FUNCTIONAL DISPLAY OF IMMUNOGLOBULIN BINDING DOMAINS ON THE SURFACE OF BIOPOLYMER BEADS

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ABSTRACT

Protein A, G and L are immunoglobulin binding proteins isolated from the cell wall of certain gram positive bacteria. The interaction between the binding domain of these proteins and the immunoglobulin molecule occur without affecting the functional Fc region of the antibody, thus making them an ideal tool for antibody purification. The capacity of protein A to bind IgG with such high affinity is the driving motivation for its industrial scale use for immunoglobulin purification such as in chromatography resins. A major disadvantage however, is the inability of protein A to bind certain subclasses of IgG as well as IgG from certain species; a pitfall that can be overcome with the display of either protein G in combination with protein A, or with protein L which binds a range of immunoglobulin based on light chain interactions. Here we display both the binding domain of protein A with the binding domain of protein G on a single platform; the surface of polyhydroxyalkanoate (PHA) biopolyester granules. We also produce a PHA granule displaying the binding domain of protein L. This was achieved via fusion and expression of the genes for these immunoglobulin binding bacterial proteins and the *phaC* gene on a single plasmid construct. The *phaC* gene codes for polyhydroxyalkanoate synthase (PhaC), a critical enzyme involved in PHA granule production in the bacterial host and which remains covalently attached to the surface of the PHA granule. When transformed into an *E.coli* strain engineered for polyhydroxyalkanoate (PHA) bead production, the functional PhaC allows for the self-assembly of intracellular PHA beads with the immunoglobulin binding proteins expressed on their surface. Based on the results of this study, these novel beads provide us with added functionalities and significantly increased immunoglobulin binding efficiency when compared to commercial standards, which could lead to an up-scaled production of novel bio polyester beads to serve as an ideal tool for immunoglobulin purification.

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"Vi veri universum vivus vici"

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Abbreviations

˚C	**Degree Celsius**
3HB	**3-hydroxybutyrate**
AGE	**Agarose Gel Electrophoresis**
BSA	**Bovine Serum Albumin**
DMSO	**Dimethyl sulfoxide**
FM	**Fluorescent microscopy**
GC/MS	**Gas chromatography mass spectrometry**
Ig	**Immunoglobulin**
kDa	**Kilo Daltons**
Maldi-TOF/MS	**Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry**
PBS	**Phosphate buffered saline**
PHA	**Polyhydroxyalkanoate**
PhaC	**PHA synthase**
PHB	**Polyhydroxybutyrate**
SDS-PAGE	**Sodium dodecyl sulfate gel electrophoresis**
WT	**Wild-type**