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Effect of Commercial Probiotics on Large Scale Culture of Black Tiger Shrimp *Penaeus monodon* (Fabricius)

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Abstract: The survival rate and growth of both the ponds (High and low dosages of probiotics), which was applied with probiotics, was higher than that of control ponds. Concentrations of nitrite, nitrate and phosphate were higher in control ponds than the probiotics treated ponds. Chlorophyll a was observed maximum in probiotics treated ponds rather than control ponds. The bacterial population decreased at the end of culture in both treated and control ponds but the load of *vibrio* sp. when compared with THB in the control pond was not showing a significant decrease. Black gill, white gut and fungal diseases were predominant in control ponds. But these diseases were meager in probiotics treated ponds. The general conclusion obtained from the present study is that the probiotics plays a vital role in growth, survival and disease resistance of the animal by maintaining good water quality parameters through out the culture period. It is clear from the microbial load data that *vibrio* sp. is dominant only in the control ponds.

Key words: Probiotics, environ-AC, *vibrio*, THB, black gill

INTRODUCTION

Aquaculture has become an important economic activity in many countries all over the world. Shrimp farming is the major aquaculture industry in Asian countries contributing 91% of the world production (FAO, 2001). Generally bacteria play two major roles as beneficial bacteria and pathogenic forms. Beneficial bacteria are helpful in nutrient recycling and organic matter degradation and thus clear the environment (Moriarty, 1997). Pathogenic bacteria are responsible for bad water quality, stress and diseases as they act as primary and secondary pathogen (Chen *et al*., 1992). One of the most common bacterial diseases, affecting farmed species in marine conditions is caused by *Vibrio* species, which consists of luminescent vibriosis such as *Vibrio harveyi*, *V. splendidus*, *V. parahaemolyticus*. To overcome the pathogenic problem, application of disinfectants and antibiotics is a common practice in shrimp culture. Instead of preventing and arresting the diseases, the unwanted and unwarranted usage of the chemicals damages the environment and develops antibiotics resistant bacteria (Karuna Sagar *et al*., 1996). In addition to resistance problems, it also impaired the growth of cultured species, posed a threat to human health and the environment at large by way of residual effects. Hence, eco-friendly treatments came in to existence and among these, probiotic application came first as it is environmentally safe and cost effective also (Moriarty, 1997). The use of probiotics in the culture of aquatic organisms is increasing with the demand for more environment-friendly aquaculture practices (Gatesoupe, 1999; Gomez-Gil *et al*., 2000; Verschuere *et al*., 2000; Irianto and Austin, 2002; Balca´zar, 2003).

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Table 1: Ponds treated with different doses of probiotics

Days of culture	High dosage pond I (kg ha^{-1})	Low dosage pond II (kg ha^{-1})
0	25	12.5
15	25	12.5
30	20	10.0
45	10	5.0
60	10	5.0
75	10	5.0
90	10	5.0
105	10	5.0
120	10	5.0

MATERIALS AND METHODS

The study was carried out in Shri Sathiya aqua farms located on the southern banks of Uppanar Estuary at Thirunagari, southeast coast of India (March, 2006 to February, 2007). Nine ponds were selected for the present study and each pond was of 1h area. For convenient the ponds were divided into 3 groups viz., I, II and III and each group had 3 ponds. Pond I was treated with high dosage of probiotics (Environ- AC, manufactured by Wockard Biostate Ltd), pond II with low dosage and pond III was kept as control (Table 1). Every 25 kg of probiotics was mixed in 50 kg of sand as per the instruction given by the manufacture itself. It was broadcasted through out the ponds during morning hours in an interval of 15 days.

The shrimps were fed with CP feed (Charoen pokhpond aquaculture India Pvt. Ltd, Chennai). The feed ration was divided into 4 times a day as 25, 20, 30 and 25% in the morning (6.00 AM), noon (12.00 PM) evening (6.00 PM) and night (10.00 PM), respectively. The feed was broadcasted from the rope method by using floats. Sampling was done in the ponds fortnightly during early hours of the day with a cast net. Five hauls were made in each pond. The shrimps were caught per haul and their individual weights are recorded. Healthiness, survival rate, Average Body Weight (ABW) and Average Daily Growth (ADG) of the animals were estimated. The water quality parameters (salinity-refrectometer, pH-meter dissolved oxygen-dissolved oxygen meter, temperature-thermometer and transparency-secchi disc) of the probiotics treated and control ponds were regularly monitored.

