E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2023; 11(5): 242-249

www.fisheriesjournal.com
Received: 21-07-2023
Accepted: 29-08-2023

A Nagesh Babu
Department of Biotechnology Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

D Srinivasa Rao
Department of Biotechnology Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

Raghavendra Rao MV
Scientist-Emeritus, Apollo Institute of Medical Sciences and Research, Hyderabad, Telangana, India

KRS Samba Siva Rao
Department of Biotechnology Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

Corresponding Author:
Raghavendra Rao MV
Scientist-Emeritus, Apollo Institute of Medical Sciences and Research, Hyderabad, Telangana, India

In vitro evaluation of probiotics and their effect on hatchery production and farming of L. *Vannamei*

A Nagesh Babu, D Srinivasa Rao, Raghavendra Rao MV and KRS Samba Siva Rao

DOI: https://doi.org/10.22271/fish.2023.v11.i5c.2871

Abstract
There are many different vaccines available for other industries, but there are very few for the fishing industry. Antibodies are used in shrimp aquaculture to lessen illness. Antibiotic resistance has increased, tissue residues are a problem, and there are trade issues as a result of the indiscriminate use of antibiotics. Alternative approaches must be used to solve the current issue. The use of "probiotics" during treatment may be beneficial. Probiotic bacteria are commonly referred to as the bacteria that can increase output by enhancing the quality of aquaculture water and/or inhibiting waterborne infections. Photosynthetic bacteria such lactobacillus, actinomycetes, nitrobacteria, denitrifying bacteria, bifidobacterium, yeast, etc. are included in it. The phrases "probiotics", "probiont", "probiotic bacteria", or "beneficial bacteria" are interchangeable.

Keywords: Probiotics, probiont, *lactobacillus*, antibiotic resistance, *nitrobacteria*, shrimp farming, disease

1. Introduction
Probiotics are a live microbial feed supplement or cultured product that benefits the host by enhancing the balance of its gut bacteria and overall health (Paulina Markowiak and Katarzyna Slizewska, 2017) [22]. Lactobacillus P., a lactic acid-producing bacteria, was the first probiotic to be discovered. According to reports, its yearly production peaked in 2001 at 1.27 million tons (8,432 million US dollars). In the declaration on global food security approved at the November 1996 Rome World Food Summit. According to Touret, Oliveira, and Semedo-Lemsaddek (2018) [31], the fishing industry is crucial to ensuring the food security of both the present and future generations. In contrast to other nations that share a border with the Indian Ocean, India has the advantage of being able to utilize marine resources. With yearly growth rates above 11% per Annum, aquaculture emerged as one of the most promising food production and economic crucial sectors, paving the path for the "Blue Revolution."
The viability of an aquaculture system is mostly dependent on the hatcheries' ability to produce disease-free, robust post larvae. It also heavily depends on management techniques that are nourishing, environmentally friendly, and commercially successful. According to The State of World Fisheries and Aquaculture (2016) [30] by the Food and Agricultural Organization of the United States, global aquaculture production has been growing linearly for more than ten years. The rate of growth has also remained relatively stable.

2. Materials and methods
2.1 Probiotic samples
Ten samples of probiotic products used for marine shrimp farming in India were acquired from a regional distributor of aquaculture products, as indicated in Table 1. Prior to usage, all samples were kept at 4 °C.

2.2 Isolation and enumeration of bacteria in probiotic products
Bacterial colony types from PCA and MRS agar were isolated and identified to genus and species using conventional morphological and biochemical tests in accordance with Bergey's manual of determinative bacteriology (Holt *et al.*, 1994) [37].

Probiotic product samples were diluted in 0.85% NaCl and analyzed by surface of plate count agar (PCA, Difco, Detroit, MI, USA), which were incubated under aerobic condition. Bacterial isolation and enumeration were carried out in duplicate.

Table 1: Dosage probiotics at different DOC

DOC	WP/hectare (A1)	FP/kg feed (A2)	WP+FP (A3)
15-20	2 kg	10 gm	2 kg+5 gm
21-30	2 kg	10 gm	2 kg+5 gm
31-40	2 kg	10 gm	2 kg+5 gm
41-50	2 kg	10 gm	2 kg+5 gm
51-60	2 kg	10 gm	2 kg+5 gm
61-70	2 kg	10 gm	2 kg+5 gm
71-80	2 kg	10 gm	2 kg+5 gm
81-90	2 kg	10 gm	2 kg+5 gm
91-100	2 kg	10 gm	2 kg+5 gm
101-110	2 kg	10 gm	2 kg+5 gm
111-125	2 kg	10 gm	2 kg+5 gm

2.3 Isolation of lactic acid bacteria from selected probiotic samples and DNA extraction

All ten of the chosen probiotic samples yielded lactic acid bacteria. Serial dilutions were prepared in sterile physiological saline, put on MR Sagar (Biolab, Biolab Diagnostics, Midrand, South Africa), with natamycin, and incubated at 30 °C for 24-48 hours.

