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Molecular Mechanism of Alginate Polymerisation and Modifications in
Pseudomonas aeruginosa

A thesis presented in partial fulfilment of the requirements for degree

of

Doctor of Philosophy

in

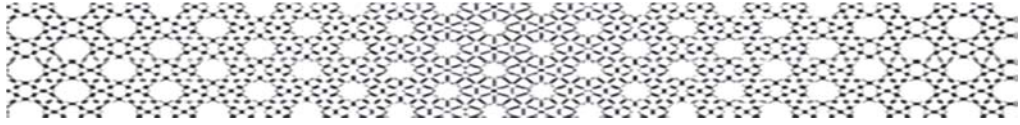
Microbiology

at Massey University

New Zealand

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2015



In Loving Memory of My Mom

Pari



*“Science is a way of thinking much more
than it is a body of knowledge”*

Carl Sagan



Abstract

Pseudomonas aeruginosa is an ubiquitous opportunistic human pathogen in immunocompromised patients. It is of particular relevance to cystic fibrosis (CF) patients where it frequently causes chronic bronchopulmonary infection and is the leading cause of morbidity and mortality. The decline in lung function is caused by the emergence of a mucoid variant showing excessive production of the exopolysaccharide, alginate. The alginate-containing biofilm matrix of this mucoid variant protects *P. aeruginosa* from the immune system and antibiotics.

Here the alginate biosynthesis/modification/secretion multiprotein complex was investigated with regard to protein-protein interactions constituting the proposed multiprotein complex and the molecular mechanisms underlying alginate polymerisation and modifications. This study sheds light on the structure and function of various alginates from a material property and biological function perspectives. The binary interactions of AlgK-AlgE, AlgX-Alg44, AlgK-Alg44 and Alg8-Alg44 were identified proposing a new model for this multiprotein complex organisation. Protein-protein interactions were found to be independent of c-di-GMP binding to PilZ_{Alg44} domain. C-di-GMP-mediated activation of alginate polymerisation was found to be different from activation mechanism proposed for cellulose synthesis. This study showed that alginate polymerisation and modifications were linked. It was shown that the molecular mass of alginate was reduced by epimerisation, while it was increased by acetylation. It was determined that previously characterized proteins AlgG (epimerase) and AlgX (acetyltransferase) have mutual auxiliary and enhancing roles. Biofilm architecture analysis showed that acetyl groups lowered viscoelasticity of alginates and promoted cell aggregation, while nonacetylated polymannuronate alginate promoted stigmergy. Experimental evidence was provided that Alg44 boosted acetylation while the periplasmic domain of this protein was critical for protein stability and regulation of alginate modifications. Full-length Alg44 was purified and it was found to be a dimer in solution. Overall, this study sheds new light on the arrangement of the proposed alginate biosynthesis/modification/secretion multiprotein complex. Furthermore, the activation mechanism and the interplay between polymerisation and modification of alginate were elucidated and new functions and interactive role of alginate-polymerising and-modifying subunits were further understood.

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 or submitted or drafted for publication in internationally peer-reviewed journals. Publications have not been arranged in chronological order.

Chapter II

Hay ID, Ur Rehman Z, Moradali MF, Wang Y, Rehm BHA (2013). Microbial Alginate Production, Modification and its Applications. *Microbial Biotechnology* **6**: 637–650.

Chapter III

Hay ID, Wang Y, Moradali MF, Ur Rehman Z, Rehm BHA (2014). Genetics and Regulation of Bacterial Alginate Production. *Environmental Microbiology* **16**: 2997-3011.

Chapter IV

Rehman ZU, Wang Y, Moradali MF, Hay ID, Rehm BHA (2013). Insight into Assembly of the Alginate Biosynthesis Machinery in *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology* **79**: 3264-3272

Chapter V

Moradali MF, Donati I, Sims IM, Ghods S, Rehm BHA (2015). Alginate Polymerisation and Modification are Linked in *Pseudomonas aeruginosa*. (*mBio* 6(3):e00453-15)

Chapter VI

Moradali MF, Rehm BHA (2015). The Role of Alg44 in Alginate Synthesis and Modification (Draft manuscript)

Contributions Moradali MF made to publications are as follows

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

Chapter III: This review was drafted by I.D.H, W.Y, M.F.M and Z.U.R and finalised by B.H.A.R

Chapter IV: Chromosomal integration of all the genes was designed and made by Z.U.R. Generation of PDO300 Δ *algK* knock-out and its complementation was done by Y.W. AlgE co-immunoprecipitation was done by Z.U.R. AlgK pull-down was done by Y.W and PDO300 Δ *alg44* Δ *algX* mutant was created by I.D.H. This mutant was re-confirmed and transformed with pBBR1MCS-5:*alg44-6his* by M.F.M. *In vivo* chemical cross-linking and Alg44 pull-down assay was performed by M.F.M. Manuscript was drafted by Z.U.R, Y.W and M.F.M and I.D.H and finalised by B.H.A.R.

