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FUNCTIONAL ANALYSIS OF A THIAMINE BIOSYNTHETIC GENE IN THE INTERACTION OF *EPICHLÖË TYPHINA* WITH PERENNIAL RYEGRASS

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Abstract

*Epichloë* *Neotyphodium* endophytes are a group of clavicipitaceous fungi that form symbiotic associations with temperate grasses. The asexual *N. lolii* form asymptomatic mutualistic associations with ryegrass whereas the sexual *E. typhina* behaves similar to a mutualist during the vegetative phase of plant growth but switches to epiphytic growth and formation of an external stroma upon development of the floral inflorescence. The aim of this project was to study the metabolic interaction between these endophytes and their perennial ryegrass host. The role of endophyte thiamine biosynthesis in host colonisation and stroma development was chosen, because of the key role this coenzyme plays in primary cellular metabolism and because thiamine biosynthetic genes are induced in several fungal-plant interactions.

The orthologue (*thil*) of *Saccharomyces cerevisiae* THI4 was isolated from *N. lolii* and *E. typhina* by PCR using degenerate primers designed to conserved regions of known thiazole biosynthetic genes. This gene is expressed *in planta* and in culture, and is alternatively spliced, with distinct patterns of the isoforms expressed under different nutritional conditions. Mutant with a deletion in the *E. typhina thil* gene was constructed and shown to have reduced hyphal density and branching compared to the wild-type on defined media lacking thiamine. Both thiamine and thiazole complemented this defect. Artificial inoculation of the mutants into plants showed that the *thil* mutant retained the ability to colonise the perennial ryegrass host and form stromata. However, the mutant had some differences in host colonisation and growth, including reduced hyphal branching and reduced detrimental effects on the host. In addition, glycogen-like deposits, which were abundant in the wild-type hyphae, were not evident in the mutants.

Unexpectedly, both the *thil* mutant and wild-type strains formed some stromata on vegetative tissue. Electron microscopic examination revealed that the cells of epiphytic
hyphae found on the vegetative tillers typically were enlarged, lacking in cytoplasm and highly vacuolated, an ultrastructure similar to that found for hyphae growing in reproductive tillers. The mutants retained the ability to form conidia on the outer layer of the stromata. Extensive vascular colonisation and hyphal ramification in the mesophyll were common characteristics of stromata bearing regions. Although the morphology and ultrastructure of stromata formed on vegetative tillers is very similar to those on reproductive tillers, one significant difference was the presence of abundant glycogen-like deposits in hyphae of vegetative tillers. Furthermore, there were dramatic differences in the levels of glycogen-like deposits in hyphae in different regions of the vegetative tillers, indicating that the energy demand changes during stroma development. This is the first report of *E. typhina* forming stroma on non-inflorescence tillers.
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# Table of contents

ABSTRACT I

ACKNOWLEDGEMENTS III

TABLE OF CONTENTS V

LIST OF FIGURES XV

LIST OF TABLES XIX

CHAPTER ONE: INTRODUCTION 1

1.1. The importance of *Epichloë/Neotyphodium* endophytes 2

1.2. Life cycles and host interactions of *Epichloë/Neotyphodium* endophytes 3
   1.2.1. Growth and life cycles of endophytes in grass host 3
   1.2.2. Host specificity and compatibility 6
   1.2.3. Mutualistic relationship and alkaloid production 7

1.3. Genetic studies of endophyte-host interactions 10
   1.3.1. Cloning and characterisation of genes for alkaloid biosynthesis 10
   1.3.2. Cloning and characterisation of other endophyte genes 13

1.4. Evolution of *Epichloë/Neotyphodium* endophytes 14
   1.4.1. *Epichloë/Neotyphodium* endophyte species 14
   1.4.2. Evolution of asexual *Neotyphodium* endophytes by interspecific hybridisation 17

   1.4.3. Evolution of asexual *Neotyphodium* endophytes by accumulation of detrimental mutations 18
   1.4.4. Evolution of asexual endophytes versus Muller’s ratchet 20
1.5. Variation in nutrition requirement and metabolism
   1.5.1. Variation in nitrogen and carbon source utilisation
   1.5.2. Thiamine requirement for endophyte growth