2.4 DNA amplification for lactobacillus identification

The 16S rRNA gene of lactic acid bacteria's V2-V3 variable region (around 200 base pairs) was amplified using the primers 534 (5' ATTACCGCGGCTGCTGG 3') and 341FGC(5'GCAGGCCCGCCGCCGCGGGGGGCGGGCAC GGG GGGCCTACGGGAGGCA 3'). Takara Bio Inc., Shiga, Japan supplied the 0.5 mM primers, 200 mM dNTP, 0.5 U Taq DNA polymerase, 1'PCR buffer, and 10 L of DNA for the PCR reaction, which was carried out in 50 L of the PCR mixture. Initial denaturation took place at 94 °C for 4 min. Thereafter, 35 cycles of denaturation at 94 °C for 30 s., annealing at 56 °C for 30 s., and elongation at 72 °C for 8 min. were performed. A Master Cycler TM Thermal Cycler (Eppendroff, Germany) was used to conduct the PCR reactions. Amplicons.

2.5 Hatchery production of shrimp post larvae

2.5.1 Selection of Brood stock

Success in the hatchery is significantly influenced by the quality of the broodstock chosen for maturation. To the best of one's ability, only large, productive, healthy, and disease-free shrimp should be used. L. *Vannamei* brood stock from the Andhra Pradesh coast of the Bay of Bengal was used for the current study. A segment of the pleopod (or telson) is removed from each shrimp and kept in 90% alcohol in a tiny bottle or tube to screen for viral infections in advance. The cut site was cleaned with pure liquid povidone PVP iodine. This was subsequently sent to a PCR lab for WSSV testing. PCR can be used to test for MBV from this.

2.6 Transportation of Broodstock and its Acclimation

2.6.1 Brood stock Preparation

The provision of high-quality brood stock that will withstand the strain of transportation and be able to generate healthy seed in the hatchery maturation system depends on the proper transportation of brood stock. Brood stock must be ready for transit so that they have the best opportunity of arriving stress-free, in good health, and alive. By adding ice that has been packed within plastic bags to the water holding the broodstock until the desired temperature is obtained, the above-collected brood stock were transferred to hatcheries in the broodstock holding tanks at temperatures between 18 and 28 °C. To lessen stress and bacterial levels, commercial feed was blended with vitamin C (2 g/kg) and a suitable probiotic formula (10 g/kg) for the brood stock. They are provided with proper aeration.

2.6.2 Broodstock Transportation

The broodstock were not fed for 12 hours prior to shipment and were only transported to the hatchery with hard shells. Placing the broodstock shrimp within twin polyethylene plastic bags inside polystyrene boxes will help you keep the water at a constant temperature of 18 to 22 °C. The packed shrimps were handled with extreme care to avoid bumping or dropping them. Transport was carried out at night with no more than two shrimp per bag, and they were loaded at a rate of 500g of shrimp per 10 liters of water. To prevent the shrimp from piercing the sack, a rubber was inserted over its rostrum.

2.6.3 Broodstock Acclimation

Only the broodstock with firm shells were brought to the hatchery, and the broodstock were not fed for 12 hours prior to shipment. Maintaining the water temperature at 18-22 °C, place the broodstock shrimp in twin polyethylene plastic bags inside polystyrene boxes. Extreme care was used when handling the packed shrimps to prevent bumping or dropping them. Maximum 2 shrimp per bag, filled with 500 g of shrimp per 10 liters of water, were delivered at night. Rubber was placed over the shrimp's rostrum to keep it from piercing the bag. The current investigation was conducted at the Krishna hatchery in the Indian state of Andhra Pradesh's Bapatla Guntur district. This hatchery is well designed, equipped and maintained for commercial production of L. *Vannamei* for the last ten years. There are four production units operating simultaneously and the annual production is around 200 - 300 million seeds.

2.4 Collection and treatment of water

The seawater for the hatchery was pumped from the sea directly using a 7 HP motor. The suction point is located about 20mt from the shoreline. The water was initially pumped into a sand-gravel filter. From the sand filter, water was lifted into chlorination tank using a 5 HP motor. Chlorination was done with 20 ppm chlorine. After 24 hrs, the chlorinated water was stored in overhead tank after passing through activated carbon filter. Subsequently the filtered water was passed through cartridge filter (0.5-1.0: m mesh size) and UV filter before filling into tanks.

The residual chlorine available in the treated seawater was determined with chlorine test kits by using O-tolidine. After knowing the availability of excess chlorine in treating sea water sodium thiosulphate (hypo) was used to neutralize the residual chlorine. The chelating agent, EDTA (10ppm) was added in treating seawater to ensure clear sea water. A 15HP air blower and a 7.5HP standby provided a continuous supply of air. The air generated by the blower was supplied with individual tanks through PVC pipes.

Samples were tested by PCR to detect the presence of WSSV and MBV. Only negative brooders were stocked in brooder maintenance tanks and fed with polychaete worms and squid

at twice in a day. Fifty percent of the water was exchanged daily. The brooders were then transferred to the maturation tanks and treated with water probiotics (Provac) of 20 ppm to control the luminous bacteria.

2.5 Spawning

To enhance artificial spawning, eyestalk ablation was performed. A pre-heated forceps was used to remove the eye and subsequently transferred into the broodstock tank. Males and females of both species were stocked in 2:1 ratio in 50 t rectangular tanks. Ripe females were transferred to 0.5 t capacity FRP tanks for spawning. After spawning, females were removed and the eggs were filtered and transferred to hatching tanks after treating with 20 ppm Iodine solution.

