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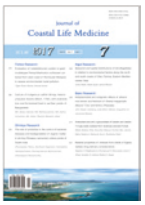

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The role of probiotics in the control of bacterial diseases and biodegradation of organic matter in shrimp (*Penaeus vannamei*) culture ponds of South India

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ABSTRACT

Objective: To utilize bacterial consortium as probiotics for the biodegradation of organic matter and to control the emergence of bacterial disease in shrimp culture pond.

Methods: Water samples were collected from shrimp culture ponds of Southern India. The physico-chemical properties of the water *viz*., pH, temperature and colour were analyzed. The bacteria were isolated and were screened for different enzyme assays. The bacterial isolates named MSWS24, MSWS30, and MSWS19 were identified and were optimized at different physical parameters for the maximum enzyme production. The treatment of bacterial pathogen using bacterial consortium was tested to analyze the degradation. Then the water quality at four different setups were analyzed and the amount of organic load was accumulated. Finally the survival rate of the shrimp was observed and total shrimp yield was examined.

Results: In this study the cell suspensions of *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus firmus* strains, were isolated from shrimp pond water, taken in equal proportions and administered as probiotic in *Penaeus vannamei* culture. The optimization of enzyme activity of the three isolates showed a particular parameter with high productivity. When compared to the untreated control group significant differences in survival of shrimp was recorded in the probiotic treated test tank. The survival percentage of shrimp in untreated T1 tank was 40% whereas in T2 tank was 80%.

Conclusions: These results suggested that administration of *Bacillus* consortium in the rearing water of shrimp confers beneficial effect on water quality, shrimp growth, disease resistance and shrimp survival.

1. Introduction

Shrimp culture represents an important and economically profitable venture and their production has grown enormously in recent years by intensive and semi-intensive methods of culture. Penaeid shrimps are one of the most important preferred species for culture in artificial impoundments[1]. Approximately more than 5 million metric tons of shrimps are annually produced, but the current global demand for both the wild (naive) and farmed shrimps are approximately more than 6.5 million metric tons per annum[2]. To overcome this, many shrimp farms are being created throughout the world to solve this increasing food demands[3]. Intensive development of these shrimp industries and extensive culture of these aqua farms has created various ecological, economical and social problems. During the last few years white spot syndrome virus (WSSV) disease has spread worldwide and caused tremendous economic loss in shrimp culture particularly in Asia[4]. Due to the continuous outbreak of this WSSV disease in *Penaeus monodon* culture leading to loss of shrimp culture in India, the farmers are seriously looking for alternative shrimp species for culture[5]. Cyanobacterial growths in ponds leads to the algal degradation due to the poor water quality. On attaining the maximum growth in pond it stops growing which is known as algal 'crash' after this occurs the periodical collapse of algal populations, the decomposition of these dead algae employs a large amount of oxygen and may cause oxygen deficiency and increased toxication of ammonia in water bodies[6]. Probiotics enhance reduction in blue-green algae, control of ammonia, reduction in nitrogen and phosphorus concentrations, nitrite, and hydrogen sulfide decomposition of organic matter, control of disease and better shrimp production[7]. The newest attempt to improve water quality in aquaculture is the application of probiotics and/or enzymes to ponds. This approach of biotechnology is also known as bioremediation, which involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and get rid of undesirable waste materials. The concept of biological disease control, particularly using microbiological modulator for disease prevention has received widespread attention[8].

Basically shrimp ponds are enclosed cultivation systems, subject to periodic water renewal to compensate for volume changes (due to

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evaporation) and salinity changes (evaporation, precipitation) and to maintain water quality. The excess feed and faecal matter may result in bacterial decomposition of organic matter in the sediment and produce excess of toxic compounds like ammonia. In addition abnormal algal growth (eutrophication) may cause stress to the animal and ultimately end with microbial diseases and high mortality[9]. In this study potential proteolytic, amylolytic and cellulolytic enzymes producing *Bacillus* sp. were isolated from shrimp culture ponds for their potential application to maintain water quality and reduce the bacterial contamination in *Penaeus vannamei* (*P. vannamei*) culture.

