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**IDENTIFICATION OF DOTHISTROMIN  
BIOSYNTHETIC PATHWAY GENES**

A thesis presented in partial fulfilment of  
the requirements for the degree of  
Masters of Science in Molecular Genetics

at

Massey University, Palmerston North  
New Zealand

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1996

## 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  $\lambda$ CGN2. 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,  $\lambda$ CGV1 and  $\lambda$ CGV2, 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 *SalI* region from clone  $\lambda$ CGV1 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  $\lambda$ CGV2 suggested that a second independent copy of a *ver-1*-like gene may also be present in the genome.

## ACKNOWLEDGEMENTS

I feel very overwhelmed and honoured when I think of all the people I would like to thank for their contribution to my project and to helping me through recent really difficult times. Firstly, I would like to thank my supervisor, Rosie Bradshaw. Her never ending faith in me, encouragement, guidance, and kindness kept me going, and enabled me to reach a goal that I wasn't sure I could reach. Thank you so much to Tania and Paul, without whom none of this would have been possible. I couldn't have won the battle without such wonderful, dedicated, patient, and loving friends. Thank you also to my parents for their love, strength, and financial support. Thank you to my brother and sister for their support. Because my project was done in two parts there are lots of people to thank for getting me started, and other people to thank for re-teaching me and building up my confidence again. Thank you to all of you. Thank you to all the people I did fourth year with, for making such a difficult year more bearable. Thank you Dianne and Karyn for getting me started on my lab work. Thank you to Carolyn for her always useful advice, and for saving lots of my sequencing gels. Thanks also to Mike and Rich for helpful tips. Thanks to Austen for his friendship. Thank you to Tash for keeping my project going while I was away. Thank you to Anita for always being really positive, especially for making the lab a fun place to be in. Special thanks must also go to Linda for her friendship, and her patience in helping me belong again in the lab. Thank you to Brendon for reminding me how to run a sequencing gel, and to Paul for teaching me sequencing the first time around. Thanks to Bran for late night company in the lab. Thank you to David for drawing my chemical structures. Thanks again to Tania, this time for helping me draw my thesis figures. Thank you to all the Friday night staff club regulars for keeping me sane and refreshed. Thank you to all the other past and present people in the MGU I haven't mentioned. Thank you to all my friends outside of the department, especially Craig and Maree for their support from afar, and Justin for always being there if I needed him. I would also like to thank my flatmates for keeping me in touch with reality over the last few months, especially Tony "I love birds, eh" Roeven for his reassurance and tolerance. I'm sure I've forgotten heaps of people who also played a really valuable part in getting me where I am today, so even if you're not on this list I would like to thank you. Thanks must also go to Molecular Genetics Unit for providing the facilities and financial assistance that enabled me to undertake this project, especially Barry for getting our lab a freezer. In addition, thank you to John Linz (Michigan State University) for providing the *Asperillus parasiticus nor-1* and *ver-1* gene clones.

# TABLE OF CONTENTS

ABSTRACT		ii
ACKNOWLEDGEMENTS		iii
TABLE OF CONTENTS		iv
LIST OF TABLES		xi
LIST OF FIGURES		xii
1.0	INTRODUCTION	1
1.1	General Features	1
1.2	Infection	2
1.3	Chemical Control	4
1.4	Resistant Strains	5
1.5	Dothistromin Toxin	6
1.6	Inactivation of Dothistromin	10
1.7	Aflatoxin Biosynthesis	13
1.8	Gene Cloning Strategies in <i>Aspergillus</i> Species	14
1.9	Organisation and Arrangement of AF/ST Biosynthetic Pathway Genes	18
1.10	Aims and Objectives	20
2.0	MATERIALS AND METHODS	22
2.1	Bacterial and Fungal Strains, $\lambda$ Clones and Vectors	22
2.2	Media	22
2.2.1	Fungal Media	22
2.2.1.1	<i>D. pini</i> Media (DM) Broth	22
2.2.1.2	<i>D. pini</i> Media (DM) Agar	22
2.2.1.3	Malt Extract Agar (MEA)	22
2.2.1.4	Malt Yeast Glucose (MYG) Agar	23

2.2.1.5	Yeast Peptone Glycerol (YPG) Agar	23
2.2.1.6	Potato Dextrose Agar (PDA)	23
2.2.1.7	Nutrient Malt Yeast (NMY) Agar	23
2.2.1.8	Minimal Medium (MM) Agar	23
2.2.1.9	Nutrient Yeast (NY) Broth	23
2.2.1.10	Nutrient Malt Yeast (NMY) Broth	23
2.2.2	Bacterial Media	26
2.2.2.1	Luria-Bertaini (LB) Media	26
2.2.2.2	TB Top Agar	26
2.2.2.3	NZCYM	26
2.3	Growth and Maintenance of Cultures	26
2.3.1	Fungal Cultures	26
2.3.2	Bacterial Cultures	27
2.4	Buffers and Solutions	27
2.4.1	TEG	27
2.4.2	Tris-Equilibrated Phenol	27
2.4.3	TE Buffer	28
2.4.4	DNase free RNase	28
2.4.5	10 x TAE Buffer	28
2.4.6	10 x Gel Loading Dye	28
2.4.7	20 x SSC	28
2.4.8	TES (10/1/100)	28
2.4.9	50 x Denhardt`s	29
2.4.10	SM Buffer	29
2.4.11	Acrylamide Mix	29
2.4.12	10 x TBE Sequencing Buffer	29

