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Role of different types of probiotics in pond ecosystem, in prawn (*Macrobrachium rosenbergii*) health and production

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Abstract
An experiment was carried out to explore the impact of probiotics on growth, survival and production of Prawn (*Macrobrachium rosenbergii*) in the Shrimp Research Station ponds where Juvenile prawn were stocked at a density of 2.5 juvenile/m^2 and was fed with quality feed (gold plus-grower) twice daily at the rate of 10.3% of prawn biomass. Three types of probiotics were supplemented viz, super PS probiotics (T_1) broadcast all over the ponds mixed with clay, super biotic probiotics (T_2) broadcast over the pond mixed with feed and super PS with super (T_3) biotic mixed broadcast over the ponds and each treatment having 3 replicates. Water quality parameters were checked periodically and found within suitable range for prawn growth without finding any stress. Growth was measured and feed was adjusted in accordance to growth rate. After the culture period of 180 days higher survival (84%) was found in T_2 followed by 81.9% in T_3 and 76.9% in T_1. average weight 70.6 g was found higher in T_2 which was significantly ($p<0.05$) different from those of T_3 (62.3 g) and T_1 (55.4 g). Food conversion ratio (FCR) was lower (2.71) in T_2 than these of 3.20 in T_1 and 2.92 in T_3. Higher yield was recorded in T_2 (1,210.4 kg/ha) than those of T_3 (1,130.46 kg/ha) and T_1 (940.3 kg/ha). The findings from the study revealed that growth, survival and yield were found higher from the ponds treated with super biotic than that of others.

Keywords: Probiotics, production, *Macrobrachium rosenbergii*

Introduction
Bangladesh is the most suitable countries in the world for giant freshwater prawn (*Macrobrachium rosenbergii*) farming due to its fertile land and agro-climatic conditions. Freshwater prawn (*Macrobrachium rosenbergii*) is widely cultured due to its advantages over in terms of size, growth, salinity tolerance and high price in international markets particularly to the USA, Europe and Japan. The export of prawn and shrimp was estimated at 50,368 metricton in 2008-09 with a value of 2,744.12 crore of which 27-30% was galda [9]. Farmers of the coastal areas are provided more attention on prawn farming because of it is more disease resistant than other shrimp species. *Macrobrachium rosenbergii* is significant potential for commerciall aquaculture [19]. One of the critical issues is soil-water quality management of farm for keeping congenial environment; Use of probiotics in aquaculture is increasing for commercial aquaculture practices [12]. Probiotics are live microorganisms that act beneficially in the host, promoting the balance of the intestinal microbiota, favoring the health of the animals [10, 11, 22] stated that an ideal probiotic, irrespective of its source must be able to colonize and multiply in the intestine of the host. There are a wide range of microalgae (*Tetraselmis*), yeast (*Debaryomyces*, *Phaffia* and *Saccharomyces*) and gram positive (*Bacillus*, *Lactococcus*, *Micrococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Streptcoccus*, *Weisslla*) and gram negative bacteria (*Aeromonas*, *Alteromonas*, *Photorhodobacterium*, *Pseudomonas* and *Vibrio*) that have been evaluated as a probiotics [12]. Several studies have exhibited promising results of the use of probiotics in fish, mollusk, crustacean and amphibian farming [7, 14, 16, 28, 33] which enables the probiotics to substitute the antibiotics as growth promoters. In fish, they are usually administered orally in order to improve the microbial flora of the intestine [23, 33]. Probiotics in aquaculture can be administrated either as a food supplement or as a additive to the water [18, 29, 30]. Though several types of probiotics (Aqua photo, Aqua mazic, Ammonil, Super biotic, Super PS) in powder or liquid forms are found in

the markets. Very little information is available in Bangladesh regarding soil- water quality and yield improvement of prawn farming using probiotics that's why this experiment was done for discovering new information and improving sustainable prawn farming technology.

Materials and Methods

Study place and time

The study was conducted in nine rectangular ponds having an area 0.052ha to 0.075 ha of Shrimp Research Station (SRS) - Bagerhat throughout a production cycle from March 2013 to September 2013. Tidal River Doratana flows by the side of the research station and the central out let of the research ponds is directly connected with this river through a canal

Experimental design

Three treatments of the experiment were: super Ps probiotics broadcast all over the ponds mixed with clay (T_1), super biotic probiotics broadcast over the pond, mixed with feed (T_2) and super Ps & super biotic probiotics mixed together broadcast over the ponds (T_3) and each treatments having 3 replicates.

