Peramine and indole-diterpene biosynthetic capability of *epichloë* endophytes as predicted by *perA* and *ltm* gene analysis

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Genes for the synthesis of the bioprotective metabolites, peramine and indole-diterpenes, were recently cloned from *Neotyphodium lolii* and *Epichloë festucae* (Tanaka *et al.* 2005; Young *et al.* 2005; Young *et al.* 2006). Peramine is synthesised by a single multifunctional non-ribosomal peptide synthetase, PerA, comprised of two adenylation and two thiolation domains, and single condensation, methylation and reductase domains (Tanaka *et al.* 2005). By contrast the indole-diterpenes are a structurally diverse group of metabolites that includes lolitrem B, the most abundant indole-diterpene found in the *N. lolii*-perennial ryegrass association. At least 10 genes are required for lolitrem B biosynthesis (*ltm*). These form a complex gene cluster composed of three mini-clusters interspersed with AT-rich retrotransposon relic sequence (Young *et al.* 2006). The aim of this study was to determine the *perA* and *ltm* gene composition and distribution among *epichloë* endophytes, including representative strains of the ten sexual *Epichloë* species and a taxonomically diverse set of strains from the asexual *Neotyphodium* species, and on the basis of this analysis predict the symbiotum chemotype.

Using primers designed to conserved regions of the A1 and A2 domains of PerA, PCR products were amplified from all strains of the 21 *Epichloë* and *Neotyphodium* species screened, indicating that *perA* sequences are widely distributed in these genera. However, not all strains have the ability to synthesise peramine in grass symbiota. DNA sequence analysis of the *perA* 3' sequence from *N. lolii* strain Lp14 identified several mutations in the 3' end of the gene including a premature stop codon at the beginning of the C domain. Several other strains were highly polymorphic in the *perA* 3' region indicating that grass symbiota containing these strains will also have a peramine negative phenotype.

By contrast *ltm* genes showed very limited distribution within the 21 *Epichloë* and *Neotyphodium* species screened, principally being confined to *E. festucae* and their asexual *Neotyphodium* derivatives. Some strains had the full *ltm* gene complement and were able to synthesise a range of lolitrems in grass symbiota. A sub-set of the *E. festucae* strains examined lacked all 10 genes, explaining the complete absence of indole-diterpenes in symbiota containing these strains. Other strains contained sequences to the eight genes that comprise clusters one and two and synthesised less complex indole-diterpenes *in planta*. Remnants of *ltm* genes were detected in some *Epichloë* species, indicating that this biosynthetic capability is ancestral, having been lost in many lineages.

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