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**The role of AlgK in alginate biosynthesis by**  
***Pseudomonas aeruginosa***

A thesis presented in partial fulfilment of the  
requirements of the degree of  
Master of Science  
in  
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**MASSEY UNIVERSITY**  
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## ABSTRACT

Alginate is a polysaccharide produced by brown seaweeds and two bacterial genera *Azotobacter* and *Pseudomonas*. While seaweed alginate finds numerous industrial and medical applications, alginate produced by *Azotobacter* and *Pseudomonas* spp., is important for cyst and biofilm formation, respectively. A member of *Pseudomonas*, *Pseudomonas aeruginosa*, is the leading cause of death in Cystic Fibrosis (CF) patients. This pathogen over-produces alginate upon infection of the CF lung, protecting it from host immune responses and antibiotics while clogging up the patients' airways leading to poor prognosis. Alginate biosynthesis occurs in four stages: (1) precursor synthesis in the cytoplasm (AlgA, D and C), (2) polymerisation at the inner membrane (Alg8 and Alg44), (3) periplasmic translocation and modification (AlgK, X, L, G, I, J and F), and (4) secretion (AlgE) across the outer membrane. The latter three stages are facilitated by a putative multi-protein complex spanning the entire envelope fraction. Currently, it is unknown how this complex is assembled and the roles certain components of the complex play in alginate biosynthesis are not clear. The periplasmic protein AlgK is a key component of this complex. This protein has multiple protein-protein interaction domains, suggesting that it could be critical for assembling functional alginate biosynthesis machinery. In the present study, an *algK* mutant was generated and used to determine the impact of AlgK's absence on (i) alginate yield and size, and (ii) the stability of other components of the alginate biosynthesis machinery. This study demonstrates that AlgK is essential for polymerisation and is required for the stability of components involved in polymerisation (Alg44), translocation (AlgX), and secretion (AlgE). We also show that AlgK interacts with periplasmic AlgX but not with inner membrane Alg44 or outer membrane AlgE. Overall, this study sheds light on the role of AlgK in alginate production and the assembly of the alginate biosynthesis machinery.

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## LIST OF ABBREVIATIONS

A full list of abbreviations used

°C	Degree Celsius
AGE	Agarose gel electrophoresis
Ap	Ampicillin
APS	Ammonium persulfate
BSA	Bovine serum albumin
Cb	Carbenicillin
Δ	Delta (deleted)
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNAase	Deoxyribonuclease
dNTPs	Deoxyribonucleotide triphosphates
EtOH	Ethanol
EDTA	Ethylenediaminetetraacetic acid
g	gravity/gram
Gm	gentamycin
GTP	Guanosine triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic
acid	
HRP	Horse radish peroxidase
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	Kilo Daltons
λ	Lambda (wavelength or type of phage)
LB	Luria-Bertani (broth)
OD	Optical density
ORF	Open reading frame
PCR	Polymerase chain reaction
PIA	Pseudomonas isolation agar
PPI	Protein-protein interaction
RNAase	Ribonuclease
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate gel electrophoresis

TBE	Tris-Borate-EDTA buffer
Tc	Tetracycline
TE	Tris-EDTA buffer
TEMED	Tetramethylethylenediamine
T <sub>m</sub>	Primer melting temperature
Tris	Trishydroxymethylaminomethane
v/v	Volume per volume
w/v	Weight per volume
X-Gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside

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