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RESEARCH ARTICLE

IMPROVED CULTIVATION OF BLACK TIGER SHRIMP BY THE APPLICATION OF MICROORGANISMS AND NOVEL BIOMOLECULES

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ABSTRACT

Studying the importance of ENVIRON-AC an analytical investigation was performed for the accuracy and acceptability of the product. Regarding the microbial status, it was found that the total bacteria were found $50\pm1.45\times10^9$ cfu/gm, where *Lactobacillus* sp. and *Bacillus* sp. were $27\pm1.45\times10^9$ and $24\pm1.45\times10^9$ cfu/gm respectively. Protease enzyme was found in highest amount (90.22 U/gm). The product also showed antimicrobial activity against *Vibrio harveyi* MTCC No. 7954. The amount of total ammonia, total nitrate and total nitrite in the pond water were 0.324±0.004, 0.020±0.006 and 0.016±0.0007 ppm respectively and which were below the safe level by the application of ENVIRON-AC. The oxygen level and pH range of the treated pond were 4.2-5.1 mg $L^{-1}$and 6.4-8.9 respectively. Average body weight of the shrimp in treated pond was 33±1.92 gm, which is 37.5% higher than control pond. *Vibrio* sp. was found very less in no. (0.03×10^5 cfu ml^{-1}) and survival rate was 91% in treated pond.

INTRODUCTION

The last decade was the Golden space and pace in the history of World Shrimp Aquaculture, where all segments in shrimp aquaculture industry made records and market forces supported this success. Aquaculture has become an important economic activity in many countries all over the world. Shrimp (or prawn) culture is widespread throughout the tropical world. The black tiger shrimp (*Penaeus monodon*) is the most widely cultured species (Boonyaratpalin, 1998). In the present day all over the world shrimp culture were frequently affected by diseases (Bondad-Reantaso *et al*., 2005), pollution of water and soil (Sansanayuth *et al*., 1996). The wastes in hatcheries or aquaculture farms can be categorized as: (i) residual food and feacal matter; (ii) residues of biocides and biostats; (iii) metabolic by-products; (iv) fertilizer derived wastes; (v) wastes produced during moulting; and (vi) collapsing algal blooms (Sharma and Scheeno, 1999). Exposure of shrimps to toxins like hydrogen sulphide, ammonia and carbon dioxide lead to stress and ultimately disease (Ravichandran *et al*., 2001).

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At present, environment friendly techniques were used to control disease outbreaks and also maintain good water quality in the culture pond. Among them applications of probiotic organisms is one of the alternative techniques against pathogenic organisms. The most predominant pathogens in aquatic body are the *Vibrio* sp. like *Vibrio harveyi, Vibrio vulnificus, Vibrio alginoliticus, Vibrio splendidus and Vibrio parahaemolyticus*. They are responsible for several types of diseases and mortalities of up to 100% (Karunasagar *et al*., 1994). To recovery from diseases of *Vibrio* sp., antibiotic or chemotherapeutic agents are first choice.

But continuous used of antibiotics or chemotherapeutic agent in aquaculture for the prophylactic treatment of diseases have potential negative consequences, particularly drug resistance arising in microorganisms through adaptation or by genetic exchange (Holmström *et al*., 2003; Le *et al*., 2005). Used of probiotics is gaining popularity as environment-friendly alternatives for antibiotics in improving shrimp health and minimizing disease caused by *Vibrio* sp. (Senok *et al*., 2005; Barman *et al*., 2011). Bioaugmentation as well as the use of probiotics for instance is a better remedy than administering antibiotics in the culture of some aquatic species (Douillet and Langdon, 1994) and is rapidly being accepted as a management strategy to prevent diseases (Gatesoupe, 1999; Corre *et al*., 2000).

