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Generation and use of *Edwardsiella ictaluri* ghosts as a vaccine against enteric septicemia of catfish (ESC)

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ABSTRACT

The enteric septicemia of channel catfish, caused by *Edwardsiella ictaluri*, is one of the most severe diseases in channel catfish worldwide. Currently, a safe and highly effective vaccine is urgently needed for the control of this disease. In this study, *E. ictaluri* ghosts (EIGs) were first generated by PhiX174 lysis gene E, and the hematological indices and relative percent of survival (RPS) were examined in channel catfish (*Ictalurus punctatus*) after immunization. Generation of ghosts in the transformant of *E. ictaluri* carrying the plasmid pBV-lysisE was enhanced by increasing the incubation temperature up to 42 °C. Lysis of *E. ictaluri* occurred 2 h after induction and the process was complete in 6 h. The efficiency of ghost induction in non-lyophilized *E. ictaluri* was 99.997%. Additionally, no bacterial growth was detected by culture on BHI plates after lyophilization of the ghosts. The immune response of channel catfish immunized with the *E. ictaluri* ghosts indicated that the number of erythrocytes and leucocytes reached a maximum value of 3.53×10^6 and 5.25×10^5 cell/ml, at day 4 post-immunization, respectively, which were both significantly higher than in the control group inoculated with PBS only ($P < 0.01$). The percentage of monocyte and neutrophils reached $7.33 \pm 1.52\%$ and $26.3 \pm 2.08\%$ at day 4 post-immunization, respectively. The phagocytic percentage (PP) and phagocytic index (PI) reached maximum values of $49.33 \pm 1.52\%$ and 5.67 ± 1.15, respectively, which were significantly higher than in control group ($P < 0.01$). The percentage of lymphocytes and the serum antibody titers also increased significantly and peaked at $61.33 \pm 0.58\%$ and a titer of 1:682.67 at day 21 post-immunization, respectively. The challenge evaluation showed that the RPS of immunized group was 89.3%. These results demonstrated that EIGs could induce significant immunological responses in channel catfish that resulted in high protection and suggest that EIGs may be a potential vaccine against ESC in channel catfish.

Statement of relevance: In the paper, we illustrated the generation method of *Edwardsiella ictaluri* ghosts and the immunological responses and protection in channel catfish after being immunized with *E. ictaluri* ghosts. The result of this paper indicated that *E. ictaluri* ghosts would be an effective approach for preventing ESC in channel catfish in the future.

1. Introduction

Edwardsiella ictaluri, a facultative intracellular Gram-negative flagellated bacterium, is the causative agent of Edwardsiellosis in many commercially important freshwater and marine fish such as channel catfish *Ictalurus punctatus* (Zhang and Arias, 2007), Zebrafish *Danio rerio* (Hawke et al., 2013) and yellow catfish *Pelteobagrus fulvidraco* (Ye et al., 2009). *E. ictaluri* causes the disease enteric septicemia (ESC) in channel catfish, that is responsible for significant economic impacts to the channel catfish industry throughout the world (Dumpala et al., 2015). ESC usually occurs in late spring, early summer and fall when the water temperatures are between 22 and 28 °C (Pridgeon et al., 2010; Williams and Lawrence, 2005). It affects all size classes of catfish, develops rapidly, spreads easily and causes high mortality (Santander et al., 2014). In general, the disease occurs as both an acute form, that is characterized by enteritis and septicemia and a chronic form that has a slower progression and causes a "hole-in-the-head" type of lesion.