Water samples of the ponds were collected in well-cleaned bottles for analysis of nutrients. The estimations were usually made within 24 h of collection. The nutrients like phosphate, nitrate and silicate and ammonia were estimated following the standard methods described by Strickland and Parsons (1972).

For microbial analysis, the water and sediment samples were collected separately from different parts of the ponds in sterile conical flask and were mixed to make a single sample. This procedure was repeated for every pond and the final samples were brought to the laboratory immediately and were analyzed for microbial counts. It was then transferred to a sterile conical flask (150 mL) containing 99 mL of sterile diluents and serial dilution was performed to get 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} suspension samples. For enumeration of Total Heterotrophic Bacteria (THB), Zobell marine agar medium (Hi-media, Mumbai) was used. For enumeration of *Vibrio* sp. TCBS media was obtained from Hi-media, Mumbai.

To know the significance between the parameters of probiotics treated and control ponds t-test was applied.

RESULTS

Survival rate of both the ponds (High and low dosages of probiotics), which was applied with probiotics, was higher than that of control ponds (Fig. 1). The results of t- test also showed significant difference between probiotics treated ponds with control ponds.

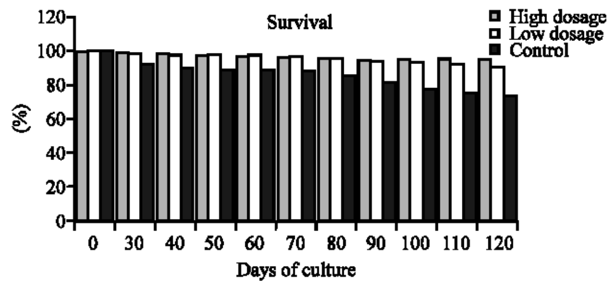

Fig. 1: Survival rate of *P. monodon* treated with different dosages of probiotics and control ponds

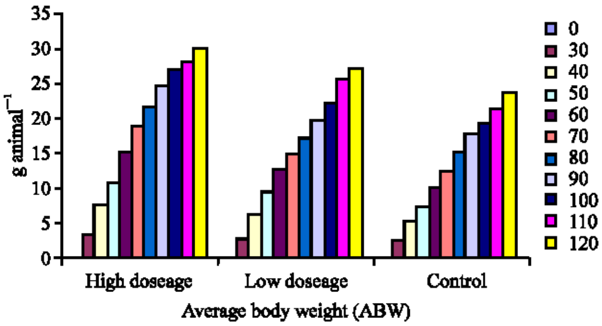

Fig. 2: Average body weight of *P. monodon* treated with different dosages of probiotics and control ponds

Maximum growth was observed in the ponds treated with different dosages of probiotics during each sampling interval and by the end of the experiment (Fig. 2). The growth was not only showed significant difference between control and treatment ponds but also between low and high dosage ponds.

There was no much difference in the average salinity for both treated and control ponds, which actually ranged from 5-36 ppt through out the culture period. The temperature for morning and after noon for both treated and control ponds were taken as average and were 26.5 and 28.3°C, respectively. In general, the average diurnal dissolved oxygen variation was similar (4.5-4.8 mg L^{-1}) for both treated and control ponds. Concentration of dissolved oxygen was higher in morning for both treated ponds and in the evening it was higher for control ponds. This showing similarity with the abundance of phytoplankton, as indicated by chlorophyll a concentration. The pH level was on alkaline side for both treated and control ponds (7.7-8.2). There was no great deviation for pH of treatment and control ponds through out the culture in morning and evening, which differed only by 0.2 units.

