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Research Article

Efficacy of Probiotic and Nitrifier Bacterial Consortium for the Enhancement of Litopenaeus Vannamei Aquaculture

Abstract

Shrimp farming is one of the most important aquaculture practices worldwide. In general, the excess feed and faecal matter deposited in the bottom of the pond undergo ammonification and result in excess of ammonia formation in pond water and sediment. In addition, eutrophication in the culture system/pond can cause stress to the animals and ultimately end with microbial diseases. The present simulation study was carried out to isolate the potential beneficial bacterial strains to improve the shrimp micro biota (GIT) and to reduce the ammonia and nitrite toxicity in shrimp grow out ponds on a laboratory scale experiment. The *Lactobacillus* sp AMET1506 obtained from AMET Microbial Culture Collection Centre, showed strongest antibacterial activity against shrimp pathogens. The *Nitrosomonas* sp AMETNM01 and *Nitrobacter* sp AMETNB03 were isolated from shrimp culture pond sediments using Winogradsky Phase I and Phase II medium. A total of 150 shrimps (*Litopenaeus vannamei*) PL (15) were obtained from a commercial shrimp hatchery located in Marakanam, Kanchipuram District, Tamil Nadu, India. After acclimation of the shrimps for seven days, the average weight of the shrimps were divided into three batches in 100 litre glass tanks (70 liter of seawater), each containing 50 post larvae. The Tank 1 was treated with commercial feed and the Tank 2 and 3 were treated through feed supplemented with 10^5 CFU g^{-1} of *Lactobacillus* sp AMET1506 for 40 days. After 30 days of culture, in tank 3 the ammonia and nitrite oxidizing bacterial strains such as, *Nitrosomonas* sp AMETNM01 and *Nitrobacter* sp AMETNB03 strains (each in the range of 10^5 ml^{-1}) were added (only once on the 31st day) and the Ammonia (NH_4^+), Nitrite (NO_2) Nitrate (NO_3) in the all the tank water were analyzed from 31st day up to 40th day. The shrimp survival (%), Individual Weight (wt/pcs) and the microbial load revealed that the tank 3 (Combination of *Lactobacillus* sp AMET1506, *Nitrosomonas* sp AMETNM01 and *Nitrobacter* sp AMETNB03) was found to be superior comparatively in probiotics than other two tanks. The present study suggests that, the use of beneficial bacterial strains in shrimp culture could prevent the aquaculture pond from undergoing eutrophication and control the shrimps from microbial diseases and ultimately enhance the production.

Introduction

Shrimp culture represents an important and economically profitable venture and their production has grown enormously in recent years by intensive and semi-intensive methods of culture. Penaeid shrimps are one of the most important preferred species for culture in artificial impoundments [1]. Approximately more than 5 million metric tons of shrimps are annually produced but the current global demand for both the wild (naive) and farmed shrimps are approximately more than 6.5 million metric tons per annum [2]. To overcome this, many shrimp farms are being created throughout the world to solve this increasing food demands [3]. However, fast development of these shrimp industries and intensive culture of these farms has created various ecological, economic and social issues. During the last few years white spot syndrome virus (WSSV) disease has spread worldwide and caused large scale mortalities and economic loss in shrimp culture particularly in Asia [4]. Due to the continuous outbreak of this WSSV disease in *Penaeus monodon* culture leading to loss of shrimp culture in India the farmers are seriously looking for alternative shrimp species for culture. In 2008, the Coastal Aquaculture Authority of India (CAA) introduced a new shrimp species *Litopenaeus vannamei* as an alternative to Penaeid species in India to culture and export. Since the *Litopenaeus vannamei* exhibits fast growth rate and its culture period is significantly shorter compared to *Penaeus monodon* and it has been selected as best alternative to *Penaeus monodon* in shrimp farming in several countries such as, East, Southeast and South Asia [5].

In general, shrimp ponds are enclosed cultivation systems, subject to periodic water renewal to compensate for volume changes (due to evaporation) and salinity changes (evaporation, precipitation) and to maintain water quality. The excess feed and faecal matter may result in bacterial decomposition of organic matter in the sediment and produce excess of toxic compounds like ammonia. In addition abnormal algal growth (eutrophication) may cause stress to the animal and ultimately end with microbial diseases and high mortality. Moreover, the effluent from shrimp ponds is often a water quality hazard, due to higher organic loading, very limited research has been carried out on the culture, growth performance and disease management of *L. vannamei.* The present study was carried out to

evaluate the effect of consortium of a *Lactobacillus* sp, *Nitrosomonas* sp and *Nitrobacter* sp in *L. vannamei* shrimp culture at laboratory scale experiments to enhance production and to set as an ecofriendly practises.