1.6. Thiamine biosynthetic pathway and genes
   1.6.1. Thiamine biosynthesis pathways
   1.6.2. Thiamine biosynthesis genes
   1.6.3. Regulation of thiamine biosynthesis
   1.6.4. Thiamine biosynthesis genes in microbe-plant interactions

1.7. Aims and objectives

CHAPTER TWO: MATERIALS AND METHODS

2.1. Biological materials
   2.1.1. Fungal and bacterial strains, plasmids and λ clones
   2.1.2. Growth and maintenance of organisms

2.2. DNA isolation, purification and quantification
   2.2.1. Large-scale isolation of endophyte DNA
   2.2.2. Small scale isolation of endophyte DNA
   2.2.3. Bacteriophage λ DNA isolation
   2.2.4. Plasmid DNA isolation using rapid boiling method
   2.2.5. Plasmid DNA isolation using an alkaline lysis method
   2.2.6. Plasmid DNA isolation using a Quantum Miniprep kit
   2.2.7. Isolation of DNA from SeaPlaque agarose gel
   2.2.8. DNA isolation from plant material
   2.2.9. Purification and precipitation of DNA
      2.2.9.1. Purification of PCR product by Concert™ Rapid PCR purification system
2.2.9.2. Purification of DNA by phenol/chloroform extraction 45
2.2.10. Detection, sizing and quantification of DNA 46
  2.2.10.1. Fluorometric quantification of DNA 46
  2.2.10.2. Agarose gel electrophoresis 46

2.3. Genomic library screening 47
  2.3.1. Genomic library plating and filter lifts 47
  2.3.2. Probe labelling and plaque DNA hybridisation 47
  2.3.3. Purification of positive lambda clones 48

2.4. DNA digestion, ligation, cloning and subcloning 49
  2.4.1. Restriction endonuclease digestion of genomic DNA 49
  2.4.2. Restriction endonuclease digestion of λ phage DNA, plasmid DNA and PCR products 49
  2.4.3. CAP-treatment of vector DNA 50
  2.4.4. Ligation of DNA fragments 50
  2.4.5. Preparation of competent cells and transformation by electroporation 51
  2.4.6. Screening for transformants 52

2.5. DNA sequencing, sequence assembly and Bioinformatic analysis 52
  2.5.1. DNA sequencing and sequence assembly 52
  2.5.2. Bioinformatic analysis 52

2.6. Southern blotting and hybridisation with Digoxigenin (DIG) labelled probe 53
  2.6.1. Southern blotting 53
  2.6.2. Hybridisation using Digoxigenin (DIG) labelled probe 54
  2.6.3. Chemiluminescent detection of DIG-labelled probes 55

2.7. Polymerase chain reaction (PCR) amplification 55
  2.7.1. Oligonucleotide primers 56
2.7.2. Routine PCR
2.7.3. Degenerate PCR
2.7.4. Thermal Asymmetric Interlaced PCR (TAIL-PCR)
2.7.5. Inverse PCR
2.7.6. Long template PCR
2.7.7. Bacterial colony PCR
2.7.8. Gel stab PCR

2.8. RT-PCR (reverse transcription PCR)
2.8.1. Isolation of total RNA with TRIzol® reagent
2.8.2. Quantification of RNA
2.8.3. DNase I treatment of RNA
2.8.4. Reverse transcription of RNA into cDNA and amplification

2.9. RACE (rapid amplification of cDNA ends)
2.9.1. 3’RACE analysis
2.9.2. 5’RACE analysis

2.10. Endophyte transformation
2.10.1. Protoplast preparation
2.10.2. Endophyte transformation
2.10.3. Single spore isolation

2.11. Examination of growth characteristics of E8 thi11 deletion mutants in culture
2.11.1. Measurement of colony diameter
2.11.2. Examination of hyphal branching and morphology
2.11.3. Examination of conidia formation
2.12. Examination of E8 thi11 deletion mutants in the endophyte-plant interaction

