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EFFECT OF PROBIOTICS ON GROWTH AND SURVIVAL OF POST LARVAE OF GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN)

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Abstract: The trend of using probiotics in aquaculture is increasing due to research results indicating their ability to increase production and prevent disease in farm animals. The development of suitable probiotics for bio-control in aquaculture would result in less reliance on chemicals and antibiotics and result in a better environment. The production of high quality larvae in scampi is very difficult, since the disease out breaks are the major constraints in scampi hatcheries. Controlling diseases through antibiotics has been widely criticized for their negative impacts, so alternate methods needed to be developed to produce high quality scampi larvae. Application of water probiotics, soil probiotics and reduction of salinity are the methods gaining importance in controlling pathogen in scampi larvae. The larval rearing tanks are divided into four chambers for our convenience. The probiotics treated tanks are considered as experimental and without probiotics treated are considered as control. The water probiotics namely C.P (Super Biotic) and Biodream (Probiotic contains *Bacillus sp*. and *Streptococcus sp.*) was added in the experimental tanks alone @ 5ppm each @ 5- 10 ppm from Zoea stage-II onwards and probiotics Zymatin @ 5ppm was added after the appearance of post larval stage in experimental tanks. In addition to probiotics 0.05 ppm of Treflan was also added both experimental and control tanks to prevent the fungal disease. Water temperature, pH, dissolved oxygen, NH_3 and H_2S were found better in trials with probiotics. In this experiment the survival rate of the nauplii of both control and experimental tanks were more or less same. The survival rate of Zoea reared in the experimental tanks was higher (84%) than that of control tanks (62%). The survival rate of all the post larval stages (PL 1- PL-10) was higher in experimental tanks than that of control tanks. Water quality parameters are also influenced by the addition of probiotics, in this experiment the dissolved oxygen was higher in experimental (6.10 ppm) and lower in control tank (5.50 ppm). The alkalinity (120 ppm) in both control and experimental tank is more or less similar. The Ammonia was higher in control tanks (0.15 ppm) compared experimental tanks (0.12 ppm).

Keywords: *Macrobrachium rosenbergii*, Probiotics, Biodream, Zymatin, Treflan, Larval Survival

INTRODUCTION

Macrobrachium rosenbergii (de Man, 1879) is a giant freshwater prawn is an important commercial species for its highest protein content, low cholesterol and for its delicious meat. In India, giant freshwater prawn is distributed throughout the revarine regions in India and mainly in the Southern region where average temperature does not change much between night and day times and seasons. Though the prawn hatchery technology has advanced over the decade, the hatchery production is more often hampered by severe mortalities caused mostly by bacteria. Increasing *Vibrio* production in larvae and rearing tanks water has been reported to reduce the survival rate of larvae and post larvae. *Luminous* species of *Vibrio harveyi* have been associated with causing mass mortalities in prawn hatcheries (Sunaryanto and Mariam, 1986; Karunasagar *et al.,* 1994) Ways of *Vibrio* entry into hatchery through seawater and feeds. The indiscriminate use of chemicals and drugs in control of diseases led to the inherence of antibiotic resistance bacteria. As a result, biological control has become an alternative means of preventing diseases outbreak. The mean principle of the biological control is to enhance the growth of beneficial micro-organisms (probiotics) that serve as an antagonosis of target species. In recent times a new biotechnological product called the 'probiotic' derived from the Greek words 'pro' and 'bios' (Sunaryanto and Mariam, 1986) it's finding application in culture systems for improving production. Fuller (1999) defined as a live micro-vial feed supplement which beneficially affects the host animal by improving its intestinal balance.

