Functional protein display on the surface of biobeads produced by recombinant *Escherichia coli*

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Shuxiong Chen

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Appendix D

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Name of Chief Supervisor: Bernd Rehm Telephone Ext: 7890

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Abstract

Polyhydroxyalkanoic acids (PHAs) are biopolyesters produced by various bacteria. They are deposited as spherical water-insoluble cytoplasmic inclusions (beads) containing an amorphous hydrophobic polyester core and surrounded by a phospholipid monolayer and embedded proteins, including PHA synthase (PhaC), the key enzyme required for PHA bead formation. Although inactive PhaC cannot produce PHA beads, fusing inactive PhaC to green fluorescent protein (GFP) leads to GFP protein bead formation. Both PHA and protein beads could serve as a versatile platform for display of desired proteins suitable for various biotechnological and medical applications.

The tuberculin skin test (TST) for diagnosing bovine tuberculosis (TB) in cattle uses the purified protein derivative (PPD) that is prepared from *Mycobacterium bovis*. However, some antigens in the PPD are also present in environmental mycobacteria. Therefore, the TST lacks specificity if animals are exposed to non-pathogenic environmental mycobacteria. In this study, three specific TB antigens, CFP10, ESAT6, and Rv3615c — which are present in pathogenic but absent in most non-pathogenic mycobacteria — were displayed on the surface of PHA beads. The results demonstrated that these triple antigen-displaying PHA beads can differentiate TB-infected from non-infected cattle, making this an attractive alternative to current skin test diagnostic reagents.

IgG binding domains displayed on GFP protein beads have a higher IgG binding ability when compared to their counterpart displayed on PHA beads. However, it is unclear whether an enhancement of IgG binding ability due to GFP protein beads could be achieved by immobilization on other fluorescent protein (FP) beads. The results showed that other FP (including yellow, red and cyan) beads displaying IgG binding domains have an approximately 1.5–2 fold greater IgG binding ability when compared to PHA beads displaying the same binding domains. To investigate whether protein beads displaying iron-binding peptides could be magnetized while maintaining IgG binding function, an iron binding peptide was displayed. The results demonstrated that protein beads displaying both IgG and iron binding peptides can be magnetised by iron oxide and retain a strong IgG binding ability. Finally, this study revealed that different cell disruption techniques could affect the morphology and functionality of FP protein bead.

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List of abbreviations

A full list of abbreviations used

^{o}C	Degree Celsius
3xFe	Triple iron-binding peptides
AGE	Agarose gel electrophoresis
Ap	Ampicillin
APS	Ammonium persulfate
BL21/	*E. coli* production strain
BL21 69/	*E. coli* production strain harbours plasmid pMCS69, encoding PhaA and PhaB enzymes
BSA	Bovine serum albumin
BCG vaccine strain	Live-attenuated *Mycobacterium bovis* Bacile Calmette-Guerin vaccine strain
CFP10	The 10-kDa culture filtrate protein
CFP10-PhaC	The 10-kDa culture filtrate protein–PHA synthase
CFP10-Rv3615c-PhaC-ESAT6	The 10-kDa culture filtrate protein-Rv3615c-PHA synthase-the 6-kDa early secretory antigenic target
Cm	Chloramphenicol
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DNAase	Deoxyribonuclease
dNTPs	Deoxyribonucleotide triphosphates
DTH	Delayed-type hypersensitivity
ECFP	Enhanced cyan fluorescent protein
ECFP-PhaC(C319A)-[GB1]3	Enhanced cyan fluorescent protein-inactive PHA synthase-triple IgG binding domain of protein G
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethanol
ESAT6	The 6-kDa early secretory antigenic target
ESAT6-PhaC	The 6-kDa early secretory antigenic target–PHA synthase