Bioaugmentation has been reported as effective in eliminating waste digestion and sludge clean-up as well (Walker and Clymo, 1996). It also improves water quality by enhancing the mineralization process and reducing the accumulation of organic wastes (Shariff, 2001). King (1986) reported that when a biofilter containing chemolithoautotrophic bacteria was used, the ammonia level decreased from pond effluents; and the use of bacterial consortia in aquaculture ponds accelerated nitrification and rapid decomposition of organic solids from the pond bottom (Ehrlich *et al*., 1988). Yang *et al*. (2011) and Kim *et al*. (2005) reported that aerobic heterotrophic *Bacillus* sp. is a good nitrogen removal bacterium. In the year 2012, Zheng *et al.*, suggested *Marinobacter* strain F6 play vital role in the nitrogen cycle of marine environments for nitrogen removal of high-salinity marine culture wastewater. The commertial probiotic product contains not only different types of probiotic bacteria but also different types of enzymes, Vitamins etc.

Matias *et al.,* (2002) mentioned that commercial microbial products could maintain good water and sediment quality, at least in the beginning of the culture period, which in turn enhanced shrimp growth and production. In the year 1995, Boyd reported that the benefits of using commercial bacterial products include the reduction of blue green algal populations thus preventing off-flavor, nitrate, nitrite, ammonia and phosphate levels, increased dissolved oxygen concentrations and promotion of organic matter decomposition. But many manufacturer being distributed their probiotic products contain inappropriate bacterial species and/or unsuitable bacterial densities for aquatic species (Moriarty *et al*., 2005; Balcazar *et al*., 2006). Keeping above points into consideration, the objective of this study was to determine the quality of commercial probiotic products for Black Tiger Shrimp (*Penaeus monodon*) based on two criteria namely, the accuracy information of products and challenge trial condition of Black Tiger Shrimp ponds for bioaugmentation.

MATERIALS AND METHODS

Commercial probiortic product

The study was done in the month of April 2011 to September 2011 for *in-vitro* and *in-vivo* experiments of commercial probiotics, ENVIRON-AC (batch No.-W110004, manufacturing date-May-2011, expiring date-April-2016), manufactured by Biostadt India Ltd., Mumbai, India. The *in-vivo* challenge trial was carried out in "Maa Tara" aqua farm, located on Contai town, (latitude 24° and longitude 87°45') Bay of Bengal region, India. Sample was stored at 4 °C before experiment.

Isolation and enumeration of bacteria in probiotic product

1 gm of commercial probiotic product was diluted in 10 mL sterile distilled water with 0.85% NaCl and well mixed for prepared 10^{-1} dilution factor and following this method up to 10^{-7} dilution factor was made up. Diluted samples were allowed to precipitate the insoluble carrier materials. Clear soup of 100 µL was taken form 10^{-7} dilution and spread on nutrient agar (NA) medium for total bacterial count and different types of selective media to isolate the specific microorganisms. Bacillus medium and Yeast Mould agar media were used for isolation of aerobic *Bacillus* species and yeast, moulds respectively and MRS for anaerobic organisms. The bacterial colony counts were recorded after incubation at 30 °C for 48 h. The organisms were isolated and identified to genus level by examining their morphological characteristics and following the identification tests of Bergey's manual of determinative bacteriology (Holt *et al*., 1994). The experiments were performed in triplicate.

Study of bacterial consortia by estimating population density

The population density of the bacterial consortia present in the tested commercial product was measured spectrophotometrically as optical density at 620 nm (λ max) in different time duration of cultivation (Barman *et al*., 2011).

Effect of pH and temperature on growth of organisms

Effect of different pH and temperature for the growth of probiotic organisms were determined by culturing the organism in different pH (6.5-9.5) and temperature (5-45 °C). The data were recorded at 620 nm after 24 h, incubation at 30 °C.

