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Recombinant outer membrane protein A induces a protective immune response against *Escherichia coli* infection in mice

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Abstract Pathogenic *Escherichia coli* (*E. coli*) is an important infectious Gram-negative bacterium causing millions of death every year. Outer membrane protein A (OmpA) has been suggested as a potential vaccine candidate for conferring protection against bacterial infection. In this study, a universal vaccine candidate for *E. coli* infection was developed and evaluated. Bioinformatics analysis revealed the OmpA protein from *E. coli* shares 96~100 %, 90~94 %, and 45 % identity with *Shigella*, *Salmonella*, and *Pseudomonas* strains, respectively. The *ompA* gene was cloned from the genomic DNA of *E. coli*, and then the OmpA protein was expressed in BL21 (DE3) using the auto-induction method. The recombinant OmpA (rOmpA) protein had an average molecular weight of 36 kDa with the purity of 93.5 %. Immunological analysis indicated that the titers of anti-rOmpA sera against rOmpA and whole cells were 1:642,000 and 1:140,000, respectively. Moreover, rOmpA not only conferred a high level of immunogenicity to protect mice against the challenge of *E. coli*, but also generated cross-protection against *Shigella* and *Salmonella*. The anti-rOmpA sera could enhance the phagocytic activity of neutrophils against *E. coli*. The survive ratios of mice immunized with rOmpA and PBS were 50 % and 20 % after 48 h post-challenge, indicating mice were protected from *E. coli* infection after immunization with rOmpA. All these results clearly indicate that rOmpA may be a promising candidate for the development of a subunit vaccine to prevent *E. coli* infection.

Keywords Outer membrane protein A · Immunogenicity · Vaccine · *Escherichia coli* · Mice

Introduction

Escherichia coli (*E. coli*), a facultative and extracellular Gram-negative bacterium, is the predominate organism in intestinal tract of human and other homoiothermal animal. Although most *E. coli* are harmless, some strains can cause diverse gastrointestinal tract diseases, such as diarrhea and emesis, and even colorectal cancer (CRC) in human and other animals (Guiral et al. 2011; Levine and Edelman 1984; Pakalniskiene et al. 2009; Arthur et al. 2012). This opportunistic pathogens entering into internal environment can also cause hematologic malignancies, such as hemolytic–uremic syndrome, early onset neonatal sepsis, and so on (Borgatta et al. 2012; Rasko et al. 2011; Stoll et al. 2011). There are 1.4~2.5 million deaths due to pathogenic *E. coli* each year (Murray et al. 2013). Moreover, some extra intestinal pathogenic *E. coli* strains are also the cause of significant economic losses in animal farms, especially in the poultry industry (Ron 2006).

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Presently, the disease caused by *E. coli* is mainly controlled by the use of antibiotics or vaccines. However, abuse of antibiotics has led to an increasing number of emergences of drug resistance to *E. coli* and destruction of intestinal flora microecology balance (Ronzio 2003; Johnson et al. 2010; Perez et al. 2014; Zhu et al. 2013). In comparison, vaccination is a more effective way of preventing infectious diseases than antibiotics. Vaccines, including inactivated vaccine and subunit vaccine, against *E. coli* strains have been studied closely in recent years. Inactivated vaccine could induce remarkable immune response associated with protection against pathogenic bacteria. Savarino et al. reported inactivated enterotoxigenic *E. coli* (ETEC) plus cholera toxin B has been proved to be safe and immunogenic in 2- to 12-year-old children as oral vaccine (Savarino et al. 1999).

The number of *E. coli* serotypes is very high, 50,000~100,000 or more (Ørskov and Ørskov 1992). *E. coli* infection is commonly caused by multiple serotypes, so it is quite expensive and time-consuming to develop vaccines against each serotype of pathogenic *E. coli* strains. In order to effectively resolve this problem, the subunit vaccines are often administered along with adjuvants and substances, which are able to increase the immunogenicity of antigens. Güereña-Burgueño et al. (2002) reported that a vaccine against ETEC could induce significant systemic immune responses in the presence of an adjuvant such as heat-labile enterotoxin (Güereña-Burgueño et al. 2002). Several studies revealed that type III secreted proteins vaccines significantly reduce fecal shedding in cattle, and prevalence of *E. coli* O157:H7 in a clinical trial was significantly reduced (Potter et al. 2004; Snedeker et al. 2012). These few vaccines available against *E. coli* provide only limited protection against homologous challenge. Therefore, there is a need for a more universal effective vaccine against *E. coli*.

