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ORIGINAL ARTICLE

Assessment of the Effect of a Disinfectant to Control *Vibrio* Strain from White Leg Shrimps Cultured in Korea

국내산 양식 흰다리새우 유래의 비브리오균에 대한 소독제의 방제 효과 검증

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ABSTRACT

White leg shrimps which were cultured in a private aquaculture farm showed abnormal swimming behavior and appetite reduction in July 2023. Then, gradual mortality was observed in the aquaculture farm. During the diagnosis, bacterial strain KNUAFVa-SHP08147 was isolated from the hepatopancreas of the dead shrimps. Based on the sequence of 16S rRNA gene, KNUAFVa-SHP08147 was proved to be *Vibrio alginolyticus,* showing 99.71 % nucleotide identity with that of *V. alginolyticus* MG54 and GS MYPK1. According to the result of the growth of KNUAFVa-SHP08147, the treatment a commercial disinfectant, Virkon™S was not proved to be effective to prohibit the growth of *Vibrio*.

Key Words : White leg shrimp, *Vibrio alginolyticus,* Commercial disinfectant

Introduction

The white leg shrimp (*Penaeus vannamei*) is known as one of the most commercially important shrimp species is cultured worldwide (Benzie, 2009). The white leg shrimp farming has expanded because it is fast growing, and tolerant to various environments and diseases (Wyban, 2009). Consequently, its aquaculture has expanded dramatically in the Asia Pacific region including South Korea (De Silva et al., 2018).

Vibrio species are among the most abundant bacteria in marine environments (Thompson et al., 2004). However, *Vibrio* is known as the most common bacterial pathogen for shrimp farming, usually causing 100% mortality (Lightner, 1983). As *Vibrio* can be problematic as opportunistic or secondary pathogens, the risk of infection can be serious to shrimps cultured under stress (Lightner, 1988). Among *Vibrio* species, *V.*

alginolyticus, *V. anguillarum*, *V. harveyi*, *V. splendidus* and *V. parahaemolyticus* have been reported as causative agents of vibriosis in shrimp (Tseng et al., 2023; Lightner, 1988; Jun et al., 2016). In general, antibiotics have been widely used for vibriosis since antibiotics are considered the standard treatment for vibriosis (Jun et al., 2016). However, the increasing incidence of antibiotic resistance limited the effect of antibiotic therapy (Jun et al., 2016).

The aim of the present study was to isolated and characterize *V. alginolyticus* from commercially cultured white leg shrimp in Korea. Also, the effect of a commercial disinfectant against *V. alginolyticus* was assessed by performing in *vitro* experiments.

Materials and methods

Cases:

White leg shrimps were cultured in a private aquaculture farm in Jeollabuk-do, Republic of Korea, and water temperature was around 27 °C. In July 2023, some shrimps showed abnormal swimming behavior, appetite reduction, and then gradual mortality. At the beginning, the mortality rate was around 0.1% per day, and increased to around 5% per day at three weeks post-outbreak.

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 supplemented with 1% NaCl 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 amplified using the 785F (5'-GGATTAGATACCCTGGTA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') primer sets and 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 *Vibrio* 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.

Assessment of the effect of a disinfectant to control bacterial growth:

A commercial disinfectant containing potassium peroxymonosulfate, Virkon^{TM}S was prepared to examine its effect to control bacterial growth. Bacterial isolates from the dead shrimps were incubated at 27 °C. Overnight culture of bacteria were diluted 1:30 and grown at 27 °C for 1 h to reach early-exponential phase. The culture was divided into two samples. One sample was treated with the disinfectant to yield a final dose of 4 ppm following the manufacturer's instruction; the other sample, non-treated sample was used as a control. Then, two prepared samples were adjusted to 30 ml by adding LB broth (supplemented with 1% NaCl). The cultures were incubated at 27 °C with shaking at 200 rpm, and the OD_{600} was monitored for 12 h in order to determine changes in the number of viable bacteria throughout the incubation period.

Results

Ten dead or moribund shrimps (average body length 128 mm, average body weight 16.38 g) were submitted to the Department of Aquaculture, Korea National University of Agriculture and Fisheries for diagnosis. The temperature of water ranged from 26.5 to 27.5 °C; the quality of water such as pH and salinity was properly controlled. The symptoms were characterized by the

presence of focal hemorrhages on the skin of legs and caudal fins. Parasitological examinations did not reveal the presence of external or gill parasites on the shrimps.Suspected common colonies were re-streaked onto LB agar and incubated at 27 °C for 24 h. Based on the results of 16S rRNA gene comparison and phylogenetic analysis, the isolate KNUAFVa-SHP08147 was identified to be *V. alginolyticus*(99% nucleotide identity, 689/691)(Fig. 1).

