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Antibacterial activity of Bacillus strains against AHPND-causing Vibrio campbellii in Pacific white shrimp

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Two *Bacillus* strains isolated from seawater in Korea show antimicrobial activity against *Vibrio* strains in shrimp



This study evaluated the antimicrobial activity of five *Bacillus* strains isolated from seawater in Jeju, South Korea against 12 *Vibrio* strains (10 AHPND strains and 2 non-AHPND strains). Photo by Darryl Jory.

Acute Hepatopancreatic Necrosis Disease (AHPND) is a bacterial disease caused by *Vibrio* spp. carrying specific toxin genes. AHPND affects the digestive tract of shrimp and the tubular cells of the hepatopancreas, disturbing digestion and resulting in mass mortality. *V. parahaemolyticus* is primarily associated with AHPND (V_{pAHPND}), but other *Vibrio* species that carry binary toxin genes, including *V. campbellii* (V_{cAHPND}), *V. owensii* (V_{oAHPND}), and *V. harveyi* (V_{hAHPND}), have been reported recently. AHPND was first reported in China (2009), and it spread to several countries, including Vietnam (2010), Malaysia (2011), Thailand (2012), Mexico (2013), the Philippines (2015), the USA (2019) and South Korea (2020). This disease is known to cause very **significant economic losses** (<https://doi.org/10.1073/pnas.1503129112>) to the shrimp aquaculture industry, estimated to exceed 1 billion dollars per year in Asia.

As antibiotic alternatives, probiotics have been frequently used in aquaculture to control bacterial diseases, especially against pathogenic *Vibrio* infections and AHPND. Researchers previously reported that shrimp treated with *Bacillus* probiotics in the form of dietary supplements showed a higher survival rate following challenge with V_{pAHPND} . In addition to their antimicrobial activity, probiotics have various advantages in aquaculture such as promoting growth, strengthening immunity and restoring water quality. Meanwhile, spore-forming *Bacillus* spp. are resistant to heat and pressure and are widely used as feed additives.

Although *V. parahaemolyticus* is the cause of most cases of AHPND, other *Vibrio* spp. – such as *V. campbellii*, *V. harveyi* and *V. owensii* – are also known to cause this disease in fields, thereby resulting in substantial economic losses on farms. However, preventative methods and studies on AHPND have

mainly focused on V_{pAHPND} , and the antimicrobial activity against V_{cAHPND} , V_{hAHPND} and V_{oAHPND} has been poorly studied.

This article – summarized from the **original publication** (<https://doi.org/10.3390/fishes7050287>). (Jeon, H.J. et al. 2022. Antibacterial Activity of *Bacillus* Strains against Acute Hepatopancreatic Necrosis Disease-Causing *Vibrio campbellii* in Pacific White Leg Shrimp. *Fishes* 2022, 7(5), 287) – presents the results of research evaluating the antimicrobial activity of five *Bacillus* strains isolated from seawater in Jeju, South Korea against 12 *Vibrio* strains (10 AHPND strains and 2 non-AHPND strains).



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Study setup

Bacillus strains that showed the strongest inhibitory effects in the dot-spot test were further subjected to the challenge test. Pacific white shrimp (*Litopenaeus vannamei*) postlarvae (PL15–PL16) were purchased from a local shrimp farm (Jeju Province, South Korea) and transported to the Laboratory of Aquatic Biomedicine, College of Veterinary Medicine, Kyungpook National University in South Korea. Shrimp were acclimated for 35 days to the experimental conditions and facilities. Then, the animals (average weight 0.2 ± 0.05 grams) were randomly distributed into 22-liter aerated seawater tanks.

For the antimicrobial activity test (challenge test), experimental shrimp (N = 56) were divided into four groups with duplicates. In group 1, the experimental shrimp (N = 14) were exposed to a suspension of *Bacillus* (B1) for 14 days via immersion at a concentration of 1.0×10^6 CFU/mL water. Then, the shrimp were challenged with a V_{cAHPND} suspension via immersion at a concentration of 2.0×10^6 CFU/mL water. In group 2, the experimental shrimp (N = 14) were exposed to a *Bacillus* (B3) suspension for 14 days via immersion at a concentration of 1.0×10^6 CFU/mL water. Then, the shrimp were challenged with a V_{cAHPND} suspension via immersion at a concentration of 2.0×10^6 CFU/mL water. In group 3, the experimental shrimp (N = 14) were exposed to the same amount of fresh broth (TSB+) without *Bacillus* strains (B1 and B3) for 14 days via immersion. Then, they were challenged with a V_{cAHPND} suspension via immersion at a concentration of 2.0×10^6 CFU/mL water. In group 4, the experimental shrimp (N = 14) were exposed to the same amount of fresh broth (TSB+) without *Bacillus* for 14 days, and then they were not challenged with V_{cAHPND} .

To confirm the presence of AHPND, dead shrimp were collected and tested using a PCR method previously described by other authors. To quantify AHPND, surviving shrimp were randomly sampled on the day of termination (day 14). The hepatopancreas of each shrimp was collected and tissue used for DNA extraction. Using the extracted DNA, quantitative PCR was performed to quantify a AHPND toxin gene (*pirA*) in the hepatopancreas in the groups.

For detailed information on the experimental design and animal husbandry; isolation of *Bacillus* spp., *Vibrio* spp. antimicrobial activity and other tests; and statistical analyses, refer to the original publication.

Effects of *Bacillus velezensis* dietary supplementation on growth and health of Pacific white shrimp



Assessing the *B. velezensis* BV007 supplement to promote growth, enhance immune response and modulate intestinal microbiota of *L. vannamei* shrimp.



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Results and discussion

In this study, we evaluated the antimicrobial activity of five *Bacillus* isolates against 12 shrimp *Vibrio* strains (10 AHPND *Vibrio* strains [9 *V. parahaemolyticus* and 1 *V. campbellii*] and 2 non-AHPND *Vibrio* strains [one *V. parahaemolyticus* and one *V. harveyi*]). *Bacillus* spp. are usually isolated from soil, fermented soybean paste (cheonggukjang), plants, and pond water, and are incubated at 30–37 degrees-C. The *Bacillus* strains described in this study were isolated from seawater and were found to grow well at 28–37 degrees-C. Additionally, all *Bacillus* strains exhibited growth in both TSA and TSA+ (supplemented with 2 percent NaCl), indicating that these strains could be applied to water with wide ranges of salinity.

In the dot-spot test, B1, B3, B5, B7 and B8 exerted inhibitory effects on at least one of tested *Vibrio* strain. In addition, these strains showed inhibitory effects against isolates from both South Korea and several other countries (Mexico and other countries in Latin America, Vietnam, Thailand and the USA). This indicates that the *Bacillus* strains used in this study can be used globally in various shrimp-farming countries to control AHPND. Improved management of AHPND, a disease that results in extensive mortality in shrimp, could increase shrimp production and decrease economic losses in shrimp farming.

In the challenge test, the B1 treatment group (100 percent) exhibited a significantly higher survival rate than the non-*Bacillus* treatment group (64.3 percent) at 60 h. In a **previous study** (<https://doi.org/10.1016/j.aquaculture.2016.12.022>), V_{CAHPND} was highly pathogenic to shrimp, similar to V_{CAHPND} , and the cumulative mortality in shrimp was as high as 100 percent within two days of V_{CAHPND} laboratory infection. In this study, two *Bacillus* strains (B1 and B3) displayed prominent antimicrobial effects within two to three days (48 to 60 hours) of V_{CAHPND} infection compared with the findings in the positive control group (V_{CAHPND} immersion without B1 and B3 treatment); thus, both strains are expected to emerge as alternatives to antibiotics for controlling V_{CAHPND} .

Moreover, based on results among the live shrimp collected on the day of experiment termination, the two *Bacillus* strains identified in this study exhibited antimicrobial activity against pathogenic V_{CAHPND} . Additionally, the histopathology of the hepatopancreas was examined after exposure to *Bacillus* spp. for 14 days in our preliminary study. The structure of the hepatopancreas was found to be similar between the *Bacillus* treatment groups and the control group (not exposed to V_c , V_{CAHPND} and *Bacillus*), indicating that *Bacillus* strains are harmless to shrimp.

Moringa leaf extract can boost Pacific white shrimp immune responses

Including moringa leaf extract at a certain level enhanced immune response, growth and resistance of *L. vannamei* against *V. alginolyticus* infection.



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The two strains (B1 and B3), which showed antimicrobial activity using the dot-spot test (in vitro) and challenge test, were finally classified as *B. velezensis* based on their whole genome-based phylogeny. Several studies have examined the probiotic effects of *B. velezensis* in various organisms. Other studies described the antibacterial activity of *B. velezensis* against *V. parahaemolyticus* isolated from shrimp and *V. anguillarum* isolated from sea bass. These results suggest that the newly isolated B1 and B3 strains will have additional advantageous characteristics in terms of their potential use in the aquaculture industry.

Based on our study results, further studies are warranted regarding the predicted presence of surfactin [a surfactant and powerful antibiotic] in the isolates because of its relatively low similarity with previously reported compounds. Moreover, the potential presence of other compounds that have been associated with the antifungal activity of some *Bacillus* strains may contribute to the potential usability of the *Bacillus* strains identified in this study.

Perspectives

Overall, our study results showed that two *Bacillus* strains isolated from seawater in Korea have antimicrobial activity against *Vibrio* strains in shrimp using dot-spot and challenge tests, and secondary metabolites derived from the B1 and B3 strains were more numerous than those previously reported for *Bacillus* spp., indicating that both strains can be used as potential candidates for the management of *Vibriosis* and AHPND, including V_{CAHPND} , in shrimp aquaculture.

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