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**Identification of genes regulating the plant-specific expression
of the *ltmM* gene in *Epichloë festucae***

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Abstract

The fungal endophyte *Epichloë festucae* forms a largely mutualistic association with the ryegrass species *Lolium perenne*. *E. festucae* produces a range of bio-protective alkaloids that protect the host grass from herbivory by both mammals and insects. One such alkaloid, Lolitrem B, is a potent mycotoxin and the causative agent of ryegrass staggers in livestock.

Ten genes required for biosynthesis of lolitrem B are encoded in the *ltm* gene cluster. The *ltm* genes are expressed in a plant-specific manner, with high levels of expression *in planta* and very low levels of expression in culture. The mechanism regulating *ltm* gene expression is unknown but it is predicted to involve signalling from the host plant.

The *ltmM* gene was chosen for use in the investigation of *ltm* gene regulation because the flanking regions do not contain retrotransposon sequence, which surrounds much of the *ltm* gene cluster. To identify fungal genes involved in the plant-induced expression of *ltmM*, a mutagenesis and screening system was developed using a *PltmM-gusA* 'knock-in' construct to detect expression from the *ltmM* promoter. *Agrobacterium tumefaciens*-mediated T-DNA mutagenesis was used to create a set of mutants with random insertions in the genome. Mutants were then screened for altered *PltmM-gusA* expression, both in culture and *in planta*. Three mutants were identified with increased *PltmM-gusA* expression in culture, however, no mutants were identified with loss of *PltmM-gusA* expression *in planta*. This indicates that a mechanism of repression is involved in the plant-induced expression of *ltmM*, either directly or indirectly.

TM mutants of interest were also observed for altered symbiosis phenotypes. Mutants were identified with reduced colonisation rates and altered hyphal growth *in planta*. Integration sites were identified for two colonisation mutants and the disrupted genes are predicted to be the CTP:cholinephosphate cytidylyltransferase (CCT) gene *PCT1* and the mitogen-activated protein kinase kinase (MAPKK) gene *mkk2*.

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Table of Contents

Abstract	i
Acknowledgements	ii
Table of Contents	iii
List of Tables	vii
List of Figures	viii
Abbreviations	ix
Chapter One Introduction	1
1.1 Plant-fungal symbiosis	2
1.2 Epichloë endophytes	2
1.2.1 <i>Epichloë festucae</i>	3
1.2.2 The <i>E. festucae</i> genome	4
1.2.3 <i>E. festucae</i> growth in planta	4
1.2.4 The benefits of mutualism	6
1.3 Bio-protective molecules	6
1.3.1 Peramine	6
1.3.2 Lolines	6
1.3.3 Ergot alkaloids	7
1.3.4 Indole-diterpenes	8
1.3.4.1 Paxilline	8
1.3.4.2 Lolitrem B	9
1.3.4.3 The <i>ltmM</i> gene	10
1.4 Regulation of plant-induced gene expression	10
1.5 <i>Agrobacterium</i>-mediated T-DNA mutagenesis	14
1.6 <i>PltmM-gusA</i> transformants	16
1.7 Aim	17
Chapter Two Material and Methods	19
2.1 Biological Material	22
2.2 Media	22
2.2.1 Luria-Bertaini media	22
2.2.2 SOC medium	22