Quantitative assay of enzymes and vitamin-C

In the current study, 1 gm of tested sample was vigorously mixed with 10 mL of distilled water and the supernatant was used as enzyme source after centrifugation for 10 min at 5000 rpm. Alpha amylase was estimated according to the method of Rick and Stegbauer (1974). The supernatant (0.5 mL) was added to a test tube containing 0.5 mL of 1.0% soluble starch solution. The tubes were incubated at 40 °C for 10 min. Then 1.0 mL of DNS (3, 5-Dinitrosalicylic acid) solution was added to the test tube and then boiled at 100 °C for 5 min. The optical density was spectrophotometrically determined at 540 nm. One enzyme unit (U) was defined as the amount of enzyme that produces 1.0 µM of glucose in 1 min under standard condition. Protease activity was measured according to the modified method of Kembhavi and Kulkami (1993). Briefly, 0.15 mL supernatant was added to a tube containing 0.3 mL of 1% (w/v) casein (dissolved in 20 mM Tris-HCl buffer, pH 7.4) and incubated at 37 °C for 30 min. Subsequently, 0.45 mL of a 10% (w/v) trichloroacetic acid solution (final concentration of 5% (w/v)) was added to stop the proteolysis. One unit of protease was defined as the amount of enzyme that hydrolyses casein to produce equivalent absorbance to 1 µM of tyrosine/min with tyrosine as standard.

Phytase activity was assayed by the method of Gulati *et al*., (2007). Phytase activity was measured in an assay mixture containing 100 µL of sodium phytate (0.5 w/v) prepared in 0.2 M sodium acetate buffer pH 5.5 and 100 µL of supernatant. The reaction was stopped by adding an equal volume (200 µl) of 15% trichloroacetic acid after 30 min of incubation at 50 °C. The liberated phosphate ions were quantified by mixing 100 µL of assay mixture with 900 µL of developing reagent containing 0.76 M H_2SO_4-2.5% ascorbic acid-0.06% ammonium molybdate (3:1:0.1). After 20 min of incubation at 50 °C, absorbance was measured at 820 nm using spectrophotometer. One unit of phytase activity was

determined as the amount of enzyme required to liberate 1 µM of phosphate per min under the assay conditions. Beta-galactosidase activity was measured according to Miller (1972). 0.5 ml supernatant was mixed with 0.5 mL Na-phosphate buffer (0.05 M, pH 6.8) and 1 drop of 0.1% SDS, and incubated at 30 ^{o}C for 2 min. Then 0.2 mL ONPG was added. When a yellow color was developed, reaction was stopped by adding 0.5 mL of 1 M Na_2CO_3. One unit of beta-galactosidase activity was defined as the activity hydrolyzing 1 µM of ONPG per min under assay condition. The concentration of vitamin-C in the tested sample was determined by 2, 4-Dinitrophenylhydrazin (2,4-DNPH) using Roe method (1961). 4 mL of sample was added to 1 mL 2, 4-Dinitrophenylhydrazin and incubated at 37 ^{o}C for 3 h. At the completion of treatment, 5 mL of 85% H_2S0_4 was added drop wise to the tube in an ice bath and allow for 30 min. the colour intensity was measures spectrophotometrically at 520 nm.

Inhibitory activity on shrimp pathogen

Different pathogenic strains from Tryptone Soy Agar (TSA) slant were inoculated into different Tryptone Soy Broth (TSB) and incubated at 30 ºC for 24 h to prepared young culture. The pathogenic strains, *Vibrio harveyi* MTCC No. 7954 and *Vibrio vulnificus* MTCC No. 1145 were diluted 10^{-3} times using sterile Normal Saline Solution (NSS) to reach the concentration of 10^{-6} cfu mL^{-1}. The diluted cultures of pathogenic strains were spread over the Nutrient Agar plates. The young culture from commercial product then poured (0.1 mL) on the different pathogenic bacterial spread plates. After overnight incubation at 30 ºC, the commercial probiotic organisms, which produced a clear inhibitory zone against the pathogenic strains and were, recorded (Ruiz *et al*., 1996).

Enumeration of *Vibrio* sp.

To count the total cfu of *Vibrio* sp. present in the trial pond, the water samples were spreaded over the Thiosulfate-citratr-bile salts-sucrose agar (TCBS, Hi-Media Laboratory, India) medium. After overnight incubation in the BOD incubator at 30 ºC, the cfu were counted.

