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**Molecular characterisation of the *EAS*
gene cluster for ergot alkaloid
biosynthesis in epichloë endophytes
of grasses**

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Abstract

Clavicipitaceous fungal endophytes of the genera *Epichloë* and *Neotyphodium* form symbioses with grasses of the family Poaceae in which they can synthesise an array of bioprotective alkaloids. Some strains produce the ergot alkaloid ergovaline, which is implicated in livestock toxicoses caused by ingestion of endophyte-infected grasses.

Cloning and analysis of a plant-induced non-ribosomal peptide synthetase (NRPS) gene from *Neotyphodium lolii* and analysis of the *E. festucae* E2368 genome sequence revealed a complex gene cluster for ergot alkaloid biosynthesis. The *EAS* cluster contained a single-module NRPS gene, *lpsB*, and other genes orthologous to genes in the ergopeptine gene cluster of *Claviceps purpurea* and the clavine cluster of *Aspergillus fumigatus*. Functional analysis of *lpsB* confirmed its role in ergovaline synthesis and bioassays with the *lpsB* mutant unexpectedly suggested that ergovaline was not required for black beetle (*Heteronychus arator*) feeding deterrence from epichloë-infected grasses. Southern analysis showed the cluster was linked with previously identified ergot alkaloid biosynthetic genes, *dmaW* and *lpsA*, at a subtelomeric location. The ergovaline genes are closely associated with transposon relics, including retrotransposons, autonomous DNA transposons and miniature inverted-repeat transposable elements (MITEs), which are very rare in other fungi.

All genes in the cluster were highly expressed *in planta* but expression was very low or undetectable in mycelia from axenic culture, including under nitrogen-, carbon- or phosphate-limited conditions. Comparative analysis of the *EAS* gene cluster in four different epichloë strains showed marked differences in gene expression and ergot alkaloid synthesis. Gene order is conserved in each strain although evidence for recombination between two MITEs and expansion or reduction of a simple sequence repeat (SSR) at a single intergenic region was observed. Heterologous expression of a candidate regulatory gene, *laeA*, from *Aspergillus nidulans*, which is a global regulator of secondary metabolism in aspergilli, did not affect *eas* gene

expression. This, along with phylogeny and microsynteny analysis, suggests there is not an orthologue of this gene in *epichloë*.

This work provides a genetic foundation for elucidating biochemical steps in the ergovaline pathway, the ecological role of individual ergot alkaloid compounds, and the regulation of their synthesis *in planta*.

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