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Research Article

INFLUENCE OF PROPHYLACTIC LACTOBACILLUS BACTERIA AGAINST GILL CHOKE DISEASE CAUSED BY *VIBRIO HARVEYI* IN PENAEID SHRIMP

Bharathi A

PG and Research Department of Zoology, Sir Theagaraya College,
Chennai. - 600021, Tamil Nadu, India.

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ABSTRACT

India is one of the world's leading shrimp producers. The shrimp hatchery industry also developed in parallel to this. Seed production is not constant due to various disease threats. Shrimp hatchery seed (*Penaeus vannamei)* production is depressed by various diseases, particularly caused by luminous bacteria (*Vibrio harveyi*), filamentous bacteria (*Leucothrix mucor*), and some viruses. Usage of various antibiotics becomes ineffective in many cases. It results in increased virulence of pathogens and poses a concern for the transfer of antibiotic resistance to human pathogens. Usage of probiotics provides a solution to these problems. The microbial species composition in hatchery tanks by adding selected bacterial species to displace deleterious normal bacteria. The virulence of luminous bacteria (*Vibrio harveyi*), can be controlled in this manner rather than an antibiotic Erythromycin. The abundance of luminous bacterial strains decreased in hatchery tanks where specially selected, probiotic strains of lactobacillus species were added. The luminous bacterial disease identified tanks were controlled by using probiotics (Lactobacillus species) than an antibiotic (Erythromycin) in a shrimp hatchery on the Chennai coast. The health of shrimp larvae and survival rate also increased to 80% after the application of the lactobacillus species as a probiotic.

Keywords: Shrimp, Luminous bacteria, Hatchery, *Vibrio harveyi*, Disease, Control and Probiotic.

INTRODUCTION

Penaeid shrimps rank among the most significant and widely farmed crustaceans globally, present in more than 60 countries. India ranks among the leading shrimp-producing nations, alongside China, Indonesia, and Thailand. Shrimp raised on farms is gaining popularity and profitability because of its unique taste and nutritional benefits (FAO, 2005). The words "shrimp" and "prawn" do not have a specific connection to any recognized taxonomic categories. While "shrimp" is occasionally used for smaller species and "prawn" typically refers to larger ones, there is no definitive separation between the two terms, and their applications are frequently misunderstood or even swapped in various countries or areas (Chan, 1998). The majority of commercially significant prawn species are classified under the penaeoidea superfamily. Research on penaeoids is extensive, and currently, there are 5 families, 23 genera and 121 species (including introduced species) identified along the Indian coastline, which includes the Lakshadweep and Andaman and Nicobar Islands, with penaeidae being the most significant family (Radhakrishnan *et al*., 2011). Vibriosis is the most common bacterial disease, leading to significant mortality in larval cultures and shrimp production (Saulnier, D., *et al*., 2000). To prevent and manage diseases, antibiotics and other chemicals have been used, which may result in antibiotic-resistant bacteria and the persistence of harmful antibiotics and chemicals in aquatic environments, thereby posing risks to human health. Therefore, rejection of shrimp containing antibiotics, steroids, and chemicals. Probiotic microbes, vaccines, and various immune-stimulation methods have been utilized more lately. Probiotics are defined as 'for life'. Probiotic refers to a live microbiological dietary supplement that creates a supportive environment for the beneficial bacteria inhabiting the digestive system. Probiotics are observed to inhibit the growth of pathogens (Moriarty, D.J.W. 1998: Robertson, P.A.W., *et al*., 2000).

*Corresponding Author: Dr. Bharathi A, Associate Professor, PG and Research Department of Zoology, Sir Theagaraya College, Chennai-600021, Tamil Nadu, India. Email: professorbharathi.stc@gmail.com.

