

# Characterisation of *Streptococcus pneumoniae* Opacity Phase Variation



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## Abstract

*Streptococcus pneumoniae* (the pneumococcus) is a common nasopharyngeal commensal in humans that can invade deeper tissues causing a range of diseases, resulting in significant global morbidity and mortality. Any given strain of *S. pneumoniae* can undergo phase variation between two different colony phenotypes, termed opaque (O) and transparent (T), as determined by their morphology on clear, solid media. In animal models, the O form is more virulent and commonly isolated from normally sterile sites, such as the blood, lungs and brain, compared to its T counterpart, which is more likely to reside in the nasopharynx. To date, the mechanism involved in the switch between opacity phenotypes is not known and there is discordance in the literature in terms of the precise molecular differences between them. This project set out to comprehensively characterise phenomic (2D-DIGE), transcriptomic (DNA microarray) and genomic (IonTorrent sequencing) differences between opacity variants in three unrelated pneumococcal strains (D39, serotype 2; WCH16, serotype 6A; and WCH43, serotype 4).

Proteomic, transcriptomic and genomic analyses revealed strain-specific differences associated with phase variation, but no single difference was consistent across the three strains tested. Nevertheless, there were examples of proteins and genes belonging to the same putative functional groups that were regulated similarly in both the O and T phase between strains. One example was the upregulation of proteins and genes related to genetic competence in the O variants. However, mutagenesis of one such gene, encoding the competence stimulating peptide receptor (*comD*), did not alter opacity phenotype. There were also inconsistencies between genes identified as differentially expressed at an mRNA and protein level, such as genes involved in cell division, and amino acid biosynthesis and acquisition. These genes were upregulated at an mRNA level in the T variants, but no such upregulation in protein expression was identified by proteomic analysis. At a genomic level, all single nucleotide polymorphisms identified by IonTorrent sequencing were independently verified. However, the insertion/deletions, particularly those associated with homopolymeric tracts were all found to be false call-outs, which is a limitation of this sequencing technology.

The role of epigenetic changes mediated by genetic rearrangements in a Type I restriction-modification (RM) system on opacity phenotype was also investigated. These rearrangements result in switching between six alternative DNA methylation site specificities impacting on genomic methylation patterns. Examination of six “locked” mutants (SpnD39IIIA-F) with monospecific DNA methylation patterns, indicated that there was an epigenetic impact on colony opacity phenotype and virulence. Importantly, these genetic rearrangements at the Type I RM locus also occurred during experimental infection. However, there were inconsistencies between the opacity phenotypes of locked mutants and the RM allele distribution in wild-type D39O and D39T. Thus, RM allele switching cannot fully account for colony opacity phase variation in pneumococci.

This study identified that pneumococcal phase variation is a complex, multifactorial phenomenon and different strains employ alternative mechanisms to attain opacity phenotypes. Furthermore, epigenetic changes impact pneumococcal opacity morphology and pathogenicity. Hence, the role of epigenetic factors in phase variation and pathogenesis should be investigated in future studies.

## Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Melissa Hui Chieh Chai

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## Abbreviations

°C	degrees Celsius
µg	microgram/s
µg/ml	microgram/milliliter
µl	microlitre/s
2D-DIGE	two-dimensional differential gel electrophoresis
A	adenine
A <sub>340</sub>	absorbance at 340 nm
A <sub>520</sub>	absorbance at 520 nm
A <sub>550</sub>	absorbance at 550 nm
A <sub>600</sub>	absorbance at 600 nm
Adc	zinc ABC transporter
AdhE	alcohol dehydrogenase
AdoMet	S-adenosyl methionine
ADP	adenosine diphosphate
AGRF	Australian Genome Research Facility Ltd
AliA	an oligopeptide ABC transporter
<i>ami</i>	aminopterin resistance operon
APC	adelaide Proteomics Centre
APIA	acid-phenol:chloroform:isoamly alcohol
ATP	adenosine triphosphate
BA	blood agar
BBB	blood-brain barrier
Blp	bacteriocin protein
bp	base pairs
C	cytosine
C+Y	semi-synthetic medium
CAP	community-acquired pneumococcal (infections)
Cbp	choline-binding protein
CD4 <sup>+</sup>	cluster of differentiation 4