Materials and Methods

Isolation and identification of *Lactobacillus* sp

The *Lactobacillus* sp (AMET1506) strain used in this study was isolated from curd sample by dilution plating on de Man, Rogosa and Sharpe (MRS) media (Himedia, India) and it was identified by biochemical examination using Bergey's Manual of Determinative Bacteriology [6]. The strain was potentially chosed for this study due to strongest antagonistic activity against different seafood bacterial pathogens such as, *E.coli, V.cholerae, V.parahaemolyticus, V. harveyi, Salmonella* sp and *Shigella* sp [7].

Mass culture of *Lactobacillus* sp (AMET1506) and preparation of probiotic feed

The strain *Lactobacillus* sp (AMET1506) was grown in MRS broth in a shaking incubator at 30°C for 24 hours. After the incubation period, the cells were harvested by centrifuging at 2000 rpm and the obtained pellet was washed twice with phosphate-buffered saline (pH 7.2) and re-suspended in the same buffer. Then, the absorbance at 600 nm was adjusted to 0.25 ± 0.05 in order to standardize the number of bacteria (10^5 CFU mL-1) by dilution plating method. The commercial shrimp feed (Blanca feed pellets, obtained from CP Aquaculture India Pvt. Ltd) was taken for the supplementation of *Lactobacillus* sp (AMET1506). In order to reach a final concentration (10^5 CFU g-1) the bacterial suspension was slowly sprayed onto the feed for mixing. The amount of *Lactobacillus* sp (AMET1506) in the feed was determined by standard plate count method on MRS agar [8].

Isolation and identification of *Nitrosomonas* sp and *Nitrobacter* sp

The sediment samples were collected from the bottom of shrimp culture pond located at nearby area of Kalpakkam, East Coast of Tamil Nadu, India. The collected sediment sample (10g) was suspended in 90 ml sterile 50% seawater blank. After vigorous shaken, it was serially diluted up to 10^{-6} and the dilutions such as, 10^{-4}, 10^{-5} and 10^{-6} were taken for isolation of *Nitrosomonas* sp using Winogradsky phase I medium (Containing (NH4)2SO4, 2.0 g; K2HPO4, 1.0 g; MgSO4.7H2O, O.5 g; NaCl,2.0 g; FeSO4.7H2O, 0.4 g; CaCO3 0.01, agar 15.0 g; Distilled water 1000 ml) and for *Nitrobacter* sp by Winogradsky phase 2 medium (Containing KNO2 0.1 g; Na2-Co3,1.0 g; NaCl, 0.5 g; FeSO4.7H2O, 0.4 g agar 15.0 g; Distilled water 1000 ml) using pour plate technique. All the plates were incubated at room temperature (27± 2°C) for 7-14 days. After the incubation period morphologically different colonies were selected and sub-cultured in the respective medium plates and they were identified by their morphological characteristics and gram reaction [9-11]. In addition, the *Nitrosomonas* sp strains were confirmed by their ability to accumulate nitrite in broth containing NH4+ as well as *Nitrobacter* sp strains by the accumulation of nitrate in broth containing NO2- [12].

Mass culture of *Nitrosomonas* sp and *Nitrobacter* sp

The potential *Nitrosomonas* sp and *Nitrobacter* sp strains were mass cultured in a 2L fermenter by using sodium nitrite (0.25mg NaNO2/l Winogradsky broth) and ammonium sulphate (5.0mg (NH4)2SO4/l Winogradsky broth) as medium for *Nitrobacter* sp and *Nitrosomonas* sp respectively with proper pH (8), temperature (28°C), agitation (200 rpm) and aeration (at the rate of 0.6 L min"1). The fermentor was covered with a black cloth to protect the culture from light inactivation. When the rate of substrate uptake and product formation declined (indicating the attainment of stationary phase) the culture was harvested by centrifuging at 8000 rpm for 20 minutes at 4°C. The obtained culture filtrate was washed with fresh medium and re suspended in corresponding medium (containing 10 µg mL^{-1} substrate) and it was stored in air tight container at 4°C. Then, the absorbance at 600 nm was adjusted to 0.25 ± 0.05 in order to standardize the number of bacteria (10^5 CFU mL-1) it was subjected to dilute plating method.