2.5	DNA Preparations	29
2.5.1	Alkaline Lysis <i>E. coli</i> Plasmid Preparation	29
2.5.2	Large Scale <i>D. pini</i> Genomic DNA Preparation	30
2.5.3	Preparation of Genomic DNA for <i>D. pini</i> Library Construction	31
2.5.4	Mini-prep of $\lambda$ Phage DNA	32
2.5.5	Extraction of DNA from Seaplaque Agarose	33
2.5.5.1	Bio 101 GeneClean kit	33
2.5.5.2	Gibco BRL GlassMax DNA Isolation Spin Cartridge System kit	33
2.5.6	Purification of DNA	34
2.6	DNA Manipulations	34
2.6.1	Restriction Enzyme Digests	34
2.6.1.1	Plasmid Digests	34
2.6.1.2	Genomic Digests	35
2.6.2	Agarose Gel Electrophoresis	35
2.6.2.1	Minigels	35
2.6.2.2	Overnight Gels	35
2.6.3	Determination of Fragment Sizes	36
2.6.4	Determination of DNA Concentration	36
2.6.4.1	Concentration Standards	36
2.6.4.2	Spectrophotometric Method	36
2.6.4.3	Fluorometric Method	37
2.7	Subcloning	37
2.7.1	Preparation of Insert DNA	38
2.7.2	Linearisation and CAP-Treatment of Vector DNA	38
2.7.3	Ligation	38
2.8	Transformation of <i>E. coli</i>	39
2.8.1	Calcium Chloride Transformation	39

2.8.1.1	Preparation of CaCl <sub>2</sub> Competent Cells	39
2.8.1.2	Transformation	39
2.8.2	Transformation of <i>E. coli</i> by Electroporation	39
2.8.2.1	Preparation of Electro-Competent <i>E. coli</i> Cells	39
2.8.2.2	Electroporation	40
2.9	DNA Hybridisations	40
2.9.1	Southern Blotting	40
2.9.2	Random-Primer Labelling of Probes	41
2.9.3	Separation of Unincorporated Nucleotides by Minispin Column Chromatography	42
2.9.4	Hybridisation of Probe DNA to Southern Blots	42
2.9.5	Autoradiography of Southern Blots	43
2.9.6	Stripping Filters	43
2.10	Genomic Library Construction	43
2.10.1	Establishing Conditions for Partial Digestion of High Molecular Weight Genomic DNA	43
2.10.2	Large Scale Preparation of Partially Digested DNA	44
2.10.3	Partial Fill-in Reaction for Genomic DNA	44
2.10.4	Ligation of Insert to Vector Arms	45
2.10.4.1	Klenow/Ligation Controls	45
2.10.4.2	Determination of Optimum Ligation Conditions	46
2.10.5	Packaging of Ligated DNA and Titration of Recombinant Phage	46
2.10.5.1	Packaging of Ligated DNA	46
2.10.5.2	Titration of Packaged Phage on LB Plates	46
2.10.6	Large Scale Packaging of Ligated DNA	47
2.10.7	Amplification of the Library	48
2.11	Library Screening by Plaque Hybridisation	49
2.11.1	Plating Phage $\lambda$	49



2.11.2	Filter Lifts	49
2.11.3	Hybridisation of Phage $\lambda$ DNA [ $\alpha$ - $^{32}$ P]dCTP Labelled DNA	50
2.12	DNA Sequencing	50
2.12.1	Preparation of DNA for Sequencing	50
2.12.1.1	Preparation of Single Stranded M13 Template DNA	50
2.12.1.2	Preparation of Template DNA for AmpliCycle Sequencing	51
2.12.2	Sequenase Version 2.0 Protocol	51
2.12.3	AmpliCycle Sequencing Protocol	52
2.12.4	Polyacrylamide Gel Electrophoresis (PAGE) of Sequencing Reactions	52
3.0	RESULTS	56
3.1	Determining Optimal Mycelial Growth Conditions in Culture	56
3.1.1	Determining Optimal Solid Media for Mycelial Growth	56
3.1.1.1	Temperature Effect on Mycelial Growth	56
3.1.1.2	Media Effect on Mycelial Growth	56
3.1.1.3	Effect of Inoculation Method and Incubation Period on Mycelial Growth	61
3.1.2	Quantitation of Mycelial Growth in Liquid Media	61
3.2	Southern Hybridisations	64
3.2.1	Detection of a <i>D. pini</i> Region Heterologous to the <i>A. parasiticus</i> <i>nor-1</i> Gene	64
3.2.2	Detection of a <i>D. pini</i> Region Heterologous to the <i>A. parasiticus</i> <i>ver-1</i> Gene	64
3.3	Library Construction	68
3.4	Isolation of $\lambda$ Clones Hybridising to the <i>A. parasiticus</i> <i>ver-1</i> Gene	74
3.4.1	Library Screening	74
3.4.2	Restriction Digestion of $\lambda$ Clones and Southern Hybridisation	74
3.5	Further Characterisation of Clone $\lambda$ CGV1	80

