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ARTICLE

Water quality improvement of *Penaeus monodon* culture pond for higher productivity through bioremediation

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ABSTRACT Intensive culturing of *Penaeus monodon* generates considerable amount of wastes leading to the deterioration of water quality, disease outbreaks and lower yields. Recently, the application of biocontrol agents were started in ponds in order to improve yield through bioremediation. Analytical investigation of a biocontrol product (PROFS), an extensively used product of Biostadt India Ltd. was performed to determine its accuracy and applicability. It was found that four different types of *Bacillus* species (69×10^9 cfu/g) were present in the product. Amylase, phytase and protease activities in the product were 3.078, 4.584 and 84.154 U/g, respectively. The biocontrol treatment was applied in the ponds five times during the cultivation (120 days) of *P. monodon* at a dose of 1 kg/ha. The amount of total ammonia, nitrate and nitrite of the treated ponds varied from 0.418 ± 0.039 to 0.079 ± 0.028 ppm, 0.029 ± 0.008 to 0.014 ± 0.004 ppm and 0.0127 ± 0.0008 to 0.0098 ± 0.00007 ppm, respectively, which were significantly lower than the values measured for untreated ponds. The product showed antimicrobial activity against *Vibrio harveyi* MTCC 7954 (inhibition zone: 4.89 mm) and *Vibrio vulnificus* MTCC 1145 (6.41 mm). Average body weight of shrimps in treated ponds was 33 ± 2.42 g, which was 39.5% higher than the values measured from control ponds. The *Vibrio* count was negligible (0.12×10^5 cfu/ml) and the survival rate was 93% in treated ponds. **Acta Biol Szeged 59(2):169-177 (2015)**

KEY WORDS

antimicrobial activity
bioremediation
biocontrol product
Penaeus monodon

Introduction

In 2011 the interesting fact was reported by The Food and Agriculture Organization of the United Nations, that out of 154 million tons of fish capture by fisheries and aquaculture, 131 million tons were consumed by human (FAO 2012). Food consumption in aquaculture farming comprised of 60 million tons ($119 billion) out of which 15 million tons were used for fish meal and fish oil production whereas the remaining amount was used for ornamental fish production. As a consequence, there is a shortage in seafood resources world-wide which may drive the growth of aquaculture, predominantly in developing countries (Kesarcodi-Watson et al. 2008). Due to space and resource limitation, traditional aquaculture has been strengthening into reticulated systems with high stocking densities of the cultured species (Balcázar et al. 2006) resulting in an artificial environment which supports the growth of pathogenic bacteria and the generation of toxic metabolites (Moriarty 1999). The unsystematic release of spent aquaculture wastes into the environment is also initiating new problems (Chávez-Crooker and Obreque-Contreras 2010). The outburst of bacterial pathogens in aquaculture systems is a complex phenomenon causing significant losses to the industry (Jeney and Jeney 1995; Irie et al. 2005). The major cause is due to poor water quality through waste accumulation during hyper-nutrification resulting from aggressive feeding rates and high protein dietary composition, both of which are common phenomena in intensive aquaculture systems (Liao and Mayo 1974; Boyd 1985; Shimeno et al. 1997). Accumulation of nitrogenous waste and its derivatives poses a threat to the environment and can predispose fish to infestation by parasites and pathogens due to a reduction in immunity (Liao and Mayo 1974; Jana and Jana 2003). Nitrogenous compounds are good stress elements for the shrimp culture. Chien (1992) suggested that the safe level of ammonia and nitrite were <1 ppm and <0.25 ppm, respectively for shrimp cultivation. The most predominant pathogens in aquatic body are *Vibrio* species like *Vibrio harveyi*, *V. vulnificus*, *V. alginolyticus*, *V. splendidus* and *V. parahaemolyticus*. They are responsible for several types of diseases and mortali-

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ties of up to 100% (Karunasagar et al. 1994). Antibiotic or chemotherapeutic agents are the first choice of treatment in the cases of diseases caused by *Vibrio* spp. However, the continuous application of antibiotics or chemotherapeutic agents in aquaculture for prophylactic treatment of diseases has potential negative effects, particularly the drug resistance commencement of microorganisms through adaptation or by genetic exchange (Holmström et al. 2003; Le et al. 2005). One of the effective alternatives in aquaculture is the water quality improvement through bioremediation (application of microbes/enzymes). It was established that bioremediators, the mixtures of macro and microorganisms have the capability to improve the water quality (Moriarty 1998). In aquaculture, lactic acid bacteria (*Lactobacillus, Carnobacterium,* etc.), *Vibrio* (*Vibrio alginolyticus*), *Bacillus* and *Pseudomonas* were proposed as biocontrol agents (Singh et al. 2001). Yang et al. (2011) and Kim et al. (2005) reported that an aerobic heterotrophic *Bacillus* species is a good bacterium for nitrogen removal. 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.

