

Shewanellaspp., A Potential Pathogen of White Leg Shrimp Cultured in Low Salinity Water in Korea

국내산 저염분 양식 흰다리새우 유래의 슈와넬라의 병원성 세균으로의 특성

전진우¹

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ABSTRACT

White leg shrimps cultured in an inland private aquaculture farm with low salinity waters showed abnormal swimming behavior and appetite reduction in July 2022. Then, gradual mortality was observed in the aquaculture farm. During the diagnosis, bacterial strain KNUAF-SHP3 was isolated from the hepatopancreas of the dead shrimps. Based on the sequence of 16S rRNA gene, KNUAF-SHP3 was proved to be *Shewanella* sp., clustering into a group with *S. algae* MARS 14 and *S. chilikensis* JC5T. According to the result of experimental infection test, all shrimps challenged with high concentrations, 2.1×10^8 CFU/ml and 2.1×10^9 CFU/ml showed apparent disease symptoms and the cumulative mortality rates reached 100% in 7 days post challenge. These results emphasized that *Shewanella* isolate in this study can be a potential pathogen of white leg shrimp cultured in low salinity water.

Key Words : White leg shrimp, Low salinity water, *Shewanella*, Potential pathogen

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Introduction

Shrimp culture in inland low salinity waters is widespread throughout the world (Roy et al., 2010). Since the white leg shrimp (*Penaeus vannamei*) can easily grow and survive in low salinity environments, it has been the candidate for low salinity culture (Roy et al., 2010). With the rapid development of culturing techniques, white leg shrimps have been

cultured in low salinity waters; its production will continue to expand, especially Asian countries such as China, Vietnam and Thailand (Roy et al., 2010; Zhou et al., 2019).

The genus *Shewanella* is rapidly expanding; *S. algae* and *S. putrefaciens* are known as the two major species among *Shewanella* spp. (Martin-Rodriguez et al., 2019; Prachumwat et al., 2020). *S. algae* and *S. putrefaciens* have been reported as

opportunistic pathogens in aquatic species (Prachumwat et al., 2020). *S. algae*, the most relevant species caused mortalities in abalone in China and Taiwan and ulcer disease in marine channel bass in China (Cai et al., 2006). *S. putrefaciens* have been reported as a pathogen for tilapia, European sea bass, rabbitfish, loach, Siberian sturgeon, hybrid sturgeon, rainbow trout and carp (Cai et al., 2006). *Shewanella* species are known to be related to shrimp (Cai et al., 2006). Of these, *S. putrefaciens* and *S. balticaare* considered to be associated with spoilage of shrimps stored at a low temperature (Zhu et al., 2017).

In the current study, we isolated and characterized *Shewanella* strain, the causative agent of mortality of white leg shrimps cultured in inland low salinity water in Korea. This is the first report of isolation of *Shewanella* sp. from shrimps cultured in inland low salinity water in Korea.

Materials and methods

Cases:

White leg shrimps were cultured in an inland private aquaculture farm in Jeollabuk-do, Republic of Korea. Salinity of water was around 10 ‰ and water temperature was around 27 °C. In July 2022, some shrimps showed abnormal swimming behavior, appetite reduction, and then gradual mortality. At the beginning, its progression was relatively slow and the mortality rate was around 0.1% per day. However, the mortality continued for two months with showing growth retardation.

Isolation and identification of the causative bacteria:

Parasitological examinations were performed for the post-mortem analysis. Sterile swabs from skin, legs and hepatopancreas of the dead shrimps were streaked onto LB agar to isolate the causative bacteria. Inoculated plates were incubated at 27 °C for 24 h. From the bacterial isolates, its genomic

DNA was extracted by the DNeasy Blood & Tissue Kit (Qiagen, Germany), following the manufacturer's instruction. The 16S ribosomal RNA (rRNA) gene of the isolates were 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).

Additionally, the obtained 16S rRNA sequences of the isolates were aligned with representative sequences from each type strain of *Shewanella* species using ClustalX (version 2.1) (Larkin et al., 2007) and BioEdit Sequence Alignment Editor (version 7.1.0.3) (Hall, 1999). Then, the datasets were phylogenetically analyzed using MEGA ver. 11.0 (Tamura et al., 2021). A neighbor-joining phylogenetic tree was constructed using a Jukes-Cantor distances matrix, and the reliability of the tree was assessed using 1,000 bootstrap replicates.

Experimental infection test:

Bacteria isolated from the shrimps were incubated at 27 °C overnight. The culture was washed twice with sterile PBS, and diluted with 10-fold gradient into different concentrations to yield the final dose from 2.1×10^6 CFU/ml to 2.1×10^9 CFU/ml. Five shrimps in each group with body weight of \pm g were kept in 10L fiber plastic tanks. The salinity of water was 10‰ and water temperature was maintained at 27 ± 0.5 °C. Four experimental groups were challenged by bath immersion method. Shrimps in control group were kept in a tank during the experiment without any treatment. The mortality rates of the shrimp were recorded daily for 7 days, and the hepatopancreas of the dead shrimp was subjected to a bacterial isolation study, as performed before.

