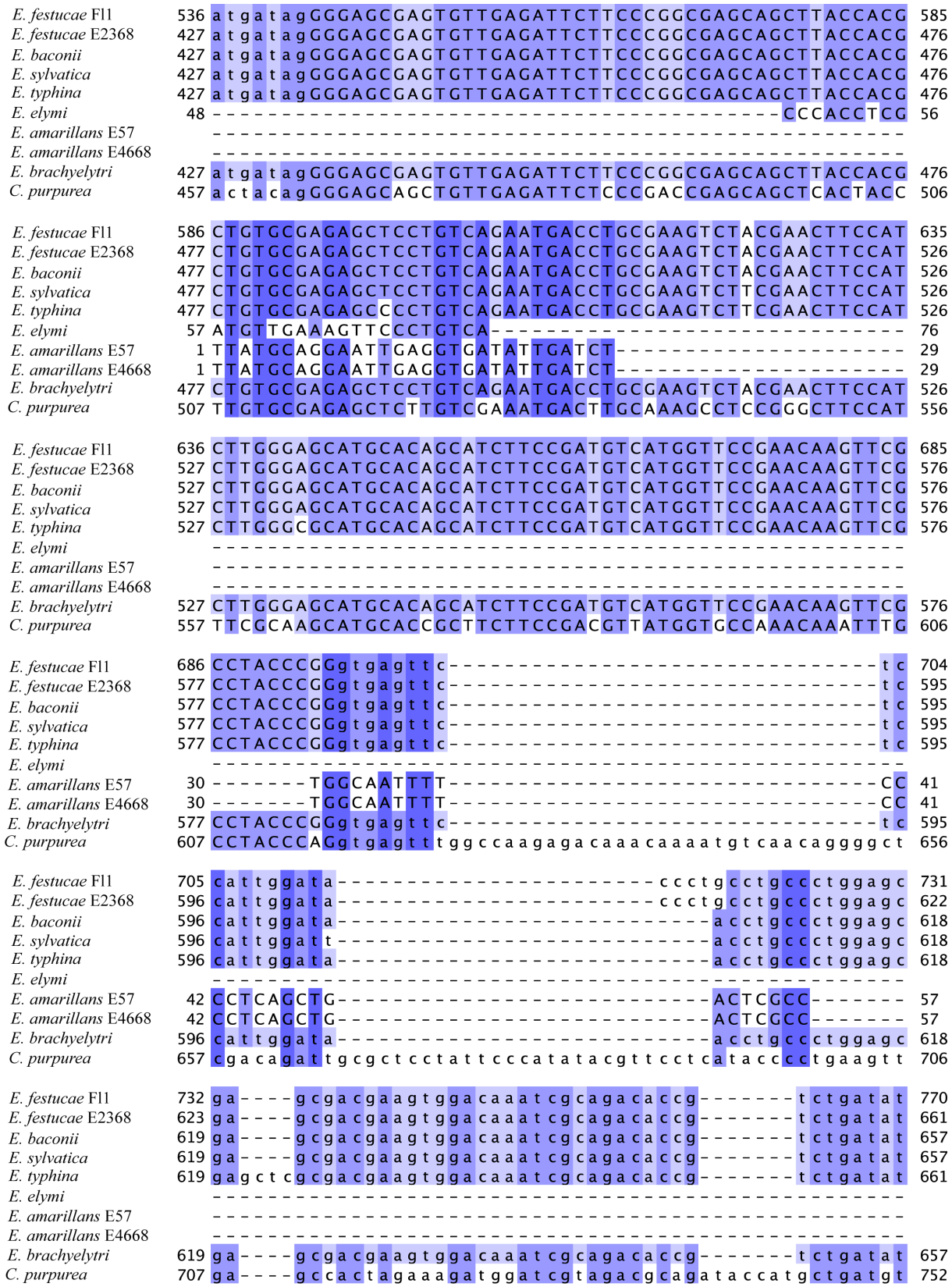
Appendix 16. Plasmid map of pNN14.

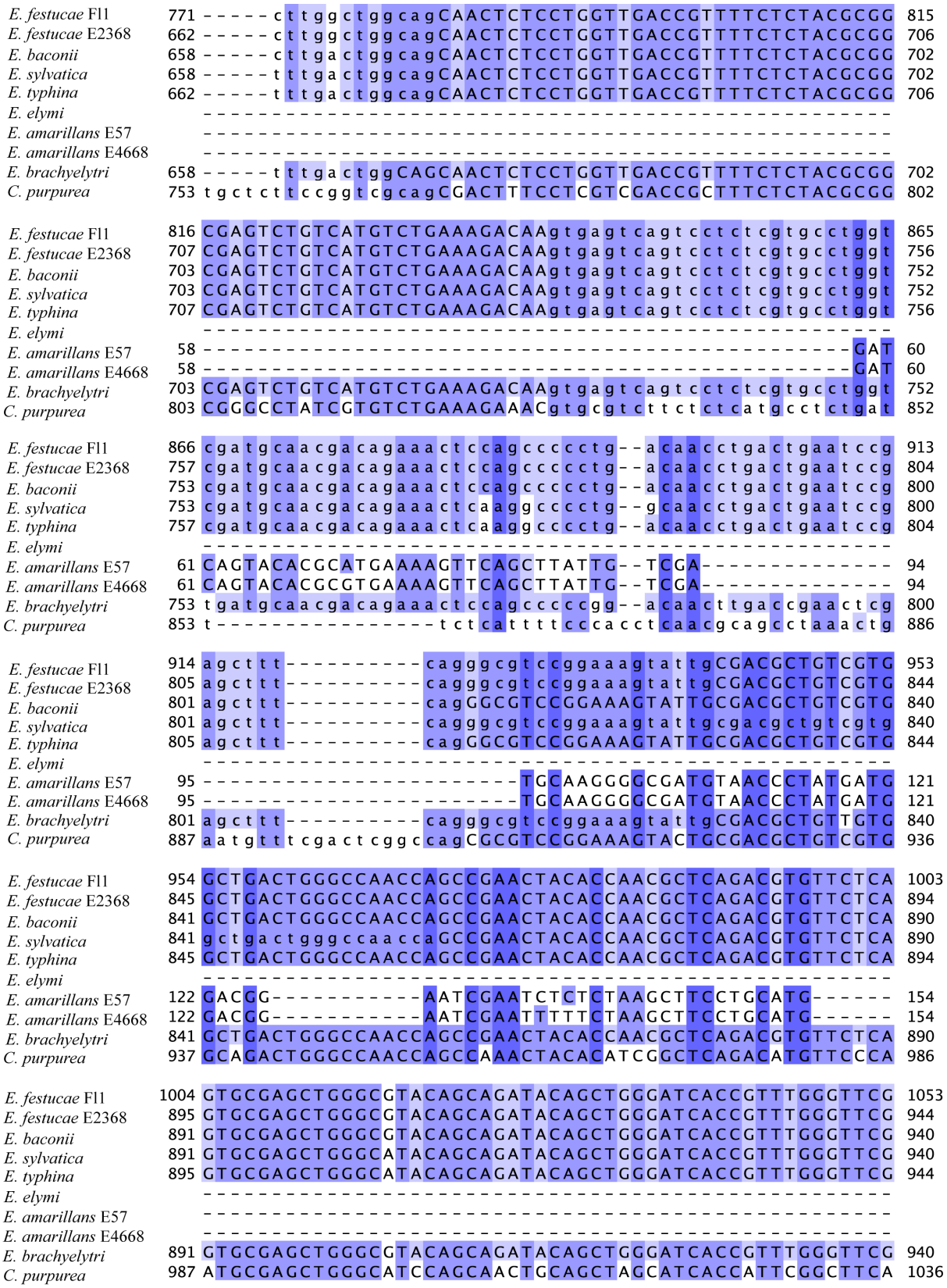


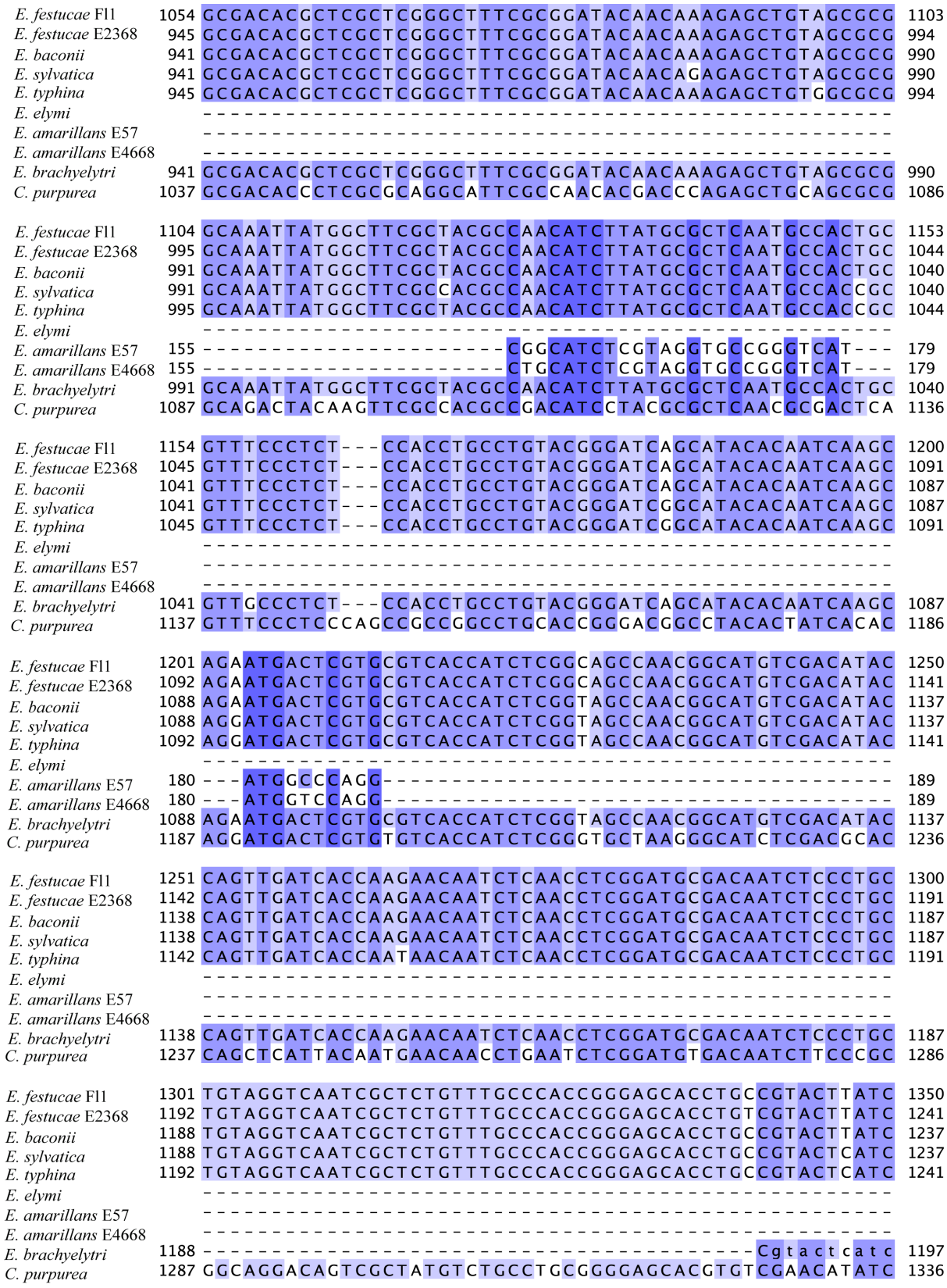
Appendix 16. Multiple sequence alignment of the *E. festucae lymA* gene with homologues from other Epichloë species and *Claviceps purpurea*. *E. festucae* (Fl1) EfM3.000820, *E. festucae* (E2368), *E. baconii, E. sylvatica, E. typhina, E. elymi, E. amarillans, E. brachyelytri, C. purpurea*. Intensity of shading indicates the level of conservation. *lymA* start codon (ATG) and stop codons (TAG and TAA) are boxed in red and green respectively. Exons and introns are indicated as capital and small letters respectively.

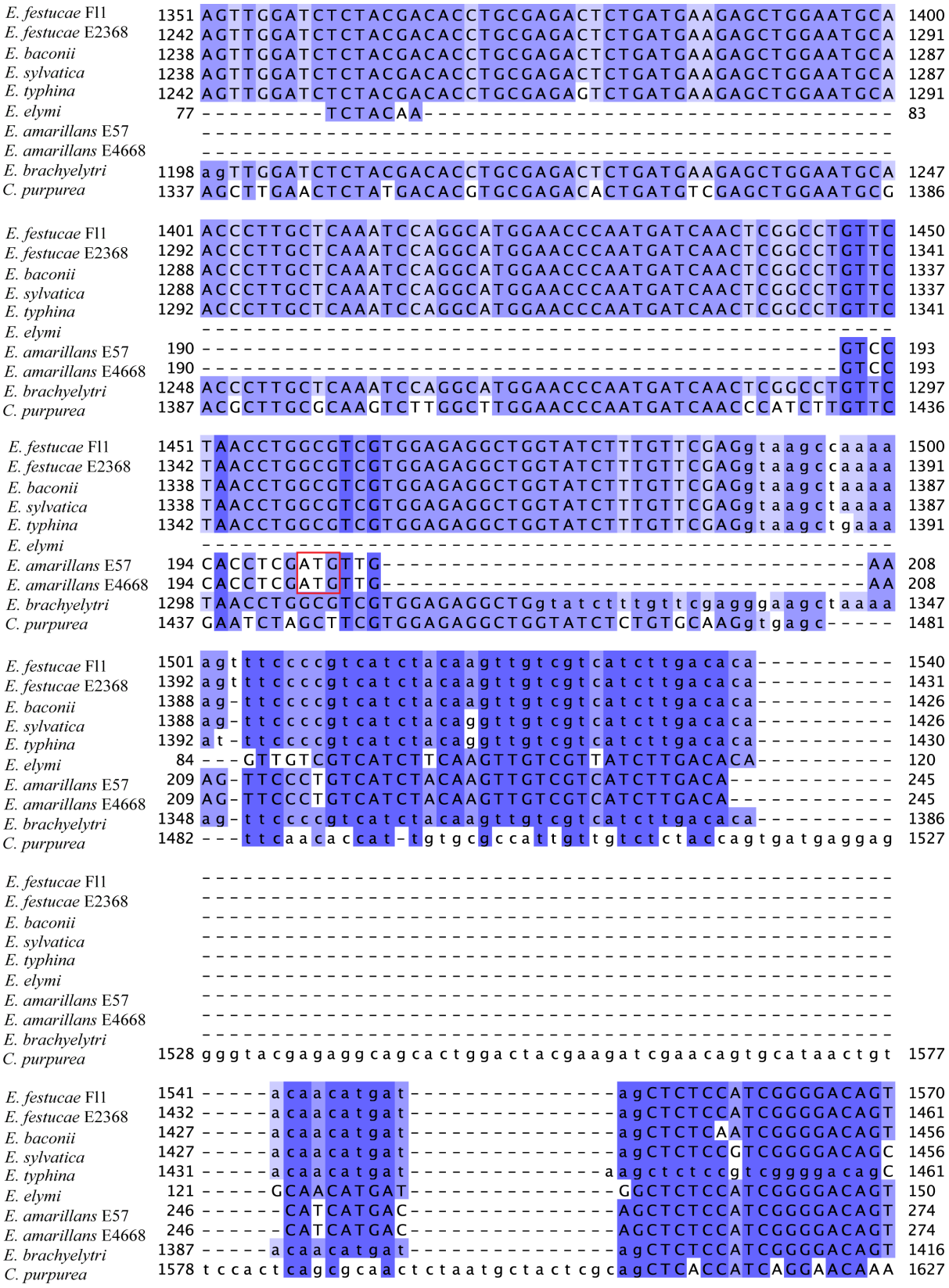

E. festucae Fl1 1 GGAAATCAGGATTTATACTACGACGTTAGCATACGCCCACTTCATGTATG 50
E. festucae E2368
E. baconii
E. sylvatica
E. typhina
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri
C. purpurea
E. festucae Fl1 51 CCTCTTATCACACGCTAGGGTGGCTGTAGTTACGAATCTTCTCTCGTCTT 100
E. festucae Fl1 101 TGGGTTTTAGCACAGATAGATGTAACTA 128
E. festucae E2368 1 GCACAGATAGATGTAACTA 19
E. baconii 1 TTTAGCAC AGATGTAACTA 19
E. sylvatica 1 TTTTAGCAC AGATGTAACTA 20
E. typhina 1 TTTTGCAC AGATGTAACTA 19
E. elymi 1 TTCCTGCAT 9
E. amarillans E57
E. amarillans E4668
E. brachyelytri 1 ATATGGCCAAGGGTCC 16
C. purpurea 1 ACAC GCATAACTTCGGCGTCGCCTCTTTCGGGGGT 35
E. festucae Fl1 129 TATTCTTCTGTCGTACGATGT GGCATTGCTCACCAATCGCGT 170
E. festucae E2368 20 TATTCTTCTGTCGTACGATGT GGCATTGCTCACCAATCGCGT 61
E. baconii 20 TACTCTCCTGTAGTACGATGT GGCATTGCTCACCAATCGTGT 61
E. sylvatica 21 TACTCTCCTGTAGTACGATGT GGCATTGCTCACCAATCGCGT 62
E. typhina 20 TACTCTCCTGTAGTACGATGT GGCATTGCTCACCAATCGCGT 61
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 17 CACCTCGATGTTGTACGATGT GGCAATGCTCAACGATTGTGT 58
C. purpurea 36 CATTCCACTCCCCGCTGCGTGTCGTTCCCGGACTTCCTCCCATACGGGGC 85
E. festucae Fl1 171 GCACCGTGTCGGAAATCCATTCCCGCAAGTGTGTATACACAAATGAGCTC 220
E. festucae E2368 62 GCACCGTGTCGGAAATCCATTCCCGCAAGTGTGTATACACAAATGAGCTC 111
E. baconii 62 GCACCGTGTCGGAAATCCATCCCCGCAAGTATGTATACACAAATGAGCTC 111
E. sylvatica 63 GCACCGTGTCGGAAATTCATCTCGGCAAGTATGTATACAC-AATGAGCTG 111
E. typhina 62 GCACCGTGTCGGAAATTCATCTCGGCAAGTATGTATACAC-AATGAGCTC 110
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 59 GCACCGTGTCGGAACTTCATCTCCGCAAGTATGTATACAC-AATGAGCTC 107
C. purpurea 86 GTGCCGTGTATGATAT GATATGTATAAGCCAAGAAGCTC 124
E. festucae Fl1 221 TTGGGTCAAATAGA GCCGTCTCTCCTTGATACCGCTGCGAGAACT 265
E. festucae E2368 112 TTGGGTCAAATAGA GCCGTCTCTCCTTGATACCGCTGCGAGAACT 156
E. baconii 112 TTGGGTCAAATAGA GCCGTCTCTCCTTGACACCGTTGCGAGAACT 156
E. sylvatica 112 TCGGGTCAAATAGA GCCGTGTCTCCTTGATACCGTTGCGAGAACT 156
E. typhina 111 TCGGGTCAAATAGA GCCGTGTCTCCTTGATACCGTTGCGAGAACT 155
E. elymi 10 GCTGCATCTCGTTGGTGCCG 29
E. amarillans E57
E. amarillans E4668
E. brachyelytri 108 TTGGGTCAAATAGA GCCGTCTCTCCTTGATAGCCTTGCGAGAACT 152
C. purpurea 125 TTAGTATCAATGGGAAGTCATCGTCTCTCTCACAGAACGTCG 166

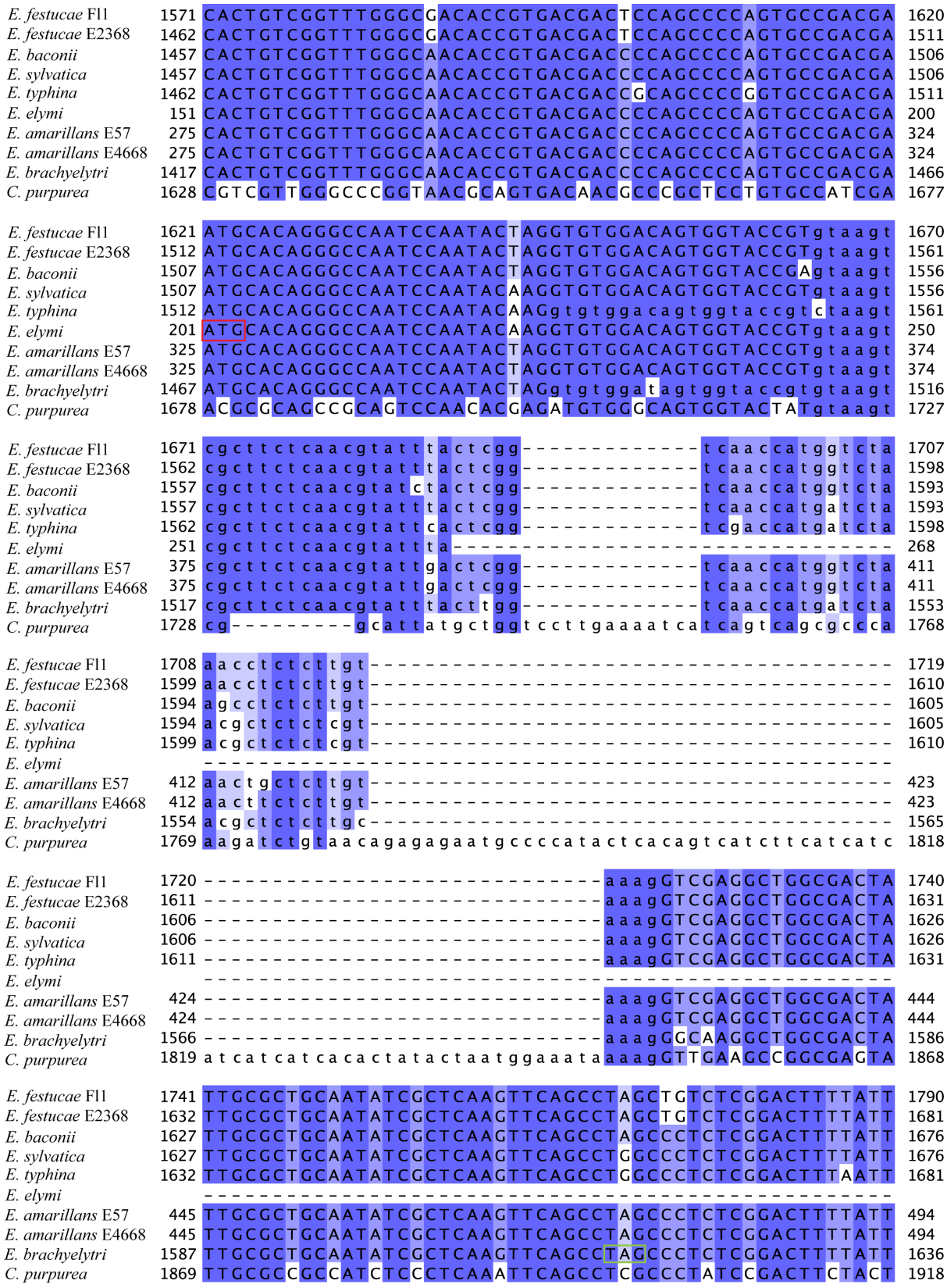

E. festucae Fl1
E. festucae E2368
E. baconii
E. sylvatica
E. typhina
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri
C. purpurea

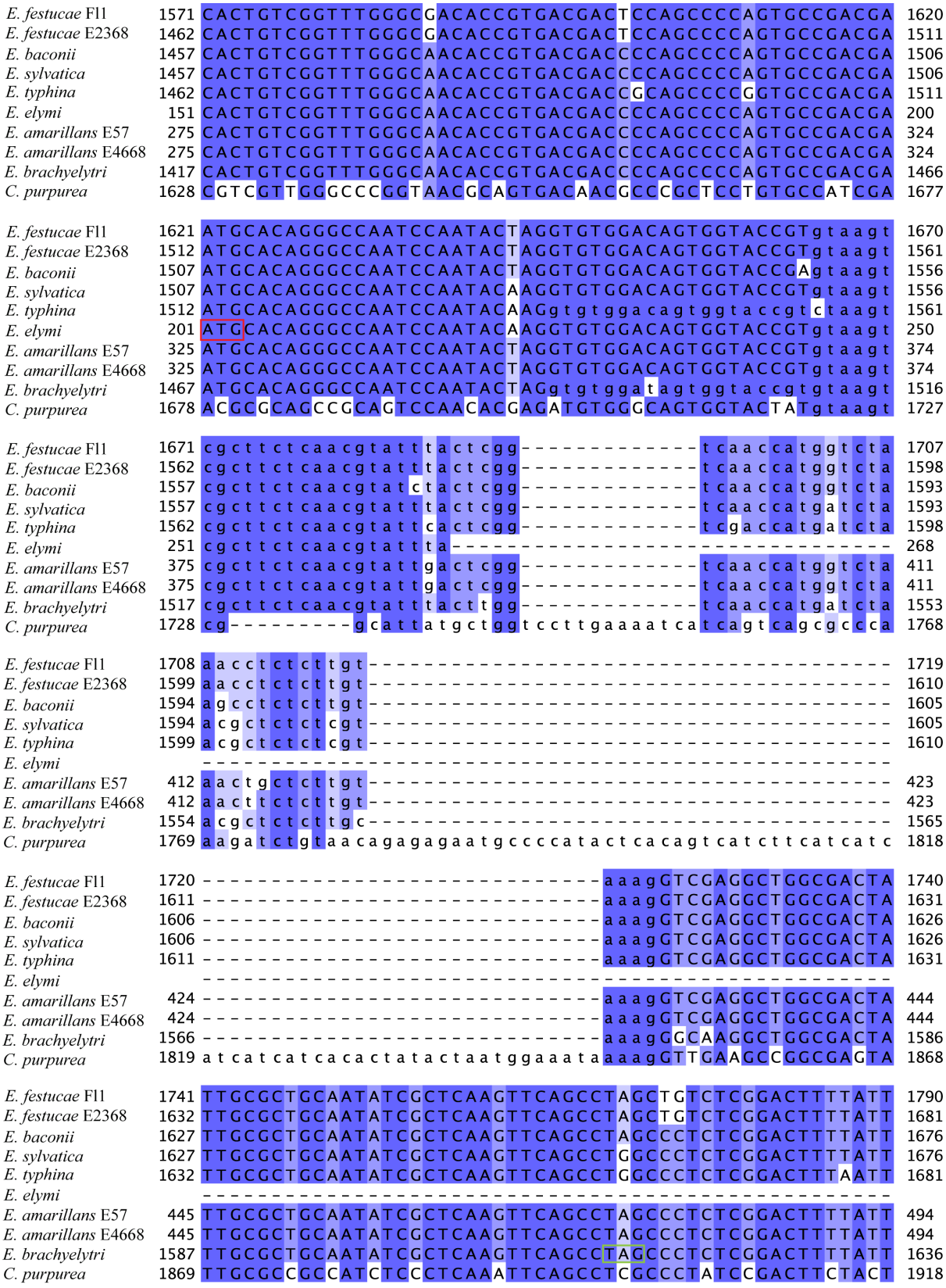

E. festucae Fl1 536 atgatagGGGAGCGAGTGTTGAGATTCTTCCCGGCGAGCAGCTTACCACG 585
E. festucae E2368 427 atgatagGGGAGCGAGTGTTGAGATTCTTCCCGGCGAGCAGCTTACCACG 476
E. baconii 427 atgatagGGGAGCGAGTGTTGAGATTCTTCCCGGCGAGCAGCTTACCACG 476
E. sylvatica 427 atgatagGGGAGCGAGTGTTGAGATTCTTCCCGGCGAGCAGCTTACCACG 476
E. typhina 427 atgatagGGGAGCGAGTGTTGAGATTCTTCCCGGCGAGCAGCTTACCACG 476
E. elymi 48 ----------CCCACCTCG 56
E. amarillans E57
E. amarillans E4668
E. brachyelytri 427 atgatagGGGAGCGAGTGTTGAGATTCTTCCCGGCGAGCAGCTTACCACG 476
C. purpurea 457 actacagGGGAGCAGCTGTTGAGATTCTCCCGACCGAGCAGCTCACTACC 506
E. festucae Fl1 586 CTGTGCGAGAGCTCCTGTCAGAATGACCTGCGAAGTCTACGAACTTCCAT 635
E. festucae E2368 477 CTGTGCGAGAGCTCCTGTCAGAATGACCTGCGAAGTCTACGAACTTCCAT 526
E. baconii 477 CTGTGCGAGAGCTCCTGTCAGAATGACCTGCGAAGTCTACGAACTTCCAT 526
E. sylvatica 477 CTGTGCGAGAGCTCCTGTCAGAATGACCTGCGAAGTCTTCGAACTTCCAT 526
E. typhina 477 CTGTGCGAGAGCCCCTGTCAGAATGACCTGCGAAGTCTTCGAACTTCCAT 526
E. elymi 57 ATGTTGAAAGTTCCCTGTCA---------- 76
E. amarillans E57 1 TTATGCAGGAATTGAGGTGATATTGATCT---------- 29
E. amarillans E4668 1 TTATGCAGGAATTGAGGTGATATTGATCT---------- 29
E. brachyelytri 477 CTGTGCGAGAGCTCCTGTCAGAATGACCTGCGAAGTCTACGAACTTCCAT 526
C. purpurea 507 TTGTGCGAGAGCTCTTGTCGAAATGACTTGCAAAGCCTCCGGGCTTCCAT 556
E. festucae Fl1 636 CTTGGGAGCATGCACAGCATCTTCCGATGTCATGGTTCCGAACAAGTTCG 685
E. festucae E2368 527 CTTGGGAGCATGCACAGCATCTTCCGATGTCATGGTTCCGAACAAGTTCG 576
E. baconii 527 CTTGGGAGCATGCACAGCATCTTCCGATGTCATGGTTCCGAACAAGTTCG 576
E. sylvatica 527 CTTGGGAGCATGCACAGCATCTTCCGATGTCATGGTTCCGAACAAGTTCG 576
E. typhina 527 CTTGGGCGCATGCACAGCATCTTCCGATGTCATGGTTCCGAACAAGTTCG 576
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 527 CTTGGGAGCATGCACAGCATCTTCCGATGTCATGGTTCCGAACAAGTTCG 576
C. purpurea 557 TTCGCAAGCATGCACCGCTTCTTCCGACGTTATGGTGCCAAACAAATTTG 606
E. festucae Fl1 686 CCTACCCGGgtgagttc---------- tc 704
E. festucae E2368 577 CCTACCCGGgtgagttc---------- tc 595
E. baconii 577 CCTACCCGGgtgagttc---------- tc 595
E. sylvatica 577 CCTACCCGGgtgagttc---------- tc 595
E. typhina 577 CCTACCCGGgtgagttc---------- tc 595
E. elymi
E. amarillans E57 30 -------TGGCAATTTT---------- CC 41
E. amarillans E4668 30 -------TGGCAATTTT---------- CC 41
E. brachyelytri 577 CCTACCCGGgtgagttc---------- tc 595
C. purpurea 607 CCTACCCAGgtgagttttggccaagagacaaacaaaatgtcaacagggggct 656
E. festucae Fl1 705 cattggata---------- ccctgcctgccctggagc 731
E. festucae E2368 596 cattggata---------- ccctgcctgccctggagc 622
E. baconii 596 cattggata---------- acctgccctggagc 618
E. sylvatica 596 cattggatt---------- acctgccctggagc 618
E. typhina 596 cattggata---------- acctgccctggagc 618
E. elymi
E. amarillans E57 42 CCTCAGCTG---------- ACTCGCC------- 57
E. amarillans E4668 42 CCTCAGCTG---------- ACTCGCC------- 57
E. brachyelytri 596 cattggata---------- acctgccctggagc 618
C. purpurea 657 cgacagattgcgctcctattcccatatacgttcctcataccctgaagtt 706
E. festucae Fl1 732 ga----gcgacgaagtggacaaatcgcagacaccg-------tctgatat 770
E. festucae E2368 623 ga----gcgacgaagtggacaaatcgcagacaccg-------tctgatat 661
E. baconii 619 ga----gcgacgaagtggacaaatcgcagacaccg-------tctgatat 657
E. sylvatica 619 ga----gcgacgaagtggacaaatcgcagacaccg-------tctgatat 657
E. typhina 619 gagctcgcgacgaagtggacaaatcgcagacaccg-------tctgatat 661
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 619 ga----gcgacgaagtggacaaatcgcagacaccg-------tctgatat 657
C. purpurea 707 ga----gccactagaaagatggatcgtagacgcagataccatgctgatgt 752

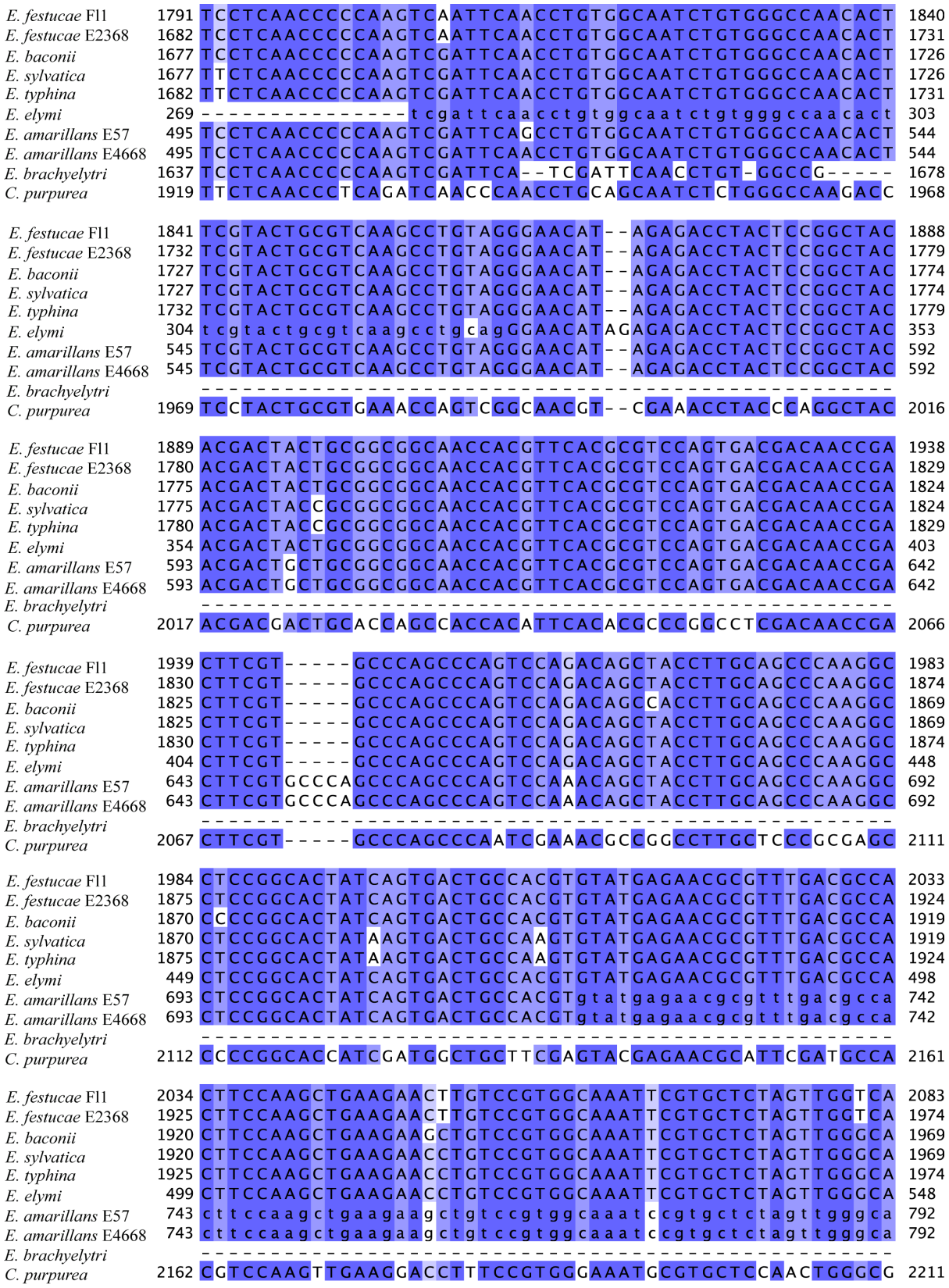

E. festucae Fl1 771 ------cttggctggcagCAACTCTCCTGGTTGACCGTTTTCTCTACGCGG 815
E. festucae E2368 662 ------cttggctggcagCAACTCTCCTGGTTGACCGTTTTCTCTACGCGG 706
E. baconii 658 ------cttgactggcagCAACTCTCCTGGTTGACCGTTTTCTCTACGCGG 702
E. sylvatica 658 -----tttgactggcagCAACTCTCCTGGTTGACCGTTTTCTCTACGCGG 702
E. typhina 662 -----tttgactggcagCAACTCTCCTGGTTGACCGTTTTCTCTACGCGG 706
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 658 -----tttgactggCAGCAACTCTCCTGGTTGACCGTTTTCTCTACGCGG 702
C. purpurea 753 tgctcttccggtcgcagCGACTTTCCTCGTCGACCGCTTTCTCTACGCGG 802
E. festucae Fl1 816 CGAGTCTGTCATGTCTGAAAGACAAgtgagtcagtcctctcgtgcctggt 865
E. festucae E2368 707 CGAGTCTGTCATGTCTGAAAGACAAgtgagtcagtcctctcgtgcctggt 756
E. baconii 703 CGAGTCTGTCATGTCTGAAAGACAAgtgagtcagtcctctcgtgcctggt 752
E. sylvatica 703 CGAGTCTGTCATGTCTGAAAGACAAgtgagtcagtcctctcgtgcctggt 752
E. typhina 707 CGAGTCTGTCATGTCTGAAAGACAAgtgagtcagtcctctcgtgcctggt 756
E. elymi
E. amarillans E57 58 ----------GAT 60
E. amarillans E4668 58 ----------GAT 60
E. brachyelytri 703 CGAGTCTGTCATGTCTGAAAGACAAgtgagtcagtcctctcgtgcctggt 752
C. purpurea 803 CGGGCCTATCGTGTCTGAAAGAACgtgcgtcttctctcatgcctctgat 852
E. festucae Fl1 866 cgatgcaacgacagaaactccagccccctg--acaacctgactgaatccg 913
E. festucae E2368 757 cgatgcaacgacagaaactccagccccctg--acaacctgactgaatccg 804
E. baconii 753 cgatgcaacgacagaaactccagccccctg--acaacctgactgaatccg 800
E. sylvatica 753 cgatgcaacgacagaaactcaaggccccctg--gcaacctgactgaatccg 800
E. typhina 757 cgatgcaacgacagaaactcaaggccccctg--acaacctgactgaatccg 804
E. elymi
E. amarillans E57 61 CAGTACACGCATGAAAAGTTCAGCTTATTG--TCGA---------- 94
E. amarillans E4668 61 CAGTACACGCGTGAAAAGTTCAGCTTATTG--TCGA---------- 94
E. brachyelytri 753 tgatgcaacgacagaaactccagccccccgg--acaacttgaccgaaactcg 800
C. purpurea 853 t----------tctcattttcccacctcaacgcagcctaaactg 886
E. festucae Fl1 914 agctttcaggggcgtccggaaagtattgCGACGCTGTCGTG 953
E. festucae E2368 805 agctttcaggggcgtccggaaagtattgCGACGCTGTCGTG 844
E. baconii 801 agctttcagGGCGTCCGGAAAGTATTGCGACGCTGTCGTG 840
E. sylvatica 801 agctttcagggcgtccggaaagtattgcgacgctgtcgtg 840
E. typhina 805 agctttcagGGCGTCCGGAAAGTATTGCGACGCTGTCGTG 844
E. elymi
E. amarillans E57 95 ----------TGCAAGGGGCGATGTAACCCTATGATG 121
E. amarillans E4668 95 ----------TGCAAGGGGCGATGTAACCCTATGATG 121
E. brachyelytri 801 agctttcaggggcgtccggaaagtattgCGACGCTGTTGTG 840
C. purpurea 887 aatgtttcgactcggccagCGCGTCCGGAAAGTACTGCGACGCTGTCGTG 936
E. festucae Fl1 954 GCTGACTGGGCCAACCAGCCGAACTACACCAACGCTCAGACGTGTTCTCA 1003
E. festucae E2368 845 GCTGACTGGGCCAACCAGCCGAACTACACCAACGCTCAGACGTGTTCTCA 894
E. baconii 841 GCTGACTGGGCCAACCAGCCGAACTACACCAACGCTCAGACGTGTTCTCA 890
E. sylvatica 841 gctgactgggccaaccaGCCGAACTACACCAACGCTCAGACGTGTTCTCA 890
E. typhina 845 GCTGACTGGGCCAACCAGCCGAACTACACCAACGCTCAGACGTGTTCTCA 894
E. elymi
E. amarillans E57 122 GACGG----------AATCGAATCTCTCTAAGCTTCCTGCATG------ 154
E. amarillans E4668 122 GACGG----------AATCGAATTTTTCTAAGCTTCCTGCATG------ 154
E. brachyelytri 841 GCTGACTGGGCCAACCAGCCGAACTACACCAACGCTCAGACGTGTTCTCA 890
C. purpurea 937 GCAGACTGGGCCAACCAGCCAAACTACACATCGGCTCAGACATGTTCCCA 986
E. festucae Fl1 1004 GTGCGAGCTGGGCGTACAGCAGATACAGCTGGGATCACCGTTTGGGTTCG 1053
E. festucae E2368 895 GTGCGAGCTGGGCGTACAGCAGATACAGCTGGGATCACCGTTTGGGTTCG 944
E. baconii 891 GTGCGAGCTGGGCGTACAGCAGATACAGCTGGGATCACCGTTTGGGTTCG 940
E. sylvatica 891 GTGCGAGCTGGGCATACAGCAGATACAGCTGGGATCACCGTTTGGGTTCG 940
E. typhina 895 GTGCGAGCTGGGCATACAGCAGATACAGCTGGGATCACCGTTTGGGTTCG 944
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 891 GTGCGAGCTGGGCGTACAGCAGATACAGCTGGGATCACCGTTTGGGTTCG 940
C. purpurea 987 ATGCGAGCTGGGCATCCAGCAACTGCAGCTAGCATCACCATTCGGCTTCA 1036

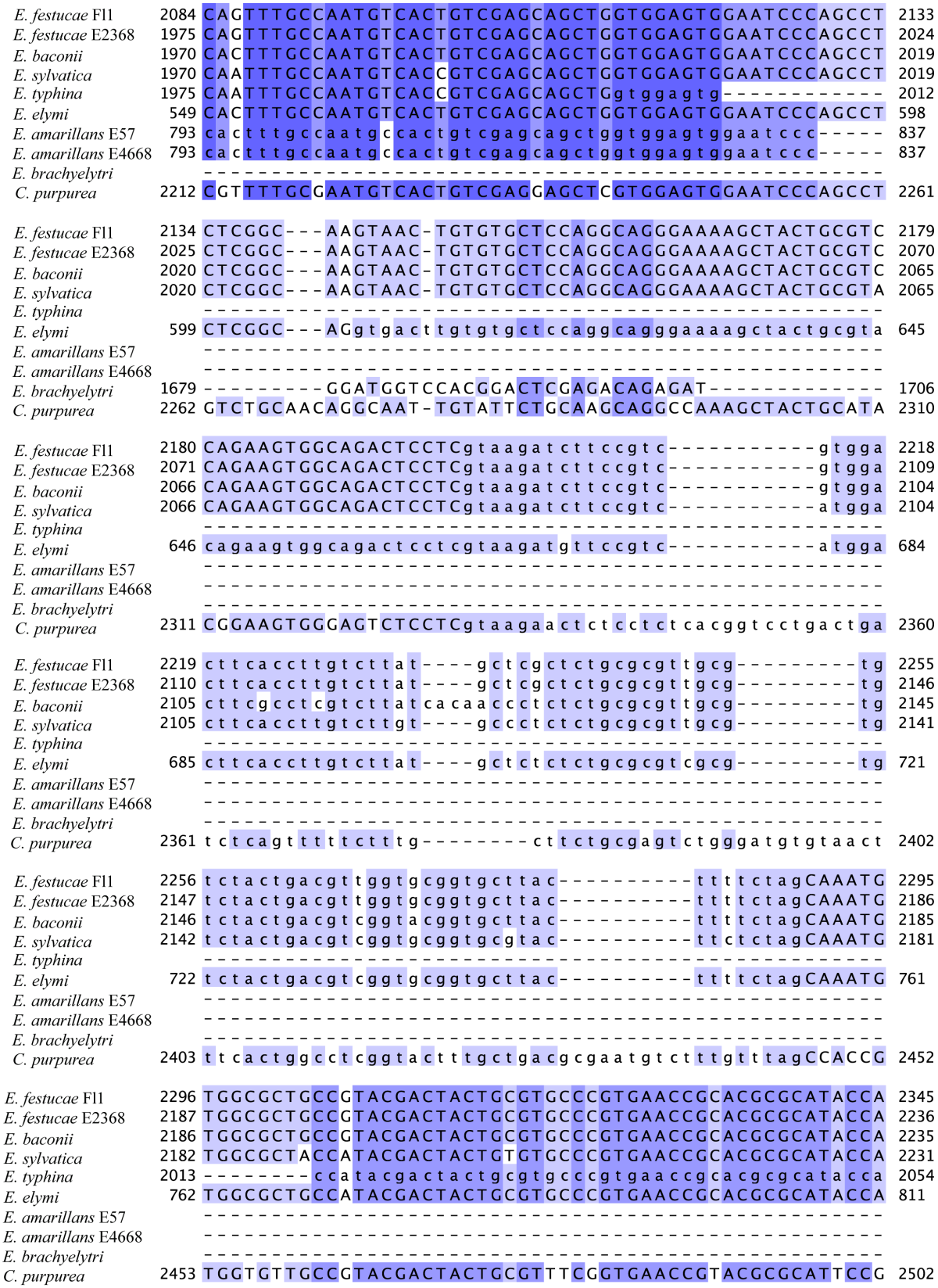

E. festucae Fl1 1054 GCGACACGCTCGCTCGGGCTTTCGCGGATACAACAAAGAGCTGTAGCGCG 1103
E. festucae E2368 945 GCGACACGCTCGCTCGGGCTTTCGCGGATACAACAAAGAGCTGTAGCGCG 994
E. baconii 941 GCGACACGCTCGCTCGGGCTTTCGCGGATACAACAAAGAGCTGTAGCGCG 990
E. sylvatica 941 GCGACACGCTCGCTCGGGCTTTCGCGGATACAACAGAGAGCTGTAGCGCG 990
E. typhina 945 GCGACACGCTCGCTCGGGCTTTCGCGGATACAACAAAGAGCTGTGGCGCG 994
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 941 GCGACACGCTCGCTCGGGCTTTCGCGGATACAACAAAGAGCTGTAGCGCG 990
C. purpurea 1037 GCGACACCCTCGCGCAGGCATTCGCCAACACGACCCAGAGCTGCAGCGCG 1086
E. festucae Fl1 1104 GCAAATTATGGCTTCGCTACGCCAACATCTTATGCGCTCAATGCCACTGC 1153
E. festucae E2368 995 GCAAATTATGGCTTCGCTACGCCAACATCTTATGCGCTCAATGCCACTGC 1044
E. baconii 991 GCAAATTATGGCTTCGCTACGCCAACATCTTATGCGCTCAATGCCACTGC 1040
E. sylvatica 991 GCAAATTATGGCTTCGCCACGCCAACATCTTATGCGCTCAATGCCACCGC 1040
E. typhina 995 GCAAATTATGGCTTCGCTACGCCAACATCTTATGCGCTCAATGCCACCGC 1044
E. elymi
E. amarillans E57 155 CGGCATCTCGTAGGTGCCGGGTCAT--- 179
E. amarillans E4668 155 CTGCATCTCGTAGGTGCCGGGTCAT--- 179
E. brachyelytri 991 GCAAATTATGGCTTCGCTACGCCAACATCTTATGCGCTCAATGCCACTGC 1040
C. purpurea 1087 GCAGACTACAAGTTCGCCACGCCGACATCCTACGCGCTCAACGCGACTCA 1136
E. festucae Fl1 1154 GTTTCCCTCT---CCACCTGCCTGTACGGGATCAGCATACACAATCAAGC 1200
E. festucae E2368 1045 GTTTCCCTCT---CCACCTGCCTGTACGGGATCAGCATACACAATCAAGC 1091
E. baconii 1041 GTTTCCCTCT---CCACCTGCCTGTACGGGATCAGCATACACAATCAAGC 1087
E. sylvatica 1041 GTTTCCCTCT---CCACCTGCCTGTACGGGATCGGCATACACAATCAAGC 1087
E. typhina 1045 GTTTCCCTCT---CCACCTGCCTGTACGGGATCGGCATACACAATCAAGC 1091
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 1041 GTTGCCCTCT---CCACCTGCCTGTACGGGATCAGCATACACAATCAAGC 1087
C. purpurea 1137 GTTTCCCTCCCAGCCGCCGGCCTGCACCGGGACGGCCTACACTATCACAC 1186
E. festucae Fl1 1201 AGAATGACTCGTGCGTCACCATCTCGGCAGCCAACGGCATGTCGACATAC 1250
E. festucae E2368 1092 AGAATGACTCGTGCGTCACCATCTCGGCAGCCAACGGCATGTCGACATAC 1141
E. baconii 1088 AGAATGACTCGTGCGTCACCATCTCGGTAGCCAACGGCATGTCGACATAC 1137
E. sylvatica 1088 AGGATGACTCGTGCGTCACCATCTCGGTAGCCAACGGCATGTCGACATAC 1137
E. typhina 1092 AGGATGACTCGTGCGTCACCATCTCGGTAGCCAACGGCATGTCGACATAC 1141
E. elymi
E. amarillans E57 180 ---ATGGCCCAGG- 189
E. amarillans E4668 180 ---ATGGTCCAGG- 189
E. brachyelytri 1088 AGAATGACTCGTGCGTCACCATCTCGGTAGCCAACGGCATGTCGACATAC 1137
C. purpurea 1187 AGGATGACTCGTGTGTCACCATCTCGGGTGCTAAGGGCATCTCGACGCAC 1236
E. festucae Fl1 1251 CAGTTGATCACCAAGAACAATCTCAACCTCGGATGCGACAATCTCCCTGC 1300
E. festucae E2368 1142 CAGTTGATCACCAAGAACAATCTCAACCTCGGATGCGACAATCTCCCTGC 1191
E. baconii 1138 CAGTTGATCACCAAGAACAATCTCAACCTCGGATGCGACAATCTCCCTGC 1187
E. sylvatica 1138 CAGTTGATCACCAAGAACAATCTCAACCTCGGATGCGACAATCTCCCTGC 1187
E. typhina 1142 CAGTTGATCACCAATAACAATCTCAACCTCGGATGCGACAATCTCCCTGC 1191
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 1138 CAGTTGATCACCAAGAACAATCTCAACCTCGGATGCGACAATCTCCCTGC 1187
C. purpurea 1237 CAGCTCATTACAATGAACAACCTGAATCTCGGATGTGACAATCTTCCCGC 1286
E. festucae Fl1 1301 TGTAGGTCAATCGCTCTGTTTGCCCACCGGGAGCACCTGCCGTACTTATC 1350
E. festucae E2368 1192 TGTAGGTCAATCGCTCTGTTTGCCCACCGGGAGCACCTGTCGTACTTATC 1241
E. baconii 1188 TGTAGGTCAATCGCTCTGTTTGCCCACCGGGAGCACCTGCCGTACTTATC 1237
E. sylvatica 1188 TGTAGGTCAATCGCTCTGTTTGCCCACCGGGAGCACCTGCCGTACTCATC 1237
E. typhina 1192 TGTAGGTCAATCGCTCTGTTTGCCCACCGGGAGCACCTGCCGTACTCATC 1241
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri 1188 Cgtactcatc 1197
C. purpurea 1287 GGCAGGACAGTCGCTATGTCTGCCTGCGGGGAGCACGTGTCGAACATATC 1336

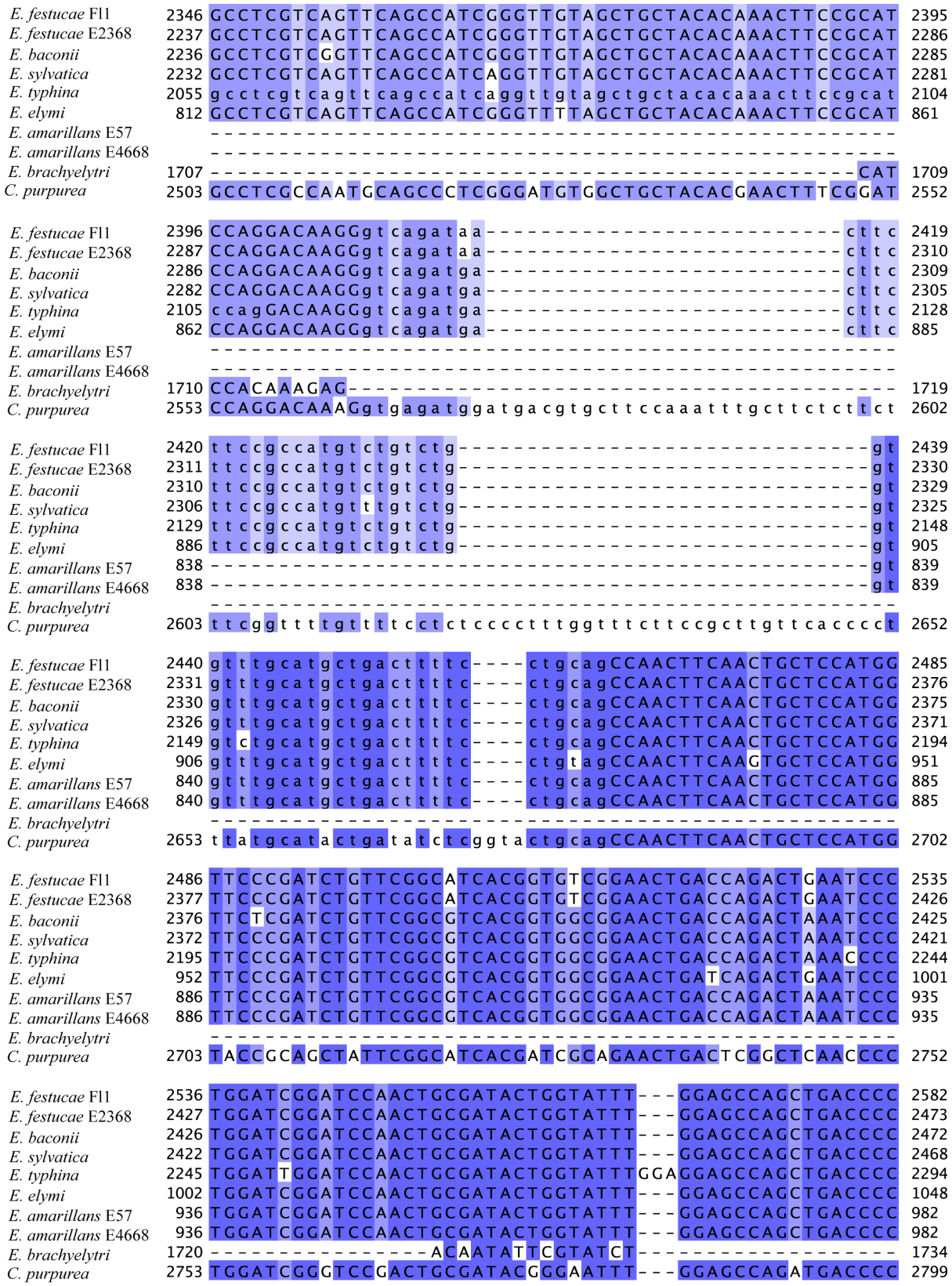

E. festucae Fl1 1351 AGTTGGATCTCTACGACACCTGCGAGACTCTGATGAAGAGCTGGAATGCA 1400
E. festucae E2368 1242 AGTTGGATCTCTACGACACCTGCGAGACTCTGATGAAGAGCTGGAATGCA 1291
E. baconii 1238 AGTTGGATCTCTACGACACCTGCGAGACTCTGATGAAGAGCTGGAATGCA 1287
E. sylvatica 1238 AGTTGGATCTCTACGACACCTGCGAGACTCTGATGAAGAGCTGGAATGCA 1287
E. typhina 1242 AGTTGGATCTCTACGACACCTGCGAGAGTCTGATGAAGAGCTGGAATGCA 1291
E. elymi 77 ----------TCTACAA---------- 83
E. amarillans E57
E. amarillans E4668
E. brachyelytri 1198 agTTGGATCTCTACGACACCTGCGAGACTCTGATGAAGAGCTGGAATGCA 1247
C. purpurea 1337 AGCTTGAACTCTATGACACGTGCGAGACACTGATGTCGAGCTGGAATGCG 1386
E. festucae Fl1 1401 ACCCTTGCTCAAATCCAGGCATGGAACCCAATGATCAACTCGGCCTGTTC 1450
E. festucae E2368 1292 ACCCTTGCTCAAATCCAGGCATGGAACCCAATGATCAACTCGGCCTGTTC 1341
E. baconii 1288 ACCCTTGCTCAAATCCAGGCATGGAACCCAATGATCAACTCGGCCTGTTC 1337
E. sylvatica 1288 ACCCTTGCTCAAATCCAGGCATGGAACCCAATGATCAACTCGGCCTGTTC 1337
E. typhina 1292 ACCCTTGCTCAAATCCAGGCATGGAACCCAATGATCAACTCGGCCTGTTC 1341
E. elymi
E. amarillans E57 190 ----------GTCC 193
E. amarillans E4668 190 ----------GTCC 193
E. brachyelytri 1248 ACCCTTGCTCAAATCCAGGCATGGAACCCAATGATCAACTCGGCCTGTTC 1297
C. purpurea 1387 ACGCTTGCGCAAGTCTTGGCTTGGAACCCAATGATCAACCCATCTTGTTC 1436
E. festucae Fl1 1451 TAACCTGGCGTCGTGGAGAGGCTGGTATCTTTGTTCGAGgtaagccaaaa 1500
E. festucae E2368 1342 TAACCTGGCGTCGTGGAGAGGCTGGTATCTTTGTTCGAGgtaagccaaaa 1391
E. baconii 1338 TAACCTGGCGTCGTGGAGAGGCTGGTATCTTTGTTCGAGgtaagctaaaa 1387
E. sylvatica 1338 TAACCTGGCGTCGTGGAGAGGCTGGTATCTTTGTTCGAGgtaagctaaaa 1387
E. typhina 1342 TAACCTGGCGTCGTGGAGAGGCTGGTATCTTTGTTCGAGgtaagctgaaa 1391
E. elymi
E. amarillans E57 194 CACCTCGATGTTG----------AA 208
E. amarillans E4668 194 CACCTCGATGTTG----------AA 208
E. brachyelytri 1298 TAACCTGGCGTCGTGGAGAGGCTGgtatcttgttcgagggaagctaaaa 1347
C. purpurea 1437 GAATCTAGCTTCGTGGAGAGGCTGGTATCTCTGTGCAAGgtgagc----- 1481
E. festucae Fl1 1501 agttcccgtcatctacaagttgtcgtcatcttgacaca---------- 1540
E. festucae E2368 1392 agttcccgtcatctacaagttgtcgtcatcttgacaca---------- 1431
E. baconii 1388 ag-ttcccgtcatctacaagttgtcgtcatcttgacaca---------- 1426
E. sylvatica 1388 ag-ttcccgtcatctacaggttgtcgtcatcttgacaca---------- 1426
E. typhina 1392 at-ttcccgtcatctacaggttgtcgtcatcttgacaca---------- 1430
E. elymi 84 ---GTTGTCGTCATCTTCAAGTTGTCGTTATCTTGACACA---------- 120
E. amarillans E57 209 AG-TTCCCTGTCATCTACAAGTTGTCGTCATCTTGACA---------- 245
E. amarillans E4668 209 AG-TTCCCTGTCATCTACAAGTTGTCGTCATCTTGACA---------- 245
E. brachyelytri 1348 ag-ttcccgtcatctacaagttgtcgtcatcttgacaca---------- 1386
C. purpurea 1482 ---ttcaacaccat-tgtgcgccattgttgtctctaccagtgatgaggag 1527
E. festucae Fl1
E. festucae E2368
E. baconii
E. sylvatica
E. typhina
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri
C. purpurea 1528 gggtacgagaggcagcactggactacgaagatcgaacagtgcataactgt 1577
E. festucae Fl1 1541 -----acaacatgat----------agCTCTCCATCGGGGACAGT 1570
E. festucae E2368 1432 -----acaacatgat----------agCTCTCCATCGGGGACAGT 1461
E. baconii 1427 -----acaacatgat----------agCTCTCAATCGGGGACAGT 1456
E. sylvatica 1427 -----acaacatgat----------agCTCTCCGTCGGGGACAGC 1456
E. typhina 1431 -----acaacatgat----------aagctctccgtcgggggacagC 1461
E. elymi 121 -----GCAACATGAT----------GGCTCTCCATCGGGGACAGT 150
E. amarillans E57 246 ------CATCATGAC----------AGCTCTCCATCGGGGACAGT 274
E. amarillans E4668 246 ------CATCATGAC----------AGCTCTCCATCGGGGACAGT 274
E. brachyelytri 1387 -----acaacatgat----------agCTCTCCATCGGGGACAGT 1416
C. purpurea 1578 tccactcagcgcaactctaatgctactcgcagCTCACCATCAGGAACAAA 1627

