



**EFFECT OF PROBIOTICS ON GROWTH AND SURVIVAL OF SHRIMP  
(*LITOPENAEUS VANNAMEI*) IN CULTURE PONDS**

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**ABSTRACT**

The present study was carried out for 120 days to know the growth and survival rate of *Litopenaeus vannamei* by applying water, soil and feed Probiotics. Four experimental ponds having one acre size shrimp ponds are selected among them one is control and the remaining are probiotic treated. The major water quality parameters like salinity, dissolved oxygen, pH, transparency, dissolved oxygen, alkalinity and total ammonia nitrogen were periodically measured by standard methods. The present results showed that the Probiotics play an important role in maintain good water quality parameters throughout the culture period, and also elevate the growth and survival percentage of shrimp.

**KEYWORDS:** Probiotics; *L. vannamei*; Water quality parameters; Growth rate; Survival rate.

**INTRODUCTION**

Aquaculture is a worldwide activity and considered as a major economic and food production sector as it is an increasingly important source of protein available for human consumption.<sup>[1]</sup> The use of probiotics as farm animal feed supplements dates back to the 1970s. They were originally incorporated into feed to increase the animal's growth and improve its health by increasing its resistance to disease.

Diseases are primary constraint to the growth of many shrimp species, which are exposed to stressful conditions, adverse environmental conditions. Consequently, wide ranges of chemicals particularly antimicrobial agents are used in shrimp farming to prevent and to treat diseases. The usage of these antimicrobial agents has increased enormously and tonnes of antibiotics are distributed in the biosphere during an antibiotic era of only about 60-year duration.<sup>[2-4]</sup>

The emergence of antibiotic-resistance among shrimp pathogens undermines the effectiveness of the prophylactic use of antibiotics in aquaculture<sup>[5,6]</sup> and increases the possibilities for passage not only of these antibiotic-resistant bacteria but also of their antibiotic resistance determinants to bacteria of terrestrial animals and human beings, including pathogens. Another problem created by the excessive use of antibiotics in industrial aquaculture is the presence of residual

antibiotics in commercialized fish, shrimp, and shellfish products.<sup>[7-10]</sup>

The use of probiotics as farm animal feed supplements dates back to the 1970s. They were originally incorporated into feed to increase the animal's growth and improve its health by increasing its resistance to disease. The results obtained in many countries have indicated that some of the bacteria used in probiotics (Lactobacilli) are capable of stimulating the immune system.<sup>[11-13]</sup>

Yasudo and Taga<sup>[14]</sup> predicted that some bacteria would be found to be useful not only as food but also as biological controllers of fish disease and activators of nutrient regeneration. It was only in the late 1980s that the first publication on biological control in aquaculture emerged, and since then the research effort has continually increased.<sup>[15]</sup>

In another experiment that was performed by Rengpipat et.al.<sup>[10]</sup> the growth and resistance to *Vibrio* in black tiger shrimp fed with a *Bacillus* probiotic (BS11) were studied. It was found that the growth and survival rates of shrimps fed on the probiotic supplement were significantly greater than those of the controls. Some strains of Gram-negative bacteria have been used as probiotics in shrimps too. Alvandi et al.<sup>[16]</sup> isolated *Pseudomonas* sp. PM11 and *Vibrio fluvialis* PM17 as candidate probions from the gut of farm-reared subadult shrimp and tested for their effect on the immunity

indicators of black tiger shrimp. The results of the study suggest that the criteria used for the selection of putative probiotic strains, such as predominant growth on primary isolation media, ability to produce extracellular enzymes and siderophores, did not bring about the desired effect in vivo and improve the immune system in shrimp.

