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**The development of polyester bead-based
particulate subunit vaccine against Johne's
disease**

A thesis presented in partial fulfillment of the
requirements of the degree of
Master of Science
in
Microbiology

Massey University, Palmerston North

Institute of Fundamental Science

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2015

Abstract

Johne's disease is the intestinal infection in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The disease is economically important in the dairy industry as infection of the calves or mature cattle can result in death. Current vaccination as a part of disease control is not only partially protective against MAP but also interferes with current diagnostic test for bovine tuberculosis. Therefore, more effective and defined vaccines are needed. In this study, vaccine candidates were developed by bioengineering *Escherichia coli* to produce polyhydroxyalkanoate (PHA) beads displaying selected vaccine candidate antigens as fusion proteins. The selected antigens were the MAP 85 antigen complex (Ag85A and Ag85B), Superoxide dismutase (SOD) and a recombinant fusion protein 74F, however, only the antigen-presenting beads with truncated Ag85A, Ag85B and SOD were successfully produced and purified. The fusion protein comprising the respective antigens was identified and confirmed to be associated with PHA beads. The PHA beads were partially purified for future characterisations such as binding of antigen specific antibodies on PHA beads *in vitro* and immunological properties in animal models.

Acknowledgement

I would like to thank to Professor Bernd Rehm who served as my supervisor for his support, encouragement and guidance throughout this research project. I also would like to thank to Alan Murray who is my co-supervisor for his helpful advice.

I would like to also thank all my colleagues at the Rehm laboratory and especially Jason Lee for advice, encouragement, patience and guidance. Special thanks to Yajie Wang for advice on this thesis. I also wish to thank to Polybatics Ltd Research division, especially Paul McDermott and Iain Hay who assisted by providing technical advice regarding cell culture and expression techniques.

My special gratitude goes to my family for their endless support.

Abbreviation

°C	Degree Celsius
µm	Micrometers
BCA	Bicinchoninic acid used in protein assay
BSA	Bovine serum albumin
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
GC/MS	Gas chromatography mass spectrometry
HA _{SCL}	Short-chain-length hydroxyalkanoic acid
HA _{MCL}	Medium-chain-length hydroxyalkanoic acid
HRP	Horse radish peroxidase
IgG	Immunoglobulin G
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	Kilo Daltons
kbp	Kilo base pairs
MALDI-TOF MS	Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry
MAP	<i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i>
MOPS	3-(N-Morpholino) propanesulfonic acid
Nile-Red	9-diethylamino-5-benzo[α]phenoxazinone
PB	Phosphate buffer saline
PBS	Phosphate buffer salt
PBST	Phosphate buffer salt tween-20
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutarate
PhaA	β-ketothiolase
PhaB	Acetoacetyl-CoA reductase
PhaC	PHA synthase
<i>PhaCAB</i>	PHA operon
PhaPs	Phasins
PhaR	Transcriptional regulator protein
PhaZ	PHA depolymerase
SDS-PAGE	Sodium dodecyl sulfate Polyacrylamide gel electrophoresis
SOD	Superoxide dismutase
TFF	Tangential flow filtration
X-GAL	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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