The pathogenicity of *V. harveyi* is associated with various factors, including the accumulation of extracellular products (ECP) that contain substances like proteases, haemolysins, and lipases. (Harris & Owens, 1999; Liu & Lee, 1999; Zhang & Austin, 2000; Teo *et al*., 2003). Thus, the present study is carried out to test the potential use of Lactobacillus as feed incorporated diet in controlling bacterial disease caused by *Vibrio harveyi* (Moriarty, D.J.W. 1998).

MATERIALS AND METHODS

The study was conducted in a commercial shrimp hatchery in East Coast Road of Chennai. The experiment was conducted to prove the efficacy of a probiotic called BiOWiSH® MultiBio 3PS (Commercial name). It is a combination of *Lactobacillus plantarum*, *Lactobacillus acidophilus, Lactobacillus rhamnosus,* and *Lactobacillus casei* according to the manufacturer. The seawater used in the tanks was filtered through a Bio-filter and mesh bag filters (1.0 µm) before being pumped into a reservoir tank for primary disinfection by chlorination (20 ppm sodium hypochlorite for 24 hours). After chlorination and particulate settling the water was filtered through a pressure filter and cartridges 1.0 µm, 0.5 µm and 0.25 µm, then disinfected using ultraviolet light (UV), and pumped to the treated seawater reservoir. Before using the seawater, the residual chlorine was checked: If chlorine was still present, sodium thiosulfate was applied, at a ratio of 1:1 of thiosulfate for each ppm of residual chlorine. General water chemistry was analysed before the experiment (Van Wyk *et al*., 1999). Luminous bacterial (*Vibrio harveyi*) infected tanks were identified and samples were collected from the neighbouring shrimp hatchery. The samples were examined under the microscope in100X. Affected animals were having luminous fibrils on the stocked eye balls. The eye balls of disease affected animals were opaque and translucent. The gills of the affected animals were dark in colour and having lot of attachment and the gill was covered like a mat. The animals were brought in to a nearby pathological lab for further confirmation. Collected samples kept it for further experiment. The *Penaeus vannamei* post larvae used in the trial were previously screened for various shrimp diseases including White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV), Infectious Myonecrosis Virus (IMNV), Acute Hepatopancreatic Necrosis Disease (AHPND), and *Enterocytozoon hepatopenaei* (EHP) using polymerase chain reaction (PCR) techniques. The experimental setup was divided in to three groups. There were six acrylic tanks (0.5 x 0.25 x 0.25 meters = 0.03125 cubic meters each) were first filled with 30 lts of sea water (30 ppt) and stocked with 100 nos of post larvae at the stage of PL_1. All tanks inoculated with luminous bacteria (*Vibrio harveyi*) from the same source. The Group "A" Tanks were maintained as such without any treatment. The Group "B" tanks were added with probiotic (BiOWiSH® MultiBio 3PS) every day morning and evening each 1.0 ppm till the end of the experiment. Water exchange and tank siphoning were identical for all treatments. All the groups were properly fed with artificial pellet feed (INVE Belgium) and freshly hatched live Artemia alternatively. The feeding interval was every four hours in a day. The experiment was continued up to 15 days. Water parameters and animal health were analyzed at the end of the experiment. The results were tabulated and interpreted for statistical analysis. Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the winter season.

Table 1. Initial Day of Experiment in Water parameter.

S. No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Water Salinity	31±1.0 ppt	31±1.0 ppt
2	Water Tem	28±1° C	28±1° C
3	Water pH	7.3±1	7.3±1
4	Alkalinity	137.0mg/L	137.0mg/L
5	Hardness	4249 mg/L	4249 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Water colour	Greenish	Greenish

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the winter season.

Table 2. Initial Day of Experiment in Animal Parameter.

S. No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Total Length	5.1±1 mm	5.1±1 mm
2	Rostral spines	2.4	2.4
3	Animal colour	Transparent	Transparent
4	Size variation	2.1%	2.1%
5	Appendages	No attachment	No attachment
6	Green colony	Nil	Nil
7	Yellow colony	72±4	72±4
8	Survival %	100	100

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the winter season.