2. Materials and methods

2.1. Sample collection

Water samples were collected from shrimp culture ponds of Southern India at Viluppuram (4 water and soil samples), Chidambaram (3 water and soil samples) and Tuticorin (5 water and soil samples) for probiont isolation and from contaminated shrimp culture pond at Kalaignanapuram, Tuticorin during an outbreak of bacterial diseases for pathogen isolation. The physico-chemical properties of the water *viz*., pH, temperature and colour were analyzed.

2.2. Isolation of probiont and pathogens from shrimp culture pond

The bacteria were isolated from water samples by serial dilution and spread plate technique. The bacterial isolates were screened for proteolytic, amylolytic and cellulolytic activity using skim milk agar, soluble starch agar and carboxymethylcellulose agar respectively. The isolates showing high proteolytic index (PI), amylolytic index (AI), and cellulolytic index (CI) were selected and named as MSWS24, MSWS30, and MSWS19.

Diseased shrimp culture pond water sample was inoculated in TCBS agar, cetrimide agar and *Aeromonas* isolation medium base with ampicillin for isolation of *Vibrio* sp., *Pseudomonas* sp., and *Aeromonas* sp., respectively.

The isolated probiont and pathogenic bacteria were identified based on morphological and biochemical characteristics as given in Bergey's manual of systematic bacteriology[10]. The stock cultures of bacteria were stored in nutrient broth containing 15% glycerol and pH 7.4 at 4 ˚C.

2.3. Optimization for protease, amylase and cellulase enzyme production

The physical and chemical parameters such as pH, temperature, incubation time, carbon source and nitrogen source were optimized for maximum enzyme production by selected probiont bacteria.

The assay for protease activity was performed as per standard colorimetric method using 1% (w/v) casein as substrate in 0.01 mol/L Tris-HCl buffer at pH 7.5 and tyrosine (0.37 mmol/L) as standard. The assay for amylase activity was performed based on standard colorimetric method using soluble starch at 1% (w/v) dissolved in 0.05 mol/L Tris-HCl buffer at pH 7.5 as a substrate and glucose as standard. The cellulase activity was determined using 1% CMC dissolved in 0.05 mol/L of sodium citrate buffer (pH 4.8) as substrate and glucose as standard[11].

2.4. Probiont preparation

The isolated *Bacillus subtilis* MSWS24 (*B. subtilis*), *Bacillus licheniformis* MSWS30 (*B. licheniformis*), and *Bacillus firmus* MSWS19 (*B. firmus*) were grown in LB broth by incubating in shaking incubator at 35 ˚C for 48 h. The density of cell suspension was calculated using UV-spectrophotometer at 600 nm and also correlated to colony-forming units (CFU) using a spread plate technique.

2.5. Lab scale cultivation of P. vannamei

Healthy *P. vannamei* (PL15) were provided by C.P Hatcheries India Private Limited, Dindivanam, Tamil Nadu, India and shrimps were acclimatized for 3 days in tanks before the start of the trial. *P. vannamei* were divided equally with 10 count of PL15 in three groups each housed in a 60 L tank and experiments were carried out employing 2 test tanks along with 1 control tank *viz*., C1: shrimp + commercial feed, T1: shrimp + commercial feed + bacterial pathogens, T2: shrimp + commercial feed + bacterial pathogens + probiotic consortium.

A good quality commercial feed with 32% protein was given 4 times a day (6 am, 11 am, 5 pm, and 10 pm) and the application was followed as per the feeding schedule of the manufacturer. Water sample collection for estimating bacterial population and nutrient level was carried out[3,12]. The T2 tank was treated with *Bacillus* consortium of MSWS24, MSWS30, and MSWS19 for 90 days. After acclimatization, shrimps were artificially infected with consortium of bacterial pathogens *Vibrio* sp., *Pseudomonas* sp., and *Aeromonas* sp.

2.6. Physical and chemical analysis of water

Temperature, salinity, dissolved oxygen, total ammonia nitrogen, nitrate, nitrite, sulphides, biological oxygen demand (BOD), chemical oxygen demand (COD) of water samples were measured and bacterial population and nutrient level were estimated. Estimation of ammonia-nitrogen, nitrite, nitrate, BOD and COD was carried out[3].

