EFFECT OF PROBIOTIC AND SUPPLEMENTED FEED ON GROWTH, SURVIVAL AND DISEASE RESISTANCE OF WHITE SHRIMP LITOPENAEUS VANNAMEI POSTLARVAE

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INTRODUCTION

Globally, aquaculture regarded as the fastest-growing food production technology with high production for several decades (Das et al., 2008; Edwards et al., 2019). In 2016, around 89 per cent of the output originated in Asia, primarily China, which attracts greater attention from global aquaculture production since 62 per cent occurred in China. After China, India takes the second position concerning annual fisheries and aquaculture production. Hence, the country promoting its aquaculture practices for more shellfishes and crustacean’s production. In 2017, fish production is expected to reach 12.60 million metric tonnes, with about 65 % coming from the inland sector and about 50% coming from cultural fisheries (FAO, 2018). Aquaculture is a vital source of food, stock augmentation, employment, and profit for millions of people worldwide (Murillo-Gurrea et al., 2001). Many microbiological pathogens, including Vibrio anguillarum, Vibrio harveyi, Vibrio parahaemolyticus, and Vibrio vulnificus, have been weakened in shrimp farming as a result of infectious disease outbreaks (MartinezPorchas and MartinezCordova, 2012). Because of its excellent survival rate, quick development in intensive culture systems, and disease resistance, the Pacific white shrimp Litopenaeus vannamei is widely farmed across the world. With about 3.8 million metric tonnes generated in 2015, intensive culture of Pacific white shrimp Litopenaeus vannamei represents substantial aquaculture operations in terms of production value and a high-value commodity (FAO, 2016). Bioencapsulation is a technology that is currently being explored for the oral administration of carotenoids, chemothapeutic vaccines, pigments, sterols, vitamins, and vital polysaturated fatty acids (PUFAs) via Artemia nauplii. The larval stages of L. vannamei exhibit complicated trophic alterations during development, particularly for penaeid. The phytoplankton-feeding zoa and carnivorous mysis stages come after the non-feeding naupliar stages. The latter stage is particularly reliant on a consistent supply of live food, which is frequently provided in hatcheries as rotifiers and Artemia nauplii. Artemia is an important live feed used in the production of shrimp larvae. They are continuous filter feeders that eat suspended particles and are not selective. These qualities allow one to increase their nutritional profile by immersing them in a solution rich in nutrients such as docosahexaenoic acid (Sorgeloos et al., 1986). The main role of aquaculturists is to provide organisms suited for the feed size to the initial feeding stage, as well as an acceptable quantity of feed organisms to ensure greater survival and quicker growth (Arulvasu and Munuswamy, 2009). Disease in shrimp cultivation was induced by a variety of biotic and abiotic causes. Farmers mostly use antibiotics as a preventative or therapeutic measure to combat illness (Hoscinifar et al., 2017). Antibiotics not only disrupt the natural flora of shrimp intestinal tracts but also render diseases resistant to medications; hence, probiotics are the most promising alternative to antibiotics (Tripathi and Giri, 2014). Because of the importance of water quality, nutritional absorption, the immune system, and the survival and growth rates of hosts, probiotics play a key role in aquaculture. Bacillus bacterium strains have produced extremely good outcomes in shrimp farming. This bacterium is a non-infectious Gram-positive spore-forming bacteria that has been utilized to ameliorate the health of shrimp (Keysami et al., 2012). Vibrio harveyi is a scintillating species commonly obtained from marine sources, has been identified as harmful to fish and various crustaceans, most notably Peneaus species. (Lavilla-Pitogo et al., 1990). The quest for sustainable, ecologically friendly aquaculture is driving up research into probiotics for marine creatures. The commercial source of probiotic dietary supplementation provided a superior growth, immune-physiology of Litopenaeus vannamei than the indigenous source of Bacillus species (Abdollahi-Arman et al., 2018). Several research have been undertaken to investigate the effect of various feeding regimens and additives on shrimp growth performance and immunological state (Bower et al., 2019). Since probiotics have been shown to promote host organism growth, nutrition, and survival. The purpose of this study was to see how enriched Artemia nauplii with the probiotic bacteria Bacillus megaterium and supplemented diet affected growth, survival, and disease resistance in postlarvae of white shrimp Litopenaeus vannamei. MATERIAL AND METHODS