Experimental study

A total of 150 *Litopenaeus vannamei* seeds (post larval stage 15, that had been acclimated to a salinity level of 17 ppt and confirmed negative for the White Spot Syndrome Virus (WSSV) and Taura Syndrome Virus (TSV) by the polymerase chain reaction), were obtained from a commercial shrimp hatchery located in Marakanam, Kanchipuram District, Tamil Nadu, India. After acclimation to a minimum period seven days, the average weight of the shrimps were divided into three batches 100 litre glass tanks (70 liter of seawater), each containing 50 post larvae. The Tank 1 was treated with commercial Blanca feed pellets and the Tank 2 and 3 were treated through the Blanca feed pellets supplemented with 10^5 CFU g^{-1} of *Lactobacillus* sp AMET1506 respectively and the experiment was carried for 30 days. During the culture period, shrimp in all groups were fed thrice daily at 9am, 1pm and 5pm with proper aeration and the water temperature was maintained at 28 ± 1°C and the salinity at 17 ppt. After 30 days of culture the average weight and the survival (%) of the shrimp and the microbial load in the culture water and shrimp intestine were recorded [13]. After 30 days of culture, in tank 3 the ammonia and nitrite oxidizing bacterial strains such as, *Nitrosomonas* sp and *Nitrobacter* sp strains (each in the range of 10^5 ml^{-1}) were added at only once on the 31st day. Then the Ammonia (NH_4^+), Nitrite (NO_2) and Nitrate (NO_3) concentration in the water was recorded from 31st day to 40th day. On 40th day the average weight and the survival (%) of the shrimp and the microbial load in the culture water and shrimp intestines were recorded in all the control and experimental tanks.

Water quality analysis

The physico-chemical parameters such as temperature, transparency, dissolved oxygen, pH, nitrite, nitrate, ammonia of water were estimated by following the methods suggested in Golterman and Clymo; Wetzel and Likens; APHA [14-16].

Microbiological analysis

Shrimps and the bottom water samples were taken from from all the control and experimental tanks. Total heterotrophic bacteria (THB), *Lactobacillus* sp and *Vibrio* sp load in the shrimp intestine and culture water was enumerated by growth on Zobell Marine

agar, MRS agar and TCBS agar (Himedia, India) respectively. For isolation of other pathogenic bacteria such as, *E.coli*, *Salmonella* sp, *Shigella* sp and *Listeria* sp MPN technique was followed using EMB agar, SS agar and PALCAM agar (Himedia, India) respectively. Moreover the ammonia and nitrite oxidizing bacterial strains such as, *Nitrosomonas* sp and *Nitrobacter* sp load also recorded on 40th day using Winogradsky phase I and phase II medium [7,12,13].

Results and Discussion

The successful shrimp farming depends upon the maintenance of water quality and the dynamic balance between beneficial probiotic and pathogenic bacteria. Water quality control and microbial community balance are necessary for successful shrimp cultivations [17]. The use of beneficial bacteria (probiotics) to control or inhibit the pathogenic bacteria by competitive processes is a more efficient disease control strategy than administering antibiotics. In this study, the *Lactobacillus* sp (AMET1506) strain was isolated from curd sample by dilution plating on de Man, Rogosa and Sharpe (MRS) media (Himedia, India) the strain was potentially chosen for this study due to its strongest antagonistic activity against different seafood bacterial pathogens such as, *E.coli, V.cholerae, V.parahaemolyticus, Salmonella* sp and *Shigella* sp [7]. Furthermore, the strain *Lactobacillus* sp AMET1506 also has proven its effectiveness against vibrio is in shrimp aquaculture [2].