Before and after seed stocking the quality of water was evaluated for standard ranges as after seed stocking, the bacterial probiont can influence the reduction of organic load. Finally initial analysis of water quality was compared with shrimp optimum water conditions during 25, 50, 75, 90 days of culture.

2.7. Shrimp activity

Visual inspection of the tank was performed on daily basis and feed consumption was monitored daily. Shrimp health and the appearance of shrimp was also monitored. The weekly growth and survival percentage (total number of live shrimp) was estimated once a week. To analyze the gut content, the shrimp was randomly sampled from a healthy, well-nourished recently fed tank. Then the shrimps were examined for visible intestinal tract (mid-gut) running a length of entire tail with full of food. A numerical grade (gut fullness index) can be used to quantify the gut fullness.

Colour changes in the appendages and chromatophores of the shrimps were monitored. The level of cramped tail syndrome on shrimps was checked. The level of incidence of white or opaque muscle condition was observed. The inflammation of tissue in appendages or extremities of the shrimp and anatomical perfections such as curve antennae, good rostrums, perfections of rostral teeth, straight intestinal tracts, arrangements of tail segments and smooth cuticle were examined.

3. Results

3.1. Collection of sample

Twelve samples were taken from three different sources (soil, sediments and water) collected at different places *viz*., Viluppuram, Chidambaram, Tuticorin districts and processed within 4–24 h. The pH of samples ranged from 7.1 to 7.5 with alkaline nature and temperature was around 28–30 ˚C. The colour was mostly green in colour.

3.2. Isolation of bacterial strains

All the strains isolated from the samples were stored in nutrient agar at 4 ˚C. Three of the twenty two potential bacteria isolated were selected by hydrolysis activity namely proteolytic MSWS24 (PI 62 mm), amylolytic MSWS30 (AI 63 mm) and cellulolytic MSWS19 (CI 45 mm). From the growth curve, it is clear that the lag phase of these strains is from 0 to 2 h, log phase is from 2 to 12 h and stationary phase is from 12 to 18 h which is comparatively longer (Table 1). It is known that the organisms showing lengthier stationary phase are capable of more metabolic than the other organisms.

Table 1
Bacterial colonies isolated from shrimp ponds.

S.No	Isolates	Hydrolysis index
1	MSWS01	PI 3.9
2	MSWS23	PI 3.9
3	MSWS10	PI 5.3
4	MSWS03	PI 3.1
5	MSWS04	PI 4.0
6	MSWS05	PI 5.4
7	MSWS30	AI 6.3
8	MSWS06	AI 4.3
9	MSWS24	AI RZ
10	MSWS08	AI 3.1
11	MSWS09	AI 3.0
12	MSWS24	PI 6.2
13	MSWS11	PI 4.2
14	MSWS12	PI 4.6
15	MSWS13	PI 5.7
16	MSWS14	PI 4.7
17	MSWS19	CI 4.5
18	MSWS15	CI 3.4
19	MSWS16	CI 3.5
20	MSWS17	CI 3.8
21	MSWS18	CI 4.2
22	MSWS20	CI 4.3

3.3. Morphological characteristics of the bacterial isolates

The selected isolates MSWS24, MSWS30 and MSWS19 were identified as Gram-positive rods, motile and endospore-forming bacteria, and based on the biochemical results, these isolates were confirmed as *B. subtilis*, *B. licheniformis*, and *B. firmus*, respectively. The bacterial pathogens isolated in selective media were identified as *Vibrio* sp., *Pseudomonas* sp., and *Aeromonas* sp. (Tables 2 and 3).

3.4. Optimization of the enzyme produced by bacteria

The three isolates were optimized at different physical parameters for testing the particular parameter which produces higher amount of enzyme. The maximum protease production by *B. subtilis* was observed at pH 7.5 as 87.60 IU/mL, at temperature 35 ˚C as 74.66 IU/mL, at incubation time 36 h as 79.14 IU/mL whereas carbon source *viz*., glucose showed 83.20 IU/mL and nitrogen source *viz*., peptone showed 81.28 IU/mL. Similarly optimal conditions for maximum amylase production by *B. licheniformis* when optimized at pH 8.0 showed enzyme activity of 104.60 IU/mL, at 35 ˚C temperature as 129.60 IU/mL, incubation time at 36 h as 102.40 IU/mL whereas carbon source *viz*., wheat bran as 119.80 IU/mL and nitrogen source *viz*., beef extract as 120.60 IU/mL. The optimum pH, temperature, incubation time, carbon source and nitrogen source for maximum cellulase production by *B. firmus* were observed at pH 8.0 as 62.80 IU/mL, at 35 ˚C as 65.20 IU/mL, at 36 h incubation as 80.10 IU/mL, whereas using galactose as carbon source gave yield as 78.70 IU/mL and nitrogen source *viz*., yeast extract as 80.50 IU/mL, respectively (Figure 1–5).

