Regulation and export of alginate in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a clinically important opportunistic human pathogen which is of particularly relevance to cystic fibrosis (CF) patients where *P. aeruginosa* pulmonary infections are the leading cause of both morbidity and mortality.

The CF lung provides a unique environment to the pathogen which induces the overproduction of the exopolysaccharide alginate by the bacteria, resulting in a thick biofilm which protects the bacteria from the host immune response and antibiotic treatment, while contributing to the clogging of the lung. Furthermore, this switch from a non-mucoid (minimal levels of alginate) to a mucoid (alginate over-producing) phenotype is widely recognised as a poor prognosis indicator for patients, after which the infection cannot be eradicated. The exact mechanisms responsible for this switch are unclear but appear to involve a complex combination of transcriptional regulation, post translational regulation and the mutation of hyper-mutable regions of the genome.

This thesis investigates the physiological role of alginate for *P. aeruginosa* as well as several of the previously poorly understood steps in the biosynthesis and regulation of this important virulence factor. The outer membrane pore, AlgE, responsible translocation of alginate across the bacterial outer membrane was characterised. Interactions between two uncharacterised proteins, AlgK and AlgX, were identified and two novel regulatory networks involved in the control of alginate biosynthesis were identified.

Preface

The format of this thesis complies with the "Submission of thesis based on publications" as described in the latest version of the Handbook for Doctoral Studies, version 6, published by the Massey University doctoral research committee (January 2010).

The following sections of this thesis have been published or are submitted for publication in internationally peer-reviewed journals. Publications do not appear in chronological order.

Chapter I

Hay ID, Rehman ZU, Ghafoor A and Rehm BHA (2010). Bacterial biosynthesis of alginates. Journal of Chemical Technology and Biotechnology **85**: 752-759

Chapter II

Hay ID, Gatland K, Campisano A, Jordens JZ and Rehm BHA (2010). Impact of alginate production on attachment and biofilm architecture of a supermucoid *Pseudomonas aeruginosa* strain. Applied and Environmental Microbiology **75**: 6022-6025

Chapter III

Hay ID, Rehman Z and Rehm BHA (2010). Membrane topology of outer membrane protein AlgE, which is required for alginate production in *Pseudomonas aeruginosa*. Applied and Environmental Microbiology **76**: 1806-1812

Chapter IV

Hay ID, Remminghorst U and Rehm BHA (2009). MucR, a novel membrane associated regulator of alginate biosynthesis in *Pseudomonas aeruginosa*. Applied and Environmental Microbiology **75**: 1110-1120

Chapter V

Hay ID, Schmidt O, Gutsche J and Rehm BHA (2011). Identification of a periplasmic AlgK-AlgX-MucD multiprotein complex in *Pseudomonas aeruginosa* involved in biosynthesis and regulation of alginate. Applied Microbiology and Biotechnology (accepted June 10 2011)

Contribution IDH made to publications by are as follows:

Chapter I: This review was drafted by IDH with critical review from ZUR & AG and finalised by BHAR.

Chapter II: Continuous flow biofilm growth, confocal microscopy and subsequent analysis were done by IDH (with assistance from KG). Solid surface attachment experiments were done by JZJ and AC.

Chapter III: *algE* deletion mutant and complementation vector were made by IDH. pEX100T:ΔalgE was made by Uwe Remminghorst. Construct expressing AlgE with deletion of extracellular loop 7 was made by IDH. FLAG tag insertions of algE were made by ZUR. Alginate quantification was done by ZUR. Outer membrane protein isolation and immunobloting was done by IDH. Manuscript was drafted by IDH , and finalised by BHAR.

Chapter IV: UR made the plasmids pEX100T:ΔmucR and pBBR1MCS-5:mucR. All other work was done by IDH. Manuscript was drafted by IDH and finalised by BHAR.

Chapter V: Plasmid pEX100T:ΔmucD was made by JG. Strains PAO1ΔmucD, PAO1ΔalgX, and PAO1ΔmucDΔalgX were made by OS. All other work was done by IDH. Manuscript was drafted by IDH and finalised by BHAR.

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

This is to certify that the above mentioned work was conducted by Iain Hay.

Signature Date Signature Date

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Prof. Bernd H.A. Rehm Iain D. Hay

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Finally thanks to Massey and IMBS for funding.

"Extraordinary claims require extraordinary evidence"-Carl Sagan

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