Table 3. Final Day of Experiment (15th day) in Water parameter.

S. No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Water Salinity	28±1.0 ppt	28±1.0 ppt
2	Water Tem	27.5±1^{o} C	27.5±1^{o} C
3	Water pH	7.8±1	8.0±1
4	Alkalinity	140.0mg/L	140.0mg/L
5	Hardness	4250 mg/L	4250 mg/L
6	Total Ammonia	0.65 ppm	0.07 ppm
7	Water colour	Transparent	Greyish

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the winter season.

Table 4. Final Day of Experiment (15th day) in Animal Parameter.

S. No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Total Length	10.3±1 mm	12.3±1 mm
2	Rostral spines	4.2 Nos	4.8 Nos
3	Animal colour	Slightly Pigmented	Pigmented
4	Size variation	5.8± 0.5%	≤ 5.0%
5	Appendages	Huge attachment	Slightly attachment
6	Green colony	TNTC	24±5 CFU
7	Yellow colony	TNTC	200±7 CFU
8	Survival %	67±2	88±3

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the summer season.

Table 5. Initial Day of Experiment in Water parameter.

S.No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Water Salinity	30±1.5 ppt	30±1.5 ppt
2	Water Tem	32±1^{o} C	32±1^{o} C
3	Water pH	7.9±1	7.9±1
4	Alkalinity	140.0mg/L	140.0mg/L
5	Hardness	4852 mg/L	4852 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Water colour	Greenish	Greenish

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the summer season.

Table 6. Initial Day of Experiment in Animal Parameter.

S. No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Total Length	6.0±2 mm	6.0±2 mm
2	Rostral spines	2.6	2.6
3	Animal colour	Transparent	Transparent
4	Size variation	2.2%	2.2%
5	Appendages	No attachment	No attachment
6	Green colony	73±5	73±5
7	Yellow colony	Nil	Nil
8	Survival %	100	100

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the summer season.

Table 7. Final Day of Experiment (15th day) in Water parameter.

S.No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Water Salinity	30±1.5 ppt	30±1.5 ppt
2	Water Tem	30±1º C	30±1º C
3	Water pH	8.0±1	8.2±1
4	Alkalinity	139.0mg/L	139.0.mg/L
5	Hardness	4851 mg/L	4851 mg/L
6	Total Ammonia	0.24 ppm	0.05 ppm
7	Water colour	Transparent	Greyish

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the summer season.

Table 8. Final Day of Experiment (15th day) in Animal Parameter.

S. No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Total Length	11.3±1 mm	14.2±1 mm
2	Rostral spines	4.6 Nos	5.4 Nos
3	Animal colour	Slightly Pigmented	Pigmented
4	Size variation	7.9± 0.5%	≤ 2.0%
5	Appendages	Huge attachment	No attachment
6	Green colony	TNTC	25±5 CFU
7	Yellow colony	TNTC	200±8 CFU
8	Survival %	64±2	94±2

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the rainy season.

Table 9. Initial Day of Experiment in Water parameter.

S. No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Water Salinity	28±1.0 ppt	28±1.0 ppt
2	Water Tem	28±5º C	28±5º C
3	Water pH	8±0.1	8±0.1

4	Alkalinity	137.0mg/L	137.0mg/L
5	Hardness	5254 mg/L	5254 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Water colour	Transparent	Transparent

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (Vibrio harveyi) in Penaeus vannamei larval production during the rainy season.

Table 10. Initial Day of Experiment in Animal Parameter.

S. No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Total Length	7±3 mm	7±3 mm
2	Rostral spines	2.7	2.7
3	Animal colour	Transparent	Transparent
4	Size variation	2.0%	2.0%
5	Appendages	No attachment	No attachment
6	Green colony	Nil	Nil
7	Yellow colony	Nil	Nil
8	Survival %	100	100

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the rainy season.

Table 11. Final Day of Experiment (15th day) in Water parameter.