Experimental design of treated pond

For the design of experiment, probiotic product applied six times in the total culture period as per instruction given by the manufacture. The product used directly due to its granular form. The commercial probiotic product was applied with 50 kg and 25 kg per hector (stocking density 25/m^3) in a alternate was in the pond during the morning times and an interval of 25 to 30 days. CP feed (Charoen Pokhpond aquaculture India Pvt. Ltd., Chennai, India) were used for the shrimp fed. Feed were applied in to four times in a day. The ratio of applied feed sample was 25, 20, 30 and 25% (of body weight) in the morning (5.00 AM), noon (11.00 AM), evening (5.00 PM) and night (10.00 PM) respectively. The floats method was used for the feed application. The sampling was done in the pond weekly in the morning with a cast net. The shrimps were caught and their individual body weight, healthiness, survival rate, moulting, appendages cutting, attachments, animal activity and gill conditions were observed.

Analysis of treated pond water parameter

Water samples were collected in sterile bottles (Borosil) and transported aseptically to the laboratory and processed immediately for analyses of different water parameters (Ammonia, Nitrate, Nitrite, Dissolved Oxygen, Salinity and pH). The estimations were made within 24 h from the sample collection.

Statistical analysis

All the analysis was carried out in triplicate. Duncan Multiple Range Test was determine the significant difference between the bacteriological and physicochemical parameter of treated and control pond water and shrimp. The correlation coefficient values of bacterial counts also calculated through Microsoft Excel package.

RESULTS

Study of bacterial diversity and population

Total plate count of the viable probiotic bacteria (cfu) present in the commercial product was estimated by allowing them to grow on NA medium. Different types of strains with various morphological characteristics were appeared after incubation at 37 °C for 24 h and their counts were recorded as $50\pm1.45\times10^9$ cfu. Thereafter, bacterial counts were also recorded in different selective media. It was noticed that there was the nearly absence of yeast or mould in the product. Only *Bacillus* and *Lactobacillus* species were found in the product. The plate counts on Bacillus medium indicated that $24\pm0.98\times10^9$ *Bacillus* sp. were present in the sample whereas the population of *Lactobacillus* sp. found in MRS medium as $27\pm1.03\times10^9$. The identification of *Bacillus* sp. and *Lactobacillus* sp. were confirmed by evaluating different biochemical characteristic following the Bergey's manual of determinative bacteriology (data not shown here). It was evidently noticed that the commercial product contained only two probiotic bacteria belongs to genus *Bacillus* and *Lactobacillus.*

Effect of cultivation time on bacterial population

The experimental commercial product containing microorganisms were cultivated in nutrient broth at 30 °C with 120 rpm for 35 h (Figure 1).

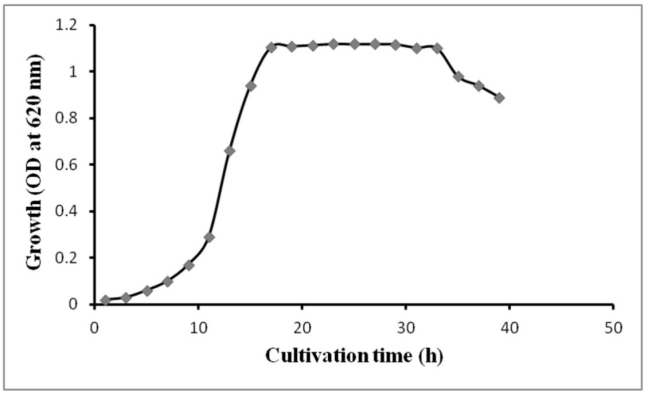

Figure 1. Effect of cultivation time on bacterial population of ENVIRON – AC (Temp. 30°C and RPM 120)

It was found that the Log phase and Stationary phase of bacteria were 5-18 h and 18-35 h respectively. From the experimental result it was observed that the bacteria of ENVIRON-AC having longer logarithmic and stationary phase.

Effect of pH and temperature on growth of bacteria

The product containing probiotic organisms were cultivated in different ranges of pH and temperature. The organisms survived in the pH range of 6.5 to 9.5 and temperature range of 20 to 40 °C. The optimum growth of the product containing organisms was 8.0 pH (OD value 1.326 at OD_{620}) and temperature 30 °C (OD value 1.217 at OD_{620}).

Enzymes and vitamin-C of the product

During study the enzyme profiles in the probiotic product (ENVIRON-AC) were estimated. The presence of a significant amount of amylase (1.050 U/gm), beta-galactosidase (0.525 U/gm), phytase (2.579 U/gm) and protease (90.220 U/gm) were obseved. Besides the amylolytic and proteolytic enzymes, a large number of Vitamin-C (0.378mg/gm) was also prasent in the product (Table 1).