2.12.1. Inoculation of endophyte into perennial ryegrass seedlings
2.12.2. Inoculation and treatment of the clonal plantlets
2.12.3. Detection of endophyte in grass by tissue-print immuno blot
2.12.4. Examination of endophyte in grass tissue by alinine blue staining
2.12.5. Re-isolation of endophyte from plant tissues
2.12.6. Extraction and assay of peramine in the symbiotic plants

2.13. Statistical analysis

2.14. Light and transmission electron microscopy

CHAPTER THREE: RESULTS

3.1. Cloning and bioinformatic analysis of the thi1 gene from N. lolii Lp19 and E. typhina E8

3.1.1. Cloning and sequencing of thi1 gene from N. lolii strain Lp19
3.1.2. Analysis of the Lp19 thi1 gene sequence
3.1.3. Cloning and sequencing of thi1 gene from E. typhina strain E8
  3.1.3.1. Amplification of E8 thi1 by routine PCR
  3.1.3.2. Amplification of E8 thi1 5’ and 3’ flanking regions by TAIL-PCR
  3.1.3.3. Amplification of an E8 thi1 3’ AT-rich region by inverse PCR
3.1.4. Analysis of the E8 thi1 gene sequence
3.1.5. Southern hybridisation analysis of thi1 region in Lp19, Lp1 and E8 strains
3.1.6. Summary and discussion

3.2. Characterisation and expression analysis of the thi1

3.2.1. Characterisation of the Lp19 thi1 mRNA
  3.2.1.1. 5’RACE analysis of the Lp19 thi1 mRNA
3.2.1.2. 3’RACE analysis of the Lp19 thiI mRNA

3.2.2. Characterisations of the E8 thiI mRNA

3.2.3. Expression and alternative splicing of the Lp19 thiI gene in culture

3.2.4. Expression and alternative splicing of the Lp19 thiI gene in planta

3.2.5. Summary and discussion

3.3. Targeted disruption of E8 thiI gene and growth characteristics of the thiI deletion mutant

3.3.1. Construction of the E8 thiI gene disruption plasmid

3.3.2. Targeted deletion of E8 thiI

3.3.3. Screening for E8 thiI deletion mutants

3.3.3.1. Screening for E8 thiI gene disrupted colonies on defined CD thiamine-free medium

3.3.3.2. Screening for E8 thiI deletion mutants by PCR

3.3.3.3. Southern blot hybridisation analysis

3.3.4. Growth characteristics of the E8 thiI deletion mutants in culture

3.3.4.1. Effect of thiamine and thiazole on growth of E8 thiI deletion mutants

3.3.4.2. Effects of thiamine concentration on the E8 thiI deletion mutants

3.3.5. Summary and discussion

3.4. Analysis of the symbiotic phenotype of E8 thiI deletion mutants in association with perennial ryegrass

3.4.1. Analysis of the symbiotic phenotype of E8 thiI deletion mutants in association with perennial ryegrass (experiments 1 and 2)

3.4.1.1. Host survival and colonisation

3.4.1.2. Endophyte growth phenotype

3.4.1.3. Host morphology and growth phenotype

3.4.1.4. Peramine concentration
3.4.1.5. Stromata development on vegetative tillers
3.4.1.6. Stromata development on reproductive tillers
3.4.1.7. Stability of the endophyte-grass associations
3.4.1.8. Analysis of the endophyte re-isolated from the plants

3.4.2. Analysis of the symbiotic phenotype of E8 *thil* deletion mutants in association with perennial ryegrass (experiment 3)

3.4.2.1. Host survival and colonization
3.4.2.2. Progressive host death
3.4.2.3. Host growth characteristics
3.4.2.4. Stromata development on vegetative tillers

3.4.3. Inoculation of E8 wild-type isolates into perennial ryegrass (experiment 4)

3.4.4. Inoculation of the E8 *thil* mutants and control endophytes into clonal plantlets (experiment 5)

3.4.5. Summary and discussion

3.5. **Microscopic examination of reproductive and vegetative tillers of perennial ryegrass with stromata**

