

**Enhanced biocontrol options for
the Australian sugar industry:
a proteomic approach**

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A thesis submitted in fulfillment of the
requirements for the degree of
Doctor of Philosophy

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November 2009

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Abstract

The filamentous fungus *Metarhizium anisopliae* is a naturally occurring biological control agent of many insects including the greyback canegrub (*Dermolepida albobirtum*), a sugarcane pest in Australia. While there have been some gene-based approaches into identifying determinants for biological control and developing improved strains, this study provides a new comparative proteomics approach into identifying key proteins produced by *M. anisopliae* during infection of greyback canegrubs.

Pathogenicity-related proteins have been identified by both liquid and solid culture approaches using proteomic technologies. Proteome maps of healthy canegrubs, canegrubs infected with *Metarhizium* and fungus only were produced and analysed. Comparative proteome analysis of proteins produced in solid culture provided a view into cellular reactions triggered in the canegrub in response to *Metarhizium* infection. Some of the proteins identified included cytoskeleton proteins, proteases, peptidases, metalloproteins and proteins involved in signal transduction. Liquid culture approach was used to display secreted proteins of *Metarhizium* growing on the whole greyback canegrubs and their isolated cuticles. Proteins identified included 64-kDa serine carboxypeptidase, 1,3 β -exoglucanase, dynamin GTPase, THZ kinase, calcineurin like phosphoesterase and phosphatidylinositol kinase. These proteins have not been previously identified from the culture supernatant of *M. anisopliae* during infection. To our knowledge, this is the first proteomic map established to study the extracellular proteins secreted by *M. anisopliae* (FI-1045), a strain currently used for biological control of greyback canegrubs.

Metarhizium anisopliae strain FI-1045 was further subjected to UV mutagenesis to select mutants that can tolerate better environmental conditions such as varying temperature and pH

ranges. *M. anisopliae* mutant strain (NM10) was isolated and bioassays against greyback canegrubs proved that the mutant strain was more virulent than the parental strain. Two-dimensional electrophoresis was employed to display secreted proteins of the *M. anisopliae* mutant strain (NM10) growing on the whole greyback canegrubs and their isolated cuticles, in order to identify various proteins involved in infection of canegrubs. Eighty six secreted proteins were identified in this approach, amongst them six proteins that have not been previously identified from the culture supernatant of *M. anisopliae* during infection. These included the 56-kDa aspartyl aminopeptidase, 29-kDa secreted aspartyl protease, cyclin-dependent protein kinase, thymidylate kinase, septin and adenylate kinase. Finally, mutant strain NM10, generated by UV-mutagenesis was stably transformed and found to be highly resistant to benomyl, a commonly used fungicide in agriculture. Benomyl resistant transformants were found to tolerate 40 times higher concentration of benomyl than then amount that inhibits the parental strain. Laboratory bioassays proved that four transformants resistant to benomyl retained virulence characteristics against greyback canegrubs.

The proteomic methods established, developed and applied in this thesis proved their strength and suitability in the visualisation, detection and identification of proteins produced by the fungus during infection of greyback canegrubs. Genetic manipulation techniques such as mutagenesis and transformation methods described in this thesis demonstrated successful steps in improving the *Metarhizium* strain while retaining pathogenicity against the greyback canegrubs. Combining the proteomics data obtained in this work with other ‘omics’ data such as genomics, transcriptomics, metabolomics and bioinformatics, will lead to a more complete understanding of the biology of canegrub infection by *Metarhizium* at the molecular level.

Declaration

This research thesis contains original work, which was performed by me. Many features of this research work have been conducted in partnership with others. These people have been acknowledged and their contributions have been recognised in the section where help was received. To the best of my knowledge it contains no material previously published and all information adapted has been promptly recognised in the text. No part of this thesis has been presented to any other institution for any other award. I consent to this thesis being made available for photocopy or for loan.

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List of manuscripts for publication

This thesis is prepared according to journal style format and on the following articles, referred to in the text by the Roman numerals listed below. Manuscripts of these articles have been sent to different journals for publication.

Manuscript I:

Manalil NS, Te'o VSJ, Braithwaite K, Brumbley S, Samson P, Grinyer J, Nevalainen H (2008) Proteomic map of the greyback canegrub, *Dermolepida albohirtum* (Waterhouse).
Under review in Australian Journal of Entomology, submitted in November 2008.

Manuscript II:

Manalil NS, Te'o VSJ, Braithwaite K, Brumbley S, Samson P, Nevalainen H (2009) A proteomic view into infection of greyback canegrubs (*Dermolepida albohirtum*) by *Metarhizium anisopliae*.
Published in Current Genetics 2009 Oct; 55(5):571-81.

