

## A gene cluster for ergovaline biosynthesis

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Some strains of *Epichloë* and *Neotyphodium* endophytes produce the ergot alkaloid ergovaline, which is implicated in livestock toxicoses caused by ingestion of endophyte-infected grasses. Cloning and analysis of a non-ribosomal peptide synthetase (NRPS) gene from *Neotyphodium lolii* revealed a cluster of genes for ergovaline biosynthesis containing a single-module NRPS, *lpsB*, and other genes *easA*, *easE*, *easF*, *easG* and *easH*. Each of the genes was highly similar to genes found in the ergot alkaloid gene clusters from *Claviceps purpurea* and *Aspergillus fumigatus*, although there are several rearrangements between the three clusters and the epichloë ergovaline genes are closely associated with several transposon relics. Southern analysis showed the *N. lolii* cluster was linked with previously identified ergovaline biosynthetic genes, *dmaW* and *lpsA*, at a subtelomeric

location. Functional analysis of *lpsB* confirmed a role in ergovaline synthesis; a targeted replacement mutant of *lpsB* was blocked in the ability to synthesise ergovaline and accumulated lysergic acid compared with wild-type, while complementation restored ergovaline production. All genes in the cluster were highly expressed *in planta* but expression was very low or undetectable in mycelia from axenic culture. Expression of *lpsB* was undetected under carbon, nitrogen and phosphate starvation conditions and exposure to a ryegrass extract also failed to induce expression, perhaps suggesting more complex signalling is required. 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*.