To control ESC epizootics the general practice is to feed diseased fish with antibiotic-medicated food (DePaola et al., 1995). However, this practice is expensive and usually ineffective, because sick fish do not feed and subsequently do not receive the treatment. Additionally, because of the potential risk of inducing antibiotic resistant strains of bacteria, many countries restrict the number and quantity of antibiotics that can be used (Roberts, 2012). Vaccination has become an increasingly important prevention strategy against infectious diseases in farmed fishes. Several attempts have been made to elicit protection against

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ESC with formalin killed preparations of *E. ictaluri*, however, no protection or only partial protection was achieved following immersion or injection immunization (Thune et al., 1997; Wise et al., 2015). Currently, a modified live *E. ictaluri* vaccine has been developed in the United States, and it is reported to be efficacious for the prevention of ESC in channel catfish (Klesius and Shoemaker, 1999; Pridgeon and Klesius, 2011; Shoemaker et al., 2002). But, live vaccines have the risk of reversion back to a virulent strain and are not permitted to be used in many countries because of this risk.

Bacterial ghosts (BGs) offer a new strategy for the study of inactivated vaccines. BGs are empty and intact bacterial envelopes of Gram-negative bacteria that are produced by controlled expression of the phage PhiX174 lysis gene E (Cai et al., 2013; Vinod et al., 2015). Protein E leads to the formation of a 40–200 nm transmembrane tunnel structure on the cell surface by fusing the internal and external membranes of Gram-negative bacteria (Peng et al., 2011). With the high internal osmotic pressure, the bacterial genome and cytoplasmic contents are expelled through the tunnel, leaving an empty cell envelope (Kwon et al., 2005). The surface structures remain in their original state and because of this BGs are a potent vaccine system and able to induce strong immune responses (Zhu et al., 2015). The use of BGs as vaccine candidates is a new approach to introduce safe and potent vectors for the prevention of infectious diseases (Szostak et al., 1996). This strategy has been used on several Gram-negative bacteria including *Yersinia enterocolitica* (Cai et al., 2013), *Salmonella enteritidis* (Peng et al., 2011) and *Edwardsiella tarda* (Kwon et al., 2005) using the plasmid-encoded protein E-mediated lysis technique, and as vaccines they possessed good immunogenicity and were able to induce a high level of efficacy. In the study reported herein, *E. ictaluri* ghosts (EIGs) were generated for the first time using the phage PhiX174 lysis gene E and channel catfish were immunized by intraperitoneal injection. After immunization various immune parameters were monitored and relative percent survival (RPS) was evaluated.

2. Materials and methods

2.1. Bacterial strains and culture conditions

E. ictaluri, *Escherichia coli* and *Staphylococcus aureus* strains were obtained from the Yangtze River Fisheries Research Institute. Transformed *E. ictaluri* and *E. coli* were grown in brain heart infusion (BHI) or Luria-Bertani medium containing 50 μg/ml ampicillin (Sigma, USA). Incubation temperatures for repression and expression of the lysis gene in transformants were 28 °C and 42 °C, respectively. Growth and lysis of bacterial cultures were monitored by measuring the optical density at 600 nm (OD_{600}) every hour.

2.2. Construction of lysis vector

The lysis E gene was amplified by polymerase chain reaction (PCR) and cloned into pBV220 vector (New England BioLabs Inc., USA) (Fig. 1A) using *Eco*RI and *Bam*HI cloning sites. Primers were designed according to the sequence of the phiX174 lysis gene E (GenBank Accession No.: J02483) and synthesized commercially (Tsingke, Wuhan, China). Primers were as follows: upstream primer LysisE-F: 5′-CGGAATTCATGGTACGCTGGACTTTGTGGG-3′ (*Eco*RI restriction site was underlined) and downstream primer LysisE-R: 5′-CGGGATCCTCACTC CTTCCGC-3′ (*Bam*HI restriction site was underlined). PCR amplifications were performed for 1 cycle of 5 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 56 °C, 1 min at 72 °C, with a final extension step of 7 min at 72 °C. PCR reactions were conducted using the iCycler thermal cycler (BioRad, USA). Each amplified PCR product was visualized on 1.0% agarose gels stained with ethidium bromide, purified with a gel extraction kit (Promega, USA) and cloned into pMD-19-T vector, named pMD-lysisE (TakaRa, Dalian, China). Both plasmids pMD-lysisE and pBV220 were digested with *Eco*RI (NEB) and *Bam*HI (NEB), and the lysis E gene was inserted into the plasmid pBV220. The resulting plasmid was designated as pBV-lysisE (Fig. 1B).