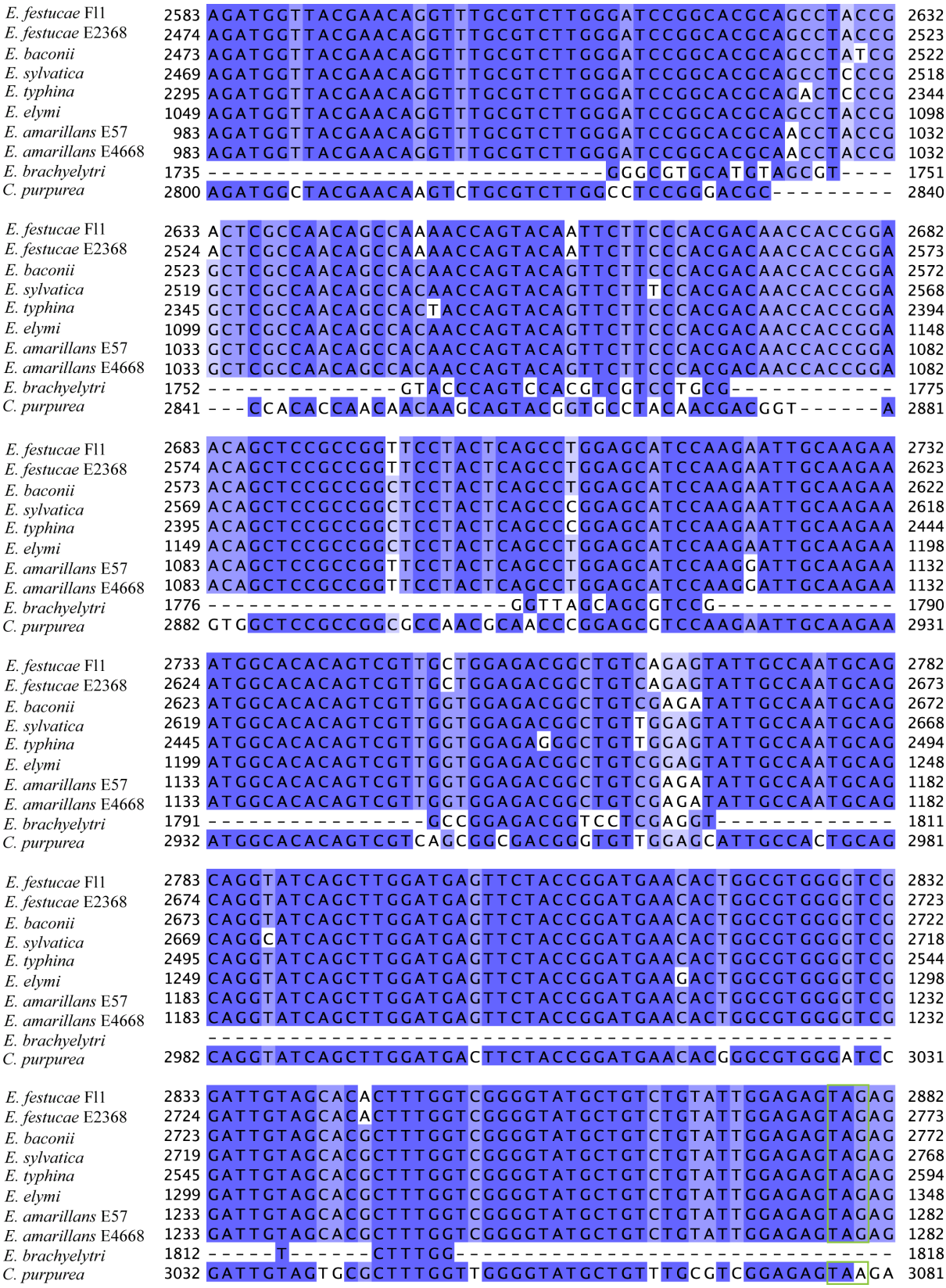

E. festucae Fl1 1571 CACTGTCGGTTTGGGCGACACCGTGACGACTCCAGCCCCAGTGCCGACGA 1620
E. festucae E2368 1462 CACTGTCGGTTTGGGCGACACCGTGACGACTCCAGCCCCAGTGCCGACGA 1511
E. baconii 1457 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 1506
E. sylvatica 1457 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 1506
E. typhina 1462 CACTGTCGGTTTGGGCAACACCGTGACGACCGCAGCCCCGGTGCCGACGA 1511
E. elymi 151 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 200
E. amarillans E57 275 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 324
E. amarillans E4668 275 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 324
E. brachyelytri 1417 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 1466
C. purpurea 1628 CGTCGTTGGGCCCGGTAACGCAGTGACAACGCCCGCTCCTGTGCCATCGA 1677
E. festucae Fl1 1621 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGTgtaagt 1670
E. festucae E2368 1512 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGTgtaagt 1561
E. baconii 1507 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGAgtaagt 1556
E. sylvatica 1507 ATGCACAGGGCCAATCCAATACAAGGTGTGGACAGTGGTACCGTgtaagt 1556
E. typhina 1512 ATGCACAGGGCCAATCCAATACAAGgtgtggacagtggtaccgtctaagt 1561
E. elymi 201 ATGCACAGGGCCAATCCAATACAAGGTGTGGACAGTGGTACCGTgtaagt 250
E. amarillans E57 325 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGTgtaagt 374
E. amarillans E4668 325 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGTgtaagt 374
E. brachyelytri 1467 ATGCACAGGGCCAATCCAATACTAGgtgtggatagtggtaccgtgtaagt 1516
C. purpurea 1678 ACGCGCAGCCGCAGTCCAACACGAGATGTGGGCAGTGGTACTATgtaagt 1727
E. festucae Fl1 1671 cgcttctcaacgtatttactcgg----------tcaaccatggtcta 1707
E. festucae E2368 1562 cgcttctcaacgtatttactcgg----------tcaaccatggtcta 1598
E. baconii 1557 cgcttctcaacgtatctactcgg----------tcaaccatggtcta 1593
E. sylvatica 1557 cgcttctcaacgtatttactcgg----------tcaaccatgatcta 1593
E. typhina 1562 cgcttctcaacgtatttcactcgg----------tcgaccatgatcta 1598
E. elymi 251 cgcttctcaacgtatttta---------- 268
E. amarillans E57 375 cgcttctcaacgtattgactcgg----------tcaaccatggtcta 411
E. amarillans E4668 375 cgcttctcaacgtattgactcgg----------tcaaccatggtcta 411
E. brachyelytri 1517 cgcttctcaacgtatttacttgg----------tcaaccatgatcta 1553
C. purpurea 1728 cg---------gcattatgctggtccttgaaaatcatcagtcagcgccca 1768
E. festucae Fl1 1708 aacctctcttgt---------- 1719
E. festucae E2368 1599 aacctctcttgt---------- 1610
E. baconii 1594 agcctctcttgt---------- 1605
E. sylvatica 1594 acgctctctcgt---------- 1605
E. typhina 1599 acgctctctcgt---------- 1610
E. elymi ----------
E. amarillans E57 412 aactgctcttgt---------- 423
E. amarillans E4668 412 aacttctcttgt---------- 423
E. brachyelytri 1554 acgctctcttgc---------- 1565
C. purpurea 1769 aagatctgtaacagagagaatgccccatactcacagtcatcttcatcatc 1818
E. festucae Fl1 1720 ----------aaagGTCGAGGCTGGCGACTA 1740
E. festucae E2368 1611 ----------aaagGTCGAGGCTGGCGACTA 1631
E. baconii 1606 ----------aaagGTCGAGGCTGGCGACTA 1626
E. sylvatica 1606 ----------aaagGTCGAGGCTGGCGACTA 1626
E. typhina 1611 ----------aaagGTCGAGGCTGGCGACTA 1631
E. elymi ----------
E. amarillans E57 424 ----------aaagGTCGAGGCTGGCGACTA 444
E. amarillans E4668 424 ----------aaagGTCGAGGCTGGCGACTA 444
E. brachyelytri 1566 ----------aaagGGCAAGGCTGGCGACTA 1586
C. purpurea 1819 atcatcatcacactatactaatggaaataaaagGTTGAAGCCGGCGAGTA 1868
E. festucae Fl1 1741 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCTGTCTCGGACTTTTATT 1790
E. festucae E2368 1632 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCTGTCTCGGACTTTTATT 1681
E. baconii 1627 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCCCTCTCGGACTTTTATT 1676
E. sylvatica 1627 TTGCGCTGCAATATCGCTCAAGTTCAGCCTGGCCCTCTCGGACTTTTATT 1676
E. typhina 1632 TTGCGCTGCAATATCGCTCAAGTTCAGCCTGGCCCTCTCGGACTTTAATT 1681
E. elymi ----------
E. amarillans E57 445 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCCCTCTCGGACTTTTATT 494
E. amarillans E4668 445 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCCCTCTCGGACTTTTATT 494
E. brachyelytri 1587 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCCCTCTCGGACTTTTATT 1636
C. purpurea 1869 TTGCGCCGCCATCTCCCTCAAATTCAGCCTCGCCCTATCCGACTTCTACT 1918

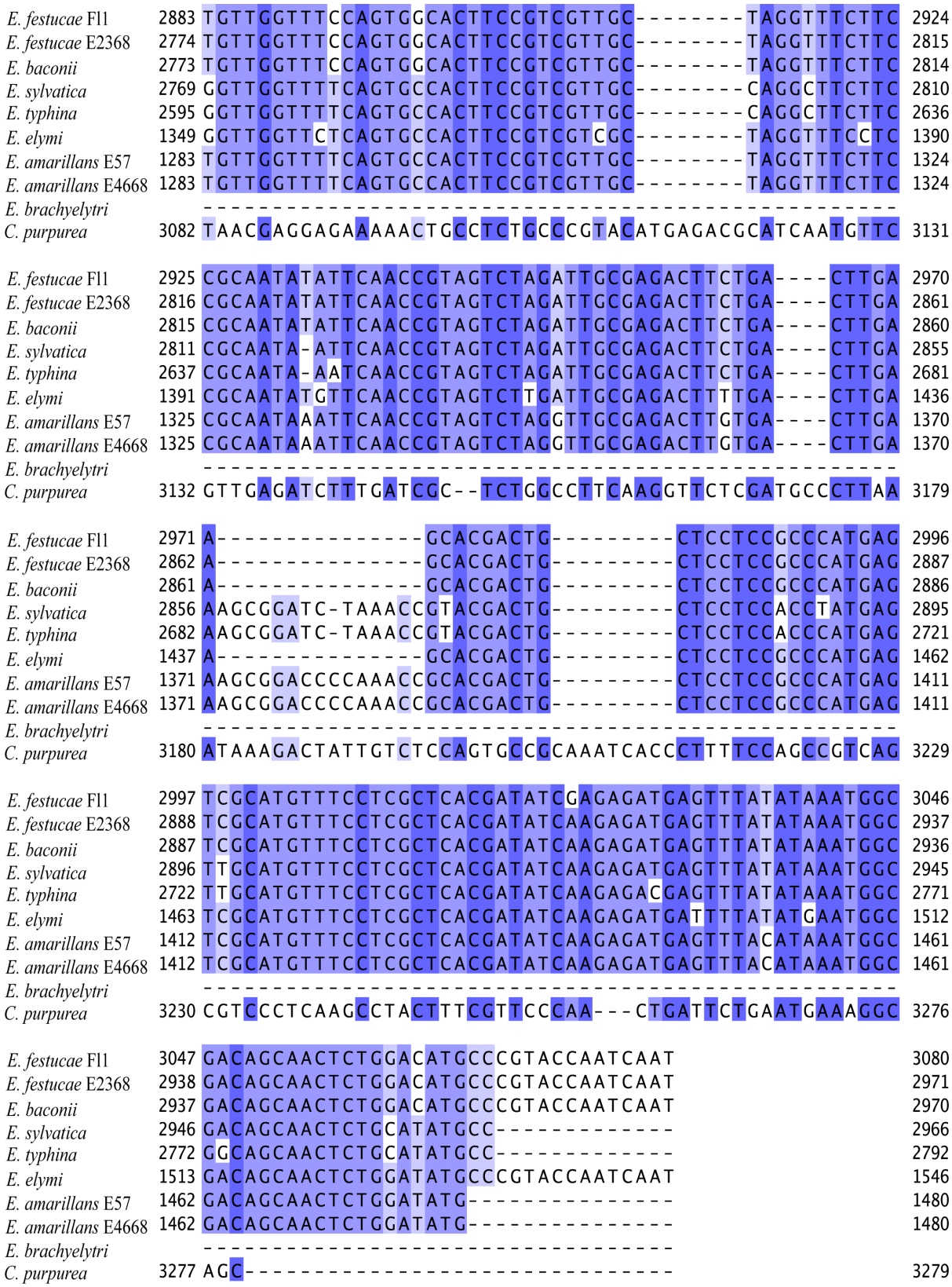

E. festucae Fl1 1571 CACTGTCGGTTTGGGCGACACCGTGACGACTCCAGCCCCAGTGCCGACGA 1620
E. festucae E2368 1462 CACTGTCGGTTTGGGCGACACCGTGACGACTCCAGCCCCAGTGCCGACGA 1511
E. baconii 1457 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 1506
E. sylvatica 1457 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 1506
E. typhina 1462 CACTGTCGGTTTGGGCAACACCGTGACGACCGCAGCCCCGGTGCCGACGA 1511
E. elymi 151 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 200
E. amarillans E57 275 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 324
E. amarillans E4668 275 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 324
E. brachyelytri 1417 CACTGTCGGTTTGGGCAACACCGTGACGACCCCAGCCCCAGTGCCGACGA 1466
C. purpurea 1628 CGTCGTTGGGCCCGGTAACGCAGTGACAACGCCCGCTCCTGTGCCATCGA 1677
E. festucae Fl1 1621 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGTgtaagt 1670
E. festucae E2368 1512 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGTgtaagt 1561
E. baconii 1507 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGAgtaagt 1556
E. sylvatica 1507 ATGCACAGGGCCAATCCAATACAAGGTGTGGACAGTGGTACCGTgtaagt 1556
E. typhina 1512 ATGCACAGGGCCAATCCAATACAAGgtgtggacagtggtaccgtctaagt 1561
E. elymi 201 ATGCACAGGGCCAATCCAATACAAGGTGTGGACAGTGGTACCGTgtaagt 250
E. amarillans E57 325 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGTgtaagt 374
E. amarillans E4668 325 ATGCACAGGGCCAATCCAATACTAGGTGTGGACAGTGGTACCGTgtaagt 374
E. brachyelytri 1467 ATGCACAGGGCCAATCCAATACTAGgtgtggatagtggtaccgtgtaagt 1516
C. purpurea 1678 ACGCGCAGCCGCAGTCCAACACGAGATGTGGGCAGTGGTACTATgtaagt 1727
E. festucae Fl1 1671 cgcttctcaacgtatttactcgg----------tcaaccatggtcta 1707
E. festucae E2368 1562 cgcttctcaacgtatttactcgg----------tcaaccatggtcta 1598
E. baconii 1557 cgcttctcaacgtatctactcgg----------tcaaccatggtcta 1593
E. sylvatica 1557 cgcttctcaacgtatttactcgg----------tcaaccatgatcta 1593
E. typhina 1562 cgcttctcaacgtatttcactcgg----------tcgaccatgatcta 1598
E. elymi 251 cgcttctcaacgtatttta---------- 268
E. amarillans E57 375 cgcttctcaacgtattgactcgg----------tcaaccatggtcta 411
E. amarillans E4668 375 cgcttctcaacgtattgactcgg----------tcaaccatggtcta 411
E. brachyelytri 1517 cgcttctcaacgtatttacttgg----------tcaaccatgatcta 1553
C. purpurea 1728 cg---------gcattatgctggtccttgaaaatcatcagtcagcgccca 1768
E. festucae Fl1 1708 aacctcttgt---------- 1719
E. festucae E2368 1599 aacctcttgt---------- 1610
E. baconii 1594 agcctcttgt---------- 1605
E. sylvatica 1594 acgctcttcgt---------- 1605
E. typhina 1599 acgctcttcgt---------- 1610
E. elymi ----------
E. amarillans E57 412 aactgcttgt---------- 423
E. amarillans E4668 412 aacttcttgt---------- 423
E. brachyelytri 1554 acgcttcttgc---------- 1565
C. purpurea 1769 aagatctgtaacagagagaatgccccatactcacagtcatcttcatcatc 1818
E. festucae Fl1 1720 ----------aaagGTCGAGGCTGGCGACTA 1740
E. festucae E2368 1611 ----------aaagGTCGAGGCTGGCGACTA 1631
E. baconii 1606 ----------aaagGTCGAGGCTGGCGACTA 1626
E. sylvatica 1606 ----------aaagGTCGAGGCTGGCGACTA 1626
E. typhina 1611 ----------aaagGTCGAGGCTGGCGACTA 1631
E. elymi ----------
E. amarillans E57 424 ----------aaagGTCGAGGCTGGCGACTA 444
E. amarillans E4668 424 ----------aaagGTCGAGGCTGGCGACTA 444
E. brachyelytri 1566 ----------aaagGGCAAGGCTGGCGACTA 1586
C. purpurea 1819 atcatcatcacactatactaatggaaataaaagGTTGAAGCCGGCGAGTA 1868
E. festucae Fl1 1741 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCTGTCTCGGACTTTTATT 1790
E. festucae E2368 1632 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCTGTCTCGGACTTTTATT 1681
E. baconii 1627 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCCCTCTCGGACTTTTATT 1676
E. sylvatica 1627 TTGCGCTGCAATATCGCTCAAGTTCAGCCTGGCCCTCTCGGACTTTTATT 1676
E. typhina 1632 TTGCGCTGCAATATCGCTCAAGTTCAGCCTGGCCCTCTCGGACTTTAATT 1681
E. elymi ----------
E. amarillans E57 445 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCCCTCTCGGACTTTTATT 494
E. amarillans E4668 445 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCCCTCTCGGACTTTTATT 494
E. brachyelytri 1587 TTGCGCTGCAATATCGCTCAAGTTCAGCCTAGCCCTCTCGGACTTTTATT 1636
C. purpurea 1869 TTGCGCCGCCATCTCCCTCAAATTCAGCCTCGCCCTATCCGACTTCTACT 1918


E. festucae Fl1 1791 TCCTCAACCCCCAAGTCAATTCAACCTGTGGCAATCTGTGGGCCAACACT 1840
E. festucae E2368 1682 TCCTCAACCCCCAAGTCAATTCAACCTGTGGCAATCTGTGGGCCAACACT 1731
E. baconii 1677 TCCTCAACCCCCAAGTCGATTCAACCTGTGGCAATCTGTGGGCCAACACT 1726
E. sylvatica 1677 TTCTCAACCCCCAAGTCGATTCAACCTGTGGCAATCTGTGGGCCAACACT 1726
E. typhina 1682 TTCTCAACCCCCAAGTCGATTCAACCTGTGGCAATCTGTGGGCCAACACT 1731
E. elymi 269 ----------tcgattcaacctgtggcaatctgtgggccaacact 303
E. amarillans E57 495 TCCTCAACCCCCAAGTCGATTCAGCCTGTGGCAATCTGTGGGCCAACACT 544
E. amarillans E4668 495 TCCTCAACCCCCAAGTCGATTCAACCTGTGGCAATCTGTGGGCCAACACT 544
E. brachyelytri 1637 TCCTCAACCCCCAAGTCGATTCA--TCGATTCAACCTGT-GGCCG----- 1678
C. purpurea 1919 TTCTCAACCCTCAGATCAACCCAACCTGCAGCAATCTCTGGGCCAAGACC 1968
E. festucae Fl1 1841 TCGTACTGCGTCAAGCCTGTAGGGAACAT--AGAGACCTACTCCGGCTAC 1888
E. festucae E2368 1732 TCGTACTGCGTCAAGCCTGTAGGGAACAT--AGAGACCTACTCCGGCTAC 1779
E. baconii 1727 TCGTACTGCGTCAAGCCTGTAGGGAACAT--AGAGACCTACTCCGGCTAC 1774
E. sylvatica 1727 TCGTACTGCGTCAAGCCTGTAGGGAACAT--AGAGACCTACTCCGGCTAC 1774
E. typhina 1732 TCGTACTGCGTCAAGCCTGTAGGGAACAT--AGAGACCTACTCCGGCTAC 1779
E. elymi 304 tcgtactgcgtcaagcctgcagGGAACATAGAGAGACCTACTCCGGCTAC 353
E. amarillans E57 545 TCGTACTGCGTCAAGCCTGTAGGGAACAT--AGAGACCTACTCCGGCTAC 592
E. amarillans E4668 545 TCGTACTGCGTCAAGCCTGTAGGGAACAT--AGAGACCTACTCCGGCTAC 592
E. brachyelytri ----------
C. purpurea 1969 TCCTACTGCGTGAAACCAGTCGGCAACGT--CGAAACCTACCCAGGCTAC 2016
E. festucae Fl1 1889 ACGACTACTGCGGCGGCAACCACGTTCACGCGTCCAGTGACGACAACCGA 1938
E. festucae E2368 1780 ACGACTACTGCGGCGGCAACCACGTTCACGCGTCCAGTGACGACAACCGA 1829
E. baconii 1775 ACGACTACTGCGGCGGCAACCACGTTCACGCGTCCAGTGACGACAACCGA 1824
E. sylvatica 1775 ACGACTACCGCGGCGGCAACCACGTTCACGCGTCCAGTGACGACAACCGA 1824
E. typhina 1780 ACGACTACCGCGGCGGCAACCACGTTCACGCGTCCAGTGACGACAACCGA 1829
E. elymi 354 ACGACTACTGCGGCGGCAACCACGTTCACGCGTCCAGTGACGACAACCGA 403
E. amarillans E57 593 ACGACTGCTGCGGCGGCAACCACGTTCACGCGTCCAGTGACGACAACCGA 642
E. amarillans E4668 593 ACGACTGCTGCGGCGGCAACCACGTTCACGCGTCCAGTGACGACAACCGA 642
E. brachyelytri ----------
C. purpurea 2017 ACGACGACTGCACCAGCCACCACATTCACACGCCCGGCCTCGACAACCGA 2066
E. festucae Fl1 1939 CTTCGT-----GCCCAGCCCAGTCCAGACAGCTACCTTGCAGCCCAAGGC 1983
E. festucae E2368 1830 CTTCGT-----GCCCAGCCCAGTCCAGACAGCTACCTTGCAGCCCAAGGC 1874
E. baconii 1825 CTTCGT-----GCCCAGCCCAGTCCAGACAGCCACCTTGCAGCCCAAGGC 1869
E. sylvatica 1825 CTTCGT-----GCCCAGCCCAGTCCAGACAGCTACCTTGCAGCCCAAGGC 1869
E. typhina 1830 CTTCGT-----GCCCAGCCCAGTCCAGACAGCTACCTTGCAGCCCAAGGC 1874
E. elymi 404 CTTCGT-----GCCCAGCCCAGTCCAGACAGCTACCTTGCAGCCCAAGGC 448
E. amarillans E57 643 CTTCGTGCCCAGCCCAGCCCAGTCCAAACAGCTACCTTGCAGCCCAAGGC 692
E. amarillans E4668 643 CTTCGTGCCCAGCCCAGCCCAGTCCAAACAGCTACCTTGCAGCCCAAGGC 692
E. brachyelytri ----------
C. purpurea 2067 CTTCGT-----GCCCAGCCCAATCGAAACGCCGGCCTTGCTCCGCGAGC 2111
E. festucae Fl1 1984 CTCCGGCACTATCAGTGACTGCCACGTGTATGAGAACGCGTTTGACGCCA 2033
E. festucae E2368 1875 CTCCGGCACTATCAGTGACTGCCACGTGTATGAGAACGCGTTTGACGCCA 1924
E. baconii 1870 CCCCGGCACTATCAGTGACTGCCACGTGTATGAGAACGCGTTTGACGCCA 1919
E. sylvatica 1870 CTCCGGCACTATAAGTGACTGCCAAGTGTATGAGAACGCGTTTGACGCCA 1919
E. typhina 1875 CTCCGGCACTATAAGTGACTGCCAAGTGTATGAGAACGCGTTTGACGCCA 1924
E. elymi 449 CTCCGGCACTATCAGTGACTGCCACGTGTATGAGAACGCGTTTGACGCCA 498
E. amarillans E57 693 CTCCGGCACTATCAGTGACTGCCACGTgtatgagaacgcgtttgacgcca 742
E. amarillans E4668 693 CTCCGGCACTATCAGTGACTGCCACGTgtatgagaacgcgtttgacgcca 742
E. brachyelytri ----------
C. purpurea 2112 CCCCGGCACCATCGATGGCTGCTTCGAGTACGAGAACGCATTCGATGCCA 2161
E. festucae Fl1 2034 CTTCCAAGCTGAAGAACTTGTCCGTGGCAAATTCGTGCTCTAGTTGGTCA 2083
E. festucae E2368 1925 CTTCCAAGCTGAAGAACTTGTCCGTGGCAAATTCGTGCTCTAGTTGGTCA 1974
E. baconii 1920 CTTCCAAGCTGAAGAAGCTGTCCGTGGCAAATTCGTGCTCTAGTTGGGCA 1969
E. sylvatica 1920 CTTCCAAGCTGAAGAACCTGTCCGTGGCAAATTCGTGCTCTAGTTGGGCA 1969
E. typhina 1925 CTTCCAAGCTGAAGAACCTGTCCGTGGCAAATTCGTGCTCTAGTTGGGCA 1974
E. elymi 499 CTTCCAAGCTGAAGAACCTGTCCGTGGCAAATTCGTGCTCTAGTTGGGCA 548
E. amarillans E57 743 cttccaagctgaagaagctgtccgtggcaaatcgtgctctagttgggca 792
E. amarillans E4668 743 cttccaagctgaagaagctgtccgtggcaaatcgtgctctagttgggca 792
E. brachyelytri ----------
C. purpurea 2162 CGTCCAAGTTGAAGGACCTTTCCGTGGGAAATGCGTGCTCCAACTGGGCG 2211

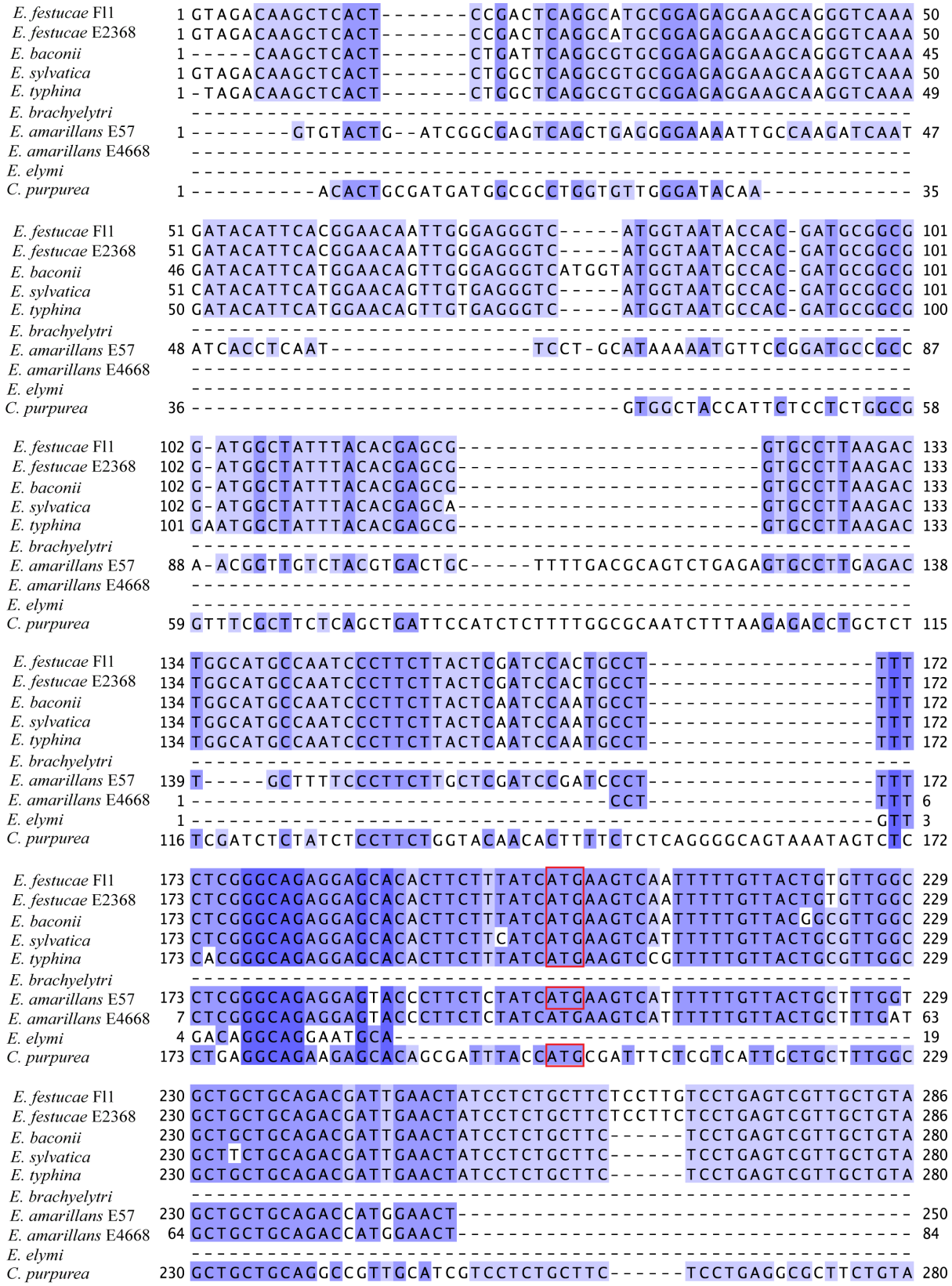

E. festucae Fl1 2084 CAGTTTGCCAATGTCACTGTCGAGCAGCTGGTGGAGTGGAATCCCAGCCT 2133
E. festucae E2368 1975 CAGTTTGCCAATGTCACTGTCGAGCAGCTGGTGGAGTGGAATCCCAGCCT 2024
E. baconii 1970 CACTTTGCCAATGTCACTGTCGAGCAGCTGGTGGAGTGGAATCCCAGCCT 2019
E. sylvatica 1970 CAATTTGCCAATGTCACCGTCGAGCAGCTGGTGGAGTGGAATCCCAGCCT 2019
E. typhina 1975 CAATTTGCCAATGTCACCGTCGAGCAGCTGgtggagtg---------- 2012
E. elymi 549 CACTTTGCCAATGTCACTGTCGAGCAGCTGGTGGAGTGGAATCCCAGCCT 598
E. amarillans E57 793 cactttgccaatgcactgtcgagcagctggtggagtggaatccc----- 837
E. amarillans E4668 793 cactttgccaatgcactgtcgagcagctggtggagtggaatccc----- 837
E. brachyelytri ----------
C. purpurea 2212 CGTTTTGCGAATGTCACTGTCGAGGAGCTCGTGGAGTGGAATCCCAGCCT 2261
E. festucae Fl1 2134 CTCGGC---AAGTAAC-TGTGTGCTCCAGGCAGGGAAAAGCTACTGCGTC 2179
E. festucae E2368 2025 CTCGGC---AAGTAAC-TGTGTGCTCCAGGCAGGGAAAAGCTACTGCGTC 2070
E. baconii 2020 CTCGGC---AAGTAAC-TGTGTGCTCCAGGCAGGGAAAAGCTACTGCGTC 2065
E. sylvatica 2020 CTCGGC---AAGTAAC-TGTGTGCTCCAGGCAGGGAAAAGCTACTGCGTA 2065
E. typhina ----------
E. elymi 599 CTCGGC---AGgtgacttgtgtgctccaggcagggaaaagctactgcgta 645
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. brachyelytri 1679 ---------GGATGGTCCACGGACTCGAGACAGAGAT---------- 1706
C. purpurea 2262 GTCTGCAACAGGCAAT-TGTATTCTGCAAGCAGGCCAAAGCTACTGCATA 2310
E. festucae Fl1 2180 CAGAAGTGGCAGACTCCTCgtaagatcttccgtc----------gtgga 2218
E. festucae E2368 2071 CAGAAGTGGCAGACTCCTCgtaagatcttccgtc----------gtgga 2109
E. baconii 2066 CAGAAGTGGCAGACTCCTCgtaagatcttccgtc----------gtgga 2104
E. sylvatica 2066 CAGAAGTGGCAGACTCCTCgtaagatcttccgtc----------atgga 2104
E. typhina ----------
E. elymi 646 cagaagtggcagactcctcgtaagatgttccgtc----------atgga 684
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. brachyelytri ----------
C. purpurea 2311 CGGAAGTGGGAGTCTCCTCgtaagaactcctcctcacggtcctgactga 2360
E. festucae Fl1 2219 cttcaccttgtcttat----gctcgctctgcgcgttgcg---------tg 2255
E. festucae E2368 2110 cttcaccttgtcttat----gctcgctctgcgcgttgcg---------tg 2146
E. baconii 2105 cttcgcctcgtcttatcacaaccctctctgcgcgttgcg---------tg 2145
E. sylvatica 2105 cttcaccttgtcttgt----gccctctctgcgcgttgcg---------tg 2141
E. typhina ----------
E. elymi 685 cttcaccttgtcttat----gctctctctgcgcgtcgcg---------tg 721
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. brachyelytri ----------
C. purpurea 2361 tctcagttttcttg--------ctctgcgagtctgggatgtgtaact 2402
E. festucae Fl1 2256 tctactgacgttggtgcggtgcttac----------tttctagCAAATG 2295
E. festucae E2368 2147 tctactgacgttggtgcggtgcttac----------tttctagCAAATG 2186
E. baconii 2146 tctactgacgtcggtacggtgcttac----------tttctagCAAATG 2185
E. sylvatica 2142 tctactgacgtcggtgcggtgcgtac----------ttctagCAAATG 2181
E. typhina ----------
E. elymi 722 tctactgacgtcggtgcggtgcttac----------tttctagCAAATG 761
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. brachyelytri ----------
C. purpurea 2403 ttcactggcctcggtactttgctgacgcgaatgtctttgttagCCACCG 2452
E. festucae Fl1 2296 TGGCGCTGCCGTACGACTACTGCGTGCCCGTGAACCGCACGCGCATACCA 2345
E. festucae E2368 2187 TGGCGCTGCCGTACGACTACTGCGTGCCCGTGAACCGCACGCGCATACCA 2236
E. baconii 2186 TGGCGCTGCCGTACGACTACTGCGTGCCCGTGAACCGCACGCGCATACCA 2235
E. sylvatica 2182 TGGCGCTACCATACGACTACTGTGTGCCCGTGAACCGCACGCGCATACCA 2231
E. typhina 2013 ---------ccatacgactactgcgtgcccgtgaaccgcacgcgcatacca 2054
E. elymi 762 TGGCGCTGCCATACGACTACTGCGTGCCCGTGAACCGCACGCGCATACCA 811
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. brachyelytri ----------
C. purpurea 2453 TGGTGTTGCCGTACGACTACTGCGTTTCGGTGAACCGTACGCGCATTCCG 2502

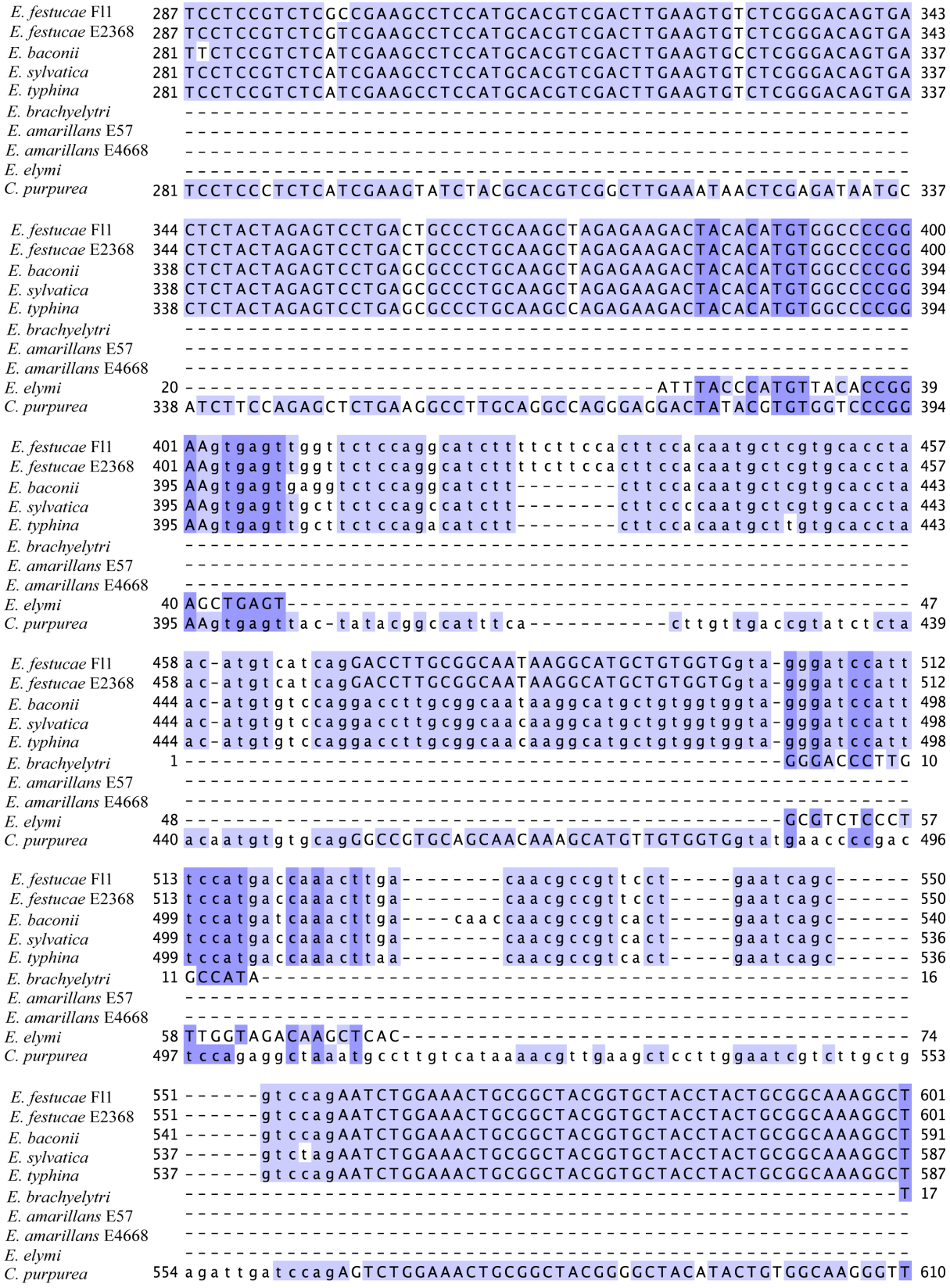

E. festucae Fl1
E. festucae E2368
E. baconii
E. sylvatica
E. typhina
E. elymi
E. amarillans E57
E. amarillans E4668
E. brachyelytri
C. purpurea

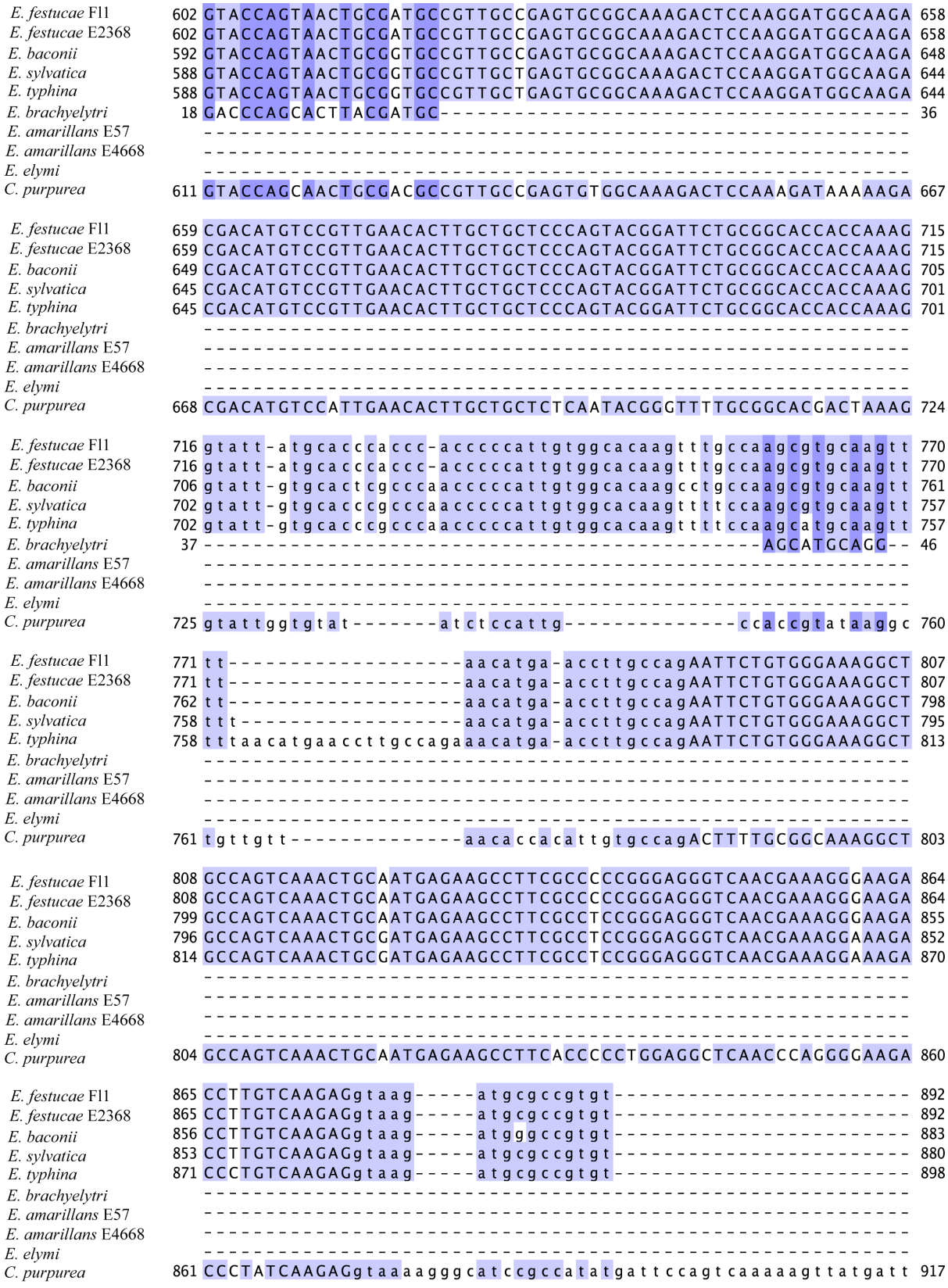

E. festucae Fl1 2583 AGATGGTTACGAACAGGTTTGCGTCTTGGGATCCGGCACGCAGCCTACCG 2632
E. festucae E2368 2474 AGATGGTTACGAACAGGTTTGCGTCTTGGGATCCGGCACGCAGCCTACCG 2523
E. baconii 2473 AGATGGTTACGAACAGGTTTGCGTCTTGGGATCCGGCACGCAGCCTATCG 2522
E. sylvatica 2469 AGATGGTTACGAACAGGTTTGCGTCTTGGGATCCGGCACGCAGCCTCCCG 2518
E. typhina 2295 AGATGGTTACGAACAGGTTTGCGTCTTGGGATCCGGCACGCAGACTCCCG 2344
E. elymi 1049 AGATGGTTACGAACAGGTTTGCGTCTTGGGATCCGGCACGCAGCCTACCG 1098
E. amarillans E57 983 AGATGGTTACGAACAGGTTTGCGTCTTGGGATCCGGCACGCAACCTACCG 1032
E. amarillans E4668 983 AGATGGTTACGAACAGGTTTGCGTCTTGGGATCCGGCACGCAACCTACCG 1032
E. brachyelytri 1735 ----------GGGCGTGCATGTAGCGT---- 1751
C. purpurea 2800 AGATGGCTACGAACAAGTCTGCGTCTTGGCCTCCGGGACGC--------- 2840
E. festucae Fl1 2633 ACTCGCCAACAGCCAAAACCAGTACAATTCTTCCCACGACAACCACCGGA 2682
E. festucae E2368 2524 ACTCGCCAACAGCCAAAACCAGTACAATTCTTCCCACGACAACCACCGGA 2573
E. baconii 2523 GCTCGCCAACAGCCACAACCAGTACAGTTCTTCCCACGACAACCACCGGA 2572
E. sylvatica 2519 GCTCGCCAACAGCCACAACCAGTACAGTTCTTTCCACGACAACCACCGGA 2568
E. typhina 2345 GCTCGCCAACAGCCACTACCAGTACAGTTCTTCCCACGACAACCACCGGA 2394
E. elymi 1099 GCTCGCCAACAGCCACAACCAGTACAGTTCTTCCCACGACAACCACCGGA 1148
E. amarillans E57 1033 GCTCGCCAACAGCCACAACCAGTACAGTTCTTCCCACGACAACCACCGGA 1082
E. amarillans E4668 1033 GCTCGCCAACAGCCACAACCAGTACAGTTCTTCCCACGACAACCACCGGA 1082
E. brachyelytri 1752 ----------GTACCCAGTCCACGTCGTCCTGCG---------- 1775
C. purpurea 2841 ---CCACACCAACAACAAGCAGTACGGTGCCTACAACGACGGT------A 2881
E. festucae Fl1 2683 ACAGCTCCGCCGGTTCCTACTCAGCCTGGAGCATCCAAGAATTGCAAGAA 2732
E. festucae E2368 2574 ACAGCTCCGCCGGTTCCTACTCAGCCTGGAGCATCCAAGAATTGCAAGAA 2623
E. baconii 2573 ACAGCTCCGCCGGCTCCTACTCAGCCTGGAGCATCCAAGAATTGCAAGAA 2622
E. sylvatica 2569 ACAGCTCCGCCGGCTCCTACTCAGCCCGGAGCATCCAAGAATTGCAAGAA 2618
E. typhina 2395 ACAGCTCCGCCGGCTCCTACTCAGCCCGGAGCATCCAAGAATTGCAAGAA 2444
E. elymi 1149 ACAGCTCCGCCGGCTCCTACTCAGCCTGGAGCATCCAAGAATTGCAAGAA 1198
E. amarillans E57 1083 ACAGCTCCGCCGGTTCCTACTCAGCCTGGAGCATCCAAGGATTGCAAGAA 1132
E. amarillans E4668 1083 ACAGCTCCGCCGGTTCCTACTCAGCCTGGAGCATCCAAGGATTGCAAGAA 1132
E. brachyelytri 1776 ----------GGTTAGCAGCGTCCG---------- 1790
C. purpurea 2882 GTGGCTCCGCCGGCGCCAACGCAACCCGGAGCGTCCAAGAATTGCAAGAA 2931
E. festucae Fl1 2733 ATGGCACACAGTCGTTGCTGGAGACGGCTGTCAGAGTATTGCCAATGCAG 2782
E. festucae E2368 2624 ATGGCACACAGTCGTTGCTGGAGACGGCTGTCAGAGTATTGCCAATGCAG 2673
E. baconii 2623 ATGGCACACAGTCGTTGGTGGAGACGGCTGTCGAGATATTGCCAATGCAG 2672
E. sylvatica 2619 ATGGCACACAGTCGTTGGTGGAGACGGCTGTTGGAGTATTGCCAATGCAG 2668
E. typhina 2445 ATGGCACACAGTCGTTGGTGGAGAGGGCTGTTGGAGTATTGCCAATGCAG 2494
E. elymi 1199 ATGGCACACAGTCGTTGGTGGAGACGGCTGTCGGAGTATTGCCAATGCAG 1248
E. amarillans E57 1133 ATGGCACACAGTCGTTGGTGGAGACGGCTGTCGAGATATTGCCAATGCAG 1182
E. amarillans E4668 1133 ATGGCACACAGTCGTTGGTGGAGACGGCTGTCGAGATATTGCCAATGCAG 1182
E. brachyelytri 1791 ----------GCCGGAGACGGTCCTCGAGGT---------- 1811
C. purpurea 2932 ATGGCACACAGTCGTCAGCGGCGACGGGTGTTGGAGCATTGCCACTGCAG 2981
E. festucae Fl1 2783 CAGGTATCAGCTTGGATGAGTTCTACCGGATGAACACTGGCGTGGGGTCG 2832
E. festucae E2368 2674 CAGGTATCAGCTTGGATGAGTTCTACCGGATGAACACTGGCGTGGGGTCG 2723
E. baconii 2673 CAGGTATCAGCTTGGATGAGTTCTACCGGATGAACACTGGCGTGGGGTCG 2722
E. sylvatica 2669 CAGGCATCAGCTTGGATGAGTTCTACCGGATGAACACTGGCGTGGGGTCG 2718
E. typhina 2495 CAGGTATCAGCTTGGATGAGTTCTACCGGATGAACACTGGCGTGGGGTCG 2544
E. elymi 1249 CAGGTATCAGCTTGGATGAGTTCTACCGGATGAAGACTGGCGTGGGGTCG 1298
E. amarillans E57 1183 CAGGTATCAGCTTGGATGAGTTCTACCGGATGAACACTGGCGTGGGGTCG 1232
E. amarillans E4668 1183 CAGGTATCAGCTTGGATGAGTTCTACCGGATGAACACTGGCGTGGGGTCG 1232
E. brachyelytri ----------
C. purpurea 2982 CAGGTATCAGCTTGGATGACTTCTACCGGATGAACACGGGCGTGGGATCC 3031
E. festucae Fl1 2833 GATTGTAGCACACTTTGGTCGGGGTATGCTGTCTGTATTGGAGAGTAGAG 2882
E. festucae E2368 2724 GATTGTAGCACACTTTGGTCGGGGTATGCTGTCTGTATTGGAGAGTAGAG 2773
E. baconii 2723 GATTGTAGCACGCTTTGGTCGGGGTATGCTGTCTGTATTGGAGAGTAGAG 2772
E. sylvatica 2719 GATTGTAGCACGCTTTGGTCGGGGTATGCTGTCTGTATTGGAGAGTAGAG 2768
E. typhina 2545 GATTGTAGCACGCTTTGGTCGGGGTATGCTGTCTGTATTGGAGAGTAGAG 2594
E. elymi 1299 GATTGTAGCACGCTTTGGTCGGGGTATGCTGTCTGTATTGGAGAGTAGAG 1348
E. amarillans E57 1233 GATTGTAGCACGCTTTGGTCGGGGTATGCTGTCTGTATTGGAGAGTAGAG 1282
E. amarillans E4668 1233 GATTGTAGCACGCTTTGGTCGGGGTATGCTGTCTGTATTGGAGAGTAGAG 1282
E. brachyelytri 1812 -----T-------CTTTGG---------- 1818
C. purpurea 3032 GATTGTAGTGCGCTTTGGTTGGGGTATGCTGTTTGCGTCGGAGAGTAAGA 3081

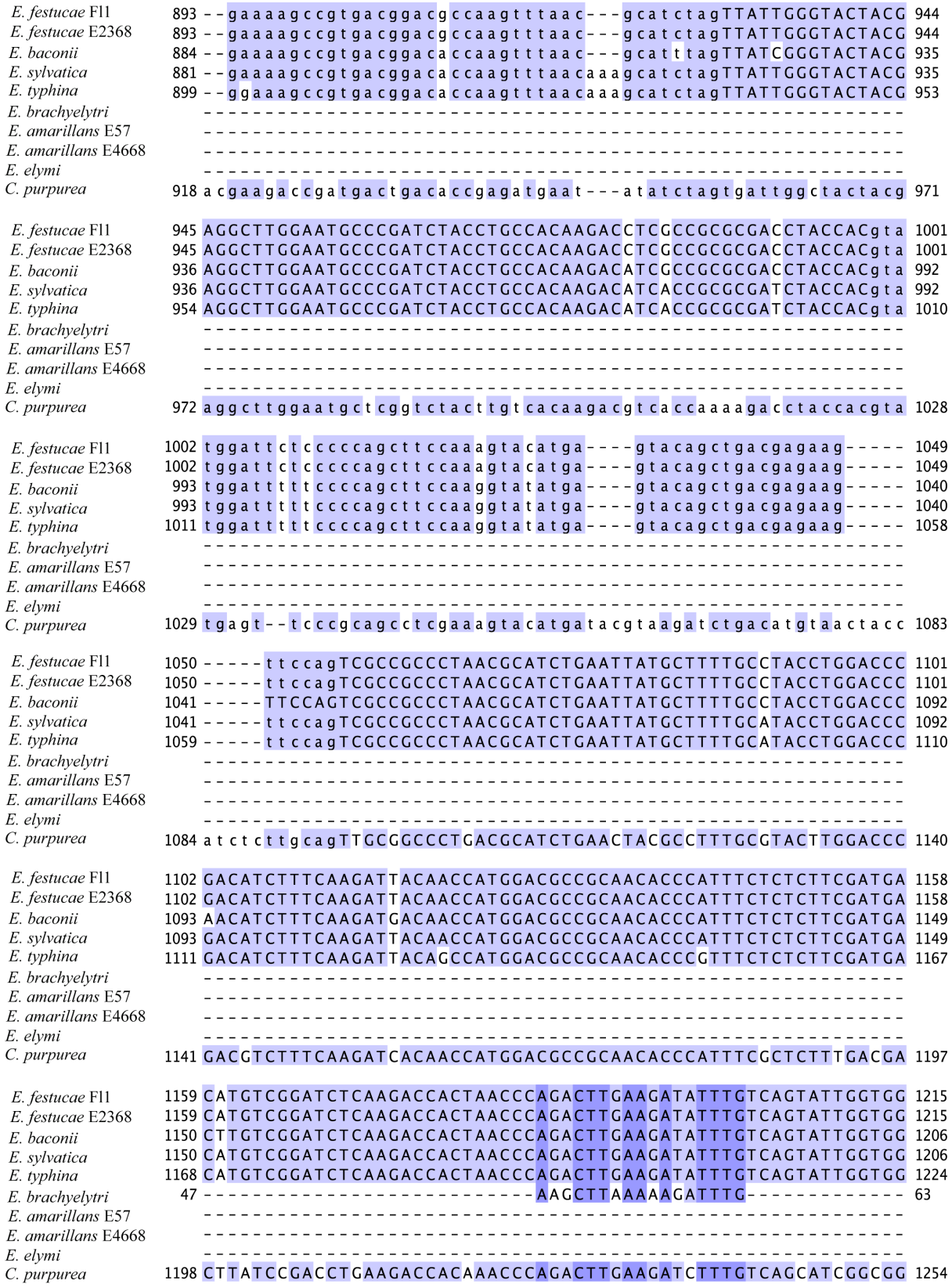

E. festucae Fl1 2883 TGTTGGTTTCCAGTGGCACTTCCGTCGTTGC-------TAGGTTTCTTC 2924
E. festucae E2368 2774 TGTTGGTTTCCAGTGGCACTTCCGTCGTTGC-------TAGGTTTCTTC 2815
E. baconii 2773 TGTTGGTTTCCAGTGGCACTTCCGTCGTTGC-------TAGGTTTCTTC 2814
E. sylvatica 2769 GGTTGGTTTTCAGTGCCACTTCCGTCGTTGC-------CAGGCTTCTTC 2810
E. typhina 2595 GGTTGGTTTTCAGTGCCACTTCCGTCGTTGC-------CAGGCTTCTTC 2636
E. elymi 1349 GGTTGGTTCTCAGTGCCACTTCCGTCGTCGC-------TAGGTTTCCTC 1390
E. amarillans E57 1283 TGTTGGTTTTCAGTGCCACTTCCGTCGTTGC-------TAGGTTTCTTC 1324
E. amarillans E4668 1283 TGTTGGTTTTCAGTGCCACTTCCGTCGTTGC-------TAGGTTTCTTC 1324
E. brachyelytri
C. purpurea 3082 TAACGAGGAGAAAAACTGCCTCTGCCCGTACATGAGACGCATCAATGTTC 3131
E. festucae Fl1 2925 CGCAATATATTCAACCGTAGTCTAGATTGCGAGACTTCTGA----CTTGA 2970
E. festucae E2368 2816 CGCAATATATTCAACCGTAGTCTAGATTGCGAGACTTCTGA----CTTGA 2861
E. baconii 2815 CGCAATATATTCAACCGTAGTCTAGATTGCGAGACTTCTGA----CTTGA 2860
E. sylvatica 2811 CGCAATA-ATTCAACCGTAGTCTAGATTGCGAGACTTCTGA----CTTGA 2855
E. typhina 2637 CGCAATA-AATCAACCGTAGTCTAGATTGCGAGACTTCTGA----CTTGA 2681
E. elymi 1391 CGCAATATGTTCAACCGTAGTCTTGATTGCGAGACTTTTGA----CTTGA 1436
E. amarillans E57 1325 CGCAATAAATTCAACCGTAGTCTAGGTTGCGAGACTTGTGA----CTTGA 1370
E. amarillans E4668 1325 CGCAATAAATTCAACCGTAGTCTAGGTTGCGAGACTTGTGA----CTTGA 1370
E. brachyelytri
C. purpurea 3132 GTTGAGATCTTTGATCGC--TCTGGCCTTCAAGGTTCTCGATGCCCTTAA 3179
E. festucae Fl1 2971 A---------------GCACGACTG---------CTCCTCCGCCCATGAG 2996
E. festucae E2368 2862 A---------------GCACGACTG---------CTCCTCCGCCCATGAG 2887
E. baconii 2861 A---------------GCACGACTG---------CTCCTCCGCCCATGAG 2886
E. sylvatica 2856 AAGCGGATC-TAAACCGTACGACTG---------CTCCTCCACCTATGAG 2895
E. typhina 2682 AAGCGGATC-TAAACCGTACGACTG---------CTCCTCCACCCATGAG 2721
E. elymi 1437 A---------------GCACGACTG---------CTCCTCCGCCCATGAG 1462
E. amarillans E57 1371 AAGCGGACCCCAAACCGCACGACTG---------CTCCTCCGCCCATGAG 1411
E. amarillans E4668 1371 AAGCGGACCCCAAACCGCACGACTG---------CTCCTCCGCCCATGAG 1411
E. brachyelytri
C. purpurea 3180 ATAAAGACTATTGTCTCCAGTGCCGCAAATCACCCTTTTCCAGCCGTCAG 3229
E. festucae Fl1 2997 TCGCATGTTTCCTCGCTCACGATATCGAGAGATGAGTTTATATAAATGGC 3046
E. festucae E2368 2888 TCGCATGTTTCCTCGCTCACGATATCAAGAGATGAGTTTATATAAATGGC 2937
E. baconii 2887 TCGCATGTTTCCTCGCTCACGATATCAAGAGATGAGTTTATATAAATGGC 2936
E. sylvatica 2896 TTGCATGTTTCCTCGCTCACGATATCAAGAGATGAGTTTATATAAATGGC 2945
E. typhina 2722 TTGCATGTTTCCTCGCTCACGATATCAAGAGACGAGTTTATATAAATGGC 2771
E. elymi 1463 TCGCATGTTTCCTCGCTCACGATATCAAGAGATGATTTTATATGAATGGC 1512
E. amarillans E57 1412 TCGCATGTTTCCTCGCTCACGATATCAAGAGATGAGTTTACATAAATGGC 1461
E. amarillans E4668 1412 TCGCATGTTTCCTCGCTCACGATATCAAGAGATGAGTTTACATAAATGGC 1461
E. brachyelytri
C. purpurea 3230 CGTCCCTCAAGCCTACTTTCGTTCCCAA---CTGATTCTGAATGAAAGGC 3276
E. festucae Fl1 3047 GACAGCAACTCTGGACATGCCCGTACCAATCAAT 3080
E. festucae E2368 2938 GACAGCAACTCTGGACATGCCCGTACCAATCAAT 2971
E. baconii 2937 GACAGCAACTCTGGACATGCCCGTACCAATCAAT 2970
E. sylvatica 2946 GACAGCAACTCTGCATATGCC---------- 2966
E. typhina 2772 GGCAGCAACTCTGCATATGCC---------- 2792
E. elymi 1513 GACAGCAACTCTGGATATGCCCGTACCAATCAAT 1546
E. amarillans E57 1462 GACAGCAACTCTGGATATG---------- 1480
E. amarillans E4668 1462 GACAGCAACTCTGGATATG---------- 1480
E. brachyelytri
C. purpurea 3277 AGC---------- 3279

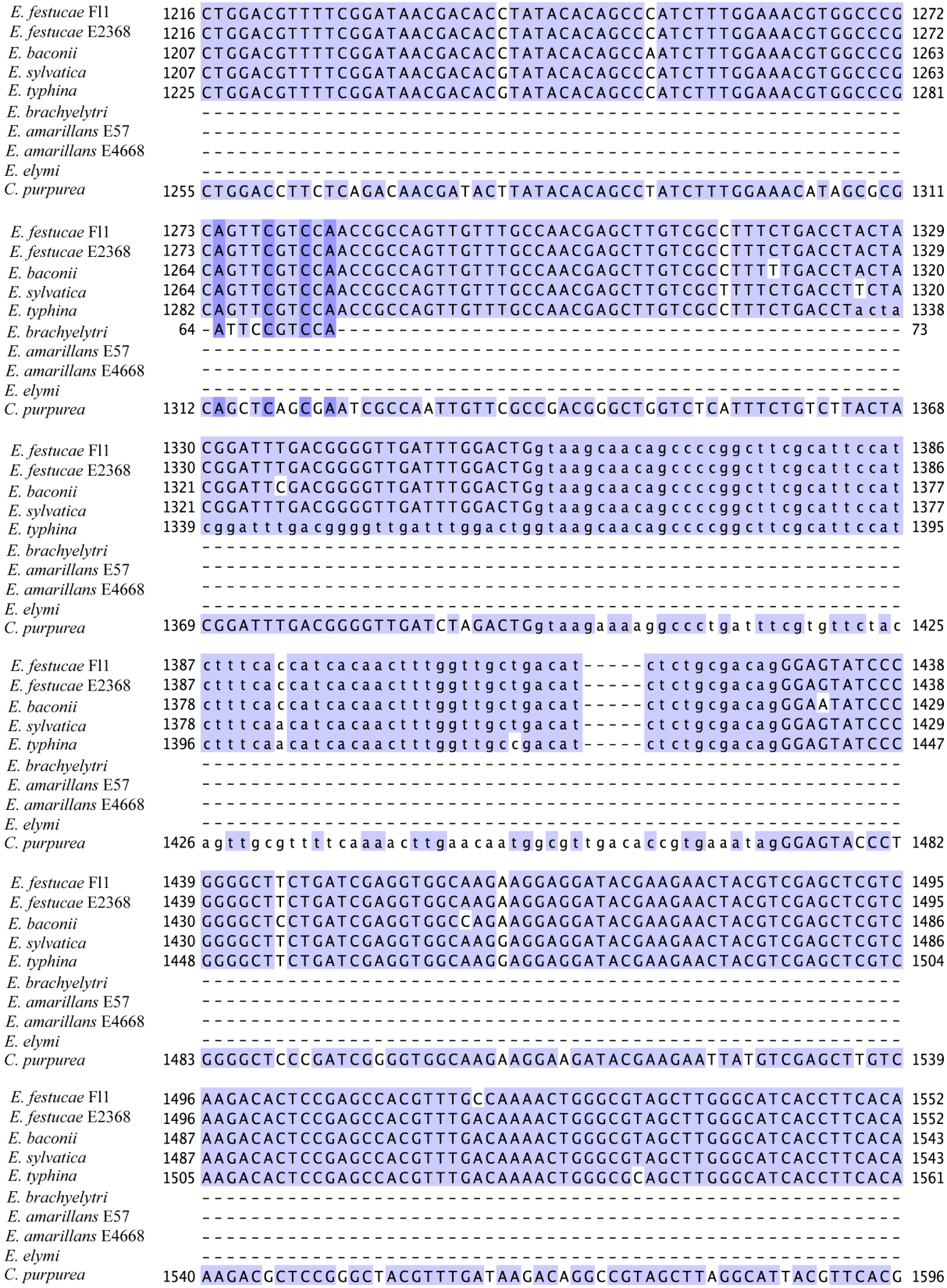
Appendix 17. 17Multiple sequence alignment of the *E. festucae chiA* gene with homologues from other *Epichloë* species and *Claviceps purpurea*. *E. festucae* (Fl1) EfM3.000810, E. *festucae* (E2368), *E. baconii, E. sylvatica, E. typhina, E. elymi, E. amarillans, E. brachyelytri, C. purpurea.* Intensity of shading indicates the level of conservation. *chiA* start codon (ATG) and stop codons (TAG and TGA) are boxed in red and green respectively. Exons and introns are indicated as capital and small letters respectively.

