

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.

**The indole-diterpene gene cluster from the ryegrass
endophyte, *Neotyphodium lolii*,
is required for the biosynthesis of lolitrem B,
a bioprotective alkaloid.**

This thesis is presented as a partial fulfillment of the requirements
for the degree of
Doctor of Philosophy (PhD),
in
Molecular Biology
at
Massey University, Palmerston North,
New Zealand

Carolyn Anne Young

2005

Abstract

Lolitrems are indole-diterpene alkaloids produced by *Epichloë* and *Neotyphodium* endophytes in association with their host grass *Lolium perenne*. Some indole-diterpene (ID) alkaloids are proposed to have insecticidal properties, but lolitrem B is known as the causative agent of the animal syndrome ryegrass staggers. Lolitrems are preferentially synthesised *in planta*, which suggests that the genes required for lolitrem biosynthesis are symbiotically expressed.

The lolitrem biosynthesis pathway has been proposed as a metabolic grid based on the identification of likely intermediates from endophyte-infected ryegrass. Closely related ID compounds are expected to serve as substrates for the same enzyme, but until recently these steps had not been validated. The identification and characterisation of a *Penicillium paxilli* gene cluster required for the synthesis of the ID paxilline has identified key enzymes required for the production of the ID backbone. Based on the similarity of lolitrem B to paxilline it was proposed that these two biosynthesis pathways would share orthologous early steps but later steps to convert paxilline to the more complex lolitrem B would require additional enzymes.

The lolitrem biosynthesis genes (*ltm*) were isolated using degenerate PCR and from candidate genes identified as ESTs in cDNA libraries. Ten *ltm* genes were identified that had functions consistent with those required for lolitrem B biosynthesis. The 10 *ltm* genes were contained on three gene clusters that are separated by repetitive AT-rich sequences that contain remnants of retrotransposons. The *ltm* clusters 1 and 2 contain eight genes, seven of which are orthologues of the characterised *P. paxilli* paxilline biosynthesis gene cluster (*pax*). Functional characterisation of *ltmM* an FAD-dependent monooxygenase and *ltmC* a prenyl transferase confirmed these two genes were required for ID biosynthesis and were orthologues of *paxM* and *paxC*, respectively. All 10 *ltm* genes have similar expression profiles and were highly expressed *in planta* where the production of lolitrem B is most prevalent. The taxonomic distribution of the *ltm* genes has established which endophyte strains are likely to produce ID compounds. This work provides the basis for elucidation of the lolitrem biochemical pathway and opens the way for determining how the plant regulates the synthesis of this important group of bioprotective molecules.

ACKNOWLEDGEMENTS

Embarking on a PhD was not an easy decision for me. I had family, I had never studied full-time, I was older than the average student and was it really something I needed to do? I finally made the decision to go for it, handed over my managerial role and I became a student!

Life has an interesting way of changing your focus by throwing a curve ball every once and a while. Mine came as a little 7lb 13oz package at about the PhD midway stage. He wasn't quite the experiment I had planned but we named him Oliver and love him dearly. This PhD could not have been completed with out the support of four very important people. The first three are my family, David, Patrick and Oliver who had the responsibility of making sure I had all the time I needed to do what I needed, even when I said I didn't need that much time. They fed, me they looked after me, they picked me up when I got low. They were there whenever I needed them. The fourth person is my supervisor Barry Scott, who feels like family. He supported my PhD decision, my pregnancy, my research and so much more. Just saying thank you to these four people is not enough. I am truly grateful for their love, their support and their encouragement.

Andrea Bryant was just the person I needed in the lab. Andrea made me smile, she looked after my plants, she kept me sane, she helped me when I needed an extra pair of hands and was unflappable when we were working together.

Thank you Geoff Jameson for correcting me. After you did such a great job on my Masters thesis I was delighted when you were prepared to read my PhD thesis. I hope that my writing style and grammar has matured for the better.

Emily Parker was invaluable for discussions on indole-diterpene biochemistry. I knew how I wanted to place the catalytic functions and Emily made sure they were realistic.

Thank you to Russell Poulter and Margy Bulter who gave me great advice on retrotransposons, especially Russell whose seminar on retrotransposons made my AT-rich regions make sense.

I would like to acknowledge Pete for his evolution expertise and the trees that have been used in Figure 3.60.

I would like to acknowledge the support I received from my co-supervisor Greg Bryan (AgResearch). I would like to thank Mike Christensen, Wayne Simpson and Anouck De Bonth for their help and guidance with all things plant like. You were my green fingers and I really appreciated it. I would also like to thank Brian Tapper and Liz Davies for the HPLC analysis and their input into what the peaks meant.

