Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
Spatial and temporal localisation of exopolysaccharide gene expression in mucoid and non-mucoid

_Pseudomonas aeruginosa_ biofilms

a thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in

Microbiology

Massey University, Manawatu,

New Zealand

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Abstract

The biofilm, or surface-associated microbial community, is the preferred method of growth for most bacteria. *Pseudomonas aeruginosa* is an ubiquitous, opportunistic pathogen capable of biofilm formation in a wide range of natural and clinical environments. In particular, biofilms formed by *P. aeruginosa* in the lungs of people with cystic fibrosis (CF) are responsible for a significant decline in the health and prognosis of these patients. Once established, *P. aeruginosa* biofilms begin to excrete an exopolysaccharide (EPS) called alginate which protects the bacterial microcolonies from antimicrobial molecules and confers a mucoid phenotype. Once this phenotypic switch has occurred, the biofilm becomes impossible to eradicate and ultimately leads to the death of the patient. Here, fluorescent signalling systems and confocal laser scanning microscopy (CLSM) have been used to spatially and temporally resolve the expression of three EPSs produced by *P. aeruginosa*; the pellicle-forming EPS (Pel), the EPS encoded by the polysaccharide synthesis locus (Psl) and alginate. In order to observe the effect (if any) of EPS production on spatial localisation of the cells within the biofilm, the biofilm-associated characteristics of three *P. aeruginosa* double-knockout mutants, each able to produce only one EPS has been observed. In analysing these biofilm structures, it was found that Pel has a role in facilitating an increased surface area of the biofilm, while Psl-producing mutants form a biofilm structure with a significantly increased biomass. By visualising fluorescent signals throughout a biofilm consisting of a mixture of the three mutants, the spatial localisation of EPS-producing bacterial populations has been observed. Here, Pel-producing mutants tended to aggregate at the attachment surface, suggesting a role in adhesion of the biofilm structure. Spatial and temporal localisation of EPS promoter activity was achieved by transforming the prototypic *P. aeruginosa* PAO1 strain with one of three plasmids encoding unstable *gfp* expression under the control of each EPS’s promoter sequence. Overall, this study has demonstrated the applications and limitations of fluorescence-based localisation of bacterial gene expression throughout *P. aeruginosa* biofilm development. Collectively, this information can help to guide future investigations into the expression and regulation of the genes associated with a biofilm phenotype, with the aim of identifying a target for effective therapy against this important pathogen.
Acknowledgements

To everyone who

believed in me when I didn’t believe in myself,

helped me find my way when I was lost

and didn’t give up on me.

You know who you are.

Thank you.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>3-D</td>
<td>3-dimensional</td>
</tr>
<tr>
<td>Ap</td>
<td>ampicillin</td>
</tr>
<tr>
<td>Ap&lt;sup&gt;R&lt;/sup&gt;</td>
<td>ampicillin resistance</td>
</tr>
<tr>
<td>bfp</td>
<td>blue fluorescent protein</td>
</tr>
<tr>
<td>bp</td>
<td>base pair(s)</td>
</tr>
<tr>
<td>c-di-GMP</td>
<td>cyclic diguanylate monophosphate</td>
</tr>
<tr>
<td>Cb</td>
<td>carbencillin</td>
</tr>
<tr>
<td>Cb&lt;sup&gt;R&lt;/sup&gt;</td>
<td>carbencillin resistance</td>
</tr>
<tr>
<td>CF</td>
<td>cystic fibrosis</td>
</tr>
<tr>
<td>cfp</td>
<td>cyan fluorescent protein</td>
</tr>
<tr>
<td>CLSM</td>
<td>confocal laser-scanning microscopy</td>
</tr>
<tr>
<td>Δ</td>
<td>delta (deletion of)</td>
</tr>
<tr>
<td>DGC</td>
<td>diguanylate cyclase</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTPs</td>
<td>deoxynucleotide triphosphates</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EPS</td>
<td>exopolysaccharide</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>g&lt;sub&gt;fp&lt;/sub&gt;</td>
<td>gene encoding green fluorescent protein</td>
</tr>
<tr>
<td>gfp</td>
<td>green fluorescent protein</td>
</tr>
<tr>
<td>Gm</td>
<td>gentamicin</td>
</tr>
<tr>
<td>Gm&lt;sup&gt;R&lt;/sup&gt;</td>
<td>gentamicin resistance</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HeRed</td>
<td><em>Heteractis crispa</em> red fluorescent protein</td>
</tr>
<tr>
<td>HSL</td>
<td>homoserine lactone</td>
</tr>
</tbody>
</table>
IFN  interferon
L  litre(s)
LB  Luria-Bertani
m  milli-
M  moles per litre
μ  micro-
Milli-Q  ultrapure, Type 1 filtered and deionised water (Millipore™)
min  minute(s)
MOPS  3-propanesulfonic acid
Mm  millimetre
nm  nanometre(s)
OD₆₀₀  optical density at 600 nm
pel  gene encoding Pel (pellicle-forming) exopolysaccharide
Pel  exopolysaccharide encoded by pel gene
PDE  phosphodiesterases
psl  polysaccharide synthesis locus
Psl  exopolysaccharide encoded by psl gene
QS  quorum sensing
RNA  ribonucleic acid
RSCV  rugose small-colony variant
s  second(s)
SDS  sodium dodecyl sulfate
TBE  tris/borate/EDTA
TCS  two-component signalling
U  unit(s)
V  volt(s)
w/w  weight by weight
w/v  weight by volume
X-gal  5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
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