Moreover, preventing the shrimp culture pond from eutrophication also controls the shrimps from microbial diseases and mortality. In general, the aaccumulation of high level of ammonia is detrimental in aquaculture systems [18]. The most commonly used ammonia "removal" process in aquaculture and wastewater treatment is nitrification. Shan and Obbard [19], reported that reduction of TAN in aquaculture system can be facilitated by providing and maintaining an optimum environment condition for nitrifying bacteria. Nitrification is the aerobic oxidation of ammonia to nitrite followed by the aerobic oxidation of nitrite to nitrate. Nitrification is a two step process in which ammonia is oxidized to nitrite by ammonia oxidizing bacteria (AOB) or ammonia oxidizing archaea (AOA) and nitrite is then oxidized to nitrate by nitrite oxidizing bacteria (NOB). In the present study, morphologically five different *Nitrosomonas* sp strains (AMETNM01 to AMETNM05) and three different *Nitrobacter* sp strains (AMETNB01 to AMETNB03) were isolated from shrimp pond sediments and water samples. From these, the *Nitrosomonas* sp AMETNM01 and *Nitrobacter* sp AMETNB03 strains were chose as potential microbes based on their effectiveness in accumulating nitrite and nitrate in broth.

The use of probiotics in aquaculture might represent a valuable mechanism to increase shrimp growth and survival rate. In general, the gastro intestinal tract (GIT) of the aquatic animal is mainly composed of gram negative bacteria [20]. So, the incorporation of beneficial gram positive (probiotic) bacteria in feed can modify the gastro intestinal tract [21]. In the present study, the potential strain *Lactobacillus* sp (AMET1506) was incorporated in the range of 10^6 CFUg-1 in shrimp feed using standard protocols. The *Lactobacillus* sp (AMET1506) incorporated feeds were fed to the shrimps in the experimental tanks (2 and 3) and the control diet was fed to the shrimps in control tank. The experiment was carried out for 30 days with zero water exchange. During the culture period the water temperature was maintained at 28 ± 1°C.

On checking the microbial load in the culture water and shrimp intestine in both control and experimental groups on 30th day, the higher total heterotrophic bacterial count was observed in shrimp intestine ($2.5 \pm 0.2 \times 10^7$) and culture water ($3.8 \pm 0.2 \times 10^7$) in control groups (tank 1) fed with unsupplemented control diet. The count decreased in (tank 2) shrimp intestine ($1.5 \pm 0.2 \times 10^8$) and culture water ($1.7 \pm 0.2 \times 10^8$) and (tank 3) shrimp intestine ($1.8 \pm 0.2 \times 10^8$) and culture water ($1.7 \pm 0.2 \times 10^8$) groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. Higher vibrio load also was observed in shrimp intestine ($0.8 \pm 0.2 \times 10^8$) and culture water ($1.0 \pm 0.2 \times 10^8$) in control groups (tank 1) fed with unsupplemented control diet. However it was slightly decreased in (tank 2) shrimp intestine ($0.1 \pm 0.2 \times 10^8$) and culture water ($0.1 \pm 0.2 \times 10^8$) and (tank 3) shrimp intestine ($0.2 \pm 0.2 \times 10^8$) and culture water ($0.2 \pm 0.2 \times 10^8$) groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. Similarly, the *Lactobacillus* sp count decreased in the shrimps intestine ($0.3 \pm 0.2 \times 10^8$) and not even a single colony was isolated from the culture water samples in control groups fed with unsupplemented control diet. However, it increased in (tank 2) shrimp intestine ($8.8 \pm 0.33 \times 10^6$) and culture water ($5.1 \pm 0.33 \times 10^6$) and (tank 3) shrimp intestine ($8.5 \pm 0.33 \times 10^6$) and culture water ($5.1 \pm 0.30 \times 10^6$) groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed (Table 1). While assessing other pathogenic microbial load in the shrimp intestine and culture water using MPN technique, the maximum load was observed in the control groups and minimum in the experimental groups, the difference was statistically significant. Not even a single colony of *Nitrosomonas* sp and *Nitrobacter* sp observed in both control and experimental groups on 30th day. Buttner et al. [22], have suggested that maintaining an optimal environment conditions such as water pH between 7 to 9 and

Table 1: Microbial load on shrimp intestine and culture water on 30th day in both control and experimental groups.

