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Effect of *Bacillus subtilis* and *Lactobacillus rhamnosus* incorporated probiotic diet on resistance of *Penaeus vannamei* against *Vibrio harveyi* infection

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Abstract:

The effect of Bacillus subtilis and L.rhamnosus incorporated diet was investigated on survival of Penaeus vannamei when challenged with V. harveyi. 25- day old shrimps were stocked in three circular tanks. Tank - 1 shrimps were fed with Lactobacillus rhamnosus incorporated diet and tank- 2 shrimps were fed with Bacillus subtilis incorporated diet. The other tank shrimps were fed with commercial diet without probiotic which served as control group. After 3 weeks of feeding trail, shrimps were challenged with V. harveyi adopting immersion method. Shrimps fed on B. subtilis incorporated diet upon challenging with V. harveyi showed 75 % survival, where as shrimps fed on Lactobacillus rhamnosus incorporated diet resulted in 68% survival rate, and negative control group shrimps which received only V. harveyi showed 100% mortality and control group fed with commercial diet without probiotic showed 95% of survival rate.

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Statistical analysis was performed using analysis of variance (ANOVA) at $P \leq 0.05$ using spss 13 version

Key words: Probiotic diet, *V. harveyi*, *Lactobacillus rhamnosus* and *Bacillus subtilis*

INTRODUCTION

Penaeid shrimps are among the most important and extensively cultured crustaceans in the world (> 60 countries). India is one of the major shrimp producing countries along with China, Indonesia and Thailand. Farm-reared shrimp is increasing in popularity and profitability due to its exclusive flavor and high nutritive value [1]. In recent times, shrimp culture has been frequently affected by viral and bacterial diseases inflicting huge loss. Bacterial diseases are considered to be a major cause of mortality in shrimp larvae culture [2]. The most prevalent bacterial disease is *Vibriosis* which causes a mass mortality both in larval cultures and shrimp production [3].

The major virulent strains of vibrio's in shrimp are *Vibrio alginolyticus, V. anguillarum, V. harveyi* and *V. parahaemolyticus.* It is common for hatchery managers to try to control bacterial infections or even the presence of potentially pathogenic bacteria in the system. For preventing and controlling diseases, antibiotics, and other chemicals were used possibly creating antibiotic resistant bacteria, persistence of antibiotics and other toxic chemicals in aquatic environment thus creating human health hazards. Thus, refusal of shrimps with antibiotics, steroids, chemicals. More recently probiotic microorganisms and vaccination or other forms of immunostimulation have also been employed. The definition of probiotics is for 'life'. Probiotic is defined as a living

microbiological dietary supplement that provides a nourishing environment to the friendly flora living in the digestive tract. Probiotics are noticed to prevent pathogens from proliferation [4, 5] improve health of culture species [6, 7] by improving the balance of intestinal microflora [8, 9]. The introduction of *Bacillus* species in proximity to pond aerators reduced chemical oxygen demand, and increased shrimp harvest [10].

Increase of shrimp or prawn survival in ponds where some strains of *Bacillus* sps were introduced [4]. The inhibitory activity of *Bacillus* species against luminous *Vibrio* species in pond sediment, but the effect on shrimp/prawn survival might be due either to a probiotic effect, or to an indirect effect on animal health. Probiotics are noticed to prevent pathogens from proliferation, improve health in culture species by improving the balance of intestinal microflora.

Thus the present study is carried out to test the potential use of Bacillus *subtilis, Lactobacillus rhamnosus* as feed incorporated diet in controlling bacterial disease caused by *Vibrio harveyi, in Litopenaeus vannamei* [4]

MATERIALS AND METHODS

Penaeus vannamei 25 (juvenile 30), 1.5 ±0.05 g (mean±SD) body weight, was acclimatized to the Laboratory conditions. The lactic acid bacterium, *Lactobacillus rhamnosus* and Bacillus *subtilis* were collected from IMTECH Chandigarh. The probiotic bacteria *L. rhamnosus* was cultured in Man-Rogosa-Sharpe broth (MRS, Himedia). *Bacillus subtilis* was cultured in Bacillus broth (Himedia) and incubated under continuous agitation of 180rpm at 37°C for 24h.

The bacterial cultures were centrifuged at 4000 rpm for 15 min at 4ºC and harvested. The collected bacteria were re suspended in normal saline solution to 5 x 10^{13} CFU/ml of *Bacillus subtilis* and 3x10^{5} CFU/ml of *L. rhamnosus*. Feed

was oven-dried at 35ºC for 1 - 2 h. The control diet was sprayed with sterile culture medium as described by [11].

