Novel polyhydroxyalkanoate bead-based vaccines against *Pseudomonas aeruginosa* infection

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Abstract

Pseudomonas aeruginosa infections are increasingly problematic due to their multiple antibiotic resistances. To date, there is no commercial vaccine against *P. aeruginosa* infection. This study used polyhydroxyalkanoate (PHA) beads as a delivery platform for selected *P. aeruginosa* antigens to produce novel particulate vaccines against *P. aeruginosa*. Genetic engineering was used to modify the PHA synthase (PhaC), the enzyme that catalyses PHA bead formation, to produce functionalised PHA beads displaying the antigens. The highly conserved PopB antigen, recently revealed as an effective stimulator of Th17 immunity was displayed on the bead surface together with and without the previously selected antigens Ag (epitopes derived from outer membrane proteins OprI, OprF and AlgE). The PHA beads were produced in two production strains: (1) the pathogen *P. aeruginosa* itself, with the benefit of co-purifying host cell proteins (HCPs) expanding the antigen repertoire that could boost the immune response, and (2) *E. coli* strain ClearColi^{TM}, a defined mutant incapable of lipopolysaccharide synthesis enabling production of endotoxin free PHA beads. Vaccination of mice with antigen-coated PHA beads (only PopB or with additional Ag) showed increased production of IL-17, a reflection of induction of a Th17 immunity. Furthermore, Th1 and Th2 mediated immunity were detected from the IgG analysis of the immunised mice with the PHA bead vaccines. Significant enhancement of Th1 immune response using the PHA bead platform was observed compared to the antigen-only counterparts. Challenge of immunised mice with pathogenic *P. aeruginosa* showed that PopB-displaying PHA beads from *P. aeruginosa* and Ag-displaying PHA beads from *E. coli* induced partially protective immunity. The promising PHA bead candidate vaccines can be conjugated with other antigens such as the exopolysaccharide Psl for induction of improved immune response towards protective immunity.

Exopolysaccharide Psl is a mannose rich polymer produced by *P. aeruginosa* in both mucoid and nonmucoid phenotypes. Psl has been revealed to play an essential role in *P. aeruginosa* pathogenicity such as biofilm formation. Previous studies had provided evidence that CdrA binds directly to Psl, functioning as a Psl cross-linker and possibly tethering Psl to the cell surface. Psl is commercially not available, hence the aim of this study was to develop a Psl production strain. This study created a *cdrA* knockout mutant that produces free Psl which may be a potential vaccine antigen against *P.*

aeruginosa infections. An isogenic knockout of *cdrA* was obtained by homologous recombination and this mutant overproduced Psl released from the cell surface. In future studies, this strain will be used to produce Psl to serve as antigen in vaccine formulations.

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"Success is not final, failure is not fatal: it is the courage to continue that counts".
-Winston Churchill-

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List of abbreviations

A full list of abbreviations used:

°C	Degree Celsius
APC	Antigen presenting cell
Ag	OprI, OprF and AlgE *P. aeruginosa* antigens
AGE	Agarose gel electrophoresis
Ag-PhaC	Ag-PHA_{SCL} synthase
Ag-$PhaC1_{Pa}$	Ag-PHA_{MCL} synthase
Amp	Ampicillin
APS	Ammonium persulfate
BL21	*E. coli* production strain
BSA	Bovine serum albumin
Cb	Carbenicillin
CDW	Cellular dry weight
Cm	Chloramphenicol
Δ	Delta (deleted)
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphates
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethanol
EDTA	Ethylenediaminetetraacetic acid
g	Gravity/gram
Gm	Gentamicin
GTP	Guanosine triphosphate
HCPs	Host cell proteins
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
IPTG	Isopropyl â-D-1-thiogalactopyranoside
kDa	Kilo Daltons
λ	Lambda (wavelength or type of phage)
λ-DNA	Phage Lambda DNA
LacZ	β-galactosidase

LB	Luria-Bertani (broth)
L	Litre
M	Molarity
ml	Millilitre
mM	Millimolar
MOPS	Morpholinopropane sulfonic acid
MS	Mass spectrometry
OD	Optical density
PCR	Polymerase chain reaction
PhaA	β-ketothiolase
PAO1	*P. aeruginosa* production strain
PHAs	Polyhydroxyalkanoic acids
PHA_{SCL}	Short chain length PHAs
PHA_{MCL}	Medium chain length PHAs
PhaB	Acetoacetyl-CoA reductase
PhaC	PHA_{SCL} synthase
PhaC-PopB	PHA_{SCL} synthase-PopB
PhaC-PopBAg	PHA_{SCL} synthase-PopBAg
$PhaC1_{Pa}$	PHA_{MCL} synthase
$PhaC1_{Pa}$-PopB	PHA_{MC} synthase-PopB
PhaE	Type III PHA synthase subunit
PhaP	Phasin
PhaR	Phasin regulatory protein
PHB	Poly(3-hydroxybutyric acid)
PIA	Pseudomonas isolation agar
PopBAg	Single fusion of PopB, OprI, OprF, and AlgE *P. aeruginosa* antigens
REs	Restriction endonucleases
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate gel electrophoresis
TBE	Tris-Borate-EDTA buffer
Tc	Tetracycline
TE	Tris-EDTA buffer

TEM	Transmission and Electron Microscopy
TEMED	Tetramethylethylenediamine
Tet	Tetracycline
TLR	Toll-like receptors
Tm	Primer melting temperature
Tris	Trishydroxymethylaminomethane
v/v	Volume per volume
w/v	Weight per volume
X-Gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
XL1-Blue	*E. coli* cloning strain

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