S.No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Water Salinity	31±1.0 ppt	31±1.0 ppt
2	Water Tem	29±2^{o} C	29±2^{o} C
3	Water pH	7.9±2	8.1±2
4	Alkalinity	139.0mg/L	139.0mg/L
5	Hardness	4860 mg/L	4860 mg/L
6	Total Ammonia	0.20 ppm	0.06 ppm
7	Water colour	Transparent	Greyish

Protective effect of a probiotic (Lactobacillus species) against a luminous pathogenic bacterium (*Vibrio harveyi*) in *Penaeus vannamei* larval production during the rainy season.

Table 12. Final Day of Experiment (15th day) in Animal Parameter.

S.No	Parameters	A (No Treatment or Control)	B (Probiotic)
1	Total Length	12±1 mm	14±1 mm
2	Rostral spines	4.5	5.8
3	Animal colour	Slightly Pigmented	Pigmented
4	Size variation	7.8± 0.5%	≤ 2.0%
5	Appendages	Huge attachment	Very mild attachment
6	Green colony	TNTC	Nil
7	Yellow colony	TNTC	50±9 CFU
8	Survival %	53±2	98±3

RESULTS AND DISCUSSION

Lower temperatures can slow the metabolic activity of both shrimp and bacteria. However, environmental stress may make shrimp post-larvae (naiads) more susceptible to opportunistic bacterial infections. Previous studies have demonstrated that the application of probiotics, particularly *Lactobacillus* spp., effectively reduces the abundance of luminous bacteria under such conditions, thereby improving shrimp survival and growth compared to untreated controls.

Growth performance and health parameters were recorded from the first week of shrimp culture. During the study period (September–June), no significant differences were observed in the water quality parameters between the control and probiotic-treated groups during the first week of the experiment (Table 1). Similarly, by the end of the second week of culture, water salinity, temperature, and hardness remained stable in both groups. However, slight variations were observed in water pH, water colour, and total ammonia concentration.

Among the biological parameters, the mean total length of shrimp was 10.3 ± 1.0 mm in the control group, whereas the probiotic-treated group exhibited a significantly higher mean total length of 12.3 ± 1.0 mm. The average number of rostral teeth also showed a slight increase in the probiotic-treated group (4.8) compared with the control group (4.2). Shrimp in the probiotic-treated group exhibited better body pigmentation than those in the control group. Furthermore, differences were observed in size distribution, green and yellow bacterial colony counts, and survival rate, with the probiotic-treated group showing superior performance compared to the control group.

Huge attachment seen in control group whereas slight atta chment of bacteria set up in treated group. The Result was depicted in the table 1,2,3,4,5,6,7,8,9,10,11 and 12. Elevated summer temperatures frequently promote swift bacterial proliferation, encompassing luminous bacteria. Adding Lactobacillus has demonstrated improved survival rates and better microbial balance, decreasing incidents of harmful bacteria. Under experimental conditions, the group administered Lactobacillus showed a notable decrease in harmful bacteria and an increase in larval survival. During the summer months (March-June), there is no change observed in table 2.2. In the second week, water temperature, salinity, and hardness were comparable in both the control and experimental tanks. The pH of water in the experimental tank was higher (8.2±1), while in the control tank it was lower (8.0±1). The total Ammonia was greater in the experimental group (0.05ppm) compared to the control tank (0.24ppm). The water in the control group is clear, while it appears greyish in the experimental group. Total length (11.3±1 mm) was observed in the control group, and (14.2±1 mm) was recorded in the experiment. In comparison to a treated tank, the rostral spine value is minimal. The probiotic group exhibits a slightly higher level of pigmentation in animal colour compared to the control group. Differences in size, green and yellow colonies, and variations between the control groups and the treated group. The group that received treatment had fewer bacteria. The survival rate in the experimental group (94±2) is significantly greater than that of the control group (64±2), as shown in table 8. Variable water conditions (such as salinity, pH, and temperature) that can affect the growth of luminous bacteria and the efficiency of probiotics. Probiotics show promise in stabilizing gut micro biota and enhancing larval resilience even with environmental shifts during the rainy season. Proper use of probiotics during wet seasons can reduce disease outbreaks triggered by luminous and various pathogenic bacteria.

