Recombinant expression of OmpA, OmpC and BamA proteins as universal vaccines against *Escherichia coli* in mice

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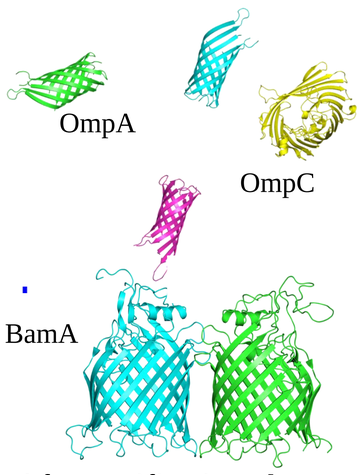
ABSTRACT



Pathogenic *Escherichia coli* (*E. coli*) is one of the primary pathogens in humans and domestic animals, and the infection is commonly caused by multiple serotypes, thus leading to an urgent need for universal vaccines. In this study, the outer membrane proteins (OmpA, OmpC and Surf_Ag_VNR domain (at aa position 448-810) of BamA) were analyzed in silico for sequence homology. The result showed that all these proteins from *E. coli* CVCC 1515 share a high homology with other *Escherichia*, *Shigella* and *Salmonella* strains. Then the proteins were expressed in BL21 (DE3) using the auto-induction method. After purification, the recombinant proteins were estimated to be approximately 40 kDa by SDS-PAGE with the purity of 93.5 % (rOmpA), 96% (rOmpC) and 93.5% (rBamA), respectively. Immunological analysis indicated that the titers of antiserum reached to 1:642,000 (anti-rOmpA), 1:240,000 (anti-rOmpC) and 1:736,000 (anti-rBamA) against the recombinant proteins and 1:140,000 (anti-rOmpA), 1:27,000 (anti-rOmpC) and 1:152,000 (anti-rBamA) against to the whole *E. coli* cells. Moreover, it also generated cross-reaction against *Shigella* and *Salmonella* stains. Opsonophagocytosis assay revealed that the antiserum induced the phagocytic activity of neutrophils against *E. coli*. Survival rate of mice vaccinated with rOmpA, rOmpC, rBamA and PBS was 50%, 50%, 80% and 20%, respectively. These data indicated that the rOmpA, rOmpC, rBamA proteins could serve as promising universal vaccine candidates for the development of protective subunit vaccine against bacterial infection. Additionally, the above protocol would provide the more feasible technical clues and choices for available control of key pathogenic *Escherichia*, *Salmonella* and *Shigella* in epidemic prevention of animal husbandry.