2.6 Design of the Experiment

Before stocking of nauplii, the larval rearing tanks were first filled with 2 t of chlorinated seawater (30ppt) and all the water quality parameters were checked. Total of 16 tanks, eight for L. *Vannamei* are controlled tanks where no probiotic was used and another four represents probiotic treated tanks for each species. The water probiotics namely Provac and Microzyme BS) were added in the experimental tanks alone at 5ppm each. In addition to probiotics 0.05 ppm of Teflon was also added both experimental and control tanks to prevent the fungal disease.

2.7 Conversions of hatching nauplii

The newly hatched nauplii from the hatching tank was harvested and transferred in polythene bags and stocked in control and experimental tanks at 2 lakhs per tank. Twenty four hours after stocking, the nauplii converted into zoeaI in experimental tanks. Where as in the control tanks, the conversion time was extended to 24 to 30hrs. First feeding was started when the zoeaI appeared. The zoeal stages (I to III) were fed with *Chaetoceros*. At 1x106 cells /ml in twice daily both control and experimental tanks. The mysis stages (I to III) were fed with algae at density of 5 x 10 4 cells/ml in both control and experimental tanks for both the species. In addition to algae, mysis stages were fed with knock *Artemia* (*Artemia* killed by hot water) at 5 to 10 g/tanks.

2.8 Control of feeding in *Artemia*

Post larval stages were fed exclusively on freshly hatched live *Artemia* nauplii at 5nos per PL per feeding. The water probiotics, Proact (Matrix Biosciences Ltd.) was added daily at 5-10ppm from zoeal stage onwards and at 15 ppm was added after the appearance of post larval stage in experimental tanks alone.

Stock cultures of *Chaetoceros* Sp were maintained at 20-24 ºC with 2000-5000 lux light intensity. Conway Walney's Medium or Guillard (F2) Medium was used for indoor culture. Culturing of *Chaetoceros*. in 2 litre glass bottles, 25 litre plastic bags, 500 litre FRP tanks and 20 – 30t cement outdoor tanks did scaling up of culture. For outdoor culture, TMRL and Skelon media were used. Once the algal culture reached into exponential phase and sufficient cell concentration was pumped into larval rearing tanks.

Before being released from their capsules, the commercial *Artemia* cysts were aerated for 30 minutes. It was thoroughly combined two liters of liquid chlorine and 120 cc of sodium hydroxide solution. Cysts were added to this solution while being continuously stirred below 40 degrees Celsius. It is clear that the cyst underwent de-capsulation because its color changed from dark brown to orange. After thorough washings in fresh water until the chlorine odor vanished, cysts were then placed to *Artemia* hatching tanks. The hatching process was sped up by constant aeration and light from a 60V lamP. Using the phototactic behavior of the Atemia nauplii, gathered newly born nauplii after 24 hours.

From the Mysis III stage on, water exchange was carried out. About 50% of the water was reduced with mesh size of 0.5 mm. the appearance of post larvae, the salinity was reduced slowly and maintained to 20ppt especially in experimental tanks, because, the effect of probiotics was well in low salinity.

2.9 Measurement of the water quality

Regular checks were made on the water quality parameters in the probiotics-treated and control tanks. Every morning, measurements of the water's salinity, temperature, pH, dissolved oxygen, ammonia, hardness, and alkalinity were made. A hand refrectometer (Erma- Japan) was used to measure the salinity of the water. Using an electronic pH pen made by the Japanese company Hanna Instrumental, the pH of the water was determined.

By using A113 Dissolved Oxygen Benchtop Meters, dissolved oxygen was estimated. The samples were titrated with the standardized sulfuric acid using the methyl red indicator after first using sodium bicarbonate and after the sulfuric acid had been standardized. By utilizing the technique developed by Solorzano and Koroleff in 1969, the ammonia level was routinely checked.

2.10 Study on the field trials

The farm

The farm chosen for the study was located in the village of Kudithipalem, which is 20 kilometers from Nellore and only a little closer to the Bay of Bengal shore. One division (A) for a distinct culture of L. *Vannamei* makes up the entire farm. Each division consists of 5 ponds totaling 0.5 hectares (2 reservoirs, 2 control ponds, A1-A3), of which 3 are utilized for shrimp farming with probiotics (A1-A3 for L. *Vannamei*) and 1 serves as a control (A4 for L. *Vannamei*) without the use of probiotics. Each division's pond serves as a reservoir. All ponds were tested for pH at the first stage of starting a culture, and any that had a reading lower than 7.5 were treated accordingly. After three days the ponds were filled with chlorination treated reservoir water. The water quality of all the ponds was checked with standard protocols (APHA standards). For the proper development of bloom 50 kg of deoiled rice bran along with 20kg of urea per pond was applied. Now these ponds are ready for seeding.

2.11 Probiotics selected

Two probiotics were selected for the present study to assess the effect of them in farming shrimP. They are Provac, a water probiotic from Matrix India Ltd and a feed probiotic Gut act from Salem microbes, Chennai. Both the formulations are rich in lacto bacillus.

2.12 Seeding

L. *Vannamei* produced in the Lila hatchery by using probiotics as described above were procured in double layered polythene bags with 1/3 water and 2/3rd oxygen with 500 pulses per bag in a fully air-conditioned vehicle. After reaching farm the seed bags were transferred to pond of their respective, and left them for about an hour to match the

conditions of both pond and seed bags.

2.13 Feeding and application of probiotics
All the seeds were fed with commercial feed (CP Ltd). For ponds A1 only water probiotic was applied and the animals in the ponds A2 was fed with a gut probiotic mixed feed whereas ponds A3 received both gut and water probiotics. The Control ponds A4 were maintained without probiotic treatment. The dosage was given in the Table.

2.14 Water quality monitoring
Both the control and treated ponds had frequent water quality parameter checks. A standard scale with centimeter markings was used to measure the depth of the water. A hand refractometer (Earma, Japan) was used to test the salinity of the water, and an electronic pH meter (Elico Ltd.) was used to determine the pH. According to accepted methods, the amounts of total alkalinity, hardness, and ammonia were estimated (APHA 2010). A thermometer was used to gauge the pond's water temperature. The amounts of dissolved oxygen were calculated using a dissolved oxygen meter. A search disc was used to measure transparency in terms of light penetration.

2.15 Growth measurement
Cast net was used to measure the growth rate of the sample. The first sampling was taken after the 40th day and number of individuals and average body weight (ABW) was measured. Sampling was regularly performed after every 10 days not only to assess the growth but also to check the healthiness of animals (Yuvaraj *et al.*, 2015) [12].

2.16 Microbial Analysis
For microbial analysis the water and sediment samples were collected separately from different parts of the ponds in sterile conical flasks and were mixed to make a single sample. This process was repeated for every pond and final samples were subjected to analysis. For an enumeration of heterotrophic bacteria, the Zobells Marine agar was used and for vibrio isolation TCBS agar was used as media. After inoculation the plates were incubated at 29 °C for 24 hrs and colonies were counted using a digital colony counter. Luminescent bacteria were identified by observing plates in dark room.
At the end of the experiment, the individuals were counted in determining the rate of survival in each tank and weight was recorded for growth studies then, the individuals were sacrificed for proximate and biochemical analysis.

3. Results and discussion
Since these live and/or dead bacterial preparations, when taken in sufficient amounts, may bestow health benefits on the host (Salminen *et al.*, 2002; Geert Huys *et al.*, 2013) [27, 9], the usage of probiotics in foods has grown in popularity. According to Fukushima *et al.* (2007) [38], Paulina Markowiak and Katarzyna Liewska (2018) [35], Torres-Rodriguez *et al.* (2007) [39], and Einar Ring *et al.* (2018) [7], probiotics can improve animal growth performances, lower serum cholesterol levels, increase nutrient utilization, and decrease the use of antibiotics, among other benefits. According to Zihui *et al.* (2012) [36], lactobacilli and bifidobacteria are typically found in probiotics utilized in diets.
The Food and Agriculture Organization/World Health Organization (FAO/WHO) published guidelines for the evaluation of probiotics in food. These guidelines state that safety and functionality properties like resistance to bile toxicity, gastric acidity, and antibiotic resistance, adhesion to intestinal cell lines, antimicrobial activity, cholesterol-lowering potential, and immunomodulation potential are extremely important and should be investigated using dependable *in vitro* screening methods. In the current investigation, isolated lactobacillus from commercial probiotics was assessed for its probiotic qualities using *in vitro* assay techniques (Neha Jain *et al.*, 2017) [20].
When L. *Vannamei* tanks' water quality was taken into account, the pH ranged from 8.2 to 8.9 in probiotic-treated ponds whereas it stayed below 8 in controls (Sreenivasulu *et al.*, 2016) [29]. Both the control and treated ponds kept the salinity at 20 ppt. In probiotic treatment tanks, the average total alkalinity levels varied from 176 ppm to 198 ppm, whereas in control tanks, the average values ranged from 167 ppm to 189 ppm. The hardness was calculated as the sum of the calcium and magnesium hardness, and the average values were shown. In control ponds, the values were found to alter from 4920 to 5600 P.m., but in treated ponds, the overall hardness was raised above levels. The values were found to change from 4920 to 5600 P.m. in control ponds whereas in treating ponds the total hardness was elevated above 5000 ppm always except in the last probiotic treatment (20 ppm).
The average DO levels in treated ponds were recorded between 3.7 to 4.6 ppm. The ammonia levels above0.1ppm a leads to mortality of both post larvae and juveniles. Hence this should be under controlled levels. In the present study the average value of ammonia in control ponds is 0.09 ppm and that of controls was recorded as o.03 ppm (Table 2) Abd El Rahman Ahmed Khattaby, (2015) [1].

Table 2: Comparison of Water quality between treated and control tanks of L. *Vannamei*

Parameter Tank No.	pH	Salinity (ppt)	Alkalinity (ppm)	Hardness (ppm)	Ammonia (ppm)	DO
VC1	7.4	20	167	5200	0.10	3.12
VC2	7.4	20	175	5180	0.11	3.92
VC3	7.7	20	179	5600	0.06	4.10
VC4	7.5	20	189	4920	0.09	3.45
VT1	8.2	20	189	5000	0.02	4.00
VT2	8.9	20	194	5200	0.05	3.97
VT3	8.0	20	176	5170	0.02	4.19
VT4	8.4	20	198	4990	0.03	4.11

3.1 L. *Vannamei* post larvae
When a comparative study was done with the L. *Vannamei* post larvae. The levels of selected enzymes were found to be increased. The levels of SOD and CAT were enhanced with the use of probiotics as increased to 0.34 (Mahmoud *et al.*, 2019) [16]. The control values have not varied much in almost all the tanks and but increase in SOD and CAT value are somewhat lower than the increase in P. *monodon* post larvae. But the PO levels in L. *Vannamei* post larvae are higher than P. *monodon* post larvae (Table 3).