Outer membrane protein A (OmpA), a major surface protein in *E. coli*, plays an important role in biofilm formation, host cell invasion, pore-forming, and multidrug resistance (Ma and Wood 2009; Martinez et al. 2014; Zakharian and Reusch 2005; Smani et al. 2014). Moreover, OmpA is also an important factor of adhesion in *E. coli* O157:H7 and other strains, which is of vital importance to pathogenicity of bacteria (Nair and Venkitanarayanan 2007; Smith et al. 2007). OmpA has been revealed to be highly immunogenic proteins and may represent a good candidate for vaccine development against bacterial infection (Li et al. 2014). Hu et al. (2013) reported that more than 30 % of the animals immunized with OmpA survived after lethal challenge, which demonstrated that OmpA significantly protect against *E. coli*, *Klebsiella pneumonia*, and *Shigella flexneri*. Yan et al. (2010) found that three recombinant OmpA-like proteins generated strong immune responses, enhanced survival, and reduced the severity of histopathological lesions, which indicated that these three OmpA-like proteins may serve as novel vaccine candidates for leptospirosis (Yan et al. 2010). OmpA in other Gram-negative bacteria has also been reported to be a potential vaccine candidate in other bacteria (Dabo et al. 2008; Maiti et al. 2011; Yan et al. 2010).

In this study, the *ompA* gene was cloned from the genomic DNA of *E. coli* strain CVCC 1515 and expressed by a prokaryotic system. The resultant recombinant OmpA (rOmpA) was used as an antigen to prepare vaccines, and protective efficacy of the rOmpA vaccine was evaluated against *E. coli* in vivo and in vitro.

Materials and methods

Bacterial strains and mice

E. coli CVCC 1515, *E. coli* CVCC 195, *Salmonella choleraesuis* CVCC 503, *Salmonella enteritidis* CVCC 3377, and *Salmonella pullorum* CVCC 1802 were purchased from China Veterinary Culture Collection Center (CVCC) (Beijing, China). *E. coli* CICC 21530 (serotype O157:H7), *Salmonella typhimurium* CICC 22596, *Pseudomonas aeruginosa* CICC 10419, CICC 21625, CICC 21636, and CICC 22630 were purchased from China Center of Industrial Culture Collection (CICC) (Beijing, China). *Shigella dysenteriae* CMCC (B) 51252, *Shigella flexneri* strain CMCC (B) 51571, and CMCC (B) 50336 were purchased from National Center for Medical Culture Collection (CMCC) (Beijing, China). *E. coli* competent strains of DH5α and BL21 (DE3) were purchased from TransGen Biotech Co., Ltd. (Beijing, China). All stains were cultured on Luria Bertani (LB) at 37 °C.

The *ompA* gene sequence of *E. coli* strain CVCC 1515 was deposited in the National Center for Biotechnology Information (NCBI) GenBank (GenBank accession no. KP031704).

Specific pathogen-free (SPF) female 6~8-week-old BALB/c mice were purchased from Vital River Laboratories (VRL, Beijing). Mice were housed in appropriate conventional animal care facilities and handled according to international guidelines required for animal experiments.

Bioinformatics analysis of the OmpA protein

Amino acid sequences of OmpA were manually aligned as described previously (Yousef Mohamad et al. 2008). Sequence of OmpA in *E. coli* strain K12 was used as a reference for blasting. Based on similarities of OmpA, five *E. coli* strains, two *Shigella* strains, five *Salmonella* strains, and four *P. aeruginosa* strains were used for phylogenetic analysis using the bootstrap method.

Expression and purification of the rOmpA protein

Genomic DNA was extracted from *E. coli* strain CVCC1515 using a TIANamp Bacteria DNA Kit (Tiangen Biotech,

Beijing, China) following the manufacturer's instructions. The primers, rOmpA F-EcoRI: 5′-GAATTCGCTCCGAA AGATAACACCTGGTACAC-3′ with the *Eco*RI restriction enzyme site and rOmpA R-NotI: 5′-GCGGCCGCAGCTTG CGGTTGAGTTACTACGTC-3′ with the *Not*I restriction enzyme site, were designed to amplify the *ompA* gene. The amplified PCR fragment was inserted into the pMD19 (Simple) T Vector (TaKaRa Biotech. (Dalian) Co., Ltd) and transformed into *E. coli* DH5α competent cells. The recombinant pMD19-ompA plasmid was isolated using the TIANprep Mini Plasmid Kit (Tiangen Biotech, Beijing, China), and digested with *Eco*RI/*Not*I.

The digested DNA fragment was then cloned into the similarly digested pET28a expression vector to generate the plasmid pET28a-ompA. The recombinant plasmids were transformed into *E. coli* BL21 (DE3) and identified by restriction enzyme digestion and DNA sequencing. The recombinant His-tagged OmpA protein was expressed in BL21 (DE3) using the auto-induction method with some modifications (Studier 2005). Briefly, the transformant cells were cultured in LB medium at 37 °C on a platform shaker at 250 rpm to an optical density at 600 nm ($OD_{600\,nm}$) of 0.40 to 0.60, and then transplanted to ZYM-5052 auto-induction media with 1 % inoculum density. The stains were cultured at 37 °C on a platform shaker with a speed of 300 rpm for 24 h.

