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An Altered Physiological State of *Pseudomonas aeruginosa* in the Biofilm Environment: Effect on the *algD* Promoter and a New Attachment-Inducible Regulatory Element

Christopher James Cooper

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For Courtney: On the 23rd of April 1999 the birth of a baby girl, whom we aptly named Courtney, had a mind numbing effect on my life and my outlook thereon. The inspiration provided by this life-altering experience is reflected in this work. May you one day achieve what your heart desires.

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An Altered Physiological State of *Pseudomonas aeruginosa* in the Biofilm Environment: Effect on the *algD* Promoter and a New Attachment-Inducible Regulatory Element

by

Christopher James Cooper

Submitted in partial fulfilment of
the requirements for the degree

Master of Science

in

the Faculty of Natural and Agricultural Sciences
Department of Microbiology and Plant Pathology,
University of Pretoria
Pretoria
South Africa

December 2002

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in it's entirety or in part been submitted at any university for a degree.

Signature:

Date:

An Altered Physiological State of *Pseudomonas aeruginosa* in the Biofilm Environment: Effect on the *algD* Promoter and a New Attachment-Inducible Regulatory Element

by

Christopher James Cooper

Promoter: Prof. V. S. Brözel

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SUMMARY

Biofilm-associated bacterial cells are known to display a unique phenotype distinct from that of free-living or planktonic cells. Suspended cells of *P. aeruginosa* PAO (DSM 1707) growing in the presence of a biofilm (surface-influenced planktonic or SIP cells) were compared to planktonic cells. The biofilm and SIP phenotypes were different to each other, and both differed from the planktonic population. The SIP population was not a mixture of planktonic and detached biofilm cells but rather a distinct physiological state. Furthermore, indirect evidence is presented for the presence of diffusible signals produced by the biofilm that give rise to the SIP phenotype. The physiological effects of a *lacZ*-based reporter vector pALacZsd on the planktonic, SIP and biofilm populations of *P. aeruginosa* were investigated. The data obtained indicate that *P. aeruginosa* cells containing the pALacZsd vector are phenotypically different to untransformed *P. aeruginosa* cells. *P. aeruginosa* cells transformed with pALacZsd were found to have more protein biomass per cellular volume than untransformed cells and plasmid DNA concentrations were found to be lower in total attached cultures when compared to planktonic cultures.

The attachment of *P. aeruginosa* to a surface with the subsequent formation of a biofilm as well as environmental stimuli causes expression or up-regulation of genes involved in the production of alginate, a bacterial exopolysaccharide produced in large quantities. The physicochemical conditions affecting up-regulation of the *P. aeruginosa* PAO (DSM1707) *algD* promoter were investigated using an *algD-lacZ* transcriptional fusion. The data presented indicate that at least five separate factors, *i.e.* osmolarity, water availability, glucose, growth as a biofilm and growth in the presence of a biofilm, influence the regulation of *algD*, either individually or in combination. In a previous study, putative attachment-inducible regulatory elements of *Pseudomonas aeruginosa* PAO were identified. One of these regulatory elements was further characterised in this study. The effect of the different physicochemical conditions found to up-regulate *algD* promoter activity were also investigated for this regulatory element. The data presented indicate that the regulatory element may contain a promoter sequence, or part thereof, that is influenced by detachment of *P. aeruginosa* from a surface.

PREFACE

The following aspects of this work have been submitted for publication:

1. Submitted for publication in *FEMS* Microbiology Letters:
   Pseudomonas aeruginosa displays two biofilm-related phenotypes distinct from the planktonic state.

