CHARACTERISATION OF A PUTATIVE DOTHISTROMIN BIOSYNTHETIC CLUSTER.

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ABSTRACT.

The fungus *Dothistroma pini* is a key pathogen in New Zealand (and international) softwood plantations, most notably *P. radiata*. The mycotoxin dothistromin produced by this saprophytic fungus is believed to play a major role in its pathogenesis.

Dothistromin shares functional groups and pathway intermediates with those of sterigmatocystin and aflatoxin, secondary metabolites of *Aspergillus* sp. As the sterigmatocystin and aflatoxin biosynthetic pathways are characterised this provided us with a model pathway and potential probes for the isolation of dothistromin genes.

The *ver*1 gene is critical to the completion of aflatoxin biosynthesis in *Aspergillus* sp. as its disruption prevented the synthesis of aflatoxin. Assuming similar enzymes act in the dothistromin biosynthetic pathway a probe for *ver*1 was obtained and used to probe a *D. pini* genomic library. This led to the isolation of two lambda clones named λCGV1 and λCGV2 (Gillman 1996). A second library screen was completed using an aflatoxin polyketide synthase (PKS) probe and led to the isolation of the lambda clone λBMKSA (Morgan 1997).

The λCGV1 clone has been studied in detail and shown to contain a gene similar to aflatoxin *ver*1 (named *dkr*1) and other potential dothistromin biosynthetic genes (Monahan 1998). This study looks in greater detail at the lambda clones λCGV2 and λBMKSA and determines whether they contain putative dothistromin biosynthetic genes and are part of the anticipated gene cluster.

In this project the lambda clone λCGV2 was partially characterised which revealed that the other potential *ver* gene showed a greater similarity to the melanin biosynthetic gene *phn* than to the aflatoxin gene *ver*-1. This implied that the clone was unlikely to contain dothistromin biosynthetic genes so no further sequence was generated. However, a partial restriction map was constructed.

The other lambda clone, λBMKSA was then further characterised. Double stranded sequence of the putative *pks* gene region was completed. The remainder of the lambda clone was subcloned and exploratory sequence revealed a gene with high similarity to *stc*W.

The next stage was to determine how the three lambda clones were related. This was approached by probing genomic Southern blots with the ends of the lambda clones to determine the presence of commonly hybridised fragments. The presence of common fragments suggests that the three clones are very close together in the genome, although the evidence which links λCGV2 and λBMKSA is stronger than the evidence that links λCGV2 and λCGV1.

This is the first evidence that the three lambda clones isolated using aflatoxin probes are close together in the genome of *D. pini.* The genes present on these lambda clones show a high degree of similarity to their aflatoxin counterparts and could potentially contain a dothistriomin biosynthetic cluster.

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TABLE OF CONTENTS.

2. MATERIALS AND METHODS.

LIST OF TABLES.

LIST OF FIGURES.