The development of polyester bead-based particulate subunit vaccine against Johne's disease

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Abstract

Johne's disease is the intestinal infection in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The disease is economically important in the dairy industry as infection of the calves or mature cattle can result in death. Current vaccination as a part of disease control is not only partially protective against MAP but also interferes with current diagnostic test for bovine tuberculosis. Therefore, more effective and defined vaccines are needed. In this study, vaccine candidates were developed by bioengineering *Escherichia coli* to produce polyhydroxyalkanoate (PHA) beads displaying selected vaccine candidate antigens as fusion proteins. The selected antigens were the MAP 85 antigen complex (Ag85A and Ag85B), Superoxide dismutase (SOD) and a recombinant fusion protein 74F, however, only the antigen-presenting beads with truncated Ag85A, Ag85B and SOD were successfully produced and purified. The fusion protein comprising the respective antigens was identified and confirmed to be associated with PHA beads. The PHA beads were partially purified for future characterisations such as binding of antigen specific antibodies on PHA beads *in vitro* and immunological properties in animal models.

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Abbreviation

°C	Degree Celsius
µm	Micrometers
BCA	Bicinchoninic acid used in protein assay
BSA	Bovine serum albumin
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
GC/MS	Gas chromatography mass spectrometry
HA_{SCL}	Short-chain-length hydroxyalkanoic acid
HA_{MCL}	Medium-chain-length hydroxyalkanoic acid
HRP	Horse radish peroxidise
IgG	Immunoglobulin G
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	Kilo Daltons
kbp	Kilo base pairs
MALDI-TOF MS	Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry
MAP	*Mycobacterium avium* subspecies *paratuberculosis*
MOPS	3-(N-Morpholino) propanesulfonic acid
Nile-Red	9-diethylamino-5-benzo[α]phenoxazinone
PB	Phosphate buffer saline
PBS	Phosphate buffer salt
PBST	Phosphate buffer salt tween-20
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutarate
PhaA	β-ketothiolase
PhaB	Acetoacetyl-CoA reductase
PhaC	PHA synthase
PhaCAB	PHA operon
PhaPs	Phasins
PhaR	Transcriptional regulator protein
PhaZ	PHA depolymerase
SDS-PAGE	Sodium dodecyl sulfate Polyacrylamide gel electrophoresis
SOD	Superoxide dismutase
TFF	Tangential flow filtration
X-GAL	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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