CONFIRMATION OF THE PRESENCE OF A DOTHISTROMIN BIOSYNTHETIC GENE CLUSTER IN THE FUNGAL FOREST PATHOGEN *DOTHISTROMA PINI.*

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ERRATUM SHEET

Figure 3.4 The term transformation event refers to each independent protoplast transformed with the disruption vector.

Figure 3.11 Dothistromin samples taken from colony 8A1 and wild type were diluted 10 fold. For qualitative comparison to undiluted dothistromin samples taken from *dotA*$^-$ colonies, % inhibition for wild type and colony 8A1 samples were multiplied by 10.

Appendix 1C and appendix 1D DNA obtained from *D. pini* and present in plasmids pR204 and pR208 is represented by

ABSTRACT

The polyketide dothistromin is a toxin produced by the fungus *Dothistroma pini* and is thought to play a role in causing *Dothistroma* needle blight on the pine *Pinus radiata.* Dothistromin is structurally similar to aflatoxin B1 (AF), a highly carcinogenic toxin with no known function, that is produced by the fungus *Aspergillus parasiticus.* The structural similarities between AF and dothistromin suggest that genes homologous to AF biosynthetic genes found in *D. pini* are dothistromin biosynthetic genes. AF biosynthetic genes in *A. parasiticus* and *A. flavus* are clustered, as are the biosynthetic genes of the structurally similar sterigmatocystin in *A. nidulans.* Dothistromin biosynthetic genes are also likely to be clustered. Two λ clones, λCGV1 and λBMKSA, containing different portions of this putative dothistromin cluster, have been isolated in previous studies.

In this study one gene contained on the clone λCGV1 coding for a putative dothistromin ketoreductase *dotA* (80.2% identical to *A. parasiticus* AF biosynthetic gene *ver-1*) was disrupted with the hygromycin B resistance gene (*hph)* using targeted disruption via homologous recombination. *dotA*$^-$ mutants were tested for dothistromin production and shown to produce at least 10 – 43 times less than the wild type strain. This confirmed that *dotA* is involved in dothistromin biosynthesis. Further more, *dotA*$^-$ mutants accumulated the intermediate versicolorin A. This finding provides evidence that λCGV1 contains a portion of the dothistromin biosynthetic gene cluster and the presence of versicolorin A suggests pathway by which dothistromin is synthesised. Other genes homologous to AF and ST biosynthetic genes contained on λCGV1 can now be disrupted in order to determine the extent of the dothistromin biosynthetic cluster on λCGV1. Dothistromin deficient mutants can also be used to determine the role of dothistromin in the pathogenicity of *D. pini.*

Further nucleotide sequencing of the clone λBMKSA revealed the promoter region and the N terminal amino acid encoding sequence of the putative dothistromin polyketide synthase PKS^{DOT}. The partial PKS^{DOT} sequence (amino acids 1-1426) is 62% identical to the *A. parasiticus* PKSA involved in AF biosynthesis. Preparations to disrupt PKS^{DOT} were made and disruption will confirm its presence on the dothistromin biosynthetic pathway.

Sequencing of λBMKSA in this study also revealed a putative dothistromin p450 mono-oxygenase gene, *dcm1,* providing more evidence that λBMKSA contains part of the dothistromin pathway. The amino acid sequence of *dcm1* is 59% identical to CYPX from the *A. parasiticus* AF cluster and 56% identical to STCB from the *A. nidulans* ST cluster. The function of these homologs has not been ascertained. The discovery of a homolog in *D. pini* (a species only thought to contain genes for the first part of the AF/ST pathway) provides information about the function of these homologs in AF and ST biosynthesis.

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