Table 3: Comparison of Antioxidant status in P. *Vannamei* post larvae between treated and control tanks

Pond No	SOD		CAT		PO	
	Control	Treated	Control	Treated	Control	Treated
1	12.23	13.17	31.22	35.42	0.18	0.21
2	14.35	15.20	34.12	35.22	0.24	0.29
3	12.54	18.14	35.00	43.40	0.29	0.31
4	12.56	22.66	34.72	51.50	0.22	0.34

3.2 B. Field trails and Farming

The farm in Kudithipalem, which is 7 kilometers from the Bay of Bengal and 22 kilometers from Nellore city, received L. *Vannamei* post larvae that had been formed from the aforementioned probiotic treatment in the hatcheries. The post larvae were shipped in double-layered polythene bags with 1/4 water with a salinity of 20, 1/4 oxygen, and about 500 post larvae each bag. They were introduced with the utmost care into their respective ponds after being transported using all the safety procedures stated in the materials and methods. Prior to the placement of post larvae into ponds, they underwent Hapa testing to determine how long they would survive in each tank.

For this the 100 PL of L. *Vannamei* were introduced into the hapa that was floating in each tank and left for about 24hrs after which the number of PLs survived were calculated. In all most all the tanks the survival rate was estimated as 96 to 97% approximately. The same survival rate was seen with probiotic treated and non-treated post larvae of L. *Vannamei*.

3.4 Water quality maintenance

3.4.1 Temperature in cultured tanks for L. *Vannamei*

When the same parameter in the pond used for *L. Vannamei* were recorded it was found that the temperature in all most all the tanks except in V3 as 29 °C and that V3 was recorded as 27 °C. Here also the temperature is not having reached to 30 ˚C even a single time during the course of culture (Table 4) William A. Wurts (2002) [34].

3.4.2. Water quality in L. *Vannamei* cultured tanks

The water quality in L. *Vannamei* cultured ponds was also monitored along with monod on cultured ponds weekly and tabulated their average values. The variation in Temperature in control as well as in probiotic treated plants is not much. In all ponds except in V3 the recorded temperature is 29 ˚C but in V3 it was 27 °C (Asian-Pacific Aquaculture, (2017) [2].

The average pH recorded in probiotic treated ponds is 8.23, 8.32 and 8.44 respectively in V2, V3 and V4 and a high pH of 8.72 was reported in control pond. With reference to salinity there exist no considerable difference in both control and test ponds (Rubia Akter *et al.*, 2017) [24].

The carbonates while measuring an integral part of alkalinity it was shown that control have higher carbonates than treated ponds. But there exists much more difference in bicarbonate levels between control and different probiotic treated ponds. In control pond it was reported as 126 ppm and the value decrease a little bit in the water probiotic treated pond (V2), then it was found to increase in both V3 and V4 to 164 and 160 ppm respectively.

While recording g hardness as measure total calcium and magnesium the variation is much more. In control pond it was reported as 3600 ppm and average 2824, 2876 and 3200 ppm were observed with V2, V3 and V4 ponds respectively. Just like in *monodon* cultured ponds here also a higher value of ammonia 0.15 poems were recorded in the control than in the treated ponds whose ammonia levels were 0.08, 0.12 and 0.10 respectively in V2, v3 and V4 in Table 5

Table 4: Water quality changes in L. *Vannamei* cultured ponds under different probiotic treatments

Pond No	Temp °C	pH	Salinity (PPT)	CO^3 (Ppm)	HCO^3 (Ppm)	Total Alkalinity (Ppm)	Total Hardness (Ppm)	Ammonia (Ppm)
V1	29	8.72	10	48	126	174	3600	0.15
V2	29	8.23	09	12	112	124	2824	0.08
V3	27	8.32	10	32	164	196	2876	0.12
V4	29	8.43	10	44	160	204	3200	0.10

In case of L. *Vannamei* culture the yellow colony count was found to decrease in control V1, V3 and V4 but the decrease is not much higher in controls than in V3 and V4. In V2 the number of yellow colonies was first increased up to 30 DOC and remained constant for the next 60 days and a slight decrease was seen at the end of 120 DOC. The green colony count in control tank V1 110 cfu/ml after 30 DOC and it was increased to 190 cfu/ml after 60 DOC and remained static after 90 DOC and once again found to decrease at the end of 120 DOC 110 CFU/mL. In V2 and V3 the number of green colonies were varied between 40 to 70 cfu/ml during entire culture period but the number almost has reached to zero in pond V4. The luminescent bacterial count was reported to be nil in all the ponds except in controls where a low cfu of luminescent bacteria was observed (Table 6).