Probiotics are live nonpathogenic microorganisms that provide colonization resistance to the pathogenic microbes and thus are effective in prevention and treatment of some diseases. Fuller<sup>[17]</sup> defined the probiotics as live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance. Probiotics, lactic acid bacteria and *Bacillus* spp. as 'bio-friendly' agents can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms.<sup>[18]</sup> The use of beneficial probiotic bacteria to displace pathogens by competitive processes is being used in the animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture.<sup>[19]</sup>

The production level of shrimp *vannamei* is low in Andhra Pradesh compared to those other countries. With the increasing intensification and commercialization of aquaculture production, disease problems inevitably emerge. In recent decades, disease prevention and control have led to a substantial increase in the use of antimicrobial drugs, pesticides and disinfectants. The abuse of antimicrobial drugs, pesticides, and disinfectants in aquaculture disease prevention and growth promotion has led to the evolution of resistant strains of bacteria as well as a question of safety.<sup>[20-22]</sup>

Typically farmers use chemicals and antibiotics in their prawn farming, and very recently they have been introduced to probiotics and employing them in their hatchery and grow-out ponds considering the demand for more disease-free, environment-friendly and sustainable aquaculture practice. There are increasing reports of research works on the application of probiotics in aquaculture<sup>[22-25]</sup> though very few studies related to the usage of probiotics in prawn culture available.

Shrimp *Litopenaeus vannamei* play an important role in the economy of East coast of Andhra Pradesh. Shrimp is of great importance in earning foreign exchange and also to meet up protein demand and to solve unemployment problem for the increasing population. One of the major problems at present in the shrimp aquaculture is the microbial disease caused by self-pond pollution.<sup>[26-27]</sup> Probiotic bacteria improve the health of shrimp by controlling pathogens and improving water quality by modifying the microbial community composition of water. The main probiotic bacteria documented in shrimp grow-out are *Bacillus* spp. strains such as *Bacillus subtilis*<sup>[28]</sup> or Gram-negative bacteria strains<sup>[10,16]</sup>. Several reviews<sup>[3,19,29]</sup> showed the detail the various developments made in the use of probiotics in aquatic

cultured species, including shrimp. Based on the previous research on probiotics it is evident that the use of probiotic bacteria in aquaculture has stupendous scope and the study on application of probiotics in aquaculture has a splendid future<sup>[30]</sup>. The present study, therefore, has been conducted with the objective of supplementing probiotics in the pond and diet of *Litopenaeus vannamei* and assessing their growth performance and survival rate the culture ponds of Nellore district at East Coast of Andhra Pradesh.

## MATERIALS AND METHODS

The Present study was carried out in the Aqua farms located of Coastal ponds of Nellore, Andhra Pradesh, East coast of India. The culture was carried out for Four months (120 days) and the required result data was collected periodically (Control, 15 days, 30 days, 45days, 60 days, 75 days, 90 days, 105 days and 120 days).

**Pond Preparation and Soil Parameters:** In any earthen pond culture system, the bottom soil plays a major role in pond yield. High organic matter content in neutral soil often promotes higher primary productivity and hence higher fish yield. Natural food organisms are one of the most important food sources in ponds. It is rich in protein, vitamins, minerals and other essential growth elements that simple supplementary feed cannot complete. Fish yield in pond can also be affected by the presence of predators, deteriorating water quality and improper pond management. Hence, pond preparation is a first step towards ensuring a better pond production.

Remove water from farming ponds and settling ponds. Remove harmful creatures (shrimp, crabs, snails, insects, fish, etc.). Dredge pond, repair banks, water drains and supplies. Create a slope in the bottom of the pond toward the drains. Thoroughly compact the pond or use canvas to prevent erosion and limit leakage. Use net to fence around the pond to avoid intermediate host species from external environment such as crabs, sand bubbler crabs, snakes, etc.

Initially ponds are re-excavated and allowed to sundry to increase the capacity of oxidation of hydrogen sulphide and to eliminate other obnoxious gases. The soil pH was recorded in the pond by pH meter. The average soil pH was calculated and required amount of lime was applied to maintain the optimum pH. Ponds were bleached at 60 ppm bleaching, containing 30% chlorine. Four multiple paddle wheel aerators (2.5 HP each) were set in the four corner positions of the pond to aerate the pond water. For plankton growing the organic compounds and minerals mix were applied in the probiotics treated and controlled ponds. Water salinity, temperature, transparency, alkalinity, dissolve oxygen, pH, Total Ammonia Nitrogen (TAN) were measured and monitored regularly. The water level was measured regularly to maintain at a constant level.