The rOmpA protein was purified and refolded according to Saleem's protocol with some minor modifications (Saleem et al. 2012). Briefly, after the fusion protein being sufficiently expressed, the cultured cells were harvested by centrifugation at 5000×*g* for 30 min at 4 °C and resuspended in lysis buffer (50 mM Tris-HCl buffer, pH 7.9, containing 5 mg of lysozyme and 5 μl of DNase I type IV stock per gram of cell paste) (8 ml/g wet weight of cell paste). The cells were sonicated for 5~6 min with an Ultrasonic Crasher Noise Isolating Chamber (SCIENTZ, Ningbo Science Biotechnol Co., Ltd., China) on ice. Inclusion bodies (IBs) were precipitated with centrifugation at 14,000×*g* for 20 min at 4 °C and washed twice in 50 ml of 50 mM Tris-HCl buffer (pH 7.9) containing 1.5 % (*v*/*v*) lauryl dimethyl amine oxide (LDAO) for each 1~1.5 g wet weight. After that, the IBs were precipitated and dissolved in denaturing buffer (10 mM Tris-HCl buffer, pH 7.5, containing 1 mM ethylenediamine tetraacetic acid (EDTA) and 8 M urea). The IBs solution was centrifuged at 14,000×*g* for 20 min to remove any undissolved material and was added dropwise to rapid stirred refolding buffer (20 mM Tris-HCl buffer, pH 7.9, containing 1 M NaCl and 5 % (*v*/*v*) LDAO) to produce a final 1:1 volume ratio. The solution was dialyzed at 4 °C against two changes of 4 l of dialysis buffer (20 mM Tris-HCl buffer, pH 7.9, containing 0.5 M NaCl and 0.1 % (*v*/*v*) LDAO) every 6 h for refolding. The fusion protein was purified using a Ni^{2+}-nitriloacetate (NTA) super flow resin column (QIAGEN, Germany) with equilibration buffer (20 mM Tris-HCl buffer, pH 7.9, containing 0.5 M NaCl, 0.1 % (*v*/*v*) LDAO and 40 mM imidazole) and elution buffer (20 mM Tris-HCl buffer, pH 7.4, containing 0.5 M NaCl, 0.1 % (*v*/*v*) LDAO and 500 mM imidazole) according to the manufacturer's instructions. Then the eluted recombinant protein was desalted using a HiPrep 26/10 desalting column with desalination buffer (20 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 0.1 % (*v*/*v*) LDAO). All proteins were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purity of proteins was calculated by the Gel-Pro Analyzer™ version 6.3 (Media Cybernetics). The purified rOmpA protein was then lyophilized with an ALPHA 1-2 LD plus freeze dryer (Christ, Germany) and kept at −20 °C.

Immunization protocols

Five SPF female Balb/c mice, 6~8 week-old were immunized with the purified rOmpA protein on day 0, day 21, and day 35 as previously reported by Reddy et al. (2010). The lyophilized rOmpA protein was resuspended in sterile-filtered PBS at 1.0 mg/ml. For the primary immunization, 25 μl of the rOmpA solution was mixed with 25 μl of complete Freund's adjuvant (Sigma-Aldrich, Inc.) and 50 μl of sterile PBS. Mice were hypodermically injected with antigen mixture (100 μl/mouse). The mice in control group were immunized with PBS instead of rOmpA.

Subsequent two intraperitoneal injections consisted of 25 μg of rOmpA emulsified with 25 μl of incomplete Freund's adjuvant (Sigma-Aldrich, Inc.). All mice were housed in individually ventilated cages (Suzhou Fengshi Laboratory Animal Equipment Co., Ltd, Suzhou) and monitored daily. Cages were changed once per week. The mice were bled from the tail vein on days 0, 5, 25, and 39, and sera were stored at −20 °C until used.

Indirect enzyme-linked immunosorbent (iELISA) assay

rOmpA detection by iELISA

One hundred microliters of rOmpA (0.2 μg/well) in coating buffer (0.015 M sodium carbonate, 0.035 M sodium bicarbonate, pH 9.6) were incubated overnight at 4 °C in 96-well plates. After washing four times with 0.01 M PBST (PBS containing 0.05 % Tween 20), the plates were blocked for 2 h at 37 °C by adding 0.01 M PBST containing 5 % BSA. After washing with PBST four times, the plates were added with serial dilutions of mice serum and incubated for 1.5 h at 37 °C and washed as above. HRP conjugated goat anti-mouse IgG was diluted 1:5000 and added into the plates. The plates were incubated for 30 min at 37 °C. Finally, 3,3′,5,5′-tetramethylbenzidine (TMB) was added to each well and incubated in the dark at room temperature for 20 min. The reaction was stopped by adding 2 mol/l H_2SO_4 (50 μl/well). The

absorbance of each well at 450 nm was read by a microplate reader (Perlong Medical, Beijing). The result was considered as positive when the ratio of the test group and negative control group was greater than 2.1.

Bacterial cell detection by iELISA

The 96-well plates were coated by incubating them for 1 h at 37 °C with 150 μl 0.1 mol/l $NaHCO_3$ containing 2.5 % glutaraldehyde and washed with sterile water four times. Bacterial cells (10^8 colony-forming unit (CFU)/ml, 100 μl/well) were then added into the plates and incubated at 37 °C for 24 h. The subsequent steps were the same as the procedure given above.

Serum bactericidal assay

Serum bactericidal assay (SBA) was performed by the protocol described previously with some modifications (Marzoa et al. 2012). Briefly, the mid-log phase bacteria were resuspended in PBS (10^5 CFU/ml). The 96-well cell culture plates were placed on ice until assayed. The volume of each reaction mixture was 100 μl, containing 50 μl of serial twofold mouse serum, 25 μl of the bacterial cells, and 25 μl of baby rabbit complement (Cedarlane, Hornby, ON, Canada). The plates were incubated at 37 °C for 45 and 90 min, and 20 μl of samples from each well were plated onto LB agar. Colony counts were recorded after 0, 45, and 90 min of incubation at 37 °C. The percentage of cells killed at each dilution was computed by using the post-incubation colony counts from the inactive complement control wells as the zero kill reference. The serum dilution giving closest to 50 % kill was reported as the titer.

Opsonophagocytosis assay in vitro

As previously described, neutrophils were isolated from mouse peritoneal fluid and adjusted to a concentration of 4×10^6 cells/ml (Luo and Dorf 2001). Briefly, 400 μl of log-phase *E. coli* CVCC 1515 cells (4×10^4 CFU/ml) was incubated with 100 μl of appropriately diluted immune or nonimmune mouse serum at 37 °C for 30 min. The bacteria were then incubated with 500 μl of neutrophils suspension and 100 μl of baby rabbit complement (Cedarlane, Hornby, ON, Canada) at 30 °C for 1 h. After incubation, neutrophils were lysed by adding sterile water into the mixture. The mixture was then serially diluted for plate count.

Feces *E. coli* counting

E. coli strain CICC 21530 was cultivated to the logarithmic phase and adjusted to 10^{11} CFU/ml. Mice were intragastric administrated after an overnight fast. Fecal samples were collected every day for up to 7 days, and viability was measured by the plate colony count.

Challenge assay

Lethal dose of 50 % (LD_{50}) was determined by the method of Reed and Muench (Reed and Muench 1938). Fourteen days after the final immunization, 10 mice from each group were injected intraperitoneally with 100 μl (10^{11} CFU/ml) logarithmic phase *E. coli* CVCC 1515 strain. Mortality was recorded each 12 h, and animals were monitored for up to 7 days post-challenge.

Statistical analysis

All statistical analyses were performed using SPSS version 22.0. One-way repeated analysis of variance (ANOVA) Dunkan method and the Mann-Whitney rank test were used to determine the significance of the differences between groups. Differences were considered significant at $p<0.05$.

Results

Bioinformatics analysis of the OmpA protein

The hylogenetic analysis result showed that OmpA is highly conserved among the *Enterobacteriaceae* family (96~100 % identity). OmpA from *E. coli* shares 96~100 %, 90~94 %, and 45 % identity with that of *Shigella*, *Salmonella*, and *Pseudomonas* strains, respectively (Fig. 1).

Cloning, expression, and purification of the rOmpA protein

The DNA fragment of *ompA* with the size of 987 bp was successfully amplified from the genomic DNA of *E. coli* and cloned into the pMD19 T vector. The digestion fragment of the *ompA* gene was inserted into the pET28a digested with the same enzymes and resultant the new recombinant pET28a-ompA plasmid. The DNA sequencing and restriction enzyme digestion results showed the pET28a-ompA plasmid was constructed successfully. The expression of rOmpA fused with His tag in *E. coli* BL21 (DE3) was auto-induced to 24 h at 37 °C. As shown in Fig. 2, the expressed rOmpA protein was present in the IBs. The molecular weight of rOmpA was approximately 36 kDa, which is consistent with theoretical molecular weight. The purification of rOmpA was performed by a Ni^{2+}-NTA affinity chromatography. After purification and refolding, the purity of rOmpA was up to 93.5 %, falling within the 80~98 % purity ranges of other protein antigens which have been shown to be the effective vaccine candidates (Frace et al. 1999; Garcõa-Garcõaa et al. 2000; Zhang and Pan 2005; Cheng et al. 2009).

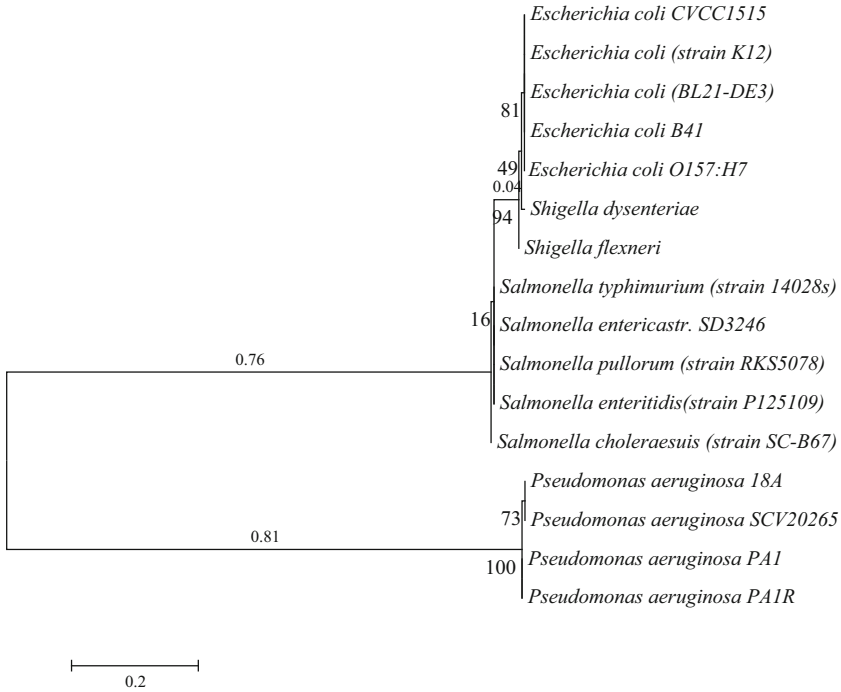

Fig. 1 A phylogenetic tree based on the *E. coli* OmpA amino acid sequences was constructed using MEGA 6.0 software and Maximum likelihood method

Immunogenicity of the rOmpA protein in mice

To assess the immunogenic property of the rOmpA protein, sera were collected from mice on days 0, 5, 25, and 39 prior to challenge and tested for reactivity with the purified rOMPs by iELISA. The results shown in Fig. 3a revealed mice immunized with rOmpA exhibited a significant immunogenicity. The initial strong and significant immune responses to rOmpA were first detected at day 25 and continued to increase after the third immunization. The titer of the OmpA sera reached their highest levels of 1:642,000 after the third immunization. Meanwhile, the OmpA sera also have a high affinity to *E. coli* CVCC 1515 (1:140,000 dilution after the third immunization). The result of sera obtained from PBS vaccinated mice clearly indicated the absence of any rOmpA specific antibody.

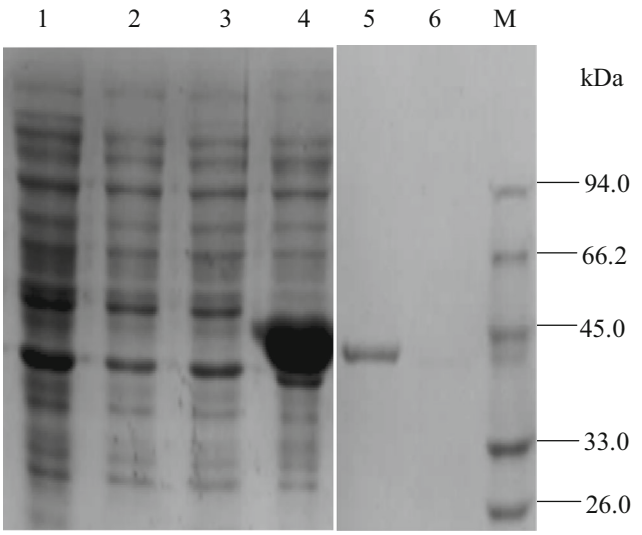

Fig. 2 Expression and purification of rOmpA. Proteins were stained with Coomassie and analyzed by SDS-PAGE. *Lane 1*, total proteins of BL21; *lanes 2*, *3*, total proteins of BL21 harboring pET28a; *lane 4*, total proteins of BL21 harboring pET28a-ompA; *lanes 5*, *6*, eluent of the purified rOmpA protein; *lane M*, molecular weight marker (14~94 kDa)

Cross-reaction properties of the anti-rOmpA sera

Cross-reaction properties of the anti-rOmpA sera were performed among *E. coli*, *Shigella*, *Salmonella*, and *P. aeruginosa* stains by the whole cell iELISA assay. Mice sera were serially diluted, and the ratio of test group and negative control group greater than 2.1 was considered as positive. As shown in Fig. 3b, *E. coli*, *Shigella*, and *Salmonella* strains except for *P. aeruginosa* stains (1:3000~9000) have a high antibody titer (>1:27,000). The results showed that OmpA was highly conserved in *E. coli*, *Shigella*, and *Salmonella*, which is in accordance with the results of bioinformatics analysis.

Bactericidal activity and phagocytosis of the anti-rOmpA sera against *E. coli* CVCC 1515 in vitro

SBA was performed to evaluate the bactericidal activity of serum. As shown in Fig. 4a, colony counts of other groups except for the control group were found to be raised in a time dependent manner. After incubation for 90 min, group 1 (rOmpA sera + complement) and the control group 2 (PBS)