Concentration of ammonia was very much lower in the ponds that were treated with probiotics than that of control ponds. Among treated ponds, the ammonium concentrations of the higher dosage ponds were less when compared to lower dosage ponds. Concentrations of nitrite, nitrate and phosphate were higher in control ponds than the other two probiotics treated ponds. Among the treated ponds lower dosage ponds was showing higher amounts of nitrite, nitrate and phosphate. Chlorophyll a was observed maximum in probiotics treated ponds rather than control ponds. Among probiotics treated ponds chlorophyll a was maximum in higher dosage ponds than lower dosage ponds (Table 2). All the nutrients studied for the present study was significantly different between control and probiotics treated ponds.

The bacterial population changed during every sampling. In general, the bacterial population of sediment was higher than that of water in both control and treatment ponds. The bacterial population

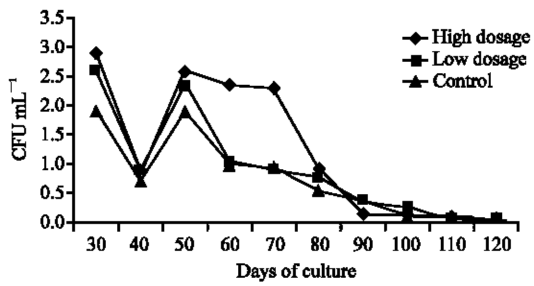

Fig. 3: Total microbial load of probiotics treated and control ponds

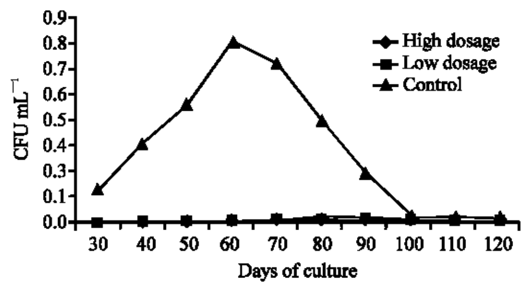

Fig. 4: *Vibrio* load of probiotics treated and control ponds

Table 2: Nutrient concentrations of both probiotics treated and control ponds

Nutrients	High dosage	Low dosage	Control
NH_4 (ppm)	0.22±0.03	0.27±0.04	1.80±0.1
NO_2 (ppm)	0.0022±0.0001	0.0030±0.0004	0.049±0.005
NO_3 (ppm)	0.0119±0.002	0.0262±0.008	0.38±0.04
PO_4 (ppm)	0.0036±0.00004	0.0042±0.0001	0.0051±0.0006
Chlorophyll a (μg L^{-1})	48.7±6.6	36.7±5.8	24.2±2.8

decreased at the end of culture in both treated and control ponds but the load of *Vibrio* sp. when compared with THB in the control pond was not showing a significant decrease. *Vibrio* sp. was contributing much in the THB load of the control ponds. However among the treated ponds high dosage pond was showing a reduced count of *Vibrio* sp. (Fig. 3 and 4).

DISCUSSION

The quality of water during the culture period will deteriorate mainly due to the accumulation of metabolic wastes of living organisms, decomposition of unutilized feed and decay of biotic materials. In general organisms are in a state of balance between potential disease causing microorganisms and their environment. Change in this equilibrium through way of impairment in water quality parameters can influence survival of organisms as they become vulnerable to disease due to stress, so also growth. Efficient removal of imbalances, which cause impairment in water quality, is difficult. However addition of some commercial preparations as probiotics is reported to effectively deal with these substances and that way helpful in maintaining water quality parameters thereby improving growth rate, weight gain and survival rate with an attractive FCR in farmed organisms (Sissons, 1989).

In the present study, the water quality parameters of the ponds, which are applied with microbial supplement through probiotics, was good because of the various roles played by the microbes. Improved water quality has especially been associated with *Bacillus* sp. The pond that was treated with the Environ-AC was abundant with *Bacillus* sp. was showing a low level of Ammonia, which was converted into nitrate through nitrite. This is corroborated by lower level of nitrate in the treated pond than the control one. Thus probiotic application lead to the nutrient enrichment through the way of microorganism, releasing nutrients by decomposing the waste material that accumulate in pond bottom as left-over feed and die-off. This nutrient enrichment leads to increased phytoplankton production and photosynthetic activity. This clear from the results obtained from the measurement of Chlorophyll a pigment as the indicator of phytoplankton.

