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# Identification of Transporter Genes from the Fungal Endophyte *Neotyphodium* *lolii*

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## **Abstract**

*Neotyphodium lolii* is an endophytic fungus that lives in the pasture grass, *Lolium perenne*. They share a mutualistic symbiotic relationship. *N. lolii* lives out its life cycle within the plant and produces secondary metabolites, including alkaloids peramine, ergovaline and lolitrem which protect the grass from insect and animal herbivory. In fungi the biosynthetic genes of secondary metabolites are often located in gene clusters. These clusters frequently contain one or more genes that code for transporter proteins responsible for the removal of toxic products from the fungal cells. Plants produce defence compounds, including antifungals to protect themselves from colonising fungi. However endophytes are able to neutralise these host toxins, one mechanism for this is possibly by efflux through transporter channels.

The goal of this study was to identify ABC and MFS genes from *N. lolii*. These two families are the largest and most diverse of transporter families, which transport a variety of substrates, including peptides, toxins, ions and sugars across membranes. Using degenerate PCR primers designed from fungal multi-drug transporter sequences, four unique ABC gene fragments were amplified from *N. lolii*. A further two ABC sequences and two MFS gene fragments were identified in a database of *N. lolii* EST sequences.

RT-PCR was used to compare expression of isolated ABC and MFS genes in *N. lolii*, growing in culture and in infected plants. Up-regulation of transporter transcripts *in planta* could suggest a role in symbiosis. Some genes were seen to have a visibly different expression pattern from others, although all genes were strongly expressed in cultured mycelia. Gene expression in the plant host was most evident in tissues more heavily infected with endophyte. To discover possible roles for the isolated transporter genes in transporting endophyte secondary metabolites a strain distribution study was completed. Five of the putative ABC and MFS genes were compared against 12 *Epichloë* and *Neotyphodium* endophytes. Amplified PCR products in the genotypes screened produced a unique pattern of gene occurrence for each of the five transporters. This added to the characterisation of the transporter genes and showed that one gene,

gABC 4c, was the most diverse in its distribution, while another ABC gene gABC 4g was present across all genotypes.

One ABC gene (gABC 4c) plus flanking DNA was sequenced in full. Bioinformatic analyses suggested that gABC 4c may be a half sized ABC transporter gene of 2 kb with four exons. An orotate phosphoribosyltransferase was identified 2 kb upstream of the ABC transporter.

Further work will be needed to confirm that the start and stop codons of this ABC transporter have been accurately predicted, as well as to verify the putative intron/ exon boundaries identified by gene prediction programmes. The role of *N. loli* ABC transporter gABC 4c has not been determined, however future research could focus on the nature of the substrate(s) transported, the sub-cellular location of the channel, and the effects of gene knockout or over-expression on the symbiosis between *N. loli* and perennial ryegrass.

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## Abbreviations

ABC:	ATP-binding cassette
BLAST:	Basic Local Alignment Search Tool
bp:	base pair
cDNA:	copy DNA
°C:	degree celsius
DNA:	deoxyribonucleic acid
DNase:	deoxyribonuclease
dNTP:	deoxynucleotide triphosphate
EST :	Expressed sequence tag
g :	gram
IPTG:	Isopropyl- $\beta$ -d-thiogalactoside
kb:	kilobase pair
L:	litre
LB:	Luria broth
M:	mole per litre
MFS:	Major Facillitator superfamily
ml:	milliliter
mM:	millimole per litre
NRPS:	Non-ribosomal peptide synthase
ORF:	Open reading frame
PCR:	Polymerase chain reaction
PKS:	Polyketide synthase
RNase:	ribonuclease
RNA:	ribonucleic acid
RT-PCR:	reverse transcriptase PCR
SDS:	sodium dodecyl sulfat
$\mu$ l:	microlitre
$\mu$ M:	micromole per litre
$\mu$ g:	microgram
v/v:	volume per volume
w/v:	weight per volume
X-Gal:	5- bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside