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Effects of probiotics on ammonia degradation in vitro and in Pacific white shrimp ponds

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Single or multispecies probiotics improved water quality by lowering total *Vibrio* counts and ammonia levels

The most common approach for maintaining aquaculture water quality is frequent water exchange, which is costly, time-consuming and may introduce pathogens into culture systems. Probiotics can play a vital role in aquaculture productivity by improving non-specific disease prevention and providing pollution-free water sources. Probiotics have been frequently proposed as environmentally friendly replacements to antibiotics.

The practical application and use of magnetic field treatments in agriculture have a wide range of applications, including seed germination, seedling development and yields of various species, and poultry production, which plays an important role in addressing the shortage of nutrition in developing countries. Magnetic treatment has been reported to enhance germination in spores of various bacteria, including *Bacillus megaterium*, *B. cereus* and *B. subtilis*, and the spores' magnetic properties may have biotechnological applications.

This study evaluated the effects of probiotics on ammonia degradation in vitro and in Pacific white shrimp ponds. Results showed that both probiotics tested were able to degrade ammonia and the magnetic field (21.56 m tesla) was efficient to improve the germination and proliferation of Bacillus spores in vitro. Photo by Darryl Jory.

This article – summarized from the **original publication** (https://doi.org/10.1186/s13568-022-01370-5) (Hassan, M. et al. 2022. Influence of probiotics on water quality in intensified *Litopenaeus vannamei* ponds under minimum-water exchange. *AMB Expr* 12, 22 (2022)) presents the results of a study that investigated the effects of two different probiotics used as water additives on ammonia (NH_3) degradation, and the effect of those probiotics on water quality in earthen ponds stocked with Pacific white shrimp (*Litopenaeus vannamei*) and a minimum-water exchange system.

Study setup

The effects of two commercial probiotics on NH_3 degradation, as well as a magnetic field (21.56 m tesla) on the germination and proliferation of spores of *Bacillus subtilis*, *B. licheniformi, Pediococcus* spp. and *Enterococcus* spp. were studied *in vitro*. Additionally, their effect on water quality maintenance in *L. vannamei* ponds was investigated.

For detailed information on the experimental designs of these trials, animal husbandry and water quality assessments; probiotics composition; In vitro evaluation of probiotics on NH_3 degradation, and evaluation of magnetic field on the propagation of *Bacillus* spores; monitoring of shrimp pond water quality and effect of probiotics; and statistical analyses, refer to the original publication.

Results and discussion

In vitro evaluation of the capability of the used probiotics in NH_3 degradation was conducted either with or without organic matter (OM); the result revealed that both probiotics reduced NH_3 in the presence of high OM (30 mg per liter) which simulates the pond conditions. And this result proves the ability of probiotics in the degradation of NH_3 in presence of OM, meanwhile, multi spp. probiotics take more time due to the various composed microorganisms.

