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# Identification and characterisation of immunoreactive antigens of mycoplasma hyopneumoniae

Jody Gorman
*University of Wollongong*

# Identification and Characterisation of Immunoreactive Antigens of *Mycoplasma hyopneumoniae*

**Jody Gorman (Wilton)**

**PhD candidate**

**University of Wollongong**

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# Declaration of Authenticity

This thesis is submitted in accordance with the regulations of the University of Wollongong in fulfillment of the degree of Doctor of Philosophy. It does not include any material previously published by another person except where due reference is made in the text. The experimental work described in this thesis is original, and has not been submitted for a degree to any other university.

Jody Gorman

# Acknowledgements

I started this thesis over 12 years ago and two jobs, two kids, two very patient supervisors, an ever-loving family, many supportive friends and one super-supportive, patient and loving husband later, I am FINALLY submitting. That makes for a very long list of acknowledgements!

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A very big thank you to those mentioned and everybody else, who loved, supported, encouraged and helped me get to this point. I don't know what life without the incredible weight of an unfinished thesis is going to feel like but I suspect it will be pretty good!

**I dedicate this thesis to my children Harrison and Ava.**

**Be everything you can be my beautiful babies!**

# List of Publications

**Wilton**, JL., Scarman, AL., Walker, MJ., and Djordjevic, SP. (1998) Reiterated repeat region variability in the ciliary adhesin gene of *Mycoplasma hyopneumoniae*. Microbiology. **144:**1931-43.

Matic, JN., **Wilton**, JL., Towers, RJ., Scarman, AL., Minion, CF., Walker, MJ., and Djordjevic, SP. (2003) The pyruvate dehydrogenase complex of *Mycoplasma hyopneumoniae* contains a novel lipoyl domain arrangement. Gene. **319:**99-106.

Djordjevic, SP., Cordwell, SJ., Djordjevic, MA., **Wilton**, JL., and Minion, FC. (2004) Proteolytic Processing of the *Mycoplasma hyopneumoniae* Cilium Adhesin. Infect Immun. **72:**2791-802.

Jenkins, C., **Wilton**, JL., Minion, FC., Falconer, L., Walker, MJ., and Djordjevic, SP. (2006) Two Domains within the Mycoplasma hyopneumoniae Cilium Adhesin Bind Heparin. Infect Immun. **74:**481-7.

**Wilton**, JL., Jenkins, C., Cordwell, SJ., Falconer, L., Minion, CF., Oneal, DC., Djordjevic, MA., Connolly, A., Barchia, I., Walker, MJ., and Djordjevic, SP. (2009) Mhp493 (P216) is a proteolytically processed, cilium and heparin binding protein of *Mycoplasma hyopneumoniae*. Mol Microbiol. **71:**566-582.

Jenkins, C., Seymour, L., Deutscher, A., Falconer, L., Stewart, K., **Wilton**, JL., Minion, FC., Rhode, M., Eamens, G., Redacliffe, L., Walker, MJ., and Djordjevic, SP. (submitted) *Mycoplasma hyopneumoniae* triggers the deposition of fibronectin at the site of colonisation.

# Abstract

*M. hyopneumoniae* is the causative agent of porcine enzootic pneumonia (PEP) and inflicts a severe economic burden on swine production. Colonisation involves adherence of *M. hyopneumoniae* to the porcine respiratory epithelia in a process believed to be mediated by surface proteins capable of binding target cells. This thesis involved the identification and characterisation of several immunoreactive *M. hyopneumoniae* antigens in an effort to increase the current understanding of the surface topography of this organism.

The first antigen identified in this research was the now well-characterised cilium adhesin of *M. hyopneumoniae*, P97. P97 was found in earlier research to contain two regions of reiterated repeats (RR1 and RR2). The repeats were found in this research to differ in number amongst strains of *M. hyopneumoniae* from different geographical localities and did not appear present in related porcine mycoplasmas *M. hyorhinis* or *M. flocculare*. Sequences like the proline rich tandem repeats of 5 and 10 amino acids found in RR1 and RR2 respectively are typically involved in protein-protein interactions. Proline rich repeats are usually highly immunogenic (Williamson, 1994) providing the recombinant 35 kDa antigen (encompassing the repeats) produced in this work with vaccine potential.

The second antigen cloned and characterised in this research was identified as a fragment of *M. hyopneumoniae* pyruvate dehydrogenase E1α (PdhA). Its intracellular location was indicated in trypsination studies of whole *M. hyopneumoniae* but its

identification in the insoluble phase as well as the aqueous phase of triton extractions raises the question of a dual function; a phenomenon reported for PdhA in *M. pneumoniae* (Layh-Scmitt *et al*., 2000) and for NrdF in *M. hyopneumonia* (Fagan *et al*., 1996).

The final antigen characterised in this research was identified as a 216 kDa adhesin-like protein of *M. hyopneumoniae* (P216). Four recombinant P216 antigens, covering 75% of the molecule, were cloned, expressed, purified and used to raise antisera in rabbits. P216 was found to be rapidly processed to cleavage fragments P120 and P85, both located on the surface of *M. hyopneumoniae*. A putative transmembrane domain was identified in P120 providing it with a mechanism for cell surface attachment. It has been hypothesized that P85 maintains its association with the cell surface through a KEKE motif and the two regions of glutamine repeats identified in its' sequence. Heparin binding of all three recombinant P216 fragments suggests that P120 contains at least two heparin-binding domains and P85 at least one, but further studies are required to define the exact location of these sites.

This research provided a foundation for continued research on P97 and P216, which remain the only two cilium adhesins of *M. hyopneumoniae* identified to date. Defining the colonisation mechanism of *M. hyopneumoniae* has implications for the treatment and prevention of disease, therefore research in this area provides an improved understanding of pathogenesis and potential vaccine development.

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# List of Abbreviations

| | |
|---|---|
| ABTS | 2,2'-azino-bis(3-ethylebenzthiazoline-6-sulfonic acid) diammonium salt |
| ANGIS | Australian National Genomic Information Service |
| ATCC | American Type Culture Collection |
| bp | base pairs |
| CSPD | disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1$^{3,7}$]decan}-4-yl)phenylphosphate |
| COG | clusters of orthologous groups |
| DAB | diaminobenzidine |
| DIG | digoxigenin |
| DMEM | Dulbecco's modified Eagle medium |
| DNA | deoxyribonucleic acid |
| $^{o}$C | degrees celsius |
| 2D | two-dimensional |
| ECM | extracellular matrix |
| EDTA | ethylenediamine tetra-acetic acid |
| ELISA | enzyme linked immunosorbent assay |
| FCS | foetal calf serum |
| g | gram |
| *g* | g forces for centrifugation |
| GAGs | glycosaminoglycans |
| h | hour |
| IFN-γ | interferon gamma |
| Ig | immunoglobulin |
| IPTG | isopropyl-β-D-thiogalactopyranoside |
| kb | kilobase pairs |
| kDa | kilo daltons |
| L | litre |
| LB | Luria Bertani |
| m | milli |
| M | molar |
| mA | milliamps |
| MALDI-TOF | matrix-assisted laser desorption ionization-time-of-flight |
| μ | micro |
| min | minute |
| mL | milliliter |
| MS | mass spectrometry |
| MSCRAMMs | microbial surface components recognizing adhesive matrix molecules |
| n | nano |
| NCBI | National Centre for Biotechnology Information |
| NrdF | R2 sub-unit of ribonucleotide reductase |
| OD | optical density |
| ORF | open reading frame |
| PBS | phosphate buffered saline |
| PBST | phosphate buffered saline with tween 20 |
| PCR | polymerase chain reaction |
| Pdh | pyruvate dehydrogenase |

| | |
|---|---|
| PEP | porcine enzootic pneumonia |
| pH | picohenry |
| PRDC | porcine respiratory disease complex |
| PRR's | proline rich repeats |
| PVDF | polyvinylidene difluoride |
| rpm | revolutions per minute |
| RR1 | repeat region 1 |
| RR2 | repeat region 2 |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| RT | room temperature |
| sec | second |
| TBE | tris-borate EDTA |
| TBS | tris buffered saline |
| TCA | tricarboxylic acid |
| TX-100 | triton X-100 |
| TX-114 | triton X-114 |
| V | volts |
| v/v | volume per volume |
| w/v | weight per volume |