Recombinant *Escherichia coli* producing an immobilised functional protein at the surface of bio-polyester beads: A novel application for a bio-bead

A thesis presented in partial fulfillment of the

requirements of the degree of

Master of Science

in

Microbiology

at Massey University, Palmerston North,

New Zealand.

Jane Adair Atwood

2008

Abstract

Polyhydroxyalkanoates (PHAs) are polyesters, produced by many bacteria and some archaea. The most commonly characterised is polyhydroxybutyrate (PHB). Produced when nutrients are growth limiting and carbon available in excess, PHA serves as a carbon-energy storage material and forms generally spherical insoluble inclusions between 50-500nm in diameter in the cytoplasm. The key enzyme for PHA synthesis is the PHA synthase and this enzyme catalyses the polymerisation of (*R*)-3-hydroxy fatty acids into PHA. PHA synthase remains covalently attached to the growing polyester chain and is displayed on the surface of the PHA granule. Other proteins associated with PHA granules include depolymerases for mobilisation or degradation of granules, regulatory proteins and phasins, proteins that aid in PHA granule stability.

PHA bio-beads displaying an IgG binding protein were produced and used to purify IgG from serum demonstrating that the PHA synthase can be engineered to display functional synthase fusion proteins at the PHA granule surface. Correctly folded eukaryotic proteins were also produced and displayed at the PHA granule surface as phasin fusion proteins. Multiple-functionality was also achievable by co-expression of various hybrid genes suggesting that this biotechnological bead production strategy might represent a versatile platform technology.

The production of functional eukaryotic proteins at the PHA bead surface represents a novel *in vivo* matrix-assisted protein folding system. Protein engineering of PHA granule surface proteins provides a novel molecular tool for the display of antigens for FACS based analysis and offers promising possibilities in the development of future biotechnological production processes. Overall, the results obtained in this study strongly enhance the applied potential of these polyester beads in biotechnology and medicine.

Acknowledgements

Approval for the research has been obtained for this work (GMO 07/MU013).

I would like to thank my supervisors, Professor Bernd Rehm and Dr Zoe Jordens for the ideas, encouragement and for all of the support during my husband Paul's illness and after his death in November 2006. Thanks to the people at IMBS and to all in the Rehm workgroup at the time who also gave me their time, support, technical advice and expertise when needed. Thanks to Pat Munro for all the scientific discussion and networking during those trips between Levin and Science Tower C. Thanks to Chris Pennington for proofreading the final draft version and finding the word horseradish funny.

Thanks to my family, especially my parents Graeme and Daphne Brockelbank who did their best to start me off in the right direction, my daughter Caitlin and my husband Paul Atwood for his unending support, encouragement, and insistence that I complete this degree.

2006 was the year that I learned much about loss, about grief, about my own strengths and weaknesses and while I do not know how I managed, I do know that due to the support of my work colleagues, my friends and my family, I was able to continue. Finally, thanks to Rory who has been there for me this year.

Life is a precious gift and my advice to you is this:

MAKE EACH DAY COUNT

(And don't forget to take photos along the way).

"When operating earthmoving equipment…….get right back out of it"

(This work is dedicated to Paul Atwood 1957-2006)

List of Tables

List of Figures

Abbreviations

AFM	Atomic force microscopy
BSA	Bovine serum albumin
CFA	Complete Freund's adjuvant
DMSO	Dimethyl sulfoxide
ELISA	Enzyme linked immuno-assay
FACS	Fluorescence activated cell sorting
FITC	Fluorescein isothiocyanate
GCMS	Gas chromatography mass spectrometry
GCPM	Gfp-PhaC and PhaP-MOG fusion protein construct
GPM	GFP-PhaP-MOG fusion protein construct
GFP	Green fluorescent protein
HRP	Horse radish peroxidase
IgG	Immunoglobulin G
IL2	Interleukin 2
kDa	Kilo Daltons
MALDI-TOF	Matrix assisted laser desorption ionisation time-of-flight mass spectrometry
MCL	Medium chain length
MOG	Myelin oligodendrocyte glycoprotein
ORF	Open reading frame
PCR	Polymerase chain reaction
PHA	Polyhydroxyalkanoate
PhaC	PHA synthase
PhaE	Type III PHA synthase subunit
PhaP	Phasin
PhaR	Phasin regulatory protein
PhaY	Hydrolase (depolymerase)
PhaZ	PHA intracellular depolymerase
PHB	Polyhydroxybutyrate
scFv	Single chain variable fragment (antibody)
SCL	Short chain length
SDS-PAGE	Sodium dodecyl sulphate gel electrophoresis