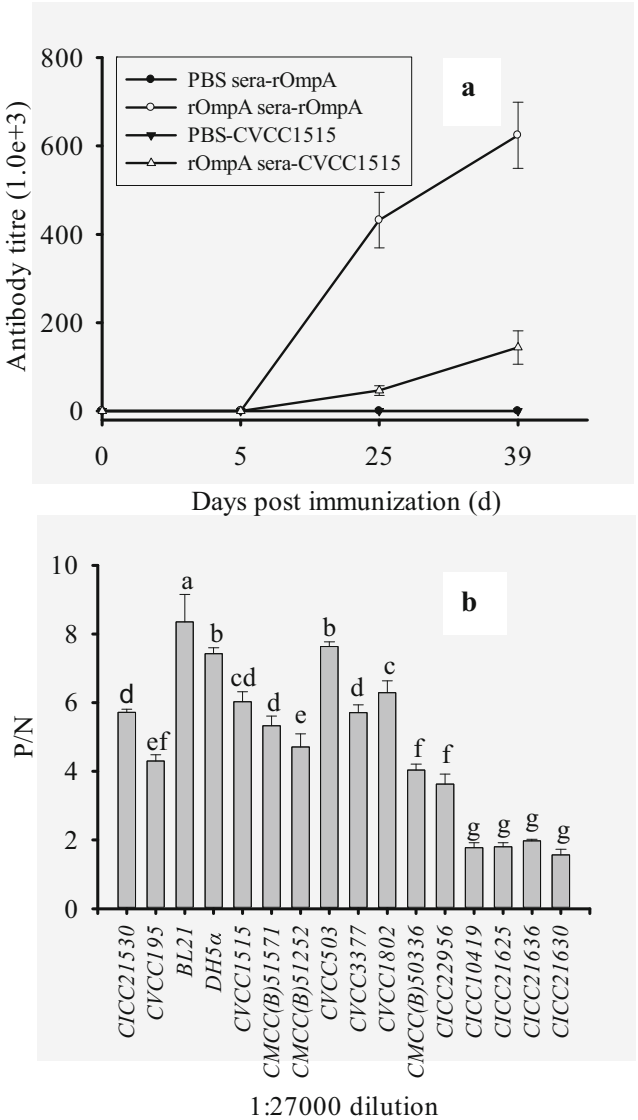

Fig. 3 Sera antibody titers of rOmpA analyzed by iELISA. Mice were vaccinated with rOmpA or PBS. Serum samples were collected at days 0, 5, 25, and 39, respectively, and antibody titers were measured by iELISA using the purified rOmpA as antigen. The error bars represent the standard deviations of triplicate measures. **a** Sera antibody titer in different groups post-vaccination. PBS sera-rOmpA, the antibody titer of PBS vaccination group against rOmpA; rOmpA sera-rOmpA, the antibody titer of rOmpA vaccination group against rOmpA; PBS sera-CVCC1515, the antibody titer of PBS vaccination group against *E. coli* CVCC 1515; rOmpA sera-CVCC1515, the antibody titer rOmpA vaccination group against *E. coli* CVCC 1515. **b** Cross-reaction properties of the anti-rOmpA sera (titer of 1:27,000) against different bacteria. Statistical significance ($p<0.05$) is indicated by a *lowercase letter*

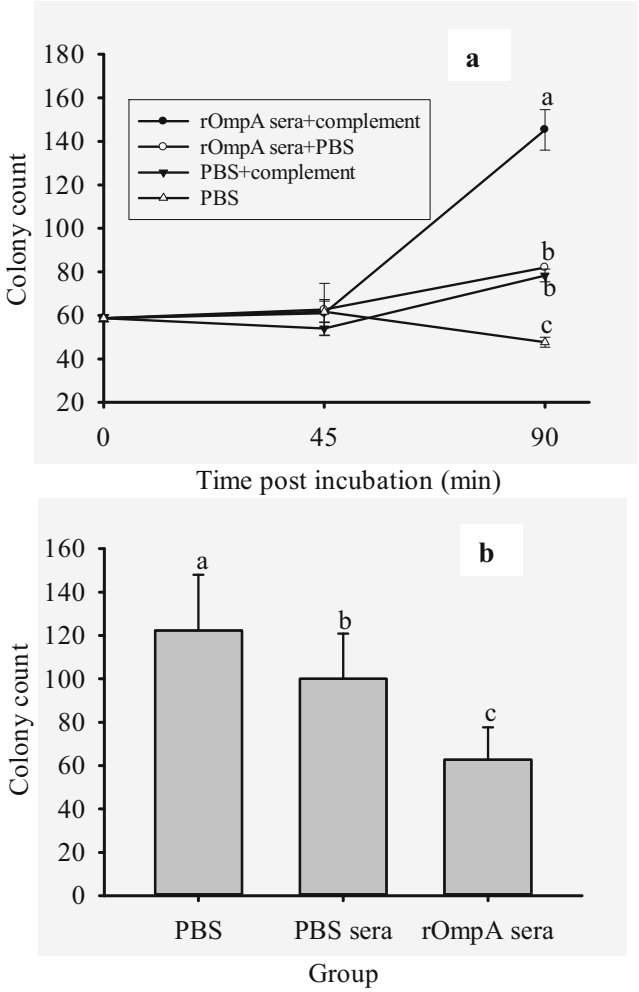

Fig. 4 Bactericidal activity and phagocytosis of the anti-rOmpAsera in vitro. **a** Bactericidal activity of sera in classic complement pathway. *E. coli* strain CVCC 1515 was incubated with rOmpA sera and complement (group 1), rOmpA sera and PBS (group 2), PBS and complement (control group 1), and PBS (control group 2), respectively. **b** Colony counts of different groups in phagocytosis. *E. coli* strain CVCC 1515 was incubated with antisera of PBS vaccination group (group 1: PBS sera), antisera of OmpA vaccination group (group 2: rOmpA sera) and PBS (control group). Statistical significance ($p<0.05$) is indicated by a *lowercase letter*

have the maximum and minimum number of colonies, respectively. The results indicated that anti-rOmpA sera had no bactericidal activity against *E. coli* CVCC 1515 in vitro, nutritional ingredient in sera maybe inversely promote the growth of *E. coli*.

