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IDENTIFICATION OF PUTATIVE DOTHISTROMIN BIOSYNTHETIC GENES.

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Molecular Biology at Massey University, Palmerston North, New Zealand.

Brendon Joseph Monahan
1998
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, λCGV1, 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 λCGV1 was determined. Analysis revealed that five genes are located within the 13.3 kb genomic region sequenced. Three of these genes (*dkrI*, *doxl* and *dteI*) display strong similarities to genes contained within the ST/AF biosynthetic gene clusters. The *dtpI* gene, located between *doxl* and *dteI*, shows similarities to transmembrane efflux pumps and is proposed to be a dothistromin toxin pump. The *ddhI* gene, located upstream of *dkrI*, shows similarities to bacterial dehydrogenases. However, the *ddhI* 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 *dkrI*, *doxl*, *dteI* and *dtpI* are expressed. However, no obvious expression was detected for the *ddhI* 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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