I would like to thank Richard Johnson (AgResearch) and German Spangenberg (Agriculture Victoria) for supplying me with some of their interesting EST sequences. I believe that the data generated with their sequences was the icing on my thesis.

Thank you to all past and present members of the Scott Base lab. Lisa, Andrea, Austen, Rohan, Brendon, Michelle, Kim, Aiko, Simon, Xuiwen, Shuguang, Christina, Emily,

Richard, Mike, Grant, and others that I may have missed. Some of you had been and gone before I started my PhD but you have still influenced my scientific career.

Thank you, to Rosie, Max, Barbara, Pat (all of them), Liz, Paul, Isabel, Chris, Catherine N, Catherine D, Kathryn, John, Gill, Trish, Lorraine, Pete, Cynthia, Neville, Vikki, and many other members of IMBS (both past and present) who have given me friendship, encouragement and support.

Lots of little thank yous are needed for; Joe Win for his advice and a printer so my thesis could be printed on A4 paper while living in the US; Brendon for the chemical structures; Chris and Mike (and many others) who entertained David while I was working; Kathryn Stowell and Robyn Marsden for their patience with the developer, making sure it was working when I needed it most; Mrs C for Oliver transport; Sanjay for the *P. paxilli* HPLC analysis; John McKay and Kirsty Allen for help with the lightcycler.

I would like to thank my Ballroom Dancing group, Leigh, Lynne and Pete, Smurf, Shirley, Wendy, Kevin, Jacinta and Bill, Vicky, Yvonne, David and others. I enjoyed the dancing, I enjoyed the coffee, but most of all I enjoyed making new friends. A special thank you to Smurf and Leigh who were required to dance with me week after week.

Thank you to my dearest friends Kate, Kirsty and Sharon for their friendship, love and support. Also thanks to my friends Rich and Linda, Mike and Kate, Kirsty and Marcus, Mark and Vanessa, Sharon and Tony, Trish and Pete, Geoff, Marion, Austen, Emily, Lisa, Rohan, Rachel and others that I may have forgotten to mention.

To Louise and all the Outtrims, Olly, Scott, Kenton and Liam. You were there for Patrick when ever I needed you. Then you were there when we REALLY needed you. Thank you for always being there.

After the arrival of Oliver, my PhD could not have advanced without the wonderful childcare from the Hoiho Centre at the Massey Childcare Centre. Oliver was so little but I knew he was in good hands. You mothered him, you babied him, you loved, you did it just right. I am especially grateful to Fleur, Anne, Raewynne, Kelly, Libby, Heather, Megan and the others who were there for Oliver when ever he needed you. Oliver would like to thank you as well and include his friends, Diana, Max, Brodie, Trent, Neitana, Jenny and many others.

I am grateful for the funding I received throughout my PhD. I was initially funded by a FRST Bright Futures Top Doctoral scholarship and then in my final year by the Bio-Protection CoRE (Lincoln). I would also like to thank IMBS for financial support for conference attendance.

Finally, I am extremely grateful to David 'the twist and turn' who was my rock during the printing process.

It is hard to believe that the end is near and it is time to move on for new life experiences. I shall miss my project but look forward to talking to those who contribute to this work in the years to come.

TABLE OF CONTENTS

Abstract	i
Acknowledgements	ii
Table of Contents	iv
List of Tables	ix
List of Figures	x
Abbreviations	xii
CHAPTER ONE INTRODUCTION	1
1.1 Fungal endophytes of ryegrass	2
1.2 Endophyte bioprotective alkaloids	6
1.2.1 Indole-diterpenes	9
1.2.2 Ergot alkaloids	10
1.2.3 Lolines	10
1.2.4 Peramine	11
1.3 Fungal secondary metabolism	12
1.4 Fungal gene clusters	12
1.5 Secondary metabolites – Isoprenoids	14
1.5.1 Gibberellins	15
1.5.2 Indole diterpenes	16
1.5.3 Trichothecenes	19
1.5.4 Aphidicolin	20
1.6 Secondary metabolites – Non-ribosomal peptides	21
1.6.1 Ergot alkaloids	21
1.6.2 HC-toxin	22
1.6.3 AM-toxin	24
1.6.4 Penicillin	24
1.6.5 Sirodesmin	25
1.7 Secondary metabolites – Polyketides	25
1.7.1 Aflatoxin and sterigmatocystin	26
1.7.2 Dothistromin	27
1.7.3 AF-toxin and AK-toxin	27
1.7.4 Fumonisin	28
1.7.5 Lovastatin	29
1.7.6 T-toxin	30