2.3. Transformation of bacteria

E. ictaluri were inoculated into 100 ml BHI broth and grown at 30 °C to an OD_{600} of 0.5 with vigorous agitation (200 rpm). The cells were washed twice with cooled PBS, and resuspended in 1 ml cooled 10% Glycerol. pBV-lysisE was mixed with 100 μl of the cell suspension and incubated on ice for 5 min, then transferred into a precooling 1 mm cuvette. Electrotransformation condition was time constant of 5 ms and a field strength of 2.1 kV/cm using a Electroporator 2510 Pulse controller (Eppendorf, Germany). After the electric shock, 800 μl of BHI medium was immediately added to the cuvette and incubated for 1 h at 28 °C

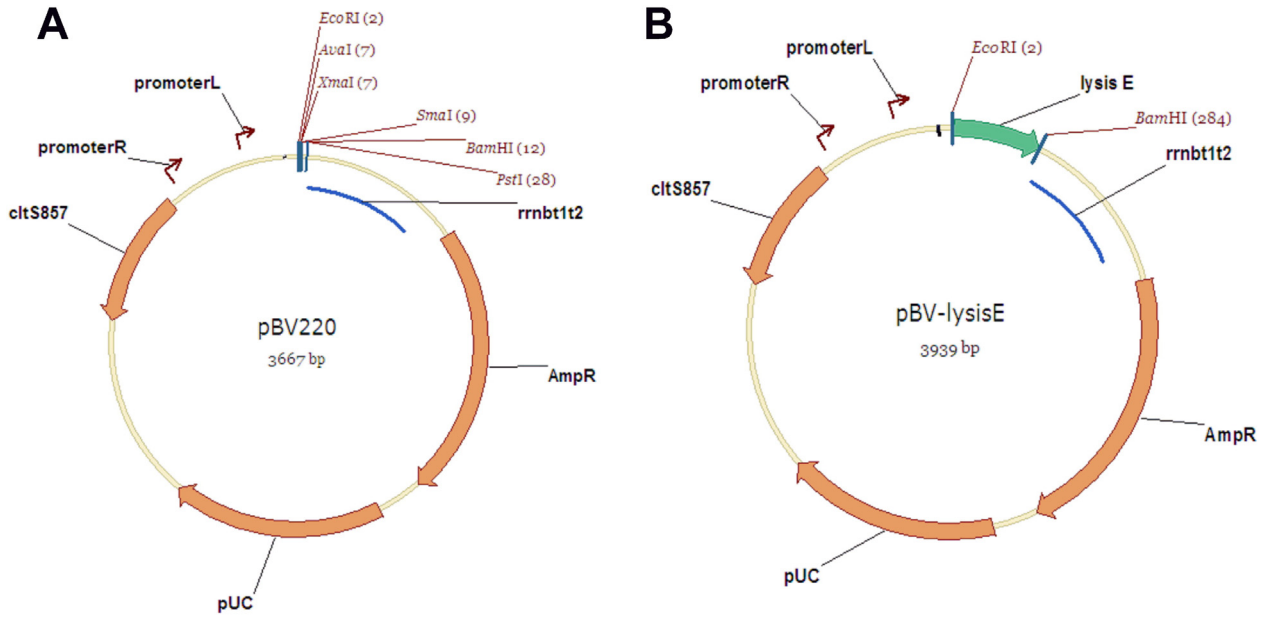

Fig. 1. The physical map of the pBV220 (A) and pBV-lysisE plasmids (B). lysisE: lysis gene E; AmpR: ampicillin acetyl transferase gene; CIts857: temperature sensitive repressor; promoterL: the leftward promoter of bacteriophage lambda; promoterR: the rightward promoter of bacteriophage lambda.