**Water Quality Parameters Monitoring:** The water salinity of the pond was measured by using a hand refractometer. The pH of the pond water was measured by using electronic pH pen (Erma-Japan). The Dissolved oxygen was estimated by modified Winkler's iodometric method as described by Strickland and Parsons.<sup>[31]</sup> Alkalinity, Total ammonia nitrogen (TAN) of the pond water were estimated by using ammonia test kit (Advance Pharma, Thailand). Water temperature was measured by using a standard centigrade thermometer. The dissolved oxygen (DO) was estimated by Do meter. Transparency was observed by using a secchi disc.

Ponds were ready for stocking after three weeks. Then PCR tested healthy *L. vannamei* seeds (PL15 - 20) which are purchased from a commercial hatchery near Nellore. Before stocking, the seeds were acclimatized to the pond environment. For this the seed bags were allowed to float on the water surface, in each pond for 15-20 minutes in order to acclimatize equal to that of the surrounding pond water temperature. The bags were opened and the pond water was introduced slowly by sprinkling into the bags for 30 minutes to equalize with pond water parameters. After acclimatization, seeds were released slowly to the ponds water. During the culture period different culture soil Probiotics Nanozyme, water Probiotics Proven and

feed probiotics Nutrigain Plus which are prepared in different combinations of probiotic bacteria and applied to the experimental culture ponds which are able to maintain the soil quality, water quality and feed consumption rate of shrimp probiotics treated ponds. The probiotics were purchased from Dr. Rag's Biolab, Nellore, Andhra Pradesh, India.

After 120 days of rearing, shrimp were harvested by pumping the pond. The feeding quantity was adjusted based on the check tray observation and body weight sampling. Four check trays were installed in each pond for monitoring feed intake. The required amount of feed was dispensed at certain interval in 24 hours period as follows- 30% in the morning at 6.00am, 25% at noon 11.00 pm, 25% at evening at 6.00 pm and 20% at night 10.00pm. Water exchange was not done for the first 30 days. After at every 15 days interval till to onset of harvesting the filtered and aerated water was added equal to that of water released from the pond. After 15 days of stocking, sampling of shrimp was done at every 15 days (Table-1), during early hours of the day with a cast net and values are recorded. Survival rate and average body weight (ABW) of the shrimp were estimated and shrimp health condition was also observed.

Average body weight of the shrimps was calculated by following formula:

$$\text{Average body weight (ABW) (g)} = \frac{\text{Total wet weight of shrimp (g)}}{\text{Total number of shrimps}}$$

The estimation of the survival rate of the animal was calculated by the following formula:

$$\text{Survival rate (\%)} = \frac{\text{No. of Approximate animals present in culture pond}}{\text{No. of seed stocked}}$$

All data were analyzed statistically using Analysis variance method. A student t-test was used to examine treatment effects on weight gain, survival, growth and production. All statistical analyses were considered significant at  $P < 0.05$ .

## RESULTS

It is observed that the variations in salinity, pH, Temperature, Dissolved Oxygen (DO<sub>2</sub>) and Total Ammonia Nitrogen (TAN) of control and Probiotic treated (PT) waters were reported and tabulated in Table-1.

Salinity of control and experimental waters were found between 15.4 to 19.1 and 15.9 to 19.1 ppt respectively during the total culture period. The average temperature of the control and PT treated water was between 29.09 to 29.1°C and 28.32 to 29.1°C during total culture period. The average pH of control and PT treated waters were found between 8.8 to 8.6 and 8.4 to 8.5 respectively

during the total culture period. The dissolved oxygen content (DO<sub>2</sub>) was recorded in between 7.0 ppm to 7.5 ppm in PT treated ponds whereas 8.7 to 7.5 ppm in unpolluted ponds. TAN was 0.5 to 2.45 mg/l in PT treated ponds and 2.7 to 2.5 mg/l in controlled ponds in the culture period (Table 1). Transparency is in between 37-40 cm in control ponds and 25 to 36 cm in probiotics treated ponds respectively during the culture period. The alkalinity was measured in controlled ponds as 92 to 120 and 112 to 122 in probiotics treated ponds.