The use of probiotics as feed supplements of farm of the animals to increase growth and improvement of healthy by increasing its resistance to disease. The results of studies carried out that the some of the bacteria used in probiotics (Lactobacillus spp.) are capable of stimulating the immune system also (Fuller, 1992). Further, probiotics are non-pathogenic and non toxic organisms without undesirable side effects when administrated to aquatic organisms (Balcazar *et al.,* 2006). Application of probiotics was found to improve the water quality parameters, improve the condition of pond bottom and stimulate the immunity of the organisms (Krishna *et al.,* 2009). According to Fuller (1992) immunity may be improved by the probiotics in

the following ways; increasing macrophage activity, showing enhanced ability to phagocytic micro-organisms or carbon particles, increasing the production of systematic antibodies usually of immunoglobulin and interferon (a nonspecific antiviral agent); increasing local antibodies and mucus surfaces at the gut wall. The role of probiotic bacteria in farming of prawn was attempted but such studies in hatchery are not that much reported especially in giant freshwater prawn, *Macrobrachium rosenbergii.* According to Maeda (1999) that probiotics led to new strategy for prevention of disease outbreak and to achieve improvement of seed quality. However, effectiveness of probiotics in aquaculture is still under observations in different regions and different cultured species. The use of microbial probiotics in aquaculture is now widely accepted (Verschure *et al.,* 2000; Iricanto and Austin, 2002; Wang *et al.,* 2005; Vine *et al.,* 2006; Wang, 2007). Hence, the beneficial effect of probiotics on the commercial seed production of Indian freshwater prawn, *M. rosenbergii* is the need of the hour. Therefore, the present study was aimed to examine the effects of probiotics on the hatchery seed production of giant freshwater prawn, *M. rosenbergii* was studied.

MATERIALS AND METHODS

The present study was carried out in a freshwater hatchery (Kakati Aqua Tech Pvt Ltd.,) located at Undavalli village near Vijayawada, Andhra Pradesh, India. The hatchery is situated about 4 km north of Vijayawada. This hatchery is well designed, equipped and maintained for commercial production of *M. rosenbergii* seeds for the past 15 years. There are five production units operating simultaneously and the annual production is around 150-250 million seeds. The sea water for the hatchery was transported from Manginipudi beech located near Machilipatnum. The suction point is located about 20 m from the shoreline and it is free from pollutants. The sea water was stored in settlement tank for a few days to control turbidly of water. After one week of ageing, this water was filtered with 100 micron net for eradication of unwanted species present in sea water. Then sea water was stored in another storage tank. From here water was pumped in to mixing tank to make up require amount of salinity for larval rearing. Here sea water was mixed with freshly collected and treated freshwater, it is collected from river Krishna and then sea water was make it up to 12 ppt. Now the water was lifted into chlorination tank using a 5 HP motor. Chlorination was done with 20 ppm chlorine. After 24hrs, the chlorinated water was stored in overhead tank after passing through activated carbon filter. Subsequently the filtered water was passed through rapid sand filter and then passed from cartridge filter (0.5 to 1.0 micro meter mesh size) and UV filter before filling into larval rearing tanks. The residual chlorine available in the treated water was determined with chlorine test kits by using O-Tolidine. After knowing the availability of excess chlorine in treated sea water sodium thio-sulphate (hypo) was used to neutralize the residual chlorine. The chelating agent, EDTA (5-10 ppm) was added in treated water to ensure clear seawater (12 ppt). A 15 HP air blower and a 10 HP standby provided continuous supply of air. The air generated by the blower was supplied to individual tank through PVC pipes. The broodstock were collected from the culture ponds (Bhimavaram and Nellore) and transported to the hatchery in oxygenated containers (this container was specially made for broodstock transportation). Initially the brooders were quarantined and maintained in a receiving section. Subsequently they were bathed in 10 ppm $Kmno_4$ washed in freshwater and maintained in separate thermacol boxes with aeration. Samples were experienced to PCR test of detect the white muscle syndrome virus. Only negative brooders were stocked in brooder maintenance tanks (Brood stock tanks) and they were fed with rice bran, mussel meat and cow liver in alternate days at twice in a day. 80% of water was exchanged daily. The brooders were then transferred to the hatching tanks and treated with water probiotics (Super Biotic) 20 ppm to control the luminous bacteria. Before stocking larvae (Zoea-I), the larval rearing tanks were first filled with 12 ppt mixed sea water and all the water quality parameters were checked.

The larval rearing tanks are divided into four chambers for our convenience. The probiotics treated tanks are considered as experimental and without probiotics treated are considered as control. The water probiotics namely CP Super Biotic and Biodream (Probiotic contains *Bacillus sp*. and *Streptococcus sp.*) was added in the experimental tanks alone @ 5ppm each. In addition to probiotics 0.05 ppm of Treflan was also added both experimental and control tanks to prevent the fungal disease. The newly hatched larvae from hatching tank was collected with the help of 100 micron nets and stocked in control and experimental tanks @ 2 lakes per tank (200 per L). Any temperature fluctuations occur during the period operation, heat chambers are used to standardize the temperature and maximize up to $3^{o}C$ in winter season. After twenty four hours of stocking, the larvae converted into Zoea-II in experimental tanks. First feeding was started when the Zoea-II. The zoeal stages (II to V) were fed with newly hatched *Artemia sp*. @ 3-9 nauplii per each larva in twice daily both control and experimental tanks. The zoea VI to XI stages was fed with supplementary diet (egg custard) along with *Artemia* for both control and experimental tanks. Post larval stages were fed exclusively on freshly hatched live Artemia nauplii @ 5 nos per PL per feeding along with supplementary diet. In general, the animal in the experimental tanks consumed more than control tanks. The water

probiotics, Super PS (C.P Aquaculture Pvt, Ltd) was added daily @ 5- 10 ppm from zoeal stage-II onwards and probiotics Zymatin @ 5ppm was added after the appearance of post larval stage in experimental tanks. The commercial *Artemia* cysts were aerated for half an hour prior to de-capsulation. Two liters of liquid chlorine and 120 ml of Sodium hydroxide solution were mixed well. Cysts were transferred to this solution with constant stirring below 40°C. The colour changed from dark brown to orange indicated that the cyst underwent de-capsulation. At this stage, cysts were transferred to *Artemia* hatching tanks after through washing in freshwater until chlorine smell disappears. Continuous aeration and illumination with a 60V lamp was provided to accelerate the hatching process. After 24 hrs, using phototactic behavior of the *Artemia* nauplii collected hatched nauplii. Water exchange was done from the Zoea-II stage onwards. Using mesh size 0.5 mm reduced up to 50% of the water. Once post larvae appeared, the salinity was reduced slowly and maintained up to '0' (zero) ppt salinity especially in experimental tanks, because the effect of probiotics was good in low salinity.

The water quality parameters of the probiotics treated and control tanks regularly monitored. Water quality parameters such as salinity, temperature, pH, dissolved oxygen, ammonia and alkalinity were estimated daily in the morning hours. The water salinity was measured by using a hard refract meter (Erma-Japan). Dissolved oxygen meter estimated the dissolved oxygen. First using sodium bicarbonate after standardized the Sulphuric acid and then the samples were titrated with the standardized Sulphuric acid by using Methyl red indicator. Ammonia level was monitored regularly adopting by ammonia kits. Water samples were taken from the rearing tanks (both control and experimental tanks) and various larval stages. Total plate count (PC) was performed by plating serial tenfold dilution in Tryptic Soy Agar contains 1% NaCl (TSAS) by the spread plate method. Each sample was plated in duplicate. The plates were incubated at 29.1°C and observed after 24 hrs. To determine luminous bacteria counts, plates were observed in a dark room. After reaching PL 10, then it was ready to sale. Before the dispatch of post larvae, the healthiness was assessed by PCR test and quality control. To maintain the reputation of the hatchery only the negative seed were sold to the farmers.

