Proteomic analysis of the biofilm and biofilm-associated phenotypes of *Pseudomonas aeruginosa* cultured in batch

by

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To my **Heavenly Father**, with whom everything is possible!

Dedicated to my father and brother

I wish that the two of you were still here… I miss you and love you always…

DECLARATION

I declare that the thesis, which I hereby submit for the degree, Philosophiae Doctor (Microbiology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another University.

Signed: …….. Date: ……..

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Pseudomonas aeruginosa is one of the most studied biofilm-forming organisms and has emerged as a model organism in the study of surface- and biofilm-induced gene expression. The transition from a planktonic to a biofilm mode of growth results in diverse changes in gene expression, which causes the attaching cells to become phenotypically and metabolically distinct from their planktonic counterparts. In this study, a proteomic approach was used to study differences in protein profiles obtained from 18-h old *P. aeruginosa* PAO1 (DSM 1707) planktonic, surface influenced planktonic (SIP) and biofilm populations grown in batch in the absence or presence of a glass wool substratum.

Glass wool as an attachment substratum not only supported growth of biofilms, but it also allowed for the separation of the biofilm biomass from the surrounding surface influenced planktonic (SIP) cells for further characterisation. Comparative analysis of the respective proteomes indicated striking differences in the protein patterns of planktonic, biofilm and SIP cells and several uniquely expressed proteins were seen on the 2-DE protein maps of the respective populations. Whereas a general down-regulation of protein expression was seen in the biofilm cells, in SIP cells, expression of the proteins was generally up-regulated. The results confirmed that the biofilm population differs from the planktonic population and indicated that the SIP population is not merely a mixture of planktonic and biofilm cells but rather a unique phenotype.

Several differentially expressed protein spots were selected and identified using a combination of N-terminal protein sequencing and peptide mass fingerprinting. The proteins comprised mostly of outer membrane or membrane-associated proteins. Based on these analyses, a mutant *P. aeruginosa* strain, deficient in outer membrane protein OprG, was generated and its ability to form biofilms on a glass wool substratum was compared with that of the wild-type *P. aeruginosa* strain. The mutant strain was attachment-proficient but biofilm-deficient, suggesting that OprG plays a role in *P. aeruginosa* biofilm development under the culturing conditions used in this study.

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LIST OF ABBREVIATIONS

%	percentage
°C	degrees Celsius
µg	microgram
µl	microlitre
µm	micrometre
2-DE	two-dimensional gel electrophoresis
A	ampere
AHL	acylated homoserine lactone
Amp^r	ampicillin resistance
bp	base pair
ca.	approximately
cfu	colony forming units
CH_3CN	acetonitrile
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
cm	centimetre
cm^2	square centimetre
CTAB	cetyltrimethylammonium bromide
DMSO	dimethyl sulfoxide
dNTP	deoxyribonucleoside-5'-triphosphate
DTE	dithioerythritol
DTT	dithiothreitol
e.g.	for example
EPS	exopolysaccharides
Fig.	figure
× *g*	centrifugal force
Gm^r	gentamicin resistance
h	hour
IEF	iso-electric focusing
IPG	immobilized pH gradient
IPTG	isopropyl β-D-thiogalactoside
kb	kilobase pairs
kDa	kilodalton
L	litre

LB broth	Luria-Bertani broth
LPS	lipopolysaccharide
M	molar
MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight
min	minute
ml	millilitre
mM	millimolar
Mr	molecular mass
MSGY	modified mineral salts medium with glucose and yeast extract
NH_4HCO_3	ammonium bicarbonate
nm	nanometer
OD	optical density
OMP	outer membrane proteins
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pI	isoelectric point
pmol	picomole
PMSF	phenylmethylsulphonyl fluoride
PVDF	Immobilon-P polyvinylidene difluoride
rpm	revolutions per minute
s	second
SB3-10	N-decyl-N,N-dimethyl-3-ammonio-1-propane sulfonate
SDS	sodium dodecyl sulphate
SIP	surface influenced planktonic
TBP	tributyl phosphine
TCA	trichloroacetic acid
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
U	units
UHQ	ultra high quality
V	volts
v/v	volume per volume
Vh	Volt-hours
W	Watt
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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RESEARCH COMMUNICATIONS

Papers published:

1. **Steyn, B.**, Oosthuizen, M.C., MacDonald, R., Theron, J. and Brözel, V.S. (2001). The use of glass wool as an attachment surface for studying phenotypic changes in *Pseudomonas aeruginosa* biofilms by two-dimensional gel electrophoresis. Proteomics 1: 871-879.

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Conference contributions:

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1. **Steyn, B.**, Oosthuizen, M.C., Theron, J. and Brözel, V.S. Determination of the proteome of *Pseudomonas aeruginosa* growing planktonically and as a biofilm. BioY2K Conference, January 2000, Grahamstown, South Africa.

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1. **Steyn, B.**, Oosthuizen, M.C., Cosette, P., Jouenne, T., Theron, J. and Brözel, V.S. Determination of the proteome of *Pseudomonas aeruginosa* growing planktonically and as a biofilm. Biofilms 2000 Conference, July 2000, Big Sky, Montana, USA.

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