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Effect of probiotics on the growth and survival of *Penaeus monodon* (Fabricius)

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Abstract: The culture of shrimps received maximum importance due to its unique taste, high nutritive value and persistent demand in the world market. In recent years, the diseases of shrimp hindered the development of shrimp culture. In the present study an attempt has been made to culture the giant tiger shrimp, *P.monodon* in 12 ponds each with 0.6ha near Vellapallam village of Sirkali taluk, Nagai district, Tamilnadu. The use of probiotic bacteria in aquaculture has tremendous scope and the study of the application of probiotics in aquaculture has a glorious future. In the present study the probiotics, *viz*., Super biotic, Super Ps, Zymetin and Mutagen was applied in culture ponds ("A" section) of *P. monodon* which is compared with "B"section ponds without probiotic treatment. Growth and survival rate of the ponds which was applied ("A" section) with probiotics was higher than that of "B" section ponds. The bacterial population decreased at the end of culture in "A" section ponds, but in "B" section the bacterial population was not showing a significant decrease. Black gill, white gut and fungal diseases were recorded in "B" section ponds. But these diseases were not that much reported in probiotic treated ("A" section) ponds. The general conclusion obtained from the present study is that the probiotic 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 bacterial colony data that green colony dominated only in the "B" section ponds. So it can be recommended that the probiotics can be very well utilized for the shrimp ponds to get maximum growth and survival.

Key words: Super biotic, shrimp, black gill, white gut, Sirkali

INTRODUCTION

In India, commercial shrimp culture started gaining roots only during the mid-eighties. It was relatively late start in India: by this time, shrimp farming has reached peak in most of the neighboring Asian countries; in some the viral disease had already taken a heavy tool. The boom period of shrimp culture in India started in 1990 and the bust came in 1995-96. The growth of the industry off late witnessed two major setbacks. The first important bottleneck is viral disease outbreak (Karuna *et al*., 1994), which withered the confidence of entrepreneurs and financial institutions. Another vital problem is pollution. The organic load in term of unutilized feed due to excessive feeding (due to over feeding), faecal matter released by shrimps and dead algae, settle at the bottom of the pond contribute pollution of the pond bottom (Yew-Hu, 1992). India is a large country with great potential for aquaculture, but the application and development of the probiotics in Indian aquaculture is very limited when compared to other countries. In recent years, the diseases of shrimp hindered the development of shrimp culture. Based on the previous research results on probiotics suggest that the use of probiotic bacteria in aquaculture has tremendous scope and the study of the application of probiotics in aquaculture have a glorious future. The role of probiotics bacteria in small culture is studied but commercial level is not that much reported especially in giant tiger shrimp, *P. monodon*. Hence the beneficial effect of probiotics on the commercial culture of Indian major candidate shrimp, *P. monodon* is very much need of the hour. Therefore, the present study was aimed to examine the effect of a probiotics viz., Super biotic, super Ps, Zymetin, Mutagen (CP aquaculture India pvt Ltd) on the shrimp, *P.monodon* culture was studied.

MATERIALS AND METHODS

The farm is located on the northern bank of Uppanar estuary in Vellapallam. The farm is situated about 7 km away from Sirkali. The southern side of the farm is elevated to a height of 3.5 m from Uppanar estuary. The total area covered is 8.8 ha of which water spread is about 7.2 ha. Totally thirteen pond is there; each culture pond size is 6 ha. One pond act as reservoir (1.0ha). The ponds are rectangular in shape. The culture is semi intensive type with stocking densities of 12 pls/m^2. The depth of pond was 1.2 m and pond bed slope 30 cm from inlet point towards outlet. The culture was carried out from May to July 2008.

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Initially all the ponds of the present study was allowed to dry and crack to increase the capacity of oxidation of hydrogen sulphide and to eliminate the fish eggs, crab larvae and other predators. Then pond bottom was scrapped 2 to 4 cm by using a tractor blade to avoid top soil. Then the pond bottom was ploughed horizontally and vertically a depth of 30 cm to remove the obnoxious gases, oxygenate the bottom soil, discolouration of the black soil to remove the hydrogen sulphide odour and to increase the fertility. The soil pH was recorded in the ponds with the help of cone type pH meter. The average pH was calculated from the collected data and required amount of lime was applied to neutralize the acid soil condition and increases the availability of nutrient.

