

<Case Report>

## Isolation and identification of *Vibrio harveyi* from chub mackerel (*Scomber japonicus*)

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**Abstract :** For several days, there was a series of mortalities of chub mackerel (*Scomber japonicus*) that were reared for public exhibition in a private aquarium in Seoul, Korea. As part of the diagnosis of the dead fish, a bacterial isolate from the kidney was cultured, identified, and confirmed to be *Vibrio (V.) harveyi* using Vitek System 2 and 16S rRNA gene sequencing. Phylogenetic analysis was also performed by the neighbor-joining method. As a result, the *V. harveyi* isolated from chub mackerels of a private aquarium in Korea, called as SNUVh-LW1, was clustered in the same group with *V. harveyi* ATCC33843.

**Keywords :** *Vibrio harveyi*, aquarium, chub mackerel

The chub mackerel (*Scomber japonicus*) is known to be distributed around the world [7]. It was reported that the Pacific populations are genetically different from the Atlantic populations [8]. The chub mackerel is considered as one of the most important fishery resources in Korea due to its low price and high production. Its fisheries have been performed mainly in the East Sea over several decades [7]. *Vibrio (V.) harveyi* belonging to the genus *Vibrio* has been reported to infect a wide range of marine organisms worldwide including fish and invertebrates [1, 3, 6]. The disease can cause mass mortalities in aquaculture species with significant economic impact.

In October 2015, several chub mackerels (32 cm average length and 786 g average body weight) which were reared for public exhibition in a private commercial aquarium in Seoul were found dead. The fish had shown common symptoms including anorexia, lethargy, and depression. At least 30 dead fish per day had been found for six days. Shortly after the death of the fish, they were submitted to the College of Veterinary Medicine, Seoul National University for diagnosis. The water temperature had ranged from 21 to 22°C, and other water parameters were optimal. At necropsy, the protrusion of intestine and the presence of yellowish fluid in the abdominal cavity were examined.

For bacterial isolation, sterile swabs from the kidney of

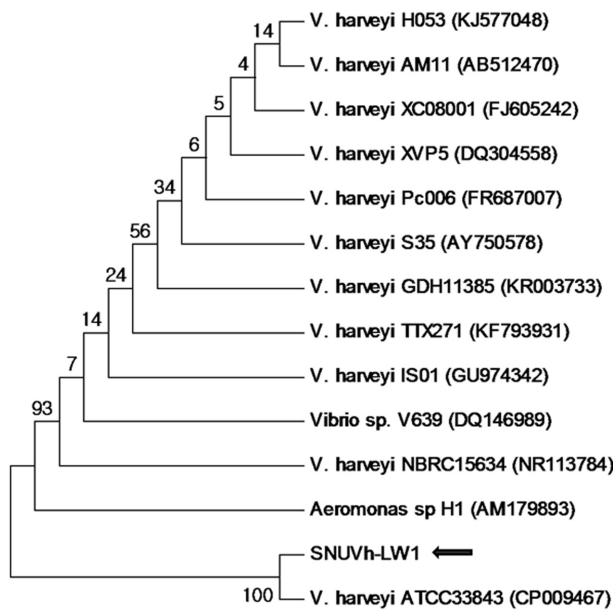
five randomly selected fish were streaked onto tryptic soy agar (TSA; BD Difco, USA), and the inoculated plate were incubated at 25°C for 24 h. Suspected common colonies were re-streaked on TSA to obtain pure cultures, which were then simply identified on the basis of microscopic analysis results, with the aid of a Vitek System 2 (bioMérieux, France) for biochemical analysis. DNA extraction was conducted using the DNeasy Blood & Tissue Kit (Qiagen, USA) according to the manufacturer's instruction. The purified DNA was submitted to the MacroGen Genomic Division (Korea) and nucleotide sequencing was conducted using an ABI PRISM Big Dye TM Terminator Cycle Sequencing Kit (Applied Biosystems, USA). Electrophoresis of the sequencing reactions was completed using an automated ABI PRISM 3730XL DNA Sequencing System (Applied Biosystems). Sequence identities were determined with the BLAST search. The 16S rRNA sequence gene of the bacterial strain acquired in the present study was aligned with other bacteria of the same species (AB512470, AM179893, AY750578, CP009467, DQ146989, DQ304558, FJ605242, FR687007, GU974342, KF793931, KJ577048, KR003733, and NR113784) using ClustalW and analysed with the MEGA6 [9]. Phylogenetic analysis was conducted with neighbor-joining (NJ) method. Bootstrap values were calculated for NJ method with 1,000 replicates. The 16S rRNA sequence of *Aeromonas* sp. H1 was used as out-

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**Fig. 1.** Neighbor-Joining tree of *Vibrio* (*V.*) *harveyi* based on the 16S rRNA sequence exploring the relationship among *V. harveyi* available in GenBank and *V. harveyi* (arrow) isolated in the present study.

group. The antibiotic susceptibility test was performed via the standard disk diffusion method [2]. The sensitivity and resistance of the isolate were determined in accordance with the guidelines of the Clinical and Laboratory Standard Institute [4] using commercial antibiotic disks (Oxoid, UK).

After the microscopic examination of isolates, a predominant bacterial strain was obtained. 16S rRNA gene sequencing was carried out on the bacterial strain; the molecular analysis identified the bacterial strain as *V. harveyi* with 100% identity. Based on the phylogenetic tree result, the *V. harveyi* isolated in the present study, called as SNUVh-LW1, was clustered in the same group with *V. harveyi* ATCC33843 (Fig. 1). Also, it indicated that SNUVh-LW1 was most closely related to *V. harveyi* ATCC33843. *V. harveyi* SNUVh-LW1 was screened for antibiotic susceptibility to primary testing antibiotic agents (cefotaxime, ceftazidime, fluoroquinolones, and tetracycline), in accordance with standardized guidelines [4]. It was resistant to fluoroquinolones and susceptible to the others.

In general, it is hard to characterize the virulence of bacterial strain toward fish without pathogenicity test. However, based on the fact that it was the only bacterial strain from the dead fish and previous reports indicated the pathogenicity of *V. harveyi* to fish, we assume that *V. harveyi* SNUVh-LW1 was the etiological factor of the mortality observed in the

present study [1, 3, 5, 10]. Thus, it is recommended to develop a proper treatment for this condition using the most effective antibiotics as *V. harveyi* SNUVh-LW1 was proved to be sensitive to all commercial antibiotics used in the present study except fluoroquinolones.

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