Table 2
Morphological and biochemical characterization.

Tests	MSWS24	MSWS30	MSWS19
Gram-stain	+	+	+
Motility	+	+	+
Oxidase	+	+	+
Catalase	+	-	-
Methyl red	-	+	+
Vogesproskauer	+	-	+
Indole	-	-	-
Nitrate	+	+	+
Citrate	+	+	+
Glucose	+	+	+
Galactose	+	+	+
Lactose	+	+	+
Urease	-	-	-
H_2S production	-	-	-
Growth at 4 °C	-	-	-
Growth at 30 °C	+	+	+
Growth at 35 °C	+	+	+
Growth at 42 °C	+	+	+
Casein hydrolysis	+	+	+
Starch hydrolysis	+	+	-
Gelatin liquefaction	+	+	+
Identification	*B. subtilis*	*B. licheniformis*	*B. firmus*

Table 3
Identification of bacterial pathogens BP1, BP2, BP3 from prawn culture pond water.

Test	BP1	BP2	BP3
TCBS agar	Yellow	Green	green
Gram-stain	-	-	-
Motility	+	+	-
Oxidase	+	+	+
Catalase	+	+	+
Methyl red	+	+	-
Vogesproskauer	-	+	+
Indole	+	+	+
Nitrate	+	+	+
Citrate	+		+
Glucose	+	+	+
Galactose	+	+	+
Lactose	-	-	+
Urease	-	-	-
H_2S production	+	-	-
Growth at 4 °C	-	-	-
Growth at 30 °C	+	+	+
Growth at 35 °C	+	+	+
Growth at 42 °C	+	+	+
Identification	*Vibrio* sp.	*Aeromonas* sp.	*Pseudomonas* sp.

3.5. Treatment of shrimp with probiotics in tanks

The probiont consortium at a cell density of 1.0×10^{11} cells/mL was applied at 20 days intervals to T1 and T2 tanks. 40% of water exchange was done during 90 days of culture as the cell density can influence the water quality (Table 4). During 90 days of culture, the shrimp growth was observed in each test and control tanks. The C1 control tank showed average shrimp weight of 18.13 g, T1 tank 14.02 g, and T2 tank 16.83 g. The growth of bacterial pathogens was analyzed in each test and control tanks. T1 tank showed bacterial pathogen consortium count of 6.0×10^2 cells/mL and T2 tank showed 3.0×10^2 cells/mL.

3.6. Water quality analysis

The initial analysis of water quality was compared with standard water quality conditions for shrimp culture. The standard water quality

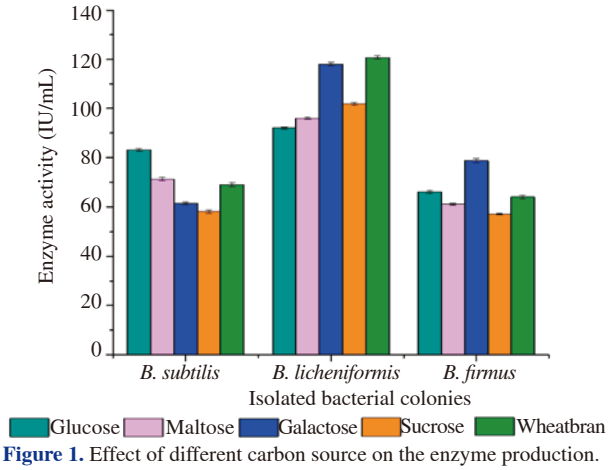

Figure 1. Effect of different carbon source on the enzyme production.