Table 1: Experimental design of the present study

Treatments	Stocking density (No./ha)	Application of probiotics	
		Probiont type	Dose
T_1 (Super Ps)	25,000	Broadcast all over the ponds (mixed with clay)	40 ml/de/10 days
T_2 (Super biotic)	do	Broadcast (mixed with feed)	10-20 g/de/day
T_3 (Super Ps + Super biotic)	do	Broadcast	15 g + 20 ml/de

Pond preparation and water management

The experimental ponds were dried up and re-excavated. The soil of ponds were treated with lime (CaO) @ 250 kg/ha. After liming, tidal water was taken into the ponds up to a depth of 50-60 cm. All the unwanted animals of water were removed using phostoxin Tablets @ 1.5 nos. /decimal after three tablets applying, lime was again used @ 25 kg/ha. Pond water was then fertilized with TSP and urea (2:1) @ 37.5 kg/ha. After production of plankton, juveniles of prawn were stocked according to the experimental design (Table 1). A total of 9 water quality parameters vis. temperature, water depth, dissolved oxygen; pH, salinity, ammonia, nitrate, total alkalinity and transparency were monitored fortnightly intervals. Notable variations in parameters were not found among the three treatments.

Juveniles stocking

On March 5-25, 2013 the juveniles (ABW, 3.0 g) were stocked in all the ponds. Before stocking, the juveniles were carefully acclimatized with the pond water.

Water and soil quality monitoring

A total of 9 water quality parameters vis. temperature, water depth, dissolved oxygen; pH, salinity, ammonia, nitrate, total alkalinity and transparency were monitored fortnightly intervals. Notable variations in parameters were not found among the three treatments. Soil samples from each pond were collected. Samples were then air-dried and ground to a fine powder and preserved in airtight polythene bags. Then the samples were sent to Soil Resources Development Institute (SRDI) Laboratory, Daulotpur, Khulna for analyzing of organic matter, salinity, pH, nitrogen, phosphorus and potassium.

Feed application and growth measurement

The stocked prawn was fed with commercial pellet (protein 34%) Quality feed (gold plus-grower) @ 5-3% of total biomass twice daily. Prawn biomass was estimated fortnightly through sampling (Number of individuals and the average body weights, Healthiness, disease syndrome) by cast net and feed was adjusted accordingly and quantity of feed was also adjusted daily by checking the feeding behavior of prawn in the check tray. Sampling was continued until harvesting.

Microbial analysis

Quality and quantity of total heterotrophic bacteria (THB) and pathogenic bacteria (*Vibrio* sp.) of pond water and sediment was monitored Fortnightly/monthly using the help of Disease Lab of Shrimp Research Station, Bagerhat. Microbial analysis was done to identify bacterial quality of the treatments.

Zymetin

Zymetin is made with beneficial bacteria used in feed. It is usually mixed with feed to increase immunity and inhibit the growth of pathogenic Bacteria in the gut. It also improves the appetite of the shrimp and increase function of digestion and assimilation for better growth and reduces FCR. It is composed mainly with *Streptococcus faecalis*, *Clostridium Butyricum*, *Bacillus mesentericus*, protease, lipase and beer yiest.

Harvesting and production parameters

After 180 days of culture period, prawn from all ponds were harvested initially by repeated netting and finally dewatering water. After complete harvest, growth, survival and production of prawn in all ponds were estimated and compared.

Super PS

Super PS is a soil probiotics made with beneficial Bacteria which is used to improve pond bottom condition, reduces harmful bacteria and keep the environment friendly for aquaculture. It is composed mainly with Rhodobacter spp. and Rhodococcus spp.

Statistical Analysis

Microsoft Excel and One-way analysis of variance (ANOVA) was used to determine any significant differences among the treatments using SPSS 16 software.

Results and Discussion

Bacterial Load

Selected probiotics (Super Ps and Super biotic, manufactured by Charoen Pokphand Foods (Public) Co. Ltd, Thailand and imported by M/S Pranti Trading (Dealer of CP Products), 23 Natun Bazar, Lanchghat Road, Khulna) had applied at a manufacturer recommended doses of 40 ml/de at 10 days intervals, 10-20 g/de/day and 15 g + 20 ml/de in T_1, T_2, T_3 treatments respectively. Bacterial load in water and sediments ranged from 1.60×10^3 - 1.76×10^3 CFU/ml and 1.63×10^4 - 2.15×10^4 CFU/gm in T_1, 1.90×10^4 - 2.76×10^4 CFU/ml and

2.33 x 10^4 - 2.88 x 10^4 CFU/gm in T_2, 1.90 x 10^3 - 2.65 x 10^4 CFU/ml and 1.33 x 10^4 - 2.55 x 10^4 CFU/gm in T_3 treatments respectively.

