Investigation of the Biosynthesis of Exopolysaccharides within the Biofilm Matrix of *Pseudomonas aeruginosa* and *Pseudomonas syringae* pv. *actinidiea*

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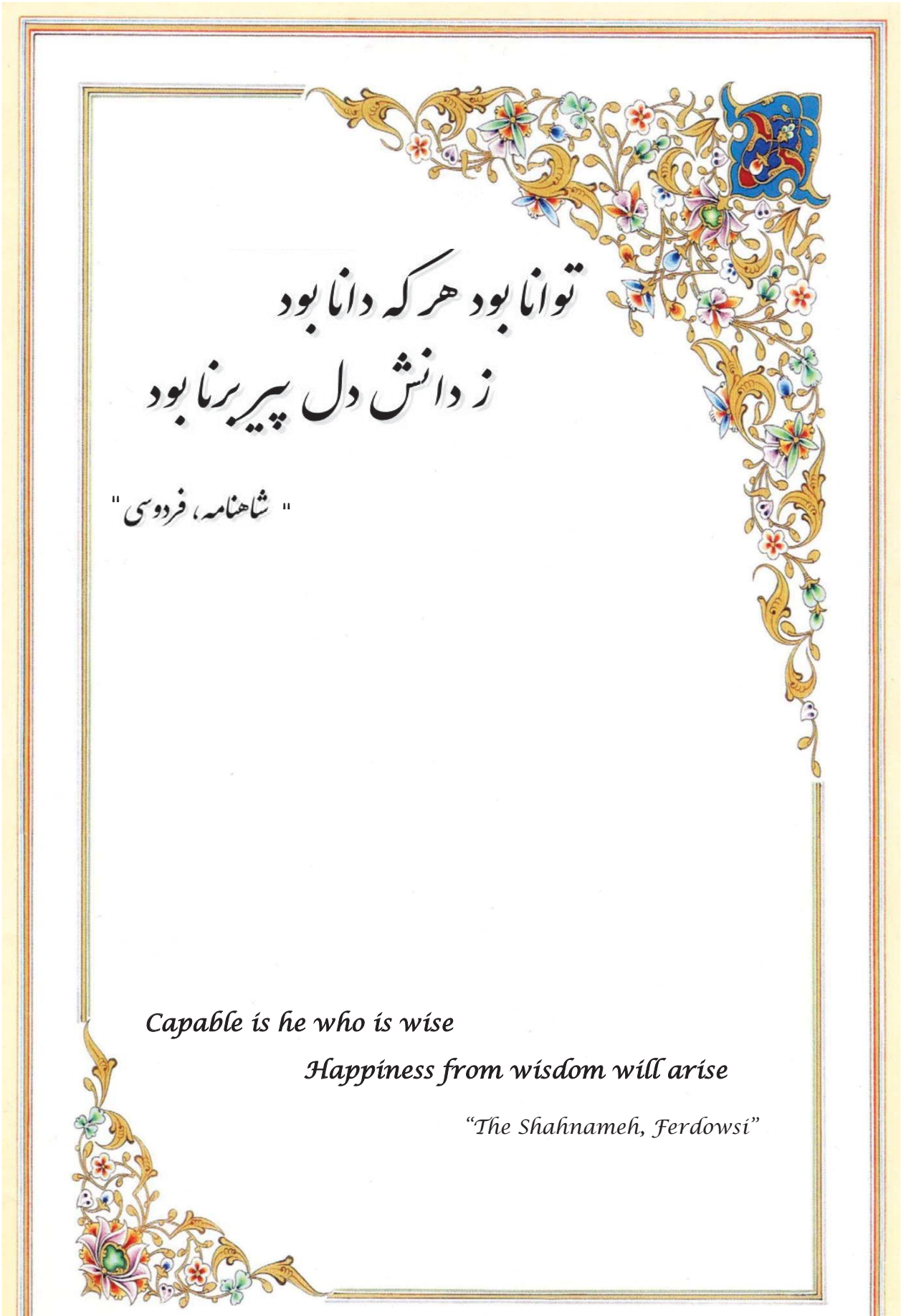

توانا بود هر که دانا بود
ز دانش دل پیر برنا بود

" شاهنامه، فردوسی "

Capable is he who is wise
Happiness from wisdom will arise

"The Shahnameh, Ferdowsi"

ABSTRACT

Polysaccharides are highly abundant natural biopolymers, which have biologically significant structural functions in living organisms. Various polysaccharides, with specific physicochemical properties, contribute to biofilm formation; defined as cell aggregations surrounded by extracellular polymeric substances. They are also important in the context of bacterial pathogenesis, while some have been harnessed for industrial and biomedical applications due to their unique chemical compositions and properties.
In present study, we aimed at studying biofilm formation by *Pseudomonas aeruginosa* and *P. syringae* pv. *actinidiae*, respectively known as human and plant pathogens. In this context we focused on the production of exopolysaccharides, which predominantly constitute the biofilm matrix of these pathogenic bacteria.