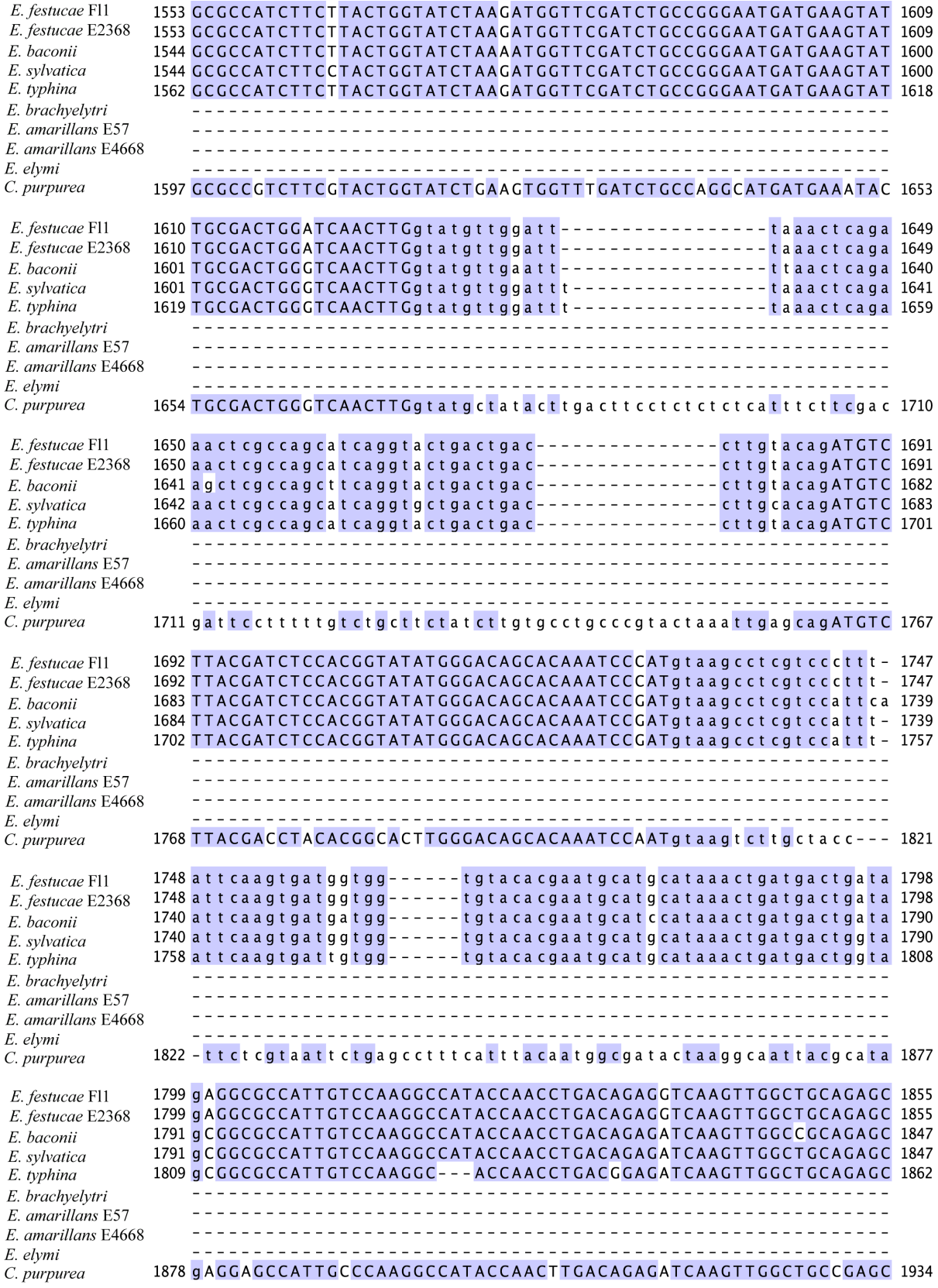

E. festucae Fl1
E. festucae E2368
E. baconii
E. sylvatica
E. typhina
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea

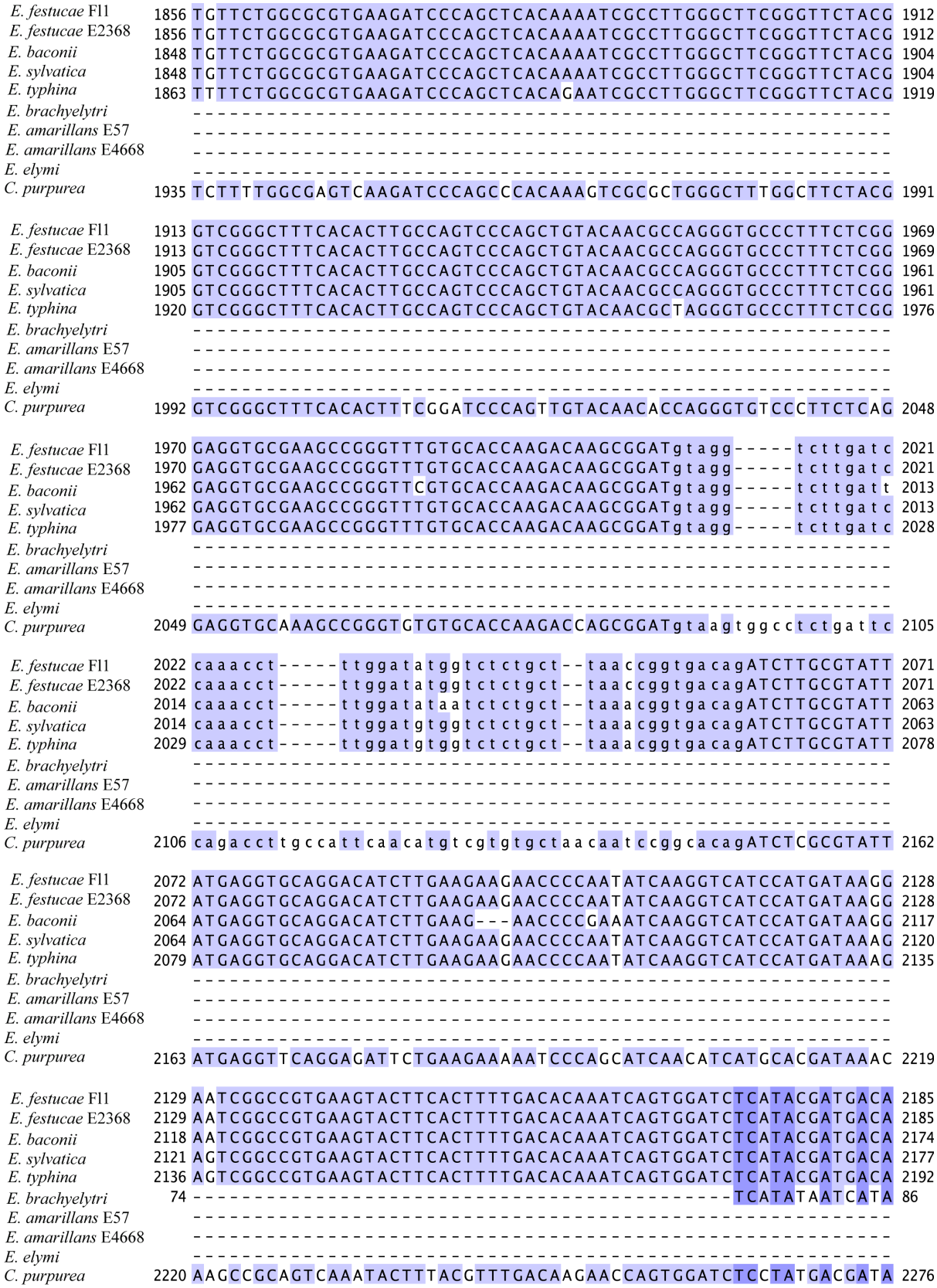

E. festucae Fl1 287 TCCTCCGTCTCGCCGAAGCCTCCATGCACGTCGACTTGAAGTGTCTCGGGACAGTGA 343
E. festucae E2368 287 TCCTCCGTCTCGTCGAAGCCTCCATGCACGTCGACTTGAAGTGTCTCGGGACAGTGA 343
E. baconii 281 TTCTCCGTCTCATCGAAGCCTCCATGCACGTCGACTTGAAGTGCCTCGGGACAGTGA 337
E. sylvatica 281 TCCTCCGTCTCATCGAAGCCTCCATGCACGTCGACTTGAAGTGTCTCGGGACAGTGA 337
E. typhina 281 TCCTCCGTCTCATCGAAGCCTCCATGCACGTCGACTTGAAGTGTCTCGGGACAGTGA 337
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 281 TCCTCCCTCTCATCGAAGTATCTACGCACGTCGGCTTGAAATAACTCGAGATAATGC 337
E. festucae Fl1 344 CTCTACTAGAGTCCTGACTGCCCTGCAAGCTAGAGAAGACTACACATGTGGCCCCGG 400
E. festucae E2368 344 CTCTACTAGAGTCCTGACTGCCCTGCAAGCTAGAGAAGACTACACATGTGGCCCCGG 400
E. baconii 338 CTCTACTAGAGTCCTGAGCGCCCTGCAAGCTAGAGAAGACTACACATGTGGCCCCGG 394
E. sylvatica 338 CTCTACTAGAGTCCTGAGCGCCCTGCAAGCTAGAGAAGACTACACATGTGGCCCCGG 394
E. typhina 338 CTCTACTAGAGTCCTGAGCGCCCTGCAAGCCAGAGAAGACTACACATGTGGCCCCGG 394
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi 20 ATTTACCCATGTTACACCGG 39
C. purpurea 338 ATCTTCCAGAGCTCTGAAGGCCTTGCAGGCCAGGGAGGACTATACGTGTGGTCCCCGG 394
E. festucae Fl1 401 AAgtgagttggttctccaggcatctttcttccacttccacaatgctcgtgcaccta 457
E. festucae E2368 401 AAgtgagttggttctccaggcatctttcttccacttccacaatgctcgtgcaccta 457
E. baconii 395 AAgtgagtgaggtctccaggcatctt--------cttccacaatgctcgtgcaccta 443
E. sylvatica 395 AAgtgagttgcttctccagccatctt--------cttccccaatgctcgtgcaccta 443
E. typhina 395 AAgtgagttgcttctccagacatctt--------cttccacaatgcttgtgcaccta 443
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi 40 AGCTGAGT 47
C. purpurea 395 AAgtgagttac-tatacggccatttca----------cttgttgaccgtatctcta 439
E. festucae Fl1 458 ac-atgtcatcagGACCTTGCGGCAATAAGGCATGCTGTGGTGgta-gggatccatt 512
E. festucae E2368 458 ac-atgtcatcagGACCTTGCGGCAATAAGGCATGCTGTGGTGgta-gggatccatt 512
E. baconii 444 ac-atgtgtccaggaccttgcggcaataaggcatgctgtggtggta-gggatccatt 498
E. sylvatica 444 ac-atgtgtccaggaccttgcggcaacaaggcatgctgtggtggta-gggatccatt 498
E. typhina 444 ac-atgtgtccaggaccttgcggcaacaaggcatgctgtggtggta-gggatccatt 498
E. brachyelytri 1 GGGACCCTTG 10
E. amarillans E57
E. amarillans E4668
E. elymi 48 GCGTCTCCCT 57
C. purpurea 440 acaatgtgtgcagGGCCGTGCAGCAACAAAGCATGTTGTGGTGgtatgaacccgac 496
E. festucae Fl1 513 tccatgaccaaacttga--------caacgccgttcct-----gaatcagc------ 550
E. festucae E2368 513 tccatgaccaaacttga--------caacgccgttcct-----gaatcagc------ 550
E. baconii 499 tccatgatcaaacttga----caaccaacgccgtcact-----gaatcagc------ 540
E. sylvatica 499 tccatgaccaaacttga--------caacgccgtcact-----gaatcagc------ 536
E. typhina 499 tccatgaccaaacttaa--------caacgccgtcact-----gaatcagc------ 536
E. brachyelytri 11 GCCATA 16
E. amarillans E57
E. amarillans E4668
E. elymi 58 TTGGTAGACAAGCTCAC 74
C. purpurea 497 tccagaggctaaatgccttgtcataaacgttgaagctccttggaatcgtcttgctg 553
E. festucae Fl1 551 ------gtccagAATCTGGAAACTGCGGCTACGGTGCTACCTACTGCGGCAAAGGCT 601
E. festucae E2368 551 ------gtccagAATCTGGAAACTGCGGCTACGGTGCTACCTACTGCGGCAAAGGCT 601
E. baconii 541 ------gtccagAATCTGGAAACTGCGGCTACGGTGCTACCTACTGCGGCAAAGGCT 591
E. sylvatica 537 ------gtctagAATCTGGAAACTGCGGCTACGGTGCTACCTACTGCGGCAAAGGCT 587
E. typhina 537 ------gtccagAATCTGGAAACTGCGGCTACGGTGCTACCTACTGCGGCAAAGGCT 587
E. brachyelytri T 17
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 554 agattgatccagAGTCTGGAAACTGCGGCTACGGGGCTACATACTGTGGCAAGGGTT 610

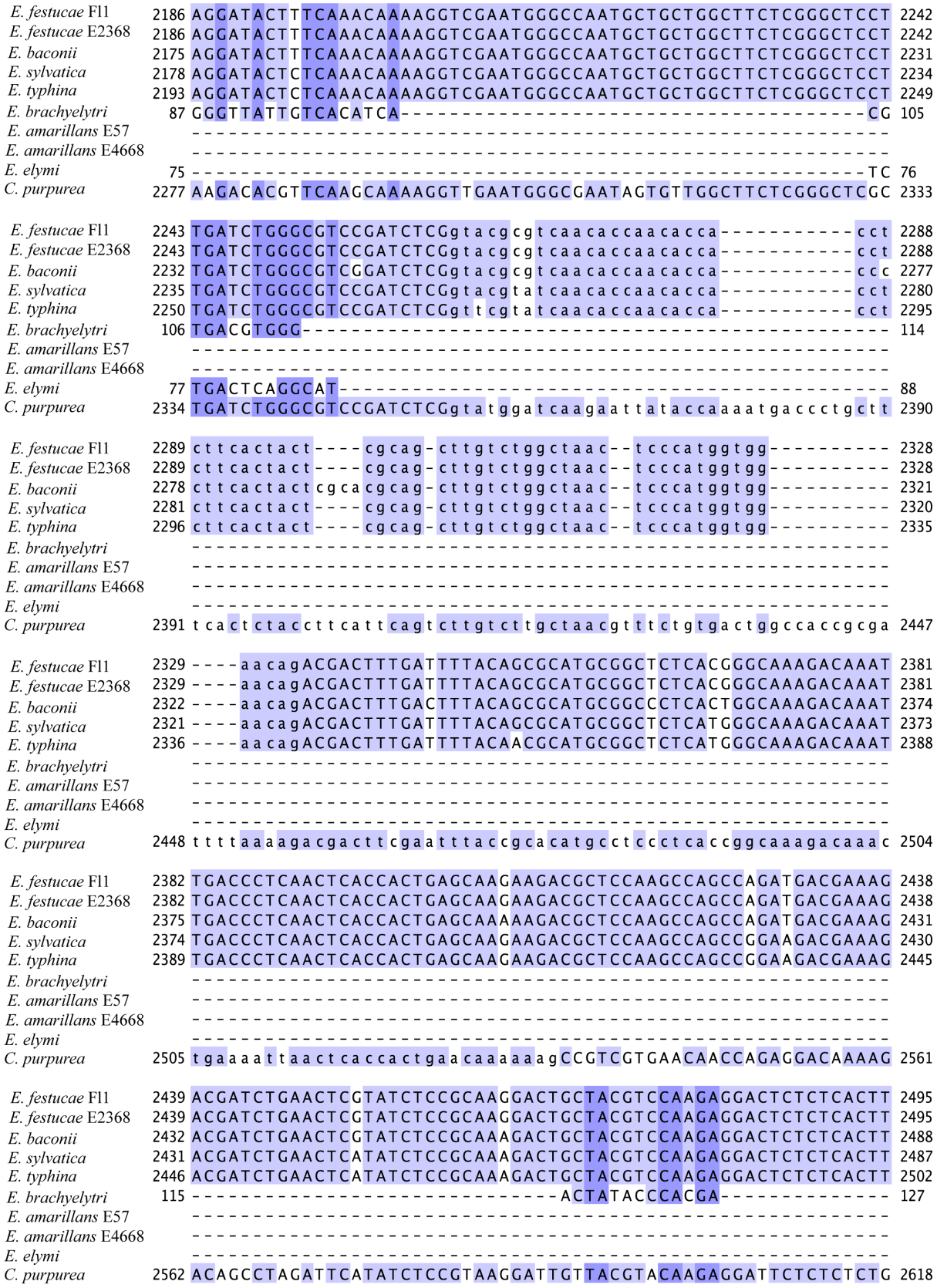

E. festucae Fl1 602 GTACCAGTAACTGCGATGCCGTTGCCGAGTGCGGCAAAGACTCCAAGGATGGCAAGA 658
E. festucae E2368 602 GTACCAGTAACTGCGATGCCGTTGCCGAGTGCGGCAAAGACTCCAAGGATGGCAAGA 658
E. baconii 592 GTACCAGTAACTGCGGTGCCGTTGCCGAGTGCGGCAAAGACTCCAAGGATGGCAAGA 648
E. sylvatica 588 GTACCAGTAACTGCGGTGCCGTTGCTGAGTGCGGCAAAGACTCCAAGGATGGCAAGA 644
E. typhina 588 GTACCAGTAACTGCGGTGCCGTTGCTGAGTGCGGCAAAGACTCCAAGGATGGCAAGA 644
E. brachyelytri 18 GACCCAGCACTTACGATGC---------- 36
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 611 GTACCAGCAACTGCGACGCCGTTGCCGAGTGTGGCAAAGACTCCAAAGATAAAAAGA 667
E. festucae Fl1 659 CGACATGTCCGTTGAACACTTGCTGCTCCCAGTACGGATTCTGCGGCACCACCAAAG 715
E. festucae E2368 659 CGACATGTCCGTTGAACACTTGCTGCTCCCAGTACGGATTCTGCGGCACCACCAAAG 715
E. baconii 649 CGACATGTCCGTTGAACACTTGCTGCTCCCAGTACGGATTCTGCGGCACCACCAAAG 705
E. sylvatica 645 CGACATGTCCGTTGAACACTTGCTGCTCCCAGTACGGATTCTGCGGCACCACCAAAG 701
E. typhina 645 CGACATGTCCGTTGAACACTTGCTGCTCCCAGTACGGATTCTGCGGCACCACCAAAG 701
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 668 CGACATGTCCATTGAACACTTGCTGCTCTCAATACGGGTTTTGCGGCACGACTAAAG 724
E. festucae Fl1 716 gtatt-atgcacccaccc-acccccattgtggcacaagtttgccaagcgtgcaagtt 770
E. festucae E2368 716 gtatt-atgcacccaccc-acccccattgtggcacaagtttgccaagcgtgcaagtt 770
E. baconii 706 gtatt-gtgcactcgcccaaccccccattgtggcacaagcctgccaagcgtgcaagtt 761
E. sylvatica 702 gtatt-gtgcacccgcccaaccccccattgtggcacaagttttccaagcgtgcaagtt 757
E. typhina 702 gtatt-gtgcacccgcccaaccccccattgtggcacaagttttccaagcatgcaagtt 757
E. brachyelytri 37 ----------AGCATGCAGG-- 46
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 725 gtattggtgtat-------atctccattg----------ccaccgtataaggc 760
E. festucae Fl1 771 tt----------aacatga-accttgccagAATTCTGTGGGAAAGGCT 807
E. festucae E2368 771 tt----------aacatga-accttgccagAATTCTGTGGGAAAGGCT 807
E. baconii 762 tt----------aacatga-accttgccagAATTCTGTGGGAAAGGCT 798
E. sylvatica 758 ttt----------aacatga-accttgccagAATTCTGTGGGAAAGGCT 795
E. typhina 758 ttaacatgaaccttgccagaaacatga-accttgccagAATTCTGTGGGAAAGGCT 813
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 761 tgttgtt----------aacaccacattgtgccagACTTTGCGGCAAAGGCT 803
E. festucae Fl1 808 GCCAGTCAAACTGCAATGAGAAGCCTTCGCCCCCGGGAGGGTCAACGAAAGGGAAGA 864
E. festucae E2368 808 GCCAGTCAAACTGCAATGAGAAGCCTTCGCCCCCGGGAGGGTCAACGAAAGGGAAGA 864
E. baconii 799 GCCAGTCAAACTGCAATGAGAAGCCTTCGCCTCCGGGAGGGTCAACGAAAGGGAAGA 855
E. sylvatica 796 GCCAGTCAAACTGCGATGAGAAGCCTTCGCCTCCGGGAGGGTCAACGAAAGGAAAGA 852
E. typhina 814 GCCAGTCAAACTGCGATGAGAAGCCTTCGCCTCCGGGAGGGTCAACGAAAGGAAAGA 870
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 804 GCCAGTCAAACTGCAATGAGAAGCCTTCACCCCCTGGAGGCTCAACCCAGGGGAAGA 860
E. festucae Fl1 865 CCTTGTCAAGAGgtaag-----atgcgccgtgt---------- 892
E. festucae E2368 865 CCTTGTCAAGAGgtaag-----atgcgccgtgt---------- 892
E. baconii 856 CCTTGTCAAGAGgtaag-----atgggccgtgt---------- 883
E. sylvatica 853 CCTTGTCAAGAGgtaag-----atgcgccgtgt---------- 880
E. typhina 871 CCCTGTCAAGAGgtaag-----atgcgccgtgt---------- 898
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 861 CCCTATCAAGAGgtaaaagggcatccgccatatgattccagtcaaaaagttatgatt 917

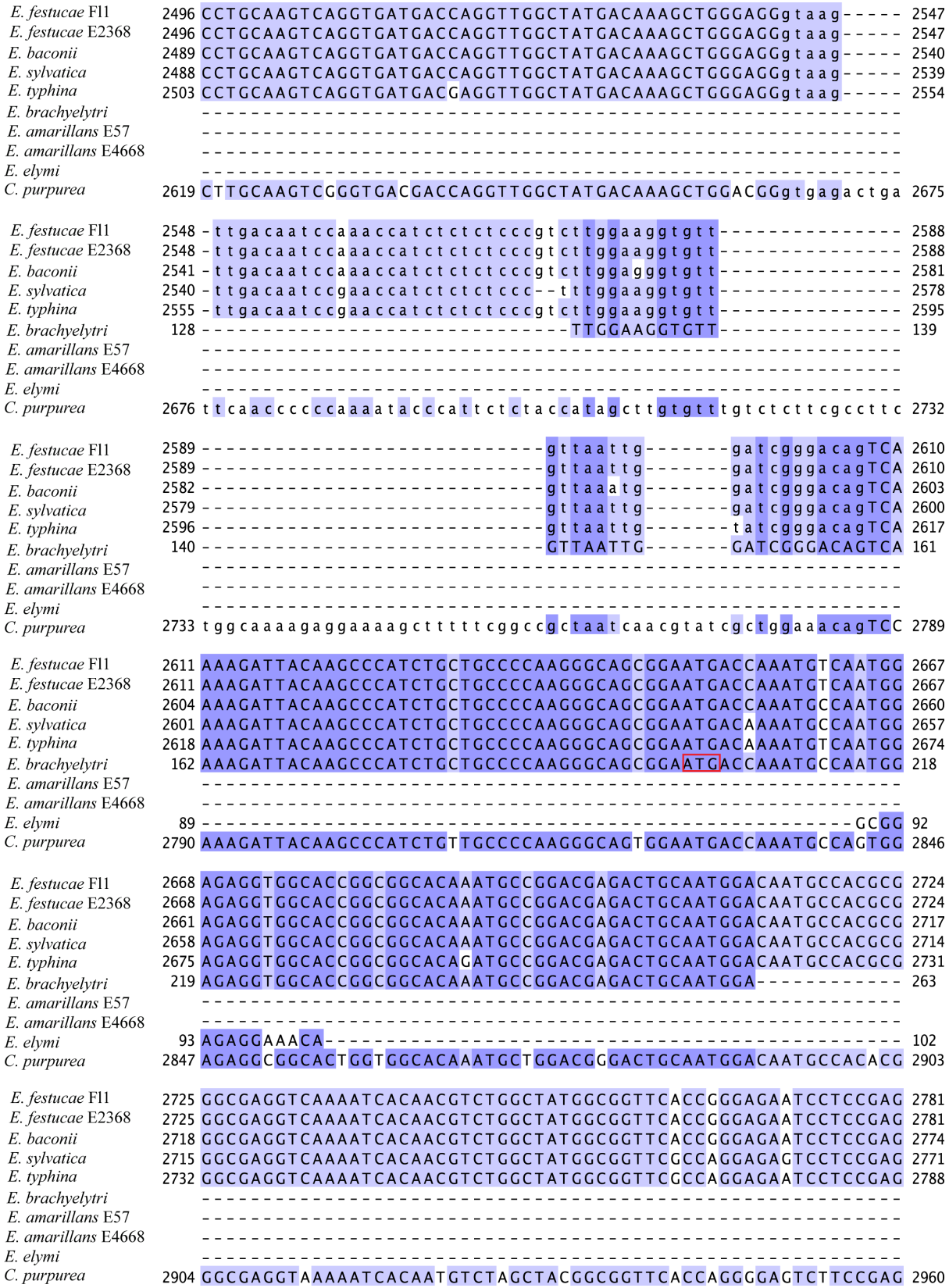

E. festucae Fl1 893 --gaaaagccgtgacggacgccaagtttaac---gcatctagTTATTGGGTACTACG 944
E. festucae E2368 893 --gaaaagccgtgacggacgccaagtttaac---gcatctagTTATTGGGTACTACG 944
E. baconii 884 --gaaaagccgtgacggacaccaagtttaac---gcatttagTTATCGGGTACTACG 935
E. sylvatica 881 --gaaaagccgtgacggacaccaagtttaacaaagcatctagTTATTGGGTACTACG 935
E. typhina 899 --ggaaagccgtgacggacaccaagtttaacaaagcatctagTTATTGGGTACTACG 953
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 918 acgaagaccgatgactgacaccgagatgaat---atatctagtgattggctactacg 971
E. festucae Fl1 945 AGGCTTGGAATGCCCGATCTACCTGCCACAAGACCTCGCCGCGCGACCTACCACgta 1001
E. festucae E2368 945 AGGCTTGGAATGCCCGATCTACCTGCCACAAGACCTCGCCGCGCGACCTACCACgta 1001
E. baconii 936 AGGCTTGGAATGCCCGATCTACCTGCCACAAGACATCGCCGCGCGACCTACCACgta 992
E. sylvatica 936 AGGCTTGGAATGCCCGATCTACCTGCCACAAGACATCACCGCGCGATCTACCACgta 992
E. typhina 954 AGGCTTGGAATGCCCGATCTACCTGCCACAAGACATCACCGCGCGATCTACCACgta 1010
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 972 aggcttggaatgctcggtctacttgtcacaagacgtcaccaaaagacctaccacgta 1028
E. festucae Fl1 1002 tggattctccccagcttccaaagtacatga----gtacagctgacgagaag----- 1049
E. festucae E2368 1002 tggattctccccagcttccaaagtacatga----gtacagctgacgagaag----- 1049
E. baconii 993 tggatttttccccagcttccaaggtatatga----gtacagctgacgagaag----- 1040
E. sylvatica 993 tggatttttccccagcttccaaggtatatga----gtacagctgacgagaag----- 1040
E. typhina 1011 tggatttttccccagcttccaaggtatatga----gtacagctgacgagaag----- 1058
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1029 tgagt--tcccgcagcctcgaaagtacatgatacgtaagatctgacatgtaactacc 1083
E. festucae Fl1 1050 -----ttccagTCGCCGCCCTAACGCATCTGAATTATGCTTTTGCCTACCTGGACCC 1101
E. festucae E2368 1050 -----ttccagTCGCCGCCCTAACGCATCTGAATTATGCTTTTGCCTACCTGGACCC 1101
E. baconii 1041 -----TTCCAGTCGCCGCCCTAACGCATCTGAATTATGCTTTTGCCTACCTGGACCC 1092
E. sylvatica 1041 -----ttccagTCGCCGCCCTAACGCATCTGAATTATGCTTTTGCATACCTGGACCC 1092
E. typhina 1059 -----ttccagTCGCCGCCCTAACGCATCTGAATTATGCTTTTGCATACCTGGACCC 1110
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1084 atctcttgcagTTGCGGCCCTGACGCATCTGAACTACGCCTTTGCGTACTTGGACCC 1140
E. festucae Fl1 1102 GACATCTTTCAAGATTACAACCATGGACGCCGCAACACCCATTTCTCTCTTCGATGA 1158
E. festucae E2368 1102 GACATCTTTCAAGATTACAACCATGGACGCCGCAACACCCATTTCTCTCTTCGATGA 1158
E. baconii 1093 AACATCTTTCAAGATGACAACCATGGACGCCGCAACACCCATTTCTCTCTTCGATGA 1149
E. sylvatica 1093 GACATCTTTCAAGATTACAACCATGGACGCCGCAACACCCATTTCTCTCTTCGATGA 1149
E. typhina 1111 GACATCTTTCAAGATTACAGCCATGGACGCCGCAACACCCGTTTCTCTCTTCGATGA 1167
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1141 GACGTCTTTCAAGATCACAACCATGGACGCCGCAACACCCATTTCGCTCTTTGACGA 1197
E. festucae Fl1 1159 CATGTCGGATCTCAAGACCACTAACCCAGACTTGAAGATATTTGTCAGTATTGGTGG 1215
E. festucae E2368 1159 CATGTCGGATCTCAAGACCACTAACCCAGACTTGAAGATATTTGTCAGTATTGGTGG 1215
E. baconii 1150 CTTGTCGGATCTCAAGACCACTAACCCAGACTTGAAGATATTTGTCAGTATTGGTGG 1206
E. sylvatica 1150 CATGTCGGATCTCAAGACCACTAACCCAGACTTGAAGATATTTGTCAGTATTGGTGG 1206
E. typhina 1168 CATGTCGGATCTCAAGACCACTAACCCAGACTTGAAGATATTTGTCAGTATTGGTGG 1224
E. brachyelytri 47 ----------AAGCTTAAAAAGATTTG---------- 63
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1198 CTTATCCGACCTGAAGACCACAAACCCAGACTTGAAGATCTTTGTCAGCATCGGCGG 1254

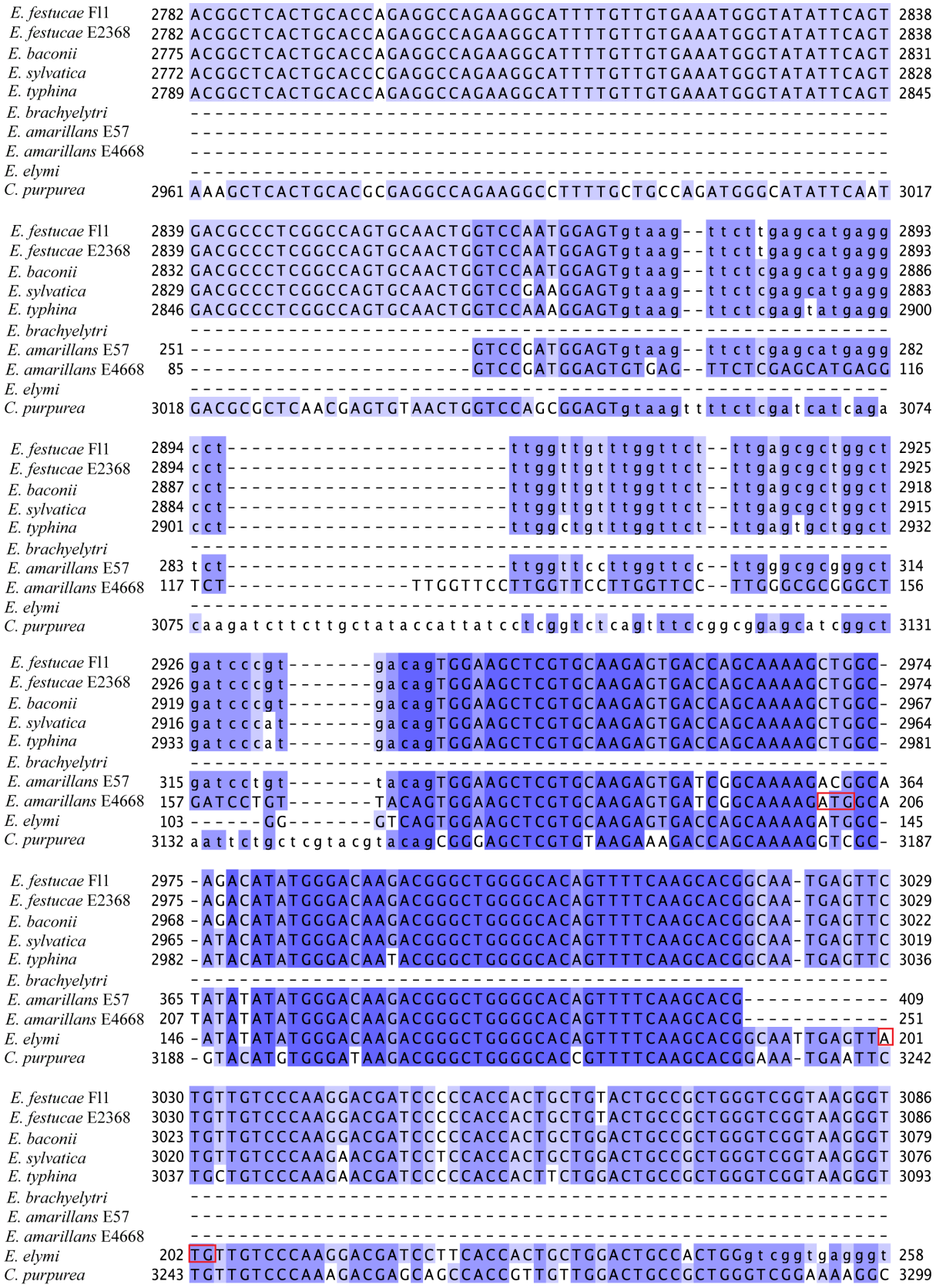

E. festucae Fl1 1216 CTGGACGTTTTCGGATAACGACACCTATACACAGCCCATCTTTGGAAACGTGGCCCG 1272
E. festucae E2368 1216 CTGGACGTTTTCGGATAACGACACCTATACACAGCCCATCTTTGGAAACGTGGCCCG 1272
E. baconii 1207 CTGGACGTTTTCGGATAACGACACCTATACACAGCCAATCTTTGGAAACGTGGCCCG 1263
E. sylvatica 1207 CTGGACGTTTTCGGATAACGACACGTATACACAGCCCATCTTTGGAAACGTGGCCCG 1263
E. typhina 1225 CTGGACGTTTTCGGATAACGACACGTATACACAGCCCATCTTTGGAAACGTGGCCCG 1281
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1255 CTGGACCTTCTCAGACAACGATACTTATACACAGCCTATCTTTGGAAACATAGCGCG 1311
E. festucae Fl1 1273 CAGTTCGTCCAACCGCCAGTTGTTTGCCAACGAGCTTGTCGCCTTTCTGACCTACTA 1329
E. festucae E2368 1273 CAGTTCGTCCAACCGCCAGTTGTTTGCCAACGAGCTTGTCGCCTTTCTGACCTACTA 1329
E. baconii 1264 CAGTTCGTCCAACCGCCAGTTGTTTGCCAACGAGCTTGTCGCCTTTTTGACCTACTA 1320
E. sylvatica 1264 CAGTTCGTCCAACCGCCAGTTGTTTGCCAACGAGCTTGTCGCTTTTCTGACCTTCTA 1320
E. typhina 1282 CAGTTCGTCCAACCGCCAGTTGTTTGCCAACGAGCTTGTCGCCTTTCTGACCTacta 1338
E. brachyelytri 64 -ATTCCGTCCA 73
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1312 CAGCTCAGCGAATCGCCAATTGTTCGCCGACGGGCTGGTCTCATTTCTGTCTTACTA 1368
E. festucae Fl1 1330 CGGATTTGACGGGGTTGATTTGGACTGgtaagcaacagccccggcttcgcattccat 1386
E. festucae E2368 1330 CGGATTTGACGGGGTTGATTTGGACTGgtaagcaacagccccggcttcgcattccat 1386
E. baconii 1321 CGGATTCGACGGGGTTGATTTGGACTGgtaagcaacagccccggcttcgcattccat 1377
E. sylvatica 1321 CGGATTTGACGGGGTTGATTTGGACTGgtaagcaacagccccggcttcgcattccat 1377
E. typhina 1339 cggatttgacggggttgatttggactggtaagcaacagccccggcttcgcattccat 1395
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1369 CGGATTTGACGGGGTTGATCTAGACTGgtaagaaaaggccctgatttcgtgttctac 1425
E. festucae Fl1 1387 ctttcaccatcacaactttggttgctgacat-----ctctgcgacagGGAGTATCCC 1438
E. festucae E2368 1387 ctttcaccatcacaactttggttgctgacat-----ctctgcgacagGGAGTATCCC 1438
E. baconii 1378 ctttcaccatcacaactttggttgctgacat-----ctctgcgacagGGAATATCCC 1429
E. sylvatica 1378 ctttcaacatcacaactttggttgctgacat-----ctctgcgacagGGAGTATCCC 1429
E. typhina 1396 ctttcaacatcacaactttggttgccgacat-----ctctgcgacagGGAGTATCCC 1447
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1426 agttgcgtttcaaaacttgaacaatggcgttgacaccgtgaaatagGGAGTACCCT 1482
E. festucae Fl1 1439 GGGGCTTCTGATCGAGGTGGCAAGAAGGAGGATACGAAGAACTACGTCGAGCTCGTC 1495
E. festucae E2368 1439 GGGGCTTCTGATCGAGGTGGCAAGAAGGAGGATACGAAGAACTACGTCGAGCTCGTC 1495
E. baconii 1430 GGGGCTCCTGATCGAGGTGGCCAGAAGGAGGATACGAAGAACTACGTCGAGCTCGTC 1486
E. sylvatica 1430 GGGGCTTCTGATCGAGGTGGCAAGGAGGAGGATACGAAGAACTACGTCGAGCTCGTC 1486
E. typhina 1448 GGGGCTTCTGATCGAGGTGGCAAGGAGGAGGATACGAAGAACTACGTCGAGCTCGTC 1504
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1483 GGGGCTCCCGATCGGGGTGGCAAGAAGGAAGATACGAAGAATTATGTCGAGCTTGTC 1539
E. festucae Fl1 1496 AAGACACTCCGAGCCACGTTTGCCAAAACTGGGCGTAGCTTGGGCATCACCTTCACA 1552
E. festucae E2368 1496 AAGACACTCCGAGCCACGTTTGACAAAACTGGGCGTAGCTTGGGCATCACCTTCACA 1552
E. baconii 1487 AAGACACTCCGAGCCACGTTTGACAAAACTGGGCGTAGCTTGGGCATCACCTTCACA 1543
E. sylvatica 1487 AAGACACTCCGAGCCACGTTTGACAAAACTGGGCGTAGCTTGGGCATCACCTTCACA 1543
E. typhina 1505 AAGACACTCCGAGCCACGTTTGACAAAACTGGGCGCAGCTTGGGCATCACCTTCACA 1561
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1540 AAGACGCTCCGGGCTACGTTTGATAAGACAGGCCGTAGCTTAGGCATTACGTTCACG 1596

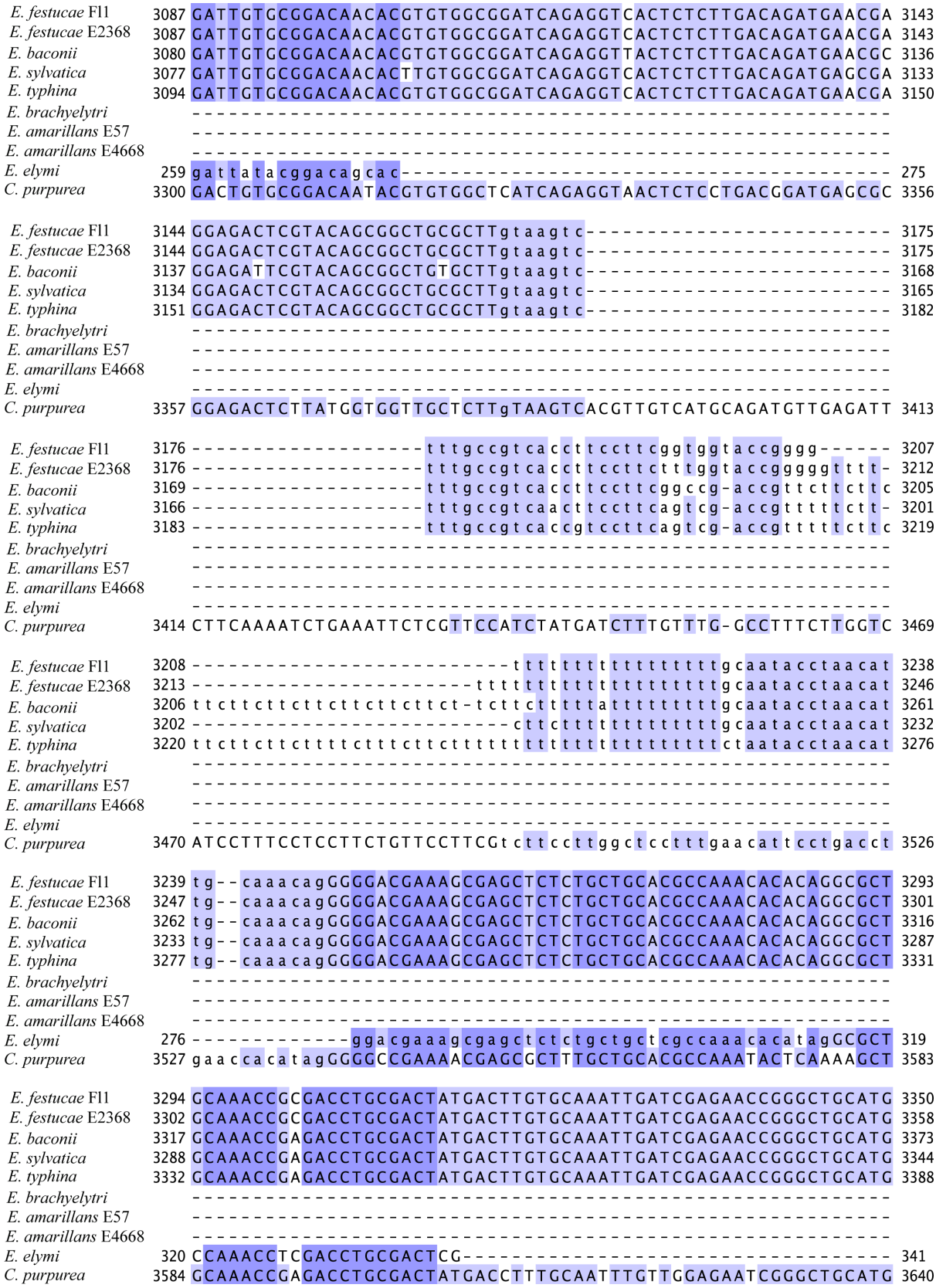

E. festucae Fl1 1553 GCGCCATCTTCTTACTGGTATCTAAGATGGTTCGATCTGCCGGGAATGATGAAGTAT 1609
E. festucae E2368 1553 GCGCCATCTTCTTACTGGTATCTAAGATGGTTCGATCTGCCGGGAATGATGAAGTAT 1609
E. baconii 1544 GCGCCATCTTCTTACTGGTATCTAAAATGGTTCGATCTGCCGGGAATGATGAAGTAT 1600
E. sylvatica 1544 GCGCCATCTTCCTACTGGTATCTAAGATGGTTCGATCTGCCGGGAATGATGAAGTAT 1600
E. typhina 1562 GCGCCATCTTCTTACTGGTATCTAAGATGGTTCGATCTGCCGGGAATGATGAAGTAT 1618
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1597 GCGCCGTCTTCGTACTGGTATCTGAAGTGGTTTGATCTGCCAGGCATGATGAAATAC 1653
E. festucae Fl1 1610 TGCGACTGGATCAACTTGgtatgttggatt----------taaactcaga 1649
E. festucae E2368 1610 TGCGACTGGATCAACTTGgtatgttggatt----------taaactcaga 1649
E. baconii 1601 TGCGACTGGGTCAACTTGgtatgttgaatt----------ttaactcaga 1640
E. sylvatica 1601 TGCGACTGGGTCAACTTGgtatgttggattt----------taaactcaga 1641
E. typhina 1619 TGCGACTGGGTCAACTTGgtatgttggattt----------taaactcaga 1659
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1654 TGCGACTGGGTCAACTTGgtatgctatacttgacttcctctctctcatttcttcgac 1710
E. festucae Fl1 1650 aactcgccagcatcaggtactgactgac----------cttgtacagATGTC 1691
E. festucae E2368 1650 aactcgccagcatcaggtactgactgac----------cttgtacagATGTC 1691
E. baconii 1641 agctcgccagcttcaggtactgactgac----------cttgtacagATGTC 1682
E. sylvatica 1642 aactcgccagcatcaggtgctgactgac----------cttgcacagATGTC 1683
E. typhina 1660 aactcgccagcatcaggtactgactgac----------cttgtacagATGTC 1701
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1711 gattcctttttgtctgcttctatcttgtgcctgcccgtactaaattgagcagATGTC 1767
E. festucae Fl1 1692 TTACGATCTCCACGGTATATGGGACAGCACAAATCCCATgtaagcctcgtccctttt- 1747
E. festucae E2368 1692 TTACGATCTCCACGGTATATGGGACAGCACAAATCCCATgtaagcctcgtccctttt- 1747
E. baconii 1683 TTACGATCTCCACGGTATATGGGACAGCACAAATCCGATgtaagcctcgtccattca 1739
E. sylvatica 1684 TTACGATCTCCACGGTATATGGGACAGCACAAATCCGATgtaagcctcgtccattt- 1739
E. typhina 1702 TTACGATCTCCACGGTATATGGGACAGCACAAATCCGATgtaagcctcgtccattt- 1757
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1768 TTACGACCTACACGGCACTTGGGACAGCACAAATCCAATgtaagtcttgctacc--- 1821
E. festucae Fl1 1748 attcaagtgatggtgg------tgtacacgaatgcatgcataaactgatgactgata 1798
E. festucae E2368 1748 attcaagtgatggtgg------tgtacacgaatgcatgcataaactgatgactgata 1798
E. baconii 1740 attcaagtgatgatgg------tgtacacgaatgcatccataaactgatgactgata 1790
E. sylvatica 1740 attcaagtgatggtgg------tgtacacgaatgcatgcataaactgatgactggta 1790
E. typhina 1758 attcaagtgattgtgg------tgtacacgaatgcatgcataaactgatgactggta 1808
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1822 -ttctcgtaattctgagcctttcatttacaatggcgatactaaggcaattacgcata 1877
E. festucae Fl1 1799 gAGGCGCCATTGTCCAAGGCCATACCAACCTGACAGAGGTCAAGTTGGCTGCAGAGC 1855
E. festucae E2368 1799 gAGGCGCCATTGTCCAAGGCCATACCAACCTGACAGAGGTCAAGTTGGCTGCAGAGC 1855
E. baconii 1791 gCGGCGCCATTGTCCAAGGCCATACCAACCTGACAGAGATCAAGTTGGCCGCAGAGC 1847
E. sylvatica 1791 gCGGCGCCATTGTCCAAGGCCATACCAACCTGACAGAGATCAAGTTGGCTGCAGAGC 1847
E. typhina 1809 gCGGCGCCATTGTCCAAGGC---ACCAACCTGACGGAGATCAAGTTGGCTGCAGAGC 1862
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1878 gAGGAGCCATTGCCCAAGGCCATACCAACTTGACAGAGATCAAGTTGGCTGCCGAGC 1934

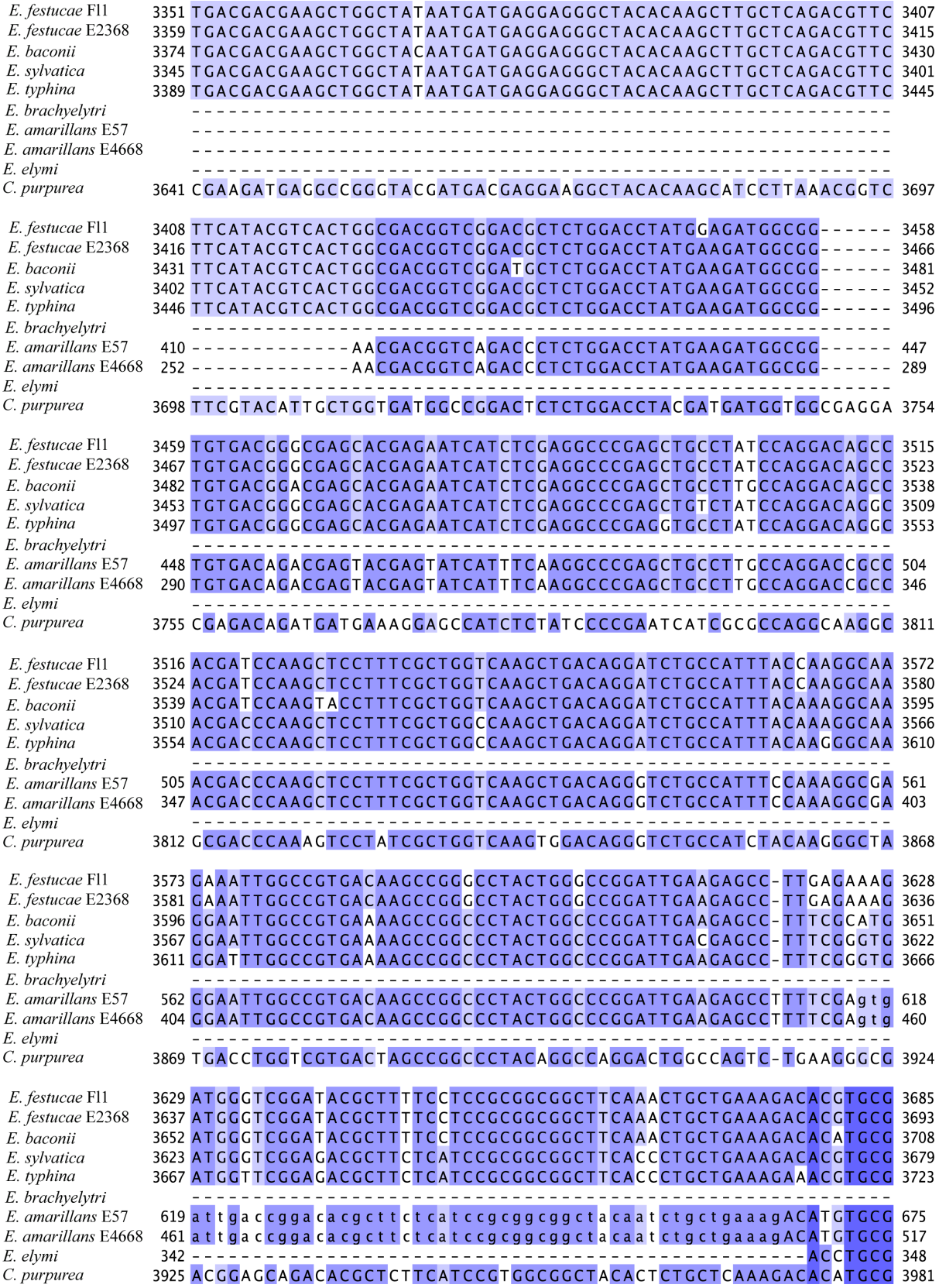

E. festucae Fl1 1856 TGTTCTGGCGCGTGAAGATCCCAGCTCACAAAATCGCCTTGGGCTTCGGGTTCTACG 1912
E. festucae E2368 1856 TGTTCTGGCGCGTGAAGATCCCAGCTCACAAAATCGCCTTGGGCTTCGGGTTCTACG 1912
E. baconii 1848 TGTTCTGGCGCGTGAAGATCCCAGCTCACAAAATCGCCTTGGGCTTCGGGTTCTACG 1904
E. sylvatica 1848 TGTTCTGGCGCGTGAAGATCCCAGCTCACAAAATCGCCTTGGGCTTCGGGTTCTACG 1904
E. typhina 1863 TTTTCTGGCGCGTGAAGATCCCAGCTCACAGAATCGCCTTGGGCTTCGGGTTCTACG 1919
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1935 TCTTTTGGCGAGTCAAGATCCCAGCCCACAAAGTCGCGCTGGGCTTTGGCTTCTACG 1991
E. festucae Fl1 1913 GTCGGGCTTTCACACTTGCCAGTCCCAGCTGTACAACGCCAGGGTGCCCTTTCTCGG 1969
E. festucae E2368 1913 GTCGGGCTTTCACACTTGCCAGTCCCAGCTGTACAACGCCAGGGTGCCCTTTCTCGG 1969
E. baconii 1905 GTCGGGCTTTCACACTTGCCAGTCCCAGCTGTACAACGCCAGGGTGCCCTTTCTCGG 1961
E. sylvatica 1905 GTCGGGCTTTCACACTTGCCAGTCCCAGCTGTACAACGCCAGGGTGCCCTTTCTCGG 1961
E. typhina 1920 GTCGGGCTTTCACACTTGCCAGTCCCAGCTGTACAACGCTAGGGTGCCCTTTCTCGG 1976
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 1992 GTCGGGCTTTCACACTTTCGGATCCCAGTTGTACAACACCAGGGTGTCCCTTCTCAG 2048
E. festucae Fl1 1970 GAGGTGCGAAGCCGGGTTTGTGCACCAAGACAAGCGGATgtagg-----tcttgatc 2021
E. festucae E2368 1970 GAGGTGCGAAGCCGGGTTTGTGCACCAAGACAAGCGGATgtagg-----tcttgatc 2021
E. baconii 1962 GAGGTGCGAAGCCGGGTTCGTGCACCAAGACAAGCGGATgtagg-----tcttgatt 2013
E. sylvatica 1962 GAGGTGCGAAGCCGGGTTTGTGCACCAAGACAAGCGGATgtagg-----tcttgatc 2013
E. typhina 1977 GAGGTGCGAAGCCGGGTTTGTGCACCAAGACAAGCGGATgtagg-----tcttgatc 2028
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2049 GAGGTGCAAAGCCGGGTGTGTGCACCAAGACCAGCGGATgtaagtggcctctgattc 2105
E. festucae Fl1 2022 caaacct-----ttggatatggtctctgct--taaccggtgacagATCTTGCGTATT 2071
E. festucae E2368 2022 caaacct-----ttggatatggtctctgct--taaccggtgacagATCTTGCGTATT 2071
E. baconii 2014 caaacct-----ttggataataatctctgct--taaacggtgacagATCTTGCGTATT 2063
E. sylvatica 2014 caaacct-----ttggatgtggtctctgct--taaacggtgacagATCTTGCGTATT 2063
E. typhina 2029 caaacct-----ttggatgtggtctctgct--taaacggtgacagATCTTGCGTATT 2078
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2106 cagaccttgccattcaacatgtcgtgtgctaacaatccggcacagATCTCGCGTATT 2162
E. festucae Fl1 2072 ATGAGGTGCAGGACATCTTGAAGAAGAACCCCAATATCAAGGTCATCCATGATAAGG 2128
E. festucae E2368 2072 ATGAGGTGCAGGACATCTTGAAGAAGAACCCCAATATCAAGGTCATCCATGATAAGG 2128
E. baconii 2064 ATGAGGTGCAGGACATCTTGAAG---AACCCCGAAATCAAGGTCATCCATGATAAGG 2117
E. sylvatica 2064 ATGAGGTGCAGGACATCTTGAAGAAGAACCCCAATATCAAGGTCATCCATGATAAAG 2120
E. typhina 2079 ATGAGGTGCAGGACATCTTGAAGAAGAACCCCAATATCAAGGTCATCCATGATAAAG 2135
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2163 ATGAGGTTCAGGAGATTCTGAAGAAAAATCCCAGCATCAACATCATGCACGATAAAC 2219
E. festucae Fl1 2129 AATCGGCCGTGAAGTACTTCACTTTTGACACAAATCAGTGGATCTCATACGATGACA 2185
E. festucae E2368 2129 AATCGGCCGTGAAGTACTTCACTTTTGACACAAATCAGTGGATCTCATACGATGACA 2185
E. baconii 2118 AATCGGCCGTGAAGTACTTCACTTTTGACACAAATCAGTGGATCTCATACGATGACA 2174
E. sylvatica 2121 AGTCGGCCGTGAAGTACTTCACTTTTGACACAAATCAGTGGATCTCATACGATGACA 2177
E. typhina 2136 AGTCGGCCGTGAAGTACTTCACTTTTGACACAAATCAGTGGATCTCATACGATGACA 2192
E. brachyelytri 74 TCATATAATCATA 86
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2220 AAGCCGCAGTCAAATACTTTACGTTTGACAAGAACCAGTGGATCTCCTATGACGATA 2276