Manuscript III:

Manalil NS, Te'o VSJ, Braithwaite K, Brumbley S, Samson P, Nevalainen H (2009) Comparative analysis of the *Metarhizium anisopliae* secretome in response to exposure to the greyback canegrubs and grub cuticles.
Accepted for publication in Mycological Research, with minor modifications in September 2009.

Manuscript IV:

Manalil NS, Te'o VSJ, Braithwaite K, Brumbley S, Samson P, Nevalainen H (2009) Proteomic analysis of the secretome of *Metarhizium anisopliae* in response to exposure to the greyback canegrubs and grub cuticles.
Under review in Current Genetics, submitted in September 2009.

Abbreviations

Abbreviations frequently used in the text are:

2D	Two-dimensional
2-DE	Two-dimensional electrophoresis
3D	Three-dimensional
AMP's	Antimicrobial peptides
BCA	Biological control agent
BHC	Benzene hexachloride
BT	<i>Bacillus thuringiensis</i>
BV	Baculoviridae
C7BzO	3-(4-Heptyl)phenyl 3-hydroxy propyl dimethyl ammonio propane sulfonate
CBB	Coomassie brillitant blue
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propane sulfonate
CID	Collision-induced dissociation
CSI	Cross species identification
DIGE	Differential gel electrophoresis
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
DTX	Destruxin
EPF	Entomopathogenic fungi
eV	Collision energy
FS	Fluorescent stains
GV	Granulosis viruses
IEF	Isoelectric focusing
IPG	Immobilised pH gradient
IPM	Integrated pest management
kDa	Kilodaltons
LC	Liquid chromatography
MALDI	Matrix assisted laser desorption ionisation
MCA	Microbial control agent
MS	Mass spectrometry
MS/MS	Mass spectrometry/mass spectrometry
m/z	Mass to charge ratio

MudPIT	Multi-dimensional protein identification technology
MW	Molecular weight
NAG	N-acetylglucosaminidase
NMR	Nuclear magnetic resonance
NVP	Nuclear polyhedrosis viruse
PAGE	Polyacrylamide gel electrophoresis
PEG	Polyethylene glycol
PCR	Polymerase chain reaction
pI	Isoelectric point
PMF	Peptide mass fingerprinting
PR	Protease
PTM	Post-translational modification
QqQ	Triple quadrupole
QTOF	Quadrupole time of flight
SDS	Sodium dodecyl sulphate
TBP	Tributylphosphine
TOF	Time of flight

Acknowledgements

This work was carried out at the Enzyme Development and Gene Expression (EDGE) lab in the department of Chemistry and Biomolecular Sciences in Macquarie University. Completion of this project involved many people's co-operation, advice and support.

First of all, I would like to express my sincere gratitude to Prof. Helena Nevalainen for giving me the opportunity to study and for her supervision, guidance, time, patience and advice throughout my PhD study. Secondly, I would like to thank my co-supervisor Dr. Junior Te'o for his supervision, advice, assistance and management of the project during my PhD study. I would also like to thank my other co-supervisor's Dr. Kathy Braithwaite, Dr. Stevens Brumbley and Dr. Peter Samson for their continuous support and guidance throughout the course of my research.

I sincerely thank Dr. Kathy Braithwaite for her advice, coordination and support during the period of my research. I would also like to thank her for correcting my manuscripts and sending me fungal cultures whenever requested. I also like to thank Dr. Peter Samson for continuous supply of canegrubs for bioassays, for correcting my thesis and also for effective communication.

I warmly thank Dr. Jasmine Grinyer for her advice, help, time and support extended throughout the time of the research. She has been a good guide in teaching me proteomic techniques. I also like to thank all members of the EDGE lab for their help during this study.

I would like to thank my parents Prof. C.S. Sampangiram and Usha. S. Ram for supporting me to pursue my studies overseas and for their continuous love and encouragement. Without

them I would have never achieved my dream. I would like to thank my sister Nagashri for looking after my parents and supporting me to complete my studies overseas.

Completing my thesis has been harder than I expected with a young family. I sincerely thank my husband Shoby Manalil for helping me complete my research. He has dealt with all my frustrations, ups and down during my study years and has been a backbone of our family. Words aren't enough to acknowledge his continuous love and support showered during the hard times of my study. I would like to thank my baby boy Ashish Manalil who was born during the course of my research and staying with grandparents in India. I also thank my in-laws for taking good care of my son in India while I am studying in Sydney. My research has been much easier to complete with your endless help and support.