EYFP	Enhanced yellow fluorescent protein
EYFP-PhaC(C319A)-[GB1]3	Enhanced yellow fluorescent protein-inactive PHA synthase-triple IgG binding domain of protein G
FPs	Fluorescent proteins
FP beads	Fluorescent protein beads
FP-PhaC(C319A)	Fluorescent protein-inactive PHA synthase
g	Gravity/gram
[GB1]3	Triple IgG binding domains of protein G
GFP	Green fluorescent protein
GFP beads	Green fluorescent protein beads
GFP-PhaC(C319A)	Green fluorescent protein-inactive PHA synthase
HcR	Far red protein HcRed
HcR-PhaC(C319A)-[GB1]3	Far red protein HcRed-inactive PHA synthase-triple IgG binding domains of protein G
HRP	Horseradish peroxidase
IFNγ	Interferon-γ
IgG	Immunoglobulin G
IPTG	Isopropyl â-D-1-thiogalactopyranoside
kDa	Kilo Daltons
λ-DNA	Phage lambda DNA
LacZ	β-galactosidase
LB	Luria-Bertani (broth)
MalE	Maltose binding domain
Maldi-TOF/MS	Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
OD	Optical density
OpdA	Organophosphohydrolases
PCR	Polymerase chain reaction
PhaA	β-ketothiolase
PHAs	Polyhydroxyalkanoic acids
PHA_{SCL}	Short chain length PHAs
PHA_{MCL}	Medium chain length PHAs

PhaB	Acetoacetyl-CoA reductase
PhaC	PHA synthase
PhaC-[GB1]3	PHA synthase-triple IgG binding domains of protein G
PhaC(C319A)	Inactive PHA synthase
PhaE	Type III PHA synthase subunit
PhaP	Phasin
PhaR	Phasin regulatory protein
PHB	Poly(3-hydroxybutyric acid)
PPDs	Purified protein derivatives
PPD-A	Avian PPD
PPD-B	Bovine PPD
REs	Restriction endonucleases
Rv3615c-PhaC	Rv3615c–PHA synthase
SD	Standard deviation
SEM	Scanning Electron Microscopy
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TB	Tuberculosis
TBE	Tris-Borate-EDTA buffer
TEM	Transmission and Electron Microscopy
TEMED	Tetramethylethylenediamine
Tet	Tetracycline
TST	Tuberculin skin test
Tris	Trishydroxymethylaminomethane
v/v	Volume per volume
w/v	Weight per volume
x	AVTS tetrapeptide extension
X-Gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
xECFP-PhaC(C319A)-[GB1]3	AVTS tetrapeptide extension-enhanced cyan fluorescent protein-inactive PHA synthase-triple IgG binding domain of protein G

xEYFP-PhaC(C319A)-[GB1]3	AVTS tetrapeptide extension-enhanced yellow fluorescent protein-inactive PHA synthase-triple IgG binding domain of protein G
xGFP-PhaC(C319A)-MalE	AVTS tetrapeptide extension-green fluorescent protein-inactive PHA synthase-maltose binding domain
xGFP-PhaC-MalE	AVTS tetrapeptide extension-green fluorescent protein- PHA synthase-maltose binding domain
xGFP-PhaC(C319A)-ZZ	AVTS tetrapeptide extension-green fluorescent protein-inactive PHA synthase-double IgG binding domains of protein A
xGFP-3xFe-PhaC(C319A)-ZZ	AVTS tetrapeptide extension-green fluorescent protein-triple iron-binding peptides-inactive PHA synthase-double IgG binding domains of protein A
xGFP-PhaC(C319A)-ZZ-3xFe	AVTS tetrapeptide extension-green fluorescent protein-inactive PHA synthase-double IgG binding domains of protein A-triple iron-binding peptides
xHcR-PhaC(C319A)-[GB1]3	AVTS tetrapeptide extension-far red protein HcRed-inactive PHA synthase-triple repeats of IgG binding domain of protein G
XL1-Blue	*E. coli* cloning strain
ZZ	Double IgG binding domain of protein A
ZZPhaC	Double IgG binding domain of protein A-active PHA synthase

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