2.2.3	Potato Dextrose media	22
2.2.4	Modified Czapek Dox media	22
2.2.5	Water agar medium	22
2.2.6	Induction media	23
2.2.6.1	Minimal salts	23
2.2.7	Media supplements	23
2.2.7.1	Antibiotics	23
2.2.7.2	Nutritional supplementants	23
2.3	Bacterial growth conditions	23
2.4	Fungal growth conditions	24
2.5	DNA isolation	24
2.5.1	Plasmid DNA	24
2.5.2	Cracking	24
2.5.3	Genomic DNA	24
2.6	DNA manipulation	25
2.6.1	DNA quantification	25
2.6.2	DNA purification and precipitation	25
2.6.2.1	Column purification	25
2.6.2.2	Phenol/chloroform purification	25
2.6.2.3	Ethanol Precipitation	25
2.6.2.4	Isopropanol precipitation	26
2.6.3	Agarose gel electrophoresis	26
2.6.4	Restriction endonuclease digest	26
2.6.5	DNA cloning	26
2.6.5.1	Cloning into pCR4 [®] -TOPO [®]	26
2.6.5.2	Transformation of TOP10 <i>E. coli</i> cells	27
2.6.6	Plasmid rescue	27
2.6.6.1	Ligation	27
2.6.6.2	Transformation of DH5 α <i>E. coli</i> cells	27
2.6.7	Thermal asymmetric interlaced PCR (<i>TAIL</i> -PCR)	27
2.6.8	Southern blotting	29
2.6.9	Radioactive hybridisation	29
2.6.10	Southern hybridisation	29
2.6.11	Stripping of membrane	30
2.7	Plant growth conditions	30
2.7.1	Sterilisation of seeds	30
2.7.2	Seedling germination and inoculation	30
2.7.3	Mature plant maintenance	30
2.8	<i>in planta</i> analysis	31
2.8.1	Immunoblot for presence of endophyte	31
2.8.2	Aniline blue staining	31
2.8.3	Isolation of endophyte from seedlings	31
2.9	Fluorescent (MUG) assay for β-glucuronidase	31

2.9.1	In culture MUG assay	31
2.9.2	<i>in planta</i> MUG assay	32
2.10	Histochemical (X-Gluc) assay for β-glucuronidase	32
2.10.1	In culture X-Gluc assay	32
2.10.2	<i>in planta</i> X-Gluc assay	32
2.11	<i>Agrobacterium</i>-mediated T-DNA mutagenesis	33
2.11.1	Growth conditions	33
2.11.2	Transformation conditions	33
2.11.3	Nuclear purification	33
Chapter Three Results		34
3.1	Analysis of <i>E. festucae</i> <i>PltmM-gusA</i> transformants	35
3.1.1	Copy number of <i>PltmM-gusA</i> insertion affects GUS levels in culture	35
3.1.2	<i>PltmM-gusA</i> expression is unaltered in KM2.49	37
3.1.3	<i>PltmM-gusA</i> does not affect hyphal growth of KM2.49 <i>in planta</i>	39
3.1.4	<i>PltmM-gusA</i> expression is not induced under altered physiological conditions	39
3.2	<i>Agrobacterium</i>-mediated T-DNA mutagenesis of KM2.49	44
3.2.1	Some TM mutants displayed altered growth and morphology phenotypes	44
3.2.2	Copy number of T-DNA insert in TM mutants	46
3.3	TM mutant screening	48
3.3.1	Identification of GUS ⁺ TM mutants in culture	48
3.3.2	Identification of GUS ⁻ TM mutants <i>in planta</i>	48
3.4	Analysis of T-DNA mutants of interest	50
3.4.1	Copy number of T-DNA insert in TM mutants of interest	50
3.4.2	TM mutants of interest displayed varied rates of colonisation	53
3.4.3	TM1066 and TM1197 express GUS in mature plants	54
3.4.4	TM1066 causes stunting of the host plant	54
3.4.5	TM944 and TM1066 have altered hyphal growth <i>in planta</i>	57
3.5	Identification of disrupted genes in TM mutants of interest	60
3.5.1	TM984 contains a disrupted CTP:cholinephosphate cytidylyltransferase	62
Chapter Four Discussion		65
4.1	Development of a mutagenesis and screening system in <i>E. festucae</i>	66
4.2	Regulation of plant-induced <i>PltmM-gusA</i> expression	67
4.3	Identification of genes involved in establishment and maintenance	69

	of the symbiosis	
4.4	Conclusion	72
4.5	Future work	73
References		74
<hr/>		
Appendix		81
<hr/>		
5.1	Physical map of pBSYT7 vector	82
5.2	<i>PltmM-gusA</i> knock-in construct	83
5.3	Southern blot of <i>PltmM-gusA</i> transformants	84
5.4	Gel photographs of Southern blots of TM mutants	85
5.5	Gel photographs of Southern blots of TM mutants of interest	86
5.6	<i>TAIL</i> -PCR conditions	87
5.7	Sequence data for TM984 LB3-T10	88
5.8	Deletion of LB of T-DNA insert in TM984	89