Antimicrobial Activity

The probiotic bacteria *L. rhamnosus* was cultured in Man-Rogosa-Sharpe broth (MRS, Himedia). *Bacillus subtilis* was cultured in Bacillus broth (Himedia) and incubated under continuous agitation of 180rpm at 37°C for 24hrs. Strains were phenotypically identified by Gram staining and the culture was centrifuged at 7000 rpm for 10 minutes. Extracellular fraction was collected 25µl of extracellular was used to perform well diffusion method against *Vibrio harveyi*, that were grown in marine agar.

Challenge Test

Twenty five days old shrimps were randomly divided into four groups of twenty animals each group. Shrimps in group I served as control. The animals in groups II, III and IV treated as experimental groups. Group II received *Bacillus subtilis* incorporated pellet feed, whereas Group III received *Lactobacillus rhamonusus* supplemented diet and group IV serve as negative control, which received only *Vibrio harveyi*, pathogen. Shrimps were fed with pellet feed thrice a day. After 3 weeks of feeding trail, shrimps were challenged with *V.harveyi* 4×10^{-4} CFU/ml adopting immersion method. The probiotics treated animals were exposed to pathogenic bacteria *V.harveyi* for 10 hrs and transferred to their respective tanks shrimps were recorded for survival rate.

RESULTS

Antimicrobial Activity

Bacillus subtilis and *Lactobacillus rhamnosus* showed antimicrobial activity against chosen pathogenic bacteria *Vibrio*

harveyi. B.subtilis showed maximum zone of inhibition against *Vibrio harveyi* (5.9 mm) compared to *Lactobacillus rhamnosus* (5.5 mm).

Challenge Test

The studies regarding challenging of *Litopenaeus vannamei* against *V.harveyi* resulted that the mortality of shrimp was reduced by *Bacillus subtilis compared to Lactobacillus rhamnosus* under in vitro conditions. Shrimps fed on *B.subtilis* incorporated diet upon challenging with *V. harveyi* showed 75 % survival, Shrimps fed on *Lactobacillus rhamnosus* incorporated diet *resulted* in 68% survival rate, where as negative control group shrimps which received only *V. harveyi showed* 100% mortality and control group fed with commercial diet without probiotic showed 95% of survival rate.

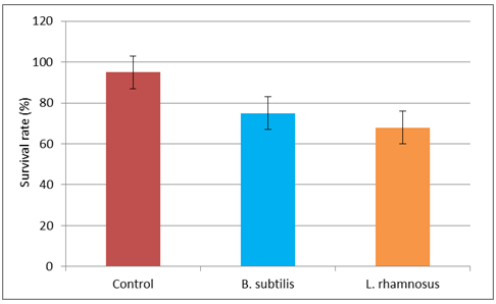

Fig 1. Survival rate of shrimp fed with probiotic bacteria

Mean error bars in the graph represent mean± standard error from the duplicate samples that were tested. Significances of differences: $P \leq 0.05$- (ANOVA)

DISCUSSION

In aquaculture, it is important to provide shrimp a healthy environment so commercial antibiotics and disinfectants are commonly employed for disease management, although this is

not advisable due to cost effectiveness, environmental hazards, and the antibiotic resistance developed by many pathogens [12]. To overcome this present situation, probiotics are widely used as effective antimicrobial agents. Probiotics are noticed to prevent pathogens from proliferation [4, 5] improve health of culture species [6] by improving the balance of intestinal micro flora [8, 9]. In the present study, two different probiotic bacteria *Bacillus subtilis, Lactobacillus rhamnosus* efficiently controlled the proliferation of pathogenic *Vibrio harveyi*. All probiotic-treated groups resulted low mortality when compared to control and negative control groups. Resistance against experimental infection with *V. harveyi* was clearly elevated in shrimps that were fed with *B. subtilis* and *L. sporogenous.* Shrimps fed with *B. subtilis* showed better survival compared to *L. rhamnosus* and control groups. It was clear from our study that the application of probiotics, *B. subtilis* and *L. rhamnosus* incorporated diet had beneficial effects on the survival rate of shrimp (*P. vannamei*) by enhancing the immune system of shrimp. Previous studies showed that supplementation of the commercial Bacillus probiotic significantly increased the survival rate of India white shrimp (*Fenneropenaeus indicus*) in the treatments over the controls [13]. [14, 15] showed better survival of blue crab (*P. trituberculatus*) when inoculated with Thalassobacter utilis in larval rearing tanks at the concentrations of 106 cells ml^{-1} and observed a survival of 27.2% in the test tanks compared with 6.8% in the control tank, with no bacteria inoculated. Use of *Lactobacillus* showed increased survival compared to control after 100 days feeding trail in *P .monodon* [7] and also showed similar results after challenging shrimps with, *V. harveyi* pathogen by immersion for 10 days; all probiotic treatment groups showed increased survival compared to control groups [8].