RESULTS

The salinity was maintained at 12 ppt for both control and experimental tanks. The pH of the control tank was 7.8 and the experimental tank was little more (8.0). The temperature (30±1.5°C) of both control and experimental tanks was similar. The dissolved oxygen was higher in experimental (6.10 ppm) and lower in control tank (5.50 ppm). The alkalinity (120 ppm) in both control and experimental tank is more or less similar. The Ammonia was higher in control tanks (0.15 ppm) rather than experimental tanks (0.12 ppm) (Table 1 and Fig1). The survival rate of the nauplii of both control and experimental tanks were more or less same. The survival rate of zoea reared in the experimental tanks was higher (84%) than that of control tanks (62%). The survival rate of all the post larval stages (PL 1- PL-10) was higher in experimental tanks than that of control tanks (Table-2 and 3 and Fig-2 and 3). Bacterial lode also plays a vital role in the production of post larvae. In the present study more number of green colonies were noticed in control tank and very less amount of green colonies were found in experimental tank (Table-4 and Fig-4)

Table.1: Water quality parameters of both control and experimental larval rearing tanks of *M.rosenbergii.*

Water Quality parameters	Control	Experimental
Salinity (ppt)	12±1	12±0
pH	7.8±0.17	8.0±0.11
Water Temperature (° C)	30±0.4	30 ± 0.2
Dissolved Oxygen (ml / L)	5.50±0.13	6.10±0.13
Alkalinity (ppm)	120±8.5	120±6.5
Ammonia (mg/L)	0.15±0.01	0.12±0.01

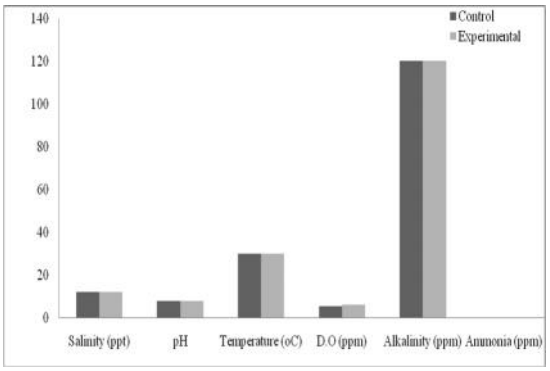

Fig.1: Water quality parameters of both control and experimental larval rearing tanks of *Macrobrachium rosenbergii.*

Table.2: Survival rate of different larval stages of *M. rosenbergii* reared in both control and experimental tanks

Stages	Survival rate (%)	
	Control	Experimental
Zoea	64±0.1	84±0.5
PL1	42±3.1	65±2.8
PL5	38±2.0	63±1.7
PL10	30±0.5	61±1.2

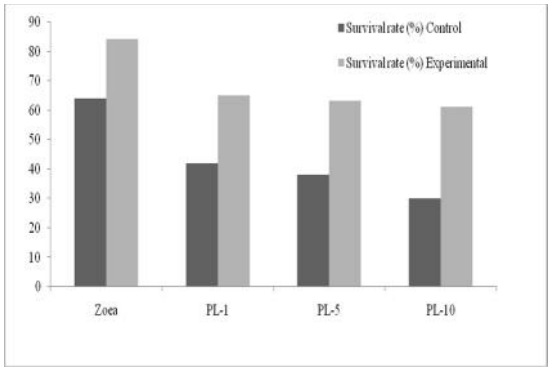

Fig.2: Survival rate of different larval stages of *M. rosenbergii* reared in both control and experimental tanks.

Table.3: Average length of *M. rosenbergii* post larvae reared in both control and experimental tanks

Stages	Length (mm)	
	Control	Experimental
PL1	5.52±0.4	5.98±0.1
PL5	5.61±0.4	6.15±1.4
PL10	5.97±0.5	6.34±1.8

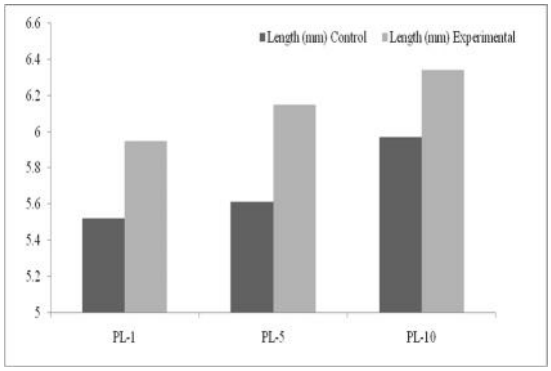

Fig.3: Average length of *M. rosenbergii* post larvae reared in both control and experimental tanks.