The aim of this study was to improve water quality of *Penaeus monodon* culture ponds for higher productivity by the application of enzyme-producing microorganisms in the culture ponds.

Materials and Methods

Commercial probiotic product

The *in vitro* and *in vivo* experiments of the study were performed in the period between April 2013 to September 2013 with the commercial probiotic/biocontrol agent PROFS (Batch No.: W120001; Manufacture (Mfg) Date: September 2012; Expiry (Exp) date: August 2014) manufactured by Biostadt India Ltd., Mumbai, India. The *in vivo* challenge trial was carried out in "Pradip Fisheries" located in Contai town (latitude 24° and longitude 87°45') in the Bay of Bengal region, India. Collected samples were stored at 4 °C before the experiments.

Isolation and enumeration of bacteria in product

One g of the commercial product was diluted in 10 ml sterile NaCl solution (0.85% in distilled water) and mixed well for the preparation of 10^{-7} dilution. Diluted samples were allowed to precipitate the insoluble carrier materials. Clear solution of 100 µl was taken from the 10^{-7} dilution and spread on nutrient agar (NA) medium for total bacterial count and different types of selective media were used for the isolation of specific microorganisms. *Bacillus* medium and yeast-mould agar medium were used for the isolation of aerobic *Bacillus* species and yeasts/moulds, respectively and Man, Rogosa and Sharpe (MRS) medium for anaerobic organisms. The bacterial colony counts were recorded after incubation at 30 °C for 24 h. The organisms were isolated and identified to the 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.

Growth pattern of the microbial consortium

Growth pattern of the bacterial consortium present in the tested commercial product was followed spectrophotometrically as optical density at 620 nm (λ max) at different time points (OD was recorded at every 3 h interval) during cultivation (Barman et al. 2011).

Effect of pH, temperature and salt concentration

Effect of different pH, temperature and salt (NaCl) concentrations on the growth of probiotic organisms were determined by culturing the organism under combinations of different pH (6.5-9.5), temperature (5-45 °C) and NaCl concentration (2-10%) values. The data were recorded at 620 nm after 24 h of incubation at 30 °C.

Quantitative assay of enzymes

One g of the tested sample was vigorously mixed with 10 ml of distilled water and the supernatant was used for enzyme measurements after centrifugation at 5000 rpm for 10 min.

Alpha-amylase was estimated according to the method of Rick and Stegbauer (1974). One unit (U) of enzyme activity was defined as the amount of enzyme that produces 1.0 µM of glucose in 1 min under standard conditions.

Protease activity was measured according to the modified method of Kembhavi and Kulkami (1993). One unit of protease activity was defined as the amount of enzyme that hydrolyses casein to produce absorbance equivalent to 1 µM of tyrosine/min with tyrosine as standard.

Phytase activity was assayed by the method of Gulati et al. (2007). 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.

Inhibitory activity on shrimp pathogen

Different pathogenic strains from Tryptone Soy Agar (TSA)