During the rainy season (July-September), there are no alterations in the control and probiotic groups during the initial week of the experiment. Changes noted in water salinity, temperature, salinity, and hardness in both the control and treated groups are presented in table 3. The pH of water in the experimental tank was higher (8.1±2) compared to the control tank, which had a lower pH (7.9±2). The overall Ammonia level was greater in the experimental group (0.06ppm) than in the control tank (0.20ppm). In the experimental group, the water has a grey tint, whereas in the control group, it appears clear. A total length of (12±1 mm) was noted in the control group and (14±1 mm) in the experimental group. The rostral spine measurement is lower in the control tank compared to the treated one. In comparison to the control group, the probiotic group displays slightly more pigmented animal coloration. The sizes of the animals vary slightly from the control group; there are considerably more bacteria on their appendages than in the experimental group. The experimental tank lacks green colonies, whereas the control tank has both yellow and green colonies, and some bacterial differences exist between the treated and control groups. The survival rate is significantly higher in the treated group (98±3) compared to the control group (53±2) as shown in table 12. An increased virulence of pathogens raises concerns about the potential transfer of antibiotic resistance to human pathogens. The use of probiotics offers a solution to these challenges. By introducing selected bacterial species to hatchery tanks, the microbial composition can be modified to displace harmful normal bacteria. This approach allows for the management of luminous bacteria (*Vibrio harveyi*) without relying on the antibiotic Erythromycin. The presence of luminous bacterial strains was reduced in hatchery tanks where specific probiotic strains of Lactobacillus were introduced. In shrimp hatcheries along the Chennai coast, the luminous bacterial disease identified in tanks was managed more effectively using probiotics (Lactobacillus species) compared to the antibiotic (Erythromycin). Following the application of the probiotics, the health and survival rate of shrimp larvae increased to 80%. Before the release of the Pérez Farfante and Kensley monograph in 1997, there was minimal published research on the genetic comparison of Penaeid shrimp species. Subsequently, 6 pertinent papers were released that address the matter (Baldwin *et al.,* 1998, Chu *et al.,* 2003, Lavery *et al*., 2004, Maggioni *et al.,* 2001, Wang *et al.,* 2004, Voloch *et al.,* 2005).

CONCLUSION

Probiotics typically have beneficial impacts on luminous bacteria in the post larval phase, enhancing survival rates and quality of water. Nonetheless, their effectiveness might vary throughout different seasons because of environmental shifts. Higher water temperatures generally boost the antimicrobial effectiveness of probiotics, while cooler conditions might necessitate modified probiotic approaches for the best outcomes. Lactobacillus bacteria are effective for the prophylactic control of *Vibrio harveyi* (gill choke disease) in *Penaeus vannamei* larvae. The conclusion is that incorporating specific *Lactobacillus* strains into the shrimp's diet or rearing water can significantly enhance their survival rates and disease resistance, serving as a safe, effective alternative to antibiotics in aquaculture. Increased Survival: Probiotic-treated shrimp showed significantly higher survival rates compared to control groups after being challenged with *V. harveyi*. Disease Resistance: The probiont improved the shrimp's immunity by modifying the gut microbiota, enhancing immune and digestive enzyme activity, and actively reducing the *Vibrio* population in the hemolymph and digestive tract. Improved Health and Growth: Probiotic supplementation led to better growth performance, improved feed conversion ratios, enhanced gut morphology, and overall better health status. Mechanism: The *Lactobacillus* strains produce antimicrobial compounds and competitively exclude the pathogen, effectively resisting the *V. harveyi* infection when challenged.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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