3.5.1	Mapping Clone $\lambda$ CGV1 Further	80
3.5.2	Subcloning of a $\lambda$ CGV1 Region Required for Sequencing	85
3.5.3	Sequence Analysis of Clone $\lambda$ CGV1	85
3.5.4	Sequence Identification	85
3.5.5	Sequence Comparison	90
3.5.6	Comparison of Intron Positions	90
3.5.7	Comparison of GC Content	90
3.5.8	Comparison of Codon Usage	93
3.6	Further Characterisation of Clone $\lambda$ CGV2	93
3.6.1	Further Hybridisation Analysis of Clone $\lambda$ CGV2	93
3.6.2	Subcloning of a $\lambda$ CGV2 Region Required for Sequencing	93
3.6.3	Sequence Analysis of Clone $\lambda$ CGV2	93
3.6.4	Sequence Identification	97
3.6.5	Comparison Between <i>D. pini</i> Sequences	97
3.6.6	Comparison of GC Content	97
3.7	Isolation and Characterisation of $\lambda$ Clone Hybridising to the <i>A. parasiticus nor-1</i> Gene	97
3.7.1	Library Screening	97
3.7.2	Restriction Digestion of Clone $\lambda$ CGN2 and Southern Hybridisation	100
4.0	DISCUSSION	104
4.1	Identification of Clone $\lambda$ CGV1	104
4.2	Role of the <i>ver-1</i> -like Genes	104
4.2.1	The Role of the <i>Aspergillus ver-1</i> and <i>ver-A</i> Genes	104
4.2.2	The Role of the <i>ThnR</i> and <i>Thr1</i> Genes	106
4.3	Duplication of the <i>ver-1</i> Gene	107

4.4	Identification of Clone $\lambda$ CGV2	108
4.5	Identification of Clone $\lambda$ CGN2	110
4.6	Potential Uses of the <i>D. pini ver-1</i> Gene	111
4.6.1	Cloning of Other Pathway Genes	111
4.6.2	Gene Disruptions	113
4.6.3	Identification of Molecular Mechanisms which Regulate Pathway Genes	114
5.0	SUMMARY AND CONCLUSIONS	116
	APPENDIX 1.0	118
	APPENDIX 2.0	122
	REFERENCES	124

## LIST OF TABLES

Table 1	Fungal and Bacterial strains, $\lambda$ Clones and Vectors	24
Table 2	Primers used in Sequencing Reactions	53
Table 3	Optimisation of Media and Method of Inoculation	62
Table 4	Quantitation of Mycelial Growth with Malt Extract Concentration	63
Table 5	Titres of Small Scale, Large Scale, and Amplified Libraries	75
Table 6	Data from Restriction Mapping of Clone $\lambda$ CGV1	81
Table 7	Data from Southern Hybridisation Analysis of Clone $\lambda$ CGV2	82
Table 8	Codon Bias Table	94
Table 9	Data from Southern Hybridisation Analysis of Clone $\lambda$ CGN2	103

## LIST OF FIGURES

Fig. 1	Structures of sterigmatocystin, aflatoxin B1, and dothistromin	8
Fig. 2	Comparison of the aflatoxin biosynthetic pathway with the proposed dothistromin biosynthetic pathway	15
Fig. 3A-F	Demonstration of media differences on mycelial growth and appearance	57
Fig. 4A-C	Southern blot of <i>D. pini</i> genomic DNA probed with <i>nor-1</i>	65
Fig. 5A-C	Southern blot of <i>D. pini</i> genomic DNA probed with <i>ver-1</i>	69
Fig. 6A-B	Profiles of partial <i>Mbo</i> I digestions of genomic DNA from <i>D. pini</i>	72
Fig. 7A-B	Mapping the position of <i>ver-1</i> on clone $\lambda$ CGV1	76
Fig. 8A-B	Mapping the position of <i>ver-1</i> on clone $\lambda$ CGV2	78
Fig. 9A-B	Further mapping analysis of clone $\lambda$ CGV1	83
Fig. 10A-B	Restriction map of clone $\lambda$ CGV1 from a <i>D. pini</i> genomic library that hybridised to <i>Aspergillus parasiticus ver-1</i>	86
Fig. 11	Partial sequence of the putative <i>D. pini ver-1</i> gene from clone $\lambda$ CGV1	88
Fig. 12	Comparison of <i>D. pini</i> sequence to other amino acid sequences	91
Fig. 13	Southern blot of $\lambda$ CGV2 probed with the <i>D. pini ver-1</i> fragment	95
Fig. 14	Partial sequence of a <i>ver-1</i> hybridising fragment from clone $\lambda$ CGV2	98
Fig. 15A-B	Southern blot of $\lambda$ CGN2 probed with <i>nor-1</i>	101