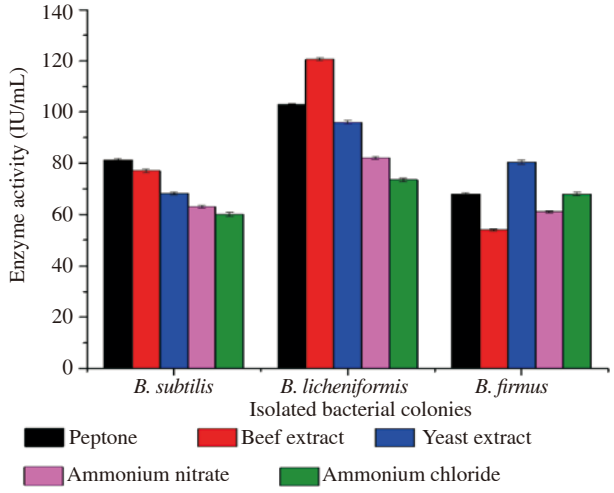

Figure 2. Effect of different nitrogen source on the enzyme production.

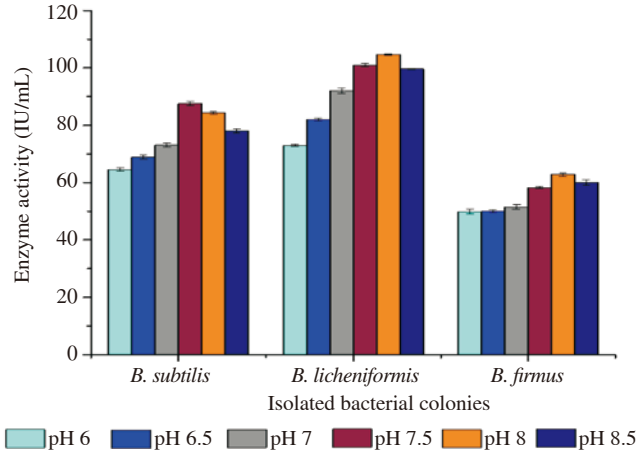

Figure 3. Effect of different pH on the enzyme production.

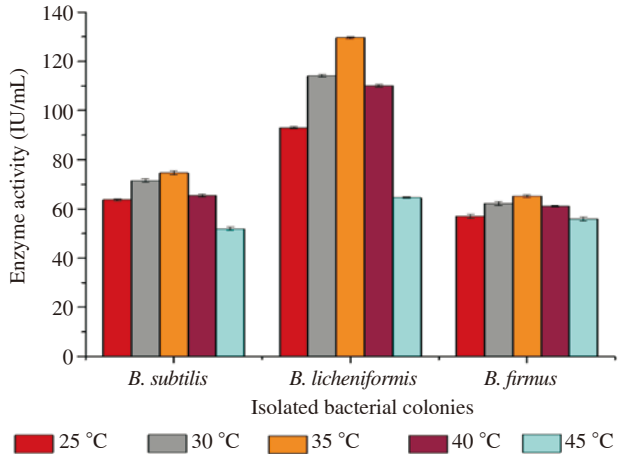

Figure 4. Effect of different temperature on the enzyme production.

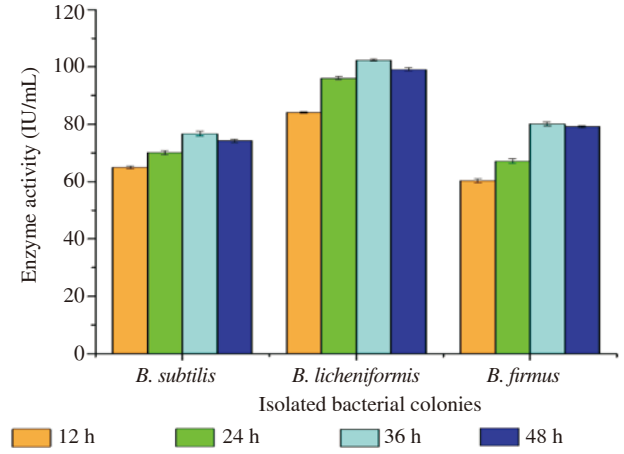

Figure 5. Effect of different time interval on the enzyme production.

Table 4

Probiont and bacterial pathogen application frequencies of shrimp culture.

