Characterisation Of The ATP-Binding Cassette Transporters Of *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is a ubiquitous, Gram-negative, rod-shaped, environmental bacterium. However, it is also a clinically significant, opportunistic human pathogen, responsible for life-threatening infections in immunocompromised persons, including those with cancer and significant burn wounds. Furthermore, *P. aeruginosa* infections are the primary cause of morbidity and mortality in individuals with the genetic disease cystic fibrosis. The success of *P. aeruginosa* as both an environmental organism and a human pathogen can be attributed to its 6.3 Mbp genome, which encodes an array of mechanisms that enable adaptation, persistence and propagation in diverse environments. Membrane transporters are critical to the ability of *P. aeruginosa* to adapt and respond to its environment. Of these proteins, the ATP-binding cassette (ABC) transporters hold a prominent role in cellular processes, facilitating the active translocation of molecules across the inner membrane. The conserved structural organisation of ABC transporters has enabled their classification based upon the vector of transport, as either efflux or import transporters. Bioinformatic analyses reveal that *P. aeruginosa* PAO1 is predicted to encode 289 proteins associated with ABC transporter functionality, with a subset shown to contribute to *in vivo* virulence.

This study presents the phenotypic characterisation of five putative ABC export proteins in *P. aeruginosa* PAO1, assessing their contribution to antibiotic efflux. Deletion mutants of each identified ABC exporter were assessed for changes in their antibiotic resistance profile. Transcriptional analysis of the ABC transporter genes in response to antibiotic treatment was also performed to detect drug-stimulated expression. These analyses revealed the PA0860, PA1113, PA1876, PA3228 and PA5231 ABC efflux proteins to have a negligible contribution to antibiotic resistance, with bioinformatic analyses subsequently utilized to propose alternative roles.

ABC importers, also known as ABC permeases, typically feature one, but sometimes multiple, solute-binding protein (SBP) component(s) that deliver the cargo molecule to the ABC transporter for cytoplasmic translocation. ABC permeases are central to the uptake of many essential nutrients in prokaryotes, including transition metal ions. Herein, this study characterises the acquisition and role of two crucial transition metal ions, molybdenum and zinc, in *P. aeruginosa*. Acquisition of either metal ion was shown to occur primarily via ABC permeases, molybdenum via ModABC, and zinc via ZnuABC. Deletion of the *modA* SBP gene abrogated molybdenum accumulation in *P. aeruginosa* and abolished the capacity for anaerobic growth or nitrate reduction. Unexpectedly, conditions that permitted nitrate

reduction were shown to inhibit biofilm formation and alter membrane fatty acid composition. By contrast, although deletion of the zinc-specific SBP gene, *znuA*, reduced cellular zinc accumulation, the mutant strain did not exhibit a phenotype corresponding with zinc depletion. Transcriptional analyses revealed that *P. aeruginosa* encodes a number of additional, previously unidentified, putative mechanisms that enable it to adapt to cellular zinc deficiency, including the use of several uncharacterized zinc acquisition systems.

Collectively, this study represents a significant advance in our understanding of *P. aeruginosa* ABC transporters and offers detailed insight into their cellular functions. Furthermore, this work highlights the remarkable adaptability of the bacterium, which enables its survival in diverse environmental and host niches.