Table 5: Microbial counts of water treated with water probiotics in L. *Vannamei* cultured pond

Pond No.	Yellow Colonies				Green colonies				Luminescent bacteria			
	30 DOC	60 DOC	90 DOC	120 DOC	30 DOC	60 DOC	90 DOC	120 DOC	30 DOC	60 DOC	90 DOC	120 DOC
V1 Con	310	310	210	200	110	190	190	110	30	Nil	40	Nil
V2	170	240	200	190	40	70	40	40	10	Nil	Nil	Nil
V3	390	290	270	250	10	30	Nil	10	20	20	Nil	Nil
V4	240	240	160	100	Nil	Nil	10	Nil	Nil	Nil	Nil	Nil

3.5 Growth patterns

The effect of Water Probiotics and Feed Probiotics on the growth patterns of P. *monodon* and P. *Venom* was studied during culture activity. In the present study the growth patterns of both shrimp were monitored in different conditions i.e. Control condition, where only feed was provided, in another condition along with normal broadcasting of feed water probiotics were used to keep the water environment clean, in another condition along with normal feed, feed probiotics were mixed broadcasted and in the Fourth condition both feed probiotics and water probiotics were introduced in the cultural environment. The following are the

four regimes.

- Control (Feed broadcasted)
- Normal Feed + Water Probiotics used
- Normal Feed + Probiotic Feed used
- Feed Probiotic + Water Probiotics used

P. *Vannamei's* growth patterns were observed under the aforementioned conditions and are shown in Tables-6. From January through April, the growth trends were tracked for 120 days on a monthly basis. The normal feeding activity without the addition of either water probiotics or feed probiotics is part of the culture conditions in the control. The development patterns also showed relatively good success in the second condition, where Water Probiotics are routinely employed to clean the water environment along with normal feeding activity, obtaining 31.87 g of average weight at the conclusion of culture activity. In a different series of tests, Feed Probiotics are combined with Feed and then dispersed throughout the cultural environment. The prawn's exhibit improved growth rates and produce an average of 33.06 g of weight. Average weight at the end of culture activity compared to the control weight of 30.12 g.

Both water probiotics and feed probiotics were utilized in the most recent experiment, and the growth patterns were recorded to be maximized. The shrimps obtained an average weight of 34.07 g in comparison to control weight. Maximum weight in the Control circumstances was 31.12 g, but it was 33.87 g in L. *Vannamei* (Table-6). Water Probiotics Used Ponds, Feed Probiotics Used Ponds, and Water and Feed Probiotics Used Ponds totaled 36.07 g.

Table 6: Comparison Stress tolerance by post larvae of P. *Vannamei* between probiotic treated and control tanks

Tank No	% Survival in Controls	% Survival in probiotic treated
VC1/VT1	77±2.58	93±2.16
VC2/VT2	76±2.16	95±1.41
VC3/VT3	79±3.56	97±1.41
VC4/VT4	86±1.83	97±2.16

In aquaculture, probiotics can be administered either as a food supplement or as an additive to the water (Moriarty, 1999) [19]. 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 (Verschuere *et al.*, 2000) [33]. Because *Bacillus* bacteria secrete many exoenzymes (Moriarty, 1996; 1999) [18, 19], these bacteria have been used widely as putative probiotics.

According to studies, these bacteria increased development and survival rates and boosted immunity when given as probiotics to shrimp larvae in rearing tanks (Rengpipat *et al.*, 1998) [40]. Some of the bacteria considered as potential probiotics have antiviral properties. Even though it is unclear exactly how these bacteria work. Two strains of Vibrio spP., NICA 1030 and NICA 1031, were obtained by Direkbusarakom *et al.* from a black tiger shrimp hatchery in 1997 [6]. IHNV and Oncorhynchus Mosaic virus (OMV) were both successfully combatted by these isolates. Some researchers have suggested that microorganisms have a beneficial effect in the digestive processes of aquatic animals. Balca'zar (2003) [41] demonstrated that the administration of a mixture of bacterial strains (*Bacillus* and *Vibrios*s P.) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens *V. harveyi* nd white spot syndrome virus. This protection was due to a 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. Because gram-positive bacteria, particularly members of the genus *Bacillus*, do secrete wide range of exoenzymes (Moriarty, 1996; 1999) [18, 19], 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. In the present study also the feed consumption was maximum in probiotics treated tanks than control tanks. In contrast, Shariff *et al.*, (2001) [42] found that treatment of P. *monotonous Litopenaeus Vannamei* with a commercial *Bacillus* probiotics did not significantly increase (P N0.05) either survival or growth. For shrimp 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 treatments than in controls.

During farming the water quality was assessed weekly and represented as an average for the total period of culture. The temperature in control as well as in treated pond of L. *Vannamei* has not crossed 30°C not even a single time during the culture period. The optimum range of temperature for the black tiger shrimp is between 28°C to 30 ºC (Ramanathan *et al.*, 2005) [43]. The temperature in the present study was 28 to 32 ºC and the low temperature 27 ºC was observed due to cloudy weather. The optimum range of temperature of P. *monodon* was between 26 to 33 ºC (Soundaria pandian, 2008 and, Prasad *et al.*, 2008) [28, 44].With reference to salinity there exist no considerable variant in probiotic treated ponds of P. *monodon* and a variation of 2 ppt was observed in control pond. But in case of L. *Vannamei* cultured tanks such a variations were not found to even in control ponds also. Saha *et al.*, (2006) [26] noticed the pH of 8.11 to 8.67 in low saline ponds. Ramakrishna, (2000) [25] and Soundara pandian *et al.*, (2008) [28] recommended pH of 7.5 to 8.5 for P. *monodon* culture. When pH is high water exchange will be a better choice (Boyd, 1995) [3].

