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Polyhydroxyalkanoate beads as a particulate vaccine against  
*Streptococcus pneumoniae* and *Neisseria meningitidis*



**A thesis presented in partial fulfilment of the requirements for the degree of**

**Doctor of Philosophy**

**in**

**Microbiology**

**at Massey University, Manawatu, New Zealand.**

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**Co-supervisors: Dr Zoe Jordens, Dr Vicente Vérez-Bencomo**

## **Abstract:**

*Streptococcus pneumoniae* and *Neisseria meningitidis* are the major causes of pneumonia and meningitis, respectively, worldwide. Capsular polysaccharide-protein vaccines (conjugate vaccines) provide protection against these diseases but not protection against infections caused by serotypes and serogroups not included in these vaccines. Proteins have been increasingly considered as antigens for vaccine development due to their more structurally conserved composition when compared to capsular polysaccharides. Proteins subunit vaccines are safe and protective; however, they have limitations such as serotype-dependent immunity, and low immunogenicity of the proteins, requiring adjuvant to be included in these formulations or delivery systems that enhance the desired immune response. In addition, complex production procedures are required, increasing production costs and therefore market prices making these vaccines inaccessible for many people affected by these diseases. Recently, bacterial storage polymer inclusions have been developed as protein antigen carriers. Polyhydroxyalkanoate, in particular 3-polyhydroxybutyrate (PHB) inclusions have been successfully bioengineered to display antigens from pathogens like *Mycobacterium tuberculosis* and Hepatitis C virus. These particulate vaccine candidates elicited both a Th1 and Th2 immunity patterns combined with a protective immune response against *Mycobacterium bovis* in mice.

This thesis focuses on the study of polyhydroxybutyrate (PHB) beads properties as a carrier/delivery system engineered to display antigens from extracellular bacteria. The antigens Pneumococcal adhesin A, Pneumolysin (proteins) and 19F capsular polysaccharide (CPS) from *Streptococcus pneumoniae*, and Neisserial adhesin A, factor H binding protein (proteins) and serogroup C CPS from *Neisseria meningitidis* were displayed on the PHB bead surface. These antigenic proteins were produced as fusion

proteins on the PHB bead surface, while the CPS was covalently attached by chemical conjugation. Mice vaccinated with these PHB beads produced strong and antigen-specific antibody levels. In addition, splenocytes from the same mice generated both IL-17A and IFN- $\gamma$  production.

The antibodies elicited against antigenic pneumococcal proteins were able to recognise the same protein in the context of an *Streptococcus pneumoniae* whole cell lysate from more than six different strains, while antibodies produced after vaccination with 19F CPS conjugate to PHB showed high opsonophagocytic titers against the homologous strain. In the case of *Neisseria meningitidis*, bactericidal antibodies were elicited in mice vaccinated with PHB beads displaying proteinaceous and CPS antigens.

Overall, this thesis shows that PHB as particulate vaccine candidate holds the promise of a broadly protective vaccine that can be produced cost-effectively for widespread application to prevent diseases caused by *Neisseria meningitidis* and *Streptococcus pneumoniae*.

*With eternal love, gratitude and in memory of my mother*

*(Mercedes Miró Alonso, 1945-1993)*



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## Preface

This thesis is written according to the regulations of the Handbook for Doctoral Study, revised in May 2016 by the Doctoral Research Committee. This thesis complies with the format of a thesis based on publication as described in the handbook.

### **Chapter 1**

#### **Introduction**

This chapter was written by Majela González Miró as an introductory chapter for this thesis only and is not intended for publication

### **Chapter 2**

#### **Self-assembled particulate PsaA as vaccine against *Streptococcus pneumoniae* infection**

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### **Chapter 3**

**Biologically assembled polyester beads displaying pneumolysin and capsular polysaccharide induce protective immunity against *Streptococcus pneumoniae***

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### **Chapter 4**

**Bioengineered polyester beads co-displaying protein and carbohydrate-based antigens enhance protective efficacy against bacterial infection**

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## **Chapter 5.**

### **General Discussion, Conclusion and Future work**

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## Abbreviations

APCs: antigen presenting cells

APS: activate polysaccharide

BCA: Bicinchoninic acid assay

CLSM: Confocal Laser Scanning Microscope

CPS: capsular polysaccharide

CFU: colony-forming unit

CON A: Concanavalin A

DC: dendritic cell

DF: Dilution Factor

DIC: Differential interference contrast

DMEM: Dulbecco's Modified Eagle's Medium

DT: Diphtheria toxoid

ELISA: enzyme-linked immunosorbent assay

FCS: fetal calf serum

fHbp: factor H binding protein

GNA2091: genome *Neisseria* antigen 2091

GNA2091-fHbp-PhaC: genome *Neisseria* antigen 2091 fuse to factor H binding protein and PhaC

HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)

Ig: Immunoglobulin

IgG: Immunoglobulin G

IgG1, IgG2a, IgG2b and IgG3: Immunoglobulin G 1,2a,2b,3

IgM: Immunoglobulin M

IL17A: cytokine 17A

INF- $\gamma$ : Interferon gamma

LB: Luria-Bertani broth (Lennox)

*M. bovis*: *Mycobacterium bovis*

*N. meningitidis*: *Neisseria meningitidis*

*S. pneumoniae*: *Streptococcus pneumoniae*

*E. coli*: *Escherichia coli*

MALDI-TOF-MS/MS: matrix assisted laser desorption ionization-time of flight mass spectrometry

MW: molecular weight  
NaBH<sub>3</sub>CN: Sodium Cyanoborohydride  
NadA: *Neisseria* adhesin A  
NadA-PhaC: NadA-PhaC fusion protein  
NIBSC: National Institute for Biological Standards and Control  
OVA: Ovalbumin  
OPA: opsonophagocytic assay  
PBS: Phosphate Buffered Saline  
PCR: polymerase chain reactions  
PHA: Polyhydroxyalkanoate  
PhA:  $\beta$ - ketothiolase  
PhaC: Polyhydroxyalkanoate synthase  
PhB: Acetoacetyl-CoA reductase  
PHB: Polyhydroxybutyrate  
Ply: Pneumolysin  
Ply-PhaC: Pneumolysin fused to PhaC  
PsaA: Pneumococcal surface adhesin A  
Psa-PhaC: Pneumococcal Surface protein A fused to PhaC  
PspA: Pneumococcal Surface protein A  
PspC: Pneumococcal Surface protein C  
rpm: revolutions per minute  
SBA: serum bactericidal activity  
SD: standard deviation  
SDS-PAGE: sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE)  
SEM: standard error of the mean  
TEM: Transmission electron microscopy.  
Th17: Lymphocyte T helper 17  
Triple TOF: mass spectrometry by Triple TOF  
TT: Tetanus toxoid  
TLR: Toll-like receptor  
<sup>0</sup>C: degrees Celsius  
<sup>1</sup>H NMR: Proton nuclear magnetic resonance  
UNICEF: United Nations Children's Fund  
WHO: World Health Organization