Microbial Load	Tank 1 Control		Tank II Experiment		Tank III Experiment	
	Shrimp Intestine	Culture Water	Shrimp Intestine	Culture Water	Shrimp Intestine	Culture Water
THB CFU/g/ml	2.5 ± 0.2 × 107	3.8 ± 0.2 × 107	1.5 ± 0.2 × 108	1.7 ± 0.2 × 108	1.8 ± 0.2 × 108	1.7 ± 0.2 × 108
Vibrio sp CFU/g/ml	0.8 ± 0.2 × 108	1.0 ± 0.2 × 108	0.1 ± 0.2 × 108	0.1 ± 0.2 × 108	0.2 ± 0.2 × 108	0.2 ± 0.2 × 108
Lactobacillus sp CFU/g/ml	0.3 ± 0.2 × 108	0±0	8.8 ± 0.33 × 106	5.1 ± 0.33 × 106	8.5 ± 0.33 × 106	5.1 ± 0.30 × 106
E.coli MPN/100 g/ml	60±0	50±0	26±0	21±0	26±0	26±0
Salmonella sp MPN/100 g/ml	12±0	9±0	-	-	-	-
Shigella sp MPN/100 g/ml	9±0	7±0	-	-	-	-
Listeria sp MPN/100 g/ml	7±0	6±0	-	-	-	-
Nitrosomonas sp MPN/100 g/ml	-	-	-	-	-	-
Nitrobacter sp MPN/100 g/ml	-	-	-	-	-	-

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temperature of about 24 to 30°C in the hatchery, will increase the utilization of TAN by nitrifying bacteria.

Nitrogen applications in excess of ponds assimilatory capacity can lead to deterioration of water quality through the accumulation of toxic nitrogenous compounds such as, ammonia and nitrite with toxicity to shrimp [23]. According to Chen et al. [24], ammonia can increase to more than 0.8 mg.L^{-1} ammonia-N during shrimp larval development in a hatchery, in spite of frequent water exchange. They found that when ammonia-N and nitrite-N increased to 808.4 and 118.1 µg.L^{-1} respectively, survival of PL was 4% in spite of water exchange. In another instance, no PLs survived when ammonia-N increased from 25.3 to 269.1 µg.L^{-1} and NO2-N increased from 0.8 to 78.3 µg.L^{-1} although one third of water was changed. So, in this study after 30 days of culture in tank 3 the ammonia and nitrite oxidizing bacterial strains such as, *Nitrosomonas* sp and *Nitrobacter* sp strains (each in the range of 10^5 ml^{-1}) were added at only once on the 31st day. When checking the microbial load in the culture water and shrimp intestine from both the control and experimental groups on 30th day, the higher total heterotrophic bacterial count was observed in shrimp intestine ($4.3 \pm 0.2 \times 10^6$) and culture water ($5.0 \pm 0.4 \times 10^6$) in control groups (tank 1) fed with unsupplemented control diet, and it was slightly decreased in (tank 2) shrimp intestine ($1.0 \pm 0.2 \times 10^8$) and culture water ($1.3 \pm 0.2 \times 10^8$) and (tank 3) shrimp intestine ($0.9 \pm 0.2 \times 10^8$) and culture water ($1.1 \pm 0.02 \times 10^8$) groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. Moreover, the higher vibrio load also observed in shrimp intestine ($4.4 \pm 0.2 \times 10^8$) and culture water ($4.5 \pm 0.4 \times 10^8$) in control groups (tank 1) fed with unsupplemented control diet, however it was slightly decreased in (tank 2) shrimp intestine ($6.3 \pm 0.2 \times 10^8$) and culture water ($7.1 \pm 0.2 \times 10^8$) and (tank 3) shrimp intestine ($5.1 \pm 0.2 \times 10^8$) and culture water ($6.1 \pm 0.02 \times 10^8$) groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. Similarly, not even a single colony was isolated from the shrimp intestine and culture water samples in control groups fed with unsupplemented control diet, but it was increased in (tank 2) shrimp intestine ($0.3 \pm 0.33 \times 10^6$) and culture water ($1.1 \pm 0.33 \times 10^6$) and (tank 3) shrimp intestine ($0.5 \pm 0.33 \times 10^6$) and culture water ($2.1 \pm 0.2 \times 10^6$) groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed.