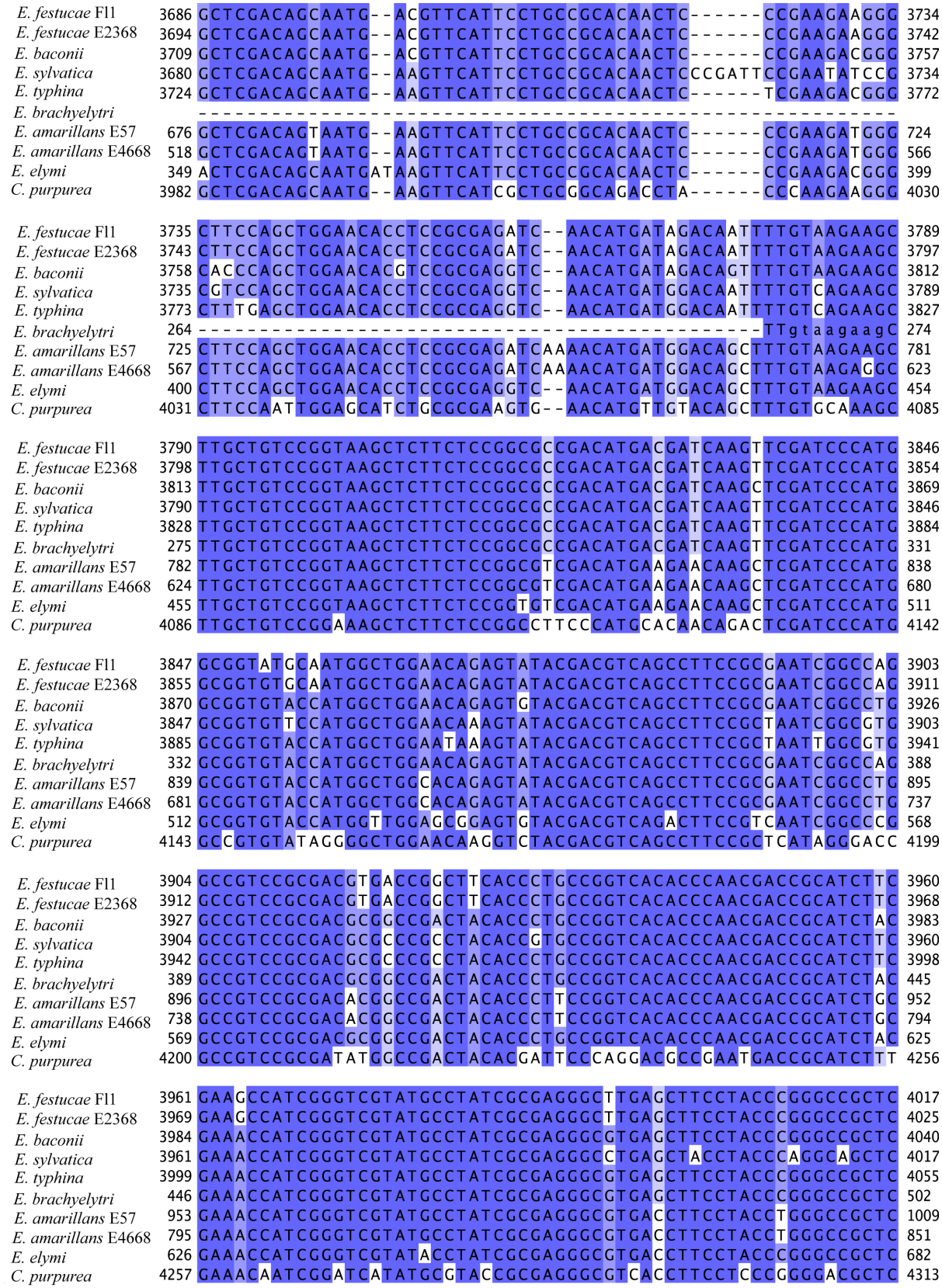

E. festucae Fl1 2186 AGGATACTTTCAAACAAAAGGTCGAATGGGCCAATGCTGCTGGCTTCTCGGGCTCCT 2242
E. festucae E2368 2186 AGGATACTTTCAAACAAAAGGTCGAATGGGCCAATGCTGCTGGCTTCTCGGGCTCCT 2242
E. baconii 2175 AGGATACTTTCAAACAAAAGGTCGAATGGGCCAATGCTGCTGGCTTCTCGGGCTCCT 2231
E. sylvatica 2178 AGGATACTCTCAAACAAAAGGTCGAATGGGCCAATGCTGCTGGCTTCTCGGGCTCCT 2234
E. typhina 2193 AGGATACTCTCAAACAAAAGGTCGAATGGGCCAATGCTGCTGGCTTCTCGGGCTCCT 2249
E. brachyelytri 87 GGGTTATTGTCACATCA----------CG 105
E. amarillans E57
E. amarillans E4668
E. elymi 75 ----------TC 76
C. purpurea 2277 AAGACACGTTCAAGCAAAAGGTTGAATGGGCGAAATAGTGTTGGCTTCTCGGGCTCGC 2333
E. festucae Fl1 2243 TGATCTGGGCGTCCGATCTCGgtacgcgtcaacaccaacacca----------cct 2288
E. festucae E2368 2243 TGATCTGGGCGTCCGATCTCGgtacgcgtcaacaccaacacca----------cct 2288
E. baconii 2232 TGATCTGGGCGTCGGATCTCGgtacgcgtcaacaccaacacca----------ccc 2277
E. sylvatica 2235 TGATCTGGGCGTCCGATCTCGgtacgtatcaacaccaacacca----------cct 2280
E. typhina 2250 TGATCTGGGCGTCCGATCTCGgttcgtatcaacaccaacacca----------cct 2295
E. brachyelytri 106 TGACGTGGG---------- 114
E. amarillans E57
E. amarillans E4668
E. elymi 77 TGACTCAGGCAT---------- 88
C. purpurea 2334 TGATCTGGGCGTCCGATCTCGgtatggatcaagaattataccaaaatgaccctgctt 2390
E. festucae Fl1 2289 cttcactact----cgcag-cttgtctggctaac--tcccatggtgg---------- 2328
E. festucae E2368 2289 cttcactact----cgcag-cttgtctggctaac--tcccatggtgg---------- 2328
E. baconii 2278 cttcactactcgcacgcag-cttgtctggctaac--tcccatggtgg---------- 2321
E. sylvatica 2281 cttcactact----cgcag-cttgtctggctaac--tcccatggtgg---------- 2320
E. typhina 2296 cttcactact----cgcag-cttgtctggctaac--tcccatggtgg---------- 2335
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2391 tcactctacctt cattcagtcttgtcttgctaacgtttctgtgactggccaccgcga 2447
E. festucae Fl1 2329 ----aacagACGACTTTGATTTTACAGCGCATGCGGCTCTCACGGGCAAAGACAAAT 2381
E. festucae E2368 2329 ----aacagACGACTTTGATTTTACAGCGCATGCGGCTCTCACGGGCAAAGACAAAT 2381
E. baconii 2322 ----aacagACGACTTTGACTTTACAGCGCATGCGGCCCTCACTGGCAAAGACAAAT 2374
E. sylvatica 2321 ----aacagACGACTTTGATTTTACAGCGCATGCGGCTCTCATGGGCAAAGACAAAT 2373
E. typhina 2336 ----aacagACGACTTTGATTTTACAACGCATGCGGCTCTCATGGGCAAAGACAAAT 2388
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2448 ttttaaaagacgacttcgaatttaccgcacatgcctccctcaccggcaaagacaaac 2504
E. festucae Fl1 2382 TGACCCTCAACTCACCACTGAGCAAGAAGACGCTCCAAGCCAGCCAGATGACGAAAG 2438
E. festucae E2368 2382 TGACCCTCAACTCACCACTGAGCAAGAAGACGCTCCAAGCCAGCCAGATGACGAAAG 2438
E. baconii 2375 TGACCCTCAACTCACCACTGAGCAAAAAGACGCTCCAAGCCAGCCAGATGACGAAAG 2431
E. sylvatica 2374 TGACCCTCAACTCACCACTGAGCAAGAAGACGCTCCAAGCCAGCCGGAAGACGAAAG 2430
E. typhina 2389 TGACCCTCAACTCACCACTGAGCAAGAAGACGCTCCAAGCCAGCCGGAAGACGAAAG 2445
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2505 tgaaaattaactcaccactgaacaaaaaagCCGTCGTGAACAACCAGAGGACAAAAG 2561
E. festucae Fl1 2439 ACGATCTGAACTCGTATCTCCGCAAGGACTGCTACGTCCAAGAGGACTCTCTCACTT 2495
E. festucae E2368 2439 ACGATCTGAACTCGTATCTCCGCAAGGACTGCTACGTCCAAGAGGACTCTCTCACTT 2495
E. baconii 2432 ACGATCTGAACTCGTATCTCCGCAAAGACTGCTACGTCCAAGAGGACTCTCTCACTT 2488
E. sylvatica 2431 ACGATCTGAACTCATATCTCCGCAAAGACTGCTACGTCCAAGAGGACTCTCTCACTT 2487
E. typhina 2446 ACGATCTGAACTCATATCTCCGCAAAGACTGCTACGTCCAAGAGGACTCTCTCACTT 2502
E. brachyelytri 115 ----------ACTATACCCACGA---------- 127
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2562 ACAGCCTAGATTCATATCTCCGTAAGGATTGTTACGTACAAGAGGATTCTCTCTCTG 2618

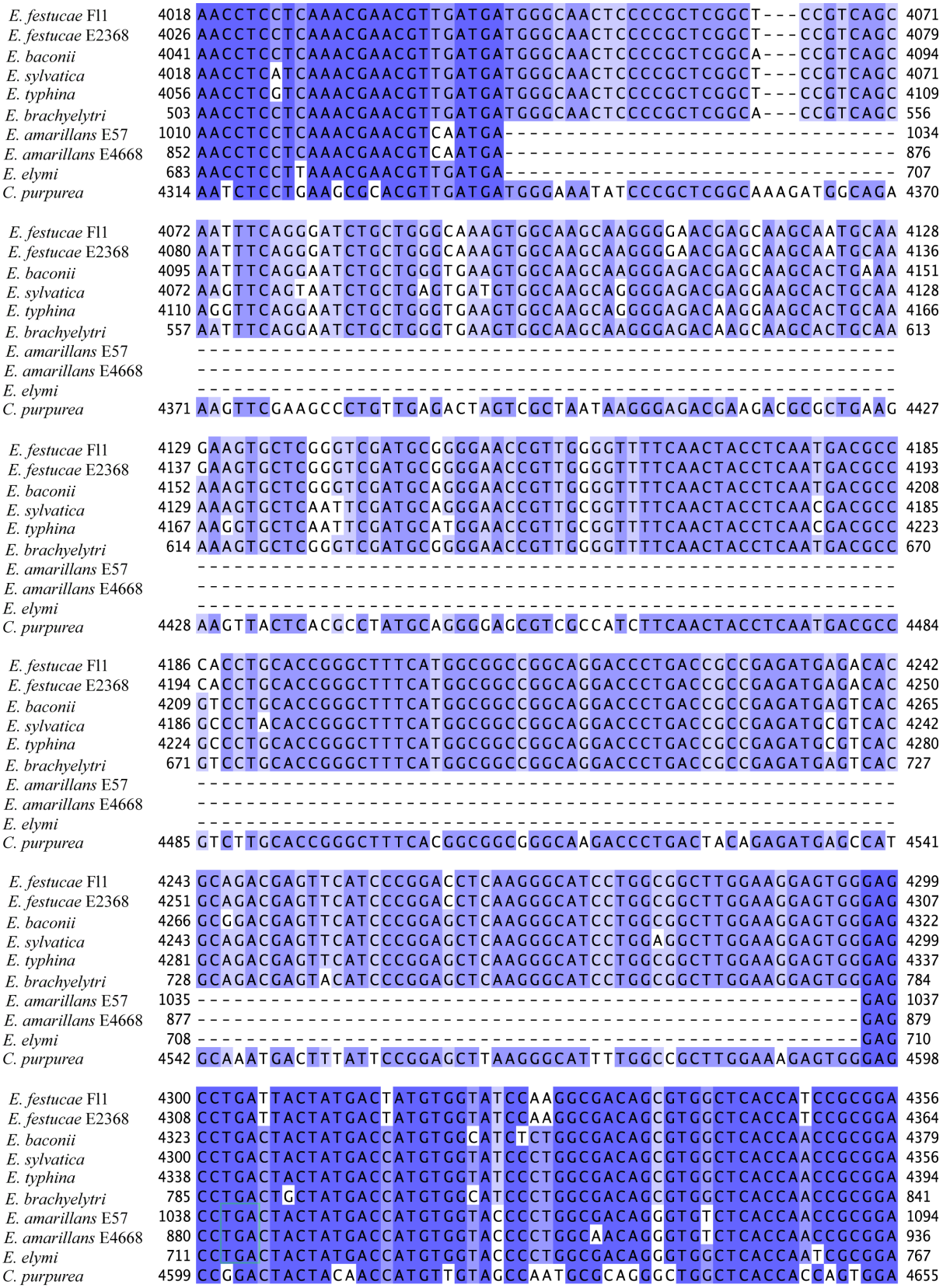

E. festucae Fl1 2496 CCTGCAAGTCAGGTGATGACCAGGTTGGCTATGACAAAGCTGGGAGGgtaag----- 2547
E. festucae E2368 2496 CCTGCAAGTCAGGTGATGACCAGGTTGGCTATGACAAAGCTGGGAGGgtaag----- 2547
E. baconii 2489 CCTGCAAGTCAGGTGATGACCAGGTTGGCTATGACAAAGCTGGGAGGgtaag----- 2540
E. sylvatica 2488 CCTGCAAGTCAGGTGATGACCAGGTTGGCTATGACAAAGCTGGGAGGgtaag----- 2539
E. typhina 2503 CCTGCAAGTCAGGTGATGACGAGGTTGGCTATGACAAAGCTGGGAGGgtaag----- 2554
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2619 CTTGCAAGTCGGGTGACGACCAGGTTGGCTATGACAAAGCTGGACGGgtgagactga 2675
E. festucae Fl1 2548 -ttgacaatccaaaccatctctctcccgtcttggaaggtgtt---------- 2588
E. festucae E2368 2548 -ttgacaatccaaaccatctctctcccgtcttggaaggtgtt---------- 2588
E. baconii 2541 -ttgacaatccaaaccatctctctcccgtcttggagggtgtt---------- 2581
E. sylvatica 2540 -ttgacaatccgaaccatctctctccc--tttggaaggtgtt---------- 2578
E. typhina 2555 -ttgacaatccgaaccatctctctcccgtcttggaaggtgtt---------- 2595
E. brachyelytri 128 ----------TTGGAAGGTGTT---------- 139
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2676 ttcaaccccccaaaataccctatctctaccatagcttgtgttttgtctcttcgccttc 2732
E. festucae Fl1 2589 ----------gttaattg-------gatcgggacagTCA 2610
E. festucae E2368 2589 ----------gttaattg-------gatcgggacagTCA 2610
E. baconii 2582 ----------gttaaatg-------gatcgggacagTCA 2603
E. sylvatica 2579 ----------gttaattg-------gatcgggacagTCA 2600
E. typhina 2596 ----------gttaattg-------tatcgggacagTCA 2617
E. brachyelytri 140 ----------GTTAATTG-------GATCGGGACAGTCA 161
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2733 tggcaaaagaggaaaagctttttcggccgctaatcaacgtatcgctggaaacagTCC 2789
E. festucae Fl1 2611 AAAGATTACAAGCCCATCTGCTGCCCCAAGGGCAGCGGAATGACCAAATGTCAATGG 2667
E. festucae E2368 2611 AAAGATTACAAGCCCATCTGCTGCCCCAAGGGCAGCGGAATGACCAAATGTCAATGG 2667
E. baconii 2604 AAAGATTACAAGCCCATCTGCTGCCCCAAGGGCAGCGGAATGACCAAATGCCAATGG 2660
E. sylvatica 2601 AAAGATTACAAGCCCATCTGCTGCCCCAAGGGCAGCGGAATGACAAAATGCCAATGG 2657
E. typhina 2618 AAAGATTACAAGCCCATCTGCTGCCCCAAGGGCAGCGGAATGACAAAATGTCAATGG 2674
E. brachyelytri 162 AAAGATTACAAGCCCATCTGCTGCCCCAAGGGCAGCGGAATGACCAAATGCCAATGG 218
E. amarillans E57
E. amarillans E4668
E. elymi 89 ----------GCGG 92
C. purpurea 2790 AAAGATTACAAGCCCATCTGTTGCCCCAAGGGCAGTGGAATGACCAAATGCCAGTGG 2846
E. festucae Fl1 2668 AGAGGTGGCACCGGCGGCACAAATGCCGGACGAGACTGCAATGGACAATGCCACGCG 2724
E. festucae E2368 2668 AGAGGTGGCACCGGCGGCACAAATGCCGGACGAGACTGCAATGGACAATGCCACGCG 2724
E. baconii 2661 AGAGGTGGCACCGGCGGCACAAATGCCGGACGAGACTGCAATGGACAATGCCACGCG 2717
E. sylvatica 2658 AGAGGTGGCACCGGCGGCACAAATGCCGGACGAGACTGCAATGGACAATGCCACGCG 2714
E. typhina 2675 AGAGGTGGCACCGGCGGCACAGATGCCGGACGAGACTGCAATGGACAATGCCACGCG 2731
E. brachyelytri 219 AGAGGTGGCACCGGCGGCACAAATGCCGGACGAGACTGCAATGGA---------- 263
E. amarillans E57
E. amarillans E4668
E. elymi 93 AGAGGAAACA---------- 102
C. purpurea 2847 AGAGGCGGCACTGGTGGCACAAATGCTGGACGGGACTGCAATGGACAATGCCACACG 2903
E. festucae Fl1 2725 GGCGAGGTCAAAATCACAACGTCTGGCTATGGCGGTTCACCGGGAGAATCCTCCGAG 2781
E. festucae E2368 2725 GGCGAGGTCAAAATCACAACGTCTGGCTATGGCGGTTCACCGGGAGAATCCTCCGAG 2781
E. baconii 2718 GGCGAGGTCAAAATCACAACGTCTGGCTATGGCGGTTCACCGGGAGAATCCTCCGAG 2774
E. sylvatica 2715 GGCGAGGTCAAAATCACAACGTCTGGCTATGGCGGTTCGCCAGGAGAGTCCTCCGAG 2771
E. typhina 2732 GGCGAGGTCAAAATCACAACGTCTGGCTATGGCGGTTCGCCAGGAGAATCCTCCGAG 2788
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2904 GGCGAGGTAAAAATCACAATGTCTAGCTACGGCGGTTCACCAGGGGAGTCTTCCGAG 2960

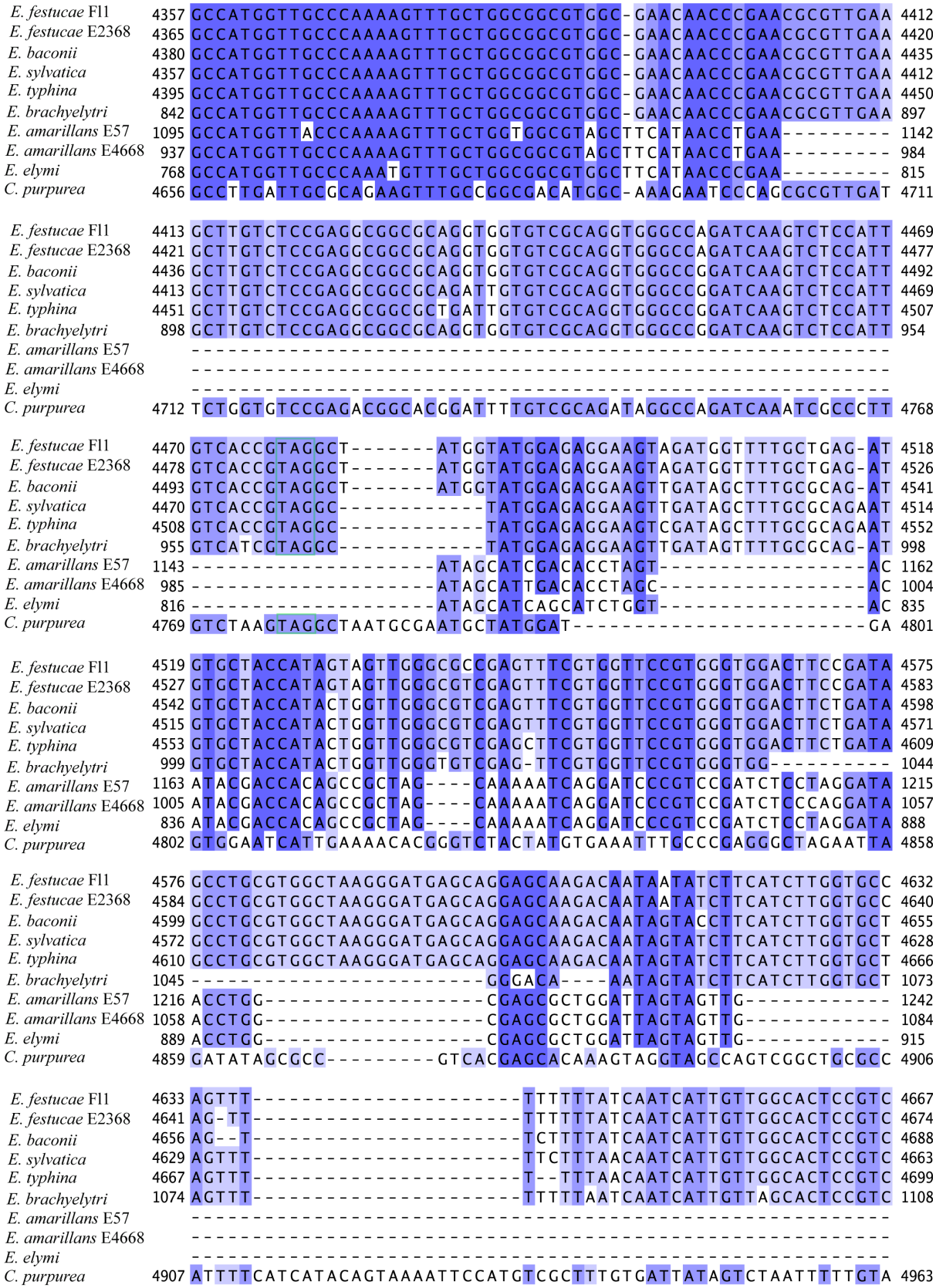

E. festucae Fl1 2782 ACGGCTCACTGCACCAGAGGCCAGAAGGCATTTTGTTGTGAAATGGGTATATTCAGT 2838
E. festucae E2368 2782 ACGGCTCACTGCACCAGAGGCCAGAAGGCATTTTGTTGTGAAATGGGTATATTCAGT 2838
E. baconii 2775 ACGGCTCACTGCACCAGAGGCCAGAAGGCATTTTGTTGTGAAATGGGTATATTCAGT 2831
E. sylvatica 2772 ACGGCTCACTGCACCCGAGGCCAGAAGGCATTTTGTTGTGAAATGGGTATATTCAGT 2828
E. typhina 2789 ACGGCTCACTGCACCAGAGGCCAGAAGGCATTTTGTTGTGAAATGGGTATATTCAGT 2845
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 2961 AAAGCTCACTGCACGCGAGGCCAGAAGGCCTTTTGCTGCCAGATGGGCATATTCAAT 3017
E. festucae Fl1 2839 GACGCCCTCGGCCAGTGCAACTGGTCCAATGGAGTgtaag--ttcttgagcatgagg 2893
E. festucae E2368 2839 GACGCCCTCGGCCAGTGCAACTGGTCCAATGGAGTgtaag--ttcttgagcatgagg 2893
E. baconii 2832 GACGCCCTCGGCCAGTGCAACTGGTCCAATGGAGTgtaag--ttctcgagcatgagg 2886
E. sylvatica 2829 GACGCCCTCGGCCAGTGCAACTGGTCCGAAGGAGTgtaag--ttctcgagcatgagg 2883
E. typhina 2846 GACGCCCTCGGCCAGTGCAACTGGTCCAAAGGAGTgtaag--ttctcgagtatgagg 2900
E. brachyelytri
E. amarillans E57 251 ----------GTCCGATGGAGTgtaag--ttctcgagcatgagg 282
E. amarillans E4668 85 ----------GTCCGATGGAGTGTGAG--TTCTCGAGCATGAGG 116
E. elymi
C. purpurea 3018 GACGCGCTCAACGAGTGTAACTGGTCCAGCGGAGTgtaagttttctcgatcatcaga 3074
E. festucae Fl1 2894 cct----------ttggttgttttggttct--ttgagcgctggct 2925
E. festucae E2368 2894 cct----------ttggttgttttggttct--ttgagcgctggct 2925
E. baconii 2887 cct----------ttggttgttttggttct--ttgagcgctggct 2918
E. sylvatica 2884 cct----------ttggttgttttggttct--ttgagcgctggct 2915
E. typhina 2901 cct----------ttggctgttttggttct--ttgagtgctggct 2932
E. brachyelytri
E. amarillans E57 283 tct----------ttggttccttggttcc--ttgggcgcgggct 314
E. amarillans E4668 117 TCT----------TTGGTTCCTTGGTTCCTTGGTTCC--TTGGGCGCGGGCT 156
E. elymi
C. purpurea 3075 caagatcttcttgctataccattatcctcggtctcagtttccggcggagcatcggct 3131
E. festucae Fl1 2926 gatcccgt-------gacagTGGAAGCTCGTGCAAGAGTGACCAGCAAAAGCTGGC- 2974
E. festucae E2368 2926 gatcccgt-------gacagTGGAAGCTCGTGCAAGAGTGACCAGCAAAAGCTGGC- 2974
E. baconii 2919 gatcccgt-------gacagTGGAAGCTCGTGCAAGAGTGACCAGCAAAAGCTGGC- 2967
E. sylvatica 2916 gatcccat-------gacagTGGAAGCTCGTGCAAGAGTGACCAGCAAAAGCTGGC- 2964
E. typhina 2933 gatcccat-------gacagTGGAAGCTCGTGCAAGAGTGACCAGCAAAAGCTGGC- 2981
E. brachyelytri
E. amarillans E57 315 gatcctgt-------tacagTGGAAGCTCGTGCAAGAGTGATCGGCAAAAGACGGCA 364
E. amarillans E4668 157 GATCCTGT-------TACAGTGGAAGCTCGTGCAAGAGTGATCGGCAAAAGATGGCA 206
E. elymi 103 ------GG-------GTCAGTGGAAGCTCGTGCAAGAGTGACCAGCAAAAGATGGC- 145
C. purpurea 3132 aattctgctcgtacgtacagCGGGAGCTCGTGTAAGAAGACCAGCAAAAGGTCGC- 3187
E. festucae Fl1 2975 -AGACATATGGGACAAGACGGGCTGGGGCACAGTTTTCAAGCACGGCAA-TGAGTTC 3029
E. festucae E2368 2975 -AGACATATGGGACAAGACGGGCTGGGGCACAGTTTTCAAGCACGGCAA-TGAGTTC 3029
E. baconii 2968 -AGACATATGGGACAAGACGGGCTGGGGCACAGTTTTCAAGCACGGCAA-TGAGTTC 3022
E. sylvatica 2965 -ATACATATGGGACAAGACGGGCTGGGGCACAGTTTTCAAGCACGGCAA-TGAGTTC 3019
E. typhina 2982 -ATACATATGGGACAATACGGGCTGGGGCACAGTTTTCAAGCACGGCAA-TGAGTTC 3036
E. brachyelytri
E. amarillans E57 365 TATATATATGGGACAAGACGGGCTGGGGCACAGTTTTCAAGCACG---------- 409
E. amarillans E4668 207 TATATATATGGGACAAGACGGGCTGGGGCACAGTTTTCAAGCACG---------- 251
E. elymi 146 -ATATATATGGGACAAGACGGGCTGGGGCACAGTTTTCAAGCACGGCAATTGAGTTA 201
C. purpurea 3188 -GTACATGTGGGATAAGACGGGCTGGGGCACCGTTTTCAAGCACGGAAA-TGAATTC 3242
E. festucae Fl1 3030 TGTTGTCCCAAGGACGATCCCCCACCACTGCTGTACTGCCGCTGGGTCGGTAAGGGT 3086
E. festucae E2368 3030 TGTTGTCCCAAGGACGATCCCCCACCACTGCTGTACTGCCGCTGGGTCGGTAAGGGT 3086
E. baconii 3023 TGTTGTCCCAAGGACGATCCCCCACCACTGCTGGACTGCCGCTGGGTCGGTAAGGGT 3079
E. sylvatica 3020 TGTTGTCCCAAGAACGATCCTCCACCACTGCTGGACTGCCGCTGGGTCGGTAAGGGT 3076
E. typhina 3037 TGCTGTCCCAAGAACGATCCCCCACCACTTCTGGACTGCCGCTGGGTCGGTAAGGGT 3093
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi 202 TGTTGTCCCAAGGACGATCCTTCACCACTGCTGGACTGCCACTGGgtcggtgagggt 258
C. purpurea 3243 TGTTGTCCCAAAGACGAGCAGCCACCGTTGTTGGACTGCCGCTGGGTCGGAAAAGGC 3299


E. festucae Fl1 3087 GATTGTGCGGACAACACGTGTGGCGGATCAGAGGTCACTCTCTTGACAGATGAACGA 3143
E. festucae E2368 3087 GATTGTGCGGACAACACGTGTGGCGGATCAGAGGTCACTCTCTTGACAGATGAACGA 3143
E. baconii 3080 GATTGTGCGGACAACACGTGTGGCGGATCAGAGGTTACTCTCTTGACAGATGAACGC 3136
E. sylvatica 3077 GATTGTGCGGACAACACTTGTGGCGGATCAGAGGTCACTCTCTTGACAGATGAGCGA 3133
E. typhina 3094 GATTGTGCGGACAACACGTGTGGCGGATCAGAGGTCACTCTCTTGACAGATGAACGA 3150
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi 259 gattatacggacagcac 275
C. purpurea 3300 GACTGTGCGGACAATACGTGTGGCTCATCAGAGGTAACTCTCCTGACGGATGAGCGC 3356
E. festucae Fl1 3144 GGAGACTCGTACAGCGGCTGCGCTTgtaagtc 3175
E. festucae E2368 3144 GGAGACTCGTACAGCGGCTGCGCTTgtaagtc 3175
E. baconii 3137 GGAGATTCGTACAGCGGCTGTGCTTgtaagtc 3168
E. sylvatica 3134 GGAGACTCGTACAGCGGCTGCGCTTgtaagtc 3165
E. typhina 3151 GGAGACTCGTACAGCGGCTGCGCTTgtaagtc 3182
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 3357 GGAGACTCTTATGGTGGTTGCTCTTgTAAGTCACGTTGTCATGCAGATGTTGAGATT 3413
E. festucae Fl1 3176 tttgccgtcaccttccttcggtggtaccgggg 3207
E. festucae E2368 3176 tttgccgtcaccttccttctttggtaccgggggtttt 3212
E. baconii 3169 tttgccgtcaccttccttcggccg-accgttcttcttc 3205
E. sylvatica 3166 tttgccgtcaacttccttcagtcg-accgttttttctt 3201
E. typhina 3183 tttgccgtcaccgtccttcagtcg-accgttttttctc 3219
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 3414 CTTCAAAATCTGAAATTCTCGTTCCATCTATGATCTTTGTTTG-GCCTTTCTTGGTC 3469
E. festucae Fl1 3208 ttttttttttttttttgcaatacctaacat 3238
E. festucae E2368 3213 ttttttttttttttttttgcaatacctaacat 3246
E. baconii 3206 ttcttcttcttcttcttcttct-tcttcttttttttttttgcaatacctaacat 3261
E. sylvatica 3202 cttctttttttttttttgcaatacctaacat 3232
E. typhina 3220 ttcttcttctttttcttcttcttttttttttttttttttttctaatacctaacat 3276
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 3470 ATCCTTTCCTCCTTCTGTTCCTTCGtcttccttggctccttttgaacattcctgacct 3526
E. festucae Fl1 3239 tg--caaacagGGGGACGAAAGCGAGCTCTCTGCTGCACGCCAAACACACAGGCGCT 3293
E. festucae E2368 3247 tg--caaacagGGGGACGAAAGCGAGCTCTCTGCTGCACGCCAAACACACAGGCGCT 3301
E. baconii 3262 tg--caaacagGGGGACGAAAGCGAGCTCTCTGCTGCACGCCAAACACACAGGCGCT 3316
E. sylvatica 3233 tg--caaacagGGGGACGAAAGCGAGCTCTCTGCTGCACGCCAAACACACAGGCGCT 3287
E. typhina 3277 tg--caaacagGGGGACGAAAGCGAGCTCTCTGCTGCACGCCAAACACACAGGCGCT 3331
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi 276 ggacgaaagcgagctctctgctgctcgccaaacacatagGCGCT 319
C. purpurea 3527 gaaccacatagGGGGCCGAAAACGAGCGCTTTGCTGCACGCCAAATACTCAAAAGCT 3583
E. festucae Fl1 3294 GCAAACCGCGACCTGCGACTATGACTTGTGCAAATTGATCGAGAACCGGGCTGCATG 3350
E. festucae E2368 3302 GCAAACCGCGACCTGCGACTATGACTTGTGCAAATTGATCGAGAACCGGGCTGCATG 3358
E. baconii 3317 GCAAACCGAGACCTGCGACTATGACTTGTGCAAATTGATCGAGAACCGGGCTGCATG 3373
E. sylvatica 3288 GCAAACCGAGACCTGCGACTATGACTTGTGCAAATTGATCGAGAACCGGGCTGCATG 3344
E. typhina 3332 GCAAACCGAGACCTGCGACTATGACTTGTGCAAATTGATCGAGAACCGGGCTGCATG 3388
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi 320 CCAAACCTCGACCTGCGACTCG 341
C. purpurea 3584 GCAAACCGAGACCTGCGACTATGACCTTTGCAATTTGTTGGAGAATCGGGCTGCATG 3640

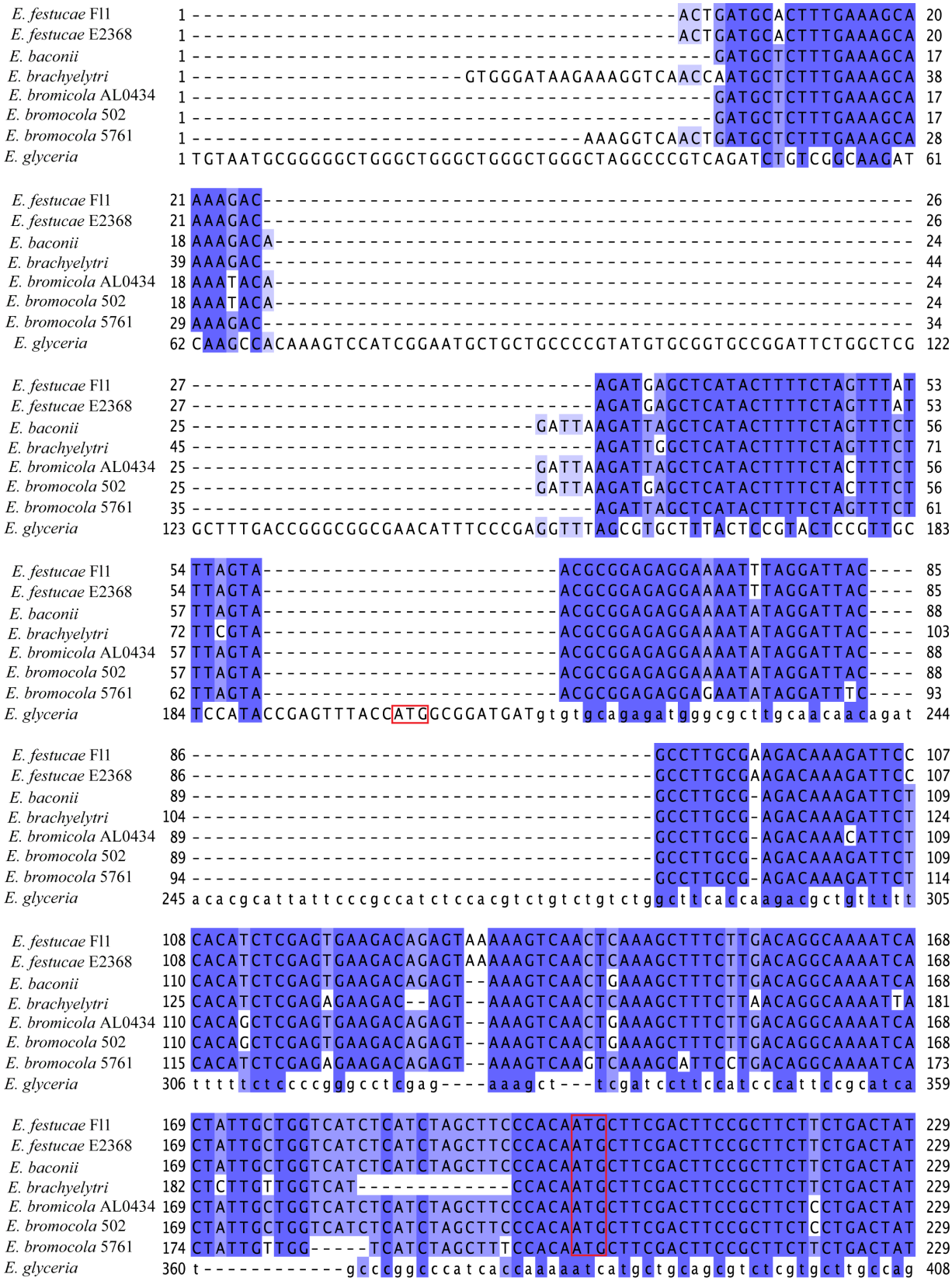

E. festucae Fl1 3351 TGACGACGAAGCTGGCTATAATGATGAGGAGGGCTACACAAGCTTGCTCAGACGTTC 3407
E. festucae E2368 3359 TGACGACGAAGCTGGCTATAATGATGAGGAGGGCTACACAAGCTTGCTCAGACGTTC 3415
E. baconii 3374 TGACGACGAAGCTGGCTACAATGATGAGGAGGGCTACACAAGCTTGCTCAGACGTTC 3430
E. sylvatica 3345 TGACGACGAAGCTGGCTATAATGATGAGGAGGGCTACACAAGCTTGCTCAGACGTTC 3401
E. typhina 3389 TGACGACGAAGCTGGCTATAATGATGAGGAGGGCTACACAAGCTTGCTCAGACGTTC 3445
E. brachyelytri
E. amarillans E57
E. amarillans E4668
E. elymi
C. purpurea 3641 CGAAGATGAGGCCGGGTACGATGACGAGGAAGGCTACACAAGCATCCTTAAACGGTC 3697
E. festucae Fl1 3408 TTCATACGTCACTGGCGACGGTCGGACGCTCTGGACCTATGGAGATGGCGG------ 3458
E. festucae E2368 3416 TTCATACGTCACTGGCGACGGTCGGACGCTCTGGACCTATGAAGATGGCGG------ 3466
E. baconii 3431 TTCATACGTCACTGGCGACGGTCGGATGCTCTGGACCTATGAAGATGGCGG------ 3481
E. sylvatica 3402 TTCATACGTCACTGGCGACGGTCGGACGCTCTGGACCTATGAAGATGGCGG------ 3452
E. typhina 3446 TTCATACGTCACTGGCGACGGTCGGACGCTCTGGACCTATGAAGATGGCGG------ 3496
E. brachyelytri
E. amarillans E57 410 ----------AACGACGGTCAGACCCTCTGGACCTATGAAGATGGCGG------ 447
E. amarillans E4668 252 ----------AACGACGGTCAGACCCTCTGGACCTATGAAGATGGCGG------ 289
E. elymi
C. purpurea 3698 TTCGTACATTGCTGGTGATGGCCGGACTCTCTGGACCTACGATGATGGTGGCGAGGA 3754
E. festucae Fl1 3459 TGTGACGGGCGAGCACGAGAATCATCTCGAGGCCCGAGCTGCCTATCCAGGACAGCC 3515
E. festucae E2368 3467 TGTGACGGGCGAGCACGAGAATCATCTCGAGGCCCGAGCTGCCTATCCAGGACAGCC 3523
E. baconii 3482 TGTGACGGACGAGCACGAGAATCATCTCGAGGCCCGAGCTGCCTTGCCAGGACAGCC 3538
E. sylvatica 3453 TGTGACGGGCGAGCACGAGAATCATCTCGAGGCCCGAGCTGTCTATCCAGGACAGGC 3509
E. typhina 3497 TGTGACGGGCGAGCACGAGAATCATCTCGAGGCCCGAGGTGCCTATCCAGGACAGGC 3553
E. brachyelytri
E. amarillans E57 448 TGTGACAGACGAGTACGAGTATCATTTCAAGGCCCGAGCTGCCTTGCCAGGACCGCC 504
E. amarillans E4668 290 TGTGACAGACGAGTACGAGTATCATTTCAAGGCCCGAGCTGCCTTGCCAGGACCGCC 346
E. elymi
C. purpurea 3755 CGAGACAGATGATGAAAGGAGCCATCTCTATCCCCGAATCATCGCGCCAGGCAAGGC 3811
E. festucae Fl1 3516 ACGATCCAAGCTCCTTTCGCTGGTCAAGCTGACAGGATCTGCCATTTACCAAGGCAA 3572
E. festucae E2368 3524 ACGATCCAAGCTCCTTTCGCTGGTCAAGCTGACAGGATCTGCCATTTACCAAGGCAA 3580
E. baconii 3539 ACGATCCAAGTACCTTTCGCTGGTCAAGCTGACAGGATCTGCCATTTACAAAGGCAA 3595
E. sylvatica 3510 ACGACCCAAGCTCCTTTCGCTGGCCAAGCTGACAGGATCTGCCATTTACAAAGGCAA 3566
E. typhina 3554 ACGACCCAAGCTCCTTTCGCTGGCCAAGCTGACAGGATCTGCCATTTACAAGGCAA 3610
E. brachyelytri
E. amarillans E57 505 ACGACCCAAGCTCCTTTCGCTGGTCAAGCTGACAGGGTCTGCCATTTCCAAAGGCGA 561
E. amarillans E4668 347 ACGACCCAAGCTCCTTTCGCTGGTCAAGCTGACAGGGTCTGCCATTTCCAAAGGCGA 403
E. elymi
C. purpurea 3812 GCGACCCAAAGTCCTATCGCTGGTCAAGTGGACAGGGTCTGCCATCTACAAGGGCTA 3868
E. festucae Fl1 3573 GAAATTGGCCGTGACAAGCCGGGCCTACTGGGCCGGATTGAAGAGCC-TTGAGAAAG 3628
E. festucae E2368 3581 GAAATTGGCCGTGACAAGCCGGGCCTACTGGGCCGGATTGAAGAGCC-TTGAGAAAG 3636
E. baconii 3596 GGAATTGGCCGTGAAAAGCCGGCCCTACTGGCCCGGATTGAAGAGCC-TTTCGCATG 3651
E. sylvatica 3567 GGAATTGGCCGTGAAAAGCCGGCCCTACTGGCCCGGATTGACGAGCC-TTTCGGGTG 3622
E. typhina 3611 GGATTTGGCCGTGAAAAGCCGGCCCTACTGGCCCGGATTGAAGAGCC-TTTCGGGTG 3666
E. brachyelytri
E. amarillans E57 562 GGAATTGGCCGTGACAAGCCGGCCCTACTGGCCCGGATTGAAGAGCCTTTTCGAgtg 618
E. amarillans E4668 404 GGAATTGGCCGTGACAAGCCGGCCCTACTGGCCCGGATTGAAGAGCCTTTTCGAgtg 460
E. elymi
C. purpurea 3869 TGACCTGGTCGTGACTAGCCGGCCCTACAGGCCAGGACTGGCCAGTC-TGAAGGGCG 3924
E. festucae Fl1 3629 ATGGGTCGGATACGCTTTTCCTCCGCGGCGGCTTCAAACTGCTGAAAGACACGTGCG 3685
E. festucae E2368 3637 ATGGGTCGGATACGCTTTTCCTCCGCGGCGGCTTCAAACTGCTGAAAGACACGTGCG 3693
E. baconii 3652 ATGGGTCGGATACGCTTTTCCTCCGCGGCGGCTTCAAACTGCTGAAAGACACATGCG 3708
E. sylvatica 3623 ATGGGTCGGAGACGCTTCTCATCCGCGGCGGCTTCACCCTGCTGAAAGACACGTGCG 3679
E. typhina 3667 ATGGTTCGGAGACGCTTCTCATCCGCGGCGGCTTCACCCTGCTGAAAGAACGTGCG 3723
E. brachyelytri
E. amarillans E57 619 attgaccggacacgcttctcatccgcggcggctacaatctgctgaaagACATGTGCG 675
E. amarillans E4668 461 attgaccggacacgcttctcatccgcggcggctacaatctgctgaaagACATGTGCG 517
E. elymi 342 ----------ACCTGCG 348
C. purpurea 3925 ACGGAGCAGACACGCTCTTCATCCGTGGCGGCTACACTCTGCTCAAAGACACATGCG 3981

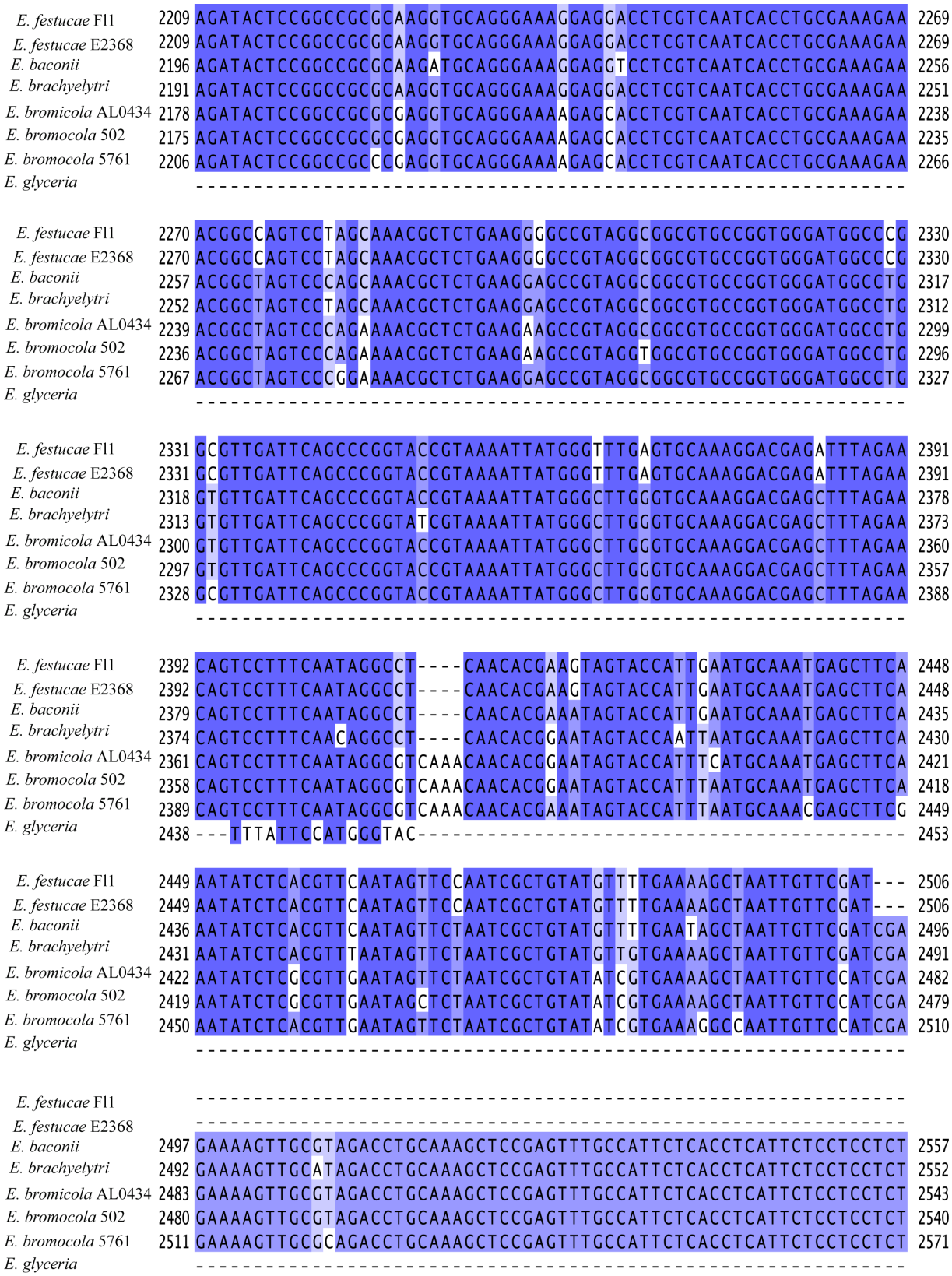

E. festucae Fl1 3686 GCTCGACAGCAATG--ACGTTCATTCCTGCCGCACAACTC------CCGAAGAAGGG 3734
E. festucae E2368 3694 GCTCGACAGCAATG--ACGTTCATTCCTGCCGCACAACTC------CCGAAGAAGGG 3742
E. baconii 3709 GCTCGACAGCAATG--ACGTTCATTCCTGCCGCACAACTC------CCGAAGACGGG 3757
E. sylvatica 3680 GCTCGACAGCAATG--AAGTTCATTCCTGCCGCACAACTCCCGATTCCGAATATCCG 3734
E. typhina 3724 GCTCGACAGCAATG--AAGTTCATTCCTGCCGCACAACTC------TCGAAGACGGG 3772
E. brachyelytri ----------
E. amarillans E57 676 GCTCGACAGTAATG--AAGTTCATTCCTGCCGCACAACTC------CCGAAGATGGG 724
E. amarillans E4668 518 GCTCGACAGTAATG--AAGTTCATTCCTGCCGCACAACTC------CCGAAGATGGG 566
E. elymi 349 ACTCGACAGCAATGATAAGTTCATTCCTGCCGCACAACTC------CCGAAGACGGG 399
C. purpurea 3982 GCTCGACAGCAATG--AAGTTCATCGCTGCGGCAGACCTA------CCCAAGAAGGG 4030
E. festucae Fl1 3735 CTTCCAGCTGGAACACCTCCGCGAGATC--AACATGATAGACAATTTTGTAAGAAGC 3789
E. festucae E2368 3743 CTTCCAGCTGGAACACCTCCGCGAGATC--AACATGATAGACAATTTTGTAAGAAGC 3797
E. baconii 3758 CACCCAGCTGGAACACGTCCGCGAGGTC--AACATGATAGACAGTTTTGTAAGAAGC 3812
E. sylvatica 3735 CGTCCAGCTGGAACACCTCCGCGAGGTC--AACATGATGGACAATTTTGTCAGAAGC 3789
E. typhina 3773 CTTTGAGCTGGAACACCTCCGCGAGGTC--AACATGATGGACAATTTTGTCAGAAGC 3827
E. brachyelytri 264 ----------TTgtaagaagC 274
E. amarillans E57 725 CTTCCAGCTGGAACACCTCCGCGAGATCAAAACATGATGGACAGCTTTGTAAGAAGC 781
E. amarillans E4668 567 CTTCCAGCTGGAACACCTCCGCGAGATCAAAACATGATGGACAGCTTTGTAAGAGGC 623
E. elymi 400 CTTCCAGCTGGAACACCTCCGCGAGGTC--AACATGATGGACAGCTTTGTAAGAAGC 454
C. purpurea 4031 CTTCCAATTGGAGCATCTGCGCGAAGTG--AACATGTTGTACAGCTTTGTGCAAAGC 4085
E. festucae Fl1 3790 TTGCTGTCCGGTAAGCTCTTCTCCGGCGCCGACATGACGATCAAGTTCGATCCCATG 3846
E. festucae E2368 3798 TTGCTGTCCGGTAAGCTCTTCTCCGGCGCCGACATGACGATCAAGTTCGATCCCATG 3854
E. baconii 3813 TTGCTGTCCGGTAAGCTCTTCTCCGGCGCCGACATGACGATCAAGCTCGATCCCATG 3869
E. sylvatica 3790 TTGCTGTCCGGTAAGCTCTTCTCCGGCGCCGACATGACGATCAAGTTCGATCCCATG 3846
E. typhina 3828 TTGCTGTCCGGTAAGCTCTTCTCCGGCGCCGACATGACGATCAAGTTCGATCCCATG 3884
E. brachyelytri 275 TTGCTGTCCGGTAAGCTCTTCTCCGGCGCCGACATGACGATCAAGTTCGATCCCATG 331
E. amarillans E57 782 TTGCTGTCCGGTAAGCTCTTCTCCGGCGTCGACATGAAGAACAAGCTCGATCCCATG 838
E. amarillans E4668 624 TTGCTGTCCGGTAAGCTCTTCTCCGGCGTCGACATGAAGAACAAGCTCGATCCCATG 680
E. elymi 455 TTGCTGTCCGGTAAGCTCTTCTCCGGTGTCGACATGAAGAACAAGCTCGATCCCATG 511
C. purpurea 4086 TTGCTGTCCGGAAAGCTCTTCTCCGGCCTTCCCATGCACAACAGACTCGATCCCATG 4142
E. festucae Fl1 3847 GCGGTATGCAATGGCTGGAACAGAGTATACGACGTCAGCCTTCCGCGAATCGGCCAG 3903
E. festucae E2368 3855 GCGGTGTGCAATGGCTGGAACAGAGTATACGACGTCAGCCTTCCGCGAATCGGCCAG 3911
E. baconii 3870 GCGGTGTACCATGGCTGGAACAGAGTGTACGACGTCAGCCTTCCGCGAATCGGCCTG 3926
E. sylvatica 3847 GCGGTGTTCCATGGCTGGAACAAAGTATACGACGTCAGCCTTCCGCTAATCGGCGTG 3903
E. typhina 3885 GCGGTGTACCATGGCTGGAATAAAGTATACGACGTCAGCCTTCCGCTAATTGGCGTG 3941
E. brachyelytri 332 GCGGTGTACCATGGCTGGAACAGAGTATACGACGTCAGCCTTCCGCGAATCGGCCAG 388
E. amarillans E57 839 GCGGTGTACCATGGCTGGCACAGAGTATACGACGTCAGCCTTCCGCGAATCGGCCTG 895
E. amarillans E4668 681 GCGGTGTACCATGGCTGGCACAGAGTATACGACGTCAGCCTTCCGCGAATCGGCCTG 737
E. elymi 512 GCGGTGTACCATGGTTGGAGCGGAGTGTACGACGTCAGACTTCCGTCAATCGGCCCG 568
C. purpurea 4143 GCCGTGTATAGGGGCTGGAACAAGGTCTACGACGTCAGCCTTCCGCTCATAGGGACC 4199
E. festucae Fl1 3904 GCCGTCCGCGACGTGACCGGCTTCACCCTGCCGGTCACACCCAACGACCGCATCTTC 3960
E. festucae E2368 3912 GCCGTCCGCGACGTGACCGGCTTCACCCTGCCGGTCACACCCAACGACCGCATCTTC 3968
E. baconii 3927 GCCGTCCGCGACGCGGCCGACTACACCCTGCCGGTCACACCCAACGACCGCATCTAC 3983
E. sylvatica 3904 GCCGTCCGCGACGCGCCCGCCTACACCGTGCCGGTCACACCCAACGACCGCATCTTC 3960
E. typhina 3942 GCCGTCCGCGACGCGCCCGCCTACACCCTGCCGGTCACACCCAACGACCGCATCTTC 3998
E. brachyelytri 389 GCCGTCCGCGACGCGGCCGACTACACCCTGCCGGTCACACCCAACGACCGCATCTAC 445
E. amarillans E57 896 GCCGTCCGCGACACGGCCGACTACACCCTTCCGGTCACACCCAACGACCGCATCTGC 952
E. amarillans E4668 738 GCCGTCCGCGACACGGCCGACTACACCCTTCCGGTCACACCCAACGACCGCATCTGC 794
E. elymi 569 GCCGTCCGCGACGCGGCCGACTACACCCTGCCGGTCACACCCAACGACCGCATCTAC 625
C. purpurea 4200 GCCGTCCGCGATATGGCCGACTACACGATTCCCAGGACGCCGAATGACCGCATCTTT 4256
E. festucae Fl1 3961 GAAGCCATCGGGTCGTATGCCTATCGCGAGGGCTTGAGCTTCCTACCCGGGCCGCTC 4017
E. festucae E2368 3969 GAAGCCATCGGGTCGTATGCCTATCGCGAGGGCTTGAGCTTCCTACCCGGGCCGCTC 4025
E. baconii 3984 GAAACCATCGGGTCGTATGCCTATCGCGAGGGCGTGAGCTTCCTACCCGGGCCGCTC 4040
E. sylvatica 3961 GAAACCATCGGGTCGTATGCCTATCGCGAGGGCCTGAGCTACCTACCCAGGCAGCTC 4017
E. typhina 3999 GAAACCATCGGGTCGTATGCCTATCGCGAGGGCGTGAGCTTCCTACCCGGGCCGCTC 4055
E. brachyelytri 446 GAAACCATCGGGTCGTATGCCTATCGCGAGGGCGTGAGCTTCCTACCCGGGCCGCTC 502
E. amarillans E57 953 GAAACCATCGGGTCGTATGCCTATCGCGAGGGCGTGACCTTCCTACCTGGGCCGCTC 1009
E. amarillans E4668 795 GAAACCATCGGGTCGTATGCCTATCGCGAGGGCGTGACCTTCCTACCTGGGCCGCTC 851
E. elymi 626 GAAACCATCGGGTCGTATACCTATCGCGAGGGCGTGACCTTCCTACCCGGGCCGCTC 682
C. purpurea 4257 GAAACAATCGGATCATATGCGTACCGCGAGGGCGTCACCTTCCTCCCGGGGACGCTC 4313