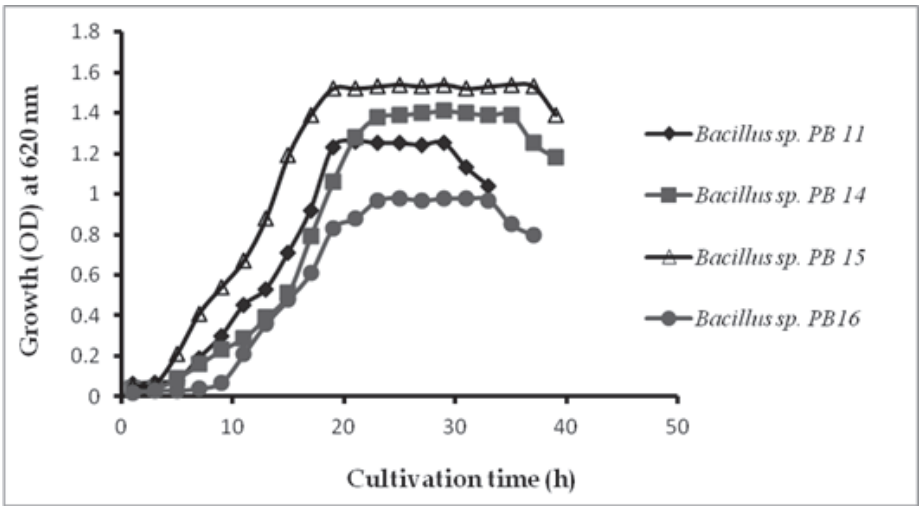

Figure 1. Growth curves of the bacterial population contained in the biocontrol product (120 rpm at 30 °C).

slants were inoculated into Tryptone Soy Broth (TSB) and incubated at 30 ºC for 24 h to prepare young cultures. The pathogenic strains, *Vibrio harveyi* MTCC 7954 and *Vibrio vulnificus* MTCC 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 NA plates. The product containing bacteria was grown in the nutrient broth at 30 °C. After incubation of 24 h the cultures were centrifuged three times with NSS at 3000 rpm for 3 min. The supernatant of young culture from the commercial product was then poured (0.1 ml) on the plates spread with different pathogenic bacteria. After overnight incubation at 30 ºC, the commercial probiotic organisms producing clear inhibition zones on the pathogenic strains were recorded (Ruiz et al. 1996).

Experimental design of treated pond

The biocontrol product was applied in the ponds five times during the 120 days cultivation of *P. monodon* at 1 kg/ha (stocking density was 28/m^3 and seeds were taken from Tropical Biomarine, Chennai, India) during the morning times and an interval of 25 to 30 days. CP feed (Charoen Pokhpond Aquaculture India Pvt. Ltd., Chennai, India) was used for the feeding of the shrimps applied four times 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 product was well mixed with feed and applied into the pond by vessel for the proper distribution. Sampling was performed from the pond weekly in the morning with a cast net. The shrimps were caught and their individual body weight, healthiness, survival rate, moulting, attachments, animal activity and gill conditions were observed.

Enumeration of Vibrio sp.

To count the total cfu of *Vibrio* spp. present in the trial pond, the water samples were spread over thiosulfate-citrate-bile salts-sucrose agar (TCBS, Hi-Media, India) medium. The cfu was counted after overnight incubation in the BOD incubator at 30 ºC.

Analysis of treated pond water parameters

Water samples were collected in sterile bottles (Borosil), transported aseptically to the laboratory and processed immediately for analyses of different water parameters (dissolved oxygen, alkalinity, salinity, water pH and sediment pH). Nitrogenous compounds (ammonia, nitrate and nitrite) were measured according to standard methods (APHA 2005). Ammonium concentrations were determined by the Nessler assay at a wavelength of 425 nm. The nitrate concentrations were determined by phenol disulfonic acid following UV spectrophotometry to measure the difference between OD at 220 nm and 2×OD at 275 nm. The nitrite concentration was determined by *N*-(1-naphthyl)-ethylene at a wavelength of 543 nm. The estimations were made within 24 h from the sample collection.

Statistical analysis

All the analyses was carried out in triplicate. Duncan Multiple Range Test was used to determine the significant difference

