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Health & Welfare

How different Bacillus strains isolated from AHPND-surviving shrimp can reduce mortality

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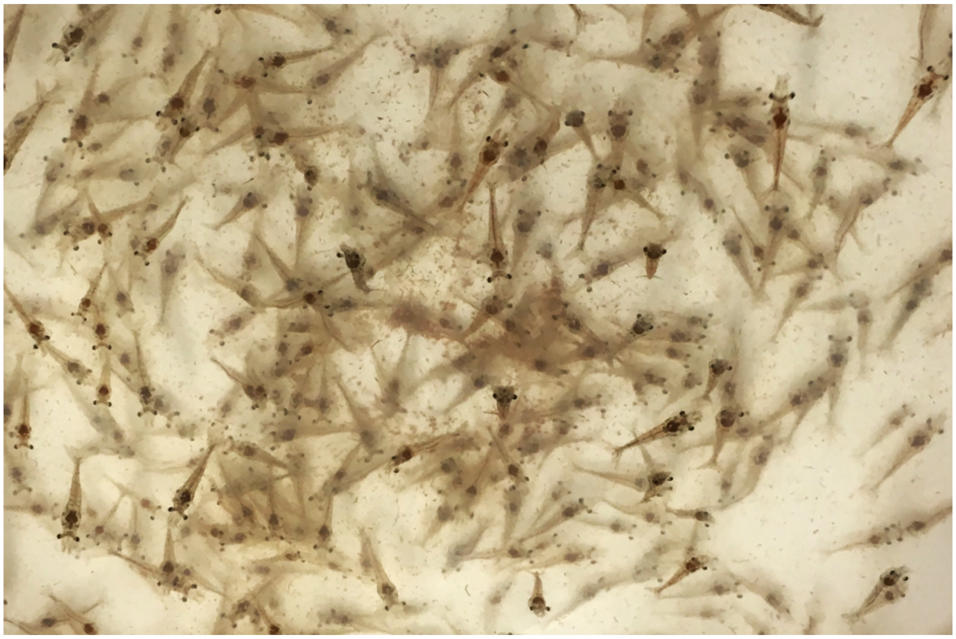
Bacillus spp. with at least two antimicrobial peptide-related genes exhibited diverse AHPND-inhibition activities



This study evaluated the potential of *Bacillus* spp. strains isolated from AHPND-surviving shrimp to reduce AHPND-related mortality in Pacific white shrimp. Photo by Darryl Jory.

Acute hepatopancreatic necrosis disease (AHPND) is one of the most serious threats to shrimp farming. AHPND is caused mainly by *Vibrio parahaemolyticus* (VP_{AHPND}) which affects penaeid shrimp, including the Pacific white shrimp (*Litopenaeus vannamei*) and black tiger shrimp (*Penaeus monodon*), with mortality up to 100 percent within 20 to 30 days of cultivation, resulting in significant economic losses in shrimp aquaculture.

In general, bacteria are promising contributors to shrimp health and are widely applied in shrimp farms for health management as an alternative strategy to reduce the risk of diseases. Probiotics – live microorganisms which when administered in adequate amounts confer a health benefit on the host – and biological control agents have been studied. Previous studies suggest that oral administration of exogenous bacteria stimulates shrimp immune reactions, and bacterial antagonism occurs in the environment, including the shrimp gut and pond water. However, the exact mechanism of the probiotic effects of bacteria on shrimp and the appropriate application are uncertain.

Bacillus spp. is one of the most studied and used bacteria as a probiotic or biocontrol agent in aquaculture. In shrimp, oral administration of spores or vegetative cells of specific strains of *Bacillus* spp. reduces the mortality of shrimp caused by bacterial infections, with induction of the host immune system and/or antagonism between the bacteria as possible mechanisms. Antimicrobial peptides (AMPs) secreted by *Bacillus* spp. inhibit the growth of other microorganisms, especially bacteria and fungi. The risk of resistance among bacteria against those AMPs is thought to be small; thus, the application of AMP-producing *Bacillus* to aquaculture fields is promising.

The stomach microbiota of penaeid shrimp may play a crucial role in protecting against bacterial infections. *Vibrio* bacteria, such as VP_{AHPND} and *Vibrio penaeicida*, colonize the shrimp's stomach during the initial stages of infection. Moreover, in contrast to mammalian animals, shrimp stomachs host a diverse range of bacteria, with variations observed in the presence or absence of AHPND development. This knowledge suggests that the bacterial community in the stomach microbiota of shrimp, similar to the intestinal microbiota of mammals, might confer beneficial effects on shrimp.

This article – summarized from the **original publication** (https://doi.org/10.3390/microorganisms11092176) (Proespraiwong, P. et al. 2023. Evaluation of *Bacillus* spp. as Potent Probiotics with Reduction in AHPND-Related Mortality and Facilitating Growth Performance of Pacific White Shrimp (*Litopenaeus vannamei*) Farms. *Microorganisms* 2023, 11(9), 2176) – reports on the results of a study on *Bacillus* spp. strains isolated from the shrimp stomach and environment and their evaluation as potential beneficial bacteria for shrimp.

Study setup

For this study, healthy Pacific white shrimp (0.5 grams) were provided by Aquatic Animal Research Mae Klong, Charoen Pokphand Foods Public Co., Ltd. (CP, Bangkok, Thailand). Shrimp were maintained in aerated aquaculture tanks at Kasetsart University until the challenge test. The shrimp were randomly screened by PCR for the presence of several pathogenic viruses including AHPND, EHP, WSSV, IHHNV, TSV and YHV. Shrimp husbandry conditions during experiments included pH 7.8–8.2, temperature 28–32 degrees-C, salinity 20 ppt, alkalinity 170–190 mg, TAN less than 1 ppm, and NO_2– less than 1 ppm.

Probiotics derived from *Bacillus* spp. were isolated from the stomachs of AHPND-surviving *L. vannamei* (22 isolates) and mangrove forest soil near the shrimp farms (10 isolates). *Bacillus* spp. were genetically identified and characterized based on the availability of antimicrobial peptide (AMP)-related genes. The phenotypic characterization of all *Bacillus* spp. was determined based on their capability to inhibit AHPND-causing strains of *Vibrio parahaemolyticus* (VP_{AHPND}).

In the lab, the efficacy of the *Bacillus* spp. against various strains of VPAHPND was evaluated. Shrimp were immersed in 1×10^5 CFU/mL *Bacillus* sp. for 10 hours and then challenged with 1×10^4 CFU/mL of different VP_{AHPND} strains in a salinity of 20 ppt. All assays were performed in triplicate for each VP_{AHPND} isolate. Then, field trials were conducted in shrimp nursery farms in several shrimp farms in Vietnam.

For detailed information on the experimental design, animal husbandry, laboratory and field trials, *Bacillus* strains isolation and characterization and data collection and analysis, refer to the original publication.

Do diets supplemented with phytobiotics protect Pacific white shrimp against AHPND?

Shrimp diets supplemented with phytobiotics and applied at critical periods could mitigate the impact of AHPND and other pathologies.

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Results and discussion

The results showed that *Bacillus* spp. without AMP-related genes were incapable of inhibiting VP_{AHPND} *in vitro*, while other *Bacillus* spp. harboring at least two AMP-related genes exhibited diverse inhibition activities. Interestingly, *B. subtilis* strain K3, isolated from shrimp, exerted remarkable inhibition against VP_{AHPND} (80 percent survival) in Pacific white shrimp and maintained a reduction in shrimp mortality within different ranges of salinity (75–95 percent survival). Moreover, with different strains of VP_{AHPND}, *B. subtilis* K3 showed outstanding protection, and the survival rate of shrimp remained stable among the tested groups (80–95 percent survival).

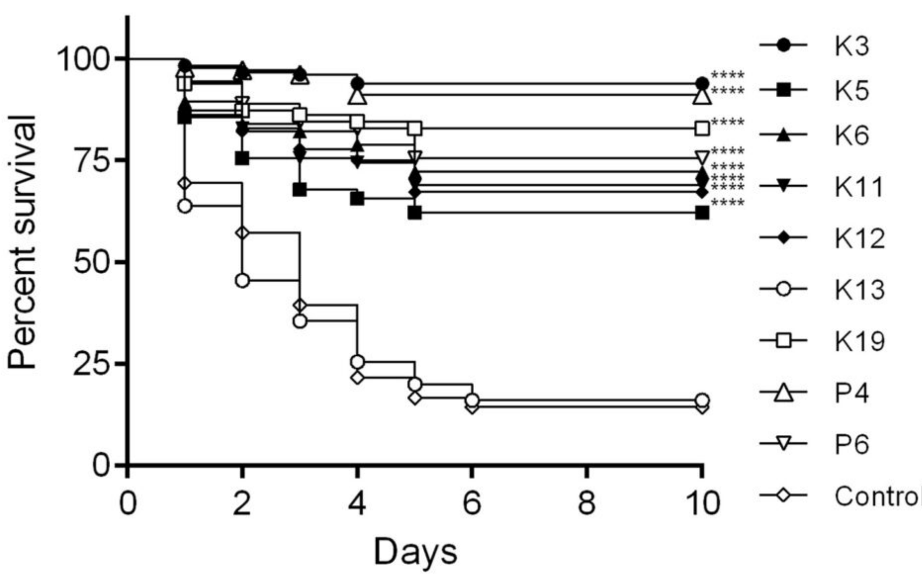

Fig. 1: Evaluation of AHPND disease control by *Bacillus* spp. Shrimp were treated with different *Bacillus* spp. isolates (isolates K3, K5, K6, K11, K12, K13, K19, P4, and P6) for 10 hours following challenge by immersion with 104 CFU/mL VPAHPND (strain RY). **** p < 0.0001 compared with the control. Adapted from the original.

Despite the apparent differences in environmental conditions between shrimp stomachs and mangrove forest soil, there were no clear patterns in the distributions of the tested AMP-related genes among *Bacillus* isolates from each origin. It is expected that the *Bacillus* present in the shrimp stomach is not specifically adapted to the shrimp stomach environment but rather that *Bacillus* that could be present in the external environment is ingested orally and colonizes in the shrimp stomach.

While some previous studies claimed that bacteria administered by feeding colonized shrimp, it is not clear whether supplied *Bacillus* in water can stably colonize the digestive tract of shrimp. A previous report showed very low colonization rates of *Bacillus* bacteria in the digestive tracts of shrimp, especially in earthen ponds. For this reason, the field trial in this study was conducted with continuous administration of the test bacterium, but the optimization of dosing methods is needed.

In general, strains of VP_{AHPND} are halophilic (thrive in high salinities), and the salinity in the water affects their virulence. Experimental infections in this study also showed different mortality rates of shrimp in a salinity-dependent manner. However, in shrimp hatcheries, it may be difficult to reduce the salinity. In contrast, inland water aquaculture of Pacific white shrimp uses low-salinity water for cultivation. In this study, at each salinity, mortality during VP_{AHPND} challenge was lower in the experimental group using *B. subtilis* K3. This indicates that the effects can be expected under a variety of environmental salinity conditions.

Fig. 2: Effect of water salinity on *B. subtilis* (K3) efficiency in controlling AHPND. Shrimp were treated with *B. subtilis* K3 for 10 hours following the challenge with VPAHPND (strain RY) at 104 CFU/mL by the immersion method. Shrimp were reared at different salinities, including 5 ppt, 20 ppt, and 40 ppt. **** p < 0.0001 compared with the control for each salinity. Adapted from the original.

This study was based on selected AMP-related genes for genetic analysis rather than whole genome analysis. We cannot deny that the isolates might harbor novel or overlooked AMP genes that contribute to bacterial inhibition. In addition, it remains unclear whether the differences in inhibitory activity between isolates *in vitro* are dependent on the amount of AMP secreted or the activity of each peptide molecule. Because of the limitations of this study, it is not certain that similar results can be obtained with other *Bacillus* isolates.

Probiotics in aquaculture are a well-known method to improve the health status of aquatic animals, and the use of probiotics to control disease has been widely discussed. However, the validity of their use should be confirmed using rational probiotic screening and selection, as well as the elucidation of their efficiency in laboratory and field trials.

Perspectives

An isolate of *Bacillus* spp. K3 obtained in this study reduced the mortality of *L. vannamei* shrimp challenged with VP_{AHPND}. The methods we used to screen potentially beneficial *Bacillus* spp. can be useful for the search for probiotics for shrimp. This study also shows one of the possible mechanisms of the beneficial effects of probiotics on shrimp.

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