Table 1. Study of enzymatic profile and vitamin-C in ENVIRON-AC

Test	Results
Amylase	1.050 (U/gm)
Beta-galactosidase	0.525 (U/gm)
Phytase	2.579 (U/gm)
Protease	90.220 (U/gm)
Vitamin-C	0.378 (mg/gm)

Ability for *Vibrio* sp. resistance

The *in-vitro* inhibitory effect of the commercial probiotics product against the pathogenic bacteria, *Vibrio harveyi* MTCC No. 7954 and *Vibrio vulnificus* MTCC No. 1145 were performed. The tested product was more effective against fish pathogen *Vibrio harveyi* MTCC No. 7954 (4 mm) than *Vibrio vulnificus* MTCC No. 1145 (3 mm).

In-vivo study of ENVIRON-AC for challenged trial

The dosages applied into the ponds for the challenged trial of ENVIRON-AC as per manufacturer instruction with feed and the controls were only feed. The shrimp and water quality were checked every 10 days interval both of treated and control ponds. The survival rate of Black Tiger Shrimps were 97% in the probiotic treated ponds which were significantly higher ($p<0.05$) than the control ponds (Figure 2) and average body weight were 33±1.92 gm in treated ponds and 24±1.58 gm in control ponds (Figure 3). The results of t-test also showed significant difference between probiotics treated ponds with control ponds. The total microbial load in treated ponds showed the highest 4.6×10^7 cfu ml^{-1} and lowest 1.6×10^7 cfu ml^{-1}, which were significantly higher ($p<0.05$) than the respective values of control ponds (Figure 4). The loads of *Vibrio* sp. were higher in control ponds (1.2×10^5 cfu ml^{-1}) than treated ponds (0.03×10^5 cfu ml^{-1}) (Figure 5). The nitrogenous element like ammonia, nitrite and nitrate were very low in treated ponds than control ponds (Table 2).

