Further Studies of Dothistromin Toxin Genes in the Fungal Forest Pathogen *Dothistroma septosporum*

A thesis presented in partial fulfillment of the requirements

for the degree of Master of Science in Biochemistry

at Massey University, Palmerston North, New Zealand

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2007

ABSTRACT

The fungal pathogen *Dothistroma septosporum* is the main causal agent of *Dothistroma* (red-band) needle blight, which is a devastating foliar disease of a wide range of pine species. Dothistromin is a difuranoanthraquinone toxin produced by *D. septosporum* and is considered as a possible virulence factor for the disease. Based on the similarity of chemical structure between dothistromin and aflatoxin (AF) /sterigmatocystin (ST) precursors, nine putative dothistromin biosynthetic genes have been identified, which are homologous to their corresponding genes in the AF/ST gene clusters. However, in contrast to all 25 AF biosynthetic genes tightly clustered in one region (70-Kb) of the genome, the dothistromin gene clusters are located on a 1.3-Mb chromosome and separated into three mini-clusters along with non-dothistromin genes.

The *dotC* gene, located in the mini-cluster 1, is predicted to encode a major facilitator superfamily (MFS) membrane transporter involved in secretion of dothistromin. In this work, by constructing DotC-eGFP fusion protein containing mutants, the subcellular localization of the DotC protein was determined to be mainly targeted to the plasma membrane. The biological function of the *dotC* gene was characterized by targeted gene disruption. The *dotC* gene disrupted mutants showed a significant reduction of dothistromin production in both the medium and mycelium. In addition, the exponential growth of *dotC* null mutants was inhibited when exogenous dothistromin was presented and these mutants also displayed more sensitivity than the wild type strain to exogenous dothistromin. The results indicated that the DotC protein is a membrane associated protein and might have a role in dothistromin production and be involved in secretion of exogenously supplied dothistromin toxin.

Two novel dothistromin biosynthetic genes, *norA/B* and *verB* (partial sequence), were identified by using degenerate PCR and *D. septosporum* genomic library screening. The putative NorA/B and VerB are postulated to encode a dehydrogenase and a desaturase, respectively and are similar to AF/ST genes. These findings further confirmed that the dothistromin shares biosynthetic pathway steps with AF/ST.

ACKNOWLEDGEMENTS

First and foremost, I would like to gratefully acknowledge my supervision Dr Rosie Bradshaw for her constant encouragement and guidance during this research. Thanks for your kindness and patience to make the last two and half years be the wonderful and inspiring time in my life.

It is a pleasure to thank my lab colleagues. Thanks for Shuguang always ready with helpful technical support and advice. Thanks for Ping sharing her knowledge to give me an easy beginning in the lab. Thanks for Arne kindly providing GFP and microscopy technical support. Thanks for Naydene for her ELISA support and English help. Thanks for Yanan and Justine, it was so happy to work together in the "fungaljungle".

I would also like to thank Dr Kathryn Stowell for giving me very useful advice when I first stepped into the IMBS. Thanks for Kathy Hamilton arranging my study course. Many thanks for other staff working in the IMBS, who gave me help over the duration of my studies.

Finally I would like to thank my parents and my wife. With your understanding and encouraging, I could easily stay by myself in Palmy, which is a quiet and small town far away from Auckland and China.

ABBREVIATIONS

ABC transporter	ATP-binding cassette transporter
AF	aflatoxin
amp^r	ampicillin resistance
bp	base pair
cm	centimeter
°C	degree celsius
dATP	deoxyadenosine triphosphate
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleotide triphosphate
Doth	dothistromin
Fig.	figure
g	gram
GFP	green fluorescent protein
eGFP	enhanced green fluorescence protein
IPTG	isopropyl-β-d-thiogalactoside
Kb	kilobase pair
L	litre
M	molar
Mb	megabase
MFS transporter	major facilitator superfamily transporter
ml	milliliter
mM	millimolar
OD_{600}	optical density at 600 nm
ORF	open reading frame
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
SDS	sodium dodecyl sulfate
ST	sterigmatocysin
TMD	transmembrane domain
µl	microlitre
µM	micromolar
µg	microgram
UV	ultraviolet
v/v	volume per volume
WT	wildtype
w/v	weight per volume
X-Gal	5- bromo-4-chloro-3-indolyl-β-D-galactopyranoside
~	approximate

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