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Molecular characterisation of PHA synthase and the \textit{in vivo} synthesis of functionalised PHA beads with surface immobilised proteins

A thesis presented in partial fulfilment of the requirements of the degree of Master of Science in Microbiology at Massey University, Palmerston North, New Zealand.

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Abstract

Polyhydroxyalkanoates (PHAs) are naturally occurring biopolymers, synthesized by a large range of bacteria and deposited as small spherical water-insoluble cytoplasmic inclusion bodies containing hydrophobic polyester core surrounded by a phospholipid monolayer and associated embedded proteins. The most common form of PHA identified in bacteria is polyhydroxybutyrate (PHB).

Formation of PHA beads requires three important enzymes with PHA synthase (PhaC) being the most important, catalysing the final stereo-selective conversion of (R)-3-hydroxyacyl-CoA thioesters into PHA. Increasingly beads are used as microbeads, which display surface immobilised proteins for a range of applications in biotechnology and medicine.

However, functionalised PHA beads are largely produced in Gram-negative bacteria which contain endotoxins that are known to co-purify with the beads and are considered undesirable in medical applications. In addition, despite extensive research towards understanding PHA synthases, to date no structural data is currently available.

Here it was shown that functionalised PHB beads can be produced in vivo for both the purification of antibodies and the display of medically relevant antigens (e.g. Hepatitis C) on the surface of PHB beads from the Gram-positive bacterium L. lactis. In addition, it was shown that PHA synthase from R. eutropha can be highly overproduced, remains largely soluble and can be purified to greater than 90% purity.

The results demonstrated and supported the use of PHB beads as a platform for the production of functionalised PHA beads suitable for a large range of biotechnological or medical applications. Although no structural data for PHA synthases are currently available, our results demonstrate progress towards obtaining a three-dimensional protein structure for PHA synthase (PhaC).
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“Success, 100% persistence and a bit of luck”

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**Abbreviations**

°C

Degree Celsius

3HA<sub>MCL</sub>

Medium chain length (R)-3-hydroxy fatty acids

3HA<sub>SCL</sub>

Short chain length (R)-3-hydroxy fatty acids

3HB

3-hydroxybutyrate

AGE

Agarose Gel Electrophoresis

BSA

Bovine serum albumin

DMSO

Dimethyl sulfoxide

FM

Fluorescent Microscopy

GAP

Bead Associated Proteins

GC/MS

Gas chromatography mass spectrometry

HRP

Horse radish peroxidise

IgG

Immunoglobulin G

IMAC

Immobilised Metal Affinity Chromatography

kDa

Kilo Daltons

LDH

Lactate dehydrogenase

Maldi-TOF<sub>MS</sub>

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry

PBS

Phosphate buffered saline

PHA

Polyhydroxyalkanoate

phaC<sub>CAB</sub>

PHA operon

PhaC

PHA synthase

PhaE

Type II PHA synthase subunit

PhaP

Phasin regulatory protein

PhaZ

PHA intracellular depolymerase

PHB

Polyhydroxybutyrate

RBS

Ribosome binding site

RE

Restriction endonuclease

SDS-PAGE

Sodium dodecyl sulfate gel electrophoresis

TEM

Transmission Electron Microscopy

TEV

Tobacco Etch Virus protease

WT

Wildtype
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