DECLARATION

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ABBREVIATIONS

°C	Degree celcius
Å	Angstroms
AA	Amino acid
ABC	ATP-binding cassette
ADP	Adenosine diphoshpate
AI	Autoinducers
Anr	Anaerobic regulator of arginine deiminase and nitrate reductase
Ap	Ampicillin
Ap^R	Ampicillin resistance cassette
Arch	Archaea
ATP	Adensosine triphosphate
b.d.	Below detection
Bap	Biofilm-associated protein
BATH	Bacterial adherence to hydrocarbons
bp	Base pairs
Cb	Carbenicillin
Cb^R	Carbenicillin resistance cassette
CDM	Chemically defined media
cDNA	Complementary DNA
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CFU	Colony forming units
Chl	Chloramphenicol
Chl^R	Chloramphenicol resistance cassette
CLD	C39-like domain
COG	Cluster of orthologous genes
CPS	Capsular polysaccharide
CTI	*Cis-trans* isomerase
Cup	Chaperone usher pathway
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucelic acid
Dnr	Dissimilartory nitrate reductase regulator
eDNA	Extracellular DNA
EDTA	2,2',2",2'"-(Ethane-1,2-diyldinitrilo)tetraacetic acid
EPS	Exopolysaccharide
FA	Fatty acid
Fnr	Fumerate and nitrate reductase regulator
FRT	Flp recombinase target
Fur	Ferric uptake regulator
g	Relative centrifugal force
gDNA	Genomic DNA
GEO	Gene expression omnibus
GI	Gene identifier
Gm	Gentamicin
Gm^R	Gentamicin resistance cassette
GPC	Gel permeation chromatography
h	Hours
His	Histidine
HK	Histidine kinase
ICP-MS	Inductively coupled plasma-mass spectroscopy
ID	Identification

IG	Intergenic
IM	Inner membrane
IMAC	Immobilised ion affinity chromatography
Kan	Kanamycin
Kan^R	Kanamycin resistance cassette
K_D	Dissociation constant
kDa	Kilodalton
L	Litre
LB	Luria bertani (Lennox) broth
LIC	Ligation independent cloning
LPS	Lipopolysaccharide
M	Molar
MATE	Multi-drug and toxic compound extrusion family
Mbp	Mega base pairs
MCS	Multiple cloning site
MDR	Multi-drug resistance
MES	2-(*N*-morpholino)ethanesulfonic acid
MFP	Membrane fusion protein
MFS	Major facilitator superfamily
mg	Milligrams
MGD	Mo-*bis* molybdopterin guanine dinucleotide
MIC	Minimal inhibitory concentration
min	Minutes
mL	Millilitres
mM	Millimolar
Mo	Molybdate, MoO_4^{2-}
MoCo	Molybdenum cofactor
MOPS	3-(*N*-morpholino)propanesulfonic acid
mRNA	Messenger RNA
MS-SIFT	Selected-ion flow mass spectrometry
Myc	Mycobacterium
n	Number of replicates
NBD	Nucleotide binding domain
NCBI	National Centre for Biotechnology Information
ng	Nanogram
nM	Nanomolar
nm	Nanometres
nt	Nucelotide
OD	Optical density
OM	Outer membrane
OMP	Outer membrane protein
Pi	Inorganic phosphate
PAR	4-(2-pyridylazo)resorcinol
PBP	Penicillin binding protein
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDB	Protein data bank
Pht	Polyhistidine triad protein
PIB-1	*Pseudomonas* imipenem β-lactamase
PIPES	Piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)
ppb	Parts per billion
PQS	*Pseudomonas* quinolone signal
PUM	Potassium urea magnesium
qPCR	Quantitative PCR

qRT-PCR	Quantitative reverse transcription polymerase chain reaction
QS	Quorum sensing
RIN	RNA integrity number
RNA	Ribonucleic acid
RND	Resistance-nodulation division
RR	Response regulator
rRNA	Ribosomal RNA
RTX	Repeat-in-toxin
s.e.m.	Standard error of the mean
SBP	Solute binding protein
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	Seconds
SEM	Scanning electron microscopy
SMR	Small multi-drug resistance family
SWM	Putative flagellar system-associated repeat
T1SS	Type 1 secretion system
TAE	Tris acetate EDTA
TCS	Two-component systems
TM	Transmembrane
TMD	Transmembrane domain
TPEN	*N*,*N*,*N*′,*N*′-Tetrakis(2-pyridylmethyl)ethylenediamine
tRNA	Transfer RNA
UTI	Urinary tract infection
V	Volt
VOC	Volatile organic compounds
W	Tungstate, WO_4^{2-}
ZIP	Zrt, Irt-like protein
Zur	Zinc uptake regulator
µg	Microgram
µM	Micromolar
µm	Micrometer

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