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Expanding the Functional Proteome of *Mycoplasma pneumoniae*

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Certificate of Original Authorship and Declaration

I, Michael Widjaja, 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. This research is supported by an Australian Government Research Training Program Scholarship.

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.

For the following, I, Michael Widjaja, declare that more than 50% of the content in the journal article (Chapter 4) and manuscripts (Chapters 5 and 6) included in this thesis:

- Planning of each chapter was conducted by my supervisors and I.
- Experiments performed and analysed by myself (unless stated).
- The initial draft prepared and written by myself. Any subsequent changes in response to co-authors and editor's reviews were performed by me.

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Publications

1. Berry I.J.[†], Jarocki V.M.[†], Tacchi J.L., Raymond B.B., **Widjaja M.**, Padula M.P., and Djordjevic S.P. (2017) 'N-terminomics identifies widespread endoproteolysis and novel methionine excision in a genome-reduced bacterial pathogen'. *Scientific Reports* (Submitted).

2. (Chapter 5): **Widjaja M.[†]**, Harvey K.L.[†], Hagemann L.[†], Berry I.J., Jarocki V.M., Raymond B.B., Tacchi J.L., Gründel A., Steele J.R., Padula M.P., Charles I.G., Dumke R.[¥], and Djordjevic S.P.[¥] (2017). 'Elongation factor Tu is a multifunctional and processed moonlighting protein'. *Scientific Reports* (Submitted).

3. Trussart M., Yus E., Martinez S., Baù D., Tahara Y.O., Pengo T., **Widjaja M.**, Kretschmer S., Swoger J., Djordjevic S., Turnbull L., Whitchurch C., Miyata M., Marti-Renom M.A., Lluch-Senar M., and Serrano L. (2017). 'Defined chromosome structure in the genome-reduced bacterium *Mycoplasma pneumoniae*'. *Nature Communications*.

4. Tacchi J.L., Raymond B.B., Haynes P.A., Berry I.J., **Widjaja M.**, Bogema D.R., Woolley L.K., Jenkins C., Minion F.C., Padula M.P., and Djordjevic S.P. (2016). 'Post-translational processing targets functionally diverse proteins in *Mycoplasma hyopneumoniae*'. *Open Biology*.

5. (Chapter 4): **Widjaja M.**, Berry I.J., Pont E.J., Padula M.P., and Djordjevic S.P. (2015). 'P40 and P90 from Mpn142 are targets of multiple processing events on the surface of *Mycoplasma pneumoniae*'. *Proteomes*.

6. Raymond B.B., Jenkins C., Seymour L.M., Tacchi J.L., **Widjaja M.**, Jarocki V.M., Deutscher A.T., Turnbull L., Whitchurch C.B., Padula M.P., and Djordjevic S.P. (2014). 'Proteolytic processing of the cilium adhesin MHJ_0194 (P123) in *Mycoplasma hyopneumoniae* generates a functionally diverse array of cleavage fragments that bind multiple host molecules'. *Cellular Microbiology*.

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Abbreviations

Standard abbreviations

Adenine and thymine	A+T
Community Acquired Respiratory Distress Syndrome	CARDS
Dihydrolipoyl dehydrogenase	Pdh-D
Dihydrolipoyllysine acetyltransferase	Pdh-C
Elongation factor Tu	Ef-Tu
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH
Guanine and cytosine	G+C
High Molecular Weight protein	HMW
Isoelectric Point	pI
Liquid chromatography tandem mass spectrometry	LC-MS/MS
Open Reading Frame	ORF
Phosphate Buffered Saline	PBS
Potential of hydrogen	pH
Pyruvate dehydrogenase E1 α subunit	Pdh-A
Pyruvate dehydrogenase E1 β subunit	Pdh-B
Short Linear Motifs	SLiMs
Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis	SDS-PAGE

Unit abbreviations

Base pair	bp	Molar	M
Dalton	Da	Mole percent	Mol%
Gram	g	Percent	%
Hour	h	Percentage weight per volume	w/v
Litre	L	Percentage weight per weight	v/v
Metre	m	Relative centrifugal force	RCF
Minute	min	Second	s

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Thesis Preface

The growing incidence of antibiotic resistance globally is a significant public health issue and as previously susceptible bacteria continue to develop resistance, we need to develop novel strategies to counter this trend. *Mycoplasma pneumoniae* is a genome reduced bacteria that is one of the major causes of bacterial pneumonia in close contact settings such as schools and hospitals. Children, the elderly, and the immuno-suppressed are commonly infected due to an under developed or impaired immune system. A successful vaccine against this respiratory pathogen is yet to be developed and treatment options are limited. Additionally, children are limited to one class of antibiotics due to the permanent side effects of other agents.

Antibiotic resistance within *M. pneumoniae* was detected over a decade ago and has now spread to most of the Northern Hemisphere. Though infections are not typically fatal, *M. pneumoniae* can cause secondary co-infections; some of which can be fatal. The work presented within this thesis expands the functional proteome of *M. pneumoniae*, with the goal of discovering potential novel therapeutic or vaccine targets. This was initially achieved by examining the full repertoire of proteins exposed on the surface of *M. pneumoniae*. This thesis then addresses which host antigens these proteins potentially interact with during infection. Although a single protein was not chosen as a vaccine target, the result of the work presented here report a list of potential targets that participate in the colonisation of the respiratory epithelium. This thesis highlights that the interactions between *M. pneumoniae* and host epithelium are complex, and involve a wide range of diverse proteins.

This thesis begins with an introduction to *M. pneumoniae* and what is currently known about the proteins involved during the interaction with the human host.