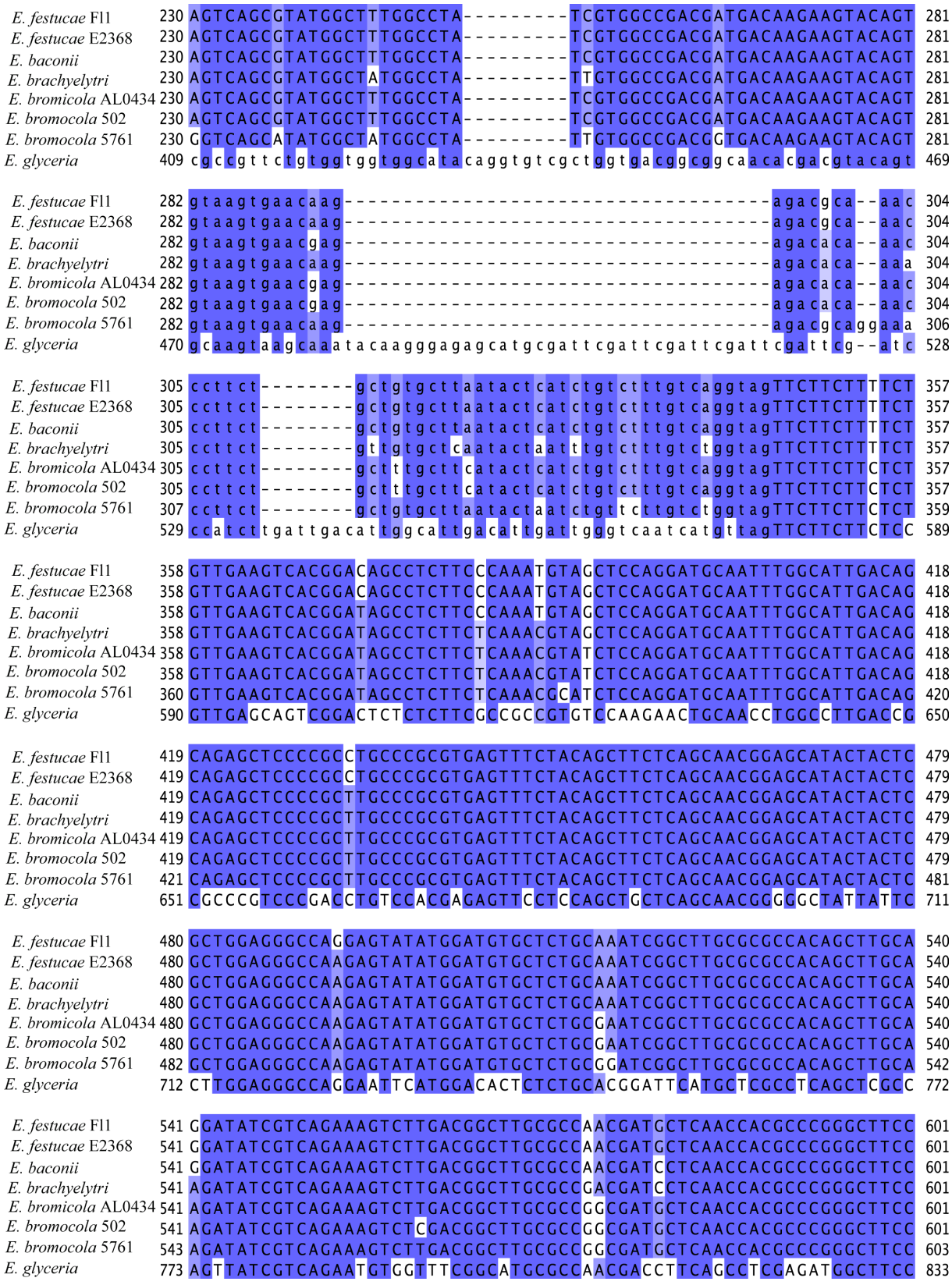

E. festucae Fl1 4018 AACCTCCTCAAACGAACGTTGATGATGGGCAACTCCCCGCTCGGCT---CCGTCAGC 4071
E. festucae E2368 4026 AACCTCCTCAAACGAACGTTGATGATGGGCAACTCCCCGCTCGGCT---CCGTCAGC 4079
E. baconii 4041 AACCTCCTCAAACGAACGTTGATGATGGGCAACTCCCCGCTCGGCA---CCGTCAGC 4094
E. sylvatica 4018 AACCTCATCAAACGAACGTTGATGATGGGCAACTCCCCGCTCGGCT---CCGTCAGC 4071
E. typhina 4056 AACCTCGTCAAACGAACGTTGATGATGGGCAACTCCCCGCTCGGCT---CCGTCAGC 4109
E. brachyelytri 503 AACCTCCTCAAACGAACGTTGATGATGGGCAACTCCCCGCTCGGCA---CCGTCAGC 556
E. amarillans E57 1010 AACCTCCTCAAACGAACGTCAATGA---------- 1034
E. amarillans E4668 852 AACCTCCTCAAACGAACGTCAATGA---------- 876
E. elymi 683 AACCTCCTTAAACGAACGTTGATGA---------- 707
C. purpurea 4314 AATCTCCTGAAGCGCACGTTGATGATGGGAAATATCCCGCTCGGCAAAGATGGCAGA 4370
E. festucae Fl1 4072 AATTTCAGGGATCTGCTGGGCAAAGTGGCAAGCAAGGGGAACGAGCAAGCAATGCAA 4128
E. festucae E2368 4080 AATTTCAGGGATCTGCTGGGCAAAGTGGCAAGCAAGGGGAACGAGCAAGCAATGCAA 4136
E. baconii 4095 AATTTCAGGAATCTGCTGGGTGAAGTGGCAAGCAAGGGAGACGAGCAAGCACTGAAA 4151
E. sylvatica 4072 AAGTTCAGTAATCTGCTGAGTGATGTGGCAAGCAGGGAGACGAGGAAGCACTGCAA 4128
E. typhina 4110 AGGTTCAGGAATCTGCTGGGTGAAGTGGCAAGCAGGGAGACAAGGAAGCACTGCAA 4166
E. brachyelytri 557 AATTTCAGGAATCTGCTGGGTGAAGTGGCAAGCAAGGGAGACAAGCAAGCACTGCAA 613
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. elymi ----------
C. purpurea 4371 AAGTTCGAAGCCCTGTTGAGACTAGTCGCTAATAAGGGAGACGAAGACGCGCTGAAG 4427
E. festucae Fl1 4129 GAAGTGCTCGGGTCGATGCGGGGAACCGTTGGGGTTTTCAACTACCTCAATGACGCC 4185
E. festucae E2368 4137 GAAGTGCTCGGGTCGATGCGGGGAACCGTTGGGGTTTTCAACTACCTCAATGACGCC 4193
E. baconii 4152 AAAGTGCTCGGGTCGATGCAGGGAACCGTTGGGGTTTTCAACTACCTCAATGACGCC 4208
E. sylvatica 4129 AAAGTGCTCAATTCGATGCAGGGAACCGTTGCGGTTTTCAACTACCTCAACGACGCC 4185
E. typhina 4167 AAGGTGCTCAATTCGATGCATGGAACCGTTGCGGTTTTCAACTACCTCAACGACGCC 4223
E. brachyelytri 614 AAAGTGCTCGGGTCGATGCGGGGAACCGTTGGGGTTTTCAACTACCTCAATGACGCC 670
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. elymi ----------
C. purpurea 4428 AAGTTACTCACGCCTATGCAGGGAGCGTCGCCATCTTCAACTACCTCAATGACGCC 4484
E. festucae Fl1 4186 CACCTGCACCGGGCTTTCATGGCGGCCGGCAGGACCCTGACCGCCGAGATGAGACAC 4242
E. festucae E2368 4194 CACCTGCACCGGGCTTTCATGGCGGCCGGCAGGACCCTGACCGCCGAGATGAGACAC 4250
E. baconii 4209 GTCCTGCACCGGGCTTTCATGGCGGCCGGCAGGACCCTGACCGCCGAGATGAGTCAC 4265
E. sylvatica 4186 GCCCTACACCGGGCTTTCATGGCGGCCGGCAGGACCCTGACCGCCGAGATGCGTCAC 4242
E. typhina 4224 GCCCTGCACCGGGCTTTCATGGCGGCCGGCAGGACCCTGACCGCCGAGATGCGTCAC 4280
E. brachyelytri 671 GTCCTGCACCGGGCTTTCATGGCGGCCGGCAGGACCCTGACCGCCGAGATGAGTCAC 727
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. elymi ----------
C. purpurea 4485 GTCTTGCACCGGGCTTTCACGGCGGCGGGCAAGACCCTGACTACAGAGATGAGCCAT 4541
E. festucae Fl1 4243 GCAGACGAGTTCATCCCGGACCTCAAGGGCATCCTGGCGGCTTGGAAGGAGTGGGAG 4299
E. festucae E2368 4251 GCAGACGAGTTCATCCCGGACCTCAAGGGCATCCTGGCGGCTTGGAAGGAGTGGGAG 4307
E. baconii 4266 GCGGACGAGTTCATCCCGGAGCTCAAGGGCATCCTGGCGGCTTGGAAGGAGTGGGAG 4322
E. sylvatica 4243 GCAGACGAGTTCATCCCGGAGCTCAAGGGCATCCTGGAGGCTTGGAAGGAGTGGGAG 4299
E. typhina 4281 GCAGACGAGTTCATCCCGGAGCTCAAGGGCATCCTGGCGGCTTGGAAGGAGTGGGAG 4337
E. brachyelytri 728 GCAGACGAGTACATCCCGGAGCTCAAGGGCATCCTGGCGGCTTGGAAGGAGTGGGAG 784
E. amarillans E57 1035 ----------GAG 1037
E. amarillans E4668 877 ----------GAG 879
E. elymi 708 ----------GAG 710
C. purpurea 4542 GCAAATGACTTTATTCCGGAGCTTAAGGGCATTTGGCCGCTTGGAAAGAGTGGGAG 4598
E. festucae Fl1 4300 CCTGATTACTATGACTATGTGGTATCCAAGGCGACAGCGTGGCTCACCATCCGCGGA 4356
E. festucae E2368 4308 CCTGATTACTATGACTATGTGGTATCCAAGGCGACAGCGTGGCTCACCATCCGCGGA 4364
E. baconii 4323 CCTGACTACTATGACCATGTGGCATCTCTGGCGACAGCGTGGCTCACCAACCGCGGA 4379
E. sylvatica 4300 CCTGACTACTATGACCATGTGGTATCCCTGGCGACAGCGTGGCTCACCAACCGCGGA 4356
E. typhina 4338 CCTGACTACTATGACCATGTGGTATCCCTGGCGACAGCGTGGCTCACCAACCGCGGA 4394
E. brachyelytri 785 CCTGACTGCTATGACCATGTGGCATCCCTGGCGACAGCGTGGCTCACCAACCGCGGA 841
E. amarillans E57 1038 CCTGACTACTATGACCATGTGGTACCCCTGGCGACAGGGTGTCTCACCAACCGCGGA 1094
E. amarillans E4668 880 CCTGACTACTATGACCATGTGGTACCCCTGGCAACAGGGTGTCTCACCAACCGCGGA 936
E. elymi 711 CCTGACTACTATGACCATGTGGTACCCCTGGCGACAGGGTGGCTCACCAATCGCGGA 767
C. purpurea 4599 CCGGACTACTACAACCATGTTGTAGCCAATGCGCAGGGCTGGCTCACCACCAGTGGA 4655

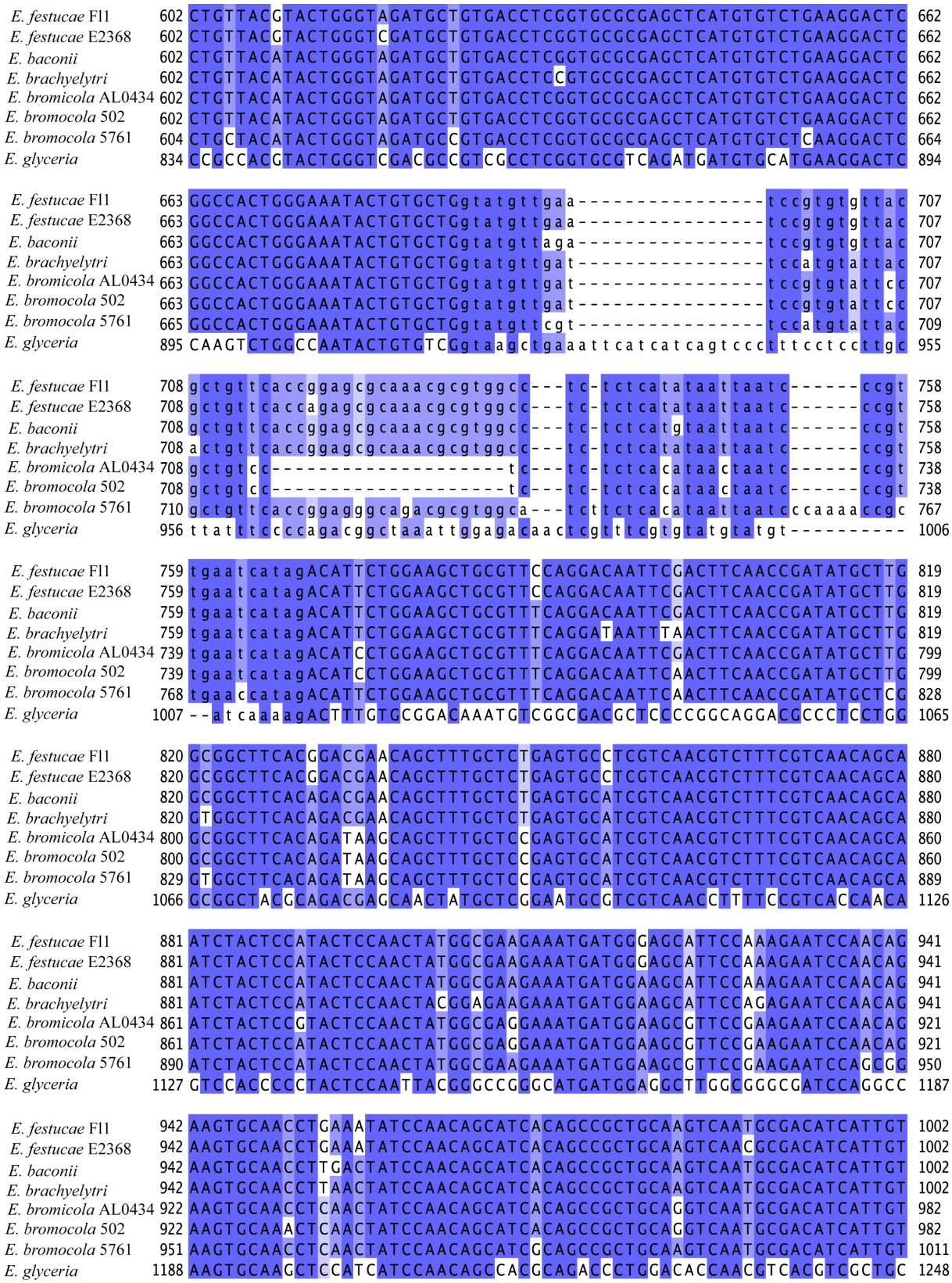

E. festucae Fl1 4357 GCCATGGTTGCCCAAAAGTTTGCTGGCGGCGTGGC-GAACAACCCGAACGCGTTGAA 4412
E. festucae E2368 4365 GCCATGGTTGCCCAAAAGTTTGCTGGCGGCGTGGC-GAACAACCCGAACGCGTTGAA 4420
E. baconii 4380 GCCATGGTTGCCCAAAAGTTTGCTGGCGGCGTGGC-GAACAACCCGAACGCGTTGAA 4435
E. sylvatica 4357 GCCATGGTTGCCCAAAAGTTTGCTGGCGGCGTGGC-GAACAACCCGAACGCGTTGAA 4412
E. typhina 4395 GCCATGGTTGCCCAAAAGTTTGCTGGCGGCGTGGC-GAACAACCCGAACGCGTTGAA 4450
E. brachyelytri 842 GCCATGGTTGCCCAAAAGTTTGCTGGCGGCGTGGC-GAACAACCCGAACGCGTTGAA 897
E. amarillans E57 1095 GCCATGGTTACCCAAAAGTTTGCTGGTGGCGTAGCTTCATAACCTGAA--------- 1142
E. amarillans E4668 937 GCCATGGTTGCCCAAAAGTTTGCTGGCGGCGTAGCTTCATAACCTGAA--------- 984
E. elymi 768 GCCATGGTTGCCCAAATGTTTGCTGGCGGCGTGGCTTCATAACCCGAA--------- 815
C. purpurea 4656 GCCTTGATTGCGCAGAAGTTTGCCGGCGACATGGC-AAAGAATCCCAGCGCGTTGAT 4711
E. festucae Fl1 4413 GCTTGTCTCCGAGGCGGCGCAGGTGGTGTCGCAGGTGGGCCAGATCAAGTCTCCATT 4469
E. festucae E2368 4421 GCTTGTCTCCGAGGCGGCGCAGGTGGTGTCGCAGGTGGGCCAGATCAAGTCTCCATT 4477
E. baconii 4436 GCTTGTCTCCGAGGCGGCGCAGGTGGTGTCGCAGGTGGGCCGGATCAAGTCTCCATT 4492
E. sylvatica 4413 GCTTGTCTCCGAGGCGGCGCAGATTGTGTCGCAGGTGGGCCGGATCAAGTCTCCATT 4469
E. typhina 4451 GCTTGTCTCCGAGGCGGCGCTGATTGTGTCGCAGGTGGGCCGGATCAAGTCTCCATT 4507
E. brachyelytri 898 GCTTGTCTCCGAGGCGGCGCAGGTGGTGTCGCAGGTGGGCCGGATCAAGTCTCCATT 954
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. elymi ----------
C. purpurea 4712 TCTGGTGTCCGAGACGGCACGGATTTTGTCGCAGATAGGCCAGATCAAATCGCCCTT 4768
E. festucae Fl1 4470 GTCACCGTAGGCT-------ATGGTATGGAGAGGAAGTAGATGGTTTTGCTGAG-AT 4518
E. festucae E2368 4478 GTCACCGTAGGCT-------ATGGTATGGAGAGGAAGTAGATGGTTTTGCTGAG-AT 4526
E. baconii 4493 GTCACCGTAGGCT-------ATGGTATGGAGAGGAAGTTGATAGCTTTGCGCAG-AT 4541
E. sylvatica 4470 GTCACCGTAGGC----------TATGGAGAGGAAGTTGATAGCTTTGCGCAGAAT 4514
E. typhina 4508 GTCACCGTAGGC----------TATGGAGAGGAAGTCGATAGCTTTGCGCAGAAT 4552
E. brachyelytri 955 GTCATCGTAGGC----------TATGGAGAGGAAGTTGATAGTTTTGCGCAG-AT 998
E. amarillans E57 1143 ----------ATAGCATCGACACCTAGT----------AC 1162
E. amarillans E4668 985 ----------ATAGCATTGACACCTAGC----------AC 1004
E. elymi 816 ----------ATAGCATCAGCATCTGGT----------AC 835
C. purpurea 4769 GTCTAAGTAGGCTAATGCGAATGCTATGGAT----------GA 4801
E. festucae Fl1 4519 GTGCTACCATAGTAGTTGGGCGCCGAGTTTCGTGGTTCCGTGGGTGGACTTCCGATA 4575
E. festucae E2368 4527 GTGCTACCATAGTAGTTGGGCGTCGAGTTTCGTGGTTCCGTGGGTGGACTTCCGATA 4583
E. baconii 4542 GTGCTACCATACTGGTTGGGCGTCGAGTTTCGTGGTTCCGTGGGTGGACTTCTGATA 4598
E. sylvatica 4515 GTGCTACCATACTGGTTGGGCGTCGAGTTTCGTGGTTCCGTGGGTGGACTTCTGATA 4571
E. typhina 4553 GTGCTACCATACTGGTTGGGCGTCGAGCTTCGTGGTTCCGTGGGTGGACTTCTGATA 4609
E. brachyelytri 999 GTGCTACCATACTGGTTGGGTGTCGAG-TTCGTGGTTCCGTGGGTGG---------- 1044
E. amarillans E57 1163 ATACGACCACAGCCGCTAG----CAAAAATCAGGATCCCGTCCGATCTCCTAGGATA 1215
E. amarillans E4668 1005 ATACGACCACAGCCGCTAG----CAAAAATCAGGATCCCGTCCGATCTCCCAGGATA 1057
E. elymi 836 ATACGACCACAGCCGCTAG----CAAAAATCAGGATCCCGTCCGATCTCCTAGGATA 888
C. purpurea 4802 GTGGAATCATTGAAAACACGGGTCTACTATGTGAAATTTGCCCGAGGGCTAGAATTA 4858
E. festucae Fl1 4576 GCCTGCGTGGCTAAGGGATGAGCAGGAGCAAGACAATAATATCTTCATCTTGGTGCC 4632
E. festucae E2368 4584 GCCTGCGTGGCTAAGGGATGAGCAGGAGCAAGACAATAATATCTTCATCTTGGTGCC 4640
E. baconii 4599 GCCTGCGTGGCTAAGGGATGAGCAGGAGCAAGACAATAGTACCTTCATCTTGGTGCT 4655
E. sylvatica 4572 GCCTGCGTGGCTAAGGGATGAGCAGGAGCAAGACAATAGTATCTTCATCTTGGTGCT 4628
E. typhina 4610 GCCTGCGTGGCTAAGGGATGAGCAGGAGCAAGACAATAGTATCTTCATCTTGGTGCT 4666
E. brachyelytri 1045 ----------GGACA----AATAGTATCTTCATCTTGGTGCT 1073
E. amarillans E57 1216 ACCTGG----------CGAGCGCTGGATTAGTAGTTG---------- 1242
E. amarillans E4668 1058 ACCTGG----------CGAGCGCTGGATTAGTAGTTG---------- 1084
E. elymi 889 ACCTGG----------CGAGCGCTGGATTAGTAGTTG---------- 915
C. purpurea 4859 GATATAGCGCC---------GTCACGAGCACAAAGTAGGTAGCCAGTCGGCTGCGCC 4906
E. festucae Fl1 4633 AGTTT----------TTTTTTATCAATCATTGTTGGCACTCCGTC 4667
E. festucae E2368 4641 AG-TT----------TTTTTTATCAATCATTGTTGGCACTCCGTC 4674
E. baconii 4656 AG--T----------TCTTTTATCAATCATTGTTGGCACTCCGTC 4688
E. sylvatica 4629 AGTTT----------TTCTTTAACAATCATTGTTGGCACTCCGTC 4663
E. typhina 4667 AGTTT----------T--TTTAACAATCATTGTTGGCACTCCGTC 4699
E. brachyelytri 1074 AGTTT----------TTTTTAATCAATCATTGTTAGCACTCCGTC 1108
E. amarillans E57 ----------
E. amarillans E4668 ----------
E. elymi ----------
C. purpurea 4907 ATTTTCATCATACAGTAAAATTCCATGTCGCTTTGTGATTATAGTCTAATTTTTGTA 4963

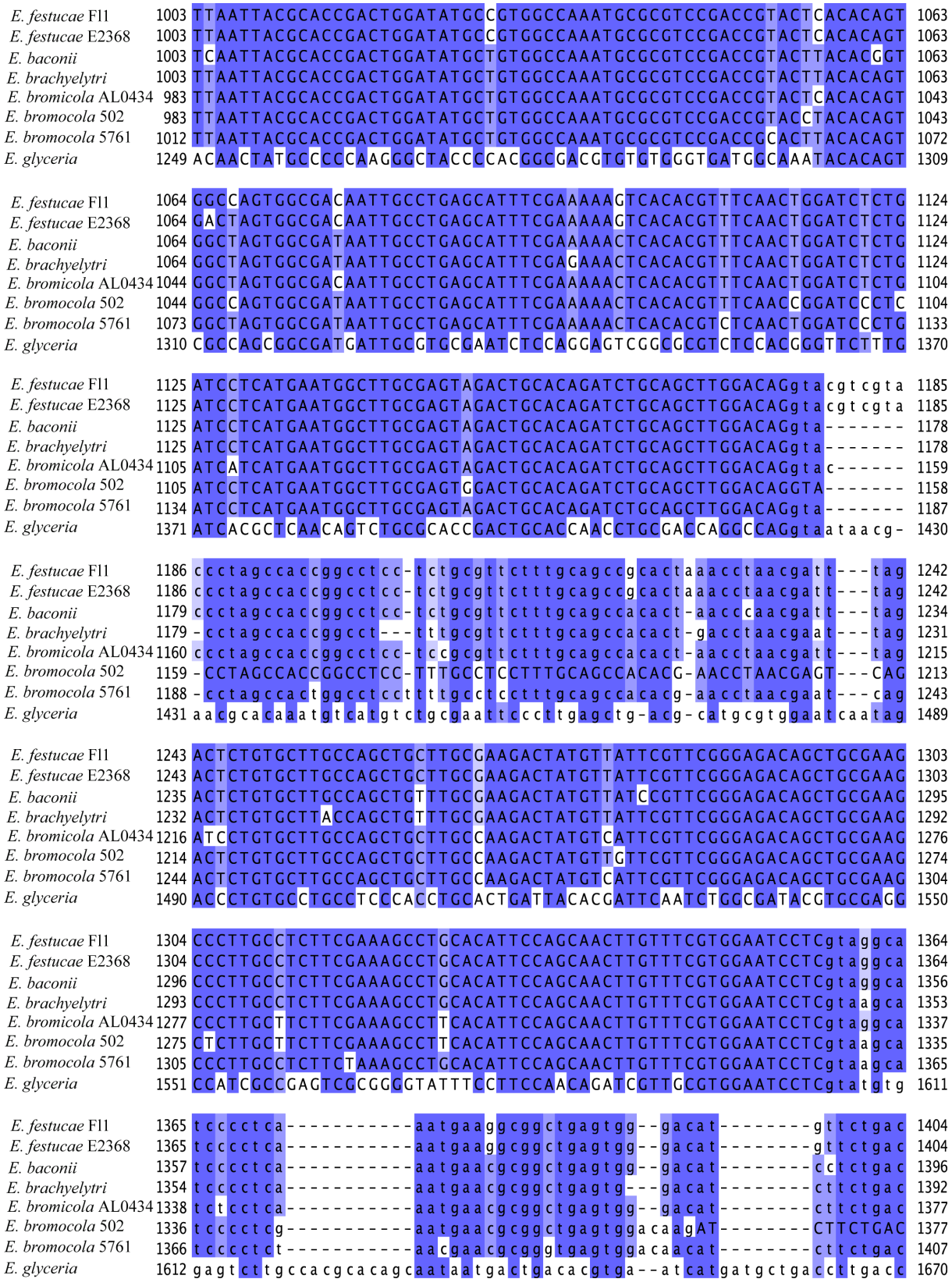
Appendix 18. Multiple sequence alignment of the *E. festucae lymB* gene with homologues from other *Epichloë* species. *E. festucae* (Fl1) EfM3.035020, E. *festucae* (E2368), *E. baconii, E. brachyelytri, E. bromicola* 5761, AL0434 and 502. Intensity of shading indicates the level of conservation. *lymB* start codon (ATG) and stop codons (TAG and TAA) are boxed in red and green respectively. Exons and introns are indicated as capital and small letters respectively.

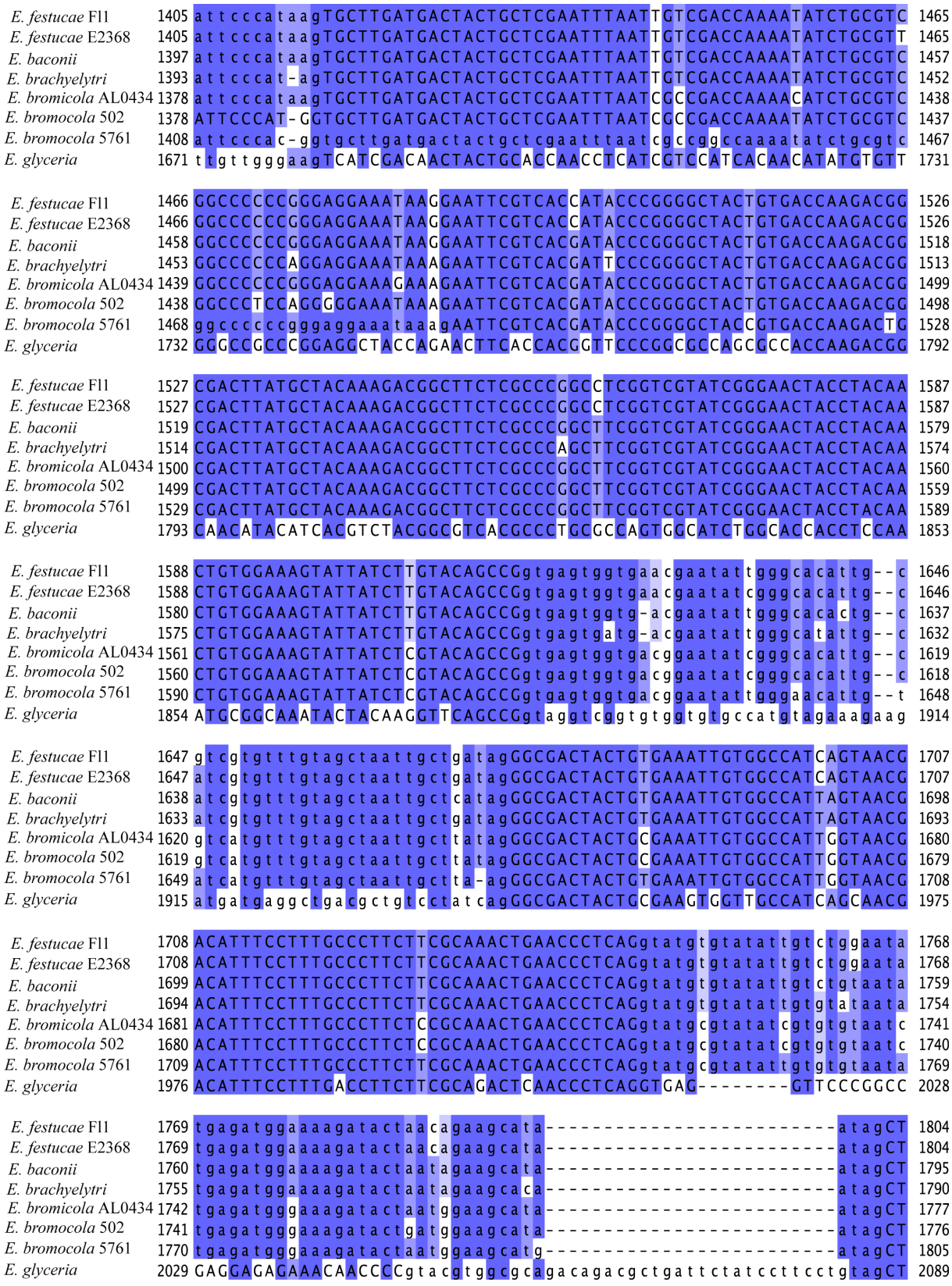

E. festucae Fl1 1 ACTGATGCACTTTGAAAGCA 20
E. festucae E2368 1 ACTGATGCACTTTGAAAGCA 20
E. baconii 1 GATGCTCTTTGAAAGCA 17
E. brachyelytri 1 GTGGGATAAGAAAGGTCAACCAATGCTCTTTGAAAGCA 38
E. bromicola AL0434 1 GATGCTCTTTGAAAGCA 17
E. bromocola 502 1 GATGCTCTTTGAAAGCA 17
E. bromocola 5761 1 AAAGGTCAACTGATGCTCTTTGAAAGCA 28
E. glyceria 1 TGTAATGCGGGGGCTGGGCTGGGCTGGGCTGGGCTAGGCCCGTCAGATCTGTCGGCAAGAT 61
E. festucae Fl1 21 AAAGAC 26
E. festucae E2368 21 AAAGAC 26
E. baconii 18 AAAGACA 24
E. brachyelytri 39 AAAGAC 44
E. bromicola AL0434 18 AAATACA 24
E. bromocola 502 18 AAATACA 24
E. bromocola 5761 29 AAAGAC 34
E. glyceria 62 CAAGCCACAAAGTCCATCGGAATGCTGCTGCCCCGTATGTGCGGTGCCGGATTCTGGCTCG 122
E. festucae Fl1 27 AGATGAGCTCATACTTTTCTAGTTTAT 53
E. festucae E2368 27 AGATGAGCTCATACTTTTCTAGTTTAT 53
E. baconii 25 GATTAAGATTAGCTCATACTTTTCTAGTTTCT 56
E. brachyelytri 45 AGATTGGCTCATACTTTTCTAGTTTCT 71
E. bromicola AL0434 25 GATTAAGATTAGCTCATACTTTTCTACTTTCT 56
E. bromocola 502 25 GATTAAGATGAGCTCATACTTTTCTACTTTCT 56
E. bromocola 5761 35 AGATTAGCTCATACTTTTCTAGTTTCT 61
E. glyceria 123 GCTTTGACCGGGCGGCGAACATTTCCCGAGGTTTAGCGTGCTTTACTCCGTACTCCGTTGC 183
E. festucae Fl1 54 TTAGTA-----ACGCGGAGAGGAAAATTTAGGATTAC---- 85
E. festucae E2368 54 TTAGTA-----ACGCGGAGAGGAAAATTTAGGATTAC---- 85
E. baconii 57 TTAGTA-----ACGCGGAGAGGAAAATATAGGATTAC---- 88
E. brachyelytri 72 TTCGTA-----ACGCGGAGAGGAAAATATAGGATTAC---- 103
E. bromicola AL0434 57 TTAGTA-----ACGCGGAGAGGAAAATATAGGATTAC---- 88
E. bromocola 502 57 TTAGTA-----ACGCGGAGAGGAAAATATAGGATTAC---- 88
E. bromocola 5761 62 TTAGTA-----ACGCGGAGAGGAGAATATAGGATTTC---- 93
E. glyceria 184 TCCATACCGAGTTTACCATGGCGGATGATgtgtgcagagatgggcgcttgcaacaacagat 244
E. festucae Fl1 86 GCCTTGCGAAGACAAAGATTCC 107
E. festucae E2368 86 GCCTTGCGAAGACAAAGATTCC 107
E. baconii 89 GCCTTGCG-AGACAAAGATTCT 109
E. brachyelytri 104 GCCTTGCG-AGACAAAGATTCT 124
E. bromicola AL0434 89 GCCTTGCG-AGACAAACATTCT 109
E. bromocola 502 89 GCCTTGCG-AGACAAAGATTCT 109
E. bromocola 5761 94 GCCTTGCG-AGACAAAGATTCT 114
E. glyceria 245 acacgcattattcccgccatctccacgtctgtctgtctggcttcaccaagacgctgttttt 305
E. festucae Fl1 108 CACATCTCGAGTGAAGACAGAGTAAAAAGTCAACTCAAAGCTTTCTTGACAGGCAAAATCA 168
E. festucae E2368 108 CACATCTCGAGTGAAGACAGAGTAAAAAGTCAACTCAAAGCTTTCTTGACAGGCAAAATCA 168
E. baconii 110 CACATCTCGAGTGAAGACAGAGT--AAAGTCAACTGAAAGCTTTCTTGACAGGCAAAATCA 168
E. brachyelytri 125 CACATCTCGAGAGAAGAC--AGT--AAAGTCAACTCAAAGCTTTCTTAACAGGCAAAATTA 181
E. bromicola AL0434 110 CACAGCTCGAGTGAAGACAGAGT--AAAGTCAACTGAAAGCTTTCTTGACAGGCAAAATCA 168
E. bromocola 502 110 CACAGCTCGAGTGAAGACAGAGT--AAAGTCAACTGAAAGCTTTCTTGACAGGCAAAATCA 168
E. bromocola 5761 115 CACATCTCGAGAGAAGACAGAGT--AAAGTCAAGTCAAAGCATTCCTGACAGGCAAAATCA 173
E. glyceria 306 ttttttctccccgggcctcgag----aaagct---tcgatccttccatccccattccgcatca 359
E. festucae Fl1 169 CTATTGCTGGTCATCTCATCTAGCTTCCCACAATGCTTCGACTTCCGCTTCTTCTGACTAT 229
E. festucae E2368 169 CTATTGCTGGTCATCTCATCTAGCTTCCCACAATGCTTCGACTTCCGCTTCTTCTGACTAT 229
E. baconii 169 CTATTGCTGGTCATCTCATCTAGCTTCCCACAATGCTTCGACTTCCGCTTCTTCTGACTAT 229
E. brachyelytri 182 CTCTTGTTGGTCAT----------CCACAATGCTTCGACTTCCGCTTCTTCTGACTAT 229
E. bromicola AL0434 169 CTATTGCTGGTCATCTCATCTAGCTTCCCACAATGCTTCGACTTCCGCTTCTCCTGACTAT 229
E. bromocola 502 169 CTATTGCTGGTCATCTCATCTAGCTTCCCACAATGCTTCGACTTCCGCTTCTCCTGACTAT 229
E. bromocola 5761 174 CTATTGTTGG-----TCATCTAGCTTTCCACAATGCTTCGACTTCCGCTTCTTCTGACTAT 229
E. glyceria 360 t----------gcccggcccatcaccaaaaatcatgctgcagcgtctcgtgcttgccag 408

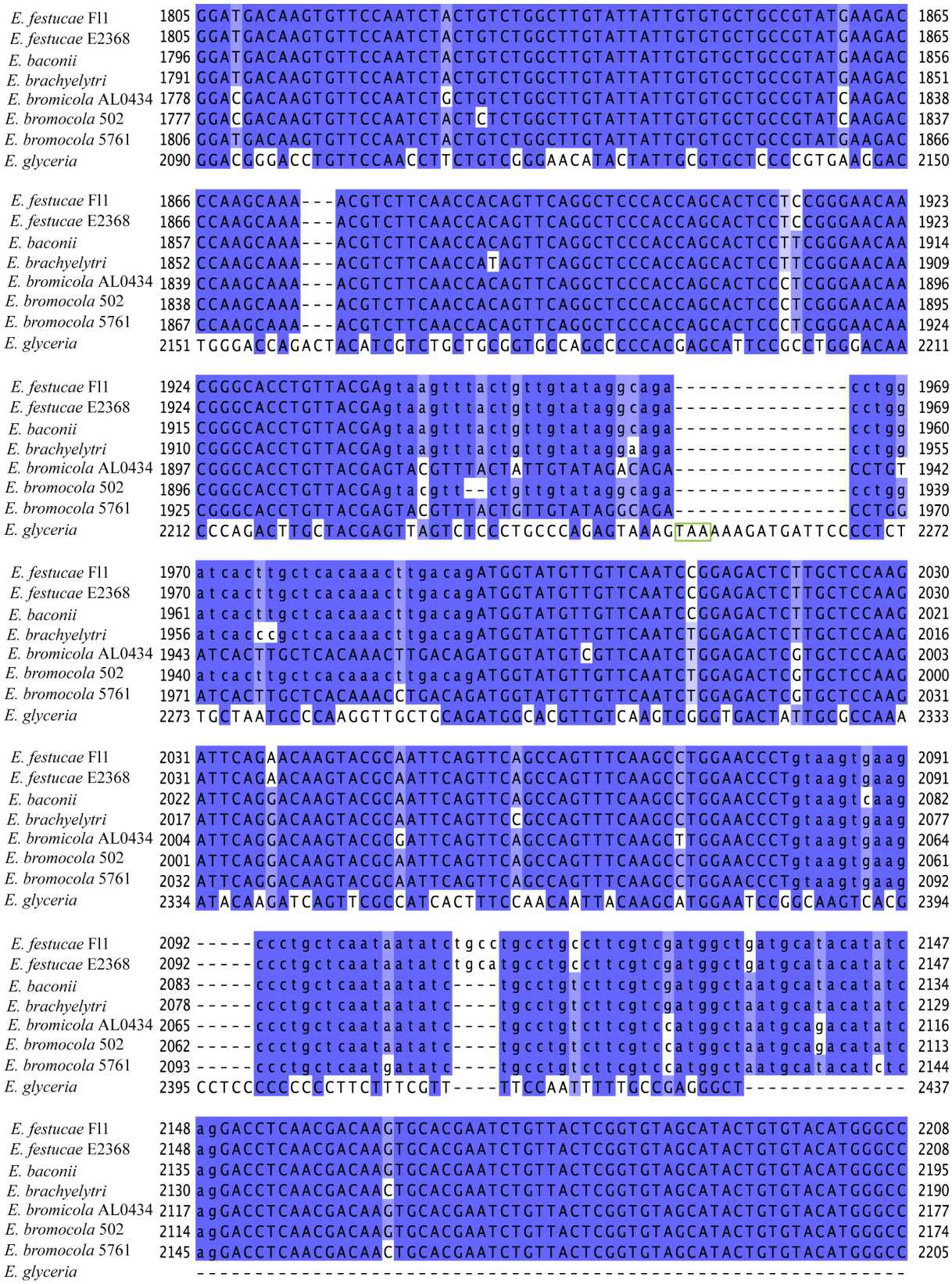

E. festucae Fl1 2209 AGATACTCCGGCCGCGCAAGGTGCAGGGAAAGGAGGACCTCGTCAATCACCTGCGAAAGAA 2269
E. festucae E2368 2209 AGATACTCCGGCCGCGCAAGGTGCAGGGAAAGGAGGACCTCGTCAATCACCTGCGAAAGAA 2269
E. baconii 2196 AGATACTCCGGCCGCGCAAGATGCAGGGAAAGGAGGTCCTCGTCAATCACCTGCGAAAGAA 2256
E. brachyelytri 2191 AGATACTCCGGCCGCGCAAGGTGCAGGGAAAGGAGGACCTCGTCAATCACCTGCGAAAGAA 2251
E. bromicola AL0434 2178 AGATACTCCGGCCGCGCGAGGTGCAGGGAAAAGAGCACCTCGTCAATCACCTGCGAAAGAA 2238
E. bromocola 502 2175 AGATACTCCGGCCGCGCGAGGTGCAGGGAAAAGAGCACCTCGTCAATCACCTGCGAAAGAA 2235
E. bromocola 5761 2206 AGATACTCCGGCCGCCCGAGGTGCAGGGAAAAGAGCACCTCGTCAATCACCTGCGAAAGAA 2266
E. glyceria ----------
E. festucae Fl1 2270 ACGGCCAGTCCTAGCAAACGCTCTGAAGGGGCCGTAGGCGGCGTGCCGGTGGGATGGCCCG 2330
E. festucae E2368 2270 ACGGCCAGTCCTAGCAAACGCTCTGAAGGGGCCGTAGGCGGCGTGCCGGTGGGATGGCCCG 2330
E. baconii 2257 ACGGCTAGTCCCAGCAAACGCTCTGAAGGAGCCGTAGGCGGCGTGCCGGTGGGATGGCCTG 2317
E. brachyelytri 2252 ACGGCTAGTCCTAGCAAACGCTCTGAAGGAGCCGTAGGCGGCGTGCCGGTGGGATGGCCTG 2312
E. bromicola AL0434 2239 ACGGCTAGTCCCAGAAAACGCTCTGAAGAAGCCGTAGGCGGCGTGCCGGTGGGATGGCCTG 2299
E. bromocola 502 2236 ACGGCTAGTCCCAGAAAACGCTCTGAAGAAGCCGTAGGTGGCGTGCCGGTGGGATGGCCTG 2296
E. bromocola 5761 2267 ACGGCTAGTCCCGGAAAACGCTCTGAAGGAGCCGTAGGCGGCGTGCCGGTGGGATGGCCTG 2327
E. glyceria ----------
E. festucae Fl1 2331 GCGTTGATTCAGCCCGGTACCGTAAAATTATGGGTTTGAGTGCAAAGGACGAGATTTAGAA 2391
E. festucae E2368 2331 GCGTTGATTCAGCCCGGTACCGTAAAATTATGGGTTTGAGTGCAAAGGACGAGATTTAGAA 2391
E. baconii 2318 GTGTTGATTCAGCCCGGTACCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2378
E. brachyelytri 2313 GTGTTGATTCAGCCCGGTATCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2373
E. bromicola AL0434 2300 GTGTTGATTCAGCCCGGTACCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2360
E. bromocola 502 2297 GTGTTGATTCAGCCCGGTACCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2357
E. bromocola 5761 2328 GCGTTGATTCAGCCCGGTACCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2388
E. glyceria ----------
E. festucae Fl1 2392 CAGTCCTTTCAATAGGCCT----CAACACGAAGTAGTACCATTGAATGCAAATGAGCTTCA 2448
E. festucae E2368 2392 CAGTCCTTTCAATAGGCCT----CAACACGAAGTAGTACCATTGAATGCAAATGAGCTTCA 2448
E. baconii 2379 CAGTCCTTTCAATAGGCCT----CAACACGAAATAGTACCATTGAATGCAAATGAGCTTCA 2435
E. brachyelytri 2374 CAGTCCTTTCAACAGGCCT----CAACACGGAATAGTACCAATTAATGCAAATGAGCTTCA 2430
E. bromicola AL0434 2361 CAGTCCTTTCAATAGGCGTCAAACAACACGGAATAGTACCATTTCATGCAAATGAGCTTCA 2421
E. bromocola 502 2358 CAGTCCTTTCAATAGGCGTCAAACAACACGGAATAGTACCATTTAATGCAAATGAGCTTCA 2418
E. bromocola 5761 2389 CAGTCCTTTCAATAGGCGTCAAACAACACGAAATAGTACCATTTAATGCAAACGAGCTTCG 2449
E. glyceria 2438 ---TTTATTCCATGGGTAC---------- 2453
E. festucae Fl1 2449 AATATCTCACGTTCAATAGTTCCAATCGCTGTATGTTTTGAAAAGCTAATTGTTCGAT--- 2506
E. festucae E2368 2449 AATATCTCACGTTCAATAGTTCCAATCGCTGTATGTTTTGAAAAGCTAATTGTTCGAT--- 2506
E. baconii 2436 AATATCTCACGTTCAATAGTTCTAATCGCTGTATGTTTTGAATAGCTAATTGTTCGATCGA 2496
E. brachyelytri 2431 AATATCTCACGTTTAATAGTTCTAATCGCTGTATGTTGTGAAAAGCTAATTGTTCGATCGA 2491
E. bromicola AL0434 2422 AATATCTCGCGTTGAATAGTTCTAATCGCTGTATATCGTGAAAAGCTAATTGTTCCATCGA 2482
E. bromocola 502 2419 AATATCTCGCGTTGAATAGCTCTAATCGCTGTATATCGTGAAAAGCTAATTGTTCCATCGA 2479
E. bromocola 5761 2450 AATATCTCACGTTGAATAGTTCTAATCGCTGTATATCGTGAAAGGCCAATTGTTCCATCGA 2510
E. glyceria ----------
E. festucae Fl1 ----------
E. festucae E2368 ----------
E. baconii 2497 GAAAAGTTGCGTAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2557
E. brachyelytri 2492 GAAAAGTTGCATAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2552
E. bromicola AL0434 2483 GAAAAGTTGCGTAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2543
E. bromocola 502 2480 GAAAAGTTGCGTAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2540
E. bromocola 5761 2511 GAAAAGTTGCGCAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2571
E. glyceria ----------

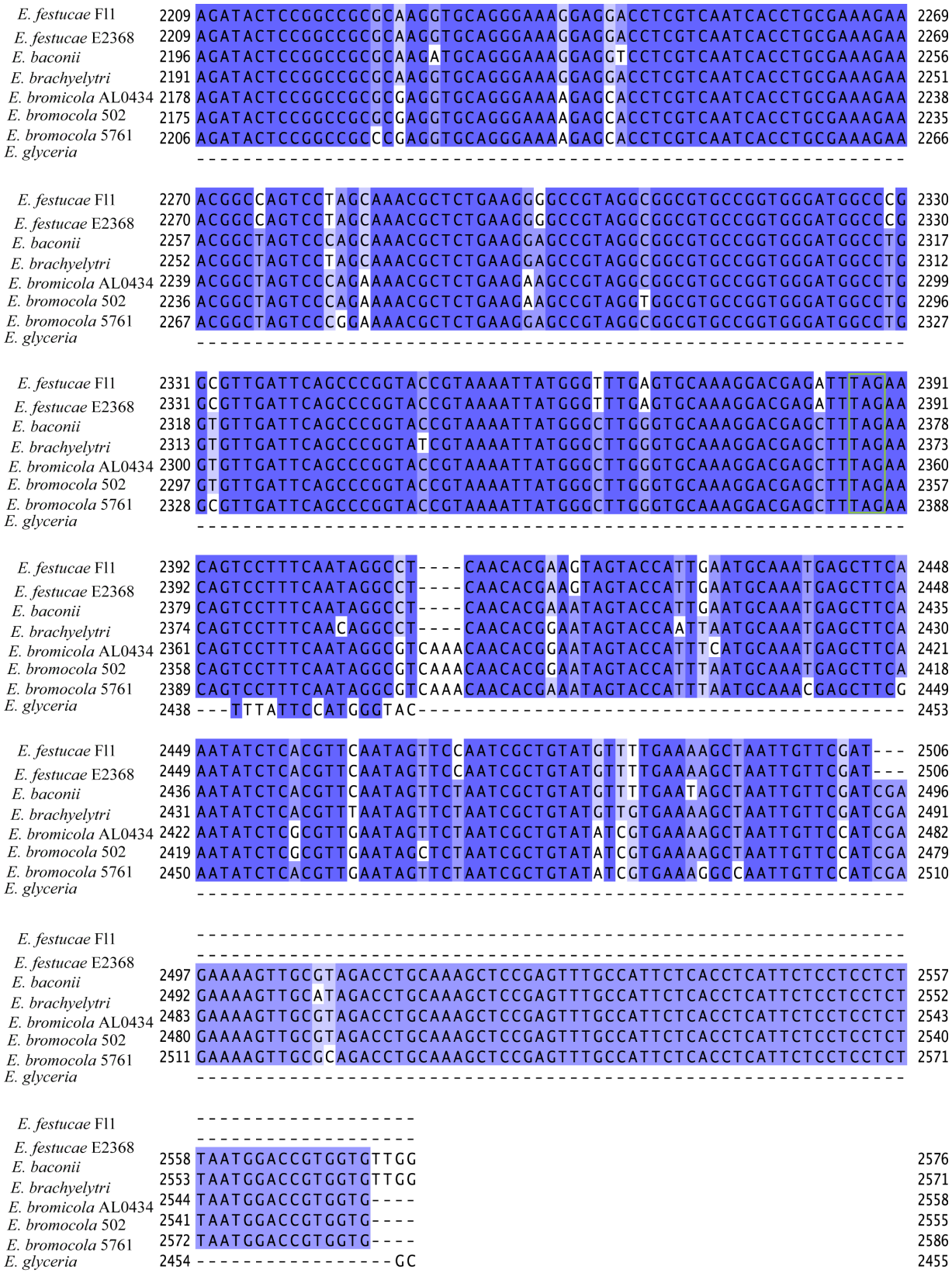

E. festucae Fl1 230 AGTCAGCGTATGGCTTTGGCCTA---------TCGTGGCCGACGATGACAAGAAGTACAGT 281
E. festucae E2368 230 AGTCAGCGTATGGCTTTGGCCTA---------TCGTGGCCGACGATGACAAGAAGTACAGT 281
E. baconii 230 AGTCAGCGTATGGCTTTGGCCTA---------TCGTGGCCGACGATGACAAGAAGTACAGT 281
E. brachyelytri 230 AGTCAGCGTATGGCTATGGCCTA---------TTGTGGCCGACGATGACAAGAAGTACAGT 281
E. bromicola AL0434 230 AGTCAGCGTATGGCTTTGGCCTA---------TCGTGGCCGACGATGACAAGAAGTACAGT 281
E. bromocola 502 230 AGTCAGCGTATGGCTTTGGCCTA---------TCGTGGCCGACGATGACAAGAAGTACAGT 281
E. bromocola 5761 230 GGTCAGCATATGGCTATGGCCTA---------TTGTGGCCGACGGTGACAAGAAGTACAGT 281
E. glyceria 409 cgccgttctgtggtggtggcatacaggtgtcgctggtgacggcggcaacacgacgtacagt 469
E. festucae Fl1 282 gtaagtgaacaag----------------------------------agacgca--aac 304
E. festucae E2368 282 gtaagtgaacaag----------------------------------agacgca--aac 304
E. baconii 282 gtaagtgaacgag----------------------------------agacaca--aac 304
E. brachyelytri 282 gtaagtgaacaag----------------------------------agacaca--aaa 304
E. bromicola AL0434 282 gtaagtgaacgag----------------------------------agacaca--aac 304
E. bromocola 502 282 gtaagtgaacgag----------------------------------agacaca--aac 304
E. bromocola 5761 282 gtaagtgaacaag----------------------------------agacgcaggaaa 306
E. glyceria 470 gcaagtaagcaaatacaagggagagcatgcgattcgattcgattcgattcgattcg--atc 528
E. festucae Fl1 305 ccttct--------gctgtgcttaatactcatctgtctttgtcaggtagTTCTTCTTTTCT 357
E. festucae E2368 305 ccttct--------gctgtgcttaatactcatctgtctttgtcaggtagTTCTTCTTTTCT 357
E. baconii 305 ccttct--------gctgtgcttaatactcatctgtctttgtcaggtagTTCTTCTTTTCT 357
E. brachyelytri 305 ccttct--------gttgtgctcaatactaatttgtctttgtctggtagTTCTTCTTTTCT 357
E. bromicola AL0434 305 ccttct--------gcttgcttcatactcatctgtctttgtcaggtagTTCTTCTTCTCT 357
E. bromocola 502 305 ccttct--------gcttgcttcatactcatctgtctttgtcaggtagTTCTTCTTCTCT 357
E. bromocola 5761 307 ccttct--------gctgtgcttaatactaatctgttcttgtctggtagTTCTTCTTCTCT 359
E. glyceria 529 ccatcttgattgacattggcattgacattgattgggtcaatcatgttagTTCTTCTTCTCC 589
E. festucae Fl1 358 GTTGAAGTCACGGACAGCCTCTTCCCAAATGTAGCTCCAGGATGCAATTTGGCATTGACAG 418
E. festucae E2368 358 GTTGAAGTCACGGACAGCCTCTTCCCAAATGTAGCTCCAGGATGCAATTTGGCATTGACAG 418
E. baconii 358 GTTGAAGTCACGGATAGCCTCTTCCCAAATGTAGCTCCAGGATGCAATTTGGCATTGACAG 418
E. brachyelytri 358 GTTGAAGTCACGGATAGCCTCTTCTCAAACGTAGCTCCAGGATGCAATTTGGCATTGACAG 418
E. bromicola AL0434 358 GTTGAAGTCACGGATAGCCTCTTCTCAAACGTATCTCCAGGATGCAATTTGGCATTGACAG 418
E. bromocola 502 358 GTTGAAGTCACGGATAGCCTCTTCTCAAACGTATCTCCAGGATGCAATTTGGCATTGACAG 418
E. bromocola 5761 360 GTTGAAGTCACGGATAGCCTCTTCTCAAACGCATCTCCAGGATGCAATTTGGCATTGACAG 420
E. glyceria 590 GTTGAGCAGTCGGACTCTCTCTTCGCCGCCGTGTCCAAGAACTGCAACCTGGCCTTGACCG 650
E. festucae Fl1 419 CAGAGCTCCCCGCCTGCCCGCGTGAGTTTCTACAGCTTCTCAGCAACGGAGCATACTACTC 479
E. festucae E2368 419 CAGAGCTCCCCGCCTGCCCGCGTGAGTTTCTACAGCTTCTCAGCAACGGAGCATACTACTC 479
E. baconii 419 CAGAGCTCCCCGCTTGCCCGCGTGAGTTTCTACAGCTTCTCAGCAACGGAGCATACTACTC 479
E. brachyelytri 419 CAGAGCTCCCCGCTTGCCCGCGTGAGTTTCTACAGCTTCTCAGCAACGGAGCATACTACTC 479
E. bromicola AL0434 419 CAGAGCTCCCCGCTTGCCCGCGTGAGTTTCTACAGCTTCTCAGCAACGGAGCATACTACTC 479
E. bromocola 502 419 CAGAGCTCCCCGCTTGCCCGCGTGAGTTTCTACAGCTTCTCAGCAACGGAGCATACTACTC 479
E. bromocola 5761 421 CAGAGCTCCCCGCTTGCCCGCGTGAGTTTCTACAGCTTCTCAGCAACGGAGCATACTACTC 481
E. glyceria 651 CGCCCGTCCCGACCTGTCCACGAGAGTTCCTCCAGCTGCTCAGCAACGGGGGCTATTATTC 711
E. festucae Fl1 480 GCTGGAGGGCCAGGAGTATATGGATGTGCTCTGCAAATCGGCTTGCGCGCCACAGCTTGCA 540
E. festucae E2368 480 GCTGGAGGGCCAAGAGTATATGGATGTGCTCTGCAAATCGGCTTGCGCGCCACAGCTTGCA 540
E. baconii 480 GCTGGAGGGCCAAGAGTATATGGATGTGCTCTGCAAATCGGCTTGCGCGCCACAGCTTGCA 540
E. brachyelytri 480 GCTGGAGGGCCAAGAGTATATGGATGTGCTCTGCAAATCGGCTTGCGCGCCACAGCTTGCA 540
E. bromicola AL0434 480 GCTGGAGGGCCAAGAGTATATGGATGTGCTCTGCGAATCGGCTTGCGCGCCACAGCTTGCA 540
E. bromocola 502 480 GCTGGAGGGCCAAGAGTATATGGATGTGCTCTGCGAATCGGCTTGCGCGCCACAGCTTGCA 540
E. bromocola 5761 482 GCTGGAGGGCCAAGAGTATATGGATGTGCTCTGCGGATCGGCTTGCGCGCCACAGCTTGCA 542
E. glyceria 712 CTTGGAGGGCCAGGAATTCATGGACACTCTCTGCACGGATTCATGCTCGCCTCAGCTCGCC 772
E. festucae Fl1 541 GGATATCGTCAGAAAGTCTTGACGGCTTGCGCCAACGATGCTCAACCACGCCCGGGCTTCC 601
E. festucae E2368 541 GGATATCGTCAGAAAGTCTTGACGGCTTGCGCCAACGATGCTCAACCACGCCCGGGCTTCC 601
E. baconii 541 GGATATCGTCAGAAAGTCTTGACGGCTTGCGCCAACGATCCTCAACCACGCCCGGGCTTCC 601
E. brachyelytri 541 AGATATCGTCAGAAAGTCTTGACGGCTTGCGCCGACGATCCTCAACCACGCCCGGGCTTCC 601
E. bromicola AL0434 541 AGATATCGTCAGAAAGTCTTGACGGCTTGCGCCGGCGATGCTCAACCACGCCCGGGCTTCC 601
E. bromocola 502 541 AGATATCGTCAGAAAGTCTCGACGGCTTGCGCCGGCGATGCTCAACCACGCCCGGGCTTCC 601
E. bromocola 5761 543 AGATATCGTCAGAAAGTCTTGACGGCTTGCGCCGGCGATGCTCAACCACGCCCGGGCTTCC 603
E. glyceria 773 AGTTATCGTCAGAATGTGGTTTCGGCATGCGCCAACGACCTTCAGCCTCGAGATGGCTTCC 833


