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

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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 albohirtum*), 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. ansiopliae* (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.

Nirupama Shoby Manalil

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.
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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	*Bacillus thuringiensis*
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

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