Water culture is one of the important processes during the culture period. In deed, if the PLs are stocked into a pond with poor algal populations, they will become stressed. That not only greatly reduces PL growth, but weakens the animals, making them much more prone to disease and subsequent death. For blooming, the pond is fertilized with inorganic or organic fertilizers.

The initial water levels in all ponds were maintained at 120 cm level (Twelve culture pond and one reservoir pond). After filling, one day was permitted for sedimentation. After sedimentation process, chlorination was done for all ponds (dosage for 1 ha for every 1meter water level/600 kg chlorine). Blooming process was started after 72 hrs of chlorination.

The organic fertilizers such as rice bran; groundnut oil cake, dry cow dung and yeast were soaked over night and applied the extract to all the ponds. The same procedure was continued for three days. After three days the watercolour turned to light green. Then water level was maintained to 120 cm of the ponds and added urea and super phosphate to improve the primary production. Fertilization enhanced the optimal algal bloom in the ponds and the transparency in the ponds ranged from 33 to 36 cm. During the culture period four types of lime was used to maintain the pH and algal bloom. During the water culture chain dragging was done daily before stocking of seeds.

The *P. monodon* (PL16 pass the PCR test and stress test) seeds were purchased from Venture hatchery, Marakkanam and were transported in oxygenated double-layered polythene bags with crushed ice packs between inner and outer covers of the bag and packed in a carton. The seeds were brought to the farm site and bags were kept in the pond water for some time to adjust the temperature. Then the pond water was added slowly into the seed bag to adjust the salinity and pH. Subsequently the seeds were released slowly in to the ponds. The stocking density per pond was 12/m^2 (72,000 PLs / pond).

The "A" section ponds (A1, A2, A3, A4, A5, A6) was treated with both water and soil probiotics (super biotic–water and super Ps – soil probiotics, manufactured by Charoen pokhpond aquaculture India Pvt. Ltd, Chennai). For "B" section ponds (B1, B2, B3, B4, B5, B6,) were free from probiotics treatment (Table 1). For water quality management 1.5 kg of super biotic mixed with 200liter water (8 hour fermentation process was done by aerating through aquarium aerator). It was broadcasted throughout the pond during morning hours in an interval of 15 days. However, it was only 10 days once after 50th DOC onwards. For bottom soil quality management 10 litre super Ps probiotic was mixed in 50 kg of dry sand then it was broadcasted throughout the pond during morning hours in an interval of 15 days. However, it was only 10 days once after 50th DOC onwards.

Table 1: Dosage of water and soil probiotics in ponds

Days of culture Before stocking	Ponds (A1,A2,A3,A4,A5,A6) Water probiotics (Super biotic)1.5 kg	Ponds (A1,A2,A3,A4,A5,A6) Soil probiotics (Super Ps) 10 lit
1	1.5 kg	10 lit
15	1.5 kg	10 lit
30	1.5 kg	10 lit
45	1.5 kg	10 lit
55	1.5 kg	10 lit
65	1.5 kg	10 lit
75	1.5 kg	10 lit
85	1.5 kg	10 lit
95	1.5 kg	10 lit
105	1.5 kg	10 lit
115	1.5 kg	10 lit
125	1.5 kg	10 lit

The water quality parameters were recorded in control and probiotics treated ponds regularly. The water level was measured by using a standard scale with cm marking. The water salinity was measured by using a hand refractometer (Erma-Japan). The pH of the pond water was measured by using electronic pH pen manufactured by Hanna Instrumental Company, Japan. Water temperature was measured in the pond itself using a standard centigrade thermometer. Dissolved oxygen meter estimated the dissolved oxygen. Transparency was measured in terms of light penetration using a secchi disc.