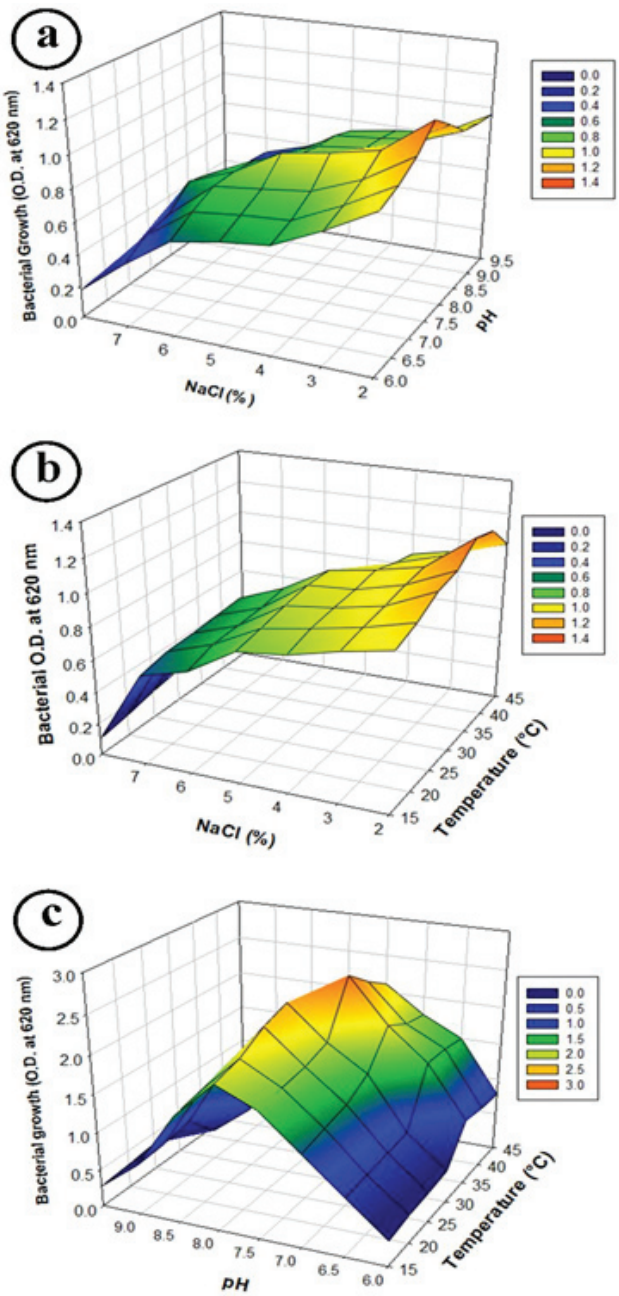

Figure 2. Study of the total bacterial growth of the biocontrol product with different physiological conditions. (a) pH and NaCl concentration, (b) NaCl concentration and temperature and (c) pH and temperature.

between the bacteriological and physicochemical parameters of treated and control pond water and shrimp. The correlation coefficient values of bacterial counts were also calculated using the Microsoft Excel package.

Table 1. Enumeration of cultivable bacterial population from the biocontrol product.

Name of the organisms	Bacterial population (cfu/g)
Total bacteria	69×10^9
Bacillus sp. PB 11	9×10^9
Bacillus sp. PB 14	21×10^9
Bacillus sp. PB 15	13×10^9
Bacillus sp. PB 16	29×10^9

Results

Microbial population of the biocontrol agent

Total plate count of the viable probiotic bacteria present in the commercial product was estimated based on growth on NA medium. Different types of strains with various morphological characteristics appeared after incubation at 37 °C for 24 h and their counts were recorded as 69×10^9 cfu (Table 1). Thereafter, bacterial counts were also recorded in different selective media. It was noticed that four different types of *Bacillus* species were found in the biocontrol product with the nearly absence of yeasts and moulds. The plate counts indicated that *Bacillus* sp. PB 11, *Bacillus* sp. PB 14, *Bacillus* sp. PB 15 and *Bacillus* sp. PB 16 were present at 9×10^9, 21×10^9, 13×10^9 and 29×10^9, respectively (Table 1). The identification of these isolates was made by biochemical characteristics following the Bergey's manual of determinative bacteriology (data not shown). It was evidently noticed that the commercial product contained only species from the genus *Bacillus*.

Study of growth pattern of the microbial consortium in the biocontrol product

Microorganisms from the experimental biocontrol product were cultivated in nutrient broth at 30 °C with 120 rpm for 39 h (Fig. 1). Within the bacterial consortium it was found that *Bacillus* sp. PB 15 has the longest logarithmic phase (3 h to 19 h) and a longer stationary phase (19 h to 37 h) than the other three bacteria.

Effect of pH, temperature and salt concentration on the growth of the organisms

The probiotic organisms contained in the product were grown in different ranges of pH, temperature and NaCl. The organisms survived in the pH range of 6.5 to 9.5 and the optimum growth was observed in a slightly alkaline environment at pH 8.0 (Fig. 2 a,b). The organisms are able to grow in the alkaline pond water and play a vital role for decreasing the water pH

Table 2. Inhibitory effects of the biocontrol product on different pathogenic bacterial strains.