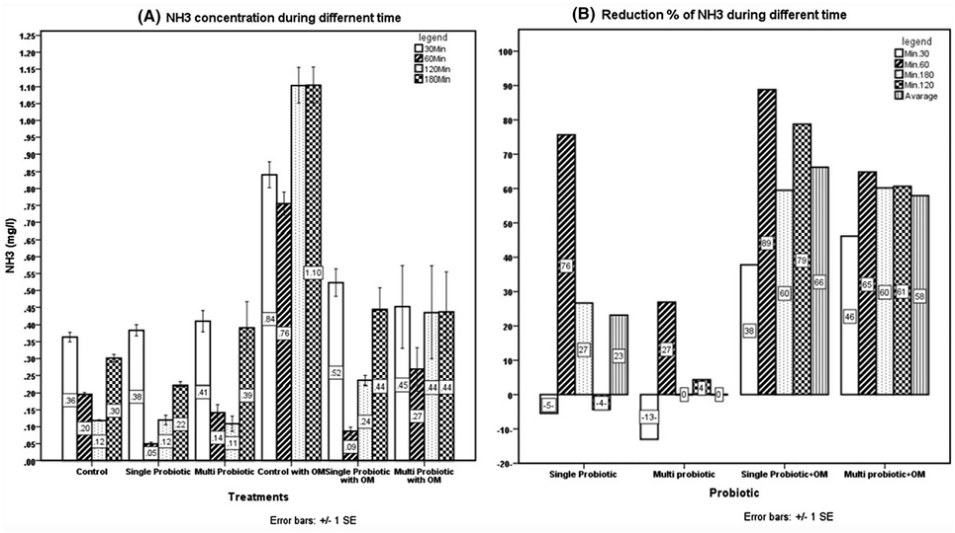

Fig. 1: Effect of probiotics on unionized ammonia (NH3) levels in the presence or absence of organic matter. Means are statistically different ($P \leq 0.01$).

In natural conditions, for their proper activation, probiotics should be introduced to the pond two to seven days before stocking of the aquatic animals, which varies depending on the type of probiotic. To shorten this period, we chose single-spp. (*Bacillus*) probiotics to investigate the prospective influence of magnetic field on spore germination. Magnetic field exposure increased the number of viable *Bacillus* count within six hours, with the maximum effect at 36 hours. One possible explanation for the magnetic field effect is that it promotes germination by enhancing their environment as a physical treatment and for regulating the quorum sensing (biological ability to detect and respond to cell population density) process. Spore germination, which is primarily characterized by spore rehydration and resistance loss, is only the very first stage in the process leading to the initiation of the first cell division and the establishment of a daughter population.

