Effect of dietary supplementation of probiotics *Bacillus subtilis* on growth performance and physio-immune responses in *Penaeus vannamei* cultured in low saline waters

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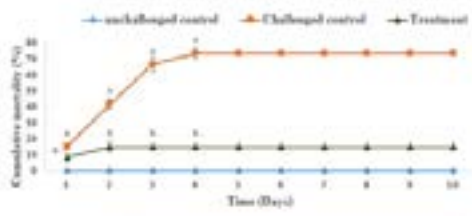
The study was conducted to examine the effect of probiotics, *Bacillus subtilis*, on growth performance, digestive enzyme activity, histopathological changes, and immune resistance against *Vibrio alginolyticus* in *Penaeus vannamei* grown under low saline waters. The study includes control (without B. subtilis) and experimental (with *B. subtilis*) diets that were fed four times daily at 10% of animal body weight, for 6 weeks in duplicate ponds (100 m2). *B. subtilis* incorporated diet-fed group showed significantly higher ($P < 0.05$) weight gain (4.96 ± 0.06 g), final weight (4.98 ± 0.08 g), average daily growth (0.12 ± 0.00 g), specific growth rate (12.26 ± 0.03 %/day), and survival rate (92.21 ± 0.34%) compared to control group. Similarly, *B. subtilis* supplemented diet-fed group significantly higher ($P < 0.05$) digestive enzyme activities of lipase (844.92 ± 122.19 U/mg protein), amylase (8.70 ± 1.04 U/mg protein), protease (2.44 ± 0.50 U/mg protein) and cellulase (28.50 ± 2.67 U/mg protein). A challenge study was conducted at the end of the trial, using *Vibrio alginolyticus*, which found higher (73.33 ± 3.33%) cumulative mortality in the control group compared to the *B. subtilis* treated group (15.00 ± 2.88%). Immune and antioxidant enzyme activities phenoloxidase (10.15 ± 0.42 U/mg protein), SOD (38.52 ± 1.40 U/mg protein), and catalase (0.34 ± 0.04 U/mg protein) was significantly higher ($P < 0.05$) in the B. subtilis treated group after challenging with *V. alginolyticus*. Interestingly, histopathology of the *B. subtilis* supplemented group showed no deformity in the hepatopancreas and intestine. Altogether, the study revealed that the inclusion of *B. subtilis* in the *P. vannamei* diet enhances the growth performance, physio-metabolic activities, and immunity against Vibrio pathogen, indicating *B. subtilis* as a promising probiotic for the shrimp aquaculture industry.

Key words: probiotics, cumulative mortality, immunity, digestive enzymes, histopathology.



Cumulative mortality of *P. vannamei* grown under low saline water fed control and *B. subtilis* supplemented diets and challenging with *V. alginolyticus*

Recent Publications

1. Tamilatasu A, Ahilan B, Gopalakannan A, Somu SLR (2021) Evaluation of probiotic potential of *Bacillus* strains on growth performance and physiological responses in *Penaeus vannamei*. Aquac Res 52(7):3124-3136.
2. Amoah, K., Huang, Q.C., Tan, B.P., Zhang, S., Chi, S.Y., Yang, Q.H., Liu, H.Y. and Dong, X.H., 2019. Dietary supplementation of probiotic *Bacillus coagulans* ATCC 7050, improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp, *Litopenaeus vannamei. Fish & Shellfish Immunology*, 87, pp.796-808.
3. Kavitha M, Raja M, Perumal P (2018) Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). Aquacult Rep 11:59-69.
4. Chai PC, Song XL, Chen GF, Xu H, Huang J (2016) Dietary supplementation of probiotic *Bacillus* PC465 isolated from the gut of *Fenneropenaeus chinensis* improves the health status and resistance of *Litopenaeus vannamei* against white spot syndrome virus. Fish Shellfish Immunol 54:602-611.
5. Li CC, Yeh ST, Chen JC (2010) Innate immunity of the white shrimp *Litopenaeus vannamei* weakened by the combination of a *Vibrio alginolyticus* injection and low-salinity stress. Fish Shellfish Immunol