3.5.1. Microscopic examinations of reproductive tillers with stromata

3.5.1.1. Microscopic examinations of reproductive tillers with stromata of wild-type *E. typhina* E8

3.5.1.2. Microscopic examinations of reproductive tillers with stromata of E8 *thil* deletion mutant

3.5.2. Microscopic examination of vegetative tillers with stromata

3.5.2.1. Distribution of epiphytic hyphae and hyphal ramification

3.5.2.2. Vascular bundle colonisation

3.5.2.3. Hyphal ultrastructure

3.5.2.4. Abundant glycogen deposits in wild-type E8 hyphae

3.5.2.5. Alkaloid bismuth staining of wild-type E8 hyphae in the vegetative
CHAPTER FOUR: DISCUSSION, CONCLUSION AND FUTURE WORK 261

4.1. Characteristics and expression of endophyte thi1 262
   4.1.1. Endophyte thi1 function 262
   4.1.2. Endophyte thi1 expression in culture 264
   4.1.3. Endophyte thi1 expression in planta 267

4.2. Morphological change of the E8 thi1 mutants 268
   4.2.1. Morphological changes of the thi1 mutants 268
   4.2.2. Morphological changes of the ectopic transformants 271

4.3. Effects of wild-type E8 and E8 thi1 deletion mutants on the host 272
   4.3.1. Host colonisation and incompatibility between E. typhina E8 and perennial ryegrass 272
   4.3.1.1. Infectivity and hyphal growth in the host 272
   4.3.1.2. Host incompatibility 274
   4.3.2. Effects of wild type E. typhina E8 on growth and development of the plant host 276
   4.3.3. Effect of thi1 deletion in the endophyte-host interactions 279
   4.3.4. Effects of host genotypes and environmental factors on the symbiotic phenotypes 282

4.4. Stromata development on vegetative tillers 285
   4.4.1. Stromata development on vegetative tillers 285
   4.4.2. Nutrient translocation and conversion in hyphae in the stroma region 288

4.5. Conclusion and future work 291

APPENDIX 293
Appendix 1. Abbreviations 294

Appendix 2. Growth response of endophytes to thiamine 295

Appendix 3. Vector maps 297

Appendix 4. Media, common solutions and buffers 302
   Appendix 4.1. Media 302
   Appendix 4.2. Antibiotics and other supplement stocks 303
   Appendix 4.3. Buffers and solutions for DNA isolation and detection 304
   Appendix 4.4. Buffers and solutions for endophyte protoplast preparation and transformation 305
   Appendix 4.5. Buffers and solutions for Southern blotting, hybridisation and detection 305
   Appendix 4.6. Buffers and solutions for RNA working 306
   Appendix 4.7. Buffers and solutions for artificial infection, isolation or microscopic analysis of endophytes in planta 307

Appendix 5. Sequence data 308

Appendix 6. List of raw sequence data, plant data and statistical analysis on CD 310