Biocontrol product	Pathogens	Diameter of inhibition zone (mm)
PROFS	*Vibrio harveyi* MTCC No. 7954	4.89
	Vibrio vulnificus MTCC No. 1145	6.41

towards neutral, which is the best environmental condition for shrimp culture. The optimum temperature for bacterial growth was 35 °C and the bacteria could survive in a broad range from 25 to 45 °C (Fig. 2 b,c).

Quantitative assay of enzymes

During the study the enzyme profiles in the probiotic product (PROFS) were examined. The presence of significant amounts of amylase (3.078 U/g), phytase (4.584 U/g) and protease (84.154 U/g) activities could be observed.

Inhibitory activity on shrimp pathogens

V. harveyi and *V. vulnificus*, the most common pathogens in shrimp cultivation were included in the inhibitory test. The *in vitro* inhibitory effect of the commercial product showed that it was more effective against *V. vulnificus* (MTCC 1145) than *V. harveyi* (MTCC 7954) (Table 2).

In vivo study of the biocontrol product for challenged trial

For the challenged trial, the dosages of the biocontrol product applied into the ponds were according to the manufacturer's instructions along with feed, while only feed was applied for the control ponds. The shrimp and water quality of treated and control ponds were checked every 10 days interval. The survival rate of black tiger shrimps was 93% in the probiotics-treated ponds, which was significantly higher ($p<0.05$) than that in the control ponds (Fig. 3). The average body weight was 33±2.42 g in the treated ponds and 24±1.42 g in the control ponds (Fig. 4). The results of t-test also showed significant difference between probiotics-treated ponds and control ponds. The loads of *Vibrio* spp. were higher in the control ponds (1.48×10^5 cfu/ml) than in treated ponds (0.12×10^5 cfu/ml) (Fig. 5). The nitrogenous compounds like ammonia, nitrite and nitrate were significantly lower ($p<0.05$) in treated ponds (Table 3). The different water quality parameters of the treated ponds were better when compared with the control of *P. monodon* culture (Table 4).

Discussion

In almost all the ecosystems, microbes play a remarkable role to recycle nutrients and boost up the immunity to the individuals of the community. Particularly in the aquaculture system the beneficial organisms also regulate the growth of pathogenic organisms and algal bloom and accelerate the process of mineralization, sediment decomposition and nitrification to get a high yield of fish (Fast and Menasveta 2000; Gomez-Gil et al. 2000; Jana and Jana 2003).

In the present study, the commercial probiotic and biocontrol product contained four different types of *Bacillus* sp. and digestive enzymes like amylase, protease and phytase. Carbohydrates, proteins and lipids are the major diet for the growth,

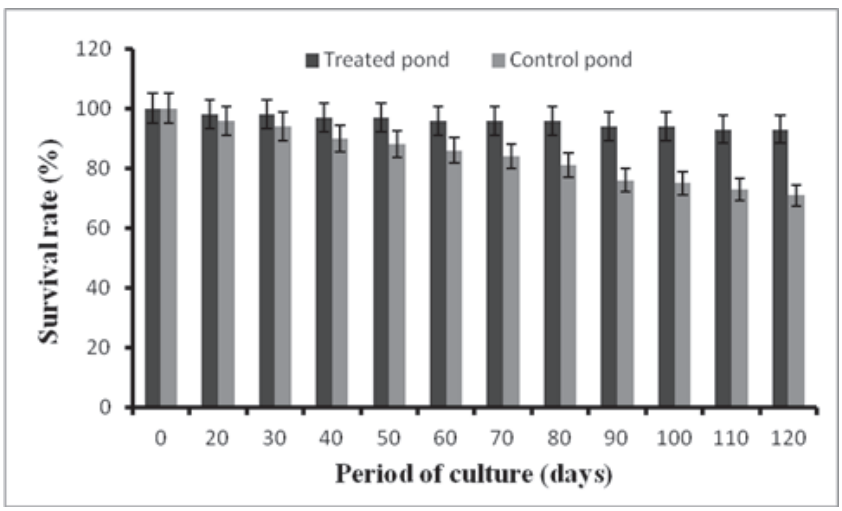

Figure 3. Effect of the biocontrol product on the survival rate of *P. monodon* culture.

