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
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.
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
I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its 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  
**Co-Promoter:** Dr. J. Theron  
**Department:** Microbiology and Plant Pathology  
**Degree:** MSc (Microbiology)

**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.
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* (PA01)  
2. Expression of the *Pseudomonas aeruginosa* (PA01) *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* PA01  
**Presenter:** V. S. Brözel

**Conference:** Eleventh Biennial Conference of the SASM, Grahamstown, South Africa, January 2000  
**Presentation:** Characterisation of the *Pseudomonas aeruginosa* (PA01) *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* (PA01) growing as a biofilm  
**Poster:** Comparison of a novel putative promoter to *algD*, a biofilm attachment-inducible promoter of *Pseudomonas aeruginosa* PA01  
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LIST OF ABBREVIATIONS

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
μm³ 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
ddH₂O double distilled water
etc. etcetera
EtOH ethanol
EPS extracellular polymeric substance
Fig. figure
FISH fluorescent in situ hybridisation
g gram
GFP green fluorescent protein
V₀ cellular volume at zero growth rate
G guanine
h hour
kDa kiloDalton
LPS lipopolysaccharide
l litre
<table>
<thead>
<tr>
<th>Acronym</th>
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<tr>
<td>LB</td>
<td>Luria Bertani</td>
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<tr>
<td>LB-S</td>
<td>Luria Bertani broth without NaCl</td>
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<tr>
<td>LB + EtOH</td>
<td>LB-S containing 2.5 or 2.9% (v/v) ethanol</td>
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<tr>
<td>LB + NaCl</td>
<td>LB-S containing 0.7 M added NaCl</td>
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<tr>
<td>mRNA</td>
<td>messenger RNA</td>
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<td>plus and minus one standard deviation from the mean</td>
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