1.8	Regulation	31
1.9	Autoresistance	32
1.10	Evolution of Clusters	33
1.11	Stability of the fungal genome	34
1.11.1	Transposable elements	34
1.11.2	Repeat-induced point mutations	35
1.11.3	Gene duplications and deletions	35
1.12	Cloning gene clusters	36
1.13	Aims	39
 CHAPTER TWO MATERIALS AND METHODS		 41
2.1	Biological material	42
2.2	Growth of cultures	42
2.2.1	Aspergillus Complete Media (ACM)	42
2.2.2	CD + Yeast Extract Media (CDYE)	42
2.2.3	LB Media	42
2.2.4	Media Supplements	42
2.2.5	Potato Dextrose Media	42
2.2.6	SOC Media	46
2.2.7	Trace Elements	46
2.2.8	Top Agarose	46
2.2.9	Fungal Growth conditions	46
2.2.10	Bacterial Growth Conditions	46
2.3	DNA isolation	47
2.3.1	Plasmid DNA	47
2.3.2	Genomic DNA	47
2.3.3	Lambda DNA	48
2.4	DNA manipulation	49
2.4.1	DNA quantification	49
2.4.2	Restriction endonuclease digestion of DNA	49
2.4.3	DNA purification and precipitation	50
2.4.4	Subcloning	51
2.4.5	Agarose gel electrophoresis	52
2.4.5.1	Standard electrophoresis	52
2.4.5.2	Pulse field gel electrophoresis	52
2.4.6	Southern blotting	53
2.4.7	Radioactive hybridisation	53
2.4.7.1	Standard Southern hybridisation	55
2.4.7.2	Low-stringency Southern hybridisation	55
2.4.7.3	Northern hybridisation	55
2.4.7.4	Stripping radioactive membranes	56

2.5	Library screening	56
2.6	RNA isolation and analysis	58
2.6.1	Isolation of RNA	58
2.6.2	cDNA analysis	58
2.6.3	Northern blotting	59
2.7	DNA sequencing and Bioinformatics	59
2.8	PCR analysis	60
2.8.1	Standard PCR conditions	64
2.8.2	Degenerate PCR	64
2.8.3	Inverse PCR (IPCR)	64
2.8.4	Colony PCR	64
2.8.5	Real-time PCR analysis	65
2.8.6	Long template	66
2.8.7	High Fidelity enzymes	66
2.9	Fungal Transformations	66
2.9.1	Fungal protoplasts	66
2.9.2	Fungal transformation	67
2.9.3	Nuclear purification of transformants	67
2.10	Plant growth, inoculations and analyses	68
2.10.1	Seedling inoculations	68
2.10.2	Immunoblotting to screen for endophyte infection	68
2.10.3	Aniline blue staining of endophyte	69
2.11	Alkaloid Analysis	69
2.11.1	Lolitre B analysis	69
2.11.2	Ergovaline and peramine extractions	70
2.11.3	Ergovaline analysis	70
2.11.4	Peramine analysis	71
2.11.5	Paxilline	71
CHAPTER THREE	RESULTS	72
3.1	<i>N. lolii</i> and <i>E. festucae</i> contain two GGPP synthases	73
3.2	The <i>ggs1</i> gene	79
3.2.1	The <i>N. lolii ggs1</i> gene	79
3.2.2	The <i>E. festucae</i> and <i>E. typhina ggs1</i> gene	85
3.2.3	Expression of <i>ggs1</i>	88
3.3	The <i>ItmG</i> gene	88
3.4	The <i>ItmG</i> gene is contained within a gene cluster	92
3.4.1	<i>ItmM</i> an FAD dependent monooxygenase	92
3.4.2	<i>ItmK</i> a P450 monooxygenase	95
3.5	Expression analysis of the <i>Itm</i> genes	96