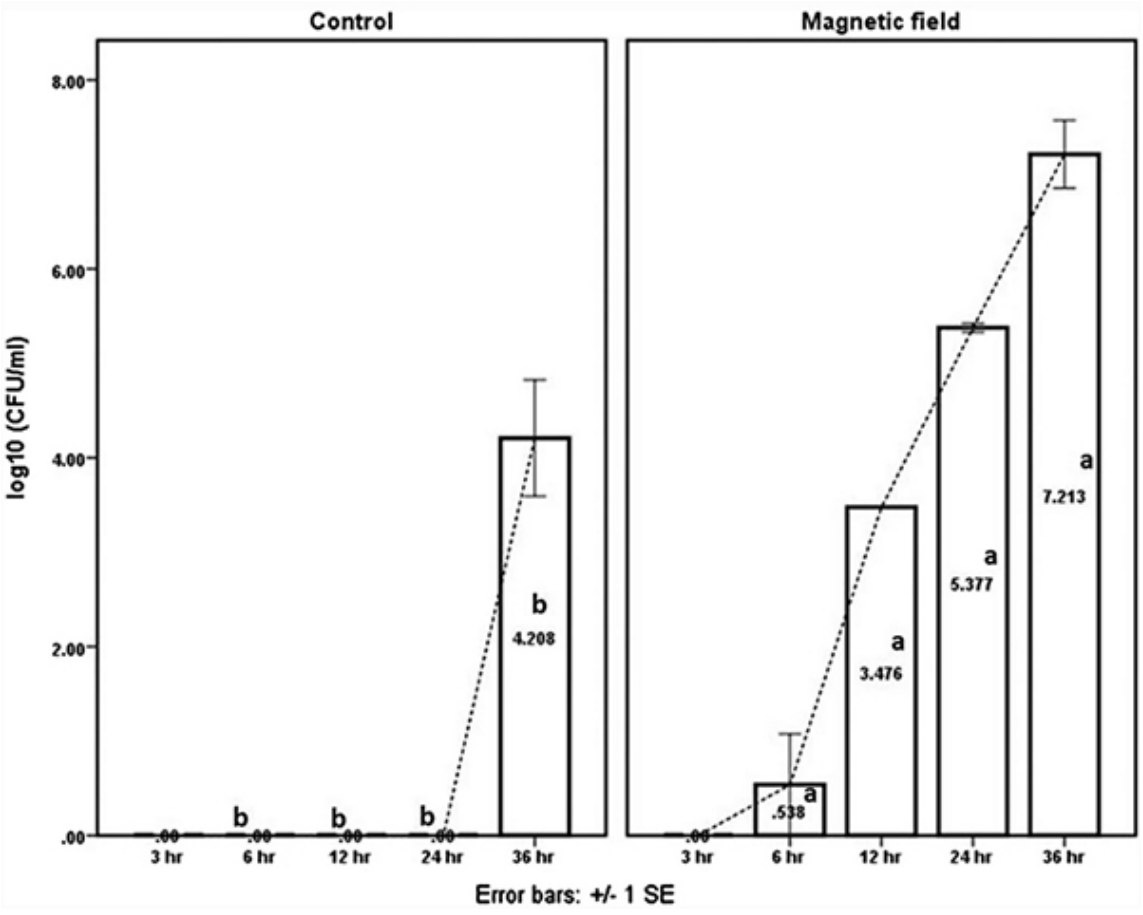

Fig. 2: Effect of magnetic field on the germination and propagation of Bacillus spores.

Microorganisms in intensive aquaculture play a key role in influencing productivity, nutrient cycling, disease outbreaks and environmental protection. *Vibrio* is a common bacteria found in a variety of aquatic and marine ecosystems; of the more than 100 *Vibrio* species discovered, approximately 12 types can cause human infections, while others cause diseases in marine animals. In general, dissolved organic carbon (DOC) has been shown to have a significant impact on *Vibrio* ecology. *Vibrio* spp. depend on organic matter for carbon sources and use a variety of them for nutrition; additionally, *Vibrio* spp. may integrate, consume, and produce organic matter, altering its chemical characteristics and bioavailability. Our data showed that both probiotics tested significantly reduced total *Vibrio* counts (TVC), with a negative correlation.

Many probiotic bacteria actively assimilate or break down organic matter or toxic material, hence improving environmental quality. Gram-positive (bacteria that give a positive result in the Gram stain test) *Bacillus* spp. convert organic matter back to CO_2 more efficiently than gram-negative bacteria, which convert a higher amount of organic carbon to bacterial biomass or slime. Gram-positive bacteria in the production pond can decrease dissolved and particulate organic carbon buildup during the culture cycle while enhancing more stable phytoplankton blooms through increased CO_2 production.

The dissolved oxygen (DO) levels in our study were significantly enhanced by the different probiotic microorganism species used as water additives. Other researchers have reported similar findings, and that *Bacillus* probiotics can help keep DO levels in the optimal range for various cultured species.

In this study, the use of both probiotics resulted in a significant reduction in water pH and, as a result, NH_3 levels, and this association was confirmed by the strong negative correlation between probiotics and both pH and NH_3. This decrease in NH_3 levels compared to the control group is consistent with those reported by other researchers. Furthermore, introducing probiotics to pond water may have enhanced the population of nitrifying bacteria, allowing ammonia to be converted to nitrite and subsequently to nitrate.

Overall, our data showed that water temperature, pH, dissolved oxygen and NH_3 were improved in our trials with probiotics, and their daily administration demonstrated their positive effect in maintaining a healthy environment for shrimp and prawn larvae in improved green-water ponds.

Perspectives

We showed that the use of probiotics (single spp. or multi spp.) as water additives with minimum water exchange improved water quality by lowering TVC and NH_3 levels and increasing DO levels. *In vitro* testing of the ability of the applied probiotics in NH_3 degradation demonstrated that both probiotics could reduce NH_3 in the presence of high organic matter (30 mg per liter), simulating pond conditions.

Furthermore, *in-vitro* investigation of the influence of magnetic field on spore germination and proliferation revealed that magnetic field exposure increased the number of *Bacillus* spores within six hours, with the maximum effect at 36 hours, which will reduce the time required for activation of spores of *Bacillus* probiotics before stocking of shrimp postlarvae. Further studies are needed to evaluate the effects of different magnetic fields strengths in aquaculture.

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