During the first 3-4 weeks of culture, water exchange is not required. Subsequently the water was exchanged five days once or depends upon the water and shrimp quality. The purpose of the water exchange is to maintain the water quality and also to stimulate moulting of the shrimp, resulting in acceleration of growth and production.

Feed management plays a major role in the shrimp culture. CP (Novo) feed was used during the entire cycle and distributed manually with the help of a boat. During the first month after stocking, feeding rates were based on estimated survival and feeding tables and distributed four times per day. After 30th DOC, daily rations were adjusted using feed trays and increased to five times per day there after.

The use of feed trays is extremely important in the control of feeding. They provide information regarding the feed consumption, the health and survival of the

Table 2: Dosage of feed probiotic (1or 2 meal / day / sugar binder)

Doc	Super biotic (4 hours fermentation must)	Zymetin	Mutagen
15-20	20 gr/kg		
20-30	10 gr/kg		
31-40		10 gr/kg	
41-50		10 gr/kg	
51-60		10 gr/kg	10 gr/kg
61-70		10 gr/kg	10 gr/kg
71-80	10 gr/kg		10 gr/kg
81-90	10 gr/kg		10 gr/kg
91-100	10 gr/kg		10 gr/kg
101-110			10 gr/kg
110-120	10 gr/kg	10 gr/kg	10 gr/kg
120-140	10 gr/kg	10 gr/kg	

shrimp and also the condition of the pond bottom. If the shrimp consumed all the feed within the given time, we have to reduce the feed to prevent over feeding. Left over feed can cause the pond bottom to decay and water becomes deteriorated easily, the shrimp will be weak and stressed. They will also avoid feeding and easily get sick and eventually die. As in water and soil probiotics feed probiotics were also used in the present study. The dosage and duration details of the feed probiotics are presented in Table 2.

Cast net was used to measure the growth rate of shrimps. The first sampling was taken after 40th days of culture and number of individuals and the average body weights were recorded in each sampling. Five hauls were made in each pond. Healthiness, survival rate, Average Body weight (ABW) and Average daily growth (ADG) of the animals were estimated. Sampling was regularly performed every ten days until harvest. Ammonia level was monitored regularly.

For microbial analysis, the water and sediment samples were collected separately from different parts of the pond 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* spp TCBS media was obtained from Hi-media, Mumbai.

Isolation and enumeration: Enumeration of the microbes was done by adopting spread plate method. In this method, sterile media were poured into petri dishes aseptically and allowed to solidify. One milliliter of serially diluted sample was pipette out into sterile petri-dish. It was made spread in the plate first by rotating it in clockwise and then anti-clockwise directions for three times and then spread with the help of a 'L'-rod. The plates were incubated in an inverted position at 28±2ºC.

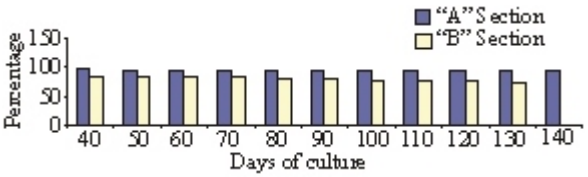

Fig. 1: Survival rate of *P. monodon* reared in probiotics and control ponds

After the incubation period of 2 to 3 days colonies were counted. The plates were examined and counted the number of colonies per plate. The microbial load in the given sample was calculated using the following formula and it is expressed as Colony Forming Units (CFU) per gram of the sample.

A bag net was fitted on outlet canal with a 20 numbers mesh of width 1m and length of 4 m. The water level in the ponds was reduced from 1m to 60 cm and then out let was opened and shrimps were caught and collected.

RESULTS

The salinity was recorded maximum (28 ppt) in the month of June and minimum (10 ppt) was during March. In general minimum salinity was recorded in probiotics treated ponds and maximum was in control ponds. The pH was recorded during the culture period from Feb to June 2007. The average pH was between 7.6 to 8.2. The pH was minimum in all months of the probiotics treated ponds. However, it was maximum in control ponds. The dissolved oxygen was recorded maximum (4.2 ppm) during the month of March and minimum (3.2 ppm) was during June. It is maximum in probiotics treated ponds and minimum in control ponds. The temperature did not show much variation in probiotics treated and control (25-30ºC) ponds. The transparency also did not show much difference in probiotics and control ponds (35-45 cm). The ammonia was totally absent in probiotics treated ponds of all months. But the ammonia was recorded maximum (0.4) in the month of June and minimum (0.2) was during May in control ponds.