Experimental animal and Probiotic strain

Litopenaeus vannamei postlarvae were acquired from Royal hatcheries, Chennai, Tamil Nadu, India. The postlarvae were transported in plastic bags containing 5 ppt seawater to the experimental setup kept at our laboratory. Before the trials, postlarvae were cultivated in laboratory tanks filled with aerated seawater at room temperature for 2–3 days. Bacillus megaterium was selected as the probiotic, which was obtained from the Central Institute of Brackishwater Aquaculture, Indian Council of Agricultural Research, Chennai, Tamil Nadu, India. The probiotic strain was grown in Tryptone soy broth (TSB) using a shaking incubator at 30°C for 24 hours. Using a spectrophotometer, the cell densities of the suspensions were calculated at 600nm.
Artemia Cyst Collection and Hatching

Artemia cysts were collected from (100 µm scoop net) Kelambakkam saltpan, Tamil Nadu, India. Cysts were hatched using the standard procedures adopted from previous publications (Sorgeloos et al., 1986). Artemia cyst of one gram was hydrated in freshwater for a period of one hour in a beaker with vigorous aeration. The cysts were collected after one hour and rinsed with tap water and then transferred into the decapsulating solution (4% sodium hypochlorite). After 5 to 10 min, the entire cysts turned to an orange-pink colour indicating decapsulation. The cysts were collected in a 100 µm sieve and rinsed with fresh water to remove all traces of the hypochlorite solution. The decapsulated cysts were transferred to 1l litre seawater (salinity 30 ppt) and vigorously aerated for 24 hours. The hatched nauplii were siphoned out by exposing them to light. The nauplii were washed completely and used for the enrichment trials (Figure 1A - E).

Enrichment Procedure

Enrichment of the second instar nauplii was carried out by the standard procedure of Sorgeloos and Kulasekarapandian (1984). First instar Artemia nauplii appeared after 24 h incubation. Cell suspensions of B. megaterium were enriched after 12 h incubation. The second instar stage Artemia nauplii were segregated from the container through a 120 µm sieve and transferred to a glass beaker at a mass of 200,000 nauplii / L of seawater. The three different concentrations of Bacillus megaterium, 5 × 10^7 cfu / mL (Experiment 1), 5 × 10^8 cfu / mL (Experiment 2) and 5 × 10^9 cfu / mL (Experiment 3). After 6 hours of enrichment period, the Artemia nauplii were picked and rinsed with seawater. Unenriched Artemia nauplii served as control. The Artemia nauplii were reaped from the enrichment glass containers. They were washed rigorously with fresh water and stored for further use. Bacillus strain present in the gut of transparent nauplii was observed under a light microscope (Figure 1F).

Experimental setup

Experiments were accomplished by dividing the Litopenaeus vannamei postlarvae into four groups such as Control, Experiment 1(E1), Experiment 2 (E2) and Experiment 3 (E3). A total count of 180 postlarvae individuals with a weight of 0.58 ± 0.27 mg on average were randomly divided into 12 tanks with 15 individuals per tank placed. Three replicate groups of postlarvae were fed with unenriched and enriched Artemia nauplii.

Preparation of the experimental diet

Pellet feed obtained from Central Institute of Brackishwater Aquaculture, Indian Council of Agricultural Research, Chennai, Tamil Nadu, India. After 24 h culture, the probiotic bacteria were collected by centrifuge at 10,000 rpm for 15 minutes. The cells collected were washed and re-suspended in phosphate-buffered saline (PBS pH 7.4). The prepared suspension containing probiotic bacteria were dried and stored at 4°C. Experimental diets E1, E2 and E3 supplemented with Bacillus megaterium were 5 × 10^7 cfu, 5 × 10^8 cfu and 5 × 10^9 cfu cells and supplement (without probiotic) were prepared once every 15 days.