The growth patterns were also observed during the culture period type of increments in growth were observed in L. *Vannamei* culture also. A maximum weight of 36.07 gm was obtained when treated with feed and water probiotics. The feed conversion ratio were found to decrease when compared to control ponds. The best FCR was obtained at the last treatment of probiotic for L. *Vannamei*.

Regular use of Probiotics in feed of fish, in U.K and other European countries has been reported to have resulted in several health benefits (JairavanichPaisal *et al.*, 1997; Moriarty, 1997) [14, 17]. In shrimp hatcheries, Probiotics are reported to have controlled the high incidence of diseases in larvae leading to dramatic improvement in shrimp health (Ukeles & Bishop, 1975) [32]. Atlantic salmon fed with Probiotic enjoyed increased survival and reduced mortality, because of Vibriosis, Furunculosis and Enteric Red mouth disease (Zorriehzahra *et al.*, 2017) [23]. FAO has now

designated the use of Probiotics as a major means for the improvement of aquatic environmental quality (Nkemakolam *et al.*, (2011) [8]. Most studies on the effects of Probiotics on cultured aquatic animals have emphasized a reduction in mortality (or) the improved resistance against putative pathogens (Irianto & Austin, 2002) [13].

4. Conclusion

Therefore more information on the host/microbe interactions *in vivo* and development of monitoring tools are still needed for better understanding of the composition and functions of the indigenous micro biota as well as of microbial cultures of 'Probiotics'. Finally probiotics may influence the hatchery production and farming of L. *Vannamei.*

5. References

1. Abd El RAK. Effect of Different Managements of Fish Ponds on Fish Productivity and Water Quality; PhD. Thesis; c2015.
2. Asian-Pacific Aquaculture. Effects of Pro YG Tablets on Growth Performance, Survival Rate and Vibrio Control of *Litopenaeus Vannamei* Culture Ponds; c2017.
3. Boyd CE. Water quality management in shrimp farming. Fisheries World. 1995;3:33-38.
4. Boyd CE. Water quality standards: pH. The Advocate; c2001, 42.
5. Balcazar JL, de Blas I, Ruiz-Zarzuela I, Cunningham D, Vendrell D, Muzquiz JL, *et al.* The role of probiotics in aquaculture. Vet. Microbio L. 2006;114:173-186.
6. Direkbusarakom S, Yoshimizu MY, Ezura L, Ruangpan Y, Danayado L. Vibrio sp. The dominant flora in shrimp hatchery against some fish pathogens. Poster session of the 2nd Asia-Pacific marine biotechnology conference and 3rd Asia-pacific conference on algal biotechnology. Phuket Thailand. 1997 May;14:7-10.
7. Ring E, Hoseinifar SH, Ghosh K, Van Doan H, Bo RB, Song SK, *et al.* Lactic Acid Bacteria in Finfish-an Update, Front Microbiol. 2018;9:1-37.
8. Nwogu NA, DayoOlaji E, Eghomwanre AF. Application of probiotics in Nigeria aquaculture: Current status, challenges and prospects: Nkemakolam Akajiaku Nwogu. 2011;2(7):215-219.
9. Huys G, Botteldoorn N, Delvigne F, De Vuyst L, Heyndrickx M, Pot B, *et al.* Microbial characterization of probiotics-Advisory report of the Working Group 8651 Probiotics of the Belgian Superior Health Council (SHC), MolNutr Food Res. 2013;57(8):1479-1504.
10. Gilles LM. Environmental factors affect immuneresponse and resistance in Crustaceans. The Advocate; c2001, 18.
11. Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds. Aqt Info; c2019.
12. Yuvaraj D, Karthik R, Muthezhilan R. Crop Rotation as a Better Sanitary Practice for the Sustainable Management of *Litopenaeus Vannamei* Culture, Asian Journal of Crop Science. 2015;7(3):219-232.
13. Irianto A, Austin B. Probiotics in aquaculture. J Fish Dis. 2002;25:633-642.
14. Jiravanichpaisal P, Chuaychuwong P, Menasveta P. The use of *Lactobacillus sp* as the probiotic bacteria in the giant tiger shrimp (*Penaeus monodon* Fabricius). Poster session of the 2nd Asia-Pacific marine biotechnology conference and 3rd Asia-pacific conference on algal biotechnology. Phuket Thailand. 1997 May;16:7-10.
15. Li Z, Qing Z, Huaquan Y. The effect of the probiotics to the shrimp ponds. Aquaculture of China (in Chinese). 1997;5:30-31.
16. Mahmoud AO, DawoodaFawzy I, Mahmoud MFI. Salemb Hanaa A. Abdel-Daima. Modulation of digestive enzyme activity, blood health, oxidative responses and growth-related gene expression in GIFT by heat-killed Lactobacillus plantarum (L-137); Aquaculture. 2019;505:127-136.
17. Moriarty DJW. Managing microbial in aquaculture with probiotic bacteria: biotechnology for sustainable aquaculture. Poster session of the 2nd Asia-Pacific marine biotechnology conference and 3rd Asia-pacific conference on algal biotechnology. Phuket Thailand. 1997 May;40:7-10.
18. Moriarty DJW. Microbial biotechnology: A keyingredient for sustainable Aquaculture. Infofish int. 1996;4:29-33.
19. Moriarty, David JW. Disease Control in Shrimp Aquaculture with probiotic bacteria. Microbial Interactions in Aquaculture. Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology; c1999.
20. Jain N, Mehata A, Bharti V. Screening, characterization, and *in vitro* evaluation of probiotic properties of Lactobacillus strains in Asian Journal of Pharmaceutical and Clinical Research. 2017;10(8):288-293.
21. Saarela M, Mogensen G, Fonde´ RN, Matto J, Sandholm TM. Probiotic bacteria: safety, functional and technological properties, Journal of Biotechnology. 2000;84:197-215.
22. Markowiak P, Śliżewska K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health, Nutrients. 2017;9(9):1-30.
23. Zorriehzahra MJ, Adel M, Delshad TS. Enteric redmouth disease: Past, present and future: A review; Iranian Journal of Fisheries Sciences. 2017;16(4)1135-1156.
24. Akter R, Md. Rahman M, Islam RHM, Islam S, Khan KUA. Role of different types of probiotics in pond ecosystem, in prawn (*Macrobrachium rosenbergii*) health and production. International Journal of Fisheries and Aquatic Studies. 2017;5(3):83-87.
25. Ramakrishna R. Culture of the tiger shrimp *Penaeus monodon* (Fabricus) in low saline waters. M.Sc., dissertation, Annamalai University; c2000. p. 1-31.
26. Saha S, Roy RN, Sen SK, Ray AK. Characterization of cellulose producing bacteria from the digestive tract of tilapia, *Oreochromis Mossambica* (Peters) and grass carp, *Ctenopharyngodon idella* (Valenciennes). Aquat. Res. 2006;37:380-388.
27. Salminen MK, Tynkkynen H, Rautelin M, Saxelin M. Lactobacillus bacteremia during a rapid increase in probiotic use of Lactobacillus rhamnosus GG in Finland. Clin. Infect. Dis. 2002;35:1155-1160.
28. Soundarapandian P, Gunalan B. Recenttechnology for the survival and production of gianttiger shrimp *Penaeus monodon* along south east coastof India. Int. J Zoo L. Res. 2008;4(1):21-27.
29. Sreenivasulu P, Joshi SDSD, Narendra K, Venkata Rao G, Satya KA. Bacillus pumilus as a potential probiotic for shrimp culture International Journal of Fisheries and Aquatic Studies. 2016;4(1):107-110.
30. The State of world fisheries and aquaculture. Food and Agricultural Organization United States; c2016.
31. Touret T, Oliveira M, Semedo-Lemsaddek T. Putative

