Molecular Mechanism of Export of Alginate in *Pseudomonas aeruginosa*

A thesis presented in partial fulfilment of the requirements for degree

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Read in the name of your lord who created man out of clot. Read and your lord is the most generous who taught by the pen. Taught man that which he knew not.

(Holy Quran)

Abstract

Pseudomonas aeruginosa is an opportunistic pathogen; infecting insects, plants and humans. It is of particular relevance to cystic fibrosis (CF) patients where it causes pulmonary infection and the leading cause of morbidity and mortality.

The CF lung environment selects for a variant of *P. aeruginosa* characterised by the overproduction of an exopolysaccharide called alginate. It has been hypothesized that outer membrane protein AlgE forms a channel through which alginate is secreted into the extracellular environment. Furthermore, studies have suggested that proteins involved in the polymerisation, modification and export of alginate form a multiprotein complex that span the bacterial envelope. The aim of this thesis was to investigate the role of AlgE in polymerisation and secretion of alginate. For this purpose *algE* knockout mutant was created in PDO300. Results showed that AlgE does not have a role in alginate polymerisation however it has a role in secretion of alginate and stability of the alginate biosynthesis machinery. By performing FLAG epitope insertion mutagenesis the topology of AlgE was verified and site-directed mutagenesis further showed that the positive electrostatic field inside the AlgE lumen is required for efficient secretion of negatively charged alginate. By employing mutual stability analysis, evidence was provided for the existence of trans-envelope multiprotein complex required for alginate biosynthesis. Co-immuniprecipitaion assay suggest that AlgE interacts with periplasmic located AlgK and, most probably, this interaction is mediated by the peripasmic turn 4 of AlgE. Pull-down assays further showed that AlgK interacts with another periplasmic protein AlgX which in turn interacts with the inner membrane protein Alg44. Based on mutual stability analysis it was proposed that Alg44 interacts with Alg8 which might interacts with AlgG as well. Our results also support the existence of internal promoters for AlgE and AlgG.

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 7, published by the Massy University doctoral research committee (January 2011).
The following sections of this thesis have been published in internationally peer-reviewed journals.

Chapter II

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

Chapter III

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

Chapter IV

Rehman ZU, Rehm BHA (2013). The dual roles of *Pseudomonas aeruginosa* AlgE in secretion of the virulence factor, alginate, and formation of the secretion complex Applied and Enviornmental Microbiology **79:** 2002-2011.

Chapter V

Rehman ZU, Wang Y, Moradali MF, Hay ID, Rehm BHA (2012). Insight into assembly of the alginate biosynthesis machinery in *Pseudomonas aeruginosa*. Applied and Enviornmental Microbiology (just accepted)

Contributions Rehman ZU made to publications are as follows

Chapter II: This review was drafted by I.D.H, Z.U.R and A.G and finalised by B.H.A.R

Chapter III: Plasmid pEX100T:Δ*algE* was made by U.R. Plasmid pBBR1MCS5:*algE* was made by IDH. PDO300Δ*algE* was made by Z.U.R and complementation was done by Z.U.R. FLAG tag variants of *algE* were made by Z.U.R. Alginate quantification was done by Z.U.R. Outer membrane protein isolation and immunoblotting was done by I.D.H. Manuscript was drafted by I.D.H and Z.U.R and finalised B.H.A.R.

ChapterIV: All of the experimental work was done by Z.U.R. Manuscript was drafted by Z.U.R and finalised by B.H.A.R.

Chapter V: Plasmids for chromosomal integration of all the genes was designed and made by Z.U.R. Chromosomal integration of *alg8*, *alg44*, *algE*, *algX*, and *mucD* was done by Z.U.R. For these strains isolation of envelope fraction, immunoblotting and alginate quantification was done by Z.U.R. AlgE co-immunoprecipitation was done by Z.U.R. PDO300Δ*algK* knock-out and its complementation was done by Y.W. AlgK pull-down was done by Y.W and Alg44 pull-down was performed by M.F.M. Strains PAO1Δ*algE* and PDO300 Δ*alg44*Δ*algX* were created by I.D.H. Manuscript was mainly drafted by Z.U.R and finalised B.H.A.R.

DNA sequencing was provided by external services.

This is to certify that above mentioned work was conducted by Zahid Ur Rehman.

Signature Date Signature Date

Prof. Bernd H.A. Rehm Zahid ur Rehman

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