Survival rate of probiotics treated ponds was higher than that of control ponds. At the end of culture period the survival rate of probiotics treated ponds was maximum (95.2%) and minimum in control ponds (75.1%) (Fig. 1).

Maximum growth was observed in probiotics treated ponds during each sampling interval and by the end of the experiment than control ponds (Table 3)

The bacterial population changed during every sampling. In general, the bacterial population of sediment was higher than that of water in both "A" and "B" section ponds. The bacterial population decreased at the end of culture in probiotics treated ponds, but the load of *Vibrio* spp when compared with THB in control ponds was not showing a significant decrease. *Vibrio* sp. was

Table 3: Average body weight of *P. monodon* reared in the control and probiotics treated ponds

PONDS	Days of culture (DOC)										
	40	50	60	70	80	90	100	111	122	130	141
	Average body weight (ABW) (g)										
Probiotics treated	6.8	10	12.6	15.2	17.9	20.7	22.4	25.1	28.4	31.2	34.1
Control	5.1	7	9.2	11.4	13.3	15.6	18.2	20.3	22.5	*	*

contributing much in the THB load of the control ponds. In general, the probiotics treated ponds was showing a reduced count of *Vibrio* sp. (Fig. 2 and 3)

DISCUSSION

There has been a considerable increase in the culture of brackish water shrimp due to its taste, market demand both national and international markets. In order to prevent many problems due to shrimp culture, sustainable shrimp farming is the need of the hour. Ideal pond size for shrimp culture was 1 or less than 1 ha (Ramanathan *et al.*, 2005). In the present investigation also 12 ponds were used for shrimp culture and each pond size was 0.6 ha. Even though shrimps are bottom dwelling organisms, the depth and volume of water in a pond has certain physical and biological consequences.

The present study was undertaken to ascertain the efficiency of probiotics (Super biotic, Super Ps, Zymetin, Mutagen) on the growth and survival of the most important cultivable shrimp species, *P. monodon* in addition to its influence on important water quality parameters. Important water quality parameters monitored during the study were, salinity, dissolved oxygen, pH and ammonia levels. The volumes of water behave like a buffer, which prevents weather fluctuations from influencing the environment in which shrimp lives. The ideal water depth is between 0.8 to 1.5 m depending upon the stage of culture. It is recommended that a minimum depth of 1m be maintained at operational level. In the present study 100 cm water level was maintained in all ponds throughout the culture period. When a pond is ready for operation, the optimum stocking density of seeds in a pond determined in accordance with the production capacity of the pond and the culture system, which included the soil and water quality, food availability and seasonal variations, target production and farmers experience (Ramanathan *at al.*, 2005). The stocking density between 10-20 PLs/m^2 is ideal for successful shrimp farms (Ramanathan *et al.*, 2005; Soundarapandian and Gunalan, 2008). In the present study the seeds were stocked at the stocking density of 12/ m^2 in all ponds.

The maintenance of good water quality is essential for optimum growth and survival of shrimps. The levels of physical, chemical and biological parameters control the quality of pond waters. The level of metabolites in pond water can have an adverse effect on the growth. Good water quality is characterized by adequate oxygen and limited level of metabolites. Excess feed, faecal

