The role of small molecule signalling in biofilm migration of *Pseudomonas aeruginosa*

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Certificate of Original Authorship

I certify that the work in this Thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the Thesis has been written by me. Any help that I have received in my research work and the preparation of the Thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the Thesis.

Signature of Student:

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Date: 22/1/2014

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List of abbreviations

Abbreviation	Meaning
ABS	Adult bovine serum
AC	Adenylate cyclase
AHL	*N*-acyl homoserine lactones
AHS	Adult serum albumin
AMP	Adenosine 5´ monophosphate
Ap^R	Ampicillin resistant
ASGM1	Glycolipid asialoGM1
ATP	Adenosine 5' triphosphate
BM	Base Media
BMA	Base media agar
Bp	Basepairs
BSA	Bovine serum albumin
CAMHB	Cation-adjusted mueller hinton broth
cAMP	3'-5'-cyclic adenosine monophosphate
CAUTI	Catheter-associated urinary tract infections
c-di-GMP	Bis-(3´-5´)-cyclic dimeric guanosine monophosphate
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis transmembrane conductance regulator
CLSM	Confocal scanning light microscopy
CRP	Catabolic repressor protein
DAG	Diacylglycerol
DGC	diguanylate cyclase
diH_2O	Deionised water
DKP	Diketopiperazine
DMSO	Dimethyl sulfoxide
DSF	Diffusible signal factors
DTT	Dithiothreitol
eATP	Extracellular adenosine 5' triphosphate
eDNA	Extracellular deoxyribonucleic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assays
EPS	Extracellular polymeric substances
g	Grams
GlcNAc	*N*-acetyl glucosamine
hr	Hour/s
HCl	Hydrochloric acid
Hpt	Histidine phosphotransfer
icAMP	Intracellular 3'-5'-cyclic adenosine monophosphate
Ig	Immunoglobulin
Kb	Kilobases

kg	Kilograms
Km^R	Kanamycin resistance
9L	Litre
LB	Luria Bertoni
LBA	Luria Bertoni agar
LCFA	Long-chain fatty acid
LPS	Lipopolysaccharide
M	Molar
Min	Minute/s
MCP	Methyl-accepting chemotaxis protein
MDCK	Madin-Darby Canine Kidney
mL	Millilitres
mM	Millimolar
mm	Millimetres
MQ	MilliQ
ms	Milliseconds
Na_2EDTA	EDTA disodium salt dehydrate
nM	Nanomolar
nm	nanometres
NO	Nitric oxide
OMV	outer-membrane vesicle
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline + Tween 20 (0.05%)
PC	Phosphatidylcholine
PCR	Polymerase chain reaction
PDE	phosphodiesterase
PE	Phosphatidylethanolamine
PIA	Pseudomonas isolation agar
PQS	*Pseudomonas* quinolone signal
PVDF	Polyvinylidene fluoride
QS	Quorum sensing
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SD	Standard deviation
SDS	Sodium dodecyl sulphate
Sec	Second/s
SEM	Standard error of mean
SNP	Single nucleotide polymorphism
SOLiD	Sequencing by Oligonucleotide Ligation and Detection
Spt	Serine phosphotransfer
T3SS	Type III secretion system
TBE	Tris Borate EDTA

TCA	Tricarboxylic acid
TE	Tris-EDTA buffer
TEM	Transmission electron microscopy
Tet^R	Tetracycline resistance
tfp	Type IV pili
TLR	Toll-like receptor
Tpt	Threonine phosphotransfer
Vfr	Virulence factor regulator
μM	Micromolar

Abstract

Pseudomonas aeruginosa is a Gram-negative pathogen which exploits damaged epithelium to cause acute and chronic infections in a range of immunocompromised individuals. The chronic nature of infections caused by *P. aeruginosa* is often associated with the formation of biofilms. Extension and retraction of type IV pili (tfp) mediates a form of surface translocation, termed twitching motility, which is involved in active biofilm expansion and sessile biofilm formation. In *P. aeruginosa* the biogenesis, assembly and twitching motility function of tfp is controlled by a number of complex regulatory systems, however the signals that these systems respond to are not well characterised. The aim of this Thesis was to understand how intracellular and extracellular signals control *P. aeruginosa* twitching motility-mediated biofilm expansion.

In this Thesis five independent *fimL* mutants, that had presumably acquired extragenic suppressor mutations which restored twitching motility ability, were characterised. All *fimL* revertants were found to have increased levels of intracellular cyclic AMP (icAMP). While an extragenic suppressor mutation in the cAMP phosphodiesterase CpdA was shown to be responsible for the increase in icAMP levels and restoration of twitching motility in one *fimL* revertant, the site of suppressor mutation(s) in the remaining four revertants was not identified. These results suggest that twitching motility reversion in *fimL* mutants occurs via at least two mechanisms and that an increase in icAMP levels is correlated with twitching motility.

Extracellular ATP (eATP) is released by damaged epithelial cells which acts as a "danger" signal to recruit host immune system factors to repair the damage. As *P. aeruginosa* has a propensity for damaged epithelia the effect of eATP on *P. aeruginosa* biofilm expansion and formation was investigated. The results presented in this Thesis demonstrate that eATP inhibits *P. aeruginosa* twitching motility-mediated biofilm expansion and stimulates sessile biofilm formation, which may provide a potential advantage for *P. aeruginosa* within an infection setting. Additionally, our results suggest that high levels of endogenously-produced bacterial eATP acts to coordinate *P. aeruginosa* multicellular behaviours.

This Thesis also reports the identification of a novel extracellular signal *N*-acetylglucosamine, which stimulates *P. aeruginosa* twitching motility. Additionally, the twitching motility response of *P. aeruginosa* to the host derived signals serum albumin, mucin and oligopeptides was characterised in detail. These analyses implicated the CheW-homolog, ChpC which is a component of the Chp chemosensory system, in this response.

Overall the results presented in this Thesis provide insight into the regulation *P. aeruginosa* twitching motility by a number of intracellular and extracellular signals. Our results suggest that the adaptive response of *P. aeruginosa* to these signals is likely to have significant implications in the success of this pathogen within an infection setting.