**Table 1: Water quality parameters of control and Probiotic treated ponds during culture period.**

Treated Days	Salinity		pH		Temperature		DO2		TAN	
	Control	PT	Control	PT	Control	PT	Control	PT	Control	PT
Control	19.1 ±0.14	19.1 ±0.14	8.6 ±0.4	8.5 ±0.3	29.1 ±0.99	29.1 ±0.99	7.5 ±0.22	7.5 ±0.12	2.5 ±0.31	2.4 ±0.22
15 days	12.01 ±0.15	19.8 ±0.16	8.5 ±0.2	7.9 ±0.4	29.3 ±0.88	29.9 ±0.55	7.4 ±0.41	7.1 ±0.32	2.8 ±0.25	0.6 ±0.12
30days	10.25 ±0.16	18.9 ±0.18	8.6 ±0.6	8.2 ±0.4	28.5 ±0.45	27.6 ±0.56	7.6 ±0.42	7.3 ±0.19	2.4 ±0.43	0.8 ±0.16
45days	17.9 ±0.49	18.5 ±0.71	8.5 ±0.3	8.1 ±0.2	27.8 ±0.48	27.2 ±0.56	6.9 ±0.36	6.8 ±0.22	2.6 ±0.22	0.7 ±0.34
60 days	18.8 ±0.65	19.2 ±0.72	8.0 ±0.4	7.8 ±0.6	28.35 ±0.65	28.15±0.44	7.9 ±0.26	7.3 ±0.18	2.5 ±0.25	0.6 ±0.11
75days	17.4 ±0.42	18.3 ±0.52	8.7 ±0.5	8.2 ±0.4	29.86 ±0.28	28.86±0.35	8.5 ±0.35	7.9 ±0.55	2.4 ±0.12	0.5 ±0.22
90days	15.2 ±0.37	16.5 ±0.43	8.3 ±0.4	8.1 ±0.3	30.25 ±0.36	29.18±0.15	8.3 ±0.24	7.5 ±0.32	2.6 ±0.34	0.6 ±0.12
105days	15.8 ±0.13	16.4 ±0.24	8.5 ±0.4	8.2 ±0.2	31.45 ±0.66	28.36±0.44	8.6 ±0.34	8.0 ±0.22	2.7 ±0.42	0.5 ±0.14
120days	15.4 ±0.26	15.9 ±0.31	8.8 ±0.2	8.4 ±0.6	29.09 ±0.27	28.32±0.15	8.7 ±0.52	7.0 ±0.43	2.4 ±0.6	0.7 ±0.43

Values mean ± SD of six individuals values. Values are significant at P < 0.05

PT= Probiotic Treated; TAN = Total Ammonia Nitrogen.

**Table -2: Variations in water quality parameters such as Transparency, Alkalinity, and Body weight and Survival rate of control and Probiotic treated shrimp during culture period.**

Treated Days	Transparency		Alkalinity		Body weight		Survival rate	
	Control	PT	Control	PT	Control	PT	Control	PT
Control	39±0.5	26±0.3	92±1.2	112±1.5	25.16±1.5	30.25±2.5	65.34±1.2	70.25±2.7
15 days	39±0.4	28±0.6	110±1.5	115±1.3	25.61±1.3	32.17±1.6	64.23±1.3	71.23±1.5
30days	38±0.3	25±0.5	96±1.3	113±1.8	25.81±1.4	33.86±1.4	63.56±1.1	73.25±1.6
45days	38±0.5	35±0.4	90±1.5	120±1.7	26.15±1.8	35.12±1.9	64.25±1.3	74.85±2.6
60 days	37±0.6	29±0.5	110±1.4	118±1.6	26.52±1.6	36.25±2.3	65.34±1.6	79.75±2.5
75days	40±0.8	28±0.6	98±1.5	117±1.5	27.34±1.4	38.81±1.9	63.25±1.4	84.15±2.4
90days	39±0.6	35±0.6	115±1.4	122±1.9	28.12±1.3	39.12±1.4	64.22±1.6	86.34±1.8
105days	36±0.7	36±0.4	120±1.6	118±1.5	28.52±1.4	40.08±1.5	64.25±1.3	89.52±1.6
120days	39±0.5	34±0.8	95±1.3	119±1.7	29.25±1.6	42.35±1.6	64.85±1.7	95.34±2.5

