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**Molecular and cellular analysis of the
endophyte *Neotyphodium uncinatum* and its
association with *Festulolium***

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Abstract

Epichloë and *Neotyphodium* endophytes are well known for the fitness benefits they impart on the cool-season grasses they inhabit. The production of secondary metabolites, in particular lolines, which deter insect predation, is one such benefit and is of particular interest in pastoral grass development. The identification, testing and implementation of novel endophyte-grass associations resulting in high production of lolines is highly valued in the development of grass cultivars in New Zealand.

An in depth analysis of the two simple sequence repeats (SSR) used to identify endophyte species showed that the repeat structure is unique for some endophyte species and that ancestral relationships of interspecific hybrids may be inferred from the repeat structure. One family of SSRs was found to be enriched in exonic regions of a number of genes and may be an important factor in gene innovation and adaptation. Levels of loline production by *N. uncinatum* was found to be strain specific with the highest production by the strain, U10. *N. uncinatum* colonising intergenic hybrids of *Festuca pratensis* and *Lolium perenne* (Festulolium) displayed incompatibility in older tissue through cell wall thickening, degeneration of cytoplasm and production of dense inclusions around hyphae and in the plant intercellular space. The production of dense inclusions actively degrading hyphae indicated a plant response to hyphal colonisation.

Results of this study indicate the importance of repeat structure in strain identification, repeat elements in genes, the testing of loline alkaloids *in planta* and the barriers to establishing novel endophyte-grass associations.

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Common Abbreviations

Amp	Ampicillin
bp	Base pairs
BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide database search using a nucleotide query
d	Days
°C	Degrees Celsius
cDNA	Complementary DNA
cv	Cultivar
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetra-acetic acid
g	Gravity
gDNA	Genomic DNA
h	Hours
H ₂ O	Dihydrogen Monoxide
kb	Kilo base pairs
LB	Luria-Bertani medium
M	Molar
min	Minutes
µg	Micro-gram
mg	Milli-gram
µL	Micro-litre
mL	Mill-litre
µM	Micro-molar
mM	Milli-molar
NCM	Nitrocellulose membrane
NRPS	Non-ribosomal peptide synthetase
NAL	<i>N</i> -Acetylloine
NFL	<i>N</i> -Formylloline
NML	<i>N</i> -Methylloine
NANL	<i>N</i> -Acetylnorololine
PCR	Polymerase chain reaction

PD	Potato dextrose medium
RNA	Ribonucleic Acid
rpm	Revolutions per minute
RT-PCR	Reverse transcriptase PCR
SDS	Sodium dodecyl sulfate
SNP	Single nucleotide polymorphism
SSR	Simple Sequence Repeat
TBE	Tris Borate EDTA buffer
v/v	Volume/volume ratio
WGA	Wheat Germ Agglutinin
w/v	Weight/volume ratio