Table.4: Bacterial loads (*Vibrio*) in the rearing water and *M. rosenbergii* larval stages of both control and experimental tanks

Stages	Control (Green colony)		Experimental (Green colony)	
	Water (CFU/ml)	Larvae (CFU/ml)	Water (CFU/ml)	Larvae (CFU/ml)
Zoea	183.6	236.9	102	133.9
PL 1	226.6	669.5	76.5	154.5
PL 5	180.25	124.8	51.0	72.1
PL 10	>257.5	>156	20.4	41.2

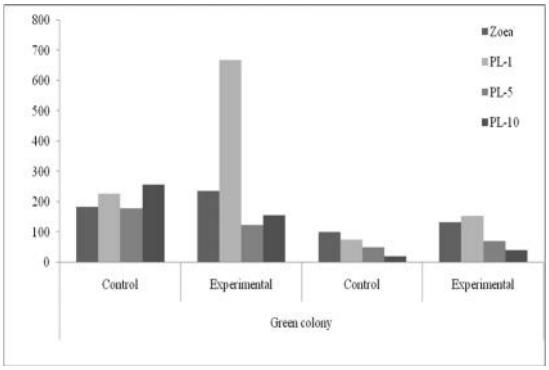

Fig.4: Bacterial loads (*Vibriosis*) in the rearing water and *M. rosenbergii* larval stages of both control and experimental tanks

DISCUSSION

There has been a considerable increase in the culture of freshwater prawn, *M. rosenbergii* due to its taste, market demand for both national and international markets. However, in scientific shrimp farming and heavy demand for healthy and quality seeds throughout the year. Thus successful scampi culture is reliant in stocking disease free, healthy seed raised in the hatcheries. The present study was undertaken to ascertain the efficiency of probiotics on the survival of the most important cultivable prawn larval farms, *M. rosenbergii* in addition to its influence on important water quality parameters. Important water quality parameters monitored during the present study were, salinity, pH, temperature, dissolved oxygen, alkalinity and ammonia. Water quality plays an important role in aquaculture production. A complete understanding of the relationship between water quality and aquatic productivity is absolutely essential for optimum growth and production. 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. Generally organisms are in a state of balance between potential disease causing microorganisms and their environment. Change in this equilibrium through the 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 such as probiotics is reported to effectively deal with these substances and that way helpful in maintaining water quality parameters thereby improving growth rate and survival rate. In the present study, the water quality parameters of hatchery, which is 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 rationale is that gram-positive bacteria are better converters of organic matter back to CO_2

that gram-negative bacteria. During the production cycle, high levels of gram-positive bacteria can minimize the buildup of dissolved and particulate organic carbon. A similar observation was found in the present study. The tank was treated with probiotics (Super Biotic, Biodream, super PC and Zymetin) was abundant with *Bacillus sp*. was showing a low level of ammonia, which was converted into nitrate through nitrite. Water temperature is probably the most important environmental variables for larval rearing, because it directly affects metabolism, oxygen consumption, growth, moulting and survival. In general, a sudden change of temperature affects the larval immune system. The optimum range of temperature for the giant freshwater prawn larvae were reared in between 28 to 32°C. The temperature in the present study was 30°C. There was no marked difference in temperature between control and experimental tanks of the present study. Salinity is the most important factor influencing many functional responses of the organisms as metabolism, growth, migration, osmotic behavior, reproduction etc. Marine organisms maintain their internal salt concentration (salt concentration of blood and body fluid) by osmoregulation. They need considerable energy for osmoregulation to maintain their internal salt balance in relation to the external medium in which they are living.