probiotic lactic acid bacteria isolated from sauerkraut fermentations; PLoS One. 2018;13(9):1-16.
32. Ukeles R, Bishop J. Enhancement of phytoplankton growth by marine bacteria. Journal of phycology. 1975;11:142-149.
33. Verschuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. Microbio L. MoL. Bio L. Rev. 2000;64:655-671.
34. William A. Wurts. Alkalinity and Hardness in Production Ponds, in World Aquaculture. 2002;33(1):16-17.
35. Markowiak P, Śliżewska K. The role of probiotics, prebiotics and synbiotics in animal nutrition; Gut Pathog. 2018;10:21.
36. Zhihui Yu, Zhang X, Shengyu Li, Changying Li, Da Li, Zhennai Yang, *et al.* Evaluation of probiotic properties of Lactobacillus plantarum strains isolated from Chinese sauerkraut; World J Microbiol Biotechnol. 2012;29:489-498.
37. Holt RD, Lawton JH. The ecological consequences of shared natural enemies. Annual review of Ecology and Systematics. 1994 Nov;25(1):495-520.
38. Fukushima T, Aida T. Ionic liquids for soft functional materials with carbon nanotubes. Chemistry: A European Journal. 2007 Jun 15;13(18):5048-58.
39. Torres-Rodriguez A, Donoghue AM, Donoghue DJ, Barton JT, Tellez G, Hargis BM, *et al.* Performance and condemnation rate analysis of commercial turkey flocks treated with a Lactobacillus spp.-based probiotic. Poultry science. 2007 Mar 1;86(3):444-6.
40. Rengpipat S, Phianphak W, Piyatiratitivorakul S, Menasveta P. Effects of a probiotic bacterium on black tiger shrimp Penaeus monodon survival and growth. Aquaculture. 1998 Sep 1;167(3-4):301-13.
41. Masi CM, Suarez-Balcazar Y, Cassey MZ, Kinney L, Piotrowski ZH. Internet access and empowerment: A community-based health initiative. Journal of General Internal Medicine. 2003 Jul;18(7):525-30.
42. Shariff ZU. Modern herbal therapy for common ailments. Spectrum Books; c2001.
43. Ramanathan V, Chung C, Kim D, Bettge T, Buja L, Kiehl JT, *et al.* Atmospheric brown clouds: Impacts on South Asian climate and hydrological cycle. Proceedings of the National Academy of Sciences. 2005 Apr 12;102(15):5326-33.
44. Prasad PV, Staggenborg SA, Ristic Z. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Response of crops to limited water: Understanding and modelling water stress effects on plant growth processes. 2008 Dec 22;1:301-55.