Results

Seven dead shrimps (average body length mm,

symptoms, with pale hepatopancreas and atrophied muscle. In the both groups, the cumulative mortality rates reached 100 % in 7 days. However, shrimps in other experimental groups including

control group showed no symptoms, and no mortality was observed (Fig. 2). The bacteria were re-isolated from all of the dead shrimp, indicating that the mortalities were caused by *Shewanella* sp.

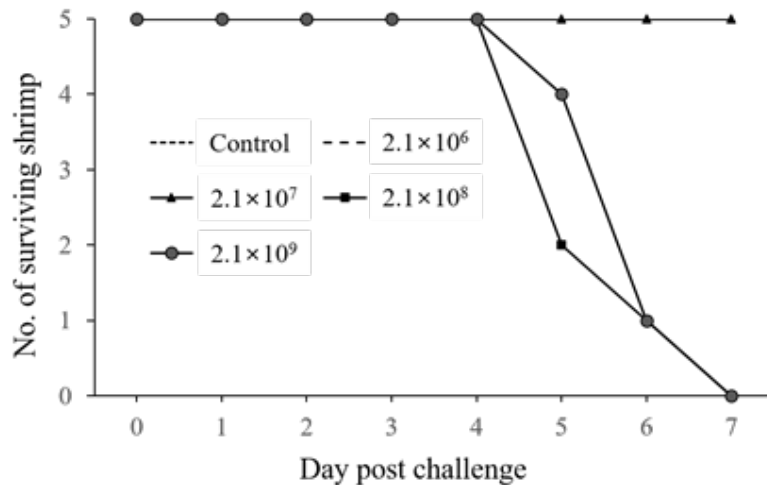


Fig. 2. Cumulative mortality of white leg shrimps after the inoculation of *Shewanella* strain KNUAF-SHP3 with different concentrations

Discussion

The genus *Shewanella* is known that its identification is tricky and the taxonomic resolution is unclear (Martin-Rodriguez et al., 2019). Especially, *S. algae* and *S. chilikensis* are closely-related; several clinical isolates were known to be assigned to same cluster although they should be reclassified (Martin-Rodriguez et al., 2019). In the present study, the isolate KNUAF-SHP3 was clustered into the group with *S. algae* MARS 14 and *S. chilikensis* JC5T. This result emphasized that more study using high resolution molecular markers is required for species identification.

So far, *Shewanella* has been considered as pathogenic to humans and most of the research focused on its risk as a human pathogen (Martin-Rodriguez et al., 2019). However, it has been reported that *Shewanella* strain is lethal to shrimps cultured in low salinity water with

causing early mortality syndrome (Prachumwat et al., 2020). Although the production of white leg shrimps cultured in low salinity water has increased in Asia (Roy et al., 2010; Zhou et al., 2019), the research on their diseases was not actively carried out in Korea. This is a meaningful study considering that it is the first report of isolation of *Shewanella* sp. from shrimps cultured in low salinity water in Korea. More research on the virulence factor in *Shewanella* spp. is recommended because little information on virulence factors has been reported (Prachumwat et al., 2020).

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요 약

2022년 7월, 국내의 저염분 해수 흰다리새우 양식장에서 양식 중이던 흰다리새우가 이상유영과 식욕부진을 보이며 지속적으로 폐사하였다. 일일 평균 폐사율이 0.1%로 완만한 폐사를 보였으나, 2달 넘게 폐사가 지속되었고 성장 지체로 인한 경제적인 피해가 가중되었다. 질병 진단 과정 중, 폐사 새우의 간췌장에서 세균(KNUAF-SHP3)이 분리되었다. 본 세균의 16S rRNA 유전자의 시퀀스 분석 결과, 분리주 KNUAF-SHP3은 *Shewanella*에 속하는 균으로 동정이 되었고, *S. algae* MARS 14, *S. chilikensis* JC5T와 근연 관계를 보이며 같은 그룹으로 분류되었다. 실험 동물을 활용한 인위 감염 실험에서는 상대적으로 고농도(2.1×10^8 CFU/ml와 2.1×10^9 CFU/ml)를 적용한 군에서는 모든 새우들이 감염 증상을 보이며 감염 후 7일 내에 100% 폐사율을 나타내었다. 본 연구는 *Shewanella* 세균이 저염분 해수 양식 흰다리새우에서의 잠재적인 기회 감염균이 될 수 있음을 기술하고 있다.