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**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

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**MASSEY
UNIVERSITY**
TE KUNENGA KI PŪREHUROA

UNIVERSITY OF NEW ZEALAND

Yajie Wang

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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.

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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.

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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

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