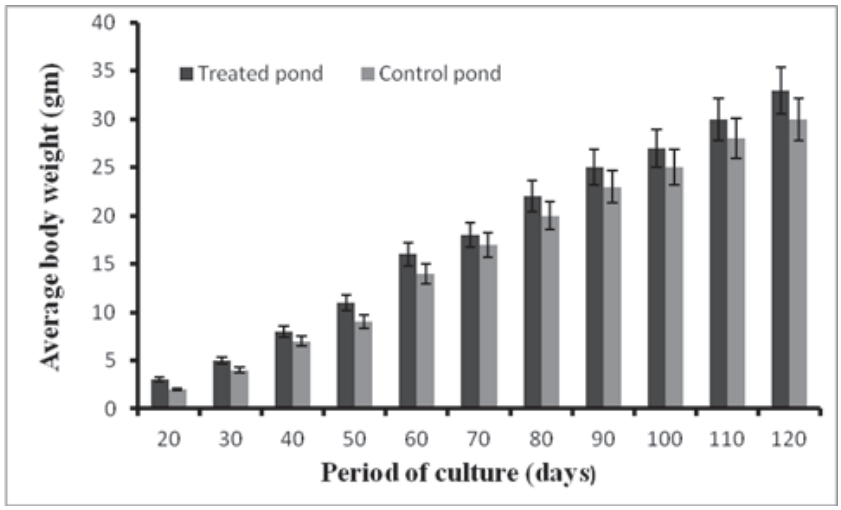

Figure 4. Effect of the biocontrol product on average body weight of *P. monodon*.

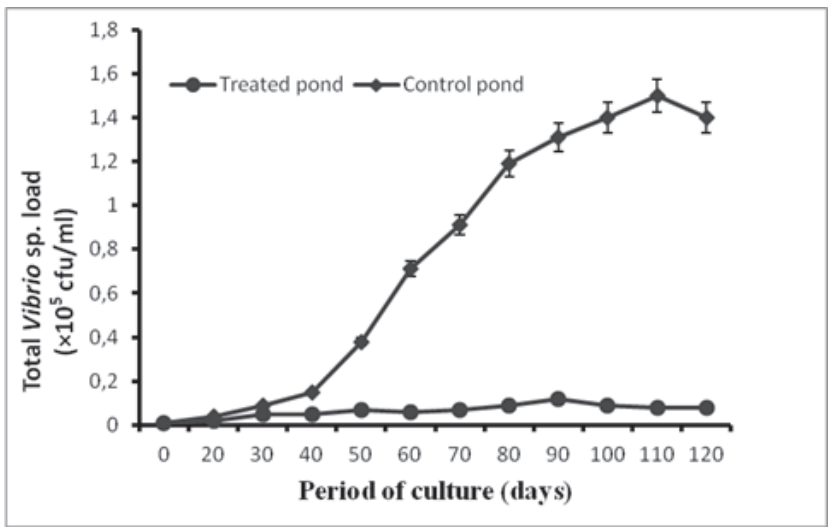

Figure 5. Study of total *Vibrio* sp. population in the shrimp culture pond treated with the biocontrol product.

function and structure of shrimp. The nutritional demand of protein, carbohydrate and lipid generally varies between 28-57%, 23-32% and 6-7.5% respectively and it depends on shrimp species (Akiyama et al. 1991; Shiau 1998). *B. subtilis* and *B. megaterium* produced different enzymes like amylase, cellulase, lipase, protease, lactase and catalase (Leonel and Olmos 2006). These enzymes help to digest and absorb the nutrient efficiently by the digestive system. In this study PROFS contained different types of enzymes such as amylase (3.078 U/g), phytase (4.584 U/g) and protease (84.154 U/g). Normally shrimp feed contained almost 40-50% protein, 20-30% carbohydrate, 5-10% fat and others ingredients. 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 is the utilization of starch and complex polysaccharides, whereas phytase releases not only phosphorus from phytate but also releases minerals and amino acids that are also bound, paving the way for maximum utilization of nutrients. Protease enzymes help in the utilization of animal and plant proteins.

The total ammonia level of the culture pond treated by the biocontrol product is much lower than in the untreated pond. The maximum total ammonia ranges of treated and control ponds were 0.418 ±0.038 ppm and 1.72±0.020 ppm, respectively. When treated with the biocontrol product, during

Table 3. Analysis of nitrogenous compounds throughout the ponds with *P. monodon* culture.