Values mean ± SD of six individuals values. Values are significant at P < 0.05

The average final body weight of the harvested shrimp was 25.16±1.5 g and 42.35±1.6 g in control and probiotics treated ponds. The average survival rate of shrimp was recorded as 63.25±1.7% in control ponds and 95.34±2.5 % in probiotics treated ponds. Average production per hectare was yielded @ 2658±125.34 kg in controlled ponds and 5679.52±106.52 kg in probiotics treated ponds (Table 2).

## DISCUSSION

The detailed available information on the efficiency of commercially available probiotics on the growth and survival of the cultivable shrimp species, *L. vannamei* is not commensurate and this study was conducted to observe the role of some of probiotics on the growth and survival of the cultivable shrimp species, *L. vannamei* and also to monitor its influence on important water quality parameters. Salinity, temperature, dissolved oxygen, pH and total ammonia nitrogen (TAN) are

important water quality parameters considered during the study.

During the culture period different culture soil and water Probiotics *Nanozyme* (prepared at different combinations of selective bacterial sps., enzymes, vitamins, minerals, aminoacids and herbal ingredients), water Probiotics – *Proven* (prepared at different combinations of *Bacillus* sps, *Aspergillus* sps, *Alcaligenes* sps, *Saccharomyces* sps), and feed probiotics *Nutrigain plus* (prepared at different combinations of *Bacillus subtilis*, *Streptococcus thermophilus*, *Clostridium butyricum*, *Bacillus mesentericus*, *Lactobacillus acidophilus*, *Lactobacillus sporogenes*, Fructo-oligosaccharides, Protease, Lipase, Beer yeast, Vitamin B<sub>12</sub>, Vitamin C ) were prepared in different combinations and applied to the experimental culture ponds which are able to maintain the soil quality, water quality and feed consumption of shrimp.

Water quality parameters were found to be more suitable in probiotics ponds than in controlled ponds those matches with the report of Jiravanichpaisal et al.<sup>[32]</sup> Maintenance of good water quality is essential for optimum growth and survival of shrimps.<sup>[33,34]</sup> In the present study the salinity of probiotic ponds ranged from 10-19 ppt. According to Soundarapandian et al.<sup>[35]</sup> salinity is an important parameter in maintaining optimum growth and survival of shrimps. Even though, *L. vannamei* is euryhaline aquatic species; it is comfortable when exposed to optimum salinity. At high salinity the shrimp grows slowly but remains healthy and resistant to diseases. If the salinity is low, the shell becomes weak and prone to diseases. Muthu,<sup>[36]</sup> Soundarapandian and Gunalan<sup>[37]</sup> and Karthikeyan<sup>[38]</sup> practically proved that an ideal salinity range of 10-35 ppt for *P. monodon* culture. While Chanratchkool et al.<sup>[39]</sup> perpetuated the salinity of pond water is maintained in between 10-30 ppt.

Soundarapandian et al.<sup>[35]</sup> promulgated in their study that pH is one of the vital environmental characteristics, which affects the metabolism and other physiological process of shrimps. In their study pH range was 7.6 to 8.2 for the probiotics treated and control ponds. In the present study pH range was 8-8.8 in probiotic ponds and 7.6-8.8 in controlled ponds. Ramanathan et al.<sup>[40]</sup> concluded that the optimum range of pH 6.8 to 8.7 is to be maintained for maximum growth and production of shrimp. The pH of shrimp culture pond water is mostly influenced by many factors, including pH of source water, acidity of bottom soil and shrimp culture inputs and biological activity.<sup>[41]</sup> According to the studies of Wang et al.<sup>[42]</sup> the recommended range of pH for the American white prawn *Litopenaeus vannamei* culture is 7.6 to 8.6.