Table 1 Growth and survival of Litopenaeus vannamei postlarvae fed with enriched Artemia nauplii for 30 days

| Experiments | Initial length (mm) | Final length (mm) | Initial weight (mg) | Final weight (mg) | SGR (%) | Survival rate (%) |
|-------------|---------------------|------------------|---------------------|-------------------|---------|-------------------|
| Control     | 5.54 ± 1.38         | 20.98 ±102       | 0.56 ± 0.27         | 19.00 ± 12.24     | 12.44 ± 1.70 | 71.11 ± 3.85     |
| E1          | 5.36 ± 1.12         | 23.68 ±52        | 0.60 ± 0.27         | 24.80 ± 1.30      | 13.12 ± 1.59 | 84.44 ± 3.85     |
| E2          | 5.28 ± 1.05         | 25.22±14         | 0.52 ± 0.29         | 27.00±1.20        | 14.00 ± 1.83 | 93.33 ± 0.00     |
| E3          | 5.76 ±1.26          | 21.14±086        | 0.64 ± 0.27         | 22.10±1.04        | 12.49 ± 1.54 | 80.00 ± 6.66     |

Table 1 represent means ± standard deviation of the samples with different superscript letters that are significantly different from each other (p < 0.05). Mean without letter are not significantly different. Control: unenriched Artemia nauplii. E1: Artemia nauplii enriched with 5 × 10^7 cfu cells. E2: Artemia nauplii enriched with 5 × 10^8 cfu cells and E3: Artemia nauplii enriched with 5 × 10^9 cfu cells and SGR: specific growth rate.

Similarly, after 15-days completion all the growth indicators measured in the experiment were significantly different (p<0.05). The effects of probiotic B. megaterium sprayed pellet feed in L. vannamei postlarvae are presented in Table 2. The present investigation is the consecutive process of the previous experiment.
Since the same PL utilized in this experiment, the initial average length and weight showed a substantial variation between the groups ($p<0.05$). The final length of shrimp PL were 31.32, 33.01, 37.30 and 32.00 mm in control, E1, E2 and E3. The data were statistically significant ($p<0.05$) between experimental groups at various doses compared to the control group. Similarly, weight gain in L. vannamei post larvae fed with probiotic supplemented feed varied significantly ($p<0.05$) compared to the post larvae fed with pellet feed. The post larvae of L. vannamei fed with probiotic supplemented feed attained maximum weight in E2 followed by E3 and E1 were 48.10, 32.41 and 31.52 mg respectively, whereas control showed comparatively lower weight of 25.11 mg. The highest specific growth rate found in E2 group followed by E1 and E3 were 3.31, 1.82 and 1.70 % respectively. In comparison to the control and other groups, the E2 probiotic supplemented group demonstrated a significant difference ($p<0.05$). Compared to the group provided control feed and the probiotic-supplemented feed had a greater survival rate. In probiotic supplemented feed survival rate were ranged from 95.00, 100.00 and 90.00% in E1, E2 and E3 respectively. The highest survival rate of 100% was recorded in shrimp’s PL fed with probiotic supplemented feed while the control feed exhibited the survival rate of 80%.

Biochemical parameters of shrimp PL

The results obtained with biochemical constituents such as protein, lipid and carbohydrate in Litopenaeus vannamei postlarvae enriched Artemia nauplii with various concentration of probiotics are given in Figure 2A. Protein content in postlarvae of E1 (5 × 10^7 cfu), E2 (5 × 10^8 cfu) and E3 (5 × 10^9 cfu) of probiotic were observed to be 57.40 mg/g, 63.40 mg/g and 56.30 mg/g respectively. The results showed a moderate difference in enriched groups compared to that of control (55.40 mg/g). The total lipid content of post larva in experimental groups such as E1, E2 and E3 were observed to be 9.20, 11.50 and 9.90 mg/g respectively. However, lipid content was moderately increased in the E2 group than that of the control and other experimental group. The carbohydrate content in postlarvae fed with enriched Artemia in E1, E2 and E3 concentrations was recorded as 12.78, 16.32 and 12mg/g. However, the carbohydrate content was less in the unenriched group. Maximum carbohydrate content was recorded in E2 (5 × 10^7 cfu) of enriched Artemia fed to postlarvae followed by the other concentration, such as E1 (5 × 10^7 cfu) and E3 (5 × 10^9 cfu) compared to unenriched Artemia fed postlarvae group. Among all groups, the E2 is statistically significant group ($p<0.05$).

