Efficacy of a vaccine against *Streptococcus parauberis* infection in starry flounder *Platichthys stellatus* Pallas

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Starry flounder, which are recently increasingly cultured in Korea, are known to highly vulnerable to *Streptococcus parauberis* infection. Five groups of starry flounder (*n*=30 for each group) were vaccinated with *S. parauberis* formalin-killed whole cells by intraperitoneal injection at a final concentration of 0, 0.01, 0.1, 1 and 10 mg fish⁻¹. Specific antibody production of 1 and 10 mg fish⁻¹ administered groups significantly increased at four weeks post immunization. All vaccinated groups showed higher survival rates than a control group when five groups of fish were challenged with *S. parauberis* at a dose of 1.14×10⁴ cfu fish⁻¹ and 1.14×10² cfu fish⁻¹, respectively. In particular, 0.1 or higher concentrations of formalin killed bacterial cells are able to confer the fish high protection against *S. parauberis* infection.

*Key words*: *Streptococcus parauberis*, *Platichthys stellatus*, Vaccine, Agglutination antibody titer, Challenge test

Starry flounder, *Platichthys stellatus*, enjoy a broad geographic distribution across the North Pacific Ocean, where they can be found in marine, brackish, and freshwater influenced areas given their ability to tolerate low salinity (Orcutt, 1950; Kramer et al., 1995). Starry flounder aquaculture has steadily grown since starting in Korea about 5 years ago. Streptococcal infections caused by *Streptococcus* spp., *Lactococcus* spp. and *Enterococcus* spp. are of serious concern in cultured fisheries (Austin and Austin, 1999). The first *S. parauberis* infection occurred in Spanish turbot (*Scophthalmus maximus*) farms between 1993 and 1996 (Toranzo et al., 1994; Domenech et al., 1996), and since then the pathogen has continued to cause problems in aquaculture industries worldwide. In recent years, *S. parauberis* has increasingly been isolated from diseased olive flounder in Korea (Baeck et al., 2006; Joeng et al., 2006; Cho et al., 2007; Kang et al., 2007). The infection has also resulted in severe economic losses to the starry flounder aquaculture industry (Cho et al., 2008). Starry flounder infected with *S. parauberis* exhibit show varying disease signs, including exophthalmia, abscesses and hemorrhages around the eyes, and abdomen distention, leading to a high long-term cumulative mortality (Cho et al., 2008).

In this study, the protection effects of a vaccine against *S. parauberis* infection were investigated. Specifically,
starry flounder were immunized with formalin-killed cells (FKCs) of the pathogen by intraperitoneal (i.p) injection.

Materials and Methods

1. Fish and environment

Starry flounder (average body wt. = 53.1±7.1 g) were obtained from a commercial fish farm in Korea, 500 of which were maintained in 10 tanks (250 L) and provided with fresh sea water at an exchange rate of 25L h⁻¹. Their health status was examined immediately upon arrival in the aquaria and at 1 week thereafter. The fish were fed with commercial dry pellets (Jeilfeed Co., Ltd., Korea) twice daily. Water temperature (average = 20.9±0.3°C) was measured once a day during the experiment.

2. Vaccine and treatment

*S. parauberis* strain PH0710 (Cho *et al.*, 2008), isolated from diseased starry flounder in December 2007 (Fig. 1), was used for vaccine manufacturing. The bacteria were massively cultured in Todd-Hewitt broth (1% final salt concentration; DB, USA) at 30°C for 48 h. The bacterial culture was inactivated by adding formalin (Fluka, Germany) to a final concentration of 1% (v/v) and incubated for 24 h at room temperature. The bacterial pellet obtained by centrifugation was washed three times with sterile saline (0.85% NaCl), and concentration of bacterial suspension was adjusted to 100 mg ml⁻¹ in saline. One hundred microliter of the vaccine was injected into the abdominal cavity of starry flounder (n=30 for each concentration, respectively) at concentrations of 10, 1, 0.1 and 0.01 mg fish⁻¹, while a control group (n=30) was injected intraperitoneally with equal volume of saline. The experiment was duplicated.

3. Agglutination antibody titer

Sera were collected from caudal vein of 8 fish in each experimental group at 0, 1, 2, 4, 6, 8 and 10 weeks after immunization. Agglutinating antibody titers to *S. parauberis* were measured by using the micro-agglutination test (Roberson, 1990). Briefly, 2-fold serial dilutions of each serum in sterile saline ranging from 1:20 to 1:2,560 were added to a 96 well microplate. Then, the