Characterization of a putative pilus assembly and secretion system in *Pseudomonas aeruginosa* DSM1707

by

ANTOINETTE VAN SCHALKWYK

Submitted in partial fulfilment of the requirements of the degree
Master of Science
in the Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

November 2003

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Characterization of a putative pilus assembly and secretion system in *Pseudomonas aeruginosa* DSM1707

by

ANTOINETTE VAN SCHALKWYK

Submitted in partial fulfilment of the requirements of the degree
Master of Science
in the Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

November 2003

ACKNOWLEDGEMENTS

To my Heavenly Father, for every day and every bug.

To my parents, Livio and Jacques, for being there every day.

To Jacques Theron, for believing in me and the Htp system.

To Prof. Brőzel, R. MacDonald, B. Steyn, M. Oosthuizen, and A. Hall, for helping me with their expertise.

To Dr Thierry Jouenne, and every-one at Rouen, for helping me with 2D gells and sequencing.

To the NRF-CNRS, for funding the project and providing the means to travel.

To the 9th floor, for being my home away from home, especially my big brothers Francois and Boet.

SUMMARY

Characterization of a putative pilus assembly and secretion system in *Pseudomonas aeruginosa* DSM1707

by

ANTOINETTE VAN SCHALKWYK

Supervisor: Prof. J. Theron
Department of Microbiology and Plant Pathology
University of Pretoria

Co-supervisor: Prof. V.S. Brözel
Department of Microbiology and Plant Pathology
University of Pretoria

for the degree M.Sc

Pseudomonas aeruginosa, an ubiquitous environmental bacterium and an opportunistic human pathogen, forms biofilms through a series of interactions between the cells and adherence to surfaces. Adherence of *P. aeruginosa* to surfaces is often mediated by surface appendages such as flagella and type IV pili. In this study, a gene cluster in *P. aeruginosa* was identified *in silico* that encoded predicted protein products with homology to those encoded by two recently described novel pilus biogenesis and assembly systems of *Actinobacillus actinomycetemcomitans* and *Caulobacter crescentus*, respectively. Both these systems are involved in the production of a novel class of pili, which, in *A. actinomycetemcomitans*, are associated with the ability of the bacterium to bind non-specifically to inert surfaces. The homologous genes in *P. aeruginosa*, which have not been characterized previously, were named *htp* for homologous to type IV pilus biogenesis genes.

To determine the functional importance of the *htp* gene cluster in *P. aeruginosa*, the *htpD*, *htpE* and *htpDEF* open reading frames (ORFs), which are highly conserved in the respective pilus biogenesis systems, were targeted for insertional inactivation. Whereas HtpD may function as an NTPase, the amino acid sequence of HtpE and HtpF indicate membrane localization, but no obvious functions. The respective *htp* ORFs were inactivated in *P. aeruginosa* strain DSM1707 by homologous recombination with appropriately constructed allelic exchange vectors to generate mutant strains DSMHtpD, DSMHtpE and DSMHtpDEF. The DSMHtpDEF mutant strain was found to be severely growth-impaired and was

consequently excluded from further analysis. Comparative analysis of the wild-type *P. aeruginosa* DSM1707 and mutant DSMHtpD and DSMHtpE strains revealed that whereas the DSMHtpE strain generally resembled the wild-type strain, the DSMHtpD strain was impaired in its ability to grow as a biofilm, and electron microscopic studies revealed that the cells of DSMHtpD were notably longer compared to the wild-type DSM1707 and mutant DSMHtpE cells. Furthermore, two-dimensional gel electrophoretic analysis of the extracellular proteins of the wild-type *P. aeruginosa* DSM1707 and mutant DSMHtpD strains revealed differences between the extracellular proteomic profiles.

Based on the results obtained during the course of this investigation, it can be proposed that the newly identified *htp* system of *P. aeruginosa* plays a role in the ability of this bacterium to successfully colonize abiotic surfaces. The more severe perturbations resulting from inactivation of the *htpD* ORF furthermore suggests that the encoded putative NTPase protein plays an important role in the putative *htp* pilus biogenesis/secretion system. Thus, it would appear that multiple factors are available to *P. aeruginosa* to facilitate its binding to various surfaces and possibly for interbacterial adhesion. The existence of different attachment mechanisms could reflect the complex needs of *P. aeruginosa* during colonization of diverse environmental niches.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

A	Absorbance
amp^r	ampicillin resistance
ATP	adenosine triphosphate
bp	base pair
ca.	approximately
°C	degrees Celsius
ddH_2O	deionized distilled water
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside-5'-triphosphate
DTT	dithiothreitol
EDTA	ethylenediaminetetra-acetic acid
e.g.	for example
EtOH	ethanol
Fig.	figure
$gent^r$	gentamicin resistance
h	hour
IPTG	isopropyl β-D-thiogalactoside
kan^r	kanamycin resistance
kb	kilobase pairs
kDa	kilodalton
lacZ	β-galactosidase gene
LB-broth	Luria-Bertani broth
l	litre
M	molar
mA	milliampere
MCS	multiple cloning site
mg	milligram
min	minute
ml	millilitre
mM	millimolar
nm	nanometer
nt	nucleotide
NH_4OAc	ammonium acetate

OD	optical density
ORF	open reading frame
PCR	polymerase chain reaction
PEG	polyethylene glycol
pmol	picomole
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
SDS	sodium dodecyl sulphate
TE	Tris-EDTA
tet^r	tetracycline resistance
TN-medium	tryptone-nitrate medium
2D	two-dimensional
2-DE	two-dimensional electrophoresis
U	units
µg	microgram
µl	microlitre
UHQ	ultra high quality
UV	ultraviolet
V	volts
v/v	volume per volume
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

LIST OF FIGURES

FIGURE NUMBER

LIST OF TABLES

TABLE NUMBER