3.6	Functional analysis of <i>ItmM</i>	99
3.6.1	The <i>ItmM</i> deletion construct	102
3.6.2	Alkaloid analysis of the <i>ItmM</i> knockouts	105
3.6.3	Complementation of <i>ItmM</i>	115
3.6.4	Lolitrems analysis of mutant associations containing <i>ItmM</i> or <i>paxM</i>	120
3.6.5	Complementation of <i>P. paxilli paxM</i> deletion	124
3.7	The regions flanking the three <i>Itm</i> genes	127
3.7.1	Extending the left-hand flanking sequence	129
3.7.2	Extending the right hand flanking sequence	131
3.7.3	Using F11 to jump across the retrotransposon platform	131
3.7.4	The Lp19 <i>pks</i> is separated from <i>ItmK</i> by a retrotransposon platform	133
3.8	Definition of the retrotransposon platform	137
3.9	Isolation of two additional <i>Itm</i> gene clusters	141
3.9.1	<i>Itm</i> cluster 2	148
3.9.2	<i>Itm</i> Cluster 3	155
3.9.3	Expression profiles of the 10 <i>Itm</i> genes	157
3.9.4	Functional analysis of <i>ItmC</i>	163
3.9.5	<i>Itm</i> clusters 1, 2 and 3 form a large platform	169
3.9.6	The <i>Itm</i> platform is genetically unstable	175
3.10	Phylogenetic distribution of the <i>Itm</i> genes	177
3.11	<i>Itm</i> homologues detected in other fungi	183
CHAPTER FOUR DISCUSSION		191
4.1	Endophytes that produce lolitrems B contain two <i>ggs</i> genes	192
4.2	Identification of 10 <i>Itm</i> genes	194
4.3	The three <i>Itm</i> gene clusters are contained on a 100 kb 'platform'	203
4.4	Regulation of the <i>Itm</i> genes	204
4.5	Predicting indole diterpene (ID) phenotypes	207
4.6	Features of the <i>N. lolii</i> genome	211
4.7	Summary	214
APPENDIX		
5.1	Multiple Sequence Alignments	216
5.1.1	Geranylgeranyl diphosphate synthases	217
5.1.2	FAD dependent monooxygenases	220
5.1.3	P450 monooxygenases	221
5.1.4	Prenyl transferases, <i>paxC</i> -like	223
5.1.5	Prenyl transferases, <i>dmaW</i> -like	225

5.1.6	PaxB-like	227
5.1.7	GGPP synthases (required for ID production)	228
5.2	Vector Maps	229
5.2.1	pGEM-T	230
5.2.2	pGEM-T easy	231
5.2.3	pUC19	232
5.2.4	pUC118	233
5.2.5	pPN1688	234
5.2.6	pII99	235
5.3	Sequence data	236
5.3.1	'ggs1' <i>ggs1</i> sequences	
5.3.2	'CAG' The CAG repeat contained within <i>ggs1</i>	
5.3.3	'Cluster 1' The <i>N. lolii</i> and <i>E. festucae ltm</i> cluster 1	
5.3.4	'Retro' Sequences of lambda clones isolated with the Rua probe	
5.3.5	'Cluster 2 and 3' The <i>N. lolii ltm</i> cluster 2 and 3	
5.3.6	'PRG tub' Perennial ryegrass tubulin genes	
5.3.7	'Cluster 1' MacVector file	
5.3.8	'Cluster 2 and 3' MacVector file	
5.4	HPLC analysis	237
5.4.1	HPLC analysis to confirm the TLC data in Fig 3.30	238
5.4.2	HPLC analysis to confirm the TLC data in Fig 3.50	239
5.5	Melting curve data	240
5.5.1	Melting curve analysis of the plant gene amplicon shown in Fig. 3.25	241
5.5.2	Melting curve analysis of the endophyte gene amplicon shown in Fig. 3.25	242
REFERENCES		243
PAPER	YOUNG CA, BRYANT MK, CHRISTENSEN MJ, TAPPER BA, BRYAN GT, SCOTT B (2005) MOLECULAR GENETICS AND GENOMICS (IN PRESS)	