Homology and phylogenesis analysis

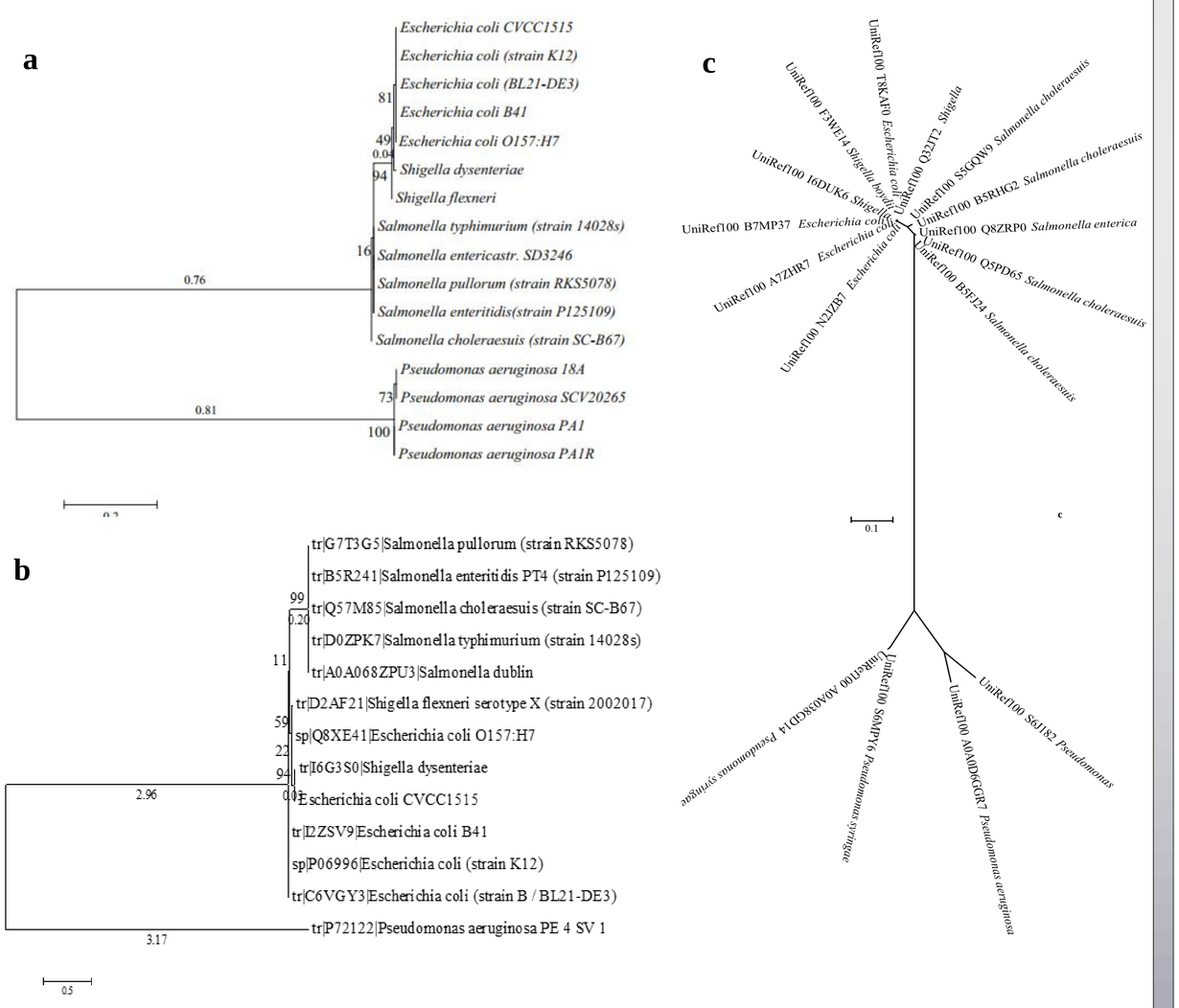


Fig 1 Phylogenesis analysis of the a) OmpA, b) OmpC and c) BamA proteins in *E. coli*, *Salmonella*, *Shigella* and *Pseudomonas*

Expression, purification of the proteins

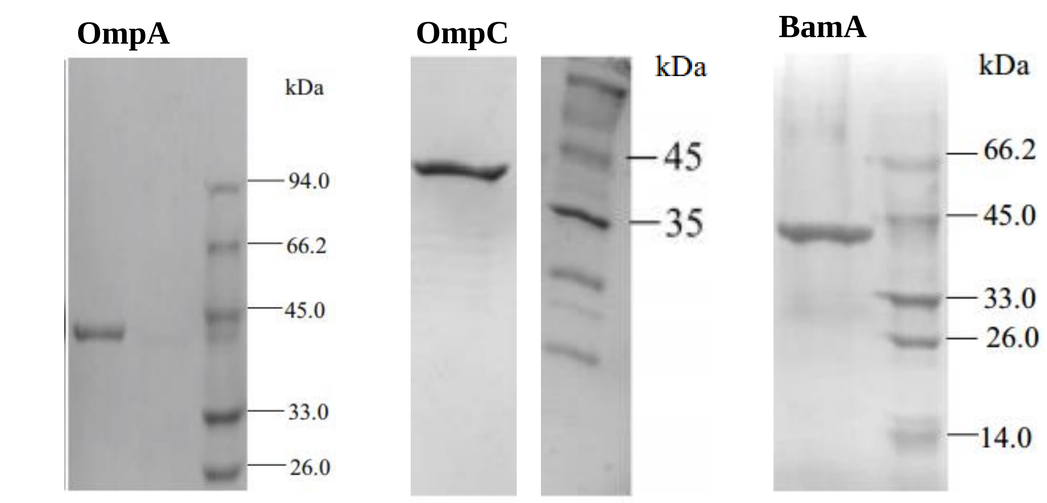


Fig 2 Purification of the rOmpA, rOmpC and rBamA proteins. The proteins were estimated to be approximately 40 kDa by SDS-PAGE with the purity of 93.5 % (rOmpA), 96% (rOmpC) and 93.5% (rBamA)