Table 2 Growth and survival of Litopenaeus vannamei postlarvae fed with probiotic supplemented feed for 15 days

| Experiments | Initial length (mm) | Final length (mm) | Initial weight (mg) | Final weight (mg) | SGR (%) | Survival rate (%) |
|-------------|---------------------|-------------------|---------------------|-------------------|---------|-------------------|
| Control     | 20.98 ± 1.02        | 31.32 ± 0.23      | 19.00 ± 1.22        | 25.11 ± 0.01      | 1.13 ± 0.21 | 80.00 ± 3.00      |
| E1          | 23.68 ± 1.52        | 33.01 ± 0.26      | 24.80 ± 1.30        | 31.52 ± 0.04      | 1.82 ± 0.65 | 95.00 ± 3.85      |
| E2          | 25.22 ± 1.14        | 37.30 ± 0.28      | 27.00 ± 1.20        | 48.10 ± 0.03      | 3.31 ± 0.33 | 100.00 ± 0.00     |
| E3          | 21.14 ± 0.86        | 32.00 ± 0.20      | 22.10 ± 1.04        | 32.41 ± 0.05      | 1.70 ± 0.37 | 90.00 ± 0.00      |

Values represent the means ± standard deviation of the samples with different superscript letters are significantly different from each other ($p<0.05$). Means without letters do not differ substantially. Control: unenriched Artemia nauplii, E1: Artemia nauplii enriched with 5 × 10^7 cfu cells, E2: Artemia nauplii enriched with 5 × 10^8 cfu cells and E3: Artemia nauplii enriched with 5 × 10^9 cfu cells and SOR: specific growth rate.

DISCUSSION

Probiotics were used as a growth regulator for aquaculture in order to improve the health of host against diseases. Many studies have shown the advantages of probiotics for aquatic animals, including how they can promote development, improve feed digestion, boost immunological responses, and regulate water quality (Balcacza et al., 2006; Suzer et al., 2008). In this present study we determined the growth, survival and biochemical of Pacific white shrimp L. vannamei PL fed with various ranges of B. megaterium. Probiotics are increasingly being given to aquaculture animals in live form. Minimizing health issues in the host animal, boosting their immunity, and lowering the pathogen in aquaculture are all regarded as beneficial practices. Additionally, instar-II unenriched Artemia nauplii have lower energy levels due to nutrient deficiencies. B. megaterium supplementation replenishes the energy and nutrients necessary for PL in experimental groups (Visudthiphol et al., 2018). Additionally, enriching Artemia with other high-HUFA substances, including fish oil, has been demonstrated to improve shrimp development performance (Immanuel, 2001).
According to the results of the current study, varying concentrations of Bacillus megaterium have positive impacts on PL growth as measured by length, weight, SGR, and survival of enrichment groups. Arulvasu et al., (2012) had previously shown that specific growth and survival rate was improved by feeding enriched Artemia nauplii to fish fry. Pocelia latipinnata. Analyzing the growth results of experimental groups, B. megaterium restored growth of PL fed with enriched Artemia was higher than that of the non-enriched group. According to earlier research, crustaceans provided probiotic-treated feed grew more quickly than those supplied untreated feed (Zarne-Nejad et al., 2006). In this study, different concentrations of probiotic B. megaterium enriched with Artemia nauplii administered to post larvae V. lamsani led to a significant rise (P<0.05) of length, weight, SGR and survival rates. Comparable results have been revealed in M. rosenbergii fed with bio-encapsulated Lactobacillus reuteri, L. sporegenes and L. acidophilus (Venkat et al., 2004).