LIST OF TABLES

1.1	Summary of alkaloid production by endophytes	8
1.2	Fungal secondary metabolites, their locus and structure type	13
2.1	Biological material	43
2.2	Primer combinations for amplification of radioactive hybridisation probes	54
2.3	Primers cited within this thesis	61
3.1	Identification of orthologues to <i>N. lolii ggs1</i> , <i>orf1</i> and <i>orf2</i>	83
3.2	Sequence analysis across the polymorphic CAG repeat	89
3.3	The <i>ltm</i> genes, intron analysis and comparisons to database sequences	94
3.4	The endophyte biomass <i>in planta</i>	100
3.5	Rates of infection and alkaloid production of perennial ryegrass associations containing <i>ltmM</i> mutants	107
3.6	Alkaloid analysis of endophyte infected ryegrass	111
3.7	Endophyte biomass <i>in planta</i>	118
3.8	Rates of infection and lolitrem production for the associations containing the complementation strains	122
3.9	EST sequences of potential <i>ltm</i> genes	142
3.10	A list of the lambda clones identified from screening the Lp19 λ GEM-12 genomic library with the <i>ltmC</i> , <i>ltmP</i> and <i>ltmJ</i> fragments	149
3.11	The <i>ltm</i> genes from clusters 2 and 3, intron analysis and comparisons to database sequences	151
3.12	Sequence identity of the <i>ltm</i> genes to their <i>pax</i> and <i>atm</i> homologues	152
3.13	Over-represented motifs from the <i>ltm</i> genes	165
3.14	<i>ltm</i> genotypes of <i>Epichloë</i> and <i>Neotyphodium</i> endophytes	184
3.15	Homologues of <i>ltm</i> genes contained in <i>N. crassa</i> , <i>A. nidulans</i> , <i>M. grisea</i> , and <i>F. graminearum</i>	187
4.1	Description of the predicted catalytic activities of the Ltm proteins	198

LIST OF FIGURES

1.1	Phylogenetic relationship of the <i>Epichloë</i> and <i>Neotyphodium</i> endophytes	4
1.2	Chemical structures of endophyte alkaloids	7
1.3	Proposed biosynthetic pathway to paxilline	18
1.4	Predicted catalytic steps from paspaline to lolitrem B	38
3.1	Schematic diagram of fungal geranylgeranyl diphosphate synthase sequences used for degenerate primer design	74
3.2	Degenerate PCR to isolate <i>ggs</i> fragments	75
3.3	Sequence of the two isolated <i>ggs</i> fragments	77
3.4	Southern analysis with the two <i>ggs</i> DNA fragments	78
3.5	Restriction enzyme map of the <i>ggs1</i> gene and flanking region	80
3.6	The chromosomal location of the <i>ggs1</i> gene	81
3.7	Schematic diagram showing microsynteny of the <i>ggs1</i> locus between the fungal species, <i>N. lolii</i> , <i>M. grisea</i> , <i>N. crassa</i> , <i>A. nidulans</i> and <i>F. graminearum</i>	84
3.8	Isolation of <i>ggs1</i> from <i>E. festucae</i> and <i>E. typhina</i>	86
3.9	Schematic diagram of the endophyte <i>ggs1</i> genes	87
3.10	Expression analysis of the <i>ggs1</i> gene	90
3.11	Genomic map of the λ CY218 region	91
3.12	Gene structures of the <i>ltm</i> genes	93
3.13	Expression analysis of the <i>ltm</i> genes	97
3.14	Endophyte biomass <i>in planta</i>	98
3.15	Determination of minimum inhibitory concentration of hygromycin and geneticin	101
3.16	The <i>ltmM</i> -deletion construct	103
3.17	PCR screening for an <i>ltmM</i> deletion	104
3.18	Southern analysis of the <i>ltmM</i> -deletion transformants	106
3.19	Visual inspection of the perennial ryegrass plants infected with F11 carrying a deleted <i>ltmM</i> gene	109
3.20	Light microscopy of perennial ryegrass leaves infected with F11 <i>ltmM</i> -deletion mutants	110
3.21	HPLC analysis for lolitrem B in the <i>ltmM</i> -deletion mutants	112
3.22	HPLC analysis for ergovaline in the <i>ltmM</i> -deletion mutants	113
3.23	HPLC analysis for peramine in the <i>ltmM</i> -deletion mutants	114
3.24	Standard curves for estimation of endophyte DNA biomass	116
3.25	Real-time PCR analysis to determine endophyte DNA biomass	117
3.26	<i>ltmM</i> and <i>paxM</i> complementation constructs	119
3.27	Southern analysis of the <i>ltmM</i> complementation transformants	121
3.28	HPLC analysis for lolitrem B in the <i>ltmM</i> -deletion mutants complemented with <i>ltmM</i> or <i>paxM</i>	123
3.29	Constructs for complementation of the <i>paxM</i> -deletion mutant	125
3.30	TLC analysis of the <i>paxM</i> complementations	126
3.31	A physical map of the Lp19 <i>ltm</i> cluster	128
3.32	IPCR to rescue left hand flanking sequence	130
3.33	Restriction enzyme map differences between Lp19 and F11 at the <i>ltm</i> junctions	132
3.34	Physical and genetic map of the <i>E. festucae</i> , F11, <i>ltm</i> locus	134
3.35	Southern analysis of the <i>pks</i> pseudogene	135

