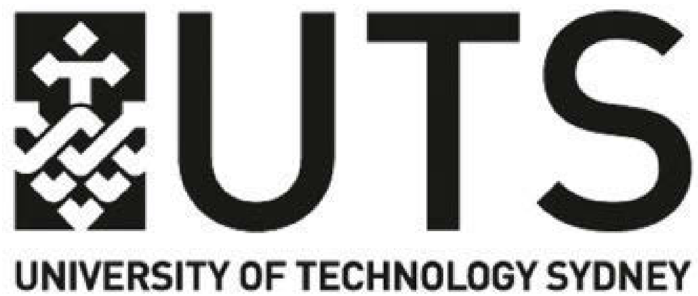

Characterisation of explosive cell lysis in *Pseudomonas aeruginosa*

Thesis submitted to
the University of Technology Sydney,
in fulfillment of the requirements for the degree of
Doctor of Philosophy

Amelia L. Hynen

2017

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.

This research was supported by an Australian Government Research Training Program Scholarship.

Signature of Student:

Date:

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List of Abbreviations

°C	Degree Celsius
3D	Three-dimensional
3D-SIM	Three-dimensional structured illumination microscopy
α	Alpha
AHL	N-acyl-L-homo-serine lactone
Amp^R	Ampicillin resistance
ATP	Adenosine tri-phosphate
β	Beta
bp	Base pairs
BSA	Bovine serum albumin
CAMHB	Cation-adjusted Mueller Hinton broth
CAUTI	Catheter-associated urinary tract infection
CF	Cystic Fibrosis
CFP	Cyan fluorescent protein
CFU/mL	Colony forming units per millilitre
CIP	Ciprofloxacin
CL	Colistin
CSP	Competence-stimulating peptide
Δ	Delta
diH_2O	Deionised water
DNA	Deoxyribonucleic acid
DNase I	Deoxyribonuclease I
dNTPs	Deoxynucleotide triphosphates
DTT	Dithiothreitol
ECL	Explosive cell lysis
EDTA	Ethylenediaminetetraacetic acid
eDNA	Extracellular deoxyribonucleic acid
EHEC	Enterohaemorrhagic *Escherichia coli*
EthHD-2	Ethidium homodimer-2
exDNA	Exogenous deoxyribonucleic acid
g	Grams
g	G-force

g/mL	Grams per millilitre
g/L	Grams per litre
GFP	Green fluorescent protein
GM	Gentamicin
Gm^R	Gentamicin resistance
h	Hour/hours
IHF	Integration host factor
kb	Kilobase
kg	Kilogram
kPa	Kilo-Pascals
kV	Kilovolts
L	Litre
LB	Luria Bertani (Lennox)
LBA	Luria Bertani agar
M	Molar
MIC	Minimal inhibitory concentration
MBC	Minimal bactericidal concentration
mg	Milligram
mg/mL	Milligram per millilitre
min	Minute/minutes
min/kb	Minute per kilobase
mL	Millilitre
mM	Millimolar
mm	Millimetre
MMC	Mitomycin C
MQ	MilliQ
µF	Microfarad
µg	Micrograms
µg/µL	Micrograms per microlitre
µg/mL	Micrograms per millilitre
µL	Microlitre
µm	Micrometre
µM	Micromolar
MVs	Membrane vesicles

NA	Numerical aperture
Na_2EDTA	EDTA disodium salt dehydrate
ng	Nanogram
OD	Optical density
P	P-value
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCD	Programmed cell death
PFU/mL	Plaque forming units per millilitre
pH	Potential of hydrogen
PQS	Pseudomonas quinolone signalling molecule
QS	Quorum sensing
RNA	Ribonucleic acid
RO	Reverse osmosis
ROS	Reactive oxygen species
rpm	Revolutions per min
s	Second/s
SDS	Sodium dodecyl sulphate
sec	Second/s
s.e.m.	Standard error of the mean
SOB	Super optimal broth
SOS	‘Save-our-souls’
TBE	Tris Borate EDTA
TE	Tris-EDTA buffer
TMGG	Twitching motility gellan gum
U	Units
U/mL	Units per millilitre
UV	Ultra violet
v	Version
VBM	Vogel-Bonner media
VBMA	Vogel-Bonner media agar
v/v	Volume per volume
w/v	Weight per volume
YFP	Yellow fluorescent protein

Abstract

Pseudomonas aeruginosa is a Gram-negative pathogen commonly associated with nosocomial infections and implanted medical devices. It causes both acute and chronic infections, which are often associated with the formation of biofilms. Extracellular DNA (eDNA) is a major component of these biofilms and is involved in adhesion, intercellular connectivity and facilitating cell migration. eDNA is also essential for the formation of biofilms, however the mechanism of its production at the early stages of development is unknown.

At the initiation of this Thesis, it was observed that during the early stages of submerged and interstitial biofilm development, a small proportion of cells spontaneously transform into round cells before rapidly lysing and releasing eDNA and cellular content into the extracellular milieu. This phenomenon is termed 'explosive cell lysis'. This process accounts for all eDNA produced in these biofilms as well as a number of other 'public goods' including cytosolic proteins and membrane vesicles (MVs) that may benefit the biofilm community. As eDNA, MVs and extracellular proteins contribute to biofilm development and virulence, explosive cell lysis may be a critical process for *P. aeruginosa*. Therefore, it is important to identify the mechanism mediating this process.

The aim of this Thesis was to identify and characterise the genes and cellular processes that lead to explosive cell lysis and eDNA release, and to better understand the role of eDNA in early stages of biofilm development. This Thesis identifies an endolysin of a cryptic bacteriophage as the enzyme responsible for this phenomenon and reports that explosive cell lysis is also under the control of the SOS response regulator RecA. Investigations into the induction of explosive cell lysis determined that intrinsic stressors like the formation of inclusion bodies and exogenous stressors that cause DNA damage induce explosive cell lysis and eDNA release. This Thesis also identifies that explosive cell lysis is mediated by the action of three independent holins that work in coordination to translocate the endolysin responsible for explosive cell lysis. Exploration into the role of explosive cell lysis in submerged biofilm formation determined that explosive cell lysis is essential for the formation of microcolonies, microcolony formation is spatially and temporally correlated to

explosive cell lysis, and sequential explosive events are required for building microcolonies.

Overall the results presented in this Thesis add to our understanding of the complex nature of eDNA production during biofilm development and have identified a novel role for cryptic bacteriophage.