The optimum range of temperature for the black tiger shrimp is between 26 to 30°C (Ramanathan *et al*., 2005). The temperature in the present study was 26.5 to 28.3°C. There was no marked difference in temperature between control and probiotics treated ponds. Muthu (1980) and Karthikeyan (1994) recommended a salinity range of 10-35 ppt was ideal for *P. monodon* culture.

Oxygen dissolved in the culture medium is an important factor not only for the respiration of aquatic organisms but also to maintain favorable chemical and hygienic environment of the water body. When oxygen level is very low and anaerobic conditions exist, nitrate is reduced by denitrifies into ammonia, which will be toxic. Continuous aeration was provided during the present study and therefore the oxygen level did not vary between the control and experimental ponds and was in the range of 4.5-4.8 mg L^{-1}. The optimum range of pH 6.8 to 8.7 was maintained for maximum growth and production of penaid species (Ramanathan *et al*., 2005). Reddy (2000) was recommended pH of 7.5 to 8.5 for *P. monodon* culture.

Ammonia is the principal end product of protein catabolism of organisms and it is excreted through gills. It is also formed by decay of organic matter. Under anaerobic conditions, sulphate is also reduced to ammonia. In the present study, there was no such change because the culture medium was continuously aerated. Collapse of cyanobacteria bloom also leads to increase ammonia. Under farm conditions, the ammonia level should be less than 1 ppm. In the present study both in the control and experimental setups, the levels were well below this mark. However it could be seen that there was a built up of ammonia in the control tanks with the progress of the experiments. But in the experimental ponds, the ammonia levels decreased during the course of the experiment. This is mainly due to the microorganisms (nitrosomonas) present in the probiotics, which initiate nitrosification. Due to this process ammonia is converted into nitrite, which is further acted upon by the nitrobacter and converted as nitrate through the process of nitrification. In control ponds the shrimps were affected by black gill disease however this is absent in probiotics treated ponds. This is mainly due to the absence of probiotics in the control ponds. Ravi *et al*. (1998) described the benefits of probiotics in maintaining water quality and enhancing growth rate in Indian White Prawn, *P. indicus*.

Shrimp aquaculture production in much of the world is depressed by disease, particularly caused by luminous Vibrio and/or viruses. Abundance of luminous Vibrio strains decreased in ponds and tanks where specially selected, probiotic strains of *Bacillus* species were added. A farm on Negros, in the Philippines, which had been devastated by luminous Vibrio disease while using heavy doses of antibiotics in feed, achieved survival of 80-100% of shrimp in all ponds treated with probiotics (Moriarty, 1997). The probiotics treated ponds in the present study had either a very low abundance or a complete absence of luminous and very good survival was achieved. This result is comparable with the study of Dalmin *et al*. (2001). Colonization of the gastrointestinal tract of animals by probiotics is possible only after birth and before the definitive installation of a very competitive indigenous microbiota. After this installation, only the addition of high doses of probiotic provokes its artificial and temporary dominance. In mature animals, the population of probiotic organisms in the gastrointestinal tract shows a sharp decrease (Fuller, 1992). Application of microbial supplement in the treated ponds hindered the growth of *Vibrio* sp. like *V. alginolyticus* and *V. harveyi* because of the colonization of the beneficial microbes like *Bacillus* sp., *Pseudomonas* sp., *Lactobacillus* sp. and *Saccharomyces* sp. in the shrimp gut. Since the shrimps in the control pond were dominated with *Vibrio* spp., which caused vibriosis can be attributed as the reason for low survival when compared with the treated one. The occurrence of vibriosis in the control pond was concluded by presence of luminescence in the nighttime and occurrence of dead animals in the check tray. In the present study, white gut disease was reported in control ponds, which ultimately leads fungal disease. The shrimps failed to take feed once the animals are affected by white gut disease, naturally animals activities slowed down and become sluggishness finally leads to fungal disease. The white gut and fungal disease are not observed in probiotics treated ponds.

The general conclusion obtained from the present study is that the probiotics plays a vital role in growth, survival and disease resistance of the animal by maintaining good water quality parameters throughout the culture period. It is clear from the microbial load data that *Vibrio* sp. is dominant in the control ponds. Besides *Vibrio* sp., the shrimps in the control ponds also affected by black gill, white gut and fungal diseases.

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