Virkon^{TM}S was used for the assessment of the effect of a commercial disinfectant to control *Vibrio* growth (Fig. 2A). According the result of the growth of *V. alginolyticus* KNUAFVa-SHP08147, the growth of bacteria treated by Virkon^{TM}S was slightly retarded after treatment(Fig. 2B).

However, the effect was not proved to be significant. The OD^{600} values of both groups continued to increase after 9 h post-inoculation without any difference of values.

Discussion

Vibriosis has been a major shrimp disease and

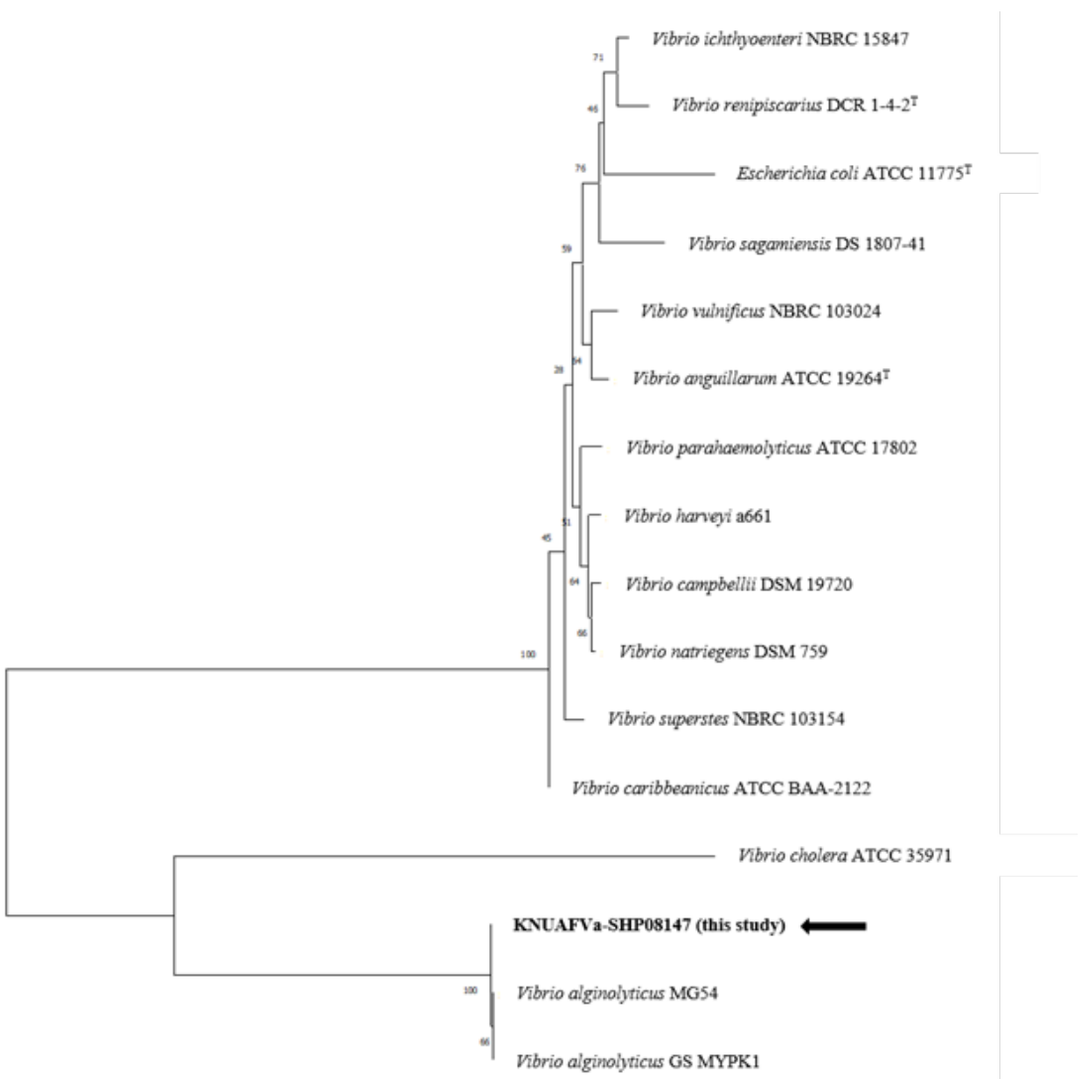

Fig. 1. The 16S rRNA phylogenetic tree of fifteen known bacteria and the isolate KNUAFVa-SHP08147 constructed using neighbor-joining method. The scale bar represents 0.01 nucleotide substitutions per site

