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Polyhydroxyalkanoate granules: surface protein topology and rational design of functionalised biobeads

A thesis presented to Massey University in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Microbiology

at Massey University, Manuwatu, New Zealand.

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2014

Acknowledgements

Prof. Bernd Rehm for the topics and ideas to work on as well as all the guidance in the various aspects of science.

Dr Zoe Jordens helped and supported me though the process.

Friends in the lab who have come and gone over the last four years, a close 'bunch' we were and great to bounce new theories off.

Financial support from Massey University and the Institute of Fundamental Sciences.

Preface

All chapters published as separate manuscripts are listed below. These contributions do not appear in chronological order.

Chapter 2

Draper, J.; Du, J.; Hooks, D. O.; Lee, J. W.; Parlane, N. A.; Rehm, B. H. A. **Polyhydroxyalkanoate inclusions: Polymer synthesis, self-assembly and display technology.** In *Bionanotechnology: Biological Self-assembly and Its Applications*; Rehm, B. H. A., Ed.; Caister Academic Press: Norfolk, **2013**; pp. 1–36.

Chapter 3

Hooks, D. O.; Venning-Slater, M.; Du, J.; Rehm, B. H. A. **Polyhydroxyalkanoate Synthase Fusions as a Strategy for Oriented Enzyme Immobilisation.** *Molecules* **2014**, *19*, 8629–8643.

Chapter 4

Hooks, D. O.; Blatchford, P. A.; Rehm, B. H. A. **Bioengineering of bacterial polymer inclusions catalyzing the synthesis of N-acetyl neuraminic acid.** *Appl. Environ. Microbiol.* **2013**, *79*, 3116–3121.

Chapter 5

Hooks, D. O.; Rehm, B. H. A. **Insights into the surface topology of polyhydroxyalkanoate synthase: self-assembly of functionalized supramolecular inclusions.** *Applied Microbiology and Biotechnology* **2015**, 1-9.

Chapter 6

Hooks, D. O.; Rehm, B. H. A. **Surface display of highly-stable *Desulfovibrio vulgaris* carbonic anhydrase on polyester beads for CO₂ capture.** *Biotechnology Letters* 2015, 1-6.

Listed below are contributions to each published chapter performed by David Hooks:

Chapter 2: This book chapter was partly written by David Hooks focussing on the genetics of PhaC, its catalytic mechanism, and application in enzyme immobilisation as well as contributions to other sections.

Chapter 3: This review was partly written and extensively edited by David Hooks focussing on the *in vivo* and *in vitro* methods of PhaC-fusion immobilisation along with current and potential applications.

Chapter 4: All experiments were performed by David Hooks. Paul Blatchford is acknowledged for construction of several plasmids as well as sample collection for GCMS analysis. Manuscript prepared by David Hooks.

Chapter 5: All experiments performed and manuscript prepared by David Hooks.

Chapter 6: All experiments performed and manuscript prepared by David Hooks.

DNA sequencing, GCMS, and MALDI-TOF/MS were provided by external services.

This is to certify that the above research was performed by David Hooks:

(Signature, Date)

Prof. Bernd H. A. Rehm

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David O. Hooks

Abstract

This thesis examined aspects of the polyhydroxyalkanoate (PHA) biobead system for immobilisation of proteins. Three separate studies have expanded the scope of this platform technology into different applications. New flexible regions along the length of the PhaC protein were discovered and functionalised with IgG binding domains. The bioremediation and fine-chemical synthesis aspects of the PHA biobeads were developed with active enzymes of interest immobilised to the bead surface. Additionally, functional dual fusion of enzymes to both the N- and C-terminus of PhaC was demonstrated for the first time. The enhanced scope of the PHA biobeads will lead to further applications in fields such as protein purification, vaccines, and diagnostics.

The first study assessed the ability of the PHA synthase (PhaC) based immobilisation system to tolerate dual enzyme fusions allowing the recapitulation of a biosynthetic pathway. *N*-acetyl neuraminic acid aldolase and *N*-acetyl glucosamine 2-epimerase allow for the synthesis of the medically relevant fine-chemical *N*-acetyl neuraminic acid (Neu5Ac). Ultimately, biobeads establishing the entire Neu5Ac synthesis pathway were able to convert up to 22% of the initial *N*-acetyl glucosamine into Neu5Ac which compares favourably with the theoretical maximum from chemi-enzymatic synthesis of 33%.

Despite intense research interest, the structure of PhaC has not yet been solved. Structural information of the exposed regions of granule-associated PhaC was gathered by the application of biotinylation labels. Six amino acid sites were found to be surface

exposed and four were able to tolerate FLAG-tag insertion. Three of these sites were chosen to functionalise with the IgG binding domain. These beads were able to mediate the binding and elution of IgG, with a maximum capacity of 16 mg IgG/g wet PHA beads.

The enhanced carbonic anhydrase from *Desulfovibrio vulgaris* str. "Miyazaki F" (DvCA) was fused the N-terminus of PhaC and immobilised on the surface of PHA beads. The DvCA beads had a specific activity of 114 U/mg enzyme. PHA-immobilised DvCA retained 54% of its initial activity after incubation at 90 °C for 1 h and 77% of its initial activity after incubation at pH 12 for 30 min. This stability indicates its usefulness in the challenging industrial environments where it may be deployed.

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