

Protection of tilapia (*Oreochromis mosambicus*) from edwardsiellosis by vaccination with *Edwardsiella tarda* ghosts

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Abstract

The vaccine potential of *Edwardsiella tarda* ghosts produced by gene *E* mediated lysis was investigated using tilapia (*Oreochromis mosambicus*). Tilapia immunized with *E. tarda* ghosts (ETG) and formalin killed *E. tarda* (FKC) vaccines showed significantly higher serum agglutination titers than control fish. Fish immunized with ETG showed no significant differences with fish immunized with FKC in serum agglutination titers, but showed significantly higher bactericidal activity than fish immunized with FKC. Furthermore, fish immunized with ETG showed higher protection than fish immunized with FKC. As this promising type of a non-living whole cell envelope preparation seems to be favorable over conventional vaccines, we suggest *E. tarda* ghosts as a new vaccine candidate.

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1. Introduction

Edwardsiella tarda, a Gram negative, motile, flagellated and rod-shaped bacterium, is the causative agent of edwardsiellosis and leads to extensive losses in many commercially important freshwater and marine fish [1,2]. Because antibiotic resistance of *E. tarda* has been reported widely in the world [3–5], there is urgent need to find other approaches for the treatment and prevention of this infection.

Over the last decade vaccination has become important for the prevention of infectious diseases in farmed fish [6]. Although several attempts have been made to induce protection against *E. tarda* [7–11], the protection efficiency was variable among the studies.

Recently the outer membrane proteins of pathogenic bacteria have been studied in relation to inducing protective humoral and cell-mediated immunity [12–16]. However, traditional inactivation of bacteria by heat or formalin may

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influence the physico-chemical characteristics of surface antigens, and immune responses against the modified antigens may not be protective against live bacteria.

The genetic inactivation of pathogenic Gram-negative bacteria by the controlled expression of cloned bacteriophage Φ X174 lysis gene *E* offers a promising new approach in non-living vaccine technology [17,18]. Expression of plasmid-encoded gene *E* leads to the formation of a transmembrane tunnel structure through the cell envelope of Gram-negative bacteria, which consequently leads to the loss of cytoplasmic contents. The resultant bacterial ghosts have been known to retain the functional and antigenic determinants of the envelope with their living counterparts and thus represent ideal vaccine candidates [19].

We have recently generated *E. tarda* ghosts by gene *E* mediated lysis [20]. In the present study, we further investigated the vaccine potential of *E. tarda* ghosts in tilapia. To our knowledge, this is the first study on ghost bacterial vaccines in fish.

2. Materials and methods

2.1. Fish

Juvenile tilapia, weighing 100 ± 20 g, were obtained from the fish farm in Pukyong National University, Korea. For the immunization experiments, fish were stocked into either three 100 litre aquaria at a density of 25 fish per aquarium, or a single 500 litre aquarium at a density of 75 fish. Fish were acclimated for 2 weeks prior to initiating the experiments.

2.2. Bacterial strain

Edwardsiella tarda FSW910410 provided by National Fisheries Research & Development Institute, Korea, was grown in Luria Broth (LB, Difco Laboratories, Detroit, USA) at 27 °C. Transformed *E. tarda* was grown in LB containing 50 µg/ml ampicillin (Sigma Chemical Co., St Louis, MO, USA). Incubation temperatures for repression and expression of lysis gene *E* in transformants were 27 °C and 42 °C, respectively. Growth and lysis of bacterial cultures were monitored by measuring the optical density at 600 nm (OD₆₀₀).

2.3. Production of *E. tarda* ghosts (ETG)

Lyophilized *E. tarda* ghosts were produced as described previously [20]. Briefly, *E. tarda* harboring the lysis plasmid p λ P_R-cI-Elysis was induced for lysis after growth under culture conditions by elevation of temperature from 27 °C to 42 °C. At the end of lysis, ghosts were harvested, washed and resuspended in PBS and then lyophilized. The efficiency of *E*-mediated killing of *E. tarda* was estimated by plating samples of appropriate dilutions of lyophilized ETG on LB agar containing 50 µg/ml ampicillin and results were compared with those from samples obtained prior to onset of lysis. Results indicated a 100% killing efficiency as no colony-forming units were found on plates with lyophilized ETG preparations at any dilution.

2.4. Production of formalin-killed *E. tarda* (FKC)

E. tarda was grown for 24 h at 27 °C in tryptic soy broth (TSB, Sigma) containing 1.5% NaCl. For FKC preparation, formalin was added to a 24 h culture of the bacterium to make the final concentration 0.5%. After 24 h incubation, cells were washed three times with phosphate buffered saline (PBS, pH 7.2) and resuspended in 10 ml PBS. The suspensions were streaked on tryptic soy agar containing 1.5% NaCl for checking sterility and stored at 4 °C until use.

2.5. Immunization and collection of blood samples

ETG and FKC preparations were reconstituted with PBS. In vaccine experiment I, groups of fish (25 fish/group) stocked into 3 aquaria were immunized twice intraperitoneally (i.p.), 2 weeks apart, with 1.4×10^6 cells of either

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