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Research Article

A STUDY TO FIND OUT THE PROPHYLITIC EFFECT OF LACTOBACILLUS SPECIES AGAINST GILL CHOKE DISEASE CAUSED BY *LEUCOTHRIX MUCOR* (FILAMENTOUS BACTERIUM) IN *PENAEUS VANNAMEI* DURING LARVAL STAGES

Sri Uma Shankari A N, Bharathi A, Shanthi R, Padmavathy S, Safiq Rahman M and Arokiya Doss S

PG & Research Department of Zoology, Sir Theagaraya College, Chennai, Tamil Nadu, India

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ABSTRACT

India is one of the world's leading shrimp-producing countries. The shrimp hatchery industry also developed in parallel to this. Seed production is not in a constant manner 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 increases in the virulence of pathogens and, furthermore, is a cause for concern in promoting 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 filamentous bacteria (*Leucothrix mucor*) is controlled during this experiment. In this study, it reveals that the probiotic not only controls the pathogenic bacteria (*Leucothrix mucor*), but also it improves the water quality and animal health.

Keywords: *Penaeus vannamei*, Lactobacillus species, *Leucothrix mucor*, Animal health, Probiotic.

INTRODUCTION

Shrimp farming is a rapidly growing sector within global aquaculture, focused on the marine or freshwater shrimp for commercial purpose (Tacon, 1990). Aquaculture produces around 360 species, with the Pacific white shrimp (*Litopenaeus vannamei*) being one of the most widely cultivated species globally. About 70% of all penaeid shrimp cultivated globally are found in underdeveloped nations (FAO, 2010) (Lin and Chen, 2003; FAO, 2010). Probiotic play an important role in growth, total length of penaeid shrimp (Biedenbach *et al*.,1989). Shrimp farming has faced challenge like diseases outbreak, soil and water degradation and impact on sensitive coastal ecosystems. The most commonly farmed species are *Litopenaeus vannamei* (Pecific white shrimp) and *Penaeus monodon* (gaint tiger prawn), valued for their fast growth, adaptability, and high market value. Shrimp farming is widely practiced in tropical and sub-tropical region, especially in countries like India, Vietnam, Thailand, Ecuador, and Indonesia (FAO 2017). The application of probiotics, particularly Lactobacillus species, has garnered attention in aquaculture for their potential to mitigate diseases in shrimp, including those affecting the gills of *Litopenaeus vannamei* post larvae by increasing the immune response (Vine *et al*., 2006). Filamentous bacteria a long thread like structure can be the part of the natural microbial community or occasionally become problematic. They may reduce water flow, clog gills, interfere with feeding, or cause opportunistic disease under stressed condition (Lowell HaysCourtesy-2020).

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

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

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 analyzed before the experiment (Van Wyk *et al*., 1999). Filamentous bacterial (*Leucothrix mucor*) infected tanks were identified and samples were collected from the neighboring shrimp hatchery. The samples were examined under the microscope in100X. Affected animals were having filamentous 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 filamentous bacteria (*Leucothrix mucor*) 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.

Table 1. 1st week of Experiment. Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the winter season (Water parameter).

S. No	Parameters	A (Control)	B (Probiotic)
1	Water Salinity	30±1.0 ppt	30±1.0 ppt
2	Water Tem	27±1o C	27±1o C
3	Water pH	7.4±1	7.4±1
4	Alkalinity	138.0mg/L	138.0mg/L
5	Hardness	4250 mg/L	4250 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Water colour	Greenish	Greenish

Table 2. 1st week of Experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the winter season (Animal parameter).

1	Total Length	5.0±1 mm	5.0±1 mm
2	Rostral spines	2.5	2.5
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	72±5	72±5
8	Survival %	100	100

Table 3. 2nd week of experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the winter season. (Water parameter).