CFU	colony forming units
Cgl	competence protein
ChoP	pneumococcal cell-wall phosphorylcholine
CiaRH	two-component signal-transducing system
<i>cibA</i>	competence induced bacteriocin A gene
CNS	central nervous system
Com	competence protein
<i>comDE</i>	two-component signal-transducing system, competence
<i>cps</i>	capsular biosynthesis genes
CPS	capsular polysaccharide
CR	coding repeat
CreX	Cre tyrosine recombinase
CRP	C-reactive protein
CSF	cerebrospinal fluid
CSP	competence-stimulating peptide
Dam	deoxyadenosine methyltransferase
<i>dexB</i>	glucan 1,6-alpha-glucosidase
<i>divIVA</i>	cell division initiation gene
DNA	deoxyribonucleic acid
DnaJ	chaperone protein DnaJ
DnaJ/K	molecular chaperone
DOC	sodium deoxycholate
ECM	extracellular matrix
EDTA	ethylene-diamine-tetra-acetic-acid disodium salt
EF-G	elongation factor G
EF-Ts	elongation factor Ts
emPAI	exponentially modified protein abundance index
Erm/Ery	erythromycin
Fba	fructose-bisphosphate aldolase
FDR	false discovery rate
fH	factor H

FRR/EF-4	ribosome-recycling factor
<i>fucK</i>	putative L-fuculose kinase
FusA	translation elongation factor G
g/L	grams per litre
G	guanine
g	relative centrifugal force
G-3-P	D-glyceraldehyde-3-phosphate
<i>galU</i>	UTP-Glc-1-phosphate uridylyltransferase
GAPDH	glycerolaldehyde-3-phosphate dehydrogenase
Gent	gentamicin
GlcNAc	N-acetyl-glucosamine
GlpO	Alpha-glycerophosphate oxidase
GMP reductase/ <i>guaC</i>	guanosine 5' monophosphate oxidoreductase
Gor	glutathione reductase
GroEL	heat shock protein
GroES	heat shock protein
h	hour/s
hbMEC	human brain microvascular endothelial cells
Hic	factor H-binding inhibitor of complement
HIV	human immunodeficiency virus
Hyl	hyaluronate lyase
<i>hsd</i>	host-specificity-determinant gene
<i>hsdM</i>	host-specificity-determinant gene for modification
<i>hsdR</i>	host-specificity-determinant gene for restriction
<i>hsdS</i>	host-specificity-determinant gene for sequence specificity
i.n.	intranasal
i.p.	intraperitoneal
i.v.	intravenous
IEF	isoelectric focusing
IL-1	interleukin-1
IL-12	interleukin-12

<i>ileS</i>	isoleucyl-tRNA synthetase
IMP	inosine phosphate
INDEL	insertion/deletion
INF- $\gamma$	interferon $\gamma$
IPD	invasive pneumococcal disease
IPS	internal pool standard
IPTG	isopropyl- $\beta$ -D-thio-galactopyranoside
ISP	Ion Sphere <sup>TM</sup> Particles
kb	kilobase/s
kDA	kilodalton/s
KEGG	Kyoto Encyclopedia of Genes and Genomes
kg	kilogram/s
l	litre/s
LB	Luria Bertani broth
LC-ESI-IT MS	liquid chromatography electrospray ionisation ion-trap mass spectrometry
LctO	lactate oxidase
Ldh	lactate dehydrogenase
<i>lgtA</i>	beta-N-acetylglucosaminyltransferase
Liv	branched-chain amino acid ABC transporter
LPS	lipopolysaccharide
Lrp	leucine response regulatory protein
LuxS	S-ribosylhomocysteine lyase
<i>lysM</i>	lysine motif
LytA	N-acetylmuramoyl-L-alanine amidase
M	molar
Mal	maltose operon transcriptional protein
Mb	megabase/s
MDR	multi-drug-resistant
mg	milligram/s
mg/ml	milligram/millilitre