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	-	0.280 ±0.003	0.293 ±0.003	0.342 ±0.004	0.679 ±0.008	1.317 ±0.016	1.381 ±0.016	0.978 ±0.011	1.573 ±0.019	1.417 ±0.017	1.694 ±0.020	1.72 ±0.020
	Treatment	-	0.081 ±0.005	0.091 ±0.005	0.079 ±0.004	0.082 ±0.005	0.113 ±0.007	0.325 ±0.020	0.219 ±0.013	0.418 ±0.038	0.214 ±0.013	0.281 ±0.017	0.216 ±0.013
Total nitrate level (ppm)	Control	-	0.34 ±0.01	0.36 ±0.01	0.39 ±0.011	0.42 ±0.012	0.56 ±0.016	0.51 ±0.015	0.41 ±0.012	0.55 ±0.016	0.43 ±0.012	0.68 ±0.02	0.71 ±0.02
	Treatment	-	0.016 ±0.004	0.019 ±0.005	0.014 ±0.003	0.019 ±0.005	0.021 ±0.005	0.018 ±0.004	0.016 ±0.004	0.029 ±0.008	0.015 ±0.004	0.016 ±0.004	0.015 ±0.004
Total nitrite level (ppm)	Control	-	0.0187 ±0.0001	0.0198 ±0.0015	0.0338 ±0.0002	0.0429 ±0.0003	0.066 ±0.0005	0.0824 ±0.0006	0.114 ±0.0008	0.281 ±0.0021	0.338 ±0.0025	0.4317 ±0.0033	0.626 ±0.0047
	Treatment	-	0.0099 ±0.0006	0.0109 ±0.0068	0.0098 ±0.0006	0.0104 ±0.0006	0.0109 ±0.0068	0.011 ±0.0006	0.0119 ±0.0007	0.0127 ±0.0008	0.0118 ±0.0007	0.0121 ±0.0007	0.0122 ±0.0007

Table 5. Analysis of different water quality parameters in ponds with *P. monodon* culture.

Test parameter	Pond	Period of shrimp cultivation (days)										
		0	20	30	40	50	60	70	80	90	100	110	120
Dissolved oxygen (mg/l)	Control	-	5.3 ±0.039	5.1 ±0.038	4.8 ±0.036	4.7 ±0.035	4.4 ±0.033	4.5 ±0.033	4.2 ±0.031	3.8 ±0.028	3.2 ±0.024	3.1 ±0.023	3.1 ±0.023
	Treatment	-	5.2 ±0.057	5.4 ±0.059	5.1 ±0.056	5.3 ±0.058	5.1 ±0.056	4.9 ±0.054	4.8 ±0.053	4.6 ±0.051	4.9 ±0.054	5.1 ±0.056	5.2 ±0.057
Temperature (°C)	Control	-	30.00 ±0.351	29.00 ±0.339	31.50 ±0.368	29.00 ±0.386	34.00 ±0.409	30.00 ±0.351	27.00 ±0.397	28.00 ±0.386	28.00 ±0.421	31.00 ±0.397	30.00 ±0.386
	Treatment	-	30.00 ±0.958	29.00 ±0.9262	31.50 ±1.006	29.00 ±1.022	34.00 ±1.117	30.00 ±0.958	27.00 ±1.054	28.00 ±1.022	28.00 ±1.149	31.00 ±1.085	30.00 ±1.054
Salinity (ppt)	Control	-	24.54 ±1.659	24.48 ±1.655	24.14 ±1.632	22.42 ±1.516	20.26 ±0.137	17.50 ±1.183	16.12 ±1.090	15.75 ±1.065	14.08 ±0.952	13.16 ±0.89	12.50 ±0.845
	Treatment	-	24.55 ±1.669	24.48 ±1.664	24.15 ±1.642	22.00 ±1.496	20.15 ±1.370	17.50 ±1.19	16.16 ±1.098	15.82 ±1.075	14.21 ±0.966	13.45 ±0.914	12.78 ±0.869
Water pH	Control	-	7.77 ±0.523	8.10 ±0.545	8.24 ±0.554	8.64 ±0.581	8.90 ±0.599	8.13 ±0.614	9.31 ±0.626	8.89 ±0.665	8.98 ±0.671	8.87 ±0.664	8.95 ±0.664
	Treatment	-	7.67 ±0.733	7.85 ±0.75	7.92 ±0.757	7.84 ±0.749	8.25 ±0.788	7.78 ±0.743	8.47 ±0.809	8.10 ±0.869	8.56 ±0.818	8.48 ±0.81	8.21 ±0.784

