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**IDENTIFICATION OF PUTATIVE DOTHISTROMIN BIOSYNTHETIC
GENES.**

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ABSTRACT.

Dothistromin is a polyketide-derived mycotoxin produced by the *Pinus* pathogen *Dothistroma pini*, and is thought to be important in the development of the necrotic disease *Dothistroma* needle blight. Targeted disruption of dothistromin biosynthetic genes will allow the direct assessment of the role of the toxin in *D. pini* pathogenicity. Dothistromin displays structural and biochemical similarities to the aflatoxins (AF) and sterigmatocystin (ST) which are produced by various *Aspergillus* species. In our laboratory, knowledge from the well characterised ST/AF pathway is being used to isolate and characterise genes likely to be involved in dothistromin production.

The *D. pini* lambda clone, λ CGV1, was isolated from a *D. pini* genomic library by heterologous hybridisation with a fragment of the *Aspergillus parasiticus ver1* gene (Gillman, 1996). In this study, the complete nucleotide sequence of λ CGV1 was determined. Analysis revealed that five genes are located within the 13.3 kb genomic region sequenced. Three of these genes (*dkr1*, *dox1* and *dte1*) display strong similarities to genes contained within the ST/AF biosynthetic gene clusters. The *dtp1* gene, located between *dox1* and *dte1*, shows similarities to transmembrane efflux pumps and is proposed to be a dothistromin toxin pump. The *ddh1* gene, located upstream of *dkr1*, shows similarities to bacterial dehydrogenases. However, the *ddh1* coding sequence contains a premature stop codon (encoding a product of 63 amino acids), indicating that the product may be non-functional.

Expression analysis of each gene identified in this study confirmed that *dkr1*, *dox1*, *dte1* and *dtp1* are expressed. However, no obvious expression was detected for the *ddh1* gene. Southern blot analysis confirmed the genomic clustering of the genes and indicated that a single copy of each gene was present in the *D. pini* genome.

Due to the biogenetic relationship between dothistromin and ST/AF biosynthesis, and because genes identified in this study show similarities to genes involved in ST/AF production, it is thought that these genes are likely to be involved in dothistromin biosynthesis and constitute part of a dothistromin biosynthetic gene cluster.

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