IDENTIFICATION OF DOTHISTROMIN BIOSYNTHETIC PATHWAY GENES

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ABSTRACT

Dothistromin is a polyketide-derived toxic secondary metabolite produced by the filamentous fungus *Dothistroma pini* which causes the disease *Dothistroma* needle blight in *Pinus radiata*. Dothistromin is considered to be an important component in the disease process, although its exact function is yet to be identified. By isolating and identifying genes involved in dothistromin biosynthesis, and subsequently obtaining mutants blocked or altered in the synthesis of dothistromin, the role of this toxin in pathogenicity will be able to be assessed. Dothistromin is structurally related to the mycotoxins, aflatoxin (AF) from *Aspergillus parasiticus* and *A. flavus*, and sterigmatocystin (ST) from *A. nidulans*. Three intermediates in the ST and AF biosynthetic pathways (averantin, averufin, and versicolorin B) are thought to also be intermediates dothistromin biosynthesis. Due to these similarities, cloned AF pathway genes were used as heterologous probes in Southern hybridisation analysis to provide a direct method for identifying dothistromin biosynthetic genes.

A fragment of the *A. parasiticus nor-1* gene, encoding a reductase involved in the conversion of norsolorinic acid (NA) to averantin (AVN) in the AF biosynthetic pathway, was used as a probe to detect a region of sequence similarity to *D. pini* genomic DNA. A *D. pini* genomic library was then constructed and screened, resulting in clone λCGN2. However, Southern hybridisation analysis suggested that this clone did not contain a homologue of the *nor-1* gene from *A. parasiticus*.

A fragment of the *Aspergillus parasiticus ver-1* gene, encoding a reductase involved in the conversion of versicolorin A (VA) to ST in the AF biosynthetic pathway, was also used as a probe to detect a region of sequence similarity to *D. pini* genomic DNA. The *D. pini* genomic library was then screened. Two clones, λCGV1 and λCGV2, were isolated and Southern hybridisation analysis confirmed that these clones contained sequences hybridising to the *A. parasiticus ver-1* gene fragment. Fragments of these clones which hybridised were then sequenced and compared to the GenBank database. The amino acid coding sequence of a 0.8 kb *Sal*I region from clone λCGV1 exhibited a high degree of similarity with the *A. nidulans verA* and *A. parasiticus ver-1* genes, involved in the ST and AF biosynthetic pathways, and the *Magnaporthe grisea ThnR*, and *Colletotrichum lagenarium Thr1* genes, involved in melanin biosynthesis. This data suggested a *ver-1* homologue is present in the *D. pini* genome. Limited sequence analysis of a 2.1 kb region from clone λCGV2 suggested that a second independent copy of a *ver-1*-like gene may also be present in the genome.

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