E. coli CVCC 1515 cells were incubated with neutrophils and sera from mice immunized with rOmpA (group 2) or PBS (group 1). As shown in Fig. 4b, after incubation for 30 min, the number of viable bacterial cells in group 1 (PBS sera) was decreased by 18 % compared with control group (PBS), which showed that neutrophils have phagocytic activity. Bacterial cells in group 2 (rOmpA sera) was decreased by 37.33 % compared to group 1 (PBS sera), and 59.66 % to control group (PBS) with p value <0.05, showing that the anti-rOmpA sera can enhance the phagocytic activity of neutrophils against *E. coli* in vitro.

Efficacy of the anti-rOmpA sera against *E. coli* in vivo

Mice were administered intragastrically with *E. coli* CICC 21530 (10^{11} CFU), and feces were collected for plate

counting. The shedding of *E. coli* in feces after the administration of *E. coli* CICC 21530 is shown in Fig. 5a. There was a dramatic decrease in the first 3 days after administration in both groups. In mice immunized with rOmpA, the shedding of *E. coli* in feces was significantly ($p<0.05$) decreased after 5 days post-administration and was about 10^7 CFU/g at 7 days post-administration. After 4 days of administration, fecal shedding of *E. coli* in the control group (PBS) was increased continuously and was up to 10^8 CFU/g at 7 days post-administration.

Two weeks after the third vaccination, control and rOmpA-immunized mice were challenged with *E. coli* CVCC1515 by intraperitoneal injection, and the protective efficacy of rOmpA was evaluated in terms of survival number. Survival ratio of mice immunized with rOmpA was higher than that of control mice. After 36 h post-challenge, the survival ratio of rOmpA group was kept to 50 %, but the survival ratio of control group was 20 % after 48 h post-challenge (Fig. 5b). The results indicated that the vaccination with rOmpA protected mice from lethal *E. coli* infection.

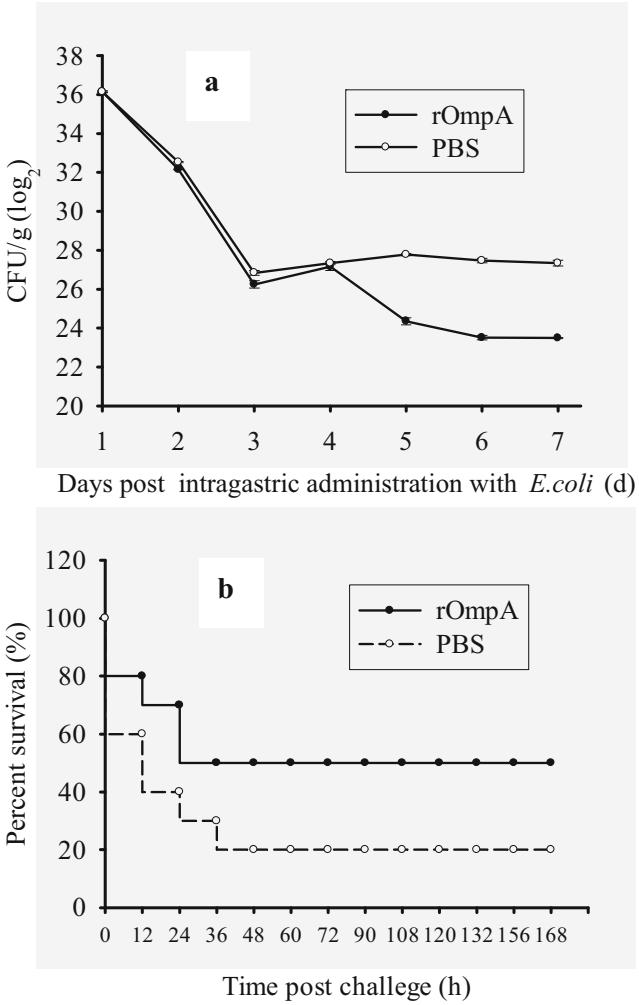

Fig. 5 Protection efficacy of the anti-rOmpA sera against *E. coli* in vivo. The mice immunized with rOmpA or PBS (control) were administered intragastrically with *E. coli* CICC 21530 (10^9 CFU) and observed for 7 days after challenge. **a** Viable count of fecal from the mice vaccinated with rOmpA or PBS. **b** Survival percent of the mice immunized with rOmpA or PBS

Discussion

OmpA is a major structural protein of the outer membrane of *E. coli* and a highly immunogenic bacterial component due to their exposed epitopes on the cell surface. Due to the different serotypes of pathogenic *E. coli*, the development of a versatile vaccine that provides heterologous protection for *E. coli* has been a growing concern. Previous reports have demonstrated the immunogenic potential of OmpA as novel vaccine candidates against Leptospirosis (Yan et al. 2010), *Riemerella anatipestifer* (Huang et al. 2002), *Edwardsiella tarda* (Maiti et al. 2011), and *Shigella* (Pore et al. 2011). The goal of the present study was to develop a universal vaccine in *E. coli* strains.