E. festucae Fl1 602 CTGTTACGTACTGGGTAGATGCTGTGACCTCGGTGCGCGAGCTCATGTGTCTGAAGGACTC 662
E. festucae E2368 602 CTGTTACGTACTGGGTCGATGCTGTGACCTCGGTGCGCGAGCTCATGTGTCTGAAGGACTC 662
E. baconii 602 CTGTTACATACTGGGTAGATGCTGTGACCTCGGTGCGCGAGCTCATGTGTCTGAAGGACTC 662
E. brachyelytri 602 CTGTTACATACTGGGTAGATGCTGTGACCTCCGTGCGCGAGCTCATGTGTCTGAAGGACTC 662
E. bromicola AL0434 602 CTGTTACATACTGGGTAGATGCTGTGACCTCGGTGCGCGAGCTCATGTGTCTGAAGGACTC 662
E. bromocola 502 602 CTGTTACATACTGGGTAGATGCTGTGACCTCGGTGCGCGAGCTCATGTGTCTGAAGGACTC 662
E. bromocola 5761 604 CTGCTACATACTGGGTAGATGCCGTGACCTCGGTGCGCGAGCTCATGTGTCTCAAGGACTC 664
E. glyceria 834 CCGCCACGTACTGGGTCGACGCCGTCGCCTCGGTGCGTCAGATGATGTGCATGAAGGACTC 894
E. festucae Fl1 663 GGCCACTGGGAAATACTGTGCTGgtatgttgaa----------tccgtgtgttac 707
E. festucae E2368 663 GGCCACTGGGAAATACTGTGCTGgtatgttgaa----------tccgtgtgttac 707
E. baconii 663 GGCCACTGGGAAATACTGTGCTGgtatgttaga----------tccgtgtgttac 707
E. brachyelytri 663 GGCCACTGGGAAATACTGTGCTGgtatgttgat----------tccatgtattac 707
E. bromicola AL0434 663 GGCCACTGGGAAATACTGTGCTGgtatgttgat----------tccgtgtattcc 707
E. bromocola 502 663 GGCCACTGGGAAATACTGTGCTGgtatgttgat----------tccgtgtattcc 707
E. bromocola 5761 665 GGCCACTGGGAAATACTGTGCTGgtatgttcgt----------tccatgtattac 709
E. glyceria 895 CAAGTCTGGCCAATACTGTGTCGgtaagctgaaattcatcatcagtccctttcctcctgc 955
E. festucae Fl1 708 gctgttcaccggagcgcaaacgcgtggcc---tc-tctcatataattaatc------ccgt 758
E. festucae E2368 708 gctgttcaccagagcgcaaacgcgtggcc---tc-tctcatataattaatc------ccgt 758
E. baconii 708 gctgttcaccggagcgcaaacgcgtggcc---tc-tctcatgtaattaatc------ccgt 758
E. brachyelytri 708 actgttcaccggagcgcaaacgcgtggcc---tc-tctcatataattaatc------ccgt 758
E. bromicola AL0434 708 gctgtcc----------tc---tc-tctcacataactaatc------ccgt 738
E. bromocola 502 708 gctgtcc----------tc---tc-tctcacataactaatc------ccgt 738
E. bromocola 5761 710 gctgttcaccggagggcagacgcgtggca---tcttctcacataattaatcccaaaaccgc 767
E. glyceria 956 ttatttcccagacggctaaattggagacaactcgtttcgtgtatgtatgt---------- 1006
E. festucae Fl1 759 tgaatcatagACATTCTGGAAGCTGCGTTCCAGGACAATTCGACTTCAACCGATATGCTTG 819
E. festucae E2368 759 tgaatcatagACATTCTGGAAGCTGCGTTCCAGGACAATTCGACTTCAACCGATATGCTTG 819
E. baconii 759 tgaatcatagACATTCTGGAAGCTGCGTTTCAGGACAATTCGACTTCAACCGATATGCTTG 819
E. brachyelytri 759 tgaatcatagACATTCTGGAAGCTGCGTTTCAGGATAATTTAACTTCAACCGATATGCTTG 819
E. bromicola AL0434 739 tgaatcatagACATCCTGGAAGCTGCGTTTCAGGACAATTCGACTTCAACCGATATGCTTG 799
E. bromocola 502 739 tgaatcatagACATCCTGGAAGCTGCGTTTCAGGACAATTCAACTTCAACCGATATGCTTG 799
E. bromocola 5761 768 tgaaccatagACATTCTGGAAGCTGCGTTTCAGGACAATTCAACTTCAACCGATATGCTCG 828
E. glyceria 1007 --atcaaaagACTTTGTGCGGACAAATGTCGGCGACGCTCCCCGGCAGGACGCCCTCCTGG 1065
E. festucae Fl1 820 GCGGCTTCACGGACGAACAGCTTTGCTCTGAGTGCCTCGTCAACGTCTTTCGTCAACAGCA 880
E. festucae E2368 820 GCGGCTTCACGGACGAACAGCTTTGCTCTGAGTGCCTCGTCAACGTCTTTCGTCAACAGCA 880
E. baconii 820 GCGGCTTCACAGACGAACAGCTTTGCTCTGAGTGCATCGTCAACGTCTTTCGTCAACAGCA 880
E. brachyelytri 820 GTGGCTTCACAGACGAACAGCTTTGCTCTGAGTGCATCGTCAACGTCTTTCGTCAACAGCA 880
E. bromicola AL0434 800 GCGGCTTCACAGATAAGCAGCTTTGCTCCGAGTGCATCGTCAACGTCTTTCGTCAACAGCA 860
E. bromocola 502 800 GCGGCTTCACAGATAAGCAGCTTTGCTCCGAGTGCATCGTCAACGTCTTTCGTCAACAGCA 860
E. bromocola 5761 829 GTGGCTTCACAGATAAGCAGCTTTGCTCCGAGTGCATCGTCAACGTCTTTCGTCAACAGCA 889
E. glyceria 1066 GCGGCTACGCAGACGAGCAACTATGCTCGGAATGCGTCGTCAACCTTTTCCGTCACCAACA 1126
E. festucae Fl1 881 ATCTACTCCATACTCCAACTATGGCGAAGAAATGATGGGAGCATTCCAAAGAATCCAACAG 941
E. festucae E2368 881 ATCTACTCCATACTCCAACTATGGCGAAGAAATGATGGGAGCATTCCAAAGAATCCAACAG 941
E. baconii 881 ATCTACTCCATACTCCAACTATGGCGAAGAAATGATGGAAGCATTCCAAAGAATCCAACAG 941
E. brachyelytri 881 ATCTACTCCATACTCCAACTACGGAGAAGAAATGATGGAAGCATTCCAGAGAATCCAACAG 941
E. bromicola AL0434 861 ATCTACTCCGTACTCCAACTATGGCGAGGAAATGATGGAAGCGTTCCGAAGAATCCAACAG 921
E. bromocola 502 861 ATCTACTCCATACTCCAACTATGGCGAGGAAATGATGGAAGCGTTCCGAAGAATCCAACAG 921
E. bromocola 5761 890 ATCTACTCCATACTCCAACTATGGCGAAGAAATGATGGAAGCGTTCCGAAGAATCCAGCGG 950
E. glyceria 1127 GTCCACCCCTACTCCAATTACGGGCCGGGCATGATGGAGGCTTGGCGGGCGATCCAGGCC 1187
E. festucae Fl1 942 AAGTGCAACCTGAAATATCCAACAGCATCACAGCCGCTGCAAGTCAATGCGACATCATTGT 1002
E. festucae E2368 942 AAGTGCAACCTGAAATATCCAACAGCATCACAGCCGCTGCAAGTCAACGCGACATCATTGT 1002
E. baconii 942 AAGTGCAACCTTGACTATCCAACAGCATCACAGCCGCTGCAAGTCAATGCGACATCATTGT 1002
E. brachyelytri 942 AAGTGCAACCTTAACTATCCAACAGCATCACAGCCGCTGCAAGTCAATGCGACATCATTGT 1002
E. bromicola AL0434 922 AAGTGCAACCTCAACTATCCAACAGCATCACAGCCGCTGCAGGTCAATGCGACATCATTGT 982
E. bromocola 502 922 AAGTGCAAACTCAACTATCCAACAGCATCACAGCCGCTGCAGGTCAATGCGACATCATTGT 982
E. bromocola 5761 951 AAGTGCAACCTCAACTATCCAACAGCATCGCAGCCGCTGCAAGTCAATGCGACATCATTGT 1011
E. glyceria 1188 AAGTGCAAGCTCCATCATCCAACAGCCACGCAGACCCTGGACACCAACGTCACGTCGCTGC 1248

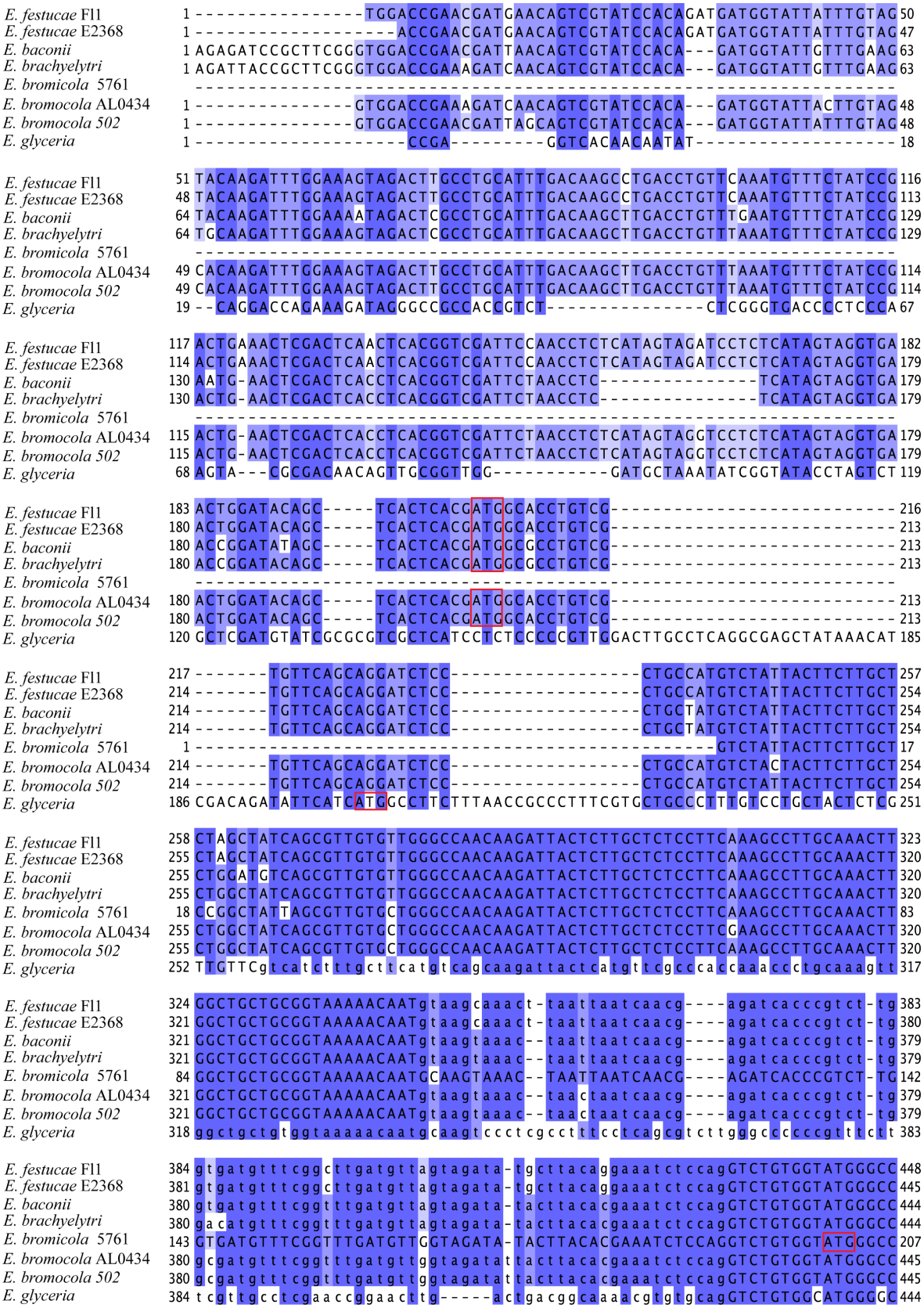

E. festucae Fl1 1003 TTAATTACGCACCGACTGGATATGCCGTGGCCAAATGCGCGTCCGACCGTACTCACACAGT 1063
E. festucae E2368 1003 TTAATTACGCACCGACTGGATATGCCGTGGCCAAATGCGCGTCCGACCGTACTCACACAGT 1063
E. baconii 1003 TCAATTACGCACCGACTGGATATGCTGTGGCCAAATGCGCGTCCGACCGTACTTACACGGT 1063
E. brachyelytri 1003 TTAATTACGCACCGACTGGATATGCTGTGGCCAAATGCGCGTCCGACCGTACTTACACAGT 1063
E. bromicola AL0434 983 TTAATTACGCACCGACTGGATATGCTGTGGCCAAATGCGCGTCCGACCGTACTCACACAGT 1043
E. bromocola 502 983 TTAATTACGCACCGACTGGATATGCTGTGGCCAAATGCGCGTCCGACCGTACCTACACAGT 1043
E. bromocola 5761 1012 TTAATTACGCACCGACTGGATATGCTGTGGCCAAATGCGCGTCCGACCGCACTTACACAGT 1072
E. glyceria 1249 ACAACTATGCCCCAAGGCTACCCCACGGCGACGTGTGTGGGTGATGGCAAATACACAGT 1309
E. festucae Fl1 1064 GGCCAGTGGCGACAATTGCCTGAGCATTTCGAAAAAGTCACACGTTTCAACTGGATCTCTG 1124
E. festucae E2368 1064 GACTAGTGGCGACAATTGCCTGAGCATTTCGAAAAAGTCACACGTTTCAACTGGATCTCTG 1124
E. baconii 1064 GGCTAGTGGCGATAATTGCCTGAGCATTTCGAAAAACTCACACGTTTCAACTGGATCTCTG 1124
E. brachyelytri 1064 GGCTAGTGGCGATAATTGCCTGAGCATTTCGAGAAACTCACACGTTTCAACTGGATCTCTG 1124
E. bromicola AL0434 1044 GGCTAGTGGCGACAATTGCCTGAGCATTTCGAAAAACTCACACGTTTCAACTGGATCTCTG 1104
E. bromocola 502 1044 GGCCAGTGGCGATAATTGCCTGAGCATTTCGAAAAACTCACACGTTTCAACCGGATCCCTC 1104
E. bromocola 5761 1073 GGCTAGTGGCGATAATTGCCTGAGCATTTCGAAAAACTCACACGTCTCAACTGGATCCCTG 1133
E. glyceria 1310 CGCCAGCGGCGATGATTGCGTGCGAATCTCCAGGAGTCGGCGCGTCTCCACGGGTTCTTTG 1370
E. festucae Fl1 1125 ATCCTCATGAATGGCTTGCGAGTAGACTGCACAGATCTGCAGCTTGGACAGgtacgtcgta 1185
E. festucae E2368 1125 ATCCTCATGAATGGCTTGCGAGTAGACTGCACAGATCTGCAGCTTGGACAGgtacgtcgta 1185
E. baconii 1125 ATCCTCATGAATGGCTTGCGAGTAGACTGCACAGATCTGCAGCTTGGACAGgta------- 1178
E. brachyelytri 1125 ATCCTCATGAATGGCTTGCGAGTAGACTGCACAGATCTGCAGCTTGGACAGgta------- 1178
E. bromicola AL0434 1105 ATCATCATGAATGGCTTGCGAGTAGACTGCACAGATCTGCAGCTTGGACAGgtac------ 1159
E. bromocola 502 1105 ATCCTCATGAATGGCTTGCGAGTGGACTGCACAGATCTGCAGCTTGGACAGGTA------- 1158
E. bromocola 5761 1134 ATCCTCATGAATGGCTTGCGAGTAGACTGCACAGATCTGCAGCTTGGACAGgta------- 1187
E. glyceria 1371 ATCACGCTCAACAGTCTGCGCACCGACTGCACCAACCTGCGACCAGGCCAGgtaataacg- 1430
E. festucae Fl1 1186 ccctagccaccggcctcc-tctgcgttctttgcagccgcactaaacctaacgatt---tag 1242
E. festucae E2368 1186 ccctagccaccggcctcc-tctgcgttctttgcagccgcactaaacctaacgatt---tag 1242
E. baconii 1179 ccctagccaccggcctcc-tctgcgttctttgcagccacact-aacccaacgatt---tag 1234
E. brachyelytri 1179 -cctagccaccggcct---tttgcgttctttgcagccacact-gacctaacgaat---tag 1231
E. bromicola AL0434 1160 ccctagccaccggcctcc-tccgcgttctttgcagccacact-aacctaacgatt---tag 1215
E. bromocola 502 1159 -CCTAGCCACCGGCCTCC-TTTGCCTCCTTTGCAGCCACACG-AACCTAACGAGT---CAG 1213
E. bromocola 5761 1188 -cctagccactggcctcctttgcctctttgcagccacacg-aacctaacgaat---cag 1243
E. glyceria 1431 aacgcacaaatgtcatgtctgcgaatttccttgagctg-acg-catgcgtggaatcaatag 1489
E. festucae Fl1 1243 ACTCTGTGCTTGCCAGCTGCTTGCGAAGACTATGTTATTCGTTCGGGAGACAGCTGCGAAG 1303
E. festucae E2368 1243 ACTCTGTGCTTGCCAGCTGCTTGCGAAGACTATGTTATTCGTTCGGGAGACAGCTGCGAAG 1303
E. baconii 1235 ACTCTGTGCTTGCCAGCTGTTTGCGAAGACTATGTTATCCGTTCGGGAGACAGCTGCGAAG 1295
E. brachyelytri 1232 ACTCTGTGCTTACCAGCTGTTTGCGAAGACTATGTTATTCGTTCGGGAGACAGCTGCGAAG 1292
E. bromicola AL0434 1216 ATCCTGTGCTTGCCAGCTGCTTGCCAAGACTATGTCATTCGTTCGGGAGACAGCTGCGAAG 1276
E. bromocola 502 1214 ACTCTGTGCTTGCCAGCTGCTTGCCAAGACTATGTTGTTCGTTCGGGAGACAGCTGCGAAG 1274
E. bromocola 5761 1244 ACTCTGTGCTTGCCAGCTGCTTGCCAAGACTATGTCATTCGTTCGGGAGACAGCTGCGAAG 1304
E. glyceria 1490 ACCCTGTGCCTGCCTCCCACCTGCACTGATTACACGATTCAATCTGGCGATACGTGCGAGG 1550
E. festucae Fl1 1304 CCCTTGCCTCTTCGAAAGCCTGCACATTCCAGCAACTTGTTTCGTGGAATCCTCgtaggca 1364
E. festucae E2368 1304 CCCTTGCCTCTTCGAAAGCCTGCACATTCCAGCAACTTGTTTCGTGGAATCCTCgtaggca 1364
E. baconii 1296 CCCTTGCCTCTTCGAAAGCCTGCACATTCCAGCAACTTGTTTCGTGGAATCCTCgtaggca 1356
E. brachyelytri 1293 CCCTTGCCTCTTCGAAAGCCTGCACATTCCAGCAACTTGTTTCGTGGAATCCTCgtaagca 1353
E. bromicola AL0434 1277 CCCTTGCTTCTTCGAAAGCCTTCACATTCCAGCAACTTGTTTCGTGGAATCCTCgtaggca 1337
E. bromocola 502 1275 CTCTTGCTTCTTCGAAAGCCTTCACATTCCAGCAACTTGTTTCGTGGAATCCTCgtaagca 1335
E. bromocola 5761 1305 CCCTTGCCTCTTCTAAAGCCTGCACATTCCAGCAACTTGTTTCGTGGAATCCTCgtaagca 1365
E. glyceria 1551 CCATCGCCGAGTCGCGGGGTATTTCCTTCCAACAGATCGTTGCGTGGAATCCTCgtatgtg 1611
E. festucae Fl1 1365 tccctca----------aatgaaggcggctgagtgg--gacat--------gttctgac 1404
E. festucae E2368 1365 tccctca----------aatgaaggcggctgagtgg--gacat--------gttctgac 1404
E. baconii 1357 tccctca----------aatgaacgcggctgagtgg--gacat--------cctctgac 1396
E. brachyelytri 1354 tccctca----------aatgaacgcggctgagtg---gacat--------cttctgac 1392
E. bromicola AL0434 1338 tctcctca----------aatgaacgcggctgagtgg--gacat--------cttctgac 1377
E. bromocola 502 1336 tccctcg----------aatgaacgcggctgagtggacaagAT--------CTTCTGAC 1377
E. bromocola 5761 1366 tccctct----------aacgaacgcgggtgagtggacaacat--------cttctgac 1407
E. glyceria 1612 gagtcttgccacgcacagcaataatgactgacacgtga--atcatgatgctgaccttgacc 1670

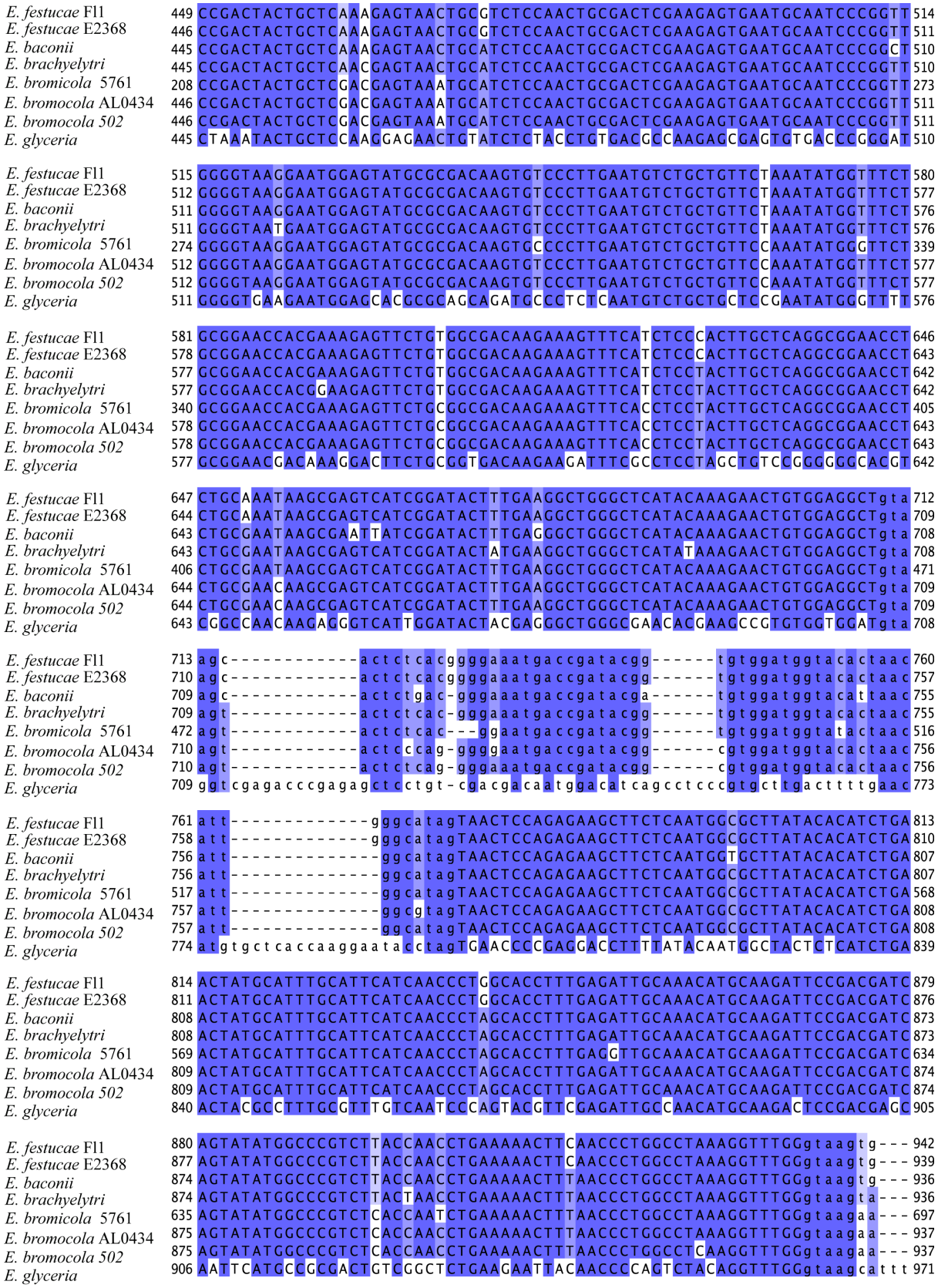

E. festucae Fl1 1405 attcccataagTGCTTGATGACTACTGCTCGAATTTAATTGTCGACCAAAATATCTGCGTC 1465
E. festucae E2368 1405 attcccataagTGCTTGATGACTACTGCTCGAATTTAATTGTCGACCAAAATATCTGCGTT 1465
E. baconii 1397 attcccataagTGCTTGATGACTACTGCTCGAATTTAATTGTCGACCAAAATATCTGCGTC 1457
E. brachyelytri 1393 attcccat-agTGCTTGATGACTACTGCTCGAATTTAATTGTCGACCAAAATATCTGCGTC 1452
E. bromicola AL0434 1378 attcccataagTGCTTGATGACTACTGCTCGAATTTAATCGCCGACCAAAACATCTGCGTC 1438
E. bromocola 502 1378 ATTCCCAT-GGTGCTTGATGACTACTGCTCGAATTTAATCGCCGACCAAAATATCTGCGTC 1437
E. bromocola 5761 1408 attccac-ggtgcttgatgactactgctcgaatttaatcgccggccaaaatatctgcgtc 1467
E. glyceria 1671 ttgttgggaagTCATCGACAACTACTGCACCAACCTCATCGTCCATCACAACATATGTGTT 1731
E. festucae Fl1 1466 GGCCCCCCGGGAGGAAATAAGGAATTCGTCACCATACCCGGGGCTACTGTGACCAAGACGG 1526
E. festucae E2368 1466 GGCCCCCCGGGAGGAAATAAGGAATTCGTCACCATACCCGGGGCTACTGTGACCAAGACGG 1526
E. baconii 1458 GGCCCCCCGGGAGGAAATAAGGAATTCGTCACGATACCCGGGGCTACTGTGACCAAGACGG 1518
E. brachyelytri 1453 GGCCCCCCAGGAGGAAATAAAGAATTCGTCACGATTCCCGGGGCTACTGTGACCAAGACGG 1513
E. bromicola AL0434 1439 GGCCCCCCGGGAGGAAAGAAAGAATTCGTCACGATACCCGGGGCTACTGTGACCAAGACGG 1499
E. bromocola 502 1438 GGCCCTCCAGGGGGAAATAAAGAATTCGTCACGATACCCGGGGCTACTGTGACCAAGACGG 1498
E. bromocola 5761 1468 ggcccccgggaggaaataaagAATTCGTCACGATACCCGGGGCTACCGTGACCAAGACTG 1528
E. glyceria 1732 GGGCCGCCCGGAGGCTACCAGAACTTCACCACGGTTCCCGGCGCCAGCGCCACCAAGACGG 1792
E. festucae Fl1 1527 CGACTTATGCTACAAAGACGGCTTCTCGCCCGGCCTCGGTCGTATCGGGAACTACCTACAA 1587
E. festucae E2368 1527 CGACTTATGCTACAAAGACGGCTTCTCGCCCGGCCTCGGTCGTATCGGGAACTACCTACAA 1587
E. baconii 1519 CGACTTATGCTACAAAGACGGCTTCTCGCCCGGCTTCGGTCGTATCGGGAACTACCTACAA 1579
E. brachyelytri 1514 CGACTTATGCTACAAAGACGGCTTCTCGCCCAGCTTCGGTCGTATCGGGAACTACCTACAA 1574
E. bromicola AL0434 1500 CGACTTATGCTACAAAGACGGCTTCTCGCCCGGCTTCGGTCGTATCGGGAACTACCTACAA 1560
E. bromocola 502 1499 CGACTTATGCTACAAAGACGGCTTCTCGCCCGGCTTCGGTCGTATCGGGAACTACCTACAA 1559
E. bromocola 5761 1529 CGACTTATGCTACAAAGACGGCTTCTCGCCCGGCTTCGGTCGTATCGGGAACTACCTACAA 1589
E. glyceria 1793 CAACATACATCACGTCTACGGCGTCACGCCCTGCGCCAGTGGCATCTGGCACCACCTCCAA 1853
E. festucae Fl1 1588 CTGTGGAAAGTATTATCTTGTACAGCCGgtgagtggtgaacgaatattgggcacattg--c 1646
E. festucae E2368 1588 CTGTGGAAAGTATTATCTTGTACAGCCGgtgagtggtgaacgaatatcgggcacattg--c 1646
E. baconii 1580 CTGTGGAAAGTATTATCTTGTACAGCCGgtgagtggtg-acgaatattgggcacactg--c 1637
E. brachyelytri 1575 CTGTGGAAAGTATTATCTTGTACAGCCGgtgagtgatg-acgaatattgggcatattg--c 1632
E. bromicola AL0434 1561 CTGTGGAAAGTATTATCTCGTACAGCCGgtgagtggtgacggaatatcgggcacattg--c 1619
E. bromocola 502 1560 CTGTGGAAAGTATTATCTCGTACAGCCGgtgagtggtgacggaatatcgggcacattg--c 1618
E. bromocola 5761 1590 CTGTGGAAAGTATTATCTCGTACAGCCGgtgagtggtgacggaatattgggaacattg--t 1648
E. glyceria 1854 ATGCGGCAAATACTACAAGGTTCAGCCGgtaggtcggtgtggtgtgccatgtagaaagaag 1914
E. festucae Fl1 1647 gtcgtgtttgtagctaattgctgatagGGCGACTACTGTGAAATTGTGGCCATCAGTAACG 1707
E. festucae E2368 1647 atcgtgtttgtagctaattgctgatagGGCGACTACTGTGAAATTGTGGCCATCAGTAACG 1707
E. baconii 1638 atcgtgtttgtagctaattgctcatagGGCGACTACTGTGAAATTGTGGCCATTAGTAACG 1698
E. brachyelytri 1633 atcgtgtttgtagctaattgctgatagGGCGACTACTGTGAAATTGTGGCCATTAGTAACG 1693
E. bromicola AL0434 1620 gtcatgtttgtagctaattgcttatagGGCGACTACTGCGAAATTGTGGCCATTGGTAACG 1680
E. bromocola 502 1619 gtcatgtttgtagctaattgcttatagGGCGACTACTGCGAAATTGTGGCCATTGGTAACG 1679
E. bromocola 5761 1649 atcatgtttgtagctaattgctta-agGGCGACTACTGTGAAATTGTGGCCATTGGTAACG 1708
E. glyceria 1915 atgatgaggctgacgctgtcctatcagGGCGACTACTGCGAAGTGGTTGCCATCAGCAACG 1975
E. festucae Fl1 1708 ACATTTCCTTTGCCCTTCTTCGCAAACTGAACCCTCAGgtatgtgtatattgtctggaata 1768
E. festucae E2368 1708 ACATTTCCTTTGCCCTTCTTCGCAAACTGAACCCTCAGgtatgtgtatattgtctggaata 1768
E. baconii 1699 ACATTTCCTTTGCCCTTCTTCGCAAACTGAACCCTCAGgtatgtgtatattgtctgtaata 1759
E. brachyelytri 1694 ACATTTCCTTTGCCCTTCTTCGCAAACTGAACCCTCAGgtatgtgtatattgtgtataata 1754
E. bromicola AL0434 1681 ACATTTCCTTTGCCCTTCTCCGCAAACTGAACCCTCAGgtatgcgtatatcgtgtgtaatc 1741
E. bromocola 502 1680 ACATTTCCTTTGCCCTTCTCCGCAAACTGAACCCTCAGgtatgcgtatatcgtgtgtaatc 1740
E. bromocola 5761 1709 ACATTTCCTTTGCCCTTCTTCGCAAACTGAACCCTCAGgtatgcgtatattgtgtgtaata 1769
E. glyceria 1976 ACATTTCCTTTGACCTTCTTCGCAGACTCAACCCTCAGGTGAG--------GTTCCCGGCC 2028
E. festucae Fl1 1769 tgagatggaaaagatactaacagaagcata----------atagCT 1804
E. festucae E2368 1769 tgagatggaaaagatactaacagaagcata----------atagCT 1804
E. baconii 1760 tgagatggaaaagatactaatagaagcata----------atagCT 1795
E. brachyelytri 1755 tgagatggaaaagatactaatagaagcaca----------atagCT 1790
E. bromicola AL0434 1742 tgagatgggaaagatactaatggaagcata----------atagCT 1777
E. bromocola 502 1741 tgagatgggaaagatactgatggaagcata----------atagCT 1776
E. bromocola 5761 1770 tgagatgggaaagatactaatggaagcatg----------atagCT 1805
E. glyceria 2029 GAGGAGAGAAACAACCCCgtacgtggcgcagacagacgctgattctatccttcctgtagCT 2089

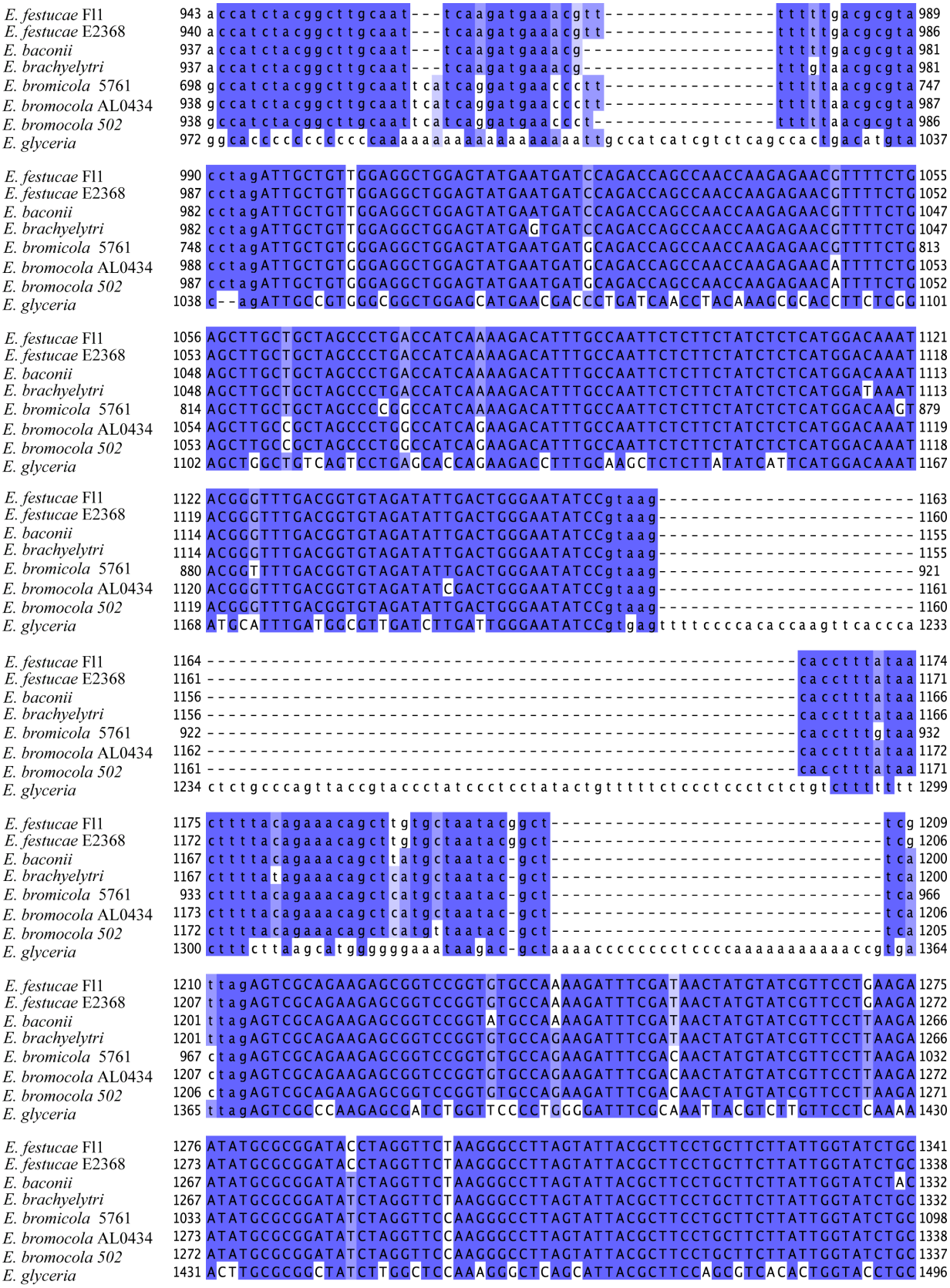

E. festucae Fl1 1805 GGATGACAAGTGTTCCAATCTACTGTCTGGCTTGTATTATTGTGTGCTGCCGTATGAAGAC 1865
E. festucae E2368 1805 GGATGACAAGTGTTCCAATCTACTGTCTGGCTTGTATTATTGTGTGCTGCCGTATGAAGAC 1865
E. baconii 1796 GGATGACAAGTGTTCCAATCTACTGTCTGGCTTGTATTATTGTGTGCTGCCGTATGAAGAC 1856
E. brachyelytri 1791 GGATGACAAGTGTTCCAATCTACTGTCTGGCTTGTATTATTGTGTGCTGCCGTATGAAGAC 1851
E. bromicola AL0434 1778 GGACGACAAGTGTTCCAATCTGCTGTCTGGCTTGTATTATTGTGTGCTGCCGTATCAAGAC 1838
E. bromocola 502 1777 GGACGACAAGTGTTCCAATCTACTCTCTGGCTTGTATTATTGTGTGCTGCCGTATCAAGAC 1837
E. bromocola 5761 1806 GGATGACAAGTGTTCCAATCTACTGTCTGGCTTGTATTATTGTGTGCTGCCGTATGAAGAC 1866
E. glyceria 2090 GGACGGGACCTGTTCCAACCTTCTGTCGGGAACATACTATTGCGTGCTCCCCGTGAAGGAC 2150
E. festucae Fl1 1866 CCAAGCAAA---ACGTCTTCAACCACAGTTCAGGCTCCCACCAGCACTCCTCCGGGAACAA 1923
E. festucae E2368 1866 CCAAGCAAA---ACGTCTTCAACCACAGTTCAGGCTCCCACCAGCACTCCTCCGGGAACAA 1923
E. baconii 1857 CCAAGCAAA---ACGTCTTCAACCACAGTTCAGGCTCCCACCAGCACTCCTTCGGGAACAA 1914
E. brachyelytri 1852 CCAAGCAAA---ACGTCTTCAACCATAGTTCAGGCTCCCACCAGCACTCCTTCGGGAACAA 1909
E. bromicola AL0434 1839 CCAAGCAAA---ACGTCTTCAACCACAGTTCAGGCTCCCACCAGCACTCCCTCGGGAACAA 1896
E. bromocola 502 1838 CCAAGCAAA---ACGTCTTCAACCACAGTTCAGGCTCCCACCAGCACTCCCTCGGGAACAA 1895
E. bromocola 5761 1867 CCAAGCAAA---ACGTCTTCAACCACAGTTCAGGCTCCCACCAGCACTCCCTCGGGAACAA 1924
E. glyceria 2151 TGGGACCAGACTACATCGTCTGCTGCGGTGCCAGCCCCCACGAGCATTCCGCCTGGGACAA 2211
E. festucae Fl1 1924 CGGGCACCTGTTACGAgtaagtttactgttgtataggcaga---------------cctgg 1969
E. festucae E2368 1924 CGGGCACCTGTTACGAgtaagtttactgttgtataggcaga---------------cctgg 1969
E. baconii 1915 CGGGCACCTGTTACGAgtaagtttactgttgtataggcaga---------------cctgg 1960
E. brachyelytri 1910 CGGGCACCTGTTACGAgtaagtttactgttgtataggaaga---------------cctgg 1955
E. bromicola AL0434 1897 CGGGCACCTGTTACGAGTACGTTTACTATTGTATAGACAGA---------------CCTGT 1942
E. bromocola 502 1896 CGGGCACCTGTTACGAgtacgtt--ctgttgtataggcaga---------------cctgg 1939
E. bromocola 5761 1925 CGGGCACCTGTTACGAGTACGTTTACTGTTGTATAGGCAGA---------------CCTGG 1970
E. glyceria 2212 CCCAGACTTGCTACGAGTTAGTCTCCCTGCCCAGAGTAAAGTAAAAAGATGATTCCCCTCT 2272
E. festucae Fl1 1970 atcacttgctcacaaacttgacagATGGTATGTTGTTCAATCCGGAGACTCTTGCTCCAAG 2030
E. festucae E2368 1970 atcacttgctcacaaacttgacagATGGTATGTTGTTCAATCCGGAGACTCTTGCTCCAAG 2030
E. baconii 1961 atcacttgctcacaaacttgacagATGGTATGTTGTTCAATCCGGAGACTCTTGCTCCAAG 2021
E. brachyelytri 1956 atcaccgctcacaaacttgacagATGGTATGTTGTTCAATCTGGAGACTCTTGCTCCAAG 2016
E. bromicola AL0434 1943 ATCACTTGCTCACAAACTTGACAGATGGTATGTCGTTCAATCTGGAGACTCGTGCTCCAAG 2003
E. bromocola 502 1940 atcacttgctcacaaacttgacagATGGTATGTTGTTCAATCTGGAGACTCGTGCTCCAAG 2000
E. bromocola 5761 1971 ATCACTTGCTCACAAACCTGACAGATGGTATGTTGTTCAATCTGGAGACTCGTGCTCCAAG 2031
E. glyceria 2273 TGCTAATGCCCAAGGTTGCTGCAGATGGCACGTTGTCAAGTCGGGTGACTATTGCGCCAAA 2333
E. festucae Fl1 2031 ATTCAGAACAAGTACGCAATTCAGTTCAGCCAGTTTCAAGCCTGGAACCCTgtaagtgaag 2091
E. festucae E2368 2031 ATTCAGAACAAGTACGCAATTCAGTTCAGCCAGTTTCAAGCCTGGAACCCTgtaagtgaag 2091
E. baconii 2022 ATTCAGGACAAGTACGCAATTCAGTTCAGCCAGTTTCAAGCCTGGAACCCTgtaagtcaag 2082
E. brachyelytri 2017 ATTCAGGACAAGTACGCAATTCAGTTCCGCCAGTTTCAAGCCTGGAACCCTgtaagtgaag 2077
E. bromicola AL0434 2004 ATTCAGGACAAGTACGCGATTCAGTTCAGCCAGTTTCAAGCTTGGAACCCTgtaagtgaag 2064
E. bromocola 502 2001 ATTCAGGACAAGTACGCAATTCAGTTCAGCCAGTTTCAAGCCTGGAACCCTgtaagtgaag 2061
E. bromocola 5761 2032 ATTCAGGACAAGTACGCAATTCAGTTCAGCCAGTTTCAAGCCTGGAACCCTgtaagtgaag 2092
E. glyceria 2334 ATACAAGATCAGTTCGCCATCACTTTCCAACAATTACAAGCATGGAATCCGGCAAGTCACG 2394
E. festucae Fl1 2092 -----ccctgctcaataatatctgcctgcctgccttcgtcgatggctgatgcatacatatc 2147
E. festucae E2368 2092 -----ccctgctcaataatatctgcatgcctgccttcgtcgatggctgatgcatacatatc 2147
E. baconii 2083 -----ccctgctcaataatatc----tgcctgtcttcgtcgatggctaatgcatacatatc 2134
E. brachyelytri 2078 -----ccctgctcaataatatc----tgcctgtcttcgtcgatggctaatgcatacatatc 2129
E. bromicola AL0434 2065 -----ccctgctcaataatatc----tgcctgtcttcgtccatggctaatgcagacatatc 2116
E. bromocola 502 2062 -----ccctgctcaataatatc----tgcctgtcttcgtccatggctaatgcagacatatc 2113
E. bromocola 5761 2093 -----ccctgctcaatgatatc----tgcctgtcttcgtccatggctaatgcatacatctc 2144
E. glyceria 2395 CCTCCCCCCCCCTTCTTTCGTT----TTCCAATTTTTGCCGAGGGCT-------------- 2437
E. festucae Fl1 2148 agGACCTCAACGACAAGTGCACGAATCTGTTACTCGGTGTAGCATACTGTGTACATGGGCC 2208
E. festucae E2368 2148 agGACCTCAACGACAAGTGCACGAATCTGTTACTCGGTGTAGCATACTGTGTACATGGGCC 2208
E. baconii 2135 agGACCTCAACGACAAGTGCACGAATCTGTTACTCGGTGTAGCATACTGTGTACATGGGCC 2195
E. brachyelytri 2130 agGACCTCAACGACAACTGCACGAATCTGTTACTCGGTGTAGCATACTGTGTACATGGGCC 2190
E. bromicola AL0434 2117 agGACCTCAACGACAAGTGCACGAATCTGTTACTCGGTGTAGCATACTGTGTACATGGGCC 2177
E. bromocola 502 2114 agGACCTCAACGACAAGTGCACGAATCTGTTACTCGGTGTAGCATACTGTGTACATGGGCC 2174
E. bromocola 5761 2145 agGACCTCAACGACAACTGCACGAATCTGTTACTCGGTGTAGCATACTGTGTACATGGGCC 2205
E. glyceria

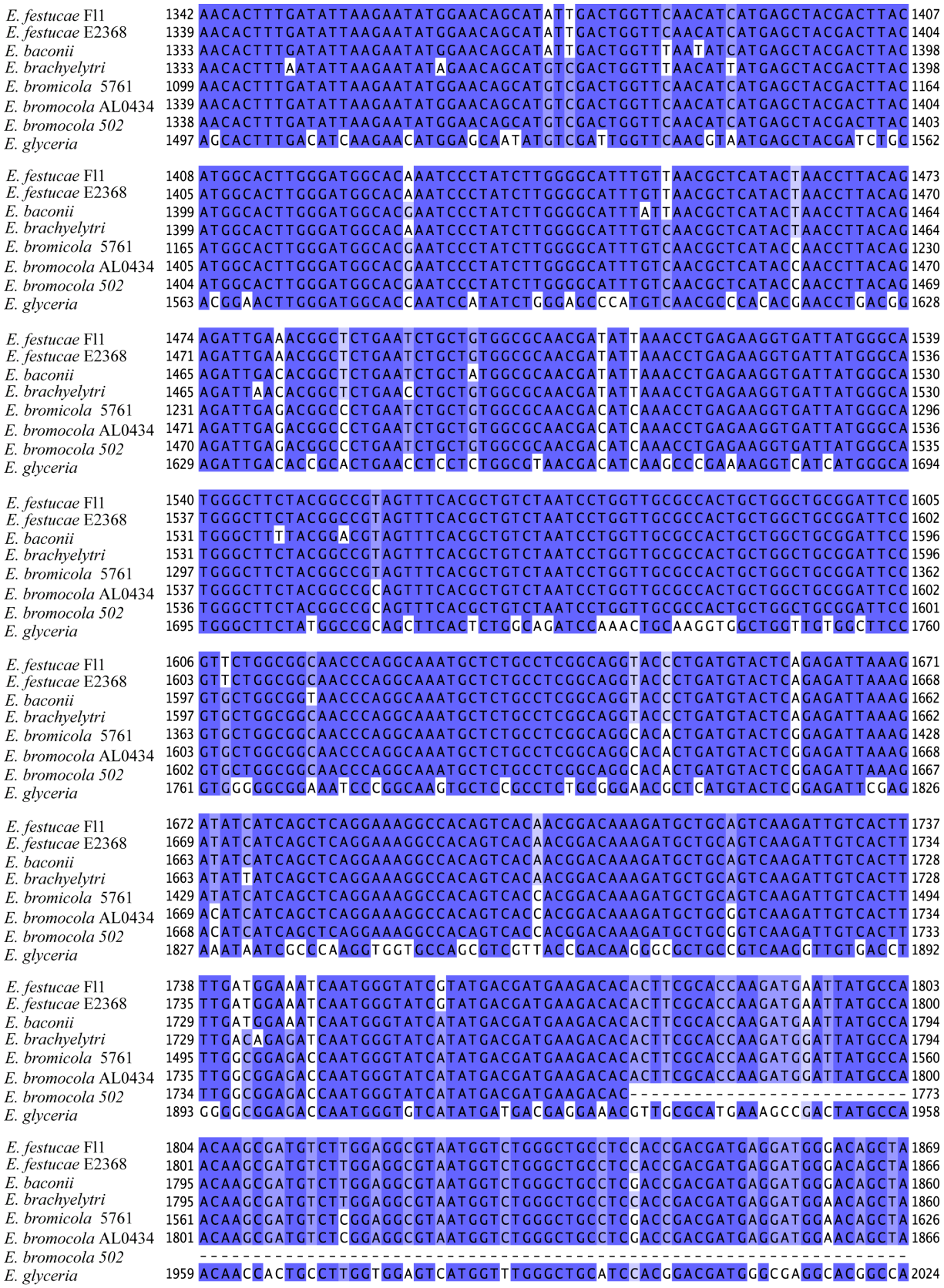

E. festucae Fl1 2209 AGATACTCCGGCCGCGCAAGGTGCAGGGAAAGGAGGACCTCGTCAATCACCTGCGAAAGAA 2269
E. festucae E2368 2209 AGATACTCCGGCCGCGCAAGGTGCAGGGAAAGGAGGACCTCGTCAATCACCTGCGAAAGAA 2269
E. baconii 2196 AGATACTCCGGCCGCGCAAGATGCAGGGAAAGGAGGTCCTCGTCAATCACCTGCGAAAGAA 2256
E. brachyelytri 2191 AGATACTCCGGCCGCGCAAGGTGCAGGGAAAGGAGGACCTCGTCAATCACCTGCGAAAGAA 2251
E. bromicola AL0434 2178 AGATACTCCGGCCGCGCGAGGTGCAGGGAAAAGAGCACCTCGTCAATCACCTGCGAAAGAA 2238
E. bromocola 502 2175 AGATACTCCGGCCGCGCGAGGTGCAGGGAAAAGAGCACCTCGTCAATCACCTGCGAAAGAA 2235
E. bromocola 5761 2206 AGATACTCCGGCCGCCCGAGGTGCAGGGAAAAGAGCACCTCGTCAATCACCTGCGAAAGAA 2266
E. glyceria ----------
E. festucae Fl1 2270 ACGGCCAGTCCTAGCAAACGCTCTGAAGGGGCCGTAGGCGGCGTGCCGGTGGGATGGCCCG 2330
E. festucae E2368 2270 ACGGCCAGTCCTAGCAAACGCTCTGAAGGGGCCGTAGGCGGCGTGCCGGTGGGATGGCCCG 2330
E. baconii 2257 ACGGCTAGTCCCAGCAAACGCTCTGAAGGAGCCGTAGGCGGCGTGCCGGTGGGATGGCCTG 2317
E. brachyelytri 2252 ACGGCTAGTCCTAGCAAACGCTCTGAAGGAGCCGTAGGCGGCGTGCCGGTGGGATGGCCTG 2312
E. bromicola AL0434 2239 ACGGCTAGTCCCAGAAAACGCTCTGAAGAAGCCGTAGGCGGCGTGCCGGTGGGATGGCCTG 2299
E. bromocola 502 2236 ACGGCTAGTCCCAGAAAACGCTCTGAAGAAGCCGTAGGTGGCGTGCCGGTGGGATGGCCTG 2296
E. bromocola 5761 2267 ACGGCTAGTCCCGGAAAACGCTCTGAAGGAGCCGTAGGCGGCGTGCCGGTGGGATGGCCTG 2327
E. glyceria ----------
E. festucae Fl1 2331 GCGTTGATTCAGCCCGGTACCGTAAAATTATGGGTTTGAGTGCAAAGGACGAGATTTAGAA 2391
E. festucae E2368 2331 GCGTTGATTCAGCCCGGTACCGTAAAATTATGGGTTTGAGTGCAAAGGACGAGATTTAGAA 2391
E. baconii 2318 GTGTTGATTCAGCCCGGTACCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2378
E. brachyelytri 2313 GTGTTGATTCAGCCCGGTATCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2373
E. bromicola AL0434 2300 GTGTTGATTCAGCCCGGTACCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2360
E. bromocola 502 2297 GTGTTGATTCAGCCCGGTACCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2357
E. bromocola 5761 2328 GCGTTGATTCAGCCCGGTACCGTAAAATTATGGGCTTGGGTGCAAAGGACGAGCTTTAGAA 2388
E. glyceria ----------
E. festucae Fl1 2392 CAGTCCTTTCAATAGGCCT----CAACACGAAGTAGTACCATTGAATGCAAATGAGCTTCA 2448
E. festucae E2368 2392 CAGTCCTTTCAATAGGCCT----CAACACGAAGTAGTACCATTGAATGCAAATGAGCTTCA 2448
E. baconii 2379 CAGTCCTTTCAATAGGCCT----CAACACGAAATAGTACCATTGAATGCAAATGAGCTTCA 2435
E. brachyelytri 2374 CAGTCCTTTCAACAGGCCT----CAACACGGAATAGTACCAATTAATGCAAATGAGCTTCA 2430
E. bromicola AL0434 2361 CAGTCCTTTCAATAGGCGTCAAACAACACGGAATAGTACCATTTCATGCAAATGAGCTTCA 2421
E. bromocola 502 2358 CAGTCCTTTCAATAGGCGTCAAACAACACGGAATAGTACCATTTAATGCAAATGAGCTTCA 2418
E. bromocola 5761 2389 CAGTCCTTTCAATAGGCGTCAAACAACACGAAATAGTACCATTTAATGCAAACGAGCTTCG 2449
E. glyceria 2438 ---TTTATTCCATGGGTAC---------- 2453
E. festucae Fl1 2449 AATATCTCACGTTCAATAGTTCCAATCGCTGTATGTTTTGAAAAGCTAATTGTTCGAT--- 2506
E. festucae E2368 2449 AATATCTCACGTTCAATAGTTCCAATCGCTGTATGTTTTGAAAAGCTAATTGTTCGAT--- 2506
E. baconii 2436 AATATCTCACGTTCAATAGTTCTAATCGCTGTATGTTTTGAATAGCTAATTGTTCGATCGA 2496
E. brachyelytri 2431 AATATCTCACGTTTAATAGTTCTAATCGCTGTATGTTGTGAAAAGCTAATTGTTCGATCGA 2491
E. bromicola AL0434 2422 AATATCTCGCGTTGAATAGTTCTAATCGCTGTATATCGTGAAAAGCTAATTGTTCCATCGA 2482
E. bromocola 502 2419 AATATCTCGCGTTGAATAGCTCTAATCGCTGTATATCGTGAAAAGCTAATTGTTCCATCGA 2479
E. bromocola 5761 2450 AATATCTCACGTTGAATAGTTCTAATCGCTGTATATCGTGAAAGGCCAATTGTTCCATCGA 2510
E. glyceria ----------
E. festucae Fl1 ----------
E. festucae E2368 ----------
E. baconii 2497 GAAAAGTTGCGTAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2557
E. brachyelytri 2492 GAAAAGTTGCATAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2552
E. bromicola AL0434 2483 GAAAAGTTGCGTAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2543
E. bromocola 502 2480 GAAAAGTTGCGTAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2540
E. bromocola 5761 2511 GAAAAGTTGCGCAGACCTGCAAAGCTCCGAGTTTGCCATTCTCACCTCATTCTCCTCCTCT 2571
E. glyceria ----------
E. festucae Fl1 ----------
E. festucae E2368 ----------
E. baconii 2558 TAATGGACCGTGGTGTTGG 2576
E. brachyelytri 2553 TAATGGACCGTGGTGTTGG 2571
E. bromicola AL0434 2544 TAATGGACCGTGGTG---- 2558
E. bromocola 502 2541 TAATGGACCGTGGTG---- 2555
E. bromocola 5761 2572 TAATGGACCGTGGTG---- 2586
E. glyceria 2454 ----------GC 2455

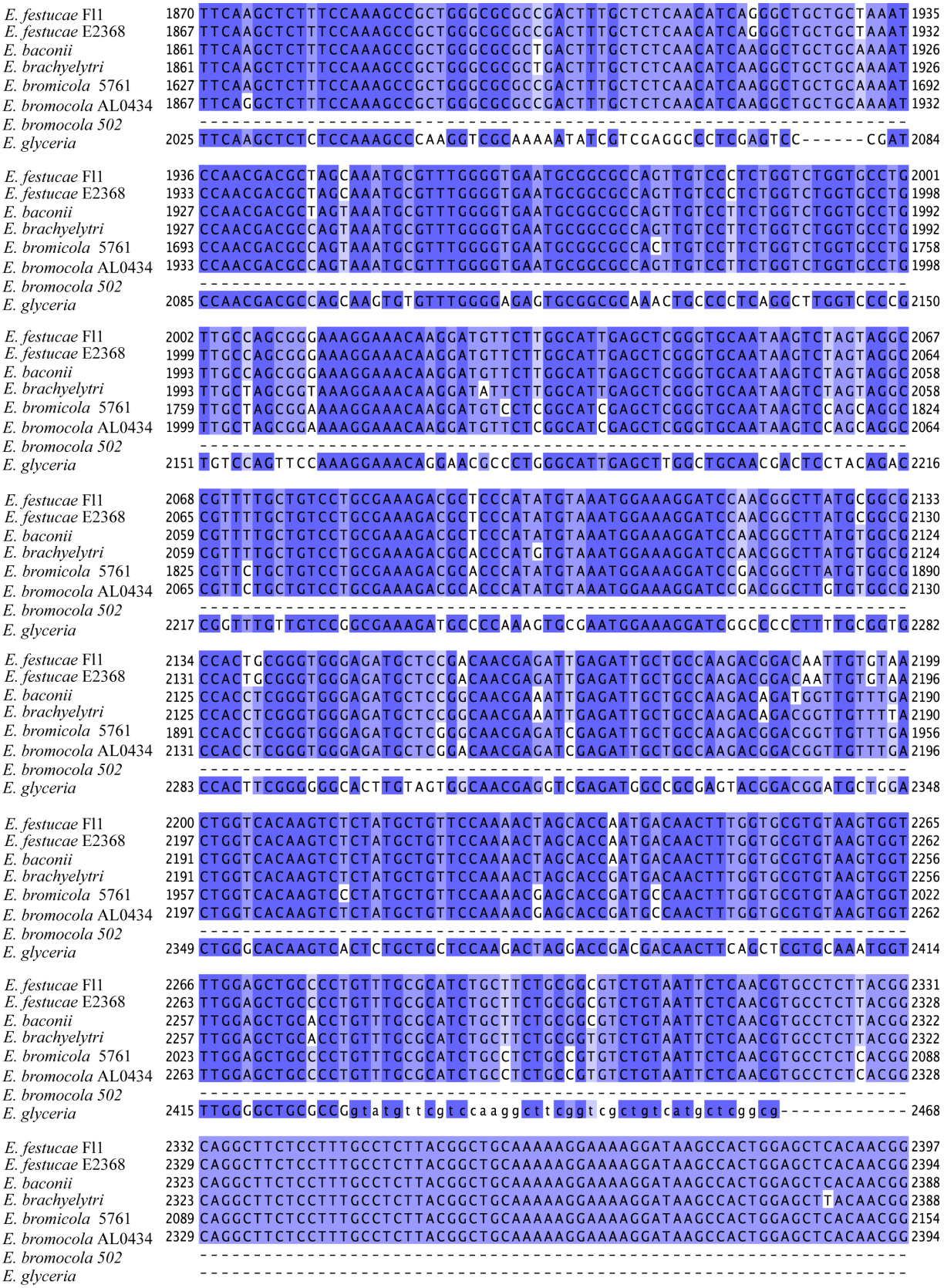
Appendix 19. Multiple sequence alignment of the *E. festucae chiB* gene with homologues from other *Epichloë* species. *E. festucae* (Fl1) EfM3.035030, E. *festucae* (E2368), *E. baconii, E. brachyelytri, E. bromicola* 5761*,* AL0434 and 502, *E. glyceria.* Intensity of shading indicates the level of conservation. *chiB* start codon (ATG) and stop codons (TAG and TAA) are boxed in red and green respectively. Exons and introns are indicated as capital and small letters respectively.

