

<Case Report>

Isolation and molecular identification of *Vibrio harveyi* from the look down fish (*Selene vomer*)

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Abstract : Gradual mortality of look down fish (*Selene vomer*) was observed in a private aquarium in Seoul, showing abnormal swimming behavior and lethargy. A bacterial pathogen from kidney was cultured, identified, and confirmed as *Vibrio harveyi* using Vitek System 2 and 16S rRNA gene sequencing. A predominant bacterial strain, SNUVh-LW2 was proved to be most closely related to isolates from China by phylogenetic analysis with minimum evolution method. Also, tetracycline was considered as the most sensitive antibiotic agent via antibiotic susceptibility test. The group of fish was treated according to the diagnostic result and no more mortality was observed.

Keywords : *Vibrio harveyi*, aquarium, look down fish

Fish of the genus *Selene*, such as look down fish (*Selene [S.] vomer*) or moon fish (*S. brownii*) are known to be one of the most morphologically derived groups on the family *Carangidae* [6, 9]. They are known to be distributed over both hard and sandy bottoms [4]. They are recognized as appreciated and highly prized species in the market due to their tasty flesh [10]. Look down fish are considered as one of the most popular fish species in commercial aquariums in Korea because of its appealing appearance: tall round body and laterally compressed shape with a unique steep head [9]. Vibriosis is a serious fish disease caused by bacteria belonging to the genus *Vibrio* [1]. Among *Vibrio* spp., *Vibrio (V.) harveyi* has been known to infect a wide range of fish and invertebrates and cause mass mortalities worldwide [1, 3, 8]. The fish infected by *V. harveyi* show the following symptoms: dark skin, hemorrhagic fins, septicemia, and ulcers on the skin [11].

In October 2015, gradual mortality had been observed in the group of look down fish for 1 week. They were reared for public exhibition in a private commercial aquarium in Seoul. The fish with a disease exhibited abnormal swimming behavior and lethargy and were found to be dead. Shortly after the death of the fish, look down fish (17 cm average length and 85 g average body weight) were submitted to the College of Veterinary Medicine, Seoul National University for diagnosis. The quality of water was properly controlled and the water temperature ranged from 23.5 to 24.5°C. Although

there were no remarkable findings on any internal organs at necropsy, the presence of yellowish fluid in the abdominal cavity were examined.

Sterile swab from the kidney of randomly selected fish was streaked onto tryptic soy agar (TSA; Becton, Dickinson and Company, USA) to isolate the causative bacteria, and the inoculated plate was incubated at 25°C for 24 h. Bacterial cultures were then simply identified on the basis of microscopic analysis results, with the aid of a Vitek System 2 (bioMérieux, France) for biochemical analysis. The genomic DNA was extracted by the DNeasy Blood & Tissue Kit (Qiagen, Germany), following the manufacturer's instruction. The purified DNA was sequenced using an ABI PRISM Big Dye TM Terminator Cycle Sequencing Kit (Applied Biosystems, USA) at the MacroGen Genomic Division (Korea). Electrophoresis of sequencing reactions was performed using the Automatic Sequencer ABI 3730XL DNA Analyzer (Applied Biosystems). The 16S ribosomal RNA gene sequence of the bacterial strain obtained in this study was aligned with those of the *Vibrio* spp.. The percentage of sequence similarity with 16S rRNA genes available in the GenBank database was determined with the BLAST search (Table 1). Sequence alignment was carried out with other bacteria of the *Vibrio* spp. (AB512470, AY911397, FJ605240, FN554614, GQ180184, HF541979, KC291498, KF793931, KJ577048, and KR003733) using ClustalW and analysed with the MEGA6 [12]. Phylo-

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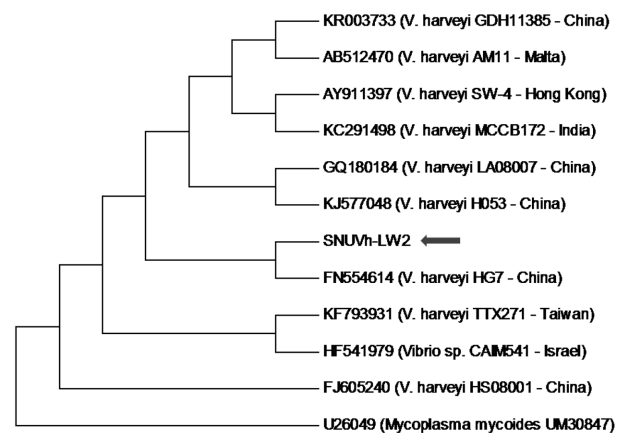
Table 1. Identity of the 16S rRNA gene sequence of *Vibrio* (*V.*) *harveyi* SNUVh-LW2 isolated in this study to those of other *Vibrio* spp.