When nutrient energy is used for osmoregulation, the growth may be reduced. For a scampi hatchery the recommended salinity range is 10-14 ppt. In the present study, filtered seawater was used and the salinity was falls in the desired range of 12 ppt for control and experimental tanks. pH of the culture is having an important say on the metabolism and other physiological processes of an organisms. It changes with accumulation of residual feed, dead algae and excreta. In the optimum range of pH, ammonia will not cause much problem. Toxicity of nitrite and hydrogen sulphide is increased in low pH. This required range if pH for scampi larval culture is 7.8-8.2 ppm. In the present study the pH level was low in the control tank (7.8) and considerably more in experimental tank (8.0) but always falls on optimum range. The results attribute that probiotics present in the experimental tanks was helpful in maintaining the pH in desired level. Oxygen dissolved in the rearing 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. It controls many of the oxidation reactions and maintains aerobic conditions in water. When oxygen level is very low and an anaerobic condition exists, nitrate is reduced into ammonia, which will be toxic. This also increases the pH. Low-level of oxygen tension hampers metabolic performances in scampi larvae and can reduce growth and molting and cause mortality. Oxygen level in the culture medium was maintained in the desired range by aeration. Continuous aeration was done during the present study and therefore the oxygen level did not vary significantly between control and experimental tanks and was in the range of 5.5 – 6.1 ppm.

The alkalinity of both control and experimental tanks are more less similar (120 ppm) in the present study. Alkalinity values were found to be in the range of from 120-140 ppm in the larval rearing of *M. rosenbergii.* In the present study both control and experimental tanks the levels of ammonia were 0.1 5 ppm and 0.12 ppm respectively. The less amount of ammonia in the experimental tank in the probiotics used, which initiate nitrification. In the present study *Artemia nauplii* was used as a feed for all larval stages of M. rosenbergii From 10th day onwards larvae were fed with egg custard as a supplementary diet along with *Artemia.* The commercial production of scampi seed has been hampered by the occurrence of infectious and non-infectious diseases. A number of microbial agents are involved in causing mortalities. Bacterial diseases are considered to be a major cause of mortality in scampi hatcheries. Bacteria, particularly *Vibrio* spp. have been reported to cause larval mortalities in southern and southeastern Asia. *Vibrio harveyi, a luminous* species, has been implicated in a number of cases causing mass mortalities. Even though disease-spreading organisms are always present in the lower, they attack larvae only when the larvae are weak due to environmental stress or nutritional deficiency. A number of factors influence the micro flora in scampi hatcheries. The natural flora present in raw seawater may be altered by filtration, chlorination and other treatment methods adopted in the hatcheries. Otta *et al*., (2001) reported that the micro flora present in the chlorinated water represents those, which survived the treatment, and those derived from biofilms formed in the water pipes and tank surfaces. During larval rearing different micro flora may enter the hatchery system through the eggs and live feeds such as algae and *Artemia*. Otta *et al*. (2001) observed a qualitative change in the micro flora between intake sea water and hatchery water, with a clear dominance of *Vibrio* sp. in the hatchery water. To avoid bacteriological problems, commercial shrimp hatcheries adopt extensive water treatments, which include filtration, chlorination, and ultraviolet treatment. Traditionally, the control of bacterial problems in freshwater scampi hatcheries has relied on the chemical compounds. According to Weston (1996) proved that the abuse of antimicrobials can result in the development of resistant strains of bacteria. More recently probiotic organisms are being used. Probiotics, which is generally used in grow-out systems and finds use in hatcheries also. A commercial probiotic product with *Lactobacillus*, *Bacillus* and *Streptococcus* was used in the present study. Water quality and

microbial load was within the permissible limits. However luminous bacteria were observed in the hatchery systems in lower counts. Fox (1988) stated that the microbial community inside the gut of some animals confers some degree of resistance or protection against diseases. In natural populations of aquatic animals, the micro flora of the gut might reflect that of the aquatic environment. But in artificial larval rearing systems, the balance is altered by the use of disinfected water, microalgae, *Artemia* nauplii, rotifers and antibacterial.