The current study demonstrates that shrimp PL fed with B. megaterium enriched feed saw increased levels of growth and survival, with improved growth performances seen compared to controls. Similar outcomes were shown in shrimp fed diets containing B. subtilis L10 and G1, which had greater survival rates of 100 and 95.5%, respectively, than the control, which had a survival rate of 86.5% (Zokaeifar et al., 2012). In the current study, post larvae survival significantly increased, which may be attributable to Bacillus’ capacity to outcompete other hazardous bacteria. In this study, E1, E2, and E3 supplemented groups outperformed controls in terms of growth and survival rate. Similar outcomes were attained when Tiliaeph Orcehromis niloticus was supplemented with the probiotic Bacillus coagulans (Wang et al., 2008). The biochemical assessment of post larvae V. lamsani fed with Artemia nauplii enriched with B. megaterium of three different concentration shows maximum level of protein in all experimental groups than control. The results suggest that E2 group shows significant changes when comparing with latter. Similar to this, Saad et al. (2009) revealed that M. rosenbergii PL’s biochemical proximate composition was greatly improved by the commercial probiotic. The findings of the current study also demonstrated that V. lamsani’s body composition is dramatically impacted by the administration of probiotic B. megaterium supplemented feed. Based on our findings, whiteleg shrimp V. lamsani post larvae fed dietary probiotic Bacillus had higher level of protein, lipid and carbohydrate content.

A variety of infections can be inhibited by probiotics. In order to research the suppression of Vibrio strains, Decamp et al., (2008) employed a commercial probiotic product that is a combination of particular Bacillus strains; the results revealed that probiotics were able to prevent the growth of Vibrio, improving shrimp larvae survival rates. A similar outcome was obtained when B. subtilis inhibited Vibrio harveyi and Vibrio damsela (Vasheeharan and Ramasamy, 2003). Similar results obtained when Vibrio harveyi pathogen challenged against the post larvae Litopenaeus vannamei fed with Artemia nauplii enriched with three different probiotic concentrations such as E1, E2 and E3 significantly improved the immunity and survival. Additionally, when added to diets, certain Bacillus species have been observed to boost shrimp growth (Dukar and Goher, 2004). The stress tolerance, survival rate, immunological responses, and disease resistance of aquatic animals were all improved by the addition of Bacillus probiotics. Additionally, B. licheniformis supplementation increased resistance to V. parahaemolyticus infection (Gao et al., 2018). (Gao et al., 2018). Similar results obtained when V. harveyi injected to the post larvae V. lamsani fed with probiotic supplemented feed showed a significantly higher level (P<0.05) of survival rate than control group. Higher survival of shrimp fed probiotic-supplemented feed may be attributable to the host’s immunological response to the probiotics. In our study, bioencapsulation and supplementation of a B. megaterium was shown to have positive effects on growth indices of V. lamsani PL. Among the experimental groups, PL fed with probiotic in E2 had improved weight, SGR and survival rate of enriched groups, showing significantly higher level of protein in all experimental groups than control, which had a survival rate of 86.5% (Zokaeifar et al., 2012). In the current study, post larvae survival significantly increased, which may be attributable to Bacillus’ capacity to outcompete other hazardous bacteria. In this study, E1, E2, and E3 supplemented groups outperformed controls in terms of growth and survival rate. Similar outcomes were attained when Tiliaeph Orcehromis niloticus was supplemented with the probiotic Bacillus coagulans (Wang et al., 2008). The biochemical assessment of post larvae V. lamsani fed with Artemia nauplii enriched with B. megaterium of three different concentration shows maximum level of protein in all experimental groups than control. The results suggest that E2 group shows significant changes when comparing with latter. Similar to this, Saad et al. (2009) revealed that M. rosenbergii PL’s biochemical proximate composition was greatly improved by the commercial probiotic. The findings of the current study also demonstrated that V. lamsani’s body composition is dramatically impacted by the administration of probiotic B. megaterium supplemented feed. Based on our findings, whiteleg shrimp V. lamsani post larvae fed dietary probiotic Bacillus had higher level of protein, lipid and carbohydrate content.

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CONCLUSION

The potential probiotic Bacillus megaterium enriched Artemia nauplii and supplemented diet enhance the growth, biochemical composition and disease resistance of V. lamsani postlarvae. Therefore, our study suggests that Bacillus megaterium can be utilised as a feed enhancer for enriching both live and supplemented diets in order to increase white shrimp, Litopenaeus vannamei postlarvae growth and production.

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