When assessing other pathogenic microbial load in the shrimp intestine and culture water using MPN technique, the maximum load was observed in the control groups and minimum in the experimental groups. Not even a single colony of *Nitrosomonas* sp and *Nitrobacter* sp observed in tank 1 and tank 2 but it was increased in (*Nitrosomonas* sp ($0.2 \pm 0.2 \times 10^8$) and Nitrobacter sp ($0.6 \pm 0.2 \times 10^8$)) culture water samples in the tank 3 experimental groups on 40th day (Table 2). After 40 days of culture, the maximum shrimp survival (%) and the mean final weight was observed in tanks 3 were treated with *Lactobacillus* sp (AMET1506) supplemented feed and where the *Nitrosomonas* sp and *Nitrobacter* sp (Figures 1, 2). The higher survival of shrimps fed with probiotic supplemented feed might be related to an immunopotentiation effect of probiotics on the shrimps immune system. It is of interest to note that, the lactic acid bacteria are the main microbes which produce extracellular compounds to stimulate the nonspecific immune response in vertebrates [25,26]. Checking the Ammonia (NH_4^+), Nitrite (NO_2) and Nitrate (NO_3) in all the tank water from 31st day to 40th day the ammonia and nitrite concentration decreased and the nitrate concentration increased in tank 3 where the *Nitrosomonas* sp and *Nitrobacter* sp were inoculated, its Confirming their nitrification roles inside the water(Figures 3-5). Padmavathi et al. [27], also observed similar results, in fish cultures of species *Pangasius sutchi*, *Catla catla* and *Labeo rohita* when treated with probiotics *Nitrosomonas* and *Nitrobacter* species. Maya et al. [28], also reported that, shrimp PLs in tanks treated with immobilized ammonia oxidizing bacteria had significantly ($P<0.05$) higher survival rate (72.44%) and specific growth rate (12.86%) compared to other treatments. Millamena [29], also reported that, maintaining low TAN concentration during experimental period can increase the shrimp growth rate (SGR) of shrimp PLs. In the light of the previous reports and also the results of the present investigation suggest that the consortium of probiotic bacteria and the nitrifiers both contribute to both shrimps survival and reduction of toxic nitrification, morbidity and mortality in *Litopenaeus vannamei* culture systems.

Conclusion

The present study has clearly demonstrated that the use of probiotic and nitrifying bacterial consortium in shrimp culture at laboratory scale experimental conditions increased the shrimp survival and reduced the ammonia and nitrite toxicity. Based on the water quality parameters and shrimp survival (%), it is concluded that the tank 3 (Consortium of *Lactobacillus* sp AMET1506, *Nitrosomonas* sp AMETNM01 and *Nitrobacter* sp AMETNB03) was found to be superior as compared to other two tanks (1 and 2). The work also suggests that, the extrapolation of the present study in fields and the use of these beneficial bacterial strains in shrimp culture will definitely prevent the aquaculture ponds from undergoing eutrophication and

Table 2: microbial load on shrimp intestine and culture water on 40th day in both control and experimental groups.

Microbial Load	Tank 1 Control		Tank Ii Experiment		Tank Iii Experiment	
	Shrimp Intestine	Culture Water	Shrimp Intestine	Culture Water	Shrimp Intestine	Culture Water
THB CFU/g/ml	4.3 ± 0.2 × 106	5.0 ± 0.4 × 106	1.0 ± 0.2 × 108	1.3 ± 0.2 × 108	0.9 ± 0.2 × 108	1.1 ± 0.02 × 108
Vibrio sp CFU/g/ml	4.4 ± 0.2 × 108	4.5 ± 0.4 × 108	6.3 ± 0.2 × 108	7.1 ± 0.2 × 108	5.1 ± 0.2 × 108	6.1 ± 0.02 × 108
Lactobacillus sp CFU/g/ml	0±0	0±0	0.3 ± 0.33 × 106	1.1 ± 0.33 × 106	0.5 ± 0.33 × 106	2.1 ± 0.2 × 106
E.coli MPN/100 g/ml	140±0	110±0	12±0	21±0	9±0	26±0
Salmonella sp MPN/100 g/ml	34±0	30±0	-	-	-	-
Shigella sp MPN/100 g/ml	34±0	40±0	-	-	-	-
Listeria sp MPN/100 g/ml	40±0	50±0	-	-	-	-
Nitrosomonas sp MPN/100 g/ml	-	-	-	-	-	0.2 ± 0.2 × 108
Nitrobacter sp MPN/100 g/ml	-	-	-	-	-	0.6 ± 0.2 × 108