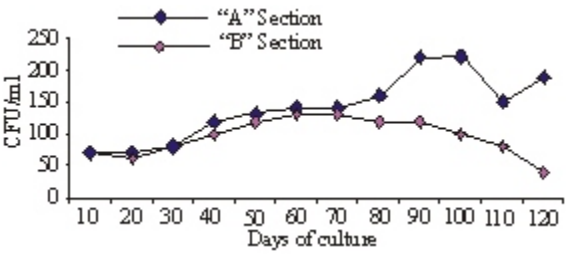

Fig. 2: Yellow colony population in control and probiotics treated ponds

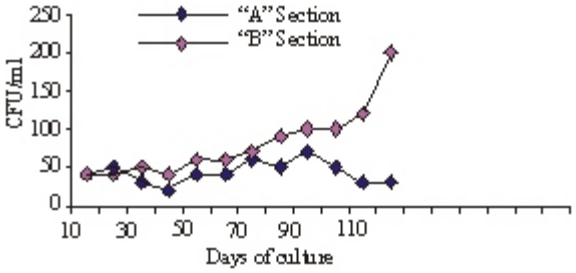

Fig. 3: Green colony population in control and probiotics treated ponds

matter and metabolites will exert tremendous influence on the water quality of the shrimp ponds. Hence critical water quality parameters are to be monitored carefully as adverse conditions may be disastrous effect on the growing shrimps (Ramanathan *et al.*, 2005; Soundarapandian and Gunalan, 2008).

Salinity is important parameters to control growth and survival of shrimps. Even though, *P. monodon* is euryhaline animals; it is comfortable when exposed to optimum salinity. At high salinity the shrimps will grow slowly but they are healthy and resistance to diseases. If the salinity is low the shell will be weak and prone to diseases. The salinity of the present study was maintained 10-28 ppt in all ponds. Muthu (1980), Soundarapandian and Gunalan (2008) and Karthikeyan (1994) recommended a salinity range of 10-35 ppt was ideal for *P. monodon* culture. While Chanratchkool *et al.* (1994) maintained the salinity of 10-30 ppt. Chen (1980) opined that salinity ranges of 15-20 ppt are optimal for culture of *P. monodon*. There are few reports (Shivappa and Hambry, 1997; Ramakrishna, 2000; Collins and Russel, 2003), which stated that *P. monodon* adapted quite well in freshwater conditions also because of its wide range of salinity tolerance.

pH is one of the vital environmental characteristics, which decides the survival and growth of shrimp under culture; it also affects the metabolism and other physiological process of shrimps. The optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production (Ramanathan *et al*., 2005). In the present study pH was ranging between 7.6 to 8.2 for the probiotics treated and control ponds. Saha *et al.* (1999) noticed the pH of 8.11 to 8.67 in low saline ponds. Ramakrishna (2000) and Soundarapandian and Gunalan (2008) was recommended pH of 7.5 to 8.5 for *P. monodon* culture. The pH of pond water is influenced by many factors, including pH of source waters and acidity of bottom soil and shrimp culture inputs and biological activity. The most common cause of low pH in water is acidic bottom soil, liming can be used to reduce soil acidity. In most common cause of high pH is high rate of photosynthesis by dense phytoplankton blooms. When pH is high water exchange will be better choice (Boyd, 2001).

Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. Oxygen affects the solubility and availability of many nutrients. Low levels of dissolved oxygen can cause damages in oxidation state of substances from the oxidized to the reduced form. Lack of dissolved oxygen can be directly harmful to shrimps and cause a substantial increase in the level of toxic metabolites. Low-level of oxygen tension hampers metabolic performances in shrimp and can be reduce growth and moulting and cause mortality (Gilles, 2001). The dissolved oxygen in all the culture ponds in the present study was ranging between 3.2 to 4.2 ppm.

Water temperature is probably the most important environmental variables in shrimp cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival. In general, a sudden change of temperature affects the shrimp immune system. The optimum range of temperature for the black tiger shrimp is between 28 to 30ºC (Ramanathan *et al*., 2005). The temperature in the present study was 25 to 30ºC and the low temperature 25ºC was observed due to cloudy weather. The optimum range of temperature of *P. monodon* was between 26 to 33ºC (Boyd, 1995; Soundarapandian and Gunalan, 2008) and temperature range of 28 to 33ºC supports normal growth (MPEDA, 2006) as observed in the present study.