The following aspects of this work have been presented as talks or posters, at international and national conferences:

Conference:	Biofilms 2000, Big Sky, Montana, USA, July 2000
Posters:	1. Characterisation of a novel biofilm regulatory element of *Pseudomonas aeruginosa* (PAO1) 2. Expression of the *Pseudomonas aeruginosa* (PAO1) *algD* promoter is affected when grown as a biofilm
Presenter:	C. J. Cooper
Conference:	101st General Meeting of the ASM, 2001, Orlando, Florida, USA, May 2001
Poster:	Comparison of a novel putative promoter (clone 65) to *algD*, a biofilm attachment-inducible promoter of *Pseudomonas aeruginosa* PAO1
Presenter:	V. S. Brözel
Conference:	Eleventh Biennial Conference of the SASM, Grahamstown, South Africa, January 2000
Presentation:	Characterisation of the *Pseudomonas aeruginosa* (PAO1) *algD* promoter
Presenter:	C. J. Cooper
Conference:	The Seventeenth Congress of the South African Genetics Society (SAGS), Pretoria, South Africa, June 2000
Presentation:	Characterisation of the *algD* promoter in *Pseudomonas aeruginosa* (PAO1) growing as a biofilm
Poster:	Comparison of a novel putative promoter to *algD*, a biofilm attachment-inducible promoter of *Pseudomonas aeruginosa* PAO1
Presenters:	C. J. Cooper and J. Smith

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following people:

Leigh-Ann, my wife, for carrying the financial burden of running the household and her constant motivation throughout my studies. Without you this thesis would not have been possible.

My parents for always encouraging me to follow my intuition and providing the grounding and upbringing necessary to succeed as an individual.

Prof. V. S. Brözel, Department of Microbiology and Plant Pathology, UP, for his constant encouragement and support. Your belief in my ability to succeed, and the constant encouragement is what ultimately led to the completion of this thesis.

Dr. J. Theron, Department of Microbiology and Plant Pathology, UP, for his constant intellectual input and genuine interest in what I was doing throughout the duration of this work.

My colleagues at UP: Marietjie, Sonya, Marleen, Boet, Julian, Marinda, Bridgitta, Francois and Joseph for their friendship, support and advice as well as making everyday life in the lab enjoyable.

Raynard MacDonald, Department of Microbiology and Plant Pathology, UP, for his friendship and his help with the FISH.

Jacques Smith for his help throughout the course of his honours year with the pALacZsd65 clone, as well as construction of the pALacJK vector.

The FRD for their initial financial support.

To everyone who directly or indirectly contributed to my achievements.

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A	absorbance
AHL	acylated homoserine lactone
AMP	adenosine mono-phosphate
ATP	adenosine tri-phosphate
α	alpha
ca.	approximately
bp	base pair
β	beta
BSA	bovine serum albumin
cm	centimetre
μm^3	cellular volume in cubic micrometers
cfu	colony forming units
CSLM	confocal scanning laser microscopy
cAMP	cyclic-AMP
CF	cystic fibrosis
Da	Dalton
d	day
°C	degrees Celsius
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleic-5'-triphosphate
DLVO	Derjaguin, Landau, Verwey, and Overbeek
DSM	Deutsche Sammlung von Mikroorganismen
DMSO	dimethyl sulphoxide
DOT	dissolved oxygen tension
ddH_2O	double distilled water
etc.	etcetera
EtOH	ethanol
EPS	extracellular polymeric substance
Fig.	figure
FISH	fluorescent *in situ* hybridisation
g	gram
GFP	green fluorescent protein
V_0	cellular volume at zero growth rate
G	guanine
h	hour
kDa	kiloDalton
LPS	lipopolysaccharide
l	litre

LB	Luria Bertani
LB-S	Luria Bertani broth without NaCl
LB + EtOH	LB-S containing 2.5 or 2.9% (v/v) ethanol
LB + NaCl	LB-S containing 0.7 M added NaCl
mRNA	messenger RNA
µl	microlitre
µm	micrometre
$mg.l^{-1}$	milligrams per litre
ml	millilitre
mm	millimetre
MMG	minimal M63 salts containing glucose
min	minute
M	molar
MWCO	molecular weight cut off
ONPG	2-nitrophenyl-β-D-galactopyranoside
OD	optical density
%	percent
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
P	probability
RT-PCR	reverse transcription followed by polymerase chain reaction
rpm	revolutions per minute
RNA	ribonucleic acid
s	second
σ	sigma
SDS	sodium dodecyl sulphate
±	plus and minus one standard deviation from the mean
cm^2	surface area in square centimetres
SIP	surface-influenced planktonic
X	times
× g	times gravity
TCA	tri-carboxylic acid
UV	ultra violet
U	unit
v/v	volume per volume
w/v	weight per volume