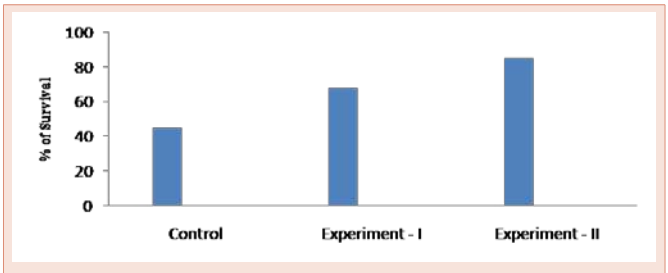

Figure 1: Survival (%) of shrimps on 40th day in the control and experimental tanks.

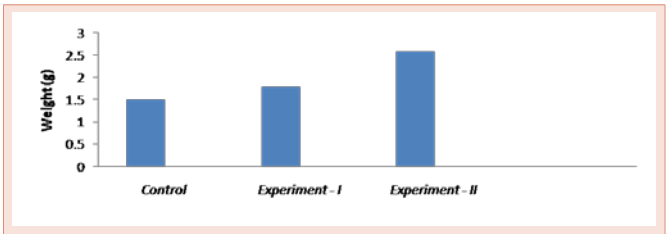

Figure 2: Mean final weight gain of shrimp on 40th day in the control and experimental tanks.

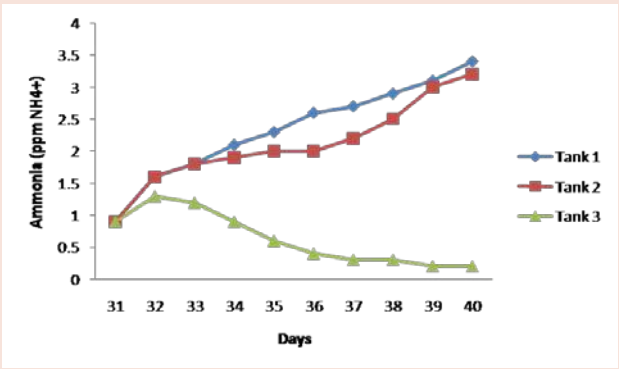

Figure 3: Ammonia (NH4+) concentration in culture water on from 31st to 40th day in control and experimental groups.

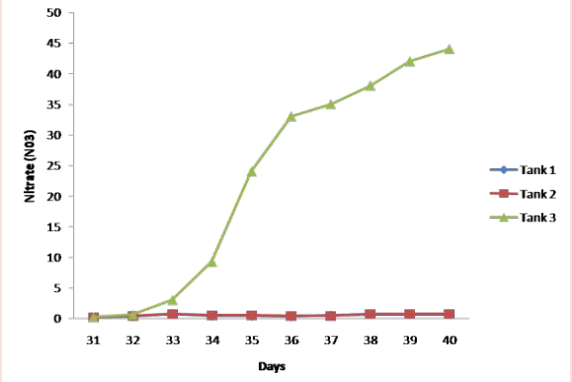

Figure 4: Nitrite (NO2) concentration in culture water on from 31st to 40th day in control and experimental groups.

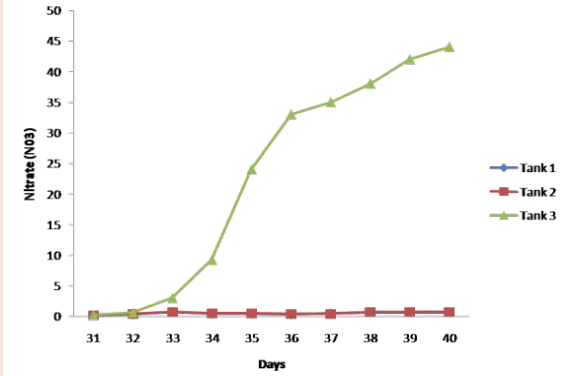

Figure 5: Nitrate (NO3) concentration in culture water on from 31st to 40th day in control and experimental groups.

can control the microbial diseases to the shrimps and enhance their production in an ecofriendly ambience without antibiotics but with probiotics and nitrifiers.

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