Understanding aspects of alginate biosynthesis and regulation by *Pseudomonas aeruginosa*

A thesis presented in partial fulfilment of the

requirements of the degree of

Doctor of Philosophy

in

Microbiology

at Massey University, Palmerston North,

New Zealand

Yajie Wang

2017

Abstract

Alginate is a medically and industrially important polymer produced by seaweeds and certain bacteria. The bacterium *Pseudomonas aeruginosa* over-produces alginate during cystic fibrosis lung infections, forming biofilms, making the infection difficult to treat. Bacteria make alginate using membrane spanning multi-protein complexes. Although alginate biosynthesis and regulation have been studied in detail, there are still major gaps in knowledge. In particular, the requirement of AlgL (a periplasmic alginate degrading enzyme) and role played by MucR (an inner membrane c-di-GMP modulator) are not well understood. Here I show that AlgL and MucR are not essential for alginate production during biofilm growth. My findings suggest that while catalytically active AlgL negatively affects alginate production, expressing catalytically inactive AlgL enhances alginate yields. Furthermore, preliminary data show AlgL is not required for the stability or functionality of the alginate biosynthesis complex, suggesting that it is a free periplasmic protein dispensable for alginate production. These findings support the prediction that the primary function of AlgL is to degrade misguided alginate from the periplasm. For MucR, I show for the first time that its sensor domain mediates nitrate-induced suppression of alginate biosynthesis. This appears to occur at multiple levels in a manner only partially dependent on c-di-GMP signaling. These results indicate that MucR is associated with the negative effect of nitrate (and possibly denitrification) on alginate production. On the basis of these results, I propose a combination of nitrate (or denitrification intermediates), exogenous lyases and antimicrobial agents could be used to eliminate established chronic biofilm infections. Furthermore, catalytically inactive AlgL and/or homologs of MucR with disabled sensor motifs could be harnessed in non-pathogenic bacteria for producing tailor-made alginates.

Acknowledgements

I would like to thank Professor Bernd Rehm, Dr. Jan Schmid and Dr. Zoe Jordens for their supervision, wisdom, guidance and support. I would also like to thank my collaborators, Dr. Iain Hay, Dr. Zahid Rehman, Dr. Fata Moradali, Dr. Ian Sims, and Dr. Ali Goudarztalejerdi. It has been a pleasure to work with you all.

I would like to thank Dr. Iain Hay and Dr. Zahid Rehman for their training and construction of various plasmids (pBBR1MCS-5:*mucR* variants), Dr. Fata Moradali for assistance with NMR, Dr. Ian Sims for SEC-MALLS analysis, and Dr. Ali Goudarztalejerdi for generation of PDO300Δ*algL* mutant. I would like to acknowledge the current and former members of the laboratory team, Shuxiong, Patricia, Jinping, Jin, Jean, Jason S, Jason L, Panan, Kampachiro, Shirin, David, Karin, Sasha, Leo, Lydia and Andy, for their friendship and collegiality; it has been fun working alongside you.

Thank you to the Massey Genome Services for DNA sequencing, Manawatu Microscopy and Imaging Centre for assistance with confocal laser scanning microscopy, Mr. Mohsen Bagheri for operating the IFS sterilization and decontamination facility, Mr. Paul Hocquard for procurement of reagents and consumables, Ms. Ann Truter, Ms. Cynthia Creswell and Debra Creswell for making administrative matters a breeze, Dr. Natisha Megan for compliance and health and safety training, Professor Kathryn Stowell for being a supportive postgraduate coordinator, Professor Simon Hall for being a supportive Head of Institute, and Professor Gill Norris and Professor Geoff Jameson for technical and moral support.

I thank the Massey University Doctoral Research Scholarship, IFS Postgraduate Scholarship and IFS Postgraduate Travel Fund for financial support.

I would like to thank all my supportive friends (you know who you are): Lucy, Paulo, Yilin, Leo, Lilian, Logan, Dam, Tuck, Jaired, Brian, Ricky, Dylan, Shao, Jennifer, Kayla, Andrew, Isaac and Iain and all my friends at Palmerston North Overseas Christian Fellowship for your friendship and Friday night and weekend fun. I would like to thank my girlfriend, Lucy, for her support.

I would like to thank my father Qiao Wang, late-mother Li Yuan Chen, step-mother Xiao Ling Chen, and half-brother George Zi Ming Wang for their unconditional love and support.

Thank you to my Creator, God the Father, my Saviour Jesus Christ, and the Holy Spirit that guide me with wisdom, strength and perseverance.

Dedication

This thesis is dedicated to my late-grandfather Jiheng Wang, a former Professor of Plant Breeding, who passed away on the 2nd of November 2016, aged 95.

Table of Contents

List of Abbreviations

^{1}H-NMR	Proton nuclear magnetic resonance
ANOVA	Analysis of variance
APS	Ammonium persulfate
BSA	Bovine serum albumin
c-di-GMP	Bis-(3´-5´)-cyclic dimeric guanosine monophosphate
CLSM	Confocal laser scanning microscopy
DGC	diguanylate cyclase
DMSO	Dimethyl sulfoxide
dNTP	Deoxynucleotide triphosphates
DSG	disuccinimidyl glutarate
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
IPTG	Isopropyl β-D-1-thiogalactopyranoside
MOPS	(3-(N-morpholino)propanesulfonic acid)
NIAC	nickel ion affinity chromatography
O.D.	Optical density
PCR	Polymerase Chain Reaction
PDE	phosphodiesterase
poly-M	polymannuronic acid
RE	Restriction endonuclease
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	Standard error
SEC-MALLS	Size Exclusion Chromatography-multi-Angle Laser Light Scattering
SLIM	Site-directed, Ligase-Independent Mutagenesis
TBE	Tris/Borate/EDTA
TBST	Tris-buffered-saline + Tween 20
TEMED	Tetramethylethylenediamine
X-GAL	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

List of Figures

List of Tables