REFERENCES 311
### List of figures

| Figure 1.1. | The asexual and sexual life cycles of *Epichloë festucae* on *Festuca rubra* | 5 |
| Figure 1.2. | Thiamine biosynthesis pathway and genes in yeast | 24 |
| Figure 3.1. | Design of degenerate primers for cloning *thi* gene | 78 |
| Figure 3.2. | Cloning and sequencing of the Lp19 *thi* gene | 80 |
| Figure 3.3. | Nucleotide and predicted polypeptide sequence of the Lp19 *thi* gene | 84 |
| Figure 3.4. | Alignment of the deduced polypeptide sequences of Lp19 and E8 *thi* genes with other homologous peptide sequences | 90 |
| Figure 3.5. | PCR amplification of E8 *thi* gene | 96 |
| Figure 3.6. | TAIL-PCR amplification of the 3' region of E8 *thi* | 98 |
| Figure 3.7. | TAIL-PCR amplification of the 3' region of E8 *thi* using arbitrary primer XZ17 | 100 |
| Figure 3.8. | Cloning the E8 *thi* gene flanking regions by TAIL-PCR | 102 |
| Figure 3.9. | Cloning the E8 *thi* 3' AT rich region by inverse PCR | 104 |
| Figure 3.10. | Nucleotide and predicted polypeptide sequence of the E8 *thi* gene | 106 |
| Figure 3.11. | Alignment of polypeptide sequences encoded by *thi* from E8 and Lp19 | 112 |
| Figure 3.12. | Alignment of the 5' upstream regions of *thi* from Lp19 and E8 | 114 |
| Figure 3.13. | Southern blot analysis of the *thi* gene in Lp19, Lp1 and E8 strains | 116 |
| Figure 3.14. | Determination 5' end of Lp19 *thi* mRNA by RACE | 123 |
| Figure 3.15. | Alignment of sequences from the Lp19 *thi* 5'RACE products | 124 |
| Figure 3.16. | Analysis of potential peptide sequences in Lp19 *thi* 5' upstream region | 126 |
| Figure 3.17. | Determination of 3' end of Lp19 *thi* mRNA by RACE | 129 |
| Figure 3.18. | Alignment of sequences from Lp19 *thi* 3'RACE products | 130 |
| Figure 3.19. | Alignment of 5' RACE sequence from E8 *thi* | 132 |

XV
Figure 3.20. Analysis of potential peptide sequences in E8 thil 5’ upstream region

Figure 3.21. Alignment of 3’RACE sequence from E8 thil

Figure 3.22. RT-PCR analysis of Lp19 thil gene expression in culture

Figure 3.23. RT-PCR analysis of Lp19 thil gene expression in planta

Figure 3.24. Construction of E8 thil gene replacement plasmid

Figure 3.25. Strategy used for disrupting the E8 thil gene

Figure 3.26. PCR analysis of E8 thil transformants

Figure 3.27. Southern blot analysis of E8 thil disruptants

Figure 3.28. Predicted outcomes for integration of replacement construct into E8 genome

Figure 3.29. Southern blot analysis of E8 thil disruptants

Figure 3.30. Effect of thiamine and thiazole on growth of E8 wild-type and thil deletion mutants

Figure 3.31. Frequency of hyphal branching of E8 wild-type and thil deletion mutants

Figure 3.32. Hyphal morphology of E8 wild-type and thil deletion mutants

Figure 3.33. Conidia production of E8 wild-type and thil deletion mutants

Figure 3.34. Effect of thiamine concentration on the growth of E8 wild-type, ectopic controls and thil deletion mutants

Figure 3.35. Effect of thiamine concentration on hyphal branching of E8 wild-type, ectopic controls and thil deletion mutants

Figure 3.36. Effect of thiamine concentration on hyphal morphology of E8 wild-type, ectopic controls and thil deletion mutants

Figure 3.37. Molecular analysis of E8 wild-type, ectopic controls and thil deletion mutants

Figure 3.38. Colony morphology of the endophyte isolates sub-cultured from CD thiamine-free medium to CD media with varied concentrations of thiamine.
Figure 3.39. Light micrographs of aniline blue stained endophytes in leaf sheaths from perennial ryegrass 192
Figure 3.40. Host phenotype of plants infected with E8 wild-type and thi1 mutants 194
Figure 3.41. Stromata on vegetative tillers 200
Figure 3.42. Stromata on reproductive tillers 202
Figure 3.43. Stromata on reproductive tillers 204
Figure 3.44. Growth of E8 thi1 mutants, E8 wild-type and ectopic controls re-isolated from the host grass 206
Figure 3.45. Microsatellite PCR analysis of E8 thi1 mutants, E8 wild-type and ectopic controls re-isolated from host grasses 208
Figure 3.46. Molecular analysis of E8 thi1 mutants, E8 wild-type and ectopic controls re-isolated from the host grasses 210
Figure 3.47. Hyphal morphology of E. typhina E8 in stroma-forming reproductive tillers 238
Figure 3.48. Hyphal morphology of E. typhina E8 in stroma-forming reproductive tillers 240
Figure 3.49. Hyphal morphology of E8 thi1 deletion mutants in stroma-forming reproductive tillers 242
Figure 3.50. Epiphytic hyphae in stroma-forming vegetative tillers 244
Figure 3.51. Hyphal ramification in the stroma-forming region of vegetative tillers 246
Figure 3.52. Vascular bundle colonisation of vegetative tillers with stromata 248
Figure 3.53. Ultrastructure of E8 wild-type hyphae on vegetative tillers. 250
Figure 3.54. Ultrastructure of hyphae of E8 thi1 deletion mutant on vegetative tillers 252
Figure 3.55. Abundant glycogen-like deposits in wild-type E8 hyphae in vegetative tillers 254
Figure 3.56. Few glycogen-like deposits in hyphae of E8 thi1 deletion mutant in vegetative tillers 256
Figure 3.57. Alkaloid bismuth staining of wild-type E8 hyphae in vegetative tillers
List of tables