List of Tables

2.1	Biological material	20
2.2	PCR primers cited within this thesis	28
3.1	Copy number and in culture expression of <i>PltmM-gusA</i> in KM transformants	35
3.2	TM mutants obtained from T-DNA mutagenesis	44
3.3	Copy number of T-DNA insert in TM mutants	46
3.4	TM mutants with GUS negative expression <i>in planta</i>	48
3.5	Copy number of T-DNA insert in TM mutants of interest	52
3.6	Survival and colonsation of endophyte-infected <i>L. perenne</i>	54

List of Figures

1.1	Schematic diagram of <i>Agrobacterium</i> -Mediated T-DNA transfer	15
3.1	Fluorescent assay for β -glucuronidase activity in <i>E. festucae</i> transformants	36
3.2	Histochemical assay for β -glucuronidase activity in <i>L. perenne</i> seedlings	38
3.3	Aniline blue stain for detection of hyphae in mature plant tissue	40
3.4	Fluorescent assay for β -glucuronidase activity in <i>E. festucae</i> grown under various carbon and nitrogen enriched conditions	41
3.5	Grow-out analysis of endophyte-infected <i>L. perenne</i> seedlings	43
3.6	TM transformants with altered growth phenotype in culture	45
3.7	Southern analysis of TM mutants	47
3.8	Histochemical assay for β -glucuronidase activity in TM mutants	49
3.9	Southern analysis of TM mutants of interest	51
3.10	Fluorescent assay for β -glucuronidase activity in endophyte-infected mature plant tissue	55
3.11	<i>L. perenne</i> plants infected with <i>E. festucae</i>	56
3.12	Aniline blue stain of TM mutants of interest in <i>L. perenne</i>	58
3.13	Gel photographs of TM984 <i>TAIL</i> -PCR product	61
3.14	Schematic diagrams of TM984 LB flanking sequence	63
3.15	ClustlW alignment of <i>E. festucae</i> gene A.1.7942	64

Abbreviations

bp	base pairs
kb	kilo bases
Mb	mega bases
AM	Arbuscular mycorrhiza
ASW	Argentine stem weevil
ATMT	<i>Agrobacterium tumefaciens</i> -mediated T-DNA (mutagenesis)
CCT	CTP:cholinephosphate cytidylyltransferase
CD	Czapek Dox
DNA	Deoxyribonucleic acid
EZ	Expansion zone
GABA	Gamma aminobutyric acid
GGPP	Geranyl geranyl diphosphate
GUS	β -glucuronidase
IM	Induction media
LB (media)	Luria-Bertaini
LB	Left border
MAPK	Mitogen-activated protein kinase
MAPKK	MAPK kinase
MAPKKK	MAPKK kinase
MUG	4-methylumbelliferyl β -D-glucuronide
NRPS	Non-ribosomal peptide synthase
PC	Phosphatidylcholine
PCR	Polymerase Chain Reaction
PD	Potato Dextrose
RB	Right border
REMI	Restriction enzyme-mediated integration
RIP	Repeat-Induced Point (mutation)
SAM	Shoot Apical Meristem
ST	Sterigmatocystin
TAIL-PCR	Thermal Assymmetric Interlaced Polymerase Chain Reaction
T-DNA	Transferred DNA
WA	Water agar
X-Gluc	5-bromo-4-chloro-3-indolyl- β -D-glucuronic acid