Days of culture	Probiotics frequency (*Bacillus* consortium)	Bacterial pathogenic consortium (*Vibrio* sp., *Aeromonas* sp., *Pseudomonas* sp.)
–3	-	2.0×10^6/mL
0	-	2.0×10^6/mL
10	-	2.0×10^6/mL
20	1.0×10^{11}/mL	2.0×10^6/mL
60		4.0×10^5/mL
61	1.0×10^{11}/mL	4.0×10^5/mL
72		5.0×10^4/mL
75	1.0×10^{11}/mL	1.0×10^4/mL
77		6.0×10^3/mL
88	1.0×10^{11}/mL	3.0×10^2/mL
90		1.0×10^2/mL

conditions are temperature 28–31 °C, salinity < 10 ppt, dissolved oxygen 3.0–7.0 mg/L, pH 7.0–8.5, total ammonia nitrogen < 0.3 mg/L, nitrate < 10.0 mg/L, nitrite < 2.0 mg/L, hydrogen sulphide 0.0 mg/L, BOD 1–2 mg/L, COD < 70 mg/L. The analysis of water showed temperature of 30 °C, salinity 8.6 ppt, dissolved oxygen 5.4 mg/L, pH 7.3, total ammonia nitrogen 0.23 mg/L, nitrate 2.0 mg/L, nitrite 0.9 mg/L, hydrogen sulphide 0.0 mg/L, BOD 1.8 mg/L, COD 34.0 mg/L (Table 5). The organic load of water after mineralization was confirmed by analysis of water quality during 90 days of shrimp culture. After seed stocking it was observed that bacterial probiont influenced the reduction of organic load.

The water samples were collected regularly at 22 days interval on (22nd, 44th, 66th, 88th day) from each tank for measurement of organic load degradation. When the water quality parameters were measured, the sample from C1 tank showed an average temperature of 32.0 °C, salinity 8.76 ppt, dissolved oxygen 5.2 mg/L, pH 7.6, total ammonia nitrogen 0.59 mg/L, nitrate 2.22 mg/L, nitrite 1.29 mg/L, hydrogen sulphide 0.44 mg/L, BOD 1.90 mg/L, and COD 38.90 mg/L. (Table 6). The T1 tank water samples had an average of temperature 32.0 °C, salinity 8.9 ppt, dissolved oxygen 5.3 mg/L, pH 7.79, total ammonia nitrogen 0.35 mg/L, nitrate 2.57 mg/L, nitrite 1.34 mg/L, hydrogen sulphide 0.43 mg/L, BOD 2.40 mg/L, COD 39.48 mg/L (Table 7). The T2 tank showed an average of temperature 31.90 °C, salinity 8.9 ppt, dissolved oxygen 8.29 mg/L, pH 7.75, total ammonia nitrogen 0.37 mg/L, nitrate 2.0 mg/L, nitrite 0.96 mg/L, hydrogen sulphide 0.20 mg/L, BOD 1.83 mg/L, and COD 36.35 mg/L (Table 8). When compared to the initial analysis of water sample, the samples from probiont treated tanks were observed to meet the optimum water quality parameter for

shrimp culture.

3.7. Survival rate of P. vannamei

The shrimp survival percentage was calculated for each test and control tanks. Table 9 shows that the survival percentages of shrimp culture in C1, T1, and T2 were 60%, 40% and 80%, respectively. The shrimp was swimming normally at the bottom of the tank, indicating good dissolved oxygen level of tank. Good water quality indicates shrimp feed consumption. The intestinal tract (mid-gut) of healthy, well-nourished recently fed shrimp was observed. The gut was visible, running a length of entire tail with full of food. A numerical grade (gut fullness index) was used to quantify the gut fullness. Tank T2 showed 85% of gut fullness and was considered as grade 2. The control C1 tank showed empty gut fullness and was considered as grade 0.

3.8. Morphological observation of treated Shrimp (P. vannamei)

The gut content was light with golden brown colour which indicates the normal conditions of shrimp culture. No colour changes were observed in the appendages and chromatophores of the shrimp. The level of cramped tail syndrome was around 5%, which indicates normal temperature, infection control from pathogens (bacteria), and good mineral balance. An stress free culture was indicated by acceptable level of incidence of white or opaque muscle condition which is 2%. Absence of inflammation of tissue in appendages or extremities of the shrimp and good anatomical perfections such as curve antennae, good rostrums, perfections of rostral teeth, straight intestinal tracts, arrangements of tail segments, smooth cuticle indicates good shrimp activity (Figure 6).

