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A new cloning system based on the OprI lipoprotein for the production of recombinant bacterial cell wall-derived immunogenic formulations

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Abstract

The conjugation of antigens with ligands of pattern recognition receptors (PRR) is emerging as a promising strategy for the modulation of specific immunity. Here, we describe a new *Escherichia coli* system for the cloning and expression of heterologous antigens in fusion with the OprI lipoprotein, a TLR ligand from the *Pseudomonas aeruginosa* outer membrane (OM). Analysis of the OprI expressed by this system reveals a triacylated lipid moiety mainly composed by palmitic acid residues. By offering a tight regulation of expression and allowing for antigen purification by metal affinity chromatography, the new system circumvents the major drawbacks of former versions. In addition, the anchoring of OprI to the OM of the host cell is further explored for the production of novel recombinant bacterial cell wall-derived formulations (OM fragments and OM vesicles) with distinct potential for PRR activation. As an example, the African swine fever virus ORF A104R was cloned and the recombinant antigen was obtained in the three formulations. Overall, our results validate a new system suitable for the production of immunogenic formulations that can be used for the development of experimental vaccines and for studies on the modulation of acquired immunity.

Highlights

► We propose a new system for expression of antigens fused with the OprI lipoprotein. ► It offers tight control of expression and metal affinity chromatography purification. ► The OprI expressed by the system is triacylated, mainly by palmitic acid residues. ► The cloned antigens can be obtained in three bacterial cell wall-derived formulations. ► Applications include studies on immunization and on modulation of acquired immunity.

Abbreviations

- ALFA, amide-linked fatty acid;
- ASFV, African swine fever virus;
- CTL, cytotoxic T lymphocyte;
- DDM, n-dodecyl-β-d-maltopyranoside;
- DM, n-decyl-β-d-maltopyranoside;
- ELFA, ester-linked fatty acid;
- FA, fatty acid;
- GLC, gas–liquid chromatography;
- IPTG, isopropyl-β-d-thiogalactopyranoside;
- MCS, multiple cloning site;
- MHC, major histocompatibility complex;
- MS, mass spectrometry;
- OG, octyl β-d-glucopyranoside;
- OM, outer membrane;
- OMF, outer membrane fragments;
- OMV, outer membrane vesicles;
- OprI, outer membrane lipoprotein I;
- PMSF, phenylmethylsulfonyl fluoride;
- PRR, pattern recognition receptors;
- TCA, trichloroacetic acid;
- TLR, Toll-like receptors

Keywords

- Outer membrane lipoprotein I;
- *Pseudomonas*;
- Bacterial outer membrane proteins;
- Cloning vector;
- Immunomodulation;
- Adjuvant