Here, we uncovered that the polysaccharide isolated from *P. syringae* pv. *actinidiae* biofilm mainly consists of rhamnose, fucose and glucose and it was cautiously introduced as a novel polysaccharide. In the context of disease control, and developing a management program, we provided some evidences for the effectiveness of chlorine dioxide and kasugamycin in the control of the bacteria living in both biofilm and planktonic modes.

Furthermore, we investigated alginate biosynthesis as major polysaccharide contributing to mucoid biofilm formation by *P. aeruginosa*. We generated various mutants producing a variety of alginates with different chemical compositions. Also, this enabled us to analyse functional relationships of protein subunits involved in multiple steps of alginate biosynthesis including alginate polymerization, modification and secretion. We present evidence that while alginate unravelled that while alginate is polymerised and translocated across the membrane by a multiprotein complex, acetylation and epimerisation events positively and negatively correlated with the polymerization of the alginate or molecular mass, respectively. Analysis of the biofilms showed that biofilm architecture and cell-to-cell interactions were differently impacted by various compositions of the alginates. Also, this study provided insights into the c-di-GMP mediated activation of alginate polymerization upon binding to c-di-GMP as well as assigning functional roles to Alg8 and Alg44 including their subcellular localization and distribution.
Here, we also used current knowledge of the alginate biosynthesis pathway to assess the production of alginate from biotechnologically accepted heterologous hosts including

Escherichia coli and *Bacillus megaterium* strains. Primarily, we evaluated the production and functionality of the minimal protein requirements in nonpathogenic heterologous hosts, required for producing alginate precursor, and proceeding into polymerization and secretion steps.

Overall, we concluded that polysaccharides play a major role in the formation of bacterial biofilms while chemical composition is a key determinant for biofilm architecture and development. This contribution to understanding the biosynthesis of bacterial polysaccharides and their properties could provide the necessary knowledge not only for developing novel therapeutics, but also for harnessing such biopolymers for various industrial applications and production via biotechnological procedures.

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PREFACE

Below lists the publication status of all chapters in this thesis.

Chapter I

General introduction

This chapter was written as an introductory chapter for this thesis by Shirin Ghods

Chapter II

Shirin Ghods, Ian M. Sims, M. Fata Moradali, Bernd H. A. Rehm. Bactericidal Compounds Controlling Growth of the Plant Pathogen *Pseudomonas syringae* pv. *actinidiae*, Which Forms Biofilms Composed of a Novel Exopolysaccharide. (Applied and environmental microbiology 81.12 (2015): 4026-4036)

This article was written by Shirin Ghods and reviewed by all other authors. The concept was conceived by Shirin Ghods and Bernd H. A. Rehm. Experimental design was performed by Shirin Ghods with the advice of Bernd H. A. Rehm. All experiments were performed by Shirin Ghods with the exception of some compositional analysis were planned and performed with the guidance and involvement of Ian M. Sims.

Chapter III

Shirin Ghods, M. Fata Moradali, Ivan Donati, Ian M. Sims, Bernd H. A. Rehm. Interplay of alginate polymerisation and modifications in *Pseudomonas aeruginosa* and their impact on biofilm formation (co-published in mBio 6 (3) (2015):e00453-15) (see Appendix)

This chapter was written by Shirin Ghods and reviewed by Bernd H.A. Rehm. The concept was conceived by Shirin Ghods and Bernd H. A. Rehm. Generation of some mutants and production of various alginates was performed by Shirin Ghods. Raw data of compositional analysis of various alginates were provided by Ivan Donati and Ian M. Sims. Interpretation of physicochemical properties of alginates was performed by Shirin Ghods and Fata Moradali and then approved by Bernd H.A Rehm. Setting up and analysis of biofilms results was performed by Shirin Ghods.