Immunological analysis *in vitro* and *in vivo*

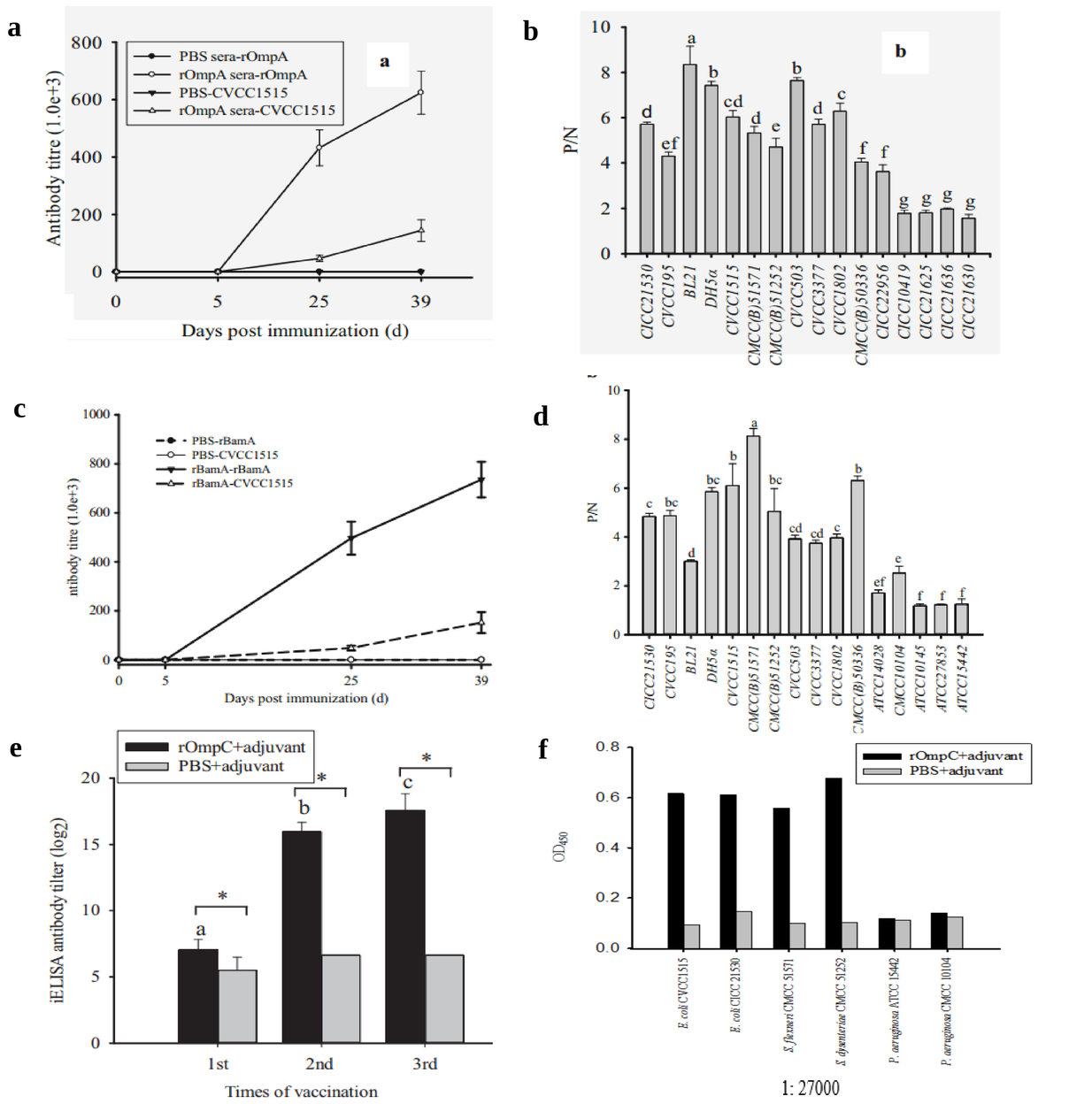


Fig 3 Antibody titers of rOmpA, rOmpC and rBamA. Antiserum titers reached to a) 1:642,000 (anti-rOmpA), c) 1:240,000 (anti-rOmpC) and e) 1:736,000 (anti-rBamA) against the proteins and b) 1:140,000 (anti-rOmpA), d) 1:27,000 (anti-rOmpC) and f) 1:152,000 (anti-rBamA) against to the whole *E. coli* cells. Moreover, it also generated cross-reaction against *Shigella* and *Salmonella* stains (b, d, f)