3.36	Linkage of <i>ItmK</i> and <i>pks</i>	136
3.37	Southern analysis to determine copy number of Tah1, Rua and the AT-rich sequence	139
3.38	Alignment of the Tah1 and Rua LTR sequences	140
3.39	Schematic diagram of PaxC and PaxP showing placement of the EST sequences	143
3.40	Southern analysis of putative <i>Itm</i> genes	146
3.41	A physical map of the <i>Itm</i> cluster 2	147
3.42	Gene structures of the <i>Itm</i> cluster 2 genes	153
3.43	Southern analysis of the AT-rich region flanking <i>ItmP</i>	156
3.44	A physical map of <i>Itm</i> cluster 3	158
3.45	Gene structures of the <i>Itm</i> cluster 3 genes	159
3.46	PCR analysis to determine cDNA dilutions with equivalent amplification of <i>tub2</i>	161
3.47	Expression analysis of the 10 <i>Itm</i> genes	162
3.48	Putative regulatory motifs of the <i>Itm</i> genes	164
3.49	Constructs for complementation of the <i>paxC</i> deletion mutant	167
3.50	TLC analysis of <i>paxC</i> complementations	168
3.51	A <i>Itm</i> gene platform	170
3.52	The chromosomal location of the <i>Itm</i> gene clusters 1, 2 and 3	171
3.53	Linkage of <i>Itm</i> cluster 1 and 2 established by Southern analysis	173
3.54	Southern analysis of the Lp19 <i>Itm</i> gene platform	174
3.55	Southern analysis of endophyte strains hybridised with genes from <i>Itm</i> clusters 2 and 3	176
3.56	Southern analysis of endophyte strains for the <i>Itm</i> genes	178
3.57	PCR screen for the <i>Itm</i> genes	180
3.58	Southern analysis on the distribution of the retrotransposons and <i>pks</i> sequences	182
3.59	Comparison of indole-diterpene gene clusters from <i>P. paxilli</i> , <i>N. lolii</i> and <i>A. flavus</i>	186
3.60	Phylogenetic analysis of the fungal GGPP synthases	190
4.1	Early steps in lolitrem B biosynthesis	196
4.2	The proposed metabolic grid to lolitrem B	197
4.3	Modifications of paspaline and the predicted indole-diterpene phenotypes Lp14, Lp19 and AR1	201

Abbreviations

bp	base pairs
kb	kilo bases
kDa	kilo daltons
pfu	plaque forming units
Mb	mega bases
ACM	Aspergillus complete media
ACP	Acyl carrier protein
AFLP	Amplified fragment length polymorphism
AT	Acyltransferase
BAC	Bacterial artificial chromosomes
BLAST	Basic Local Alignment Search Tool
cDNA	copy DNA
CD	Czapek Dox
CDYE	Czapek Dox + yeast extract
DH	Dehydratase
DMAPP	Dimethylallyl diphosphate
DMAT	Dimethylallyl tryptophan
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Enoyl reductase
EST	Expressed sequence tag
ETC	Epichloë typhina complex
FAS	Fatty acid synthesis
GGPP	geranylgeranyl diphosphate
HCl	Hydrochloric acid
HMG CoA	Hydroxymethylglutaryl coenzyme A
ID	Indole diterpene
IPCR	Inverse polymerase chain reaction
IPP	Isopentyl diphosphate
IPTG	Isopropyl bD-thiogalactopyranoside
KR	Ketoreductase
KS	Ketoacyl synthase
LB	Luria broth
LINE	Long interspersed nuclear elements
LAE	Lolium-associated clade
LTR	Long terminal repeat
MFS	Major facilitator superfamily
NRPS	Non-ribosomal peptide synthase
ORF	Open reading frame
PCR	Polymerase chain reaction
PD	Potato dextrose
PKS	Polyketide synthase
RAPD	Random amplified polymorphic DNA
REMI	Restriction enzyme mediated integration
RIP	Repeat induced point mutations
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RT	Reverse transcription

RT-PCR	Reverse transcriptase-polymerase chain reaction
SINE	Short interspersed nuclear elements
TAGKO	Transposon-arrayed gene knockouts
TE	Thioesterase
Tris	Tris (hydroxymethyl) methylamine
TLC	Thin layer chromatography
Tris-HCl	Tris (hydroxymethyl) methylamine pH changed with HCl
X-gal	5-bromo-4-chloro-3-indolyl-bD- galactoside