Chapter IV

M. Fata Moradali, Shirin Ghods, Bernd H. A. Rehm. Activation Mechanism and Cellular Localization of Membrane-Anchored Alginate Polymerase in *Pseudomonas aeruginosa.* (Applied and Environmental Microbiology 83.9 (2017): e03499-16)

This article was written by Fata Moradali and Shirin Ghods and reviewed by Bernd H. A. Rehm. The concept was conceived by Fata Moradali, Shirin Ghods and Bernd H. A. Rehm. Generation of double-gene knockout mutants, complementation, biofilm assessment, alginate quantification, and imaging was performed by Fata Moradali and Shirin Ghods. Manuscript was mainly drafted by Fata Moradali and Shirin Ghods and finalized by Bernd H. A. Rehm.

Chapter V

Shirin Ghods, Bernd H. A. Rehm. Preliminary assessment of the establishment of the alginate biosynthesis pathway in non-pathogenic heterologous hosts (Drafted manuscript, 2017)

This manuscript was written by Shirin Ghods and reviewed by Bernd H. A. Rehm. The concept was conceived by Shirin Ghods and Bernd H. A. Rehm. All Experimental design was performed by Shirin Ghods with the advice of Bernd H. A. Rehm.

This is to certify that above mentioned work was conducted by Shirin Ghods.

Signature Date	Signature Date 2/6/2017
	
Prof. Bernd H.A. Rehm	Shirin Ghods

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ABBREVIATIONS

Psa	*Pseudomonas syringae* pv. *Actinidiae*
EPS	Extracellular polymeric substances
LB	Luria Bertani
°C	Degree Celsius
mM	Millimolar
pH	Potential hydrogen
µg	Micro gram
µl	Micro litre
µg ml^{-1}	Micro gram/millilitre
Nm	Nano metre
Mm	Micro metre
Ppm	Part per million
MIC	Minimal inhibitory concentration
OD	Optical density
H	Hour
Min	Minute
CFU	Colony forming units
3D	Three-dimensional
CDM	Cell dry mass
GC-MS	Gas chromatograph mass spectrometer
HPAEC	High-performance anion-exchange chromatography
DMSO	Dimethylsulfoxide
HSQC	Heteronuclear single quantum coherence
TOCSY	Total correaltion spectroscopy
COSY	Correlation spectroscopy
NMR	Nuclear magnetic resonance spectroscopy
SSA	Solid surfaces attachment
Ap	Ampicillin
Gm	Gentamycin
Cm	Chloramphenicol
Tet	Tetracycline
Kan	Kanamycin
BSA	Bovine serum albumin
Cb	Carbenicillin
Δ Delta	Deleted
DMSO	Dimethyl sulfoxide
D_2O	Deuterium oxide
DNA	Deoxyribonucleic acid
DNAase	Deoxyribonuclease
RNAase	Ribonuclease
dNTPs	Deoxyribonucleotide triphosphates
EtOH	Ethanol
EDTA	Ethylenediaminetetraacetic acid
G	Gravity/gram
GTP	Guanosine triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP	Horse radish peroxidase
IPTG	Isopropyl â-D-1-thiogalactopyranoside
kDa	Kilodaltons
Λ	Lambda (wavelength or type of phage)

GFP	Green fluorescent protein
ORF	Open reading frame
PCR	Polymerase chain reaction
PIA	Pseudomonas isolation agar
PPI	Protein-protein interaction
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate gel electrophoresis
TBE	Tris-Borate-EDTA buffer
TE	Tris-EDTA buffer
Tm	Primer melting temperature
Tris	Tris(hydroxymethyl)aminomethane
vol/vol	Volume per volume
wt/vol	Weight per volume
X-Gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
ELISA	enzyme-linked immunosorbent assay
M	1,4-linked β-D-mannuronic acid
G	C5 epimer α-L-guluronic acid
GG-blocks	C5 epimer α-L-guluronic acid residues
PMI	Phosphomannose isomerase
GMP/GDP-MP	Guanosine diphosphate (GDP)-mannose pyrophosphorylase
PMM	Phosphomannomutase
GMD	GDP-mannose 4, 6-dehydrogenase
KEGG	Kyoto encyclopedia of genes and genomes
His	Histidine
DTT	Dithiothreitol
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide - hydrogen
PGC	Porous graphitic carbon
Q-TOF	Quadrupole time of flight
SPE	Solid extraction column
MS	Mass spectrometry
OPD	O-Phenylenediamine dihydrochloride
TCA	Tricarboxylic acid
T0	Zero Time
HRP	Horseradish peroxidase