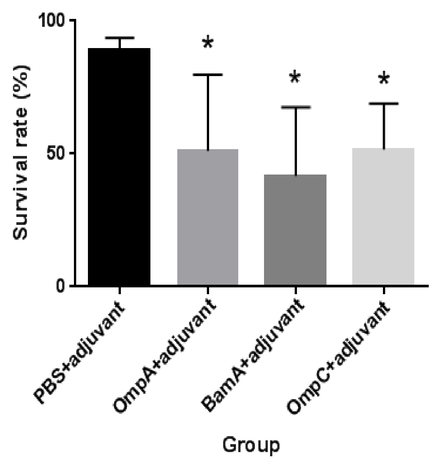


Fig 4 Opsonophagocytosis of the anti-protein sera *in vitro*

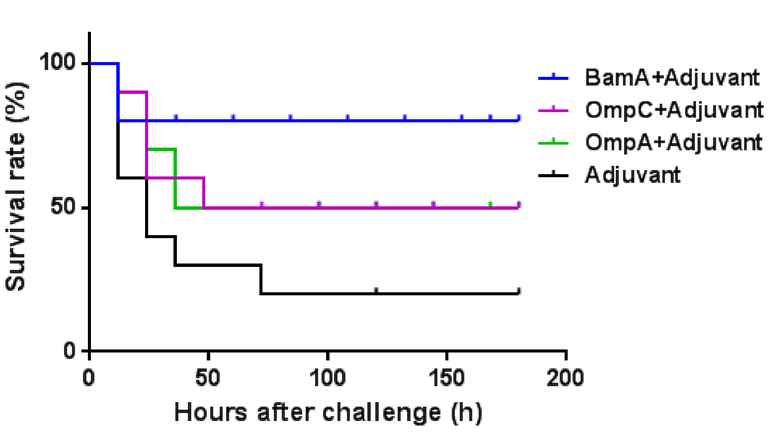


Fig 5 Protection efficacy of the anti-serum against *E. coli* CVCC 1515 *in vivo*

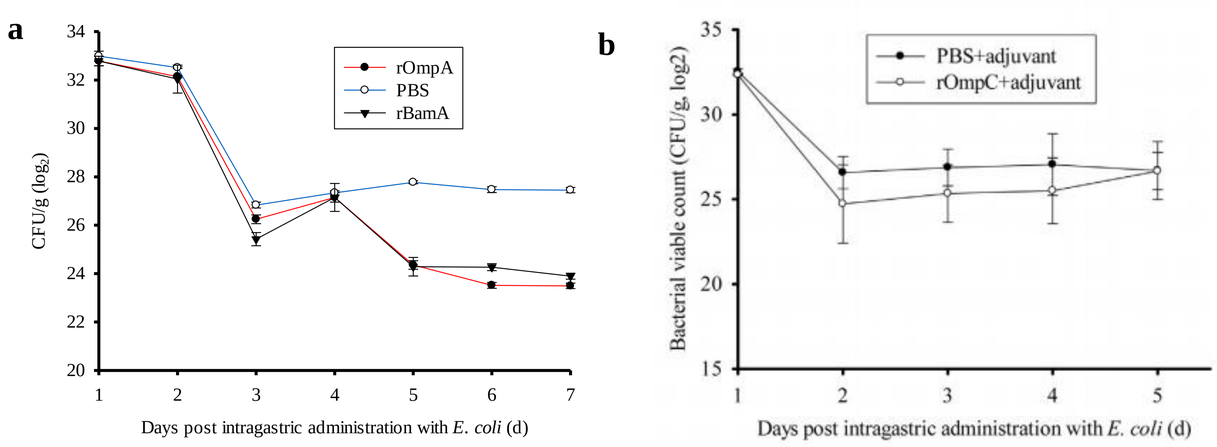


Fig 6 Protection efficacy of the anti-serum against *E. coli* CICC 21530 (serotype O157:H7) *in vivo*. Fecal shedding of the mice immunized with proteins or PBS

ACKNOWLEDGEMENT

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