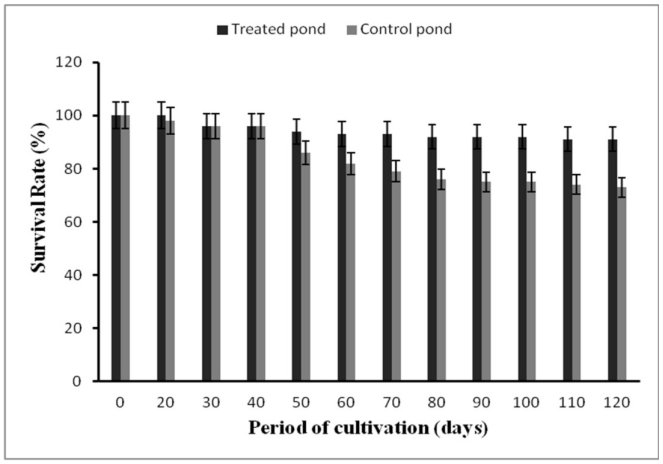

Figure 2. Effect of ENVIRON-AC on survivability rate of *P. monodon*

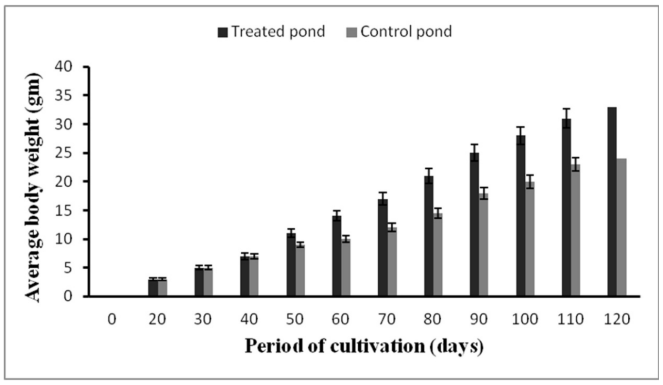

Figure 3. Effect of ENVIRON-AC on body weight of *P. monodon*

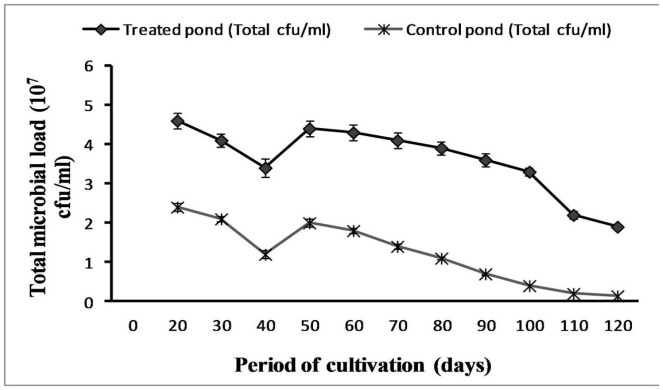

Figure 4. Study of total probiotic bacterial population in the ENVIRON-AC treated shrimp culture pond

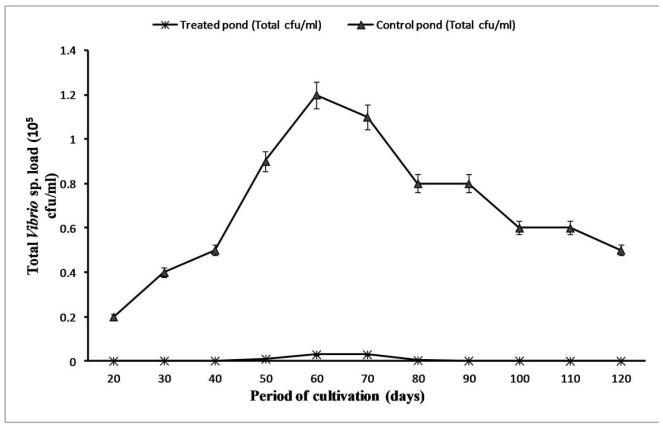

Figure 5. Study of total *Vibrio* sp. population in the ENVIRON-AC treated shrimp culture pond

Table 2. Effect of ENVIRON-AC on ammonia, nitrate and nitrite in the shrimp culture pond

Test parameter	Pond	Period of shrimp cultivation (days)											
		0	20	30	40	50	60	70	80	90	100	110	120
Total Ammonia level (ppm)	Control	-	0.118 ±0.002	0.211 ±0.003	0.249 ±0.005	0.317 ±0.004	0.478 ±0.008	0.587 ±0.003	0.618 ±0.006	0.657 ±0.008	0.689 ±0.005	0.724 ±0.002	0.788 ±0.005
	Treatment	-	0.021 ±0.002	0.039 ±0.004	0.041 ±0.003	0.078 ±0.006	0.124 ±0.004	0.189 ±0.003	0.241 ±0.008	0.324 ±0.004	0.287 ±0.002	0.294 ±0.003	0.289 ±0.002
Total Nitrate level (ppm)	Control	-	0.09 ±0.01	0.12 ±0.014	0.21 ±0.011	0.24 ±0.018	0.31 ±0.012	0.33 ±0.016	0.34 ±0.013	0.33 ±0.018	0.33 ±0.013	0.32 ±0.011	0.32 ±0.016
	Treatment	-	0.011 ±0.006	0.019 ±0.004	0.015 ±0.007	0.019 ±0.002	0.020 ±0.006	0.016 ±0.009	0.014 ±0.004	0.011 ±0.002	0.008 ±0.003	0.006 ±0.003	0.006 ±0.002
Total Nitrite level (ppm)	Control	-	0.016 ±0.0005	0.037 ±0.0003	0.038 ±0.0048	0.042 ±0.0061	0.046 ±0.0052	0.041 ±0.0008	0.038 ±0.0067	0.034 ±0.0051	0.032 ±0.0005	0.031 ±0.0004	0.026 ±0.0042
	Treatment	-	0.008 ±0.0002	0.009 ±0.0003	0.012 ±0.0038	0.015 ±0.0056	0.016 ±0.0007	0.014 ±0.0045	0.011 ±0.0037	0.008 ±0.0005	0.006 ±0.0061	0.002 ±0.0031	0.002 ±0.0036