Chapter V: Generation of double-gene knockout mutants in *alg8/alg44*, *algG/algX* and *algG/alg44*, single-gene knockout mutant in *algG* and re-confirming single-gene knockout mutants in *alg8*, *alg44* and *algX*, construction of plasmids for complementing these mutants, chromosomal integration of the genes and site-specific mutations and deletions for producing Alg8, Alg44, AlgX and AlgG variants, *in vivo* detection of protein-protein interaction network, isolation of cytoplasmic membrane and general protein analysis and all other assessments were performed by M.F.M. ¹H-NMR analysis was done and interpreted by I. D and M.F.M. SEC-MALLS analysis was performed by I.S and M.F.M. Setting up and analysis of biofilms was performed by M.F.M and S.G. Manuscript was mainly drafted by M.F.M. and finalized by B.H.A.R.

Chapter VI: All of the experimental work was done by M.F.M. Manuscript was drafted by M.F.M and finalised by B.H.A.R.

DNA sequencing was provided by external services.

This is to certify that above mentioned work was conducted by M. Fata Moradali.

Signature Date

Prof. Bernd H.A. Rehm

Signature Date

M. Fata Moradali

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Table of contents

Abstract	i
Preface	ii
Acknowledgements	ii
List of tables	vii
List of figures	viii
Chapter I	1
Introduction	1
Thesis Aims.....	4
Thesis Findings.....	4
Chapter II	6
Microbial Alginate Production, Modification and its Applications	6
Abstract.....	7
Introduction.....	8
Microbial biosynthesis of alginate	8
Alginate precursor synthesis.....	11
Polymerisation	11
Periplasmic translocation and modification.....	13
Alginate secretion.....	17
Multiprotein alginate polymerisation/secretion complex.....	18
Regulation.....	19
Applications of bacterial alginates	22
Chapter III	25
Genetics and Regulation of Bacterial Alginate Production	25
Abstract.....	26
Introduction.....	27
Genetics	28
Regulation of alginate biosynthesis	29
Regulated intramembrane proteolysis (RIP) of the MucA anti-sigma factor.....	29
Transcriptional regulation.....	34
Posttranscriptional regulation	38
Posttranslational regulation	39
Concluding remarks	41

Chapter IV	42
Insight into Assembly of the Alginate Biosynthesis Machinery in <i>Pseudomonas aeruginosa</i>	42
Abstract.....	43
Introduction.....	44
Materials and Methods	45
Results	50
Discussion.....	59
Acknowledgments.....	63
Supplementary Material	64
Chapter V	68
Alginate Polymerisation and Modification are Linked in <i>Pseudomonas aeruginosa</i>	68
Abstract.....	69
Importance	69
Introduction.....	70
Materials and Methods	72
Results	76
Discussion.....	96
Acknowledgements	99
Supplementary Materials.....	101
Chapter VI	119
The Role of Alg44 in Alginate Synthesis and Modification	119
Abstract.....	120
Introduction.....	121
Materials and Methods	122
Results	125
Discussion.....	137
Acknowledgements	138
Final Discussion and Outlook	139
References	145

List of tables	Page
Chapter II	
Table 1. Proteins involved in alginate biosynthesis.....	10
Chapter IV	
Table 1. Proposed interactions between various proteins of alginate biosynthesis and secretion complex.....	62
Table S1. Bacterial strains and plasmids used in the study.....	64
Table S2. Oligonucleotides used in this study.....	66
Chapter V	
Table 1. Composition and molecular mass analyses of alginate produced by different mutants.....	88
Table 2. Compactness and dead/live ratio calculated for analysed biofilms.....	96
Table S1. Strains and plasmids applied in this study.....	115
Chapter VI	
Table 1. Composition of alginates produced by different variants of Alg44.....	132
Table 2. Descending order of values presented in Table1.....	132