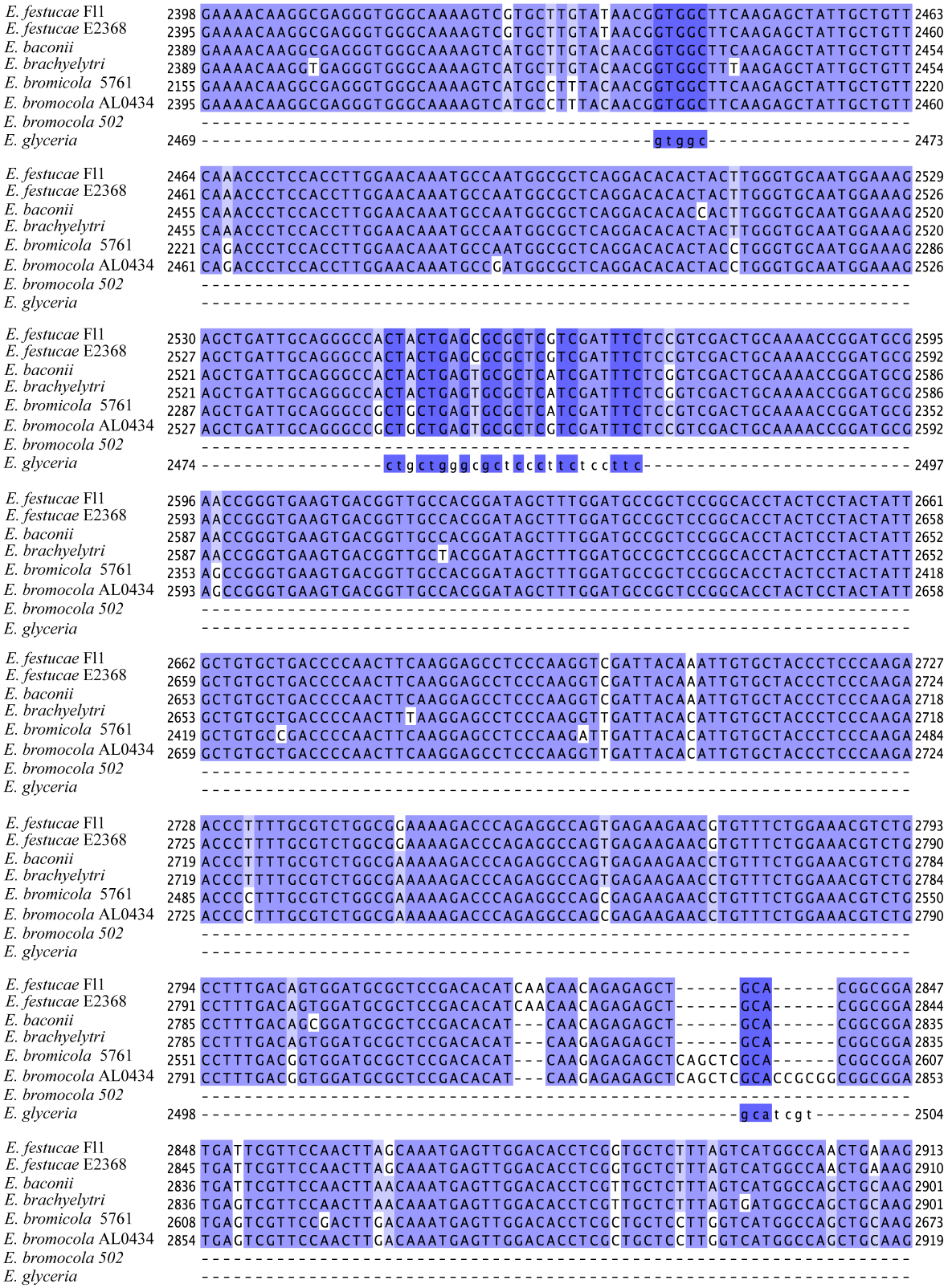

E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromicola 5761
E. bromocola AL0434
E. bromocola 502
E. glyceria

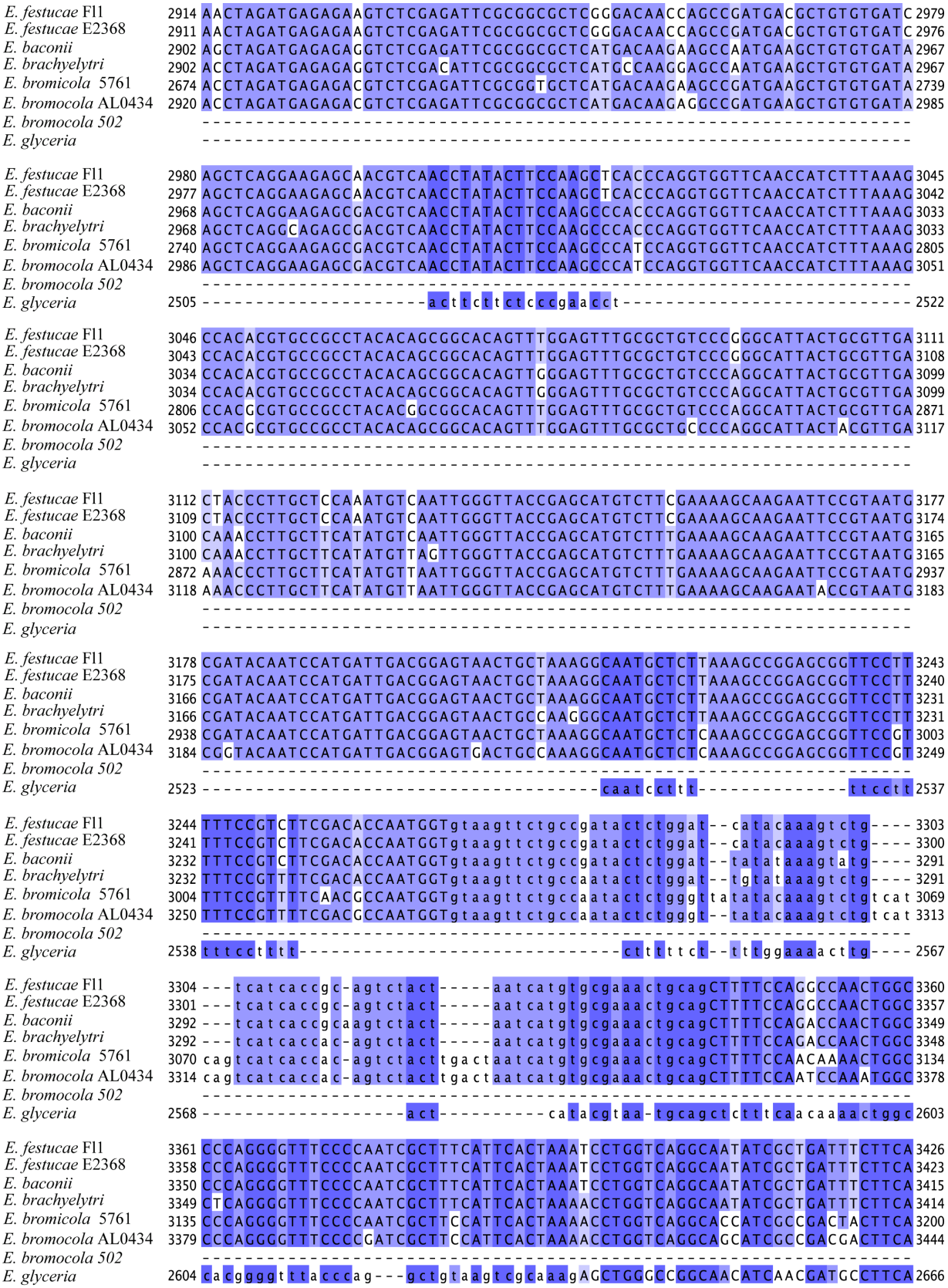

E. festucae Fl1 449 CCGACTACTGCTCAAAGAGTAACTGCGTCTCCAACTGCGACTCGAAGAGTGAATGCAATCCCGGTT 514
E. festucae E2368 446 CCGACTACTGCTCAAAGAGTAACTGCGTCTCCAACTGCGACTCGAAGAGTGAATGCAATCCCGGTT 511
E. baconii 445 CCGACTACTGCTCAAAGAGTAACTGCATCTCCAACTGCGACTCGAAGAGTGAATGCAATCCCGGCT 510
E. brachyelytri 445 CCGACTACTGCTCAACGAGTAACTGCATCTCCAACTGCGACTCGAAGAGTGAATGCAATCCCGGTT 510
E. bromicola 5761 208 CCGACTACTGCTCGACGAGTAAATGCATCTCCAACTGCGACTCGAAGAGTGAATGCAATCCCGGTT 273
E. bromocola AL0434 446 CCGACTACTGCTCGACGAGTAAATGCATCTCCAACTGCGACTCGAAGAGTGAATGCAATCCCGGTT 511
E. bromocola 502 446 CCGACTACTGCTCGACGAGTAAATGCATCTCCAACTGCGACTCGAAGAGTGAATGCAATCCCGGTT 511
E. glyceria 445 CTAAATACTGCTCCAAGGAGAACTGTATCTCTACCTGTGACGCCAAGAGCGAGTGTGACCCGGGAT 510
E. festucae Fl1 515 GGGGTAAGGAATGGAGTATGCGCGACAAGTGTCCCTTGAATGTCTGCTGTTCTAAATATGGTTTCT 580
E. festucae E2368 512 GGGGTAAGGAATGGAGTATGCGCGACAAGTGTCCCTTGAATGTCTGCTGTTCTAAATATGGTTTCT 577
E. baconii 511 GGGGTAAGGAATGGAGTATGCGCGACAAGTGTCCCTTGAATGTCTGCTGTTCTAAATATGGTTTCT 576
E. brachyelytri 511 GGGGTAATGAATGGAGTATGCGCGACAAGTGTCCCTTGAATGTCTGCTGTTCTAAATATGGTTTCT 576
E. bromicola 5761 274 GGGGTAAGGAATGGAGTATGCGCGACAAGTGCCCCTTGAATGTCTGCTGTTCCAAATATGGGTTCT 339
E. bromocola AL0434 512 GGGGTAAGGAATGGAGTATGCGCGACAAGTGTCCCTTGAATGTCTGCTGTTCCAAATATGGTTTCT 577
E. bromocola 502 512 GGGGTAAGGAATGGAGTATGCGCGACAAGTGTCCCTTGAATGTCTGCTGTTCCAAATATGGTTTCT 577
E. glyceria 511 GGGGTGAAGAATGGAGCACGCGCAGCAGATGCCCTCTCAATGTCTGCTGCTCCGAATATGGGTTTT 576
E. festucae Fl1 581 GCGGAACCACGAAAGAGTTCTGTGGCGACAAGAAAGTTTCATCTCCCACTTGCTCAGGCGGAACCT 646
E. festucae E2368 578 GCGGAACCACGAAAGAGTTCTGTGGCGACAAGAAAGTTTCATCTCCCACTTGCTCAGGCGGAACCT 643
E. baconii 577 GCGGAACCACGAAAGAGTTCTGTGGCGACAAGAAAGTTTCATCTCCTACTTGCTCAGGCGGAACCT 642
E. brachyelytri 577 GCGGAACCACGGAAGAGTTCTGTGGCGACAAGAAAGTTTCATCTCCTACTTGCTCAGGCGGAACCT 642
E. bromicola 5761 340 GCGGAACCACGAAAGAGTTCTGCGGCGACAAGAAAGTTTCACCTCCTACTTGCTCAGGCGGAACCT 405
E. bromocola AL0434 578 GCGGAACCACGAAAGAGTTCTGCGGCGACAAGAAAGTTTCACCTCCTACTTGCTCAGGCGGAACCT 643
E. bromocola 502 578 GCGGAACCACGAAAGAGTTCTGCGGCGACAAGAAAGTTTCACCTCCTACTTGCTCAGGCGGAACCT 643
E. glyceria 577 GCGGAACGACAAAGGACTTCTGCGGTGACAAGAAGATTTCGCCTCCTAGCTGTCCGGGGGGCACGT 642
E. festucae Fl1 647 CTGCAAATAAGCGAGTCATCGGATACTTTGAAGGCTGGGCTCATACAAAGAACTGTGGAGGCTgta 712
E. festucae E2368 644 CTGCAAATAAGCGAGTCATCGGATACTTTGAAGGCTGGGCTCATACAAAGAACTGTGGAGGCTgta 709
E. baconii 643 CTGCGAATAAGCGAATTATCGGATACTTTGAGGGCTGGGCTCATACAAAGAACTGTGGAGGCTgta 708
E. brachyelytri 643 CTGCGAATAAGCGAGTCATCGGATACTATGAAGGCTGGGCTCATATAAAGAACTGTGGAGGCTgta 708
E. bromicola 5761 406 CTGCGAATAAGCGAGTCATCGGATACTTTGAAGGCTGGGCTCATACAAAGAACTGTGGAGGCTgta 471
E. bromocola AL0434 644 CTGCGAACAAGCGAGTCATCGGATACTTTGAAGGCTGGGCTCATACAAAGAACTGTGGAGGCTgta 709
E. bromocola 502 644 CTGCGAACAAGCGAGTCATCGGATACTTTGAAGGCTGGGCTCATACAAAGAACTGTGGAGGCTgta 709
E. glyceria 643 CGGCCAACAAGAGGGTCATTGGATACTACGAGGGCTGGGCGAACACGAAGCCGTGTGGTGGATgta 708
E. festucae Fl1 713 agc----------actctcacgggaaatgaccgatacgg------tgtggatggtacactaac 760
E. festucae E2368 710 agc----------actctcacgggaaatgaccgatacgg------tgtggatggtacactaac 757
E. baconii 709 agc----------actctgac-gggaaatgaccgatacga------tgtggatggtacattaac 755
E. brachyelytri 709 agt----------actctcac-gggaaatgaccgatacgg------tgtggatggtacactaac 755
E. bromicola 5761 472 agt----------actctcac---ggaatgaccgatacgg------tgtggatggtatactaac 516
E. bromocola AL0434 710 agt----------actccag-ggggaatgaccgatacgg------cgtggatggtacactaac 756
E. bromocola 502 710 agt----------actctcag-gggaaatgaccgatacgg------cgtggatggtacactaac 756
E. glyceria 709 ggtcgagacccgagagctcctgt-cgacgacaatggacatcagcctcccgtgcttgactttttgaac 773
E. festucae Fl1 761 att----------ggcatagTAACTCCAGAGAAGCTTCTCAATGGCGCTTATACACATCTGA 813
E. festucae E2368 758 att----------ggcatagTAACTCCAGAGAAGCTTCTCAATGGCGCTTATACACATCTGA 810
E. baconii 756 att----------ggcatagTAACTCCAGAGAAGCTTCTCAATGGTGCTTATACACATCTGA 807
E. brachyelytri 756 att----------ggcatagTAACTCCAGAGAAGCTTCTCAATGGCGCTTATACACATCTGA 807
E. bromicola 5761 517 att----------ggcatagTAACTCCAGAGAAGCTTCTCAATGGCGCTTATACACATCTGA 568
E. bromocola AL0434 757 att----------ggcgtagTAACTCCAGAGAAGCTTCTCAATGGCGCTTATACACATCTGA 808
E. bromocola 502 757 att----------ggcatagTAACTCCAGAGAAGCTTCTCAATGGCGCTTATACACATCTGA 808
E. glyceria 774 atgtgctcaccaaggaatacctagTGAACCCCGAGGACCTTTTATACAATGGCTACTCTCATCTGA 839
E. festucae Fl1 814 ACTATGCATTTGCATTCATCAACCCTGGCACCTTTGAGATTGCAAACATGCAAGATTCCGACGATC 879
E. festucae E2368 811 ACTATGCATTTGCATTCATCAACCCTGGCACCTTTGAGATTGCAAACATGCAAGATTCCGACGATC 876
E. baconii 808 ACTATGCATTTGCATTCATCAACCCTAGCACCTTTGAGATTGCAAACATGCAAGATTCCGACGATC 873
E. brachyelytri 808 ACTATGCATTTGCATTCATCAACCCTAGCACCTTTGAGATTGCAAACATGCAAGATTCCGACGATC 873
E. bromicola 5761 569 ACTATGCATTTGCATTCATCAACCCTAGCACCTTTGAGGTTGCAAACATGCAAGATTCCGACGATC 634
E. bromocola AL0434 809 ACTATGCATTTGCATTCATCAACCCTAGCACCTTTGAGATTGCAAACATGCAAGATTCCGACGATC 874
E. bromocola 502 809 ACTATGCATTTGCATTCATCAACCCTAGCACCTTTGAGATTGCAAACATGCAAGATTCCGACGATC 874
E. glyceria 840 ACTACGCCTTTGCGTTTGTCAATCCCAGTACGTTCGAGATTGCCAACATGCAAGACTCCGACGAGC 905
E. festucae Fl1 880 AGTATATGGCCCGTCTTACCAACCTGAAAAACTTCAACCCTGGCCTAAAGGTTTGGgtaagtg--- 942
E. festucae E2368 877 AGTATATGGCCCGTCTTACCAACCTGAAAAACTTCAACCCTGGCCTAAAGGTTTGGgtaagtg--- 939
E. baconii 874 AGTATATGGCCCGTCTTACCAACCTGAAAAACTTTAACCCTGGCCTAAAGGTTTGGgtaagtg--- 936
E. brachyelytri 874 AGTATATGGCCCGTCTTACTAACCTGAAAAACTTTAACCCTGGCCTAAAGGTTTGGgtaagta--- 936
E. bromicola 5761 635 AGTATATGGCCCGTCTCACCAATCTGAAAAACTTTAACCCTGGCCTAAAGGTTTGGgtaagaa--- 697
E. bromocola AL0434 875 AGTATATGGCCCGTCTCACCAACCTGAAAAACTTTAACCCTGGCCTAAAGGTTTGGgtaagaa--- 937
E. bromocola 502 875 AGTATATGGCCCGTCTCACCAACCTGAAAAACTTTAACCCTGGCCTCAAGGTTTGGgtaagaa--- 937
E. glyceria 906 AATTCATGCCGCGACTGTCGGCTCTGAAGAATTACAACCCCAGTCTACAGGTTTGGgtaagcattt 971

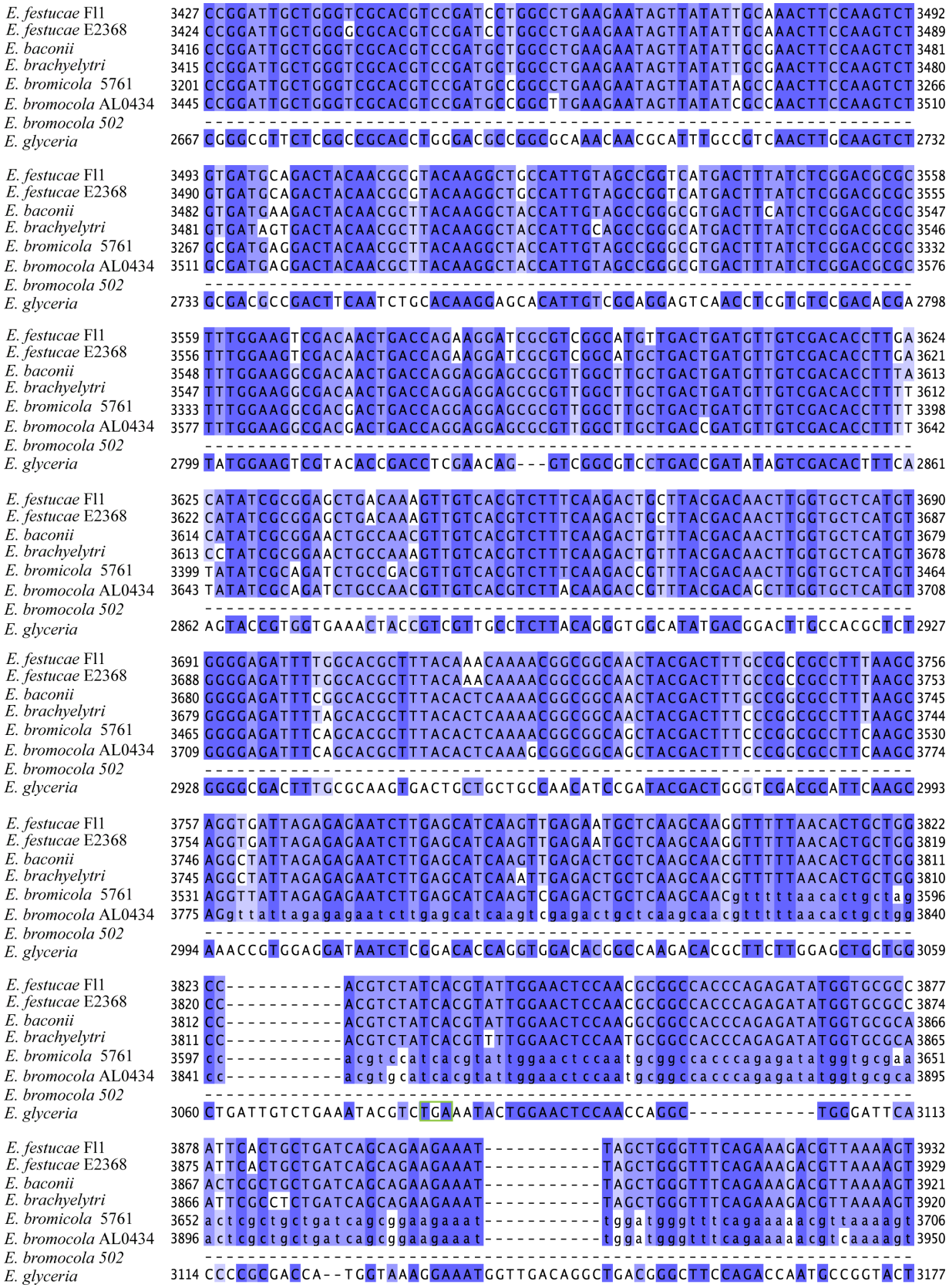

E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromicola 5761
E. bromocola AL0434
E. bromocola 502
E. glyceria

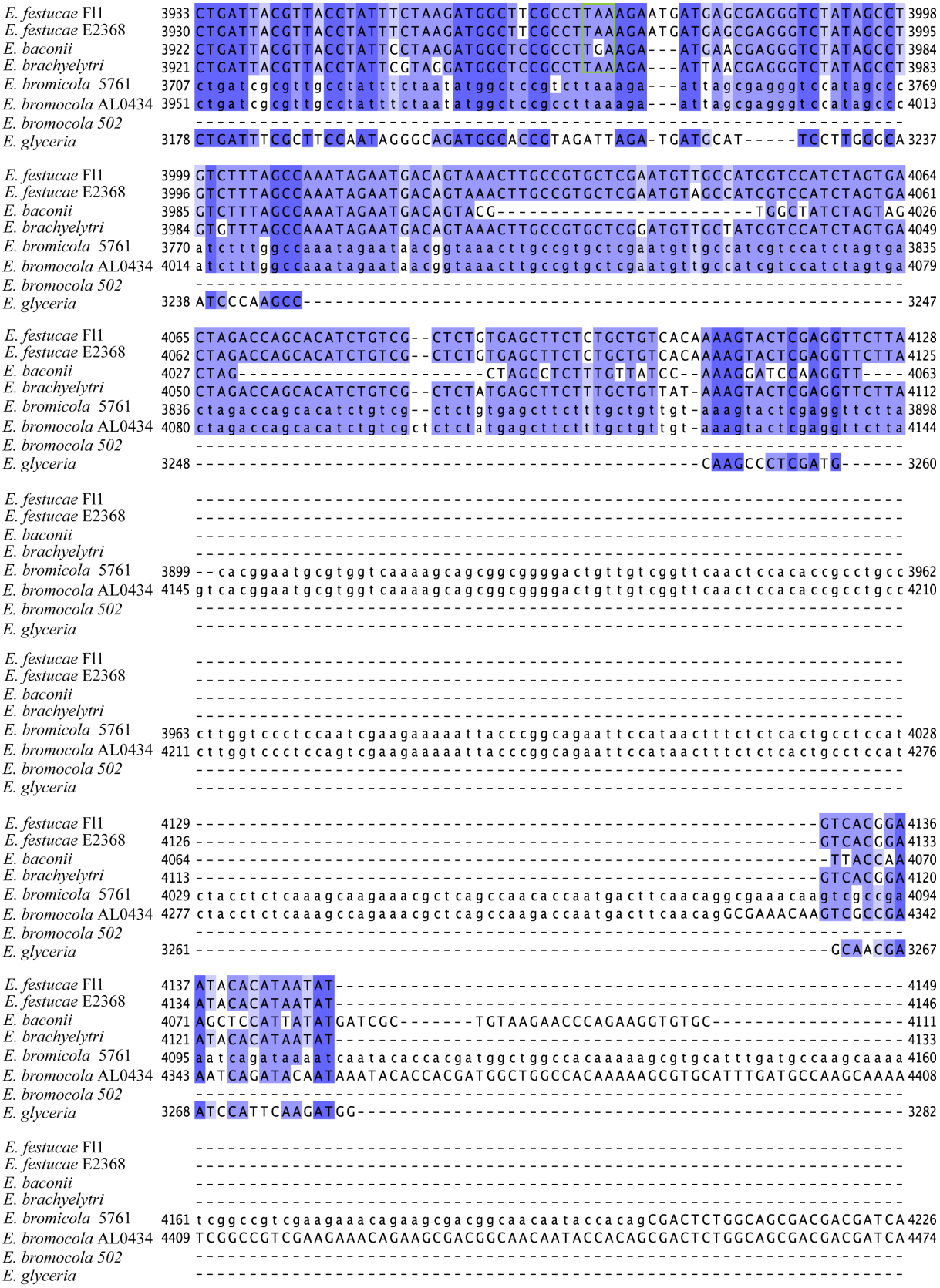

E. festucae Fl1 1342 AACACTTTGATATTAAGAATATGGAACAGCATATTGACTGGTTCAACATCATGAGCTACGACTTAC 1407
E. festucae E2368 1339 AACACTTTGATATTAAGAATATGGAACAGCATATTGACTGGTTCAACATCATGAGCTACGACTTAC 1404
E. baconii 1333 AACACTTTGATATTAAGAATATGGAACAGCATATTGACTGGTTTAATATCATGAGCTACGACTTAC 1398
E. brachyelytri 1333 AACACTTTAATATTAAGAATATAGAACAGCATGTCGACTGGTTTAACATTATGAGCTACGACTTAC 1398
E. bromicola 5761 1099 AACACTTTGATATTAAGAATATGGAACAGCATGTCGACTGGTTCAACATCATGAGCTACGACTTAC 1164
E. bromocola AL0434 1339 AACACTTTGATATTAAGAATATGGAACAGCATGTCGACTGGTTCAACATCATGAGCTACGACTTAC 1404
E. bromocola 502 1338 AACACTTTGATATTAAGAATATGGAACAGCATGTCGACTGGTTCAACATCATGAGCTACGACTTAC 1403
E. glyceria 1497 AGCACTTTGACATCAAGAACATGGAGCAATATGTCGATTGGTTCAACGTAATGAGCTACGATCTGC 1562
E. festucae Fl1 1408 ATGGCACTTGGGATGGCACAAATCCCTATCTTGGGGCATTTGTTAACGCTCATACTAACCTTACAG 1473
E. festucae E2368 1405 ATGGCACTTGGGATGGCACAAATCCCTATCTTGGGGCATTTGTTAACGCTCATACTAACCTTACAG 1470
E. baconii 1399 ATGGCACTTGGGATGGCACGAATCCCTATCTTGGGGCATTTATTAACGCTCATACTAACCTTACAG 1464
E. brachyelytri 1399 ATGGCACTTGGGATGGCACAAATCCCTATCTTGGGGCATTTGTCAACGCTCATACTAACCTTACAG 1464
E. bromicola 5761 1165 ATGGCACTTGGGATGGCACGAATCCCTATCTTGGGGCATTTGTCAACGCTCATACCAACCTTACAG 1230
E. bromocola AL0434 1405 ATGGCACTTGGGATGGCACGAATCCCTATCTTGGGGCATTTGTCAACGCTCATACCAACCTTACAG 1470
E. bromocola 502 1404 ATGGCACTTGGGATGGCACGAATCCCTATCTTGGGGCATTTGTCAACGCTCATACCAACCTTACAG 1469
E. glyceria 1563 ACGGAACTTGGGATGGCACCAATCCATATCTGGGAGCCCATGTCAACGCCACACGAACCTGACGG 1628
E. festucae Fl1 1474 AGATTGAAACGGCTCTGAATCTGCTGTGGCGCAACGATATTAAACCTGAGAAGGTGATTATGGGCA 1539
E. festucae E2368 1471 AGATTGAAACGGCTCTGAATCTGCTGTGGCGCAACGATATTAAACCTGAGAAGGTGATTATGGGCA 1536
E. baconii 1465 AGATTGACACGGCTCTGAATCTGCTATGGCGCAACGATATTAAACCTGAGAAGGTGATTATGGGCA 1530
E. brachyelytri 1465 AGATTAACACGGCTCTGAACCTGCTGTGGCGCAACGATATTAAACCTGAGAAGGTGATTATGGGCA 1530
E. bromicola 5761 1231 AGATTGAGACGGCCCTGAATCTGCTGTGGCGCAACGACATCAAACCTGAGAAGGTGATTATGGGCA 1296
E. bromocola AL0434 1471 AGATTGAGACGGCCCTGAATCTGCTGTGGCGCAACGACATCAAACCTGAGAAGGTGATTATGGGCA 1536
E. bromocola 502 1470 AGATTGAGACGGCCCTGAATCTGCTGTGGCGCAACGACATCAAACCTGAGAAGGTGATTATGGGCA 1535
E. glyceria 1629 AGATTGACACCGCACTGAACCTCCTCTGGCGTAACGACATCAAGCCCGAAAAGGTCATCATGGGCA 1694
E. festucae Fl1 1540 TGGGCTTCTACGGCCGTAGTTTCACGCTGTCTAATCCTGGTTGCGCCACTGCTGGCTGCGGATTCC 1605
E. festucae E2368 1537 TGGGCTTCTACGGCCGTAGTTTCACGCTGTCTAATCCTGGTTGCGCCACTGCTGGCTGCGGATTCC 1602
E. baconii 1531 TGGGCTTTTACGGACGTAGTTTCACGCTGTCTAATCCTGGTTGCGCCACTGCTGGCTGCGGATTCC 1596
E. brachyelytri 1531 TGGGCTTCTACGGCCGTAGTTTCACGCTGTCTAATCCTGGTTGCGCCACTGCTGGCTGCGGATTCC 1596
E. bromicola 5761 1297 TGGGCTTCTACGGCCGTAGTTTCACGCTGTCTAATCCTGGTTGCGCCACTGCTGGCTGCGGATTCC 1362
E. bromocola AL0434 1537 TGGGCTTCTACGGCCGCAGTTTCACGCTGTCTAATCCTGGTTGCGCCACTGCTGGCTGCGGATTCC 1602
E. bromocola 502 1536 TGGGCTTCTACGGCCGCAGTTTCACGCTGTCTAATCCTGGTTGCGCCACTGCTGGCTGCGGATTCC 1601
E. glyceria 1695 TGGGCTTCTATGGCCGCAGCTTCACTCTGGCAGATCCAAACTGCAAGGTGGCTGGTTGTGGCTTCC 1760
E. festucae Fl1 1606 GTTCTGGCGGCAACCCAGGCAAATGCTCTGCCTCGGCAGGTACCCTGATGTACTCAGAGATTAAAG 1671
E. festucae E2368 1603 GTTCTGGCGGCAACCCAGGCAAATGCTCTGCCTCGGCAGGTACCCTGATGTACTCAGAGATTAAAG 1668
E. baconii 1597 GTGCTGGCGGTAACCCAGGCAAATGCTCTGCCTCGGCAGGTACCCTGATGTACTCAGAGATTAAAG 1662
E. brachyelytri 1597 GTGCTGGCGGCAACCCAGGCAAATGCTCTGCCTCGGCAGGTACCCTGATGTACTCAGAGATTAAAG 1662
E. bromicola 5761 1363 GTGCTGGCGGCAACCCAGGCAAATGCTCTGCCTCGGCAGGCACACTGATGTACTCGGAGATTAAAG 1428
E. bromocola AL0434 1603 GTGCTGGCGGCAACCCAGGCAAATGCTCTGCCTCGGCAGGCACACTGATGTACTCGGAGATTAAAG 1668
E. bromocola 502 1602 GTGCTGGCGGCAACCCAGGCAAATGCTCTGCCTCGGCAGGCACACTGATGTACTCGGAGATTAAAG 1667
E. glyceria 1761 GTGGGGGCGGAAATCCCGGCAAGTGCTCCGCCTCTGCGGGAACGCTCATGTACTCGGAGATTCGAG 1826
E. festucae Fl1 1672 ATATCATCAGCTCAGGAAAGGCCACAGTCACAACGGACAAAGATGCTGCAGTCAAGATTGTCACTT 1737
E. festucae E2368 1669 ATATCATCAGCTCAGGAAAGGCCACAGTCACAACGGACAAAGATGCTGCAGTCAAGATTGTCACTT 1734
E. baconii 1663 ATATCATCAGCTCAGGAAAGGCCACAGTCACAACGGACAAAGATGCTGCAGTCAAGATTGTCACTT 1728
E. brachyelytri 1663 ATATTATCAGCTCAGGAAAGGCCACAGTCACAACGGACAAAGATGCTGCAGTCAAGATTGTCACTT 1728
E. bromicola 5761 1429 ATATCATCAGCTCAGGAAAGGCCACAGTCACCACGGACAAAGATGCTGCAGTCAAGATTGTCACTT 1494
E. bromocola AL0434 1669 ACATCATCAGCTCAGGAAAGGCCACAGTCACCACGGACAAAGATGCTGCGGTCAAGATTGTCACTT 1734
E. bromocola 502 1668 ACATCATCAGCTCAGGAAAGGCCACAGTCACCACGGACAAAGATGCTGCGGTCAAGATTGTCACTT 1733
E. glyceria 1827 AAATAATCGCCCAAGGTGGTGCCAGCGTCGTTACCGACAAGGGCGCTGCCGTCAAGGTTGTGACCT 1892
E. festucae Fl1 1738 TTGATGGAAATCAATGGGTATCGTATGACGATGAAGACACACTTCGCACCAAGATGAATTATGCCA 1803
E. festucae E2368 1735 TTGATGGAAATCAATGGGTATCGTATGACGATGAAGACACACTTCGCACCAAGATGAATTATGCCA 1800
E. baconii 1729 TTGATGGAAATCAATGGGTATCATATGACGATGAAGACACACTTCGCACCAAGATGAATTATGCCA 1794
E. brachyelytri 1729 TTGACAGAGATCAATGGGTATCATATGACGATGAAGACACACTTCGCACCAAGATGGATTATGCCA 1794
E. bromicola 5761 1495 TTGGCGGAGACCAATGGGTATCATATGACGATGAAGACACACTTCGCACCAAGATGGATTATGCCA 1560
E. bromocola AL0434 1735 TTGGCGGAGACCAATGGGTATCATATGACGATGAAGACACACTTCGCACCAAGATGGATTATGCCA 1800
E. bromocola 502 1734 TTGGCGGAGACCAATGGGTATCATATGACGATGAAGACAC---------- 1773
E. glyceria 1893 GGGGCGGAGACCAATGGGTGTCATATGATGACGAGGAAACGTTGCGCATGAAAGCCGACTATGCCA 1958
E. festucae Fl1 1804 ACAAGCGATGTCTTGGAGGCGTAATGGTCTGGGCTGCCTCCACCGACGATGAGGATGGGACAGCTA 1869
E. festucae E2368 1801 ACAAGCGATGTCTTGGAGGCGTAATGGTCTGGGCTGCCTCCACCGACGATGAGGATGGGACAGCTA 1866
E. baconii 1795 ACAAGCGATGTCTTGGAGGCGTAATGGTCTGGGCTGCCTCGACCGACGATGAGGATGGGACAGCTA 1860
E. brachyelytri 1795 ACAAGCGATGTCTTGGAGGCGTAATGGTCTGGGCTGCCTCCACCGACGATGAGGATGGAACAGCTA 1860
E. bromicola 5761 1561 ACAAGCGATGTCTCGGAGGCGTAATGGTCTGGGCTGCCTCGACCGACGATGAGGATGGAACAGCTA 1626
E. bromocola AL0434 1801 ACAAGCGATGTCTCGGAGGCGTAATGGTCTGGGCTGCCTCGACCGACGATGAGGATGGAACAGCTA 1866
E. bromocola 502 ----------
E. glyceria 1959 ACAACCACTGCCTTGGTGGAGTCATGGTTTGGGCTGCATCCACGGACGATGGGCGAGGCACGGCCA 2024

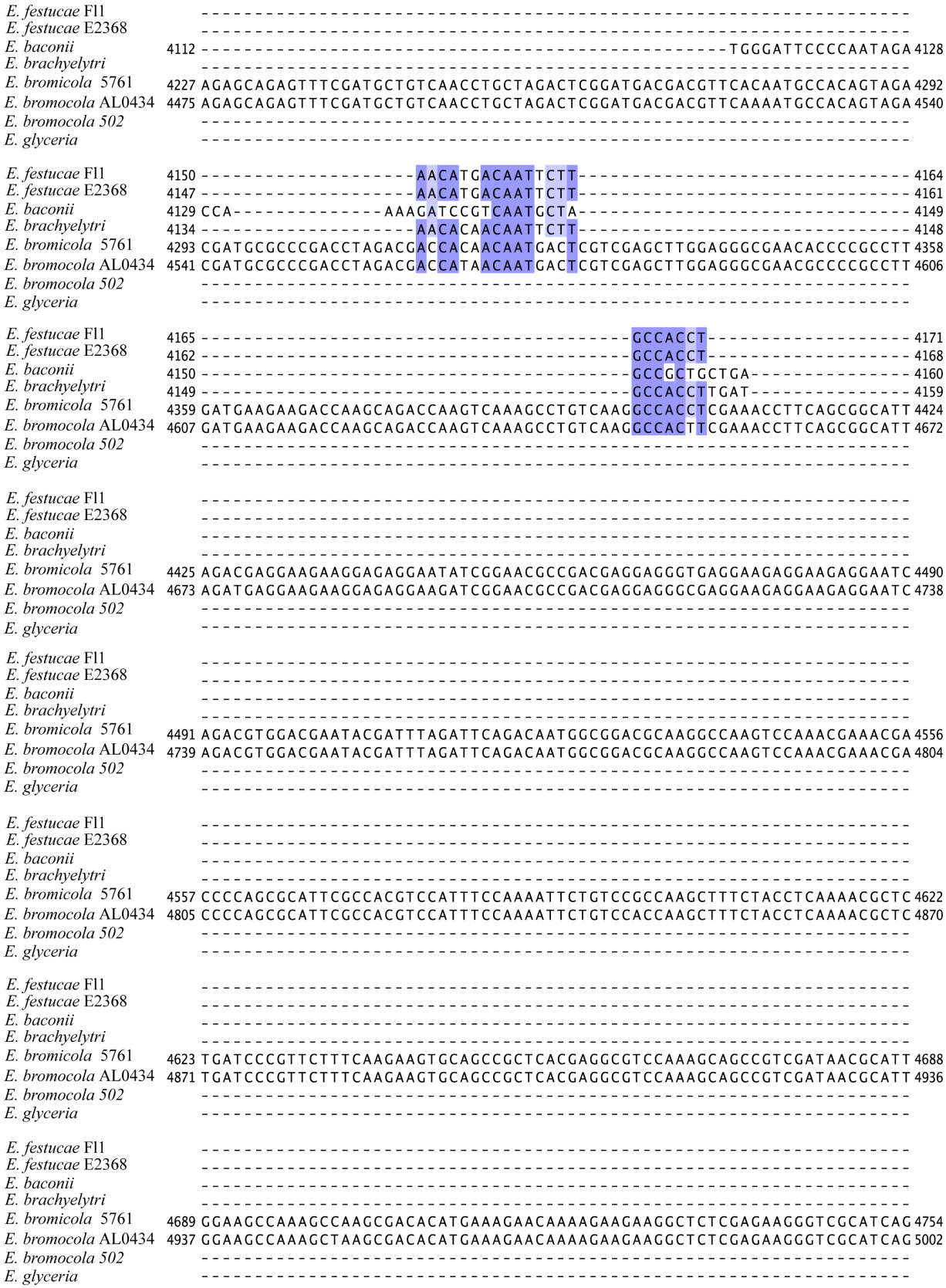

E. festucae Fl1 1870 TTCAAGCTCTTTCCAAAGCCGCTGGGCGCGCCGACTTTGCTCTCAACATCAGGGCTGCTGCTAAAT 1935
E. festucae E2368 1867 TTCAAGCTCTTTCCAAAGCCGCTGGGCGCGCCGACTTTGCTCTCAACATCAGGGCTGCTGCTAAAT 1932
E. baconii 1861 TTCAAGCTCTTTCCAAAGCCGCTGGGCGCGCTGACTTTGCTCTCAACATCAAGGCTGCTGCAAAAT 1926
E. brachyelytri 1861 TTCAAGCTCTTTCCAAAGCCGCTGGGCGCGCTGACTTTGCTCTCAACATCAAGGCTGCTGCAAAAT 1926
E. bromicola 5761 1627 TTCAAGCTCTTTCCAAAGCCGCTGGGCGCGCCGACTTTGCTCTCAACATCAAGGCTGCTGCAAAAT 1692
E. bromocola AL0434 1867 TTCAGGCTCTTTCCAAAGCCGCTGGGCGCGCCGACTTTGCTCTCAACATCAAGGCTGCTGCAAAAT 1932
E. bromocola 502
E. glyceria 2025 TTCAAGCTCTCTCCAAAGCCCAAGGTCGCAAAAATATCGTCGAGGCCCTCGAGTCC------CGAT 2084
E. festucae Fl1 1936 CCAACGACGCTAGCAAATGCGTTTGGGGTGAATGCGGCGCCAGTTGTCCCTCTGGTCTGGTGCCTG 2001
E. festucae E2368 1933 CCAACGACGCTAGCAAATGCGTTTGGGGTGAATGCGGCGCCAGTTGTCCCTCTGGTCTGGTGCCTG 1998
E. baconii 1927 CCAACGACGCTAGTAAATGCGTTTGGGGTGAATGCGGCGCCAGTTGTCCTTCTGGTCTGGTGCCTG 1992
E. brachyelytri 1927 CCAACGACGCCAGTAAATGCGTTTGGGGTGAATGCGGCGCCAGTTGTCCTTCTGGTCTGGTGCCTG 1992
E. bromicola 5761 1693 CCAACGACGCCAGTAAATGCGTTTGGGGTGAATGCGGCGCCACTTGTCCTTCTGGTCTGGTGCCTG 1758
E. bromocola AL0434 1933 CCAACGACGCCAGTAAATGCGTTTGGGGTGAATGCGGCGCCAGTTGTCCTTCTGGTCTGGTGCCTG 1998
E. bromocola 502
E. glyceria 2085 CCAACGACGCCAGCAAGTGTGTTTGGGGAGAGTGCGGCGCAAACTGCCCTCAGGCTTGGTCCCCG 2150
E. festucae Fl1 2002 TTGCCAGCGGGAAAGGAAACAAGGATGTTCTTGGCATTGAGCTCGGGTGCAATAAGTCTAGTAGGC 2067
E. festucae E2368 1999 TTGCCAGCGGGAAAGGAAACAAGGATGTTCTTGGCATTGAGCTCGGGTGCAATAAGTCTAGTAGGC 2064
E. baconii 1993 TTGCCAGCGGGAAAGGAAACAAGGATGTTCTTGGCATTGAGCTCGGGTGCAATAAGTCTAGTAGGC 2058
E. brachyelytri 1993 TTGCTAGCGGTAAAGGAAACAAGGATATTCTTGGCATTGAGCTCGGGTGCAATAAGTCTAGTAGGC 2058
E. bromicola 5761 1759 TTGCTAGCGGAAAAGGAAACAAGGATGTCCTCGGCATCGAGCTCGGGTGCAATAAGTCCAGCAGGC 1824
E. bromocola AL0434 1999 TTGCTAGCGGAAAAGGAAACAAGGATGTTCTCGGCATCGAGCTCGGGTGCAATAAGTCCAGCAGGC 2064
E. bromocola 502
E. glyceria 2151 TGTCCAGTTCCAAAGGAAACAGGAACGCCCTGGGCATTGAGCTTGGCTGCAACGACTCCTACAGAC 2216
E. festucae Fl1 2068 CGTTTTGCTGTCCTGCGAAAGACGCTCCCATATGTAAATGGAAAGGATCCAACGGCTTATGCGGCG 2133
E. festucae E2368 2065 CGTTTTGCTGTCCTGCGAAAGACGCTCCCATATGTAAATGGAAAGGATCCAACGGCTTATGCGGCG 2130
E. baconii 2059 CGTTTTGCTGTCCTGCGAAAGACGCTCCCATATGTAAATGGAAAGGATCCAACGGCTTATGTGGCG 2124
E. brachyelytri 2059 CGTTTTGCTGTCCTGCGAAAGACGCACCCATGTGTAAATGGAAAGGATCCAACGGCTTATGTGGCG 2124
E. bromicola 5761 1825 CGTTCTGCTGTCCTGCGAAAGACGCACCCATATGTAAATGGAAAGGATCCGACGGCTTATGTGGCG 1890
E. bromocola AL0434 2065 CGTTCTGCTGTCCTGCGAAAGACGCACCCATATGTAAATGGAAAGGATCCGACGGCTTGTGTGGCG 2130
E. bromocola 502
E. glyceria 2217 CGGTTTGTTGTCCGGCGAAAGATGCCCCAAAGTGCGAATGGAAAGGATCGGCCCCTTTTGCGGTG 2282
E. festucae Fl1 2134 CCACTGCGGGTGGGAGATGCTCCGACAACGAGATTGAGATTGCTGCCAAGACGGACAATTGTGTAA 2199
E. festucae E2368 2131 CCACTGCGGGTGGGAGATGCTCCGACAACGAGATTGAGATTGCTGCCAAGACGGACAATTGTGTAA 2196
E. baconii 2125 CCACCTCGGGTGGGAGATGCTCCGGCAACGAAATTGAGATTGCTGCCAAGACAGATGGTTGTTTGA 2190
E. brachyelytri 2125 CCACCTCGGGTGGGAGATGCTCCGGCAACGAAATTGAGATTGCTGCCAAGACAGACGGTTGTTTTA 2190
E. bromicola 5761 1891 CCACCTCGGGTGGGAGATGCTCGGGCAACGAGATCGAGATTGCTGCCAAGACGGACGGTTGTTTGA 1956
E. bromocola AL0434 2131 CCACCTCGGGTGGGAGATGCTCGGACAACGAGATCGAGATTGCTGCCAAGACGGACGGTTGTTTGA 2196
E. bromocola 502
E. glyceria 2283 CCACTTCGGGGGGCACTTGTAGTGGCAACGAGGTCGAGATGGCCGCGAGTACGGACGGATGCTGGA 2348
E. festucae Fl1 2200 CTGGTCACAAGTCTCTATGCTGTTCCAAAACTAGCACCAATGACAACTTTGGTGCGTGTAAGTGGT 2265
E. festucae E2368 2197 CTGGTCACAAGTCTCTATGCTGTTCCAAAACTAGCACCAATGACAACTTTGGTGCGTGTAAGTGGT 2262
E. baconii 2191 CTGGTCACAAGTCTCTATGCTGTTCCAAAACTAGCACCAATGACAACTTTGGTGCGTGTAAGTGGT 2256
E. brachyelytri 2191 CTGGTCACAAGTCTCTATGCTGTTCCAAAACTAGCACCGATGACAACTTTGGTGCGTGTAAGTGGT 2256
E. bromicola 5761 1957 CTGGTCACAAGTCCCTATGCTGTTCCAAAACGAGCACCGATGCCAACTTTGGTGCGTGTAAGTGGT 2022
E. bromocola AL0434 2197 CTGGTCACAAGTCTCTATGCTGTTCCAAAACGAGCACCGATGCCAACTTTGGTGCGTGTAAGTGGT 2262
E. bromocola 502
E. glyceria 2349 CTGGGCACAAGTCACTCTGCTGCTCCAAGACTAGGACCGACGACAACTTCAGCTCGTGCAAATGGT 2414
E. festucae Fl1 2266 TTGGAGCTGCCCCTGTTTGCGCATCTGCTTCTGCGGCGTCTGTAATTCTCAACGTGCCTCTTACGG 2331
E. festucae E2368 2263 TTGGAGCTGCCCCTGTTTGCGCATCTGCTTCTGCGGCGTCTGTAATTCTCAACGTGCCTCTTACGG 2328
E. baconii 2257 TTGGAGCTGCACCTGTTTGCGCATCTGCTTCTGCGGCGTCTGTAATTCTCAACGTGCCTCTTACGG 2322
E. brachyelytri 2257 TTGGAGCTGCACCTGTTTGCGCATCTGCTTCTGCGGTGTCTGTAATTCTCAACGTGCCTCTTACGG 2322
E. bromicola 5761 2023 TTGGAGCTGCCCCTGTTTGCGCATCTGCCTCTGCCGTGTCTGTAATTCTCAACGTGCCTCTCACGG 2088
E. bromocola AL0434 2263 TTGGAGCTGCCCCTGTTTGCGCATCTGCCTCTGCCGTGTCTGTAATTCTCAACGTGCCTCTCACGG 2328
E. bromocola 502
E. glyceria 2415 TTGGGCTGCGCCGgtatgttcgtccaaggcttcggtcgctgtcatgctcggcg------------ 2468
E. festucae Fl1 2332 CAGGCTTCTCCTTTGCCTCTTACGGCTGCAAAAAGGAAAAGGATAAGCCACTGGAGCTCACAACGG 2397
E. festucae E2368 2329 CAGGCTTCTCCTTTGCCTCTTACGGCTGCAAAAAGGAAAAGGATAAGCCACTGGAGCTCACAACGG 2394
E. baconii 2323 CAGGCTTCTCCTTTGCCTCTTACGGCTGCAAAAAGGAAAAGGATAAGCCACTGGAGCTCACAACGG 2388
E. brachyelytri 2323 CAGGCTTCTCCTTTGCCTCTTACGGCTGCAAAAAGGAAAAGGATAAGCCACTGGAGCTTACAACGG 2388
E. bromicola 5761 2089 CAGGCTTCTCCTTTGCCTCTTACGGCTGCAAAAAGGAAAAGGATAAGCCACTGGAGCTCACAACGG 2154
E. bromocola AL0434 2329 CAGGCTTCTCCTTTGCCTCTTACGGCTGCAAAAAGGAAAAGGATAAGCCACTGGAGCTCACAACGG 2394
E. bromocola 502
E. glyceria

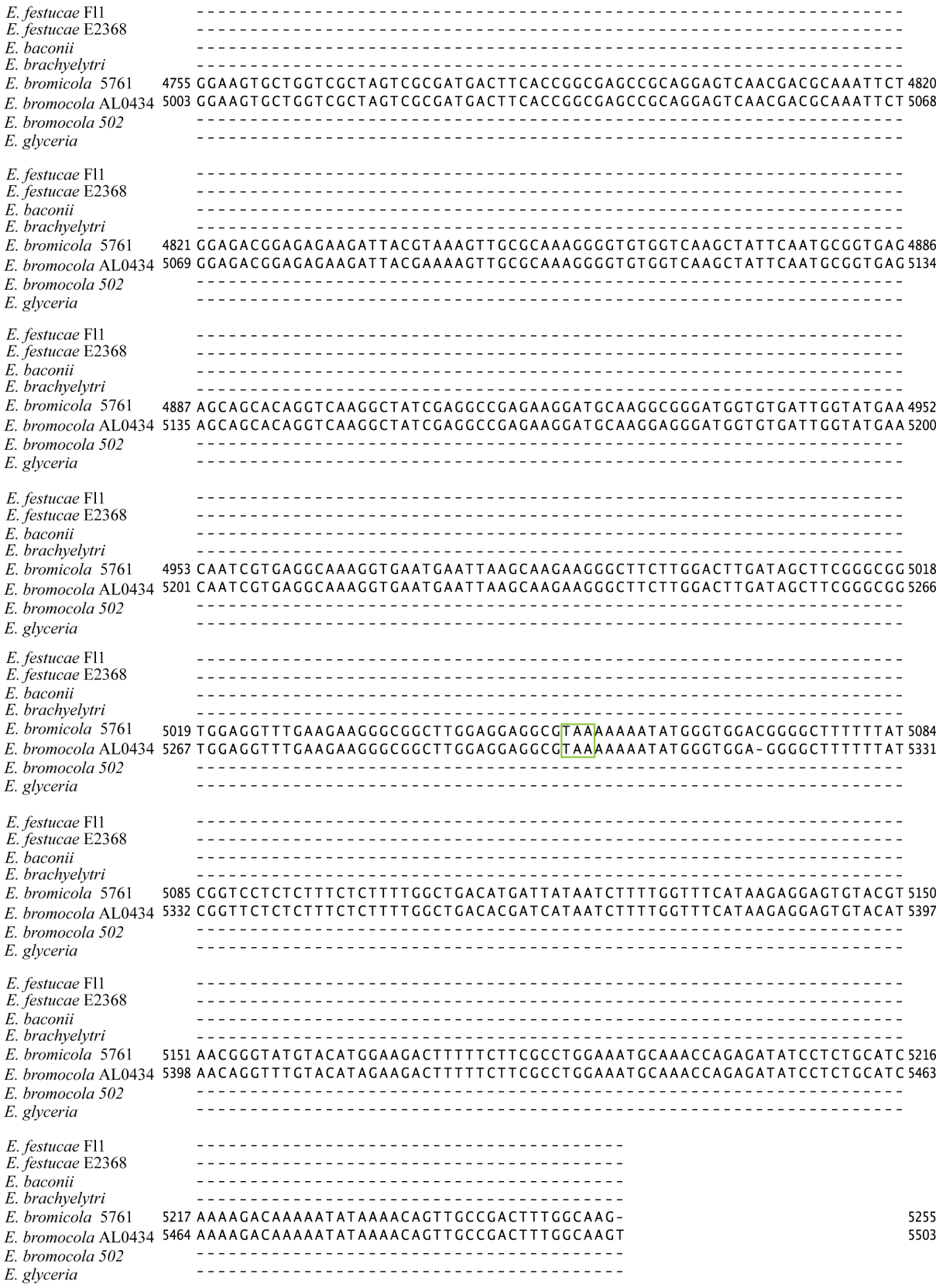

E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromicola 5761
E. bromocola AL0434
E. bromocola 502
E. glyceria


E. festucae Fl1 2914 AACTAGATGAGAGAAGTCTCGAGATTCGCGGCGCTCGGGACAACCAGCCGATGACGCTGTGTGATC 2979
E. festucae E2368 2911 AACTAGATGAGAGAAGTCTCGAGATTCGCGGCGCTCGGGACAACCAGCCGATGACGCTGTGTGATC 2976
E. baconii 2902 AGCTAGATGAGAGAGGTCTCGAGATTCGCGGCGCTCATGACAAGAAGCCAATGAAGCTGTGTGATA 2967
E. brachyelytri 2902 ACCTAGATGAGAGAGGTCTCGACATTCGCGGCGCTCATGCCAAGGAGCCAATGAAGCTGTGTGATA 2967
E. bromicola 5761 2674 ACCTAGATGAGAGACGTCTCGAGATTCGCGGTGCTCATGACAAGAAGCCGATGAAGCTGTGTGATA 2739
E. bromocola AL0434 2920 ACCTAGATGAGAGACGTCTCGAGATTCGCGGCGCTCATGACAAGAGGCCGATGAAGCTGTGTGATA 2985
E. bromocola 502
E. glyceria
E. festucae Fl1 2980 AGCTCAGGAAGAGCAACGTCAACCTATACTTCCAAGCTCACCCAGGTGGTTCAACCATCTTTAAAG 3045
E. festucae E2368 2977 AGCTCAGGAAGAGCAACGTCAACCTATACTTCCAAGCTCACCCAGGTGGTTCAACCATCTTTAAAG 3042
E. baconii 2968 AGCTCAGGAAGAGCGACGTCAACCTATACTTCCAAGCCCACCCAGGTGGTTCAACCATCTTTAAAG 3033
E. brachyelytri 2968 AGCTCAGGCAGAGCGACGTCAACCTATACTTCCAAGCCCACCCAGGTGGTTCAACCATCTTTAAAG 3033
E. bromicola 5761 2740 AGCTCAGGAAGAGCGACGTCAACCTATACTTCCAAGCCCATCCAGGTGGTTCAACCATCTTTAAAG 2805
E. bromocola AL0434 2986 AGCTCAGGAAGAGCGACGTCAACCTATACTTCCAAGCCCATCCAGGTGGTTCAACCATCTTTAAAG 3051
E. bromocola 502
E. glyceria 2505 ----------actttcttctccgaacct---------- 2522
E. festucae Fl1 3046 CCACACGTGCCGCCTACACAGCGGCACAGTTTGGAGTTTGCGCTGTCCCGGGCATTACTGCGTTGA 3111
E. festucae E2368 3043 CCACACGTGCCGCCTACACAGCGGCACAGTTTGGAGTTTGCGCTGTCCCGGGCATTACTGCGTTGA 3108
E. baconii 3034 CCACACGTGCCGCCTACACAGCGGCACAGTTGGGAGTTTGCGCTGTCCCAGGCATTACTGCGTTGA 3099
E. brachyelytri 3034 CCACACGTGCCGCCTACACAGCGGCACAGTTGGGAGTTTGCGCTGTCCCAGGCATTACTGCGTTGA 3099
E. bromicola 5761 2806 CCACGCGTGCCGCCTACACGGCGGCACAGTTTGGAGTTTGCGCTGTCCCAGGCATTACTGCGTTGA 2871
E. bromocola AL0434 3052 CCACGCGTGCCGCCTACACAGCGGCACAGTTTGGAGTTTGCGCTGCCCCAGGCATTACTACGTTGA 3117
E. bromocola 502
E. glyceria
E. festucae Fl1 3112 CTACCCTTGCTCCAAATGTCAATTGGGTTACCGAGCATGTCTTCGAAAAGCAAGAATTCCGTAATG 3177
E. festucae E2368 3109 CTACCCTTGCTCCAAATGTCAATTGGGTTACCGAGCATGTCTTCGAAAAGCAAGAATTCCGTAATG 3174
E. baconii 3100 CAAACCTTGCTTCATATGTCAATTGGGTTACCGAGCATGTCTTTGAAAAGCAAGAATTCCGTAATG 3165
E. brachyelytri 3100 CAAACCTTGCTTCATATGTTAGTTGGGTTACCGAGCATGTCTTTGAAAAGCAAGAATTCCGTAATG 3165
E. bromicola 5761 2872 AAACCCTTGCTTCATATGTTAATTGGGTTACCGAGCATGTCTTTGAAAAGCAAGAATTCCGTAATG 2937
E. bromocola AL0434 3118 AAACCCTTGCTTCATATGTTAATTGGGTTACCGAGCATGTCTTTGAAAAGCAAGAATACCGTAATG 3183
E. bromocola 502
E. glyceria
E. festucae Fl1 3178 CGATACAATCCATGATTGACGGAGTAACTGCTAAAGGCAATGCTCTTAAAGCCGGAGCGGTTCCTT 3243
E. festucae E2368 3175 CGATACAATCCATGATTGACGGAGTAACTGCTAAAGGCAATGCTCTTAAAGCCGGAGCGGTTCCTT 3240
E. baconii 3166 CGATACAATCCATGATTGACGGAGTAACTGCTAAAGGCAATGCTCTTAAAGCCGGAGCGGTTCCTT 3231
E. brachyelytri 3166 CGATACAATCCATGATTGACGGAGTAACTGCCAAGGGCAATGCTCTTAAAGCCGGAGCGGTTCCTT 3231
E. bromicola 5761 2938 CGATACAATCCATGATTGACGGAGTAACTGCTAAAGGCAATGCTCTCAAAGCCGGAGCGGTTCCGT 3003
E. bromocola AL0434 3184 CGGTACAATCCATGATTGACGGAGTGACTGCCAAAGGCAATGCTCTCAAAGCCGGAGCGGTTCCGT 3249
E. bromocola 502
E. glyceria 2523 ----------caatcctct----------ttcctt 2537
E. festucae Fl1 3244 TTTCCGTCTTCGACACCAATGGTgtaagttctgccgatactctggat--catacaaagtctg---- 3303
E. festucae E2368 3241 TTTCCGTCTTCGACACCAATGGTgtaagttctgccgatactctggat--catacaaagtctg---- 3300
E. baconii 3232 TTTCCGTCTTCGACACCAATGGTgtaagttctgccgatactctggat--tatataaagtatg---- 3291
E. brachyelytri 3232 TTTCCGTTTTCGACACCAATGGTgtaagttctgccaatactctggat--tgtataaagtctg---- 3291
E. bromicola 5761 3004 TTTCCGTTTTCAACGCCAATGGTgtaagttctgccaatactctgggttatatacaaagtctgtcat 3069
E. bromocola AL0434 3250 TTTCCGTTTTCGACGCCAATGGTgtaagttctgccaatactctgggt--tatacaaagtctgtcat 3313
E. bromocola 502
E. glyceria 2538 tttcctttt----------ctttttct--ttttggaaaacttg---- 2567
E. festucae Fl1 3304 ---tcatcaccgc-agtctact-----aatcatgtgcgaaactgcagCTTTTCCAGGCCAACTGGC 3360
E. festucae E2368 3301 ---tcatcaccgc-agtctact-----aatcatgtgcgaaactgcagCTTTTCCAGGCCAACTGGC 3357
E. baconii 3292 ---tcatcaccgcaagtctact-----aatcatgtgcgaaactgcagCTTTTCCAGACCAACTGGC 3349
E. brachyelytri 3292 ---tcatcaccac-agtctact-----aatcatgtgcgaaactgcagCTTTTCCAGACCAACTGGC 3348
E. bromicola 5761 3070 cagtcatcaccac-agtctacttgactaatcatgtgcgaaactgcagCTTTTCCAACAAAACTGGC 3134
E. bromocola AL0434 3314 cagtcatcaccac-agtctacttgactaatcatgtgcgaaactgcagCTTTTCCAATCCAAATGGC 3378
E. bromocola 502
E. glyceria 2568 ----------act----------catacgtaa-tgcagctctttcaacaaaactggc 2603
E. festucae Fl1 3361 CCCAGGGGTTTCCCCAATCGCTTTCATTCACTAAATCCTGGTCAGGCAATATCGCTGATTTCTTCA 3426
E. festucae E2368 3358 CCCAGGGGTTTCCCCAATCGCTTTCATTCACTAAATCCTGGTCAGGCAATATCGCTGATTTCTTCA 3423
E. baconii 3350 CCCAGGGGTTTCCCCAATCGCTTTCATTCACTAAATCCTGGTCAGGCAATATCGCTGATTTCTTCA 3415
E. brachyelytri 3349 CTCAGGGGTTTCCCCAATCGCTTTCATTCACTAAAACCTGGTCAGGCAATATCGCTGATTTCTTCA 3414
E. bromicola 5761 3135 CCCAGGGGTTTCCCCAATCGCTTCCATTCACTAAAACCTGGTCAGGCACCATCGCCGACTACTTCA 3200
E. bromocola AL0434 3379 CCCAGGGGTTTCCCCGATCGCTTCCATTCACTAAAACCTGGTCAGGCAGCATCGCCGACGACTTCA 3444
E. bromocola 502
E. glyceria 2604 cacggggtttacccag---gctgtaagtcgcaaagAGCTGGGCCGGCAACATCAACGATGCCTTCA 2666