DISCUSSION

The water quality and eco-friendly environment of the shrimp culturing pond deteriorate mainly due to residual food, faecal matter, metabolic by-products, residues of biocides, fertilizer derived wastes and wastes produced during moulting (Sharma and Scheeno, 1999), finally disease causing microorganisms also increased. The changes of equilibrium in the pond environment ultimately lead to enhance stress for shrimp health and decline survival rate as well as yield of production. Efficient removal of equilibrium of culture water, increase of nitrogenous wastes like ammonia, nitrite and nitrate are most important to crash the friendly environment of pond. Sissons (1989) reported that some commercial product made by shrimp beneficial microorganisms or probiotics effectively deal with these unwanted substance and that helps to maintain good water quality, improving growth rate, weight gain and good survival rate with an attractive feed conversion ratio (FCR). In the present study, the commercial probiotics product or bioaugmentor ENVIRON-AC contained *Lactobacillus* sp. and *Bacillus* sp. and played a vital role to maintain good water quality of the culture pond. Ehrlich *et al*., (1988) mentioned that addition of bacterial products in aquaculture ponds accelerates nitrification and rapid decomposition of organic solids. This explains the rapid reduction and stable levels of ammonia and particulate organic matter following a bacterial product application in this study.

The bioaugmentation agent used also contained enzymes like, amylase, protease, beta-galactosidase and phytase, which further hastens the breakdown of inorganic compounds and organic wastes into available nutrients for the bacterial agents. Carbohydrates, proteins and lipids are essential aliments of human, livestock, fish, shellfish, and mollusk including shrimp for the growth, function and structure. Protein is the major component for the commercial shrimp diet. According to Shiau (1998) the protein requirement of penaied shrimp varies from 28-57% depending on the shrimp species. In the year of 1991, Akiyama *et al.* mentioned that lipid levels in commercial shrimp diets fluctuate from 6 to 7.5% with a maximum of 10%. Leonel and Olmos, (2006) suggested that *B. subtilis* and *B. megaterium* were good exoenzyme-producing bacteria. Extracellular enzymes such as amylase, cellulase, lipase, protease, lactase and catalase biosynthesized by promising probiotic bacteria can improve nutrient digestibility and overall animal health. Therefore, information on the enzyme producing efficacy of the probiotic products is always helpful to the aquaculture industry.

In this study ENVIRON-AC contained different types of enzymes such as amylase (1.050 U/gm), beta-galactosidase (0.525 U/gm), phytase (2.579 U/gm) and protease (90.220 U/gm). The product also contained Vitamin-C (0.378 mg/gm) which is very essential for shrimp health. Normally shrimp feed contained almost 40-50% protein, 20-30% carbohydrate, 5-10% fat and others ingredient. Out of the 100% feed given in the pond, 30-40% feed is being wasted. Enzymes immediately digest these waste feed particles and control or prevent the pond from pollution of toxic gases. Function of amylase in the pond, utilization of starch and complex polysaccharides. Where beta-galactosidase acts on galactosides to reduce anti nutritional pentosans. The enzyme phytase not only releases phosphorus from the phytate but also releases minerals and amino acids that are also bound, paving the way for maximum utilization of nutrients. On the other hand function of protease is the utilization of animal and plant protein. According to Merchie *et al*., (1998) Vitamin-C increases the immunity power of the shrimp. Also it helps to increase the growth rate and survival rate.