The transparency is mainly depends on the presence of phytoplankton. The secchi disc reading should be between 30-40 cm (MPEDA, 2006). The optimum range of secchi disc reading is between 30 to 60 cm to the juvenile stage and between 25 to 40 cm to the sub adult and final stage. The transparency of the present study is 35 to 45 cm. Ramakrishna (2000) and Soundarapandian and Gunalan (2008) also observed similar transparencies (25-50 cm) for their studies on shrimp culture. The reading less than 30 cm mean that the phytoplankton density is high. If it is more than 40 cm indicates, low population of phytoplankton. For the growth of phytoplankton adequate quality of sunlight is needed. Due to low intensity of light during the culture period, the plankton bloom was less. Hence, the transparency was more.

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 condition, sulphate is also reduced to ammonia. In present study, there was no such change because the culture medium was continuously aerated. Collapse of cyanobacteria bloom also leads to increase ammonia. When the ammonia concentration in the culture medium increases, excretion of ammonia by cultured organisms decreases. Under farm conditions, the ammonia level should be less than 1 ppm. In the present study, the level of ammonia was well below this mark in “B” ponds. But in the “A” section ponds no ammonia problem was encountered. This is mainly due to the microorganisms (*Nitosomonas*) 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 nitrification. In “B” section ponds the shrimps were affected by black gill and tail rot diseases, however this is absent in probiotic used “A” section ponds. This is mainly due to the absence of probiotic in the “B” section ponds. Ravi *et al*. (1998) already described the benefits of probiotics in maintaining water quality and enhancing growth rate in Indian white shrimp, *P. indicus*.

Shrimp aquaculture production in much of the world is depressed by disease, particularly caused by luminous *Vibrio* and/or viruses. Antibiotics, which have been used in large quantities, are in many cases ineffective, or result in increases in virulence of pathogens and, furthermore, are cause for concern in promoting transfer of antibiotic resistance to human pathogens. Probiotic technology provides a solution to these problems. The microbial species composition in hatchery tanks or adding selected bacterial species to displace deleterious normal bacteria can change large aquaculture ponds. Virulence of luminous of *Vibrio* species can be controlled in this manner. 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 antibiotic in feed, achieved survival of 80-100% of shrimp in all ponds treated with probiotics (Moriarty, 1996).

Ruangpan (1991) reported in their study that the high abundance of luminescent *Vibrio* is consistent with occurrence of disease and poor or zero harvest results. *V.*

harveyi, a pathogen of *P. monodon* that causes severe losses (Baticados *et al*., 1990). The farm, which used the superbiotic probiotic bacteria, had either a very low abundance or a complete absence of luminous *Vibrio* in pond water and very good harvest result. This consistent and high productivity occurred, even though the proportion of luminescent *Vibrio* in the pond water was high in the sea water source, and the abundance of total green colony in the pond water was higher than in the water source. Furthermore, luminescent *Vibrio* were completely absent at all stages of grow out from the pond sediment in the presence of the superbiotic *Bacillius* species. In the present study also the shrimps were healthier in ponds with super biotic, super Ps, Zymetin and Mutagen.

The probiotics treated ("A" section) ponds in the present study had either a very low abundance or a complete absence of luminous bacteria 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 only after birth, and before the definitive installation of a very competitive indigenous micribiota. After this installation, only the addition of 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 probiotic ponds hindered the growth of Vibrio spp, like *V. alginolyticus* and V. harveyi because of the colonization of the beneficial microbes like *Bacillus* sp., *Pseudomonas* sp., *Lactobacillus* sp. and *Saccharomuces* sp. in the shrimp gut. Since the shrimps in the "B" section ponds were dominated with green colony, which caused Vibriosis can be attributed as the reason for low survival when compared with the "A" section ponds. This was evident from the presence of higher load of green colony, in the water and sediment of control ponds than in the probiotics used ponds. The occurrence of green colony in the "B" section ponds 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 also reported in "B" section ponds, which ultimately leads fungal diseases, naturally animal's activities slowed down and become sluggishness. The white gut and fungal disease are not observed in probiotics treated ("A" section) 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 green colony is dominant in the "B" section (without probiotics) ponds. Besides green colony, the shrimps in the "B" section ponds also affected by black gill, white gut and fungal diseases.

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