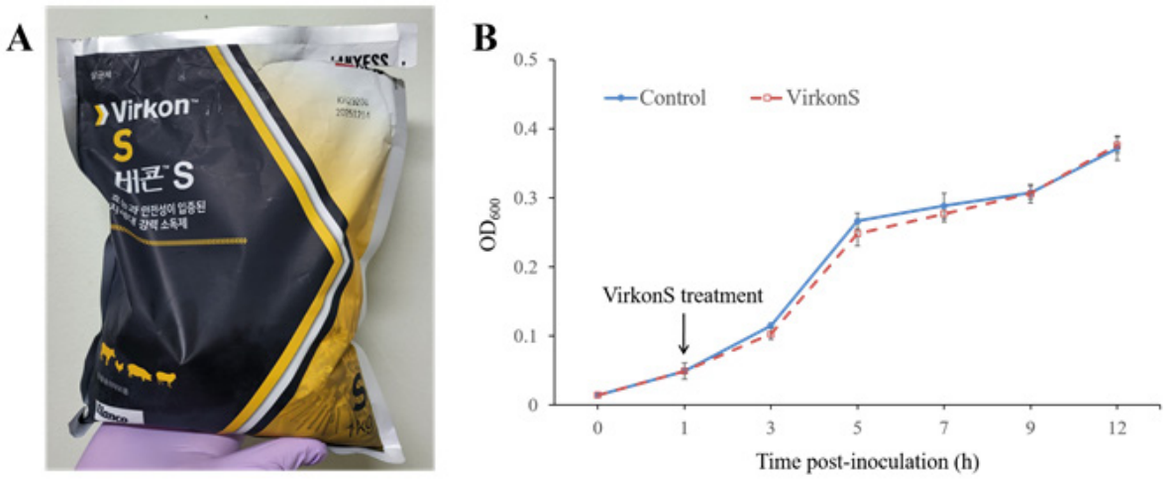

Fig. 2. A commercial disinfectant, Virkon™S used in this study (A); Growth curves of *V. alginolyticus* KNUAFVa-SHP08147 treated by Virkon™S(B)

become a serious issue in shrimp aquaculture around the world (Lightner et al., 2012; Jun et al., 2017). Although the use of antibiotics is generally the only option for aquaculturists, its effectiveness has been limited because of the antibiotic resistance (Han et al., 2015; Jun et al., 2017). In Korea, several shrimp aquaculture farms have arbitrarily used Virkon™S against vibriosis to the shrimp aquaculture systems, but they were not sure of its effectiveness. However, the current study generated by the demand of shrimp aquaculture farms indicated that the application of a commercial disinfectant, Virkon™S was not effective. It emphasizes the need of the development of effective treatment methods for vibriosis in shrimps.

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요 약

2023년 7월, 국내의 흰다리 새우 양식장에서 양식 중이던 흰다리 새우가 이상유영을 보이며 식욕부진에 시달리며 지속적으로 폐사하였다. 초기에는 일일 평균 폐사율이 0.1% 였으나, 3주 후에는 5%까지 증가하였다. 질병 진단 과정 중, 폐사 새우의 간췌장에서 세균(KNUAFVa-SHP08147)이 분리되었다. 본 세균의 16S rRNA 유전자의 시퀀스 분석 결과, 분리주 KNUAFVa-SHP08147은 *Vibrio alginolyticus*로 동정이 되었다. 본 연구에서는, 분리주를 대상으로 비브리오 방제 효과가 있다고 알려져 있는 소독제의 효능을 검증해보았다. 소독제 Virkon™S의 효능 검증을 위하여, Virkon™S를 처리한 후 KNUAFVa-SHP08147의 성장 곡선을 조사하였다. 실험 결과, Virkon™S가 KNUAFVa-SHP08147의 성장에 미치는 영향이 무의미한 것으로 드러나, 소독제 Virkon™S의 *Vibrio* 방제 효과는 없는 것으로 드러났다.