4. Discussion

For optimum growth and survival of *P. vannamei* shrimp, the maintenance of good water quality is essential. Good water quality is characterized by adequate oxygen, limited level of metabolites and significant reduction of ammonia, nitrite and nitrate ions. Excess feed, faecal matter and metabolites will exert

Table 5
Analysis of water sample during culture period.

Water parameter	Optimum level	Initial analysis of water sample in lab trail
Temperature (°C)	28–31	30.0
Salinity (ppt)	< 10.0	8.6
Dissolved oxygen (mg/L)	3–7	5.4
pH	7.0–8.5	7.3
Total Ammonia Nitrogen (mg/L)	< 0.3	0.23
Nitrate (mg/L)	< 10.0	2.0
Nitrite (mg/L)	< 2.0	0.9
Hydrogen sulphide (mg/L)	0.0	0.0
BOD (mg/L)	1–2	1.8
COD (mg/L)	< 70	34.0

Water quality analysis of C1 control tank.

Table 6
Water quality parameters of C1 control group (shrimp + commercial feed).

Water parameter	Sample withdrawal (COD)			
	Day 22	Day 44	Day 66	Day 88
Temperature (°C)	32.00	32.30	32.00	31.80
Salinity (ppt)	8.72	8.74	8.78	8.81
Dissolved oxygen (mg/L)	5.32	5.28	5.19	5.01
pH	7.56	7.61	7.68	7.71
Total ammonia nitrogen (mg/L)	0.48	0.59	0.62	0.69
Nitrate (mg/L)	2.12	2.21	2.26	2.29
Nitrite (mg/L)	1.01	1.06	1.09	2.01
Hydrogen sulphide (mg/L)	0.23	0.25	0.28	1.01
BOD (mg/L)	1.87	1.90	1.92	1.91
COD (mg/L)	36.44	37.89	40.17	41.20

Table 7
Water quality parameters of T1 (shrimp + commercial feed + bacterial pathogen).

Water parameter	Sample withdrawal			
	Day 22	Day 44	Day 66	Day 88
Temperature (°C)	32.40	31.80	31.90	31.81
Salinity (ppt)	8.89	8.91	8.93	8.96
Dissolved oxygen (mg/L)	5.46	5.43	5.23	5.11
pH (units)	7.68	7.79	7.81	7.91
Total ammonia nitrogen (mg/L)	0.31	0.34	0.37	0.39
Nitrate (mg/L)	2.20	2.47	2.49	3.12
Nitrite (mg/L)	1.08	1.12	1.18	2.01
Hydrogen sulphide (mg/L)	0.34	0.41	0.48	0.51
BOD (mg/L)	2.13	2.18	2.38	2.92
COD (mg/L)	38.80	39.11	39.91	40.10

Table 8
Water quality parameters of T2 bacterial treatment.

Water parameter	Sample withdrawal			
	Day 22	Day 44	Day 66	Day 88
Temperature (°C)	32.40	31.60	31.80	31.60
Salinity (ppt)	8.89	8.91	8.96	8.98
Dissolved oxygen (mg/L)	8.74	8.71	7.91	7.81
pH	7.71	7.71	7.74	7.85
Total ammonia nitrogen (mg/L)	0.28	0.20	0.19	0.82
Nitrate (mg/L)	2.11	2.05	1.98	1.87
Nitrite (mg/L)	1.02	1.0	0.95	0.89
Hydrogen sulphide (mg/L)	0.29	0.21	0.19	0.12
BOD (mg/L)	2.10	2.03	1.96	1.25
COD (mg/L)	34.80	43.50	33.90	33.20

Table 9
Survival percentage of shrimp culture.

