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

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


Janet Margaret Seconi

2001

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, λ CGV1 and λ BMKSA, containing different portions of this putative dothistromin cluster, have been isolated in previous studies.

In this study one gene contained on the clone λ CGV1 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 λ CGV1 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 λ CGV1 can now be disrupted in order to determine the extent of the dothistromin biosynthetic cluster on λ CGV1. 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 λ BMKSA 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 λ BMKSA in this study also revealed a putative dothistromin p450 monooxygenase gene, *dcm1*, providing more evidence that λ BMKSA 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.

ACKNOWLEDGEMENTS

Firstly to Rosie Bradshaw for being the most patient and inspiring supervisor I could ever have hoped for.

To all at the Institute of Molecular Biosciences. Thankyou for all of your advice and contributions to the project. Special thanks to fungal knockout gurus Carolyn Young and Lisa McMillan (for always being on hand to answer stupid questions) and Dr. Malcolm Chick for invaluable contributions to helping us understand the dothistromin biosynthetic pathway and isolation of the intermediates.

Thanks to those outside Massey without whom we would never have been able to complete the project. Bill Jones and Dawn Harvey at Hort Research for doing the ELISA work and to those at the Southern Regional Research Center, New Orleans, who detected the intermediates for us.

Financial help for myself and the project was received and much appreciated from the Robert C. Bruce Trust, New Zealand Plant Protection Society, Queenstown Molecular Biology Society, Massey University Affinity Card Alumni and Mum and Dad.

Personally I would like to thank all those who have been there for me over what has not been an easy two and a half years. To my family at Christian Community Church for all your prayers, support, friendship and food! I have been so blessed by all of you. To all those I ever had lunch with, Beccy, Bec, Anton, James, Rowanosaurus, Jonoasaurus, Andrea and of course those who cleaned up after us! Special thanks must go to the Kairanga whanau; Alan, Sheralee, Seamus and Paula. Thankyou so much for all those little treats that lightened my weeks, cool trips, good food, shelter when I had no home, great company and not leastly being such lovely friends.

Of course my wonderful family, Mum, Dad and Tim. Love you guys. Thanks for always loving me, understanding and being so patient. I could never have done this without your love and support.

Lastly but most definitely not leastly, I praise the Lord for his faithfulness over the last two and a half years and helping me every single day of this study!

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