List of figures	Page
Chapter II	
Fig. 1. Chemical structure of alginate.....	9
Fig. 2. Overview of bacterial alginate biosynthesis.....	13
Fig. 3. Modification of bacterial alginate.....	16
Fig. 4. Schematic representation of the alginate polymerisation/secretion complex spanning from the inner membrane to the outer membrane.....	19
Fig. 5. Overview of the regulation of alginate biosynthesis.....	21
Chapter III	
Fig. 1. MucA RIP cascade.....	31
Fig. 2. Schematic representation of various regulatory mechanisms of alginate biosynthesis.....	36
Chapter IV	
Fig. 1. Complementation of the <i>algK</i> knock-out mutant.....	50
Fig. 2. Amount of free uronic acids produced by various strains when grown in liquid culture.....	51
Fig. 3. Effect of the absence or presence of proposed subunits of the alginate biosynthesis machinery on the stability other subunits in the multiprotein complex and alginate production.....	53
Fig. 4. Co-IP and pull-down assays show AlgK interacts with AlgE and AlgX.....	56
Fig. 5. <i>In vivo</i> cross linking shows Alg44 interacts with AlgX.....	58
Fig. 6. Proposed model of the alginate polymerisation/secretion multiprotein complex.....	63
Fig. S1. Mutual stability analysis.....	67
Chapter V	
Fig. 1. Alg8-Alg44 protein-protein interaction.....	77
Fig. 2. Protein-protein interaction analysis indicates interaction of Alg44-AlgK, Alg44-AlgX, and probable Alg44-Alg44 (dimer).....	79
Fig. 3. Localization, stability, and protein-protein interaction of Alg44 variants.....	81
Fig. 4. Bacterial cellulose synthase-associated autoinhibiting mechanism does not play a role in alginate polymerisation.....	83

Fig. 5. Impact of putative alginate polymerase subunits on alginate polymerase activity, alginate polymerisation, and composition and correlation between polymerisation and modification.....	87
Fig. 6. Biofilm architecture of mutants producing acetylated and nonacetylated alginates.....	91
Fig. 7. Biofilm architecture of mutants producing epimerized and nonepimerized alginates.....	93
Fig. 8. Biofilm architecture of mutant-producing nonepimerized and nonacetylated alginates and the wild type.....	94
Fig. 9. Biofilm architecture of a mutant producing a high mannuronate molar fraction and M-block.....	95
Fig. 10. A new proposed model of alginate biosynthesis machinery complex and interactive performances of protein functionality over alginate polymerisation, acetylation, epimerisation, and length determination.....	100
Fig. S1. Alg44 stability is independent of c-di-GMP.....	111
Fig. S2. ¹ H-NMR spectra of acetylated and deacetylated samples.....	112
Fig. S3. Plots show molar mass of alginate samples versus time analysed using SEC-MALLS.....	113
Fig. S4. Viscoelastic property of alginates was impacted by molecular weight and modifications.....	114
Fig. S5. Impact of alginates on motility of <i>P. aeruginosa</i>	114

Chapter VI

Fig. 1. Clustered periplasmic residues of Alg44 which are highly conserved among alginate-producing species.....	126
Fig. 2. Point-mutation of highly conserved periplasmic residues reduced or abolished alginate production.....	127
Fig. 3. Alginate polymerisation was impaired by by site-specific mutagenesis of highly conserved periplasmic amino acid residues of Alg44.....	128
Fig. 4. Stability of Alg44 variants in envelope fraction.	129
Fig. 5. Disulfide bond may play in Alg44 interaction with other subunits when it is in native stoichiometry.....	130
Fig. 6. Assessment of heterologous production of Alg44 protein.....	133

Fig. 7. Assessment of Alg44 purification produced from homologous (left) and heterologous (right) hosts and treated with EDTA.....134

Fig. 8. Purification of Alg44 dimer using gel filtration chromatography.....136

LIST OF ABBREVIATIONS

°C Degree Celsius

Ap Ampicillin

BSA Bovine serum albumin

Cb Carbenicillin

Δ Delta (deleted)

DMSO Dimethyl sulfoxide

D₂O Deuterium oxide

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 viii

Tc Tetracycline

TE Tris-EDTA buffer

Tm Primer melting temperature

Tris Trishydroxymethylaminomethane

vol/vol Volume per volume

wt/vol Weight per volume

X-Gal 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside