Pathogenicity of *Streptococcus parauberis* to Olive Flounder *Paralichthys olivaceus*

Ji Hyung Kim¹, Dennis K. Gomez², Gun Wook Baeck³, Gee Wook Shin⁴, Gang Joon Heo⁵, Tae Sung Jung⁶ and Se Chang Park¹,²*

¹College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea.
²KRF Zoonotic Disease Priority Research Institute, Seoul National University, Seoul 151-742, Korea
³Faculty of Marine Technology, Chonnam National University, Yeosu 550-749, Korea
⁴Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, Korea
⁵College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Korea

(Received June 8, 2006)

ABSTRACT—*Streptococcus parauberis* (strain SNUFPC-050803), isolated from diseased olive flounder *Paralichthys olivaceus* in Jeju Island, Korea, was evaluated for its pathogenicity to healthy juvenile flounder (29.3 g in average body weight). When challenged with the isolate by intraperitoneal injection with tenfold serial dilutions of 4.5 × 10⁻¹ to 10⁶ CFU/fish, the cumulative mortality ranged from 10% to 80% within 14 days except for 4.5 CFU/fish and control with no mortality. Disease signs were hemorrhage around the mouth, eyes and pectoral fins, pale and friable liver with hepatomegaly and ascitic fluid in the peritoneal cavity. These signs were similar to those of naturally affected fish. *S. parauberis* was reisolated and identified by PCR method, which confirmed the pathogenicity of the bacterium to olive flounder.

Key words: streptococcosis, *Paralichthys olivaceus*, *Streptococcus parauberis*, pathogenicity

Streptococcosis causes severe diseases in both freshwater and marine fishes from cultured and wild populations¹⁻³. Among commercially important fish species, this disease has been reported worldwide in yellowtail *Seriola spp.*, Japanese eel *Anguilla japonica*, menhaden *Brevoortia patronus*, striped mullet *Mugil cephalus* and striped bass *Morone saxatilis*⁴⁻⁵. In addition, there was an important epizootic outbreak of streptococcosis in turbot *Scophthalmus maximus* cultured in Spain in 1993 that was initially thought to be caused by an *Enterococcus* species-like bacterium⁶. Domenech et al.⁷ stated that the 16S rRNA genes of fish isolates were found to belong to the species *Streptococcus parauberis*, a recognized pathogen of mammals. Recently, a streptococcal infection had also occurred from cultured olive flounder *Paralichthys olivaceus* in May 2005 at a fish farm in Jeju Island, Korea. Identification of species by culture-based, biochemical and serological tests and multiplex PCR demonstrated that the dominant strains causing streptococcosis were identified as *S. parauberis*⁸. In order to fulfill the Koch’s postulates, pathogenicity of *S. parauberis* was conducted in this present study by infection experiment in healthy cultured juvenile olive flounder.

**Materials and Methods**

**Experimental fish**

Hatchery-reared healthy juvenile olive flounders (average body weight: 29.3 g; average body length: 15.2 cm) from Wan-do, Korea, were used for the infection experiment. Prior to experiment, liver, kidney and spleen of each of ten fish randomly sampled were negative for streptococci by multiplex polymerase chain reaction (PCR) assay based on Baeck et al.⁸. Fish were acclimatized at 23–24°C for 1 week before the experiment.

**Bacterial preparation**

*S. parauberis* (SNUFPC-050803) previously isolated from cultured diseased flounder in Jeju Island⁸ and stored at −30°C was used for the infection experiment after growing for 24 h at 25°C in brain heart infusion agar plate (DIFCO, USA) supplemented with 1.5% NaCl (BHIA).

**Infection experiment**

The bacterium was serially ten-fold diluted with PBS (Invitrogen, USA). One hundred forty healthy juvenile flounders were divided into seven groups in 300 L fiber plastic tanks with a flow-through water supply at 23–24°C. The fish were challenged with 0.1 mL of the bacterial suspension by intraperitoneal (IP) injection. The final doses of infections in the experimental groups were from 10⁰–10⁴ CFU/fish. The control group was injected with 0.1 mL of PBS. After injection, these fishes were kept for 2 weeks. Dead fish were sampled everyday for isolation of bacteria from liver using BHIA and incubated at 25°C for 24 h. After cultivation, the isolates were checked by the PCR assay⁸.

**Detection of the bacterium by PCR assay**

A PCR assay was used to confirm the detection of *S. parauberis* from pure culture of bacteria. The oligonucleotide primers Spa 2152 and Spa 2870 used for the
detection of *S. parauberis* were based on the method of Baeck *et al.*\(^8\) with a target region of 718 bp.

**Results and Discussion**

Olive flounder is one of the most important aquaculture species in Korea especially in Jeju Island. Streptococcal infections appeared and caused significant losses in several aquaculture facilities in Jeju Island. It showed that *S. parauberis* is the dominant species responsible for streptococcosis in the cultured flounder. The challenged fish showed hemorrhages in the mouth, eyes and pectoral fins generally with ascitic fluid in the peritoneal cavities (Fig. 1), and the internal signs showed pale, friable liver with hepatomegaly. These signs were similar to those of naturally affected fish. Domenech *et al.*\(^7\) also reported the external lesions of affected turbot with haemorrhage in the eyes and pectoral fins. The internal signs were similarly observed from the affected turbot including pale, friable liver with hepatomegaly and sometimes there was ascitic fluid in the peritoneal cavity\(^7\). Colonies of Gram-positive short-rod (coccibacilli)-shaped bacteria isolated from the liver of an affected flounder were formed on BHIA. The bacteria grew optimally at 25°C within 24 h. The PCR products (Fig. 2) with the expected size (718 bp) confirmed that the isolates from the infected fish were *S. parauberis*. Baeck *et al.*\(^8\) also amplified in PCR several isolates of *S. parauberis* from diseased flounder with a target region of 718 bp. The mortality started at the 10\(^{th}\) day post-injection, and cumulative mortalities of the groups injected with \(4.5 \times 10^6\), \(4.5 \times 10^5\), \(4.5 \times 10^4\), \(4.5 \times 10^3\) or \(4.5 \times 10^2\) CFU/fish were 80, 70, 70, 60 or 10%, respectively. No mortality was observed at the group injected with \(4.5 \times 10\) CFU/fish and the control group (Fig. 3). Characteristic feature of the pathogenicity of *S. parauberis* showed that the mortality of the group injected with \(4.5 \times 10^3\) CFU/fish already reached 50% mortalities at the 10\(^{th}\) day post-infection, when the mor-

---

**Fig. 1.** Challenged fish injected with *S. parauberis*. Infected olive flounder showing hemorrhages in the a) eyes and pectoral fins and b) mouth.

**Fig. 2.** Representative amplification products obtained from infected olive flounder using PCR assay for the detection of *Streptococcus parauberis*. Lanes: M, 100 bp DNA ladder; P, positive control; N, negative control and 1–13, samples isolated from infected fish.

**Fig. 3.** Cumulative mortalities of olive flounder intraperitoneally injected with different doses (CFU/fish) of *S. parauberis* (SNUFPC-050803).
tality started. Confirmation with isolation in pure culture and PCR assay from affected flounder in this study clearly indicates that *S. parauberis* was the causative agent of streptococcosis that infected flounders in Jeju Island. These findings alarm fish farmers that streptococcosis caused by *S. parauberis* can contribute high mortality and economic loss to the flounder aquaculture.

**Acknowledgement**

This study was supported by the Korea Research Foundation Grant (KRF-2006-005-J02903).

**References**