Table 1.1.  *Epichloë/Neotyphodium* species and their life cycles  
Table 1.2.  Genes and enzymes involved in thiamine biosynthesis in yeast  
Table 1.3.  Homologous genes of yeast THI4 and THI5 from other fungi  
Table 2.1.  Fungal and bacterial strains, λ clones and plasmids  
Table 2.2.  Oligonucleotide primers used in this study  
Table 2.3.  Cycling conditions used for TAIL PCR in this study  
Table 3.1.  Blast X analysis of Th1 homologous polypeptide sequences  
Table 3.2.  E8 transformation frequencies (experiment 1)  
Table 3.3.  E8 transformation frequencies (experiment 2)  
Table 3.4.  E8 transformation frequencies (experiment 3)  
Table 3.5.  Colony diameter of the E8 *thil* deletion mutants, E8 wild type and ectopic controls grown on defined medium supplemented with thiazole or thiamine  
Table 3.6.  Effect of different thiamine concentrations on colony diameter of the E8 *thil* deletion mutants, E8 wild type and ectopic controls (experiment 1)  
Table 3.7.  Effect of different thiamine concentrations on colony diameter of the E8 *thil* deletion mutants, E8 wild type and ectopic controls (experiment 2)  
Table 3.8.  Seedling survival and colonisation following inoculation with E8 *thil* deletion mutants, E8 wild type and ectopic controls: experiment 1  
Table 3.9.  Seedling survival and colonisation following inoculation with E8 *thil* deletion mutants, E8 wild type and ectopic controls: experiment 2  
Table 3.10.  Number of different hyphal colonisation phenotypes  
Table 3.11.  Number of stunted plants in experiment 2  
Table 3.12.  Correlation of hyphal growth phenotypes, host stunting, and stromata development for experiment 2  

XIX
Table 3.13. Host tiller number, tiller weight, fresh weight and peramine concentration for experiment 2 198
Table 3.14. Number of plants with stromata on vegetative tillers or reproductive tiller for experiment 2 199
Table 3.15. Seedling survival and colonisation following inoculation with E8 thi1 deletion mutants, E8 wild type and ectopic controls: experiment 3 213
Table 3.16. Death rates of infected plants: experiment 3 218
Table 3.17. Number of stunted plants: experiment 3 218
Table 3.18. Effects of E8 thi1 deletion mutants, E8 wild type and ectopic controls on host fresh weight (mg): experiment 3 219
Table 3.19. Effects of E8 thi1 deletion mutants, E8 wild type and ectopic controls on host tiller number: experiment 3 220
Table 3.20. Effects of E8 thi1 deletion mutants, E8 wild type and ectopic controls on host tiller weight (mg): experiment 3 221
Table 3.21. Comparison of E8 wild types 227
Table 3.22. Clonal plant survival and colonisation following inoculation with E8 thi1 deletion mutants, E8 wild type and ectopic controls: experiments 4 and 5. 228
Table 3.23. Host tiller number, tiller weight and fresh weight for clonal plant experiment: experiment 4 and 5 229
Table 3.24. Peramine concentration for clonal plants 230