E. festucae Fl1 3427 CCGGATTGCTGGGTCGCACGTCCGATCCTGGCCTGAAGAATAGTTATATTGCAAACTTCCAAGTCT 3492
E. festucae E2368 3424 CCGGATTGCTGGGGCGCACGTCCGATCCTGGCCTGAAGAATAGTTATATTGCAAACTTCCAAGTCT 3489
E. baconii 3416 CCGGATTGCTGGGTCGCACGTCCGATGCTGGCCTGAAGAATAGTTATATTGCGAACTTCCAAGTCT 3481
E. brachyelytri 3415 CCGGATTGCTGGGTCGCACGTCCGATGCTGGCCTGAAGAATAGTTATATTGCGAACTTCCAAGTCT 3480
E. bromicola 5761 3201 CCGGATTGCTGGGTCGCACGTCCGATGCCGGCCTGAAGAATAGTTATATAGCCAACTTCCAAGTCT 3266
E. bromocola AL0434 3445 CCGGATTGCTGGGTCGCACGTCCGATGCCGGCTTGAAGAATAGTTATATCGCCAACTTCCAAGTCT 3510
E. bromocola 502
E. glyceria 2667 CGGGCGTTCTCGGCCGCACCTGGGACGCCGGCGCAAACAACGCATTTGCCGTCAACTTGCAAGTCT 2732
E. festucae Fl1 3493 GTGATGCAGACTACAACGCGTACAAGGCTGCCATTGTAGCCGGTCATGACTTTATCTCGGACGCGC 3558
E. festucae E2368 3490 GTGATGCAGACTACAACGCGTACAAGGCTGCCATTGTAGCCGGTCATGACTTTATCTCGGACGCGC 3555
E. baconii 3482 GTGATGAAGACTACAACGCTTACAAGGCTACCATTGTAGCCGGGCGTGACTTCATCTCGGACGCGC 3547
E. brachyelytri 3481 GTGATAGTGACTACAACGCTTACAAGGCTACCATTGCAGCCGGGCATGACTTTATCTCGGACGCGC 3546
E. bromicola 5761 3267 GCGATGAGGACTACAACGCTTACAAGGCTACCATTGTAGCCGGGCGTGACTTTATCTCGGACGCGC 3332
E. bromocola AL0434 3511 GCGATGAGGACTACAACGCTTACAAGGCTACCATTGTAGCCGGGCGTGACTTTATCTCGGACGCGC 3576
E. bromocola 502
E. glyceria 2733 GCGACGCCGACTTCAATCTGCACAAGGAGCACATTGTCGCAGGAGTCAACCTCGTGTCCGACACGA 2798
E. festucae Fl1 3559 TTTGGAAGTCGACAACTGACCAGAAGGATCGCGTCGGCATGTTGACTGATGTTGTCGACACCTTGA 3624
E. festucae E2368 3556 TTTGGAAGTCGACAACTGACCAGAAGGATCGCGTCGGCATGCTGACTGATGTTGTCGACACCTTGA 3621
E. baconii 3548 TTTGGAAGGCGACAACTGACCAGGAGGAGCGCGTTGGCTTGCTGACTGATGTTGTCGACACCTTTA 3613
E. brachyelytri 3547 TTTGGAAGGCGACAACTGACCAGGAGGAGCGCGTTGGCTTGCTGACTGATGTTGTCGACACCTTTT 3612
E. bromicola 5761 3333 TTTGGAAGGCGACGACTGACCAGGAGGAGCGCGTTGGCTTGCTGACTGATGTTGTCGACACCTTTT 3398
E. bromocola AL0434 3577 TTTGGAAGGCGACGACTGACCAGGAGGAGCGCGTTGGCTTGCTGACCGATGTTGTCGACACCTTTT 3642
E. bromocola 502
E. glyceria 2799 TATGGAAGTCGTACACCGACCTCGAACAG---GTCGGCGTCCTGACCGATATAGTCGACACTTTCA 2861
E. festucae Fl1 3625 CATATCGCGGAGCTGACAAAGTTGTCACGTCTTTCAAGACTGCTTACGACAACTTGGTGCTCATGT 3690
E. festucae E2368 3622 CATATCGCGGAGCTGACAAAGTTGTCACGTCTTTCAAGACTGCTTACGACAACTTGGTGCTCATGT 3687
E. baconii 3614 CATATCGCGGAACTGCCAACGTTGTCACGTCTTTCAAGACTGTTTACGACAACTTGGTGCTCATGT 3679
E. brachyelytri 3613 CCTATCGCGGAACTGCCAAAGTTGTCACGTCTTTCAAGACTGTTTACGACAACTTGGTGCTCATGT 3678
E. bromicola 5761 3399 TATATCGCAGATCTGCCGACGTTGTCACGTCTTTCAAGACCGTTTACGACAACTTGGTGCTCATGT 3464
E. bromocola AL0434 3643 TATATCGCAGATCTGCCAACGTTGTCACGTCTTACAAGACCGTTTACGACAGCTTGGTGCTCATGT 3708
E. bromocola 502
E. glyceria 2862 AGTACCGTGGTGAAACTACCGTCGTTGCCTCTTACAGGGTGGCATATGACGGACTTGCCACGCTCT 2927
E. festucae Fl1 3691 GGGGAGATTTTGGCACGCTTTACAAACAAAACGGCGGCAACTACGACTTTGCCGCCGCCTTTAAGC 3756
E. festucae E2368 3688 GGGGAGATTTTGGCACGCTTTACAAACAAAACGGCGGCAACTACGACTTTGCCGCCGCCTTTAAGC 3753
E. baconii 3680 GGGGAGATTTCGGCACGCTTTACACTCAAAACGGCGGCAACTACGACTTTGCCGGCGCCTTTAAGC 3745
E. brachyelytri 3679 GGGGAGATTTTAGCACGCTTTACACTCAAAACGGCGGCAACTACGACTTTCCCGGCGCCTTTAAGC 3744
E. bromicola 5761 3465 GGGGAGATTTCAGCACGCTTTACACTCAAAACGGCGGCAGCTACGACTTTCCCGGCGCCTTCAAGC 3530
E. bromocola AL0434 3709 GGGGAGATTTCAGCACGCTTTACACTCAAAGCGGCGGCAGCTACGACTTTCCCGGCGCCTTCAAGC 3774
E. bromocola 502
E. glyceria 2928 GGGGCGACTTTGCGCAAGTGACTGCTGCTGCCAACATCCGATACGACTGGGTCGACGCATTCAAGC 2993
E. festucae Fl1 3757 AGGTGATTAGAGAGAATCTTGAGCATCAAGTTGAGAATGCTCAAGCAAGGTTTTTAACACTGCTGG 3822
E. festucae E2368 3754 AGGTGATTAGAGAGAATCTTGAGCATCAAGTTGAGAATGCTCAAGCAAGGTTTTTAACACTGCTGG 3819
E. baconii 3746 AGGCTATTAGAGAGAATCTTGAGCATCAAGTTGAGACTGCTCAAGCAACGTTTTTAACACTGCTGG 3811
E. brachyelytri 3745 AGGCTATTAGAGAGAATCTTGAGCATCAAATTGAGACTGCTCAAGCAACGTTTTTAACACTGCTGG 3810
E. bromicola 5761 3531 AGGTTATTAGAGAGAATCTTGAGCATCAAGTCGAGACTGCTCAAGCAACgttttttaacactgctag 3596
E. bromocola AL0434 3775 AGgttattagagagaatcttgagcatcaagtcgagactgctcaagcaacgttttttaacactgctgg 3840
E. bromocola 502
E. glyceria 2994 AAACCGTGGAGGATAATCTCGGACACCAGGTGGACACGGCCAAGACACGCTTCTTGGAGCTGGTGG 3059
E. festucae Fl1 3823 CC----------ACGTCTATCACGTATTGGAACTCCAACGCGGCCACCCAGAGATATGGTGCGCC 3877
E. festucae E2368 3820 CC----------ACGTCTATCACGTATTGGAACTCCAACGCGGCCACCCAGAGATATGGTGCGCC 3874
E. baconii 3812 CC----------ACGTCTATCACGTATTGGAACTCCAAGGCGGCCACCCAGAGATATGGTGCGCA 3866
E. brachyelytri 3811 CC----------ACGTCTATCACGTTTTGGAACTCCAATGCGGCCACCCAGAGATATGGTGCGCA 3865
E. bromicola 5761 3597 cc----------acgtccatcacgtattggaactccaatgcggccacccagagatatggtgcgaa 3651
E. bromocola AL0434 3841 cc----------acgtgcatcacgtattggaactccaatgcggccacccagagatatggtgcgca 3895
E. bromocola 502
E. glyceria 3060 CTGATTGTCTGAAATACGTCTGAAATACTGGAACTCCAACCAGGC----------TGGGATTCA 3113
E. festucae Fl1 3878 ATTCACTGCTGATCAGCAGAAGAAAT----------TAGCTGGGTTTCAGAAAGACGTTAAAAGT 3932
E. festucae E2368 3875 ATTCACTGCTGATCAGCAGAAGAAAT----------TAGCTGGGTTTCAGAAAGACGTTAAAAGT 3929
E. baconii 3867 ACTCGCTGCTGATCAGCAGAAGAAAT----------TAGCTGGGTTTCAGAAAGACGTTAAAAGT 3921
E. brachyelytri 3866 ATTCGCCTCTGATCAGCAGAAGAAAT----------TAGCTGGGTTTCAGAAAGACGTTAAAAGT 3920
E. bromicola 5761 3652 actcgctgctgatcagcggaagaaat----------tggatgggtttcagaaaaacgttaaaagt 3706
E. bromocola AL0434 3896 actcgctgctgatcagcggaagaaat----------tggatgggtttcagaaaaacgtcaaaagt 3950
E. bromocola 502
E. glyceria 3114 CCCCGCGACCA--TGGTAAAGGAAATGGTTGACAGGCTGACGGGCTTCCAGACCAATGCCGGTACT 3177

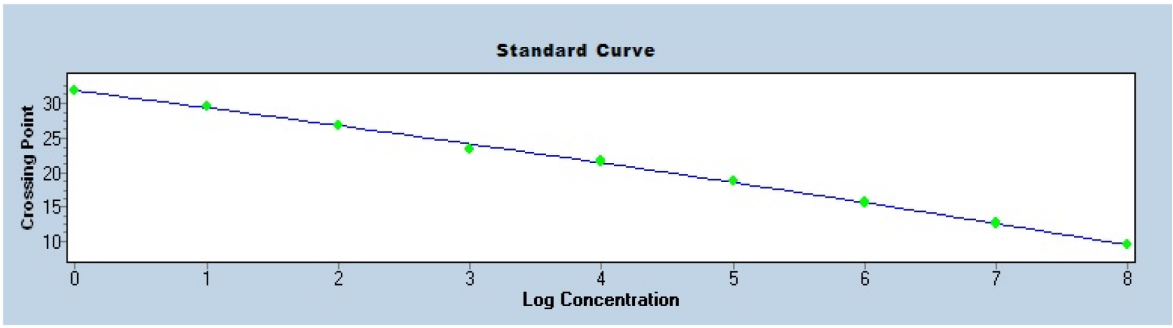

E. festucae Fl1 3933 CTGATTACGTTACCTATTTCTAAGATGGCTTCGCCTTAAAGAATGATGAGCGAGGGTCTATAGCCT 3998
E. festucae E2368 3930 CTGATTACGTTACCTATTTCTAAGATGGCTTCGCCTTAAAGAATGATGAGCGAGGGTCTATAGCCT 3995
E. baconii 3922 CTGATTACGTTACCTATTCCTAAGATGGCTCCGCCTTGAAGA---ATGAACGAGGGTCTATAGCCT 3984
E. brachyelytri 3921 CTGATTACGTTACCTATTCGTAGGATGGCTCCGCCTTAAAGA---ATTAACGAGGGTCTATAGCCT 3983
E. bromicola 5761 3707 ctgatcgcgttgcctatttctaatatggctccgtcttaaaga---attagcgagggtccatagccc 3769
E. bromocola AL0434 3951 ctgatcgcgttgcctatttctaatatggctccgccttaaaga---attagcgagggtccatagccc 4013
E. bromocola 502
E. glyceria 3178 CTGATTTCGCTTCCAATAGGGCAGATGGCACCGTAGATTAGA-TGATGCAT-----TCCTTGGGCA 3237
E. festucae Fl1 3999 GTCTTTAGCCAAATAGAATGACAGTAAACTTGCCGTGCTCGAATGTTGCCATCGTCCATCTAGTGA 4064
E. festucae E2368 3996 GTCTTTAGCCAAATAGAATGACAGTAAACTTGCCGTGCTCGAATGTAGCCATCGTCCATCTAGTGA 4061
E. baconii 3985 GTCTTTAGCCAAATAGAATGACAGTACG----------TGGCTATCTAGTAG 4026
E. brachyelytri 3984 GTGTTTAGCCAAATAGAATGACAGTAAACTTGCCGTGCTCGGATGTTGCTATCGTCCATCTAGTGA 4049
E. bromicola 5761 3770 atctttggccaaatagaataacggtaaacttgccgtgctcgaatgttgccatcgtccatctagtga 3835
E. bromocola AL0434 4014 atctttggccaaatagaataacggtaaacttgccgtgctcgaatgttgccatcgtccatctagtga 4079
E. bromocola 502
E. glyceria 3238 ATCCCAAGCC---------- 3247
E. festucae Fl1 4065 CTAGACCAGCACATCTGTCG--CTCTGTGAGCTTCTCTGCTGTCACAAAAGTACTCGAGGTTCTTA 4128
E. festucae E2368 4062 CTAGACCAGCACATCTGTCG--CTCTGTGAGCTTCTCTGCTGTCACAAAAGTACTCGAGGTTCTTA 4125
E. baconii 4027 CTAG----------CTAGCCTCTTTGTTATCC--AAAGGATCCAAGGTT---- 4063
E. brachyelytri 4050 CTAGACCAGCACATCTGTCG--CTCTATGAGCTTCTTTGCTGTTAT-AAAGTACTCGAGGTTCTTA 4112
E. bromicola 5761 3836 ctagaccagcacatctgtcg--ctctgtgagcttctttgctgttgt-aaagtactcgaggttctta 3898
E. bromocola AL0434 4080 ctagaccagcacatctgtcgctctctatgagcttctttgctgttgt-aaagtactcgaggttctta 4144
E. bromocola 502
E. glyceria 3248 ----------CAAGCCCTCGATG------ 3260
E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromicola 5761 3899 --cacggaatgcgtggtcaaaagcagcggcggggactgttgtcggttcaactccacaccgcctgcc 3962
E. bromocola AL0434 4145 gtcacggaatgcgtggtcaaaagcagcggcggggactgttgtcggttcaactccacaccgcctgcc 4210
E. bromocola 502
E. glyceria
E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromicola 5761 3963 cttggtccctccaatcgaagaaaaattacccggcagaattccataactttctctcactgcctccat 4028
E. bromocola AL0434 4211 cttggtccctccagtcgaagaaaaattacccggcagaattccataactttctctcactgcctccat 4276
E. bromocola 502
E. glyceria
E. festucae Fl1 4129 ----------GTCACGGA 4136
E. festucae E2368 4126 ----------GTCACGGA 4133
E. baconii 4064 ----------TTACCAA 4070
E. brachyelytri 4113 ----------GTCACGGA 4120
E. bromicola 5761 4029 ctacctctcaaagcaagaaacgctcagccaacaccaatgacttcaacaggcgaaacaagtcgccga 4094
E. bromocola AL0434 4277 ctacctctcaaagccagaaacgctcagccaagaccaatgacttcaacagGCGAAACAAGTCGCCGA 4342
E. bromocola 502
E. glyceria 3261 ----------GCAACGA 3267
E. festucae Fl1 4137 ATACACATAATAT---------- 4149
E. festucae E2368 4134 ATACACATAATAT---------- 4146
E. baconii 4071 AGCTCCATTATATGATCGC-------TGTAAGAACCCAGAAGGTGTGC---------- 4111
E. brachyelytri 4121 ATACACATAATAT---------- 4133
E. bromicola 5761 4095 aatcagataaaatcaatacaccacgatggctggccacaaaaagcgtgcatttgatgccaagcaaaa 4160
E. bromocola AL0434 4343 AATCAGATACAATAAATACACCACGATGGCTGGCCACAAAAAGCGTGCATTTGATGCCAAGCAAAA 4408
E. bromocola 502
E. glyceria 3268 ATCCATTCAAGATGG---------- 3282
E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromicola 5761 4161 tcggccgtcgaagaaacagaagcgacggcaacaatacccacagCGACTCTGGCAGCGACGACGATCA 4226
E. bromocola AL0434 4409 TCGGCCGTCGAAGAAACAGAAGCGACGGCAACAATACCACAGCGACTCTGGCAGCGACGACGATCA 4474
E. bromocola 502
E. glyceria


E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromicola 5761
E. bromocola AL0434
E. bromocola 502
E. glyceria
4112 TGGGATTCCCCAATAGA 4128
4227 AGAGCAGAGTTTCGATGCTGTCAACCTGCTAGACTCGGATGACGACGTTCACAATGCCACAGTAGA 4292
4475 AGAGCAGAGTTTCGATGCTGTCAACCTGCTAGACTCGGATGACGACGTTCAAAATGCCACAGTAGA 4540
4150 AACATGACAATTCTT 4164
4147 AACATGACAATTCTT 4161
4129 CCA AAAGATCCGTCAATGCTA 4149
4134 AACACAACAATTCTT 4148
4293 CGATGCGCCCGACCTAGACGACCACAACAATGACTCGTCGAGCTTGGAGGGCGAACACCCCGCCTT 4358
4541 CGATGCGCCCGACCTAGACGACCATAACAATGACTCGTCGAGCTTGGAGGGCGAACGCCCCGCCTT 4606
4165 GCCACCT 4171
4162 GCCACCT 4168
4150 GCCGCTGCTGA 4160
4149 GCCACCTTGAT 4159
4359 GATGAAGAAGACCAAGCAGACCAAGTCAAAGCCTGTCAAGGCCACCTCGAAACCTTCAGCGGCATT 4424
4607 GATGAAGAAGACCAAGCAGACCAAGTCAAAGCCTGTCAAGGCCACTTCGAAACCTTCAGCGGCATT 4672
4425 AGACGAGGAAGAAGGAGAGGAATATCGGAACGCCGACGAGGAGGGTGAGGAAGAGGAAGAGGAATC 4490
4673 AGATGAGGAAGAAGGAGAGGAAGATCGGAACGCCGACGAGGAGGGCGAGGAAGAGGAAGAGGAATC 4738
4491 AGACGTGGACGAATACGATTTAGATTCAGACAATGGCGGACGCAAGGCCAAGTCCAAACGAAACGA 4556
4739 AGACGTGGACGAATACGATTTAGATTCAGACAATGGCGGACGCAAGGCCAAGTCCAAACGAAACGA 4804
4557 CCCCAGCGCATTCGCCACGTCCATTTCCAAAATTCTGTCCGCCAAGCTTTCTACCTCAAAACGCTC 4622
4805 CCCCAGCGCATTCGCCACGTCCATTTCCAAAATTCTGTCCACCAAGCTTTCTACCTCAAAACGCTC 4870
4623 TGATCCCGTTCTTTCAAGAAGTGCAGCCGCTCACGAGGCGTCCAAAGCAGCCGTCGATAACGCATT 4688
4871 TGATCCCGTTCTTTCAAGAAGTGCAGCCGCTCACGAGGCGTCCAAAGCAGCCGTCGATAACGCATT 4936
4689 GGAAGCCAAAGCCAAGCGACACATGAAAGAACAAAAGAAGAAGGCTCTCGAGAAGGGTCGCATCAG 4754
4937 GGAAGCCAAAGCTAAGCGACACATGAAAGAACAAAAGAAGAAGGCTCTCGAGAAGGGTCGCATCAG 5002

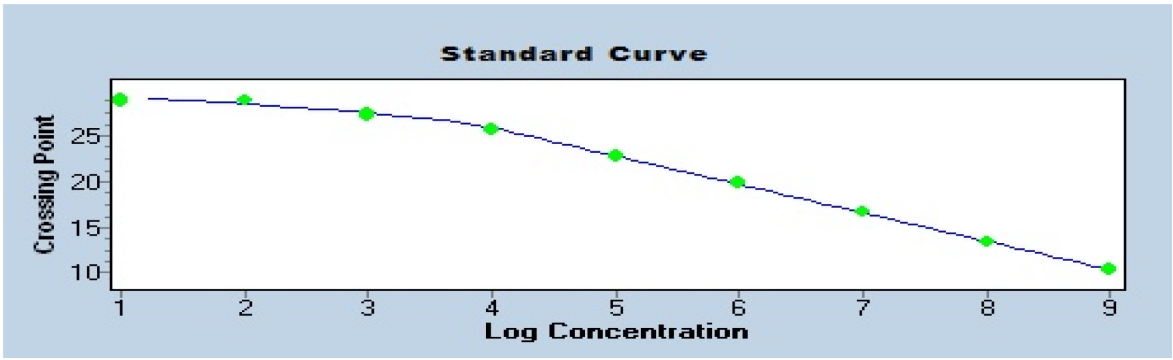

E. festucae Fl1
E. festucae E2368
E. baconii
E. brachyelytri
E. bromicola 5761 4755 GGAAGTGCTGGTCGCTAGTCGCGATGACTTCACCGGCGAGCCGCAGGAGTCAACGACGCAAATTCT 4820
E. bromocola AL0434 5003 GGAAGTGCTGGTCGCTAGTCGCGATGACTTCACCGGCGAGCCGCAGGAGTCAACGACGCAAATTCT 5068
E. bromocola 502
E. glyceria
E. bromicola 5761 4821 GGAGACGGAGAGAAGATTACGTAAAGTTGCGCAAAGGGGTGTGGTCAAGCTATTCAATGCGGTGAG 4886
E. bromocola AL0434 5069 GGAGACGGAGAGAAGATTACGAAAAGTTGCGCAAAGGGGTGTGGTCAAGCTATTCAATGCGGTGAG 5134
E. bromicola 5761 4887 AGCAGCACAGGTCAAGGCTATCGAGGCCGAGAAGGATGCAAGGCGGGATGGTGTGATTGGTATGAA 4952
E. bromocola AL0434 5135 AGCAGCACAGGTCAAGGCTATCGAGGCCGAGAAGGATGCAAGGAGGGATGGTGTGATTGGTATGAA 5200
E. bromicola 5761 4953 CAATCGTGAGGCAAAGGTGAATGAATTAAGCAAGAAGGGCTTCTTGGACTTGATAGCTTCGGGCGG 5018
E. bromocola AL0434 5201 CAATCGTGAGGCAAAGGTGAATGAATTAAGCAAGAAGGGCTTCTTGGACTTGATAGCTTCGGGCGG 5266
E. bromicola 5761 5019 TGGAGGTTTGAAGAAGGGCGGCTTGGAGGAGGCGTAAAAAAATATGGGTGGACGGGGCTTTTTTAT 5084
E. bromocola AL0434 5267 TGGAGGTTTGAAGAAGGGCGGCTTGGAGGAGGCGTAAAAAAATATGGGTGGA-GGGGCTTTTTTAT 5331
E. bromicola 5761 5085 CGGTCCTCTCTTTCTCTTTTGGCTGACATGATTATAATCTTTTGGTTTCATAAGAGGAGTGTACGT 5150
E. bromocola AL0434 5332 CGGTTCTCTCTTTCTCTTTTGGCTGACACGATCATAATCTTTTGGTTTCATAAGAGGAGTGTACAT 5397
E. bromicola 5761 5151 AACGGGTATGTACATGGAAGACTTTTTCTTCGCCTGGAAATGCAAACCAGAGATATCCTCTGCATC 5216
E. bromocola AL0434 5398 AACAGGTTTGTACATAGAAGACTTTTTCTTCGCCTGGAAATGCAAACCAGAGATATCCTCTGCATC 5463
E. bromicola 5761 5217 AAAAGACAAAAATATAAAACAGTTGCCGACTTTGGCAAG- 5255
E. bromocola AL0434 5464 AAAAGACAAAAATATAAAACAGTTGCCGACTTTGGCAAGT 5503

Appendix 20. Standard curve for *cdaB* (A) and *cdaC* (B).

A



B



Appendix 18. Genes encoding chitinase (GH18) in *E. festucae* Fl1 genome.

	Gene model	Protein name	Homologues	SP	GH 18 catalytic domain	CB domain	Divergently transcribed with a *lysM* gene
1	EfM3.061270			-	1	-	-
2	EfM3.024080			-	1	-	-
3	EfM3.020470			-	1	-	-
4	EfM3.062870		TAC6 (*T.atrivirdae*)	+	1	2	EfM3.0062860
5	EfM3.056280			-	1	-	-
6	EfM3.065380			-	1	-	-
7	EfM3.031780			-	1	-	-
8	EfM3.064090			+	1	-	-
9	EfM3.074100			+	1	-	-
10	EfM3.000810	ChiA		+	1	2	*lymA*
11	EfM3.024310			+	1	-	-
12	EfM3.035030	ChiB		+	1	2	*lymB*
13	EfM3.078090			+	1	2	EfM3.078100

Appendix 19. Differences in expression of genes encoding chitinase proteins (GH18) in *E. festucae* Fl1 genome between wild type and symbiotic mutants.

	Gene Model	Gene Name	*noxA* dir [b]	*noxA* Fold [a]	*proA* dir [b]	*proA* Fold [a]	*sakA* dir [b]	*sakA* Fold [a]
1	EfM3.061270		+	1.85	+	1.99	NA	NA
2	EfM3.024080		+	1.58	+	1.34	+	2.43
3	EfM3.020470		-	5.4	-	2.77	+	1.26
4	EfM3.062870		-	1.17	+	1.16	+	2.27
5	EfM3.056280		+	1.16	+	2.6	+	5.17
6	EfM3.065380		+	1.24	+	1.48	+	2.36
7	EfM3.031780		-	1.73	+	1.08	+	1.65
8	EfM3.064090		-	1.26	-	1.27	+	3.28
9	EfM3.074100		-	1.89	-	1.49	+	1.51
10	EfM3.000810	*chiA*	-	3.98	-	3.82	-	2.88
11	EfM3.024310		+	1.36	+	1.62	+	1.84
12	EfM3.035030	*chiB*	-	10.8	-	3.69	-	1.59
13	EfM3.078090		+	1.34	+	1.7	+	2.46

[a] Fold changes in red and green are significant whereas those in black are not significant.

[b] Direction indicates the level of expression change. Minus and plus indicate the direction of change (down regulated and up regulated respectively).

Data obtained from Eaton *et al*, 2015.

Appendix 20. Differences in expression of genes encoding chitinase proteins (GH18) in *E. festucae* Fl1 genome, *in planta* versus in culture.

	Gene Model	Gene Name	Culture_RPKM [a]	*InPlanta*_RPKM [a]	Ratio	Direction	Fold Diff
1	EfM3.061270		0.136	0.138	1.01	+	1.01
2	EfM3.024080		19.502	38.92	1.99	+	1.99
3	EfM3.020470		6.252	3.428	0.548	-	1.82
4	EfM3.062870		2.833	1.202	0.424	-	2.35
5	EfM3.056280		29.580	6.318	0.213	-	4.68
6	EfM3.065380		83.730	19.036	0.227	-	4.398
7	EfM3.031780		0.747	15.477	20.70	+	20.70
8	EfM3.064090		8.038	5.247	0.652	-	1.53
9	EfM3.074100		3.754	4.643	1.236	+	1.236
10	EfM3.000810	*chiA*	4.373	143.571	32.83	+	32.83
11	EfM3.024310		1387.39	4424.98	3.189	+	3.189
12	EfM3.035030	*chiB*	0.984	2.485	2.623	+	2.623
13	EfM3.078090		1.890	2.318	1.226	+	1.226

[a] Data obtained from T.Chujo (in preparation). Direction indicates the level of expression change. Minus and plus indicate the direction of change (down regulated and up regulated respectively). RPKM: Reads Per Kilobase of transcript per Million mapped reads.

Chapter 11. References

Adams, D.J., (2004) Fungal cell wall chitinases and glucanases. *Microbiology* **150**: 2029-2035.

Ariyawansa, K.G.S.U., (2015) Sensing and Signalling Mechanical Stress During Intercalary Growth in Epichloë Grass Endophytes. *Massey University*.

Baker, L.G., C.A. Specht, M.J. Donlin & J.K. Lodge, (2007) Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in *Cryptococcus neoformans*. *Eukaryot Cell* **6**: 855-867.

Baker, L.G., C.A. Specht & J.K. Lodge, (2011) Cell wall chitosan is necessary for virulence in the opportunistic pathogen *Cryptococcus neoformans*. *Eukaryot Cell* **10**: 1264-1268.

Banks, I.R., C.A. Specht, M.J. Donlin, K.J. Gerik, S.M. Levitz & J.K. Lodge, (2005) A chitin synthase and its regulator protein are critical for chitosan production and growth of the fungal pathogen *Cryptococcus neoformans*. *Eukaryot Cell* **4**: 1902-1912.

Becker, M., Y. Becker, K. Green & B. Scott, (2016) The endophytic symbiont *Epichloë festucae* establishes an epiphyllous net on the surface of *Lolium perenne* leaves by development of an expressorium, an appressorium-like leaf exit structure. *New Phytol* **211**: 240-254.

Becker, Y., C.J. Eaton, E. Brasell, K.J. May, M. Becker, B. Hassing, G.M. Cartwright, L. Reinhold & B. Scott, (2015) The Fungal Cell-Wall Integrity MAPK Cascade Is Crucial for Hyphal Network Formation and Maintenance of Restrictive Growth of *Epichloë festucae* in Symbiosis With *Lolium perenne*. *Molecular plant-microbe interactions : MPMI* **28**: 69-85.

Bernard, M. & J.P. Latge, (2001) *Aspergillus fumigatus* cell wall: composition and biosynthesis. *Med Mycol* **39 Suppl 1**: 9-17.

Blair, D.E., O. Hekmat, A.W. Schuttelkopf, B. Shrestha, K. Tokuyasu, S.G. Withers & D.M. van Aalten, (2006) Structure and mechanism of chitin deacetylase from the fungal pathogen *Colletotrichum lindemuthianum*. *Biochemistry* **45**: 9416-9426.

Bolton, M.D., H.P. van Esse, J.H. Vossen, R. de Jonge, I. Stergiopoulos, I.J. Stulemeijer, G.C. van den Berg, O. Borras-Hidalgo, H.L. Dekker, C.G. de Koster, P.J. de Wit, M.H. Joosten & B.P. Thomma, (2008) The novel *Cladosporium fulvum* lysin motif effector Ecp6 is a virulence factor with orthologues in other fungal species. *Molecular microbiology* **69**: 119-136.

Borkovich, K.A., L.A. Alex, O. Yarden, M. Freitag, G.E. Turner, N.D. Read, S. Seiler, D. Bell-Pedersen, J. Paietta, N. Plesofsky, M. Plamann, M. Goodrich-Tanrikulu, U. Schulte, G. Mannhaupt, F.E. Nargang, A. Radford, C. Selitrennikoff, J.E. Galagan, J.C. Dunlap, J.J. Loros, D. Catcheside, H. Inoue, R. Aramayo, M. Polymenis, E.U. Selker, M.S. Sachs, G.A. Marzluf, I. Paulsen, R. Davis, D.J. Ebbole, A. Zelter, E.R. Kalkman, R. O'Rourke, F. Bowring, J. Yeadon, C. Ishii, K. Suzuki, W. Sakai & R. Pratt, (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. *Microbiol Mol Biol Rev* **68**: 1-108.

Bowman, S.M. & S.J. Free, (2006) The structure and synthesis of the fungal cell wall. *Bioessays* **28**: 799-808.

Bowman, S.M., A. Piwowar, M. Al Dabbous, J. Vierula & S.J. Free, (2006) Mutational analysis of the glycosylphosphatidylinositol (GPI) anchor pathway demonstrates that GPI-anchored proteins are required for cell wall biogenesis and normal hyphal growth in *Neurospora crassa*. *Eukaryot Cell* **5**: 587-600.

Bultman, T.L., J.F. White, T.I. Bowdish & A.M. Welch, (1998) A new kind of mutualism between fungi and insects. *Mycol Res* **102**: 235-238.

Christensen, M. & C.R. Voissey, (2007) The biology of the endophyte/grass partnership *In Popay AJ, editor; , Thom ER, editor. , eds. Proceedings of the 6th International Symposium on Fungal Endophytes of Grasses. Grasslands Research and Practice Series No. 13. Christchurch, New Zealand: New Zealand Grassland Association*: 123-133.

Christensen, M.J., O.J.P. Ball, R.J. Bennett & C.L. Schardl, (1997) Fungal and host genotype effects on compatibility and vascular colonization by *Epichloë festucae*. *Mycol Res* **101**: 493-501.

Christensen, M.J., R.J. Bennett, H.A. Ansari, H. Koga, R.D. Johnson, G.T. Bryan, W.R. Simpson, J.P. Koolaard, E.M. Nickless & C.R. Voisey, (2008) Epichloë endophytes grow by intercalary hyphal extension in elongating grass leaves. *Fungal Genet Biol* **45**: 84-93.

Christensen, M.J., R.J. Bennett & J. Schmid, (2002) Growth of Epichloe/Neotyphodium and p-endophytes in leaves of Lolium and Festuca grasses. *Mycol Res* **106**: 93-106.

Christensen, M.J. & G.C.M. Latch, (1991) Variation among Isolates of Acremonium Endophytes (a-Coenophialum and Possibly a-Typhinum) from *Tall fescue* (*Festuca arundinacea*). *Mycol Res* **95**: 1123-1126.

Christensen, M.J., A. Leuchtmann, D.D. Rowan & B.A. Tapper, (1993) Taxonomy of Acremonium Endophytes of *Tall fescue* (Festuca-Arundinacea), *Meadow fescue* (*F. Pratensis*) and Perennial Rye-Grass (*Lolium. perenne*). *Mycol Res* **97**: 1083-1092.

Christodoulidou, A., V. Bouriotis & G. Thireos, (1996) Two sporulation-specific chitin deacetylase-encoding genes are required for the ascospore wall rigidity of *Saccharomyces cerevisiae*. *J Biol Chem* **271**: 31420-31425.

Christodoulidou, A., P. Briza, A. Ellinger & V. Bouriotis, (1999) Yeast ascospore wall assembly requires two chitin deacetylase isozymes. *FEBS Lett* **460**: 275-279.

Chung, K.R., W. Hollin, M.R. Siegel & C.L. Schardl, (1997) Genetics of Host Specificity in *Epichloe typhina*. *Phytopathology* **87**: 599-605.

Clay, K. & C. Schardl, (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am Nat* **160 Suppl 4**: S99-S127.

de Jonge, R. & B.P.H.J. Thomma, (2009) Fungal LysM effectors: extinguishers of host immunity? *Trends Microbiol* **17**: 151-157.

de Jonge, R., H.P. van Esse, A. Kombrink, T. Shinya, Y. Desaki, R. Bours, S. van der Krol, N. Shibuya, M.H. Joosten & B.P. Thomma, (2010) Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. *Science* **329**: 953-955.

de Wit, P.J., A. van der Burgt, B. Okmen, I. Stergiopoulos, K.A. Abd-Elsalam, A.L. Aerts, A.H. Bahkali, H.G. Beenen, P. Chettri, M.P. Cox, E. Datema, R.P. de Vries, B. Dhillon, A.R. Ganley, S.A. Griffiths, Y. Guo, R.C. Hamelin, B. Henrissat, M.S. Kabir, M.K. Jashni, G. Kema, S. Klaubauf, A. Lapidus, A. Levasseur, E. Lindquist, R. Mehrabi, R.A. Ohm, T.J. Owen, A. Salamov, A. Schwelm, E. Schijlen, H. Sun, H.A. van den Burg, R.C. van Ham, S. Zhang, S.B. Goodwin, I.V. Grigoriev, J. Collemare & R.E. Bradshaw, (2012) The genomes of the fungal plant pathogens *Cladosporium fulvum* and *Dothistroma septosporum* reveal adaptation to different hosts and lifestyles but also signatures of common ancestry. *PLoS genetics* **8**: e1003088.

Deising, H. & J. Siegrist, (1995) Chitin Deacetylase Activity of the Rust *Uromyces. viciae fabae* Is Controlled by Fungal Morphogenesis. *Fems Microbiol Lett* **127**: 207-211.

Dodds, P.N. & J.P. Rathjen, (2010) Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat Rev Genet* **11**: 539-548.

Eaton, C.J., M.P. Cox, B. Ambrose, M. Becker, U. Hesse, C.L. Schardl & B. Scott, (2010) Disruption of signaling in a fungal-grass symbiosis leads to pathogenesis. *Plant Physiol* **153**: 1780-1794.

Eaton, C.J., P.Y. Dupont, P. Solomon, W. Clayton, B. Scott & M.P. Cox, (2015) A Core Gene Set Describes the Molecular Basis of Mutualism and Antagonism in *Epichloe spp*. *Molecular plant-microbe interactions : MPMI* **28**: 218-231.

Eisenhaber, B., S. Maurer-Stroh, M. Novatchkova, G. Schneider & F. Eisenhaber, (2003) Enzymes and auxiliary factors for GPI lipid anchor biosynthesis and post-translational transfer to proteins. *Bioessays* **25**: 367-385.

El Gueddari, N.E., U. Rauchhaus, B.M. Moerschbacher & H.B. Deising, (2002) Developmentally regulated conversion of surface-exposed chitin to chitosan in cell walls of plant pathogenic fungi. *New Phytol* **156**: 103-112.

Erwig, L.P. & N.A. Gow, (2016) Interactions of fungal pathogens with phagocytes. *Nat Rev Microbiol* **14**: 163-176.

Fleet, G.H., (1991) Cell walls. *In Rose AH, Harrison JS. ed; The Yeasts. 2nd edn* **4**: 199-277.

Fontaine, T., C. Simenel, G. Dubreucq, O. Adam, M. Delepierre, J. Lemoine, C.E. Vorgias, M. Diaquin & J.P. Latge, (2000) Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. *J Biol Chem* **275**: 27594-27607.

Fuenzalida, J.P., T. Weikert, S. Hoffmann, C. Vila-Sanjurjo, B.M. Moerschbacher, F.M. Goycoolea & S. Kolkenbrock, (2014) Affinity protein-based FRET tools for cellular tracking of chitosan nanoparticles and determination of the polymer degree of acetylation. *Biomacromolecules* **15**: 2532-2539.

Fujikawa, T., Y. Kuga, S. Yano, A. Yoshimi, T. Tachiki, K. Abe & M. Nishimura, (2009) Dynamics of cell wall components of *Magnaporthe grisea* during infectious structure development. *Molecular microbiology* **73**: 553-570.

Fujikawa, T., A. Sakaguchi, Y. Nishizawa, Y. Kouzai, E. Minami, S. Yano, H. Koga, T. Meshi & M. Nishimura, (2012) Surface alpha-1,3-glucan facilitates fungal stealth infection by interfering with innate immunity in plants. *Plos Pathog* **8**: e1002882.

Geoghegan, I., G. Steinberg & S. Gurr, (2017) The Role of the Fungal Cell Wall in the Infection of Plants. *Trends Microbiol* **25**: 957-967.

Geoghegan, I.A. & S.J. Gurr, (2016) Chitosan Mediates Germling Adhesion in *Magnaporthe oryzae* and is required for surface sensing and germling morphogenesis. *Plos Pathog* **12**: e1005703.

Geoghegan, I.A. & S.J. Gurr, (2017) Investigating chitin deacetylation and chitosan hydrolysis during vegetative growth in *Magnaporthe oryzae*. *Cell Microbiol* **19**.

Gibson, D.G., L. Young, R.Y. Chuang, J.C. Venter, C.A. Hutchison, 3rd & H.O. Smith, (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature methods* **6**: 343-345.

Gow, N.A.R., J.P. Latge & C.A. Munro, (2017) The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiol Spectr* **5**.

Green, K.A., Y. Becker, H.L. Fitzsimons & B. Scott, (2016) An *Epichloë festucae* homologue of MOB3, a component of the STRIPAK complex, is required for the establishment of a mutualistic symbiotic interaction with *Lolium perenne*. *Mol Plant Pathol* **17**: 1480-1492.

Green, K.A., Y. Becker, A. Tanaka, D. Takemoto, H.L. Fitzsimons, S. Seiler, H. Lalucque, P. Silar & B. Scott, (2017) SymB and SymC, two membrane associated proteins, are required for *Epichloë festucae* hyphal cell-cell fusion and maintenance of a mutualistic interaction with *Lolium perenne*. *Molecular microbiology* **103**: 657-677.

Gruber, S., G. Vaaje-Kolstad, F. Matarese, R. Lopez-Mondejar, C.P. Kubicek & V. Seidl-Seiboth, (2011) Analysis of subgroup C of fungal chitinases containing chitin-binding and LysM modules in the mycoparasite *Trichoderma atroviride*. *Glycobiology* **21**: 122-133.

Grun, C.H., F. Hochstenbach, B.M. Humbel, A.J. Verkleij, J.H. Sietsma, F.M. Klis, J.P. Kamerling & J.F. Vliegenthart, (2005) The structure of cell wall alpha-glucan from fission yeast. *Glycobiology* **15**: 245-257.

Hekmat, O., K. Tokuyasu & S.G. Withers, (2003) Subsite structure of the endo-type chitin deacetylase from a deuteromycete, *Colletotrichum lindemuthianum*: an investigation using steady-state kinetic analysis and MS. *The Biochemical journal* **374**: 369-380.

Hinton, D.M. & C.W. Bacon, (1985) The distribution and ultrastructure of the endophyte of toxic *Tall fescue*. *Canadian Journal of Botany-Revue Canadienne De Botanique* **63**: 36-42.

Hopke, A., A.J.P. Brown, R.A. Hall & R.T. Wheeler, (2018) Dynamic fungal cell wall architecture in stress adaptation and immune evasion. *Trends Microbiol* **26**: 284-295.

Jashni, M.K., I.H. Dols, Y. Iida, S. Boeren, H.G. Beenen, R. Mehrabi, J. Collemare & P.J. de Wit, (2015) Synergistic action of a metalloprotease and a serine protease from *Fusarium oxysporum f. sp. lycopersici* cleaves chitin-binding tomato chitinases, reduces their antifungal activity, and enhances fungal virulence. *Molecular plant-microbe interactions : MPMI* **28**: 996-1008.

Jones, J.D. & J.L. Dangl, (2006) The plant immune system. *Nature* **444**: 323-329.

Kamakura, T., S. Yamaguchi, K. Saitoh, T. Teraoka & I. Yamaguchi, (2002) A novel gene, CBP1, encoding a putative extracellular chitin-binding protein, may play an important role in the hydrophobic surface sensing of *Magnaporthe grisea* during appressorium differentiation. *Molecular plant-microbe interactions : MPMI* **15**: 437-444.

Katoh, K., K. Misawa, K. Kuma & T. Miyata, (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* **30**: 3059-3066.

Katoh, K. & D.M. Standley, (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* **30**: 772-780.

Kayano, Y., A. Tanaka & D. Takemoto, (2018) Two closely related Rho GTPases, Cdc42 and RacA, of the endophytic fungus *Epichloë festucae* have contrasting roles for ROS production and symbiotic infection synchronized with the host plant. *Plos Pathog* **14**: e1006840.

Kinoshita, T. & N. Inoue, (2000) Dissecting and manipulating the pathway for glycosylphosphatidylinositol-anchor biosynthesis. *Curr Opin Chem Biol* **4**: 632-638.

Kollar, R., B.B. Reinhold, E. Petrakova, H.J. Yeh, G. Ashwell, J. Drgonova, J.C. Kapteyn, F.M. Klis & E. Cabib, (1997) Architecture of the yeast cell wall. ß (1,6) glucan interconnects mannoprotein, ß (1,3) glucan, and chitin. *J Biol Chem* **272**: 17762-17775.

Kombrink, A. & B.P. Thomma, (2013) LysM effectors: secreted proteins supporting fungal life. *Plos Pathog* **9**: e1003769.

Krogh, A., Larsson, B., von Heiine, G. and Sonnhammer, E. L. (2001) Predicting transmembrane protein topology with a hidden Markov model application to complete genomes. *J. Mol. Biol*., **305**: 567-580.

Kuroki, M., K. Okauchi, S. Yoshida, Y. Ohno, S. Murata, Y. Nakajima, A. Nozaka, N. Tanaka, M. Nakajima, H. Taguchi, K.I. Saitoh, T. Teraoka, M. Narukawa & T. Kamakura, (2017) Chitin-deacetylase activity induces appressorium differentiation in the rice blast fungus *Magnaporthe oryzae*. *Sci Rep* **7**: 9697.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson & D.G. Higgins, (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947-2948.

Latge, J.P., (2007) The cell wall: a carbohydrate armour for the fungal cell. *Molecular microbiology* **66**: 279-290.

Latge, J.P., (2010) Tasting the fungal cell wall. *Cell Microbiol* **12**: 863-872.

Leuchtmann, A., C.W. Bacon, C.L. Schardl, J.F. White, Jr. & M. Tadych, (2014) Nomenclatural realignment of Neotyphodium species with genus Epicholë. *Mycologia* **106**: 202-215.

Levin, E., A.R. Ballester, G. Raphael, O. Feigenberg, Y. Liu, J. Norelli, L. Gonzalez-Candelas, J. Ma, C. Dardick, M. Wisniewski & S. Droby, (2017) Identification and characterization of LysM effectors in *Penicillium expansum*. *PLoS One* **12**: e0186023.

Li, H.M., R. Sullivan, M. Moy, D.Y. Kobayashi & F.C. Belanger, (2004) Expression of a novel chitinase by the fungal endophyte in *Poa ampla*. *Mycologia* **96**: 526-536.

Lickfeld, M. & H.P. Schmitz, (2012) A network involving Rho-type GTPases, a paxillin and a formin homologue regulates spore length and spore wall integrity in the filamentous fungus *Ashbya gossypii*. *Molecular microbiology* **85**: 574-593.

Liu, T., Z. Liu, C. Song, Y. Hu, Z. Han, J. She, F. Fan, J. Wang, C. Jin, J. Chang, J.M. Zhou & J. Chai, (2012) Chitin-induced dimerization activates a plant immune receptor. *Science* **336**: 1160-1164.

Livak, K.J. & T.D. Schmittgen, (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 (ΔΔ C(T)) Method. *Methods* **25**: 402-408.

Lo Presti, L., D. Lanver, G. Schweizer, S. Tanaka, L. Liang, M. Tollot, A. Zuccaro, S. Reissmann & R. Kahmann, (2015) Fungal effectors and plant susceptibility. *Annu Rev Plant Biol* **66**: 513-545.

Ma, L.S., L. Wang, C. Trippel, A. Mendoza-Mendoza, S. Ullmann, M. Moretti, A. Carsten, J. Kahnt, S. Reissmann, B. Zechmann, G. Bange & R. Kahmann, (2018) The *Ustilago maydis* repetitive effector Rsp3 blocks the antifungal activity of mannose-binding maize proteins. *Nat Commun* **9**: 1711.

Marshall, R., A. Kombrink, J. Motteram, E. Loza-Reyes, J. Lucas, K.E. Hammond-Kosack, B.P. Thomma & J.J. Rudd, (2011) Analysis of two in planta expressed LysM effector homologs from the fungus *Mycosphaerella graminicola* reveals novel functional properties and varying contributions to virulence on wheat. *Plant Physiol* **156**: 756-769.

May, K.J., M.K. Bryant, X. Zhang, B. Ambrose & B. Scott, (2008) Patterns of expression of a lolitrem biosynthetic gene in the *Epichloë festucae*-perennial ryegrass symbiosis. *Molecular plant-microbe interactions : MPMI* **21**: 188-197.

Mentlak, T.A., A. Kombrink, T. Shinya, L.S. Ryder, I. Otomo, H. Saitoh, R. Terauchi, Y. Nishizawa, N. Shibuya, B.P.H.J. Thomma & N.J. Talbot, (2012) Effector-Mediated Suppression of Chitin-Triggered Immunity by *Magnaporthe oryzae* Is Necessary for Rice Blast Disease. *Plant Cell* **24**: 322-335.

Mishra, C., C.E. Semino, K.J. McCreath, H. de la Vega, B.J. Jones, C.A. Specht & P.W. Robbins, (1997) Cloning and expression of two chitin deacetylase genes of *Saccharomyces cerevisiae*. *Yeast* **13**: 327-336.

Moy, M., F. Belanger, R. Duncan, A. Freehoff, C. Leary, W. Meyer, R. Sullivan & J.F. White, (2000) Identification of epiphyllous mycelial nets on leaves of grasses infected by clavicipitaceous endophytes. *Symbiosis* **28**: 291-302.

Mravec, J., S.K. Kracun, M.G. Rydahl, B. Westereng, F. Miart, M.H. Clausen, J.U. Fangel, M. Daugaard, P. Van Cutsem, H.H. De Fine Licht, H. Hofte, F.G. Malinovsky, D.S. Domozych & W.G. Willats, (2014) Tracking developmentally regulated post-synthetic processing of homogalacturonan and chitin using reciprocal oligosaccharide probes. *Development* **141**: 4841-4850.

Nampally, M., B.M. Moerschbacher & S. Kolkenbrock, (2012) Fusion of a novel genetically engineered chitosan affinity protein and green fluorescent protein for specific detection of chitosan *in vitro* and *in situ*. *Appl Environ Microbiol* **78**: 3114-3119.

Nielson,H., Engelbrecht, J., Brunak, S. and von Heiin, G. (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng*, **10**: 1-6.

Peterson, T. N., Brunak, S., von Heiin, G. and Nielson, H. (2011) SignalP4.0 discrimanating signal peptides from transmembrane regions. *Nat Methods*, **8**: 785-786.

Okmen, B. & G. Doehlemann, (2014) Inside plant: biotrophic strategies to modulate host immunity and metabolism. *Curr Opin Plant Biol* **20**: 19-25.

Perez, P. & J.C. Ribas, (2004) Cell wall analysis. *Methods* **33**: 245-251.

Philipson, M.N. & M.C. Christey, (1986) The relationship of host and endophyte during flowering, seed formation, and germination of *Lolium perenne*. *New Zeal J Bot* **24**: 125-134.

Pierloni, A., Martelli, P. L., Casadio, R. (2008) PredGPI: a GPI anchor predictor. *BMC Bioinformatics*. **9**:392.

Quevillon, E., Silventoinen, V., Pillai, S., Harte, N., Mulder, N., Apweiler, R. (2005) InterProScan: protein domains identifier. *Nulcleic Acids Res*., **33**: W116-120.

Ram, A.F. & F.M. Klis, (2006) Identification of fungal cell wall mutants using susceptibility assays based on Calcofluor white and Congo red. *Nat Protoc* **1**: 2253-2256.

Rappleye, C.A., L.G. Eissenberg & W.E. Goldman, (2007) *Histoplasma capsulatum* α (1,3) glucan blocks innate immune recognition by the ß glucan receptor. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 1366-1370.

Robin, J.B., R.C. Arffa, I. Avni & N.A. Rao, (1986) Rapid visualization of three common fungi using fluorescein-conjugated lectins. *Invest Ophthalmol Vis Sci* **27**: 500-506.

Ruiz-Herrera, J. & L. Ortiz-Castellanos, (2010) Analysis of the phylogenetic relationships and evolution of the cell walls from yeasts and fungi. *Fems Yeast Res* **10**: 225-243.

Sahai, A.S. & M.S. Manocha, (1993) Chitinases of Fungi and Plants - Their Involvement in Morphogenesis and Host-Parasite Interaction. *Fems Microbiology Reviews* **11**: 317-338.

Sanchez-Vallet, A., J.R. Mesters & B.P. Thomma, (2015) The battle for chitin recognition in plant-microbe interactions. *FEMS Microbiol Rev* **39**: 171-183.

Schardl, C.L., (1996) EPICHLOE SPECIES: fungal symbionts of grasses. *Annu Rev Phytopathol* **34**: 109-130.

Schardl, C.L., (2001) *Epichloë festucae* and related mutualistic symbionts of grasses. *Fungal Genet Biol* **33**: 69-82.

Schardl, C.L., (2010) The Epichloe symbionts of the grass subfamily Pooideae. *Annals of the Missouri Botanical Garden* **97**: 646-665.

Schardl, C.L., A. Leuchtmann, K.R. Chung, D. Penny & M.R. Siegel, (1997) Coevolution by common descent of fungal symbionts (Epichloe spp) and grass hosts. *Molecular Biology and Evolution* **14**: 133-143.

Schardl, C.L., A. Leuchtmann, H.F. Tsai, M.A. Collett, D.M. Watt & D.B. Scott, (1994) Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen, *Epichloë typhina*. *Genetics* **136**: 1307-1317.

Schardl, C.L., C.A. Young, U. Hesse, S.G. Amyotte, K. Andreeva, P.J. Calie, D.J. Fleetwood, D.C. Haws, N. Moore, B. Oeser, D.G. Panaccione, K.K. Schweri, C.R. Voisey, M.L. Farman, J.W. Jaromczyk, B.A. Roe, D.M. O'Sullivan, B. Scott, P. Tudzynski, Z. An, E.G. Arnaoudova, C.T. Bullock, N.D. Charlton, L. Chen, M. Cox, R.D. Dinkins, S. Florea, A.E. Glenn, A. Gordon, U. Guldener, D.R. Harris, W. Hollin, J. Jaromczyk, R.D. Johnson, A.K. Khan, E. Leistner, A. Leuchtmann, C. Li, J. Liu, J. Liu, M. Liu, W. Mace, C. Machado, P. Nagabhyru, J. Pan, J. Schmid, K. Sugawara, U. Steiner, J.E. Takach, E. Tanaka, J.S. Webb, E.V. Wilson, J.L. Wiseman, R. Yoshida & Z. Zeng, (2013) Plant-symbiotic fungi as chemical engineers: multi-genome analysis of the clavicipitaceae reveals dynamics of alkaloid loci. *PLoS genetics* **9**: e1003323.

Scott, B., Y. Becker, M. Becker & G. Cartwright, (2012) Morphogenesis, growth and development of the grass symbiont *Epichloë festucae*. *In: Martin JP, Di Pietro A, eds. Morphogenesis and pathogenicity in fungi. Heidelberg, Germany: Springer-Verlag,* : 234-264.

Scott B, B.Y., Becker M, Cartwright G. , (2012) Morphogenesis, growth and development of the grass symbiont *Epichloë festucae*. *In: Martin JP, Di Pietro A, eds. Morphogenesis and pathogenicity in fungi. Heidelberg, Germany: Springer-Verlag,* : 243–264.

Scott, B., K. Green & D. Berry, (2018) The fine balance between mutualism and antagonism in the *Epichloë festucae*-grass symbiotic interaction. *Curr Opin Plant Biol* **44**: 32-38.

Seidl-Seiboth, V., S. Zach, A. Frischmann, O. Spadiut, C. Dietzsch, C. Herwig, C. Ruth, A. Rodler, A. Jungbauer & C.P. Kubicek, (2013) Spore germination of

Trichoderma atroviride is inhibited by its LysM protein TAL6. *FEBS J* **280**: 1226-1236.

Sonnhammer, E. L., von Heiine, G. and Krogh, A. (1998) A hidden Markov model for predicting transmembrane helices in protein sequences. *In: Proceedings of the international confernce on intelligent systems for molecular biology; ISMB. International conference on intelligent systems for molecular biology*. pp: 175-182.

Suda, Y., R.K. Rodriguez, A.E. Coluccio & A.M. Neiman, (2009) A screen for spore wall permeability mutants identifies a secreted protease required for proper spore wall assembly. *PLoS One* **4**: e7184.

Takahara, H., S. Hacquard, A. Kombrink, H.B. Hughes, V. Halder, G.P. Robin, K. Hiruma, U. Neumann, T. Shinya, E. Kombrink, N. Shibuya, B.P. Thomma & R.J. O'Connell, (2016) *Colletotrichum higginsianum* extracellular LysM proteins play dual roles in appressorial function and suppression of chitin-triggered plant immunity. *New Phytol* **211**: 1323-1337.

Takemoto, D., A. Tanaka & B. Scott, (2006) A p67Phox-like regulator is recruited to control hyphal branching in a fungal-grass mutualistic symbiosis. *Plant Cell* **18**: 2807-2821.

Tan, Y.Y., M.J. Spiering, V. Scott, G.A. Lane, M.J. Christensen & J. Schmid, (2001) In planta regulation of extension of an endophytic fungus and maintenance of high metabolic rates in its mycelium in the absence of apical extension. *Appl Environ Microbiol* **67**: 5377-5383.

Tanaka, A., G.M. Cartwright, S. Saikia, Y. Kayano, D. Takemoto, M. Kato, T. Tsuge & B. Scott, (2013) ProA, a transcriptional regulator of fungal fruiting body development, regulates leaf hyphal network development in the *Epichloë festucae-Lolium perenne* symbiosis. *Molecular microbiology* **90**: 551-568.

Tanaka, A., M.J. Christensen, D. Takemoto, P. Park & B. Scott, (2006) Reactive oxygen species play a role in regulating a fungus-perennial ryegrass mutualistic interaction. *Plant Cell* **18**: 1052-1066.

Tanaka, A., D. Takemoto, G.S. Hyon, P. Park & B. Scott, (2008) NoxA activation by the small GTPase RacA is required to maintain a mutualistic symbiotic association between *Epichloë festucae* and perennial ryegrass. *Molecular microbiology* **68**: 1165-1178.

Tanaka, A., B.A. Tapper, A. Popay, E.J. Parker & B. Scott, (2005) A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory. *Molecular microbiology* **57**: 1036-1050.

van Esse, H.P., M.D. Bolton, I. Stergiopoulos, P.J. de Wit & B.P. Thomma, (2007) The chitin-binding *Cladosporium fulvum* effector protein Avr4 is a virulence factor. *Molecular plant-microbe interactions : MPMI* **20**: 1092-1101.

Voissey, C.R., (2010) Intercalary growth in hyphae of filamentous fungi. *Fungal Biology Reviews* **Reviews** 123-131.

Wawra, S., P. Fesel, H. Widmer, M. Timm, J. Seibel, L. Leson, L. Kesseler, R. Nostadt, M. Hilbert, G. Langen & A. Zuccaro, (2016) The fungal-specific ß glucan binding lectin FGB1 alters cell-wall composition and suppresses glucan-triggered immunity in plants. *Nat Commun* **7**: 13188.

Wilson, D., (1995) Endophyte - the evolution of a term, and clarification of its use and definition. *Oikos* **73**: 274-276.

Winter, D.J., A.R. Ganley, C.A. Young, I. Lichko, C.L. Schardl, P.Y. Dupont, D. Berry, A. Ram, B.D. Scott & M.P. Cox, (2018) Repeat elements organize 3D genome structure and mediate transcription in the filamentous fungus *Epichloë festucae bioRxiv* **http://dx.doi.org/10.1101/339010**.

Yelton, M.M., W.E. Timberlake & C.A. Hondel, (1985) A cosmid for selecting genes by complementation in *Aspergillus nidulans*: Selection of the developmentally regulated yA locus. *Proceedings of the National Academy of Sciences of the United States of America* **82**: 834-838.

Young, C.A., M.K. Bryant, M.J. Christensen, B.A. Tapper, G.T. Bryan & B. Scott, (2005) Molecular cloning and genetic analysis of a symbiosis-expressed gene cluster for lolitrem biosynthesis from a mutualistic endophyte of perennial ryegrass. *Molecular genetics and genomics : MGG* **274**: 13-29.

Zdobnov, E. M. and Apweiler, R. (2001) InterProScan-an integration platform for the signature recognition methods in InterPro. *Bioinfomatics*, **17**: 847-848.