The use of *Lactobacillus sp.* as the probiotic bacteria in the giant tiger shrimp (*P. monodon;* Fabricius). They

investigated effective of *Lactobacillus* treated shrimps against vibriosis and white spot diseases [16].

CONCLUSION

B. subtilis and *L.rhamnosus* are *efficient* probiotics and can be used as feed supplement for shrimp, *Penaeus vannamei.* Use of these two probiotics resulted in higher survival rate compared to control groups. Both the probiotics are potential in controlling *Vibrio harveyi* in shrimps by producing antimicrobial compounds and enhancing immunity.

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REFERENCES

1. FAO, 2005. Fishing and culture year book. Rome pub, 33-35.
2. Wilkenfeld, J.S. 1992. Commercial hatchery status report: an industry panel viewpoint. In: Wyban, J. Ed. , Ž . Proceedings of the special session on shrimp farming. World Aquaculture Society, Baton Rouge, pp. 71–86.
3. Saulnier, D., P. Haffner, C. Goarant, P. Levy and D. Ansquer. 2000. Experimental infection models for shrimp *vibriosis* studies: a review. Aquaculture, 191: 133-144.
4. Moriarty, D.J.W. 1998. Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture. 164: 351–358.
5. Robertson, P.A.W., C.O. Dowd, C. Burrells, P. Williams and B. Austin, 2000. Use of *Carnobacterium* sp. as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture. 185: 235-243.

6. Austin, B., E. Baudet and M.B.C. Stobie, 1992. Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. J. Fish Dis. 15: 55-61.

7. Phianphak, W., S. Rengpipat, S. Piyatiratitivorakul, P. Menasveta, 1999. Probiotic use of Lactobacillus spp. for black tiger shrimp, Penaeus monodon. J. Sci. Res. Chula Univ. 24, 42–51.

8. Rengpipat, S., W. Phianphak, S. Piyatiratitivorakul, P. Menasaveta, 1998. Effects of a probiotic bacterium in black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture. 167: 301– 313.

9. Irianto, A. and B. Austin, 2002. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 25: 333-342.

10. Porubcan, R.S. 1991. Reduction in Chemical oxygen demand and improvement in Penaeus monodon yield in ponds inoculated with aerobic Bacillus bacteria. Program and Abstracts of the 22nd Annual Conference and Exposition, 16-20 June 1991, San Juan, Puerto Rico. World Aquaculture Society

11. Avakh, K.M. 2006. Assessment of putative bacteria as probiotics in juvenile freshwater prawn, *Macrobrachium rosenbergii*; Unpublished doctoral dissertation, University of Putra, Malaysia.

12. Kruse, H. and H. Soram, 1994. Tromster of multiple drug resistance plasmids between bacteria of diverse origins in natural environments. Appl Environ Microbiol. 60(1):4015–4021.

13. Ziaei-Nejad, S., M.H. Rezaeib, G.A. Takamic, D.L. Lovettd, A. Mirvaghefia and M. Shakourie, 2006. The effect of Bacillus spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquacult. 252: 516-524.

14. Nogami, K. and M. Maeda, 1992. Bacteria as biocontrol agents for rearing larvae of the crab, Portunus trituberculatus. Can. J. Fish. Aquat. Sci. 49: 2373-2376.
15. Maeda, M. 1992. Fry production with biocontrol. Isr. J. Aquac-Bamidgeh. 44: 142–143.
16. Jiravanichpaisal, P., P. Chuaychuwong and P. Menasveta. 1997. The use of *Lactobacillus* sp. as the probiotic bacteria in the giant tiger shrimp (*Penaeus monodon*; Fabricius). Poster session of the 2nd Asia-Pacific marine biotechnology conference and 3rd Asia-pacific conference on algal biotechnology. 7-10 May 1997 Phuket Thailand. P16 .