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The outer membrane proteins of *Stenotrophomonas maltophilia* are potential vaccine candidates for channel catfish (*Ictalurus punctatus*)

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ABSTRACT

Channel catfish (*Ictalurus punctatus*) is an important agricultural fish that has been plagued by *Stenotrophomonas maltophilia* (S. *maltophilia*) infections in recent years, some of them severe. The outer membrane proteins (OMPs) of *S. maltophilia* are one of the most immunogenic and highly conserved candidates for vaccine development in aquaculture. The present study investigated OMPs of *S. maltophilia* as vaccine on immune response and disease resistance against *S. maltophilia* of channel catfish and investigated the enhancement effect of natural adjuvants Propolis (Pro), FIG polysaccharide (Fcps), Glycyrrhizine (Gly) to OMPs of *S. maltophilia* for further study. The results indicated that channel catfish injected intraperitoneally with OMPs showed better immune response including leukocytes phagocytosis activity, serum bactericidal activity, complement C3, IgM level and an increasement of resistance against *S. maltophilia* compared to the control group. Moreover, Pro, Fcps and Gly could enhance the immune response of OMPs. The relative percent of survival (RPS) was 73.33%, 66.67%, 63.33%, 60%, 0% in fish injected OMPs + Pro, OMPs + Fcps, OMPs + Gly, OMPs and 0.65% normal saline, respectively. These results suggested that OMPs used as vaccine could induce and stimulate immune response and enhance disease resistance in channel catfish, especially for Pro as immunoenhancer. Results revealed that OMPs were an effective vaccine against *S. maltophilia* in channel catfish.

1. Introduction

The channel catfish (*Ictalurus punctatus*), one of the most dominant aquaculture species in China, suffer from seriously damage due to a bacterial pathogen *Stenotrophomnas maltophilia* (*S. maltophilia*) in recent years [1]. *S. maltophilia* is a member of the genus *Stenotrophomonas*, which distributes in the water, soil, plant roots, animals and peoples' body surface and so on [2,3]. It can cause common diseases such as pneumonia, bacteremia and sepsis [4]. After 2000, the bacterium have infected terrestrial, aquatic animals and even rice plants, and it could cause death [5]. When fish infect *S. maltophilia*, Geng et al. found that the clinical symptoms appeared enteritis and ascites, especially prolapse in the rectum and intussusception in the lower intestine. Moreover, the signs mentioned above appeared in over 80% diseased fish [1,6,7]. It is necessary to find a simple and effective methods of prevention or treatment of *S. maltophilia* in channel catfish.

Vaccines are green intervention to prevent *S. maltophilia* infection. Outer membrane proteins (OMPs) located on the cell walls of the specific outer membrane of gram-negative bacteria, which are one of the most important surface antigen of the bacteria [8], and have an important role in the interaction between bacteria with hosts in absorption and entry into the host to subvert hostdefense mechanisms [9]. These OMPs not only can stimulate the cellular immunity and humoral immunity, but also can induce the host to produce protective immunity [10]. In recent years, there has been already more research work about OMPs and got enough attention

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[11]. In order to enhance the immunogenicity of vaccine, adjuvants were always used. The adjuvants that have been studied in association with fish vaccines included oil-based adjuvants, aluminum-based adjuvants and natural adjuvants [12]. Most oil adjuvant and aluminum-based adjuvants have toxicity, potential adverse reaction, and high cost make them unfit to be used as a vaccine adjuvant [13]. Nowadays, natural adjuvants such as Propolis (Pro) [14], Ficuscaricapolysaccharide (Fcps) [15], glycyrrhizin (Gly) [16] and have been reported to enhance the immunity of fish. They are vaccine adjuvant with immunomodulatory, antimicrobial, anti-inflammatory, antiviral, antitumor, antioxidant, antiparasitic, and anti-diabetic activities [17].

The aim of this work was to investigate the OMPs of *S. maltophilia* may been a promising candidate vaccine to prevent *S. maltophilia* infection. We used SDS-PAGE analysis to investigate biological characteristics of the OMPs in vitro. Channel catfish were vaccinated to evaluate its immune protection in vivo. Subsequently, leukocytes phagocytosis activity, serum bactericidal activity, complement C3, and IgM level were used to assess the effect of OMPs. Moreover, as we still have to make further study of the enhancement effect of natural adjuvants Propolis (Pro), Ficuscaricapolysaccharide (Fcps) and glycyrrhizin (Gly) to OMPs of *S. maltophilia.* All the experimental results indicated that OMPs are one of the promising candidate vaccines against *S. maltophilia* in channel catfish.

2. Materials and methods

2.1. Strains and experimental materials

Stenotrophomonas maltophilia(CCF00024) was isolated from channel catfish and stored in our lab. *Staphylococcus aureus* (ATCC25923) was purchased from National Center for Clinical Laboratories. They were cultured in Luria–Bertani broth medium at 28 °C. Coomassie brilliant blue kit was purchased from Nanjing Jiancheng Bioengineering Institute. Ficuscaricapolysaccharide(Fcps) crude products were purchased from corona technology development company in Fuzhou. Rough Propolis(Pro) and Glycyrrhizine(Gly) were purchased from Chinese herbal medicine company.

2.2. Fish

Fingerling channel catfish (mean weight 50±5 g) were selected from a farm of channel catfish in Meishan (Sichuan Province, China) and fed daily on commercial feed for a minimum of 7 days in a flow water system at 28 °C. They were fed on commercial channel catfish feed daily at 3% fish body weight. Fish were randomly sampled for the examination of bacterial recovery from the blood, liver, spleen, and kidney, and no bacteria could be detected in any of the examined fish. All of the animal experiments were performed in accordance with ethical standards. Fish were usually anaesthetized with tricaine methanesulfonate (Sigma-Aldrich, St. Louis, MO, USA) prior to experimental manipulation to minimize suffering.

2.3. Bacteria culture and OMPs extracted

The culture media and extracted method used in this study have been described previously [11,18] with some modification.

The 24 h *S. maltophilia* culture was streaking 100 ml of TSB broth by plating to a TSA plate. Plates were incubated for 24 h at 28 °C. Bacteria were harvested by scraping the plate with sterile plastic loops into 10 ml centrifuge tubes containing 20 mmol sterile phosphate buffered saline (PBS), pH 7.2. The bacteria was washed three times with 20 mmol PBS and the supernatant was discarded by centrifugation at 4000g for 30 min. The bacteria were resuspended with 20 mmol PBS then repetitive freeze-thawing between room temperature and −20 °C. The sediment was collected at 13000 g for 30 min and resuspended in 2% (W/V) N-lauroylsarcosine sodium salt at 37 °C for 30 min and collected at 13000 g for 30 min. In the end, the sediment were dissolved in 20 mmol/L PBS and all extracted products were stored at −20 °C until utilised.

2.4. Biological characteristics of OMPs

The content of OMPs was determined by Coomassie brilliant blue kit according to the manual. The quality of OMPs was determined with SDS-PAGE.

2.5. Determination the safety of OMPs to channel catfish

Sixty channel catfish were divided into six groups of ten fish each and injected intraperitoneally with 0.2 ml OMPs of *S. maltophilia* (24 mg/kg, 12 mg/kg, 6 mg/kg, 3 mg/kg, 1.5 mg/kg, respectively), the control group received an identical dose of 0.65% normal saline. The clinical symptoms of challenged fish were observed to record and Karbers method was used to test the LD_{50} of channel catfish in infected fish in 14 days. LD_{50} was calculated according to the formula:$LD_{50} = \log\text{-}1[Xm\text{-}i(\Sigma p\text{-}0.5)]$ [27].

Xm is the log value of maximal does. Σp is total mortality.

2.6. The preparation of antigen

Methods the Fcps were obtained through hot water extraction and ethanol precipitation, which content was determined by phenol-vitriolic colorimetry [14,28]. Rough Pro is ground, macerated with four-five(w/v) absolute ethanol(95%) for 3 days, and stirred many times every day. The supernate was obtained by centrifugation at 5000*g* for 10 min and stored in a dark place at 4 °C until used. Gly was finished product. It was suspended in a final concentration of 0.65% sterilized saline and stored at 4 °C.

The extracted OMPs can be divided into four. One is without the adjuvant, the other three mixd with the Pro, Fcps and Gly extracts respectively. The OMPs concentration was adjusted to 0.75 mg/mL, Fcps and Gly element concentration to 50 mg/mL, Pro final concentration to 5 mg/mL [13–15]. All the adjuvanted vaccines above were stored at 4 °C until use (no more than three days).

2.7. Detection of immunogen security

Fifty channel catfish were divided into five groups of 10 fish each and injected intraperitoneally with 0.2 ml OMPs + Pro, OMPs + Fcps, OMPs + Gly, OMPs and normal saline, respectively. A period of 7 days to continuously observe.

2.8. Fish immunization and challenge

300 channel catfish were divided into five groups of 60 fish each: four vaccine-treated groups and one control group (Each group contains a parallel of 30 fish, total 60). The individual fish in the four vaccine groups, namely, OMPs + Pro, OMPs + Fcps, OMPs + Glys, OMPs, were injected intraperitoneally with 0.2 ml OMPs + Pro, OMPs + Fcps, OMPs + Gly, OMPs, respectively. The control group received an identical dose of 0.65% normal saline. 4 weeks post-vaccination, the fish in four vaccine groups were boosted with OMPs + Pro, OMPs + Fcps, OMPs + Gly, OMPs and normal saline as mentioned above.

On the 28th day after booster vaccination, 30 fish were randomly selected from each treatment group, and challenged with