UNIVERSITY OF TECHNOLOGY, SYDNEY

Proteomic Investigation of the Genome-Reduced Pathogen, *Mycoplasma hyopneumoniae*

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Declaration

I, Jessica L. Tacchi, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.
I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.
This thesis includes chapters that have been published, or are in preparation for publication in refereed journals. I declare that I was primarily responsible for data collection, analysis and interpretation and responding to editing suggestions from co-authors. The contributions from other authors are described at the beginning of each chapter.

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Abbreviations

↓	Denotes site of cleavage
1D	One-dimensional
2D	Two-dimensional
6xHis-tagged	Hexahistidine tagged
ACN	Acetonitrile
amu	atomic mass units
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BALF	Bronchoalveolar lavage fluid
BLAST	Basic Local Alignment Search Tool
BM	Basement membrane
BN	Blue native
BSA	Bovine Serum Albumin
C-terminus	Carboxyl-terminus
C7BzO	3-(4-Heptyl)phenyl-3-hydroxypropyl)dimethylammoniopropanesulfonate
CDS	Coding sequences
CN	Clear Native
CO_2	Carbon Dioxide
ddH2O	Double-distilled water
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
ECM	Extracellular matrix
EDTA	Ethylenediamine tetraacetic acid
EfTu	Elongation factor Tu
ELISA	Enzyme-linked immunosorbent assay
EMAI	Elizabeth Macarthur Agricultural Institute
ESI	Electrospray Ionisation
ExPASy	Expert Protein Analysis System
Fn	Fibronectin
FnBP	Fibronectin binding protein
FT	Fourier transform
FT ICR	Fourier transform ion cyclotron resonance
G + C	Guanine and cytosine
GAG	Glycosaminoglycan
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GeLC-MS/MS	Gel-electrophoresis-coupled liquid chromatography tandem mass spectrometry
GO	Gene Ontology
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HLB	Hydrophillic-lipophillic balanced
HRP	Horseradish peroxidase

HSP	Heat shock protein
ID	Inside/Inner Diameter
IDA	Intelligent Data Acquisition
IEF	Isoelectric focusing
Ig	Immunoglobulin
IMAC	Immobilised metal affinity chromatography
IPG	Immobilised pH gradient
IPTG	Isopropyl-β-D-thiogalactopyranoside
KCl	Potassium Chloride
kDa	Kilodalton
kVh	KiloVolt Hours
LB	Luria-Bertani
LC	Liquid chromatography
LPS	Lipopolysaccharide
LTQ	Linear Trap Quadrupole
MØ	Macrophages
MAb	Monoclonal antibody
MALDI	Matrix-assisted laser desorption/ionization
MMP	Matrix metalloproteinases
mRNA	Messenger Ribonucleic Acid
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MudPIT	Multidimensional protein identification technology
N-terminus	Amino-terminus
NH_4HCO_3	Ammonium bicarbonate
OD_{600}	Optical density at 600 nm
ORF	Open reading frame
PAGE	Polyacrylamide gel electrophoresis
PAI-1	Plasminogen activation inhibitor-1
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate buffered saline
PBS-T	Phosphate buffered saline-Tween 20
PDB	Protein Database
PEP	Porcine enzootic pneumonia
p*I*	Isoelectric point
PICS	Proteomic identification of protease cleavage site
PK15 cell	Porcine kidney epithelial-like cell
Plg	Plasminogen
PONDR	Predictor of Naturally Disordered Regions
PPLO	Pleuropneumonia-like organism
ppm	Parts per million
PRRSV	Porcine reproductive and respiratory syndrome virus
PVDF	Polyvinylidene fluroride
Q-TOF	Quadrupole time-of-flight

R1	Repeat region 1
R2	Repeat region 2
rpm	Revolutions per minute
SCX	Strong cation exchange
SDS	Sodium Dodecyl Sulphate
SEM	Scanning electron microscopy
SPE	Solid phase extraction
Sulfo-NHS-LC-biotin	Sulfosuccinimidobiotin long chain biotin
TAILS	Terminal amine isotopic labelling of substrates
TBP	Tributylphosphine
TCA	Trichloroacetic acid
TCA cycle	Tricarboxylic acid cycle
TEM	Transmission electron microscopy
TFA	Trifluoroaceteic Acid
TOF	Time of flight
tPA	Tissue-type plasminogen activator
Tris	2-Amino-2-hydroxymethyl-propane-1,3-diol
Tuf_{Sp}	Elongation factor Tu (Tuf) of *Streptococcus pneumoniae*
Tween-20	Polyoxyethylenesorbitan monolaurate
TX-100	Triton X-100
TX-114	Triton X-114, Octylphenoxy polyethoxyethanol
uPA	Urokinase-type plasminogen activator
uPAR	Urokinase-type plasminogen activator receptor
v/v	Volume per volume
w/v	Weight per volume
WHO	World Health Organisation

Publications

"Sequence TTKF ↓ QE defines the site of proteolytic cleavage in Mhp683 protein, a novel glycosaminoglycan and cilium adhesin of *Mycoplasma hyopneumoniae*."
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"*Mycoplasma hyopneumoniae* Surface proteins Mhp385 and Mhp384 bind host cilia and glycosaminoglycans and are endoproteolytically processed by proteases that recognize different cleavage motifs."
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"MHJ_0125 is an M42 glutamyl aminopeptidase that moonlights as a multifunctional adhesin on the surface of *Mycoplasma hyopneumoniae."*
Robinson MW, Buchtmann KA, Jenkins C, Tacchi JL, Raymond BBA, To J, Roy Chowdhury P, Woolley LK, Labbate M, Turnbull L, Whitchurch CB, Padula MP, Djordjevic SP. *Open Biol.* 2013.