min	minute/s
ml	millilitre/s
mM	millimolar
Mod	methyltransferase
mRNA	messenger RNA
MurC	UDP-N-aceetylmuramate—alanine ligase
NAD	$\beta$ -Nicotinamide adenine dinucleotide hydrate
NanA	neuraminidase A
ng	nanogram/s
ng/ml	nanogram/millilitre
nm	nanometers
nt	nucleotide/s
Nrd	anaerobic ribonucleoside triphosphate reductase
O	opaque variant
OD	optical density
OE-PCR	overlap-extension polymerase chain reaction
Opa	opacity outer-membrane proteins
ORF	open reading frame
p.s.i.	per square inch
PacBio	Pacific BioSciences
PAFr	platelet-activating factor receptor
PAGE	polyacrylamide gel electrophoresis
Pap	pyelonephritis-associated pilus
PbcA	C3-binding protein A
PBMC	human peripheral blood mononuclear cells
PBP2B	penicillin-binding protein 2B
PBS	phosphate buffered saline
PcpA	pneumococcal choline-binding protein A
PCR	polymerase chain reaction
PCV13	pneumococcal conjugate vaccine, 13 serotypes
PCV7	pneumococcal conjugate vaccine, 7 serotypes

PECAM-1	platelet endothelial cell adhesion molecule-1
Pfl	pyruvate formate lyase/formate acetyltransferase
PGM	personal genome machine
<i>pgm</i>	phosphoglucomutase
Pht	polyhistidine triad
PiaA	pneumococcal iron uptake
PIgR	polymeric Ig receptor
Pit	pneumococcal iron transport
PiuA	pneumococcal iron uptake
PIgR	polymeric Ig receptor
Ply	pneumolysin
PMT	photomultiplier
PNSP	penicillin-non-susceptible <i>S. pneumoniae</i>
PotD	spermidine/putrecine ABC transporter
Ppc	phosphoenolpyruvate carboxylase
PPI-1	pneumococcal pathogenicity island 1
PpmA	putative proteinase maturation protein A
PPSV23	pneumococcal polysaccharide vaccine, 23-valent
PrsP	pneumococcal serine-rich protein
PsaA	pneumococcal surface adhesin A
PspC	pneumococcal surface protein C
PurA	adenylosuccinate synthetase
Pyk	pyruvate kinase
qRT-PCR	quantitative real-time PCR
qRT-PCR	real-time reverse transcription polymerase chain reaction
<i>radC</i>	DNA repair gene
RE	restriction endonuclease
RM	restriction-modification
RNA	ribonucleic acid
<i>rpsA</i>	ribosomal protein S1 gene
RT-PCR	reverse transcription polymerase chain reaction

s	second/s
SB	serum broth
SDH	streptococcal surface dehydrogenase
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
<i>sIgA</i>	secretory component of immunoglobulin A
SMRT	single-molecule real-time
SNPs	single nucleotide polymorphisms
SpsA	secretory IgA-binding protein
SpxB	pyruvate oxidase
sRNA	short non-coding RNA
SsbB	single-stranded DNA-binding protein
SSM	slipped-strand mispairing
T	transparent variant
T	thymine
TA	teichoic acid
TAE	tris-acetate EDTA buffer
TBE	tris, borate and EDTA
THY	todd-Hewitt broth supplemented with yeast extract
THY+catalase	THY supplemented with catalase
TLR	toll-like receptor
TNF	tumour necrosis factor
TRD	target recognition domain
TSB	tryptic soy broth
TTBS	tris-buffered saline containing Tween 20
U	unit
U/ml	unit/millilitre
UDP-Glc	UDP-glucose
v/v	volume per volume
w/v	weight per volume
WT	wild-type

wzy

capsular biosynthesis gene

ZMW

zero-mode waveguide





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