Dissolved oxygen affects the solubility and availability of many nutrients. Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation and low levels of dissolved oxygen can cause damages in oxidation state of substances from the oxidized to the reduced form. Dissolved oxygen also plays an important role in the culture ponds; it keeps the pond ecosystem healthy and promotes the growth of aerobic bacteria and a low level of D.O. hampers the metabolic activities and reduces the shrimp growth.<sup>[43]</sup> In the present study dissolved oxygen was found 4.3-6.2 ppm and 4.7-6 ppm in probiotic and controlled ponds respectively. Soundarapandian et al.<sup>[35]</sup> found 3.2 to 4.2 ppm dissolved oxygen in all the culture ponds that is close to the present study. Low levels of dissolve oxygen tension hampers metabolic performances in shrimp and can be reduce growth and moulting and cause mortality.<sup>[44]</sup>

Water temperature is the most important environmental variables in shrimp cultures, because it directly affects on metabolism, oxygen consumption, growth, moulting and survival.<sup>[45]</sup> Despite its economic importance, little

quantitative information is available regarding temperature effects on growth of this species. In this study temperature range was found 24-30°C and 25-30°C in probiotic and controlled pond respectively which was maintained within reference ranges. So it was observed that probiotic does not alter water temperature or it does not have any beneficial effect on it.

It is obvious that the total Ammonia builds up in the water of the fish pond when nitrogen-containing substances decay. The two main nitrogen sources in culturable fish ponds are the waste excretions from the fish and uneaten food. One of the breakdown products of both these substances is ammonia. At farm level, Ammonia level should be less than 1 ppm<sup>[47]</sup>. In the present study total ammonia nitrogen was 0.7-0.8 and 2.0-2.9 in probiotic treated and controlled ponds respectively. Thus maintaining the ammonia level probiotic helps in maintaining good water quality and thereby keeps the shrimp disease free.

The average body weight of the harvested shrimp was 42.35±1.6 g and 29.25±1.6 g in probiotics treated and controlled ponds, respectively. Result showed that all probiotic-supplemented diets resulted in higher growth in prawn than the control diet though the amount of feeding was same in both the ponds. This result is very inspiring in shrimp culture with probiotics as size of shrimp is directly related to better foreign exchange earnings. Maeda and Nagami<sup>[48]</sup> observed that bacterial strains possessing vibrio static activity improved the growth of prawn and crab larvae.

In the present study the average survival rate was 95.34±2.5% in probiotics treated ponds and 64.85±1.7% in controlled ponds. In probiotic ponds survival rate was 19.67% more than control ponds. Here in the present study with the treatment of probiotics, survival rate of shrimp has been found more compared to the controlled ponds which are very similar to the reports of Wang et al.<sup>[50]</sup> Survival percentage showed a significant variation in the probiotic treated ponds to control pond (Table 2). This can be supported by the above results which show that the probiotic bacteria plays a significant role in maintaining good water quality parameters which promotes the high survival and growth rate of the shrimp.

According the present results it is evident that the probiotic bacteria as an agent increases survival and growth in *P. vannamei* postlarvae by competitive elimination of potential pathogenic bacteria, and also effectively reduces the need for antibiotic prophylaxis in intensive larvae culture system.

## CONCLUSION

The general conclusion of the present study is that the used probiotics play a vital role in growth and survival percentage of the aquatic animal by maintaining good water quality parameters throughout the culture period. It

is evident that the Probiotic treatment offers a promising alternative to the antibiotics for shrimp aquaculture system.

Further research is still needed to detect the mode of action of probiotics on *L.vannamei* digestibility and its effect on immune response and stress resistance at molecular level.

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