Only approximately 50 % sequence homology of PRRSV virus genotypes can be distinguished by their immunological properties (Dea et al. 2000). Our results suggest that there is a high degree of amino acid sequence conservation (identity >96 %) of OmpA among *E. coli* and *Shigella* strains (Fig. 1). The similarities of the OmpA protein from *Salmonella* and *Pseudomonas* ranged from 45 to 94 %, which implies that OmpA may be a shared antigen among these strains (Li et al. 2014).

The OmpA protein, expressed in this study, is a 329-amino acid protein composed of an N-terminal extracellular and transmembrane domain (residues ~22–194) and a C-terminal periplasmic domain (residues ~195–329) (Arora et al. 2001; Koebnik 1999). To increase epitope sequestered or masked in the interior of IBs, rOmpA was further refolded after purification. Meanwhile, a mild detergent LDAO, which dissociated the dimer into monomers, was used in desalination buffer in this study (Kruip et al. 1994). The iELISA result showed that higher affinity antisera against rOmpA (1:642,000 dilution) were observed than against *E. coli* (1:140,000 dilution) (Fig. 3a), which not only indicated membrane protein immunogenicity of the purified rOmpA protein was maintained, but also that antibodies against the extracellular domain of transmembrane proteins can specifically recognize bacteria. Additionally, it was speculated that other non-extracellular domain of rOmpA may be the epitope for anti-OmpA antibody (Shirai et al. 2012). However, the immunological characteristic of OmpA is yet to be further investigated.

Antigenic cross-reactivity of OMPs has been reported among Gram-negative bacteria (Xu et al. 2005; Lun et al. 2014). In our study, significant antibody responses to *Shigella* and *Salmonella* strains were observed in Fig. 3b,

suggesting high homology in the amino acid sequence among these strains. However, the anti-rOmpA sera weakly recognize *Pseudomonas* strains, indicating the lower homology in the amino acid sequence. These results were consistent with similarities of the amino acid sequences (Fig. 1). Additionally, the result further indicated that OmpA has a broad cross-reaction property among Gram-negative bacteria.

So far, it is found that there are three pathways of complement activation, including classical pathway (Cooper 1985), alternate pathway (Götze and Mueller-Eberhard 1971), and mannan-binding lectin (MBL) pathway (Vang Petersen et al. 2001). Complement can be activated by classical pathway during antibody recognizing and eliminating pathogen (Carroll 2004; Cooper 1985). In this study, *E. coli* could not be killed by the coexistence of antisera and complement (Fig. 4a). On the contrary, multiplication rate of *E. coli* strains in group 1 (rOmpA sera+complement) was faster than those of other three groups. Two reasons may be suggested for the dissimilar results in the four groups. One reason may be that the classic complement pathway was not be activated by the antibody, and the other one may be that adequate nutrition in sera promoted the growth of *E. coli* (Morrison and Kline 1977; Nieman 1954). However, opsonophagocytosis assay showed that antibody could significantly ($p<0.05$) accelerate the phagocytosis of phagocyte against *E. coli* (Fig. 4b).

It was known that OmpA is also an important factor of adhesion in O157:H7 and other *E. coli* strains, and it is of vital importance in pathogenicity of bacteria (Nair and Venkitanarayanan 2007; Smith et al. 2007). In this study, *E. coli* CICC 21530 was used to investigate the scavenging activity of rOmpA vaccination. The result showed that viable count was decreased in the first 3 days and increased at day 4, revealing that the strains begun to colonize in intestinal tract. However, colony count immune with rOmpA was decreased at day 5, which was significantly lower than that of the control group ($p<0.05$) (Fig. 5a). The similar result was also found in a previous report that demonstrated that immunized mice with outer membrane protein (OMP)-VP0802 had significantly more efficient clearance of *Vibrio parahaemolyticus* than that of control mice (Li et al. 2014). It is apparent that, in an effective vaccine-based therapy, specific antibodies against outer membrane proteins (OMPs) can effectually reduce the adhesion and colonization of bacteria and enhance complement-mediated clearance of circulating bacteria (Li et al. 2014).

To evaluate the potential immune protection of the anti-OmpA sera against *E. coli* infection, an active protection assay in a mouse model was performed in this study. Our results showed that mice vaccinated with rOmpA were well protected when challenged with *E. coli* CVCC 1515 (Fig. 5b). Similar results were also found in previous studies that demonstrated that anti-OmpA, OmpL, and other Omp serum had a high bactericidal activity for *Escherichia*, *Salmonella*, *Klebsiella*, *Shigella*, *Edwardsiella*, and *Leptospira* serovars (Kawai et al. 2004; Yan et al. 2010; Hu et al. 2013).

In conclusion, the present study found that the OmpA protein shared a high degree of similarity and distributed widely among Gram-negative strains. The anti-rOmpA sera had a significant cross-reaction capacity against several pathogenic strains of *Escherichia*, *Shigella*, and *Salmonella*. Vaccination with rOmpA can effectively reduce the colonization of *E. coli* and improve the survival rate of mice against *E. coli* infection. The results indicated that OmpA is the most promising candidate antigen for the development of a subunit vaccine.

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