Species - isolated area	Sequence identity (%)	GenBank Number
<i>V. harveyi</i> GDH11385 - China	680/680 (100%)	KR003733
<i>V. harveyi</i> TTX271 - Taiwan	684/686 (99%)	KF793931
<i>V. harveyi</i> LA08007 - China	679/680 (99%)	GQ180184
<i>V. harveyi</i> HS08001 - China	679/680 (99%)	FJ605240
<i>V. harveyi</i> SW-4 - Hong Kong	679/680 (99%)	AY911397
<i>V. harveyi</i> H053 - China	678/680 (99%)	KJ577048
<i>V. harveyi</i> HG7 - China	678/680 (99%)	FN554614
<i>V. harveyi</i> AM11 - Malta	678/680 (99%)	AB512470
<i>V. harveyi</i> MCCB172 - India	676/682 (99%)	KC291498
<i>Vibrio</i> spp. CAIM541 - Israel	680/681 (99%)	HF541979

genetic analysis was inferred using the Minimum Evolution (ME) algorithm in MEGA6. ME analysis was performed using a Close-Neighbor-Interchange search level 1, with evolution distance determined by Maximum Composite Likelihood. The initial tree for ME analysis was constructed using a Neighbor-Joining algorithm with complete gap deletion. Bootstrap values were calculated for ME algorithm, with 1,000 replicates. The 16S rRNA sequence of *Mycoplasma mycoides* UM30847 was used as out-group. Antibiotic susceptibility of bacterial isolate was determined by the standard disk diffusion method [2]. The sensitivity and resistance of the isolate were determined according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI) [5] using commercial antibiotic disks (Oxoid, UK).

A predominant bacterial strain, SNUVh-LW2 was isolated through the microscopic examination. 16S rRNA gene sequencing was carried out on SNUVh-LW2. Molecular analysis identified SNUVh-LW2 as *V. harveyi* with 100% identity. In the BLAST search analysis, SNUVh-LW2 showed 100% identity with *V. harveyi* GDH11385 (Table 1). Phylogenetic tree result showed that SNUVh-LW2 was clustered in the same group with *V. harveyi* HG7 (Fig. 1). *V. harveyi* GDH11385 and *V. harveyi* HG7 were isolated from China, which indicates that SNUVh-LW2 is most closely related to isolates from China. *V. harveyi* SNUVh-LW2 was screened for antibiotic susceptibility to the primary testing antibiotic agents (cefotaxime, ceftazidime, fluoroquinolones, and tetracycline) recommended by CLSI, according to the standardized guidelines [5]. It was shown to be the most sensitive to tetracycline which we recommended the aquarium to use for disease control. With the successful disease control using tetracycline, the group of look down fish seemed to enter the recovery stage and no more mortality was observed.

In this study, *V. harveyi* SNUVh-LW2 was the only bacterial strain from the group of look down fish. It was proved to be the causative agent of the gradual mortality in the group of look down fish. Korea imports a variety of aquarium fishes from other Asian countries and the number of imported species increases annually [7]. Imported fishes sometimes die of disease infections soon after arrival or during transport [7]. As

**Fig. 1.** Phylogenetic tree generated by minimum evolution analysis of the 16S rRNA sequences of *Vibrio* spp. The arrow represents *V. harveyi* isolated in this study.

SNUVh-LW2 showed the highest identity with the bacterial isolate from China, further study is required to determine whether the fish importing might be one of the sources of *V. harveyi* infection. This study is meaningful as the staff in the aquarium treated the fish according to the diagnostic result instead of the imprudent use of broad-host-range antibiotics, which is the outcome of collaboration between the college of veterinary medicine and the commercial aquarium.

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