Experiment	Initial count	Final count	Survival percentage
C1	10	6	60
T1	10	4	40
T2	10	8	80

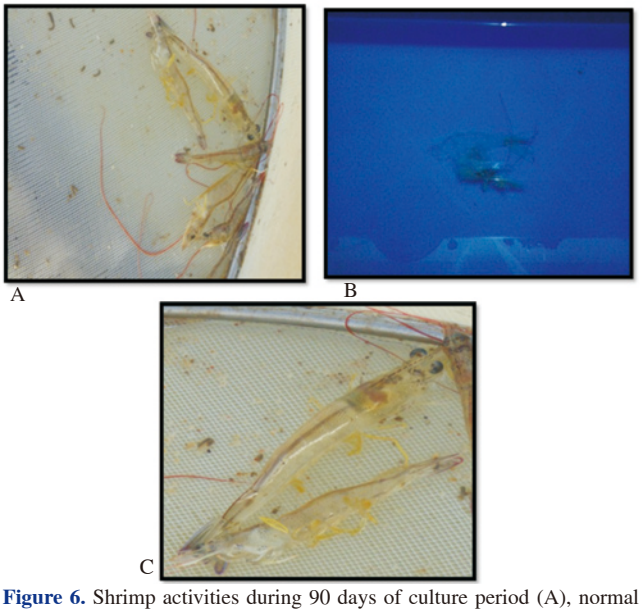

Figure 6. Shrimp activities during 90 days of culture period (A), normal softness immediately following post-molt stages (B), and no inflammation of tissue in appendages or extremities of the shrimp (C).

tremendous influence on the water quality of the shrimp ponds. Hence favourable water quality parameters must be maintained throughout shrimp culture. The current study deals with the selection of three bacterial strains *Bacillus* sp. (*B. subtilis*, *B. licheniformis*, and *B. firmus* strains) with higher activity of extracellular amylase, protease, and cellulase enzymes were isolated, taken in equal proportions and administered in a dose of 1×10^{11}/mL as probiotic for mineralization (biodegradation) of organic material in shrimp culture water. This resulted in significant increase in dissolved oxygen, control of ammonia, nitrate and nitrite and maintenance of pH and salinity. Extracellular amylase and protease enzymes were used for mineralization process in the previous study done by Baumann *et al*.[13] and was reported. Similar effects have been reported for fish and shrimp aquaculture in previous studies and *Bacillus* sp. had been developed as an agent to decompose organic material and control bacterial pathogen. This is also supported by the study of Subuntith Nimrat *et al*.[14] which showed that administration of mixed *Bacillus* sp. as probiotic significantly improved growth and survival of post larval shrimp, increased beneficial bacteria in shrimp and culture water and enhanced water quality (pH, ammonia and nitrite levels) of culture water. These effects also have been demonstrated in the Indian carp (*Labeo rohita*). Bacterial probiotic enhances the shrimp growth by release of relevant enzymes and other growth factors that facilitate nutrients to hosts and increase the feed efficiency of *P. vannamei* shrimp. It also induces resistance to bacterial diseases and control the bacterial infection by competitive inhibition, which ultimately enhances the survival of shrimp. Due to space and resource limitation, traditional aquaculture has been strengthening into reticulated systems with high stocking densities of the cultured species resulting in an artificial environment which supports the growth of pathogenic bacteria and the generation of toxic metabolites[15]. The outburst of bacterial pathogens in aquaculture systems is a complex phenomenon causing significant losses to the industry[3]. To minimize the organic load in aquaculture pond, there is a need for mineralization process. In this process macro molecules are converted into smaller and inefficient soluble molecules by protease, amylase and cellulase enzymes. These molecules are then frequently eliminated by exchange of water at 2 days interval during cultivation[16].

In the present study, the probiotic-supplemented diets resulted in an increase of final weight of shrimps showing that the addition of probiotics increased the growth performance of shrimp. It showed a significant reduction of ammonia, nitrite and nitrate ions in shrimp culture water. In comparison to untreated control group, significant differences in survival of shrimp was recorded in the probiotic treated test tanks which indicate the effective control of bacterial infection by the probiotic. In this work, the survival percentage of shrimp in untreated T1 tank was 40% whereas in T2 tank was 80%, These results suggest that administration of *Bacillus* consortium in the rearing water of shrimp confers beneficial effect on water quality, shrimp growth, disease resistance and shrimp survival.

Conflict of interest statement

We declare that we have no conflict of interest.

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