The total ammonia level of culture pond treated by ENVIRON-AC is very lower than untreated pond. Where the maximum total ammonia ranges of treated and control pond were 0.324±0.004 ppm and 0.788±0.005 ppm respectively. During the total culture period, treated with ENVIRON-AC, the maximum level of total nitrate and total nitrite were 0.020±0.006 ppm and 0.016±0.0007 ppm respectively, where control pond showed these types of nitrogenous wastes are very high (Table 2). Yang *et al*., (2011) mentioned that a nitrifying denitrifying *Bacillus* sp. have the capability to remove the ammonia. Where Wang *et al*., (2013) recommended that *Bacillus cereus* HS-N25 was a good denitrifier bacterium. Ramanathan *et al*., (2005) mentioned that 26 to 30 °C is the most appropriate temperature for the black tiger shrimp culture. The average temperature of ENVIRON-AC treated pond was 28 to 32 °C. According to Muthu (1980) and Karthikeyan *et al*., (1994) 13 to 35 ppt salinity ranges was the ideal for *P. monodon* culture whereas ENVIRON-AC treated pond carried out in 11 to 25 ppt salinity range. Dissolved oxygen also is the major factor for shrimp culture. It maintains the respiration of aquatic organisms also maintain favorable chemical and hygienic environment of the water body. Nitrate which is very toxic is reduced by denitrifies into ammonia when dissolved oxygen level is very low. The oxygen level and optimum range of pH of the treated pond were 4.2-5.1 mg L^{-1} and 6.4-8.9 respectively. Ramanathan *et al*., (2005) mentioned that 6.8-8.7 pH range was the optimum for growth and production for penaid species

where Reddy (2000) recommended 7.5 to 8.5 pH ranges was the best for *P. monodon* culture. In 1992, Chein suggested that the safe level of ammonia and nitrite were <1 ppm and <0.25 ppm respectively for the shrimp culture. The present study also showed that the highest total ammonia level of the treated pond was lower (0.324±0.004 ppm) than the safe level where control pond was greater (0.788±0.005 ppm). Where the highest total nitrite level of the treated pond was 0.016±0.0007 ppm. On the other hand in the year of 2004, Abraham *et al*. observed that the highest nitrate level throughout the shrimp culture all over West Bengal was 0.012 ppm. Where the shrimp pond treated with ENVIRON-AC showed maximum nitrate level was 0.020±0.006 ppm. Present study also showed that the total nitrate and total nitrite level were under safe condition as mentioned by Chein (1992) and the survival rate and average body weight of shrimp were very good.

In case of control pond the highest level of total ammonia, total nitrate and total nitrite were 0.788±0.005 ppm, 0.34±0.013 ppm and 0.046±0.0052 ppm respectively which were very high than treated pond. As a result the shrimps of control pond were affected by disease and on the other hand treated ponds shrimps were healthy and survival rate was high than control pond (Figure 2). Ravi *et al*., (1998) mentioned that the probiotic organisms maintain good water quality and enhancing growth rate in Indian White Prawn, *P. indicus*. The present study also support that the shrimp of treated pond showed the better body weight than untreated pond (Figure 3). Jha and Naik, (2007, 2008, 2009a, 2009b) reported that probiotic bacteria inhibit the growth of *Vibrio* sp. and decrease the formation of toxic gases like ammonia, nitrite, hydrogen sulphide etc. The shrimp ponds treated with commercial probiotic product were less incidence of *Vibrio* sp. (Figure 5), well survival rate with high growth rate than untreated pond. Nitrogenous pollutant of water like ammonia, nitrate and nitrite levels were near to the ground where untreated ponds very high. As a result the water quality of the pond treated with commercial probiotic product, ENVIRON-AC, a bioaugmentor agent, was superior and maintaining eco-friendly environment for black tiger shrimp culture. Further work is needed to determine the life span of the bioaugmentation agents added in the system in terms of their survival in ponds and their ability to maintain a clean environment for shrimp.

Conclusion

The general conclusion obtained from the present study was that the commercial probiotic product ENVIRON-AC (manufactured by Biostadt India Ltd., Mumbai) plays a vital role in growth, survival and disease resistance of the Black Tiger Shrimp by maintaining good water quality parameters in the culturing pond throughout the culture period. It was clear from the data, not only that the load of *Vibrio* sp. was dominant in the control ponds also maintaining high level of nitrogenous contaminant like ammonia, nitrate and nitrite. The shrimp of the control ponds also affected by black gill, white gut and fungal disease due to environmental tress. Where the shrimp of treated pond are fresh, healthy, no disease affected high survival rate and good water quality throughout the culture. So it is concluded that ENVIRON-AC, not only a good commercial probiotics product but also a good bioaugmentor agent which helps the pond to carry on an eco-friendly environment for Black Tiger Shrimp culture.

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