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"Non-proteolytic functions of microbial proteases increases pathological complexity."
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Jarocki VM, Santos J, Tacchi,JL, Raymond BB, Deutscher AT, Jenkins C, Padula MP, and Djordjevic SP. *Open biology* 2015.

Conference Presentations – Presenting author

- 20th Lorne Proteomics Symposium, 2015 – Poster presentation: "Post-translational processing, multifunctionality and moonlighting."
- 19th Lorne Proteomics Symposium, 2014 – Poster presentation: "Proteolytic processing of adhesin proteins on the surface of *Mycoplasma hyopneumoniae"*
- BacPath 12: The Molecular Biology of Bacterial Pathogens, Tangalooma Resort QLD, 2013 – Poster presentation: "Protein Processing and Promiscuity in Pathogenesis"
- 18th Lorne Proteomics Symposium, 2013 – Oral presentation: "It's all about cleavage: generating diversity in a genome-reduced organism"
- Proteomics and Beyond Symposium, Macquarie University, 2012 – Poster Presentation: "Mining 2D gels for a wealth of biological information"
- 17th Lorne Proteomics Symposium, 2012 – Poster presentation: "The Surface Proteome of *Mycoplasma hyopneumoniae*"
- 19th Congress of the International Organisation for Mycoplasmology, Toulouse, France 2012 – Poster presentation: "Endoproteolytic Adhesin Fragments and Geographical Moonlighting Proteins Dominate the Surfaceome of *Mycoplasma hyopneumoniae*"
- 16th Lorne Proteomics Symposium, 2011 – Poster presentation: "Surface Proteins of a Reduced-Genome Pathogen: *Mycoplasma hyopneumoniae*"
- Royal North Shore Hospital Annual Scientific Research Meeting, 2010 – Poster Presentation: "Protein Complexes of *Mycoplasma hyopneumoniae* Revealed by High-Resolution Clear Native Electrophoresis"
- HUPO 9th Annual World Congress, Sydney 2010 – Poster presentation: "Protein Complexes of *Mycoplasma hyopneumoniae* Revealed by High-Resolution Clear Native Electrophoresis"
- Australian Society for Microbiology Annual Scientific Meeting & Exhibition, Sydney 2010 – Poster presentation: "A Comprehensive Proteomics Analysis of *Mycoplasma hyopneumoniae* Strain J"
- 18th Congress of the International Organisation for Mycoplasmology, Chianciano Terme, Italy 2010 – Oral presentation: "A Comprehensive Proteomics Analysis of *Mycoplasma hyopneumoniae* Strain J"

Awards and Scholarships

- Poster Presentation Award – 20th Lorne Proteomics Symposium, 2015
- Student Oral Presentation Award – 18th Lorne Proteomics Symposium, 2013
- Best poster – 17th Lorne Proteomics Symposium, 2012
- IOM Student Travel Award – 19th Congress of the International Organisation for Mycoplasmology, Toulouse, France, 2012
- Student Travel Award to attend the Human Proteome Organization Congress (HUPO) Congress in Sydney, 2010
- Vice-Chancellor's Postgraduate Research Students Conference Funding, 2010
- Faculty of Science Post Graduate Conference Funding, 2010
- IOM Student Travel Award – 18th Congress of the International Organisation for Mycoplasmology, Chianchiano Terme, Italy, 2012
- UTS Doctoral Scholarship, 2010

Abstract

Mycoplasma hyopneumoniae is a genome-reduced bacterium and an economically significant pathogen that chronically infects the respiratory tract of swine. This infection often leads to pneumonia and secondary infections, costing agricultural industries significantly in the use of antibiotics and vaccines, which are currently largely ineffective. An improved understanding of the molecular mechanisms behind the infection process is essential to our ability to rationally design better vaccine and therapeutic interventions. With fewer than 700 predicted protein coding sequences, *M. hyopneumoniae* possesses one of the smallest genomes of any free-living organism. As such, it lends itself well to thorough proteomic interrogation.

In this thesis, a range of proteomic techniques have been used to investigate the *M. hyopneumoniae* global and surface proteome at the protein and peptide level, including surface shaving and labelling techniques, ligand and immuno-blotting and affinity chromatography, as well as N-terminal dimethyl labelling to determine true N-termini of mature proteins. This conceptually unbiased, function-oriented approach has revealed an unexpected level of complexity in the use of proteolytic processing, multifunctional proteins and moonlighting to compensate for reduced coding capacity at the genome level. While microarray and transcriptome studies suggest that under normal culture conditions, the majority of genes are transcribed; our analyses identified less than 400 detectable expressed protein products under similar conditions. A significant number of the expressed proteins were discovered to be multifunctional, post-translationally modified by proteolysis.

Surface proteome analyses identified a range of proteins to be surface exposed, despite lacking known signal peptides. Even though many of these proteins had well-characterised functions in the cytoplasm, they were also identified to have secondary functions at the cell surface, a phenomenon known as moonlighting. Many of the proteins present at the cell surface were identified to be subjected to proteolytic cleavage events. These were predominantly cell surface adhesins, many of which have already been described in the literature, however a large number of cytoplasmic

"housekeeping" proteins are also found to be post-translationally cleaved, multifunctional proteins or moonlighting proteins.

These findings can be applied to improve the rational design and development of vaccines and therapeutics for the prevention and treatment of *Mycoplasma hyopneumoniae*, as well as having wider implications for the field of biology as a whole, if similar levels of post-translational regulation can be found in other bacterial pathogens.