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

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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.

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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!

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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 diterepene
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