The post larvae reared in relatively sterile environment of a hatchery do not grow well and show poor survival when exposed to complex microbial populations which makes them susceptible to environmental stress and potential pathogenic bacteria. Regular health checks and examination of animals were carried out in the hatchery at various stages of rearing. Post larval stages were examined regularly for necrosis, luminous bacteria, white muscle syndrome, endoparasites etc. Stress tests were also conducted. Only healthy larvae, which passed the health limits, were sold to the farmers. The survival rate in the present study is 30 and 55% for control and experimental tanks respectively. This clearly shows that probiotics used in the experimental tanks control the water quality and disease causing organisms than in control tanks. The average length of PL also was higher in the larval tanks exposed to probiotic than in control tanks. In aquaculture, probiotics can be administered either as a food supplement or as an additive to the water (Moriarty, 1999). Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Verschure *et al*., 1999, 2000). Because *Bacillus* bacteria secrete many exoenzymes (Moriarty, 1996; 1999), these bacteria have been used widely as putative probiotics. Studies have shown that when these bacteria were administered as probiotics in the shrimp larval rearing tanks, growth and survival were improved and immunity was enhanced. Some bacteria used as candidate probiotics have antiviral effects. Although the exact mechanism by which these bacterial action is not known. Some researchers have suggested that microorganisms have a beneficial effect in the digestive processes of aquatic animals. Balca'zar (2003) demonstrated that the administration of a mixture of bacterial strains (*Bacillus* and *Vibrios* sp.) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens *V. harveyi* and white spot syndrome virus. This protection was due to the stimulation of the immune system, by increasing phagocytosis and antibacterial activity. Administration of the *Bacillus* bacteria to shrimp larval rearing tanks resulted in an increase in the specific activity of lipase, protease and amylase in the larval digestive tract (Moriarty, 1966; 1999). Because gram-positive bacteria, particularly members of the genus *Bacillus*, do secrete a wide range of exo-enzymes we cannot distinguish between activity due to enzyme synthesized by the larvae and activity due to enzyme synthesized by the bacteria. They observed increases in specific activities of digestive enzymes in probiotic treatments might have led to enhanced digestion and increased absorption of food, which in turn contributed to the improved survival and growth in *P. indicus*. In the present study also the feed consumption was high in probiotic treated tanks than in control tanks. Similar results were also observed by Krishna *et al.,* (2009).

The application for probiotics in aquaculture ponds appears bright there is an over-increasing demand for aquaculture production and a similar increase in the search for alternative to antibiotics, the application of probiotics intended for culture systems is now attracting considerable attention and a number of commercial products are available, particularly directed at the culture organisms. Probiotics strains already adapted through natural processed to the dynamics of an aquaculture production systems will probably lessen, farm management environmental manipulation practices required to achieve the desired antibiotic affect in final product (Kesarcodi-Watson *et al.,* 2008). Introduction such specially intended probiotics bound to favor an increase in the application probiotics particularly in area of prawn aquaculture in view of its global marketability. Krishna *et al.,* (2009) explained that the use of probiotics in biological systems in details and they give details report on sustainable aquaculture management. In the present study scampi receiving probiotic in both the hatchery and the farming stages, all of the growth parameters except total length and carapace length were significantly higher in treatment tanks than in control tanks. The correlation of higher bacterial counts with higher digestive enzyme activity and improved survival and growth parameters in treatments over controls strongly suggests that adding the probiotics during the hatchery stages and continuing its administration throughout the farming stages is necessary to maximize survival and growth in the shrimp. The general conclusion obtained from the present study is that the probiotics plays a vital role in survival and disease resistance of the larval farms by maintaining good water quality parameters throughout the cycle. It is clear from the microbial load data that *Vibrio* sp. is dominant only in the control tanks not in experimental tanks.

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