the total culture period the maximum levels of total nitrate and total nitrite were 0.029±0.008 ppm and 0.0127 ±0.0008 ppm, respectively, while the control pond revealed that the concentrations of these types of nitrogenous wastes are very high (Table 4). Yang et al. (2011) mentioned that a nitrifying-denitrifying *Bacillus* sp. has 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 °C to 30 °C is the most appropriate temperature for the black tiger shrimp culture. Due to environmental changes, in this study the maximum temperature reached 34.00±1.183 °C but the shrimp cultivation was not hampered, because the optimum growth temperature of the organisms in the product was 35 °C (Fig. 2b,c). According to Muthu (1980) and Karthikeyan (1994), 13 to 35 ppt salinity ranges were ideal for *P. monodon* culture whereas in PROFS-treated pond they can survive up to 6% NaCl concentration due to the organisms contained in the product (Fig. 2a,b). Dissolved oxygen is also a major factor for shrimp culture. It maintains the respiration of aquatic organisms and also maintains the favorable chemical and hygienic environment of the water body. Nitrate which is very toxic is reduced by denitrifiers into ammonia when dissolved oxygen level is very low. The oxygen levels of the treated ponds varied from

4.6±0.051 to 5.2±0.057 mg/l. The maximum range of water pH was 8.48±0.81. Ramanathan et al. (2005) mentioned that 6.8-8.7 pH range was the optimum for growth and production of penaid species, while Reddy (2000) recommended that the pH range of 7.5 to 8.5 was the best for *P. monodon* culture. Our *in vitro* experiment also showed that pH 8.0 was the optimum for their growth (Fig. 2 a,b).

Chien (1992) suggested that the safe levels of ammonia and nitrite were <1 ppm and <0.25 ppm, respectively for shrimp culture. The present study also showed that the highest total ammonia level of the treated pond (0.418±0.038 ppm) was lower than the level of the control pond (1.72±0.020 ppm). The highest total nitrite level of the treated pond was 0.0127±0.0008 ppm. Shrimp ponds treated with the biocontrol product showed a maximum nitrate level of 0.281±0.0021 ppm. The total nitrate levels were slightly higher than the safe levels, but the total nitrite levels were under the safe condition as mentioned by Chien (1992) and the survival rate and average body weight of shrimp were very good. In the case of control ponds the highest level of total ammonia, total nitrite and total nitrate were much higher than in the treated pond (Table 4). As a result the shrimps of the control pond were affected by disease, while shrimps in the treated ponds were healthy and their survival rate was higher than in the control pond (Fig. 3). Ravi et al. (1998) mentioned that probiotic organisms maintain good water quality and enhance the growth rate of the Indian white prawn, *P. indicus.* The present study also supports that the shrimps of the treated pond showed higher body weights than those of the untreated pond (Fig. 4). Jha and Naik (2007; 2008; 2009a; 2009b) reported that probiotic bacteria inhibit the growth of *Vibrio* spp. and decrease the formation of toxic compounds like ammonia, nitrite, hydrogen sulfide etc. The incidence of *Vibrio* spp. was lower in the shrimp ponds treated with the commercial probiotic product (Fig. 5), which increased the survival rate and the growth rate in relation to the untreated pond. Levels of nitrogenous pollutants of water like ammonia, nitrate and nitrite were very low, while those of the untreated ponds very high. As a result the water quality was superior in the pond treated with the commercial probiotic and biocontrol product, which maintained an 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.

The general conclusion obtained from the present study was that the commercial probiotic and/or biocontrol product (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 evident that the product has the ability to inhibit the growth of fish pathogens and reduce the nitrogenous contaminants in the water pool. The fresh aquatic systems promoted health, immunity and survival rate. So, it can be concluded that the product is a good commercial probiotic agent as well as a biocontrol product which helps to provide a better eco-friendly environment for culturing black tiger shrimp.

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