S No	Parameters	A (No Treatment)	B (Probiotic)
1	Water Salinity	27±1.0 ppt	27±1.0 ppt
2	Water Tem	26.5±1° C	26.5±1° C
3	Water pH	7.9±1	8.1±1
4	Alkalinity	138.0mg/L	138.0mg/L
5	Hardness	4250 mg/L	4250 mg/L
6	Total Ammonia	0.60 ppm	0.06 ppm
7	Water colour	Transparent	Grayish

Table No 4. 2nd week of experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the winter season (Animal parameter).

S No	Parameters	A (No Treatment)	B (Probiotic)
1	Total Length	10.2±1 mm	12.1±1 mm
2	Rostral spines	4.0 Nos	4.5 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	25±5 CFU
7	Yellow colony	TNTC	200±8 CFU
8	Survival %	67±2	89±3

TNTC – Too numerous to count

Table 5. 1st week of Experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the summer season. (water parameter).

S No	Parameters	A (No Treatment)	B (Probiotic)
1	Water Salinity	30±1.0 ppt	30±1.0 ppt
2	Water Tem	31±1° C	31±1° C
3	Water pH	7.8±1	7.8±1
4	Alkalinity	138.0mg/L	138.0mg/L
5	Hardness	4850 mg/L	4850 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Water colour	Greenish	Greenish

Table 6. 1st week of Experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the summer season (Animal parameter).

S No	Parameters	A (No Treatment)	B (Probiotic)
1	Total Length	6.0±1 mm	6.0±1 mm
2	Rostral spines	2.5	2.5
3	Animal colour	Transparent	Transparent
4	Size variation	2.0%	2.0%
5	Appendages	No attachment	No attachment
6	Green colony	72±5	72±5

7	Yellow colony	Nil	Nil
8	Survival %	100	100

Table 7. 2nd week of Experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the summer season (water parameter).

S No	Parameters	A (No Treatment)	B (Probiotic)
1	Water Salinity	30±1.0 ppt	30±1.0 ppt
2	Water Tem	29±1° C	29±1° C
3	Water pH	7.9±1	8.1±1
4	Alkalinity	138.0mg/L	138.0mg/L
5	Hardness	4850 mg/L	4850 mg/L
6	Total Ammonia	0.20 ppm	0.06 ppm
7	Water colour	Transparent	Grayish

Table 8. 2nd week of Experiment (15th day) Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the winter season (Animal parameter).

S No	Parameters	A (No Treatment)	B (Probiotic)
1	Total Length	11.2±1 mm	14.1±1 mm
2	Rostral spines	4.5 Nos	5.5 Nos
3	Animal colour	Slightly Pigmented	Pigmented
4	Size variation	7.8± 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 %	65±2	92±2

TNTC – Too numerous to count

Table 9. 1st week of Experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the rainy season (water parameter)

S No	Parameters	A (No Treatment)	B (Probiotic)
1	Water Salinity	27±1.0 ppt	27±1.0 ppt
2	Water Tem	28±5° C	28±5° C
3	Water pH	8±0.1	8±0.1
4	Alkalinity	136.0mg/L	136.0mg/L
5	Hardness	5250 mg/L	5250 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Water colour	Transparent	Transparent

Table 10. 1st week of Experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the rainy season(animal parameter).

S No	Parameters	A (No Treatment)	B (Probiotic)
1	Total Length	7±1 mm	7±1 mm
2	Rostral spines	2.5	2.5

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

Table 11. 2nd of Experiment Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the rainy season (water parameter).

S.No	Parameters	A (control)	B (Probiotic)
1	Water Salinity	30±1.0 ppt	30±1.0 ppt
2	Water Tem	29±1° C	29±1° C
3	Water pH	7.9±1	8.1±1
4	Alkalinity	138.0mg/L	138.0mg/L
5	Hardness	4850 mg/L	4850 mg/L
6	Total Ammonia	0.20 ppm	0.06 ppm
7	Water colour	Transparent	Grayish

Table 12. 2ndof Experiment (15th day) Evaluation of the protective effect of a probiotic (Lactobacillus species) against a filamentous pathogenic bacterium (*Leucothrix mucor*) in *Penaeus vannamei* larval production during the rainy season (animal parameter).

S.No	Parameters	A (control)	B (Probiotic)
1	Total Length	11±1 mm	13±1 mm
2	Rostral spines	4.0 Nos	5.5 Nos
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±8 CFU
8	Survival %	52±2	98±2

TNTC – Too numerous to count

Cooler temperature can slow the metabolism of both shrimp and bacteria, but stress may make larvae more vulnerable to opportunistic infections. Studies indicate that probiotic use especially with lactobacillus continues to lower filamentous bacteria under this condition, maintaining higher survival and growth rates compared to control. From the beginning of the first week of shrimp culture, the values were noted for the estimation of growth and the diseases resistant of gill diseases. Throughout the winter season (Sep-June) there is no variation in water and animal parameters in the first week of experiment in both control and probiotic groups represent in table1. At the end of the Second week of shrimp culture, there is no change in the water salinity, water temperature, salinity and hardness. But there is slight variation in water pH, water colour and total ammonia. In Animal parameter total length is 10.2± 1mm in control group were as 12.1±1mm was noted in probiotic group. Rostral spine shows light variation compare to control group the value ranges from 4.0Nos to 4.5Nos. Animal colour is slightly pigmented in control group but pigmented in probiotic group. Size variation, green colony, yellow colony and survival rate are differed from control group to treated group. Huge attachment seen in control group were as slight attachment of bacteria found in treated group.

High temperatures in summer often favor rapid bacterial growth, including filamentous bacteria Supplementation with Lactobacillus has been show to increase survival rates and enhance the microbial balance, reducing outbreaks of harmful bacteria. In experimental conditions, group treated with Lactobacillus had a significant reduction in pathogenic bacteria and boost in larval survival. In Summer season (March – June) no variation is seen in table 3. Were as in second week Water salinity, water temperature, salinity and hardness of both control and experimental tanks were similar. Water pH was higher in experimental tank (8.1±1) and lower in control tank (7.9±1). The total Ammonia was higher in experimental group (0.06ppm) rather than control tank (0.20ppm). water colour is transparent in control group and grayish in experimental group. Total length (11.2±1 mm) in control and (14.1±1 mm) was noted in the experiment. Compared to a treated tank, the rostral spine value is low. The probiotic group has somewhat more pigmented animal color than the control group. Variations in size, green and yellow colonies, and differences between control groups and treated group. The treated group had a little bacterium. The survival rate is very higher in experimental group (98±2) refer to control group (65±2)

Rainy season: Fluctuating water parameters (e.g., salinity, pH, temperature) which can influence both filamentous bacteria growth and probiotic effectiveness. Probiotic demonstrate potential to stabilize gut microbiota and improve larval resistance despite environmental changes in rainy season. Effective probiotic application during rainy seasons can mitigate diseases out breaks caused by filamentous and other pathogenic bacteria. In rainy season (Jul-Sep) no changes in control and probiotic group in the first week of experiment. Any changes observed in Water salinity, water temperature, salinity and hardness in control and treated group in Table 5-11. Water pH was higher in experimental tank (8.1±1) and lower in control tank (7.9±1). The total Ammonia was higher in experimental group (0.06ppm) rather than control tank (0.20ppm). In the experimental group, the water color is grayish, while in the control group, it is translucent. A total length of (11±1 mm) was recorded in the control group and (13±1 mm) in the experiment Table 12. The rostral spine value is low in control when compared to a treated tank. Compared to the control group, the probiotic group has a little more pigmented animal color. The animals' sizes differ slightly from those of the control group, there are significantly more bacteria attached to their appendages than in the experimental group, there are no green colonies in the experimental tank, but there are yellow colonies and green colonies in the control tank, and there are some bacterial differences between the treated group and the control groups. The survivality is higher in treated group (98±2) in control group (52±2).

CONCLUSION

Probiotics generally exert positive effects against filamentous bacteria during the post larval stage improving survival rates and water quality. However, their efficacy may fluctuate across different seasons due to environmental changes. Warmer water temperatures tend to enhance the antimicrobial activity of probiotics, whereas colder conditions may require adjusted probiotic strategies for optimal result.

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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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