Water Quality Parameters

Water quality parameters of three treatments as recorded are shown in Table 2. The salinity was recorded maximum (5.0 ppt) in the month of May-June and it was minimum (1.2 ppt) in March and August. Minimum salinity was recorded in probiotics treated ponds and maximum was in control ponds. The average pH ranged from 7.7 to 8.5. It was lower in the treated ponds than that of control ponds. The values of dissolved oxygen was maximum (4.9 mg/L) during the month of August and minimum (3.8 mg/L) was in June. It was maximum in probiotics treated ponds and control ponds. The temperature (27.6-32.8 ˚C) did not show any variation in probiotics treated ponds and control ponds. The transparency also did not show much difference in probiotics and control ponds. The range of nitrate of nitrogen of water of different treatments was 0.006-0.018 mg/L with no significant difference among them. Ammonia was minimum (0.002-0.039 mg/L) in probiotics treated ponds of all months. But ammonia was maximum (0.065 mg/L) in control ponds during last three months of culture period. In the present study, the size of selected ponds for prawn culture was 0.06 - 0.18 ha each. Ideal ponds size for prawn/shrimp culture should be 1 or less than 1 ha [29]. Water depth of 0.65-1.62m was maintained in all ponds throughout the culture period. It is recommended that a minimum depth of 1.0m be maintained at operational period. Initial water level of all culture ponds was slightly lower than the recommended level. The temperature in the present study was 27.6- 32.8 ˚C and higher temperature 32.8 ˚C was observed due to lower water in the month of April and May.

Table 2: Water quality parameters (mean ± SD with range) as recorded from the different treatments ponds during the study period.

Parameters	Treatments		
	T_1 (Broadcast all over the ponds mixed with clay)	**T_2 (Broadcast mixed with feed)**	**T_3 (Broadcast all over the ponds)**
Water depth (cm)	106.2±26.19 (49.4~139.0)	98.6±22.18 (48.5~130.6)	107.5±24.20 (53.4~141.7)
Temperature (ºC)	30.6±2.21 (28.2~35.8)	30.7±2.18 (28.1~35.6)	30.6±2.20 (28.2~35.6)
Salinity (ppt)	2.56±1.8 (0.0~5.0)	2.56±1.8 (0.0~5.0)	2.56±1.8 (0.0~5.0)
Dissolved oxygen (mg/L)	7.1±2.30 (4.9~12.4)	7.0±1.95 (4.9~9.9)	6.9±2.08 (4.9~10.9)
pH	8.36 (7.8~8.7)	8.40 (7.8~8.8)	8.34 (7.8~8.6)
Transparency (cm)	36.21±5.13 (29.3~44.0)	35.60±5.21 (30.0~44.7)	36.13±6.30 (28.0~46.7)
Total alkalinity (mg/L)	151.10±23.2 (108.3~179.0)	153.9±22.6 (101.3~175.0)	145.2±23.76 (102.7~172.0)
NO_3-N (mg/L)	0.002±0.001 (0.001~0.006)	0.003±0.001 (0.001~0.005)	0.003±0.001 (0.001~0.007)
NH_4-N (mg/L)	0.031±0.014 (0.01~0.07)	0.023±0.012 (0.01~0.05)	0.031±0.014 (0.01~0.05)

Soil sediment quality Parameters

Table 3: Mean values (ranges in parenthesis) of soil sediment quality as recorded from the probiotics reated ponds under different treatments during the study period.

Parameters	Treatments		
	T_1	**T_2**	**T_3**
pH	7.79±0.08 (7.7~8.1)	7.90±0.05 (7.8~8.1)	7.76±0.07 (7.7~8.0)
Salinity (Ec) (dec/m)	12.89±3.75 (8.65~18.12)	12.77±3.63 (8.73~17.96)	13.18±4.21 (8.81~18.15)
Organic matter (%)	2.11±0.20 (2.00~2.29)	2.21±0.11 (2.15~2.40)	2.08±0.17 (2.10~2.23)
Total nitrogen (%)	0.127±0.008 (0.119~0.137)	0.129±0.006 (0.126~0.140)	0.122±0.005 (0.115~0.125)
Phosphorus (µg/g soil)	10.33±2.73 (8.21~14.02)	11.73±3.10 (9.71~15.68)	10.0±2.55 (7.95~13.76)
Potassium (m.eq/100 g soil)	0.93±0.03 (0.90~0.98)	0.98±0.04 (0.94~1.10)	0.89±0.03 (0.87~0.90)

Variation of major sediments parameters like as pH, salinity, organic matter, total nitrogen, phosphorus and potassium are depicted in Table 3. The range of pH and salinities recorded was 7.8~8.0 and 8.62~18.63 ds/m, respectively. Remarkable variation in soil pH and salinity was not found among the probiotics treated ponds and control ponds. The highest content (2.37% and 2.39%) of organic matter was found in probiotics treated ponds and the lower of 1.75% in control ponds. Total nitrogen percentage was 0.118~0.138 and 0.124~0.139%, respectively in probiotics treated ponds, while it was within 0.102~0.116% in control ponds.

Growth, survival and Production performance

Higher survival (84%) of prawn was found in T_2 followed by 81.9% in T_3 and 76.9% in T_1. Mean body weight of prawn in T_2 was the highest of 70.6 g which was significantly ($p<0.05$) different from those of T_3 (62.3 g) and T_1 (55.4 g). Food conversion ratio (FCR) was lower (2.71) in T_2 than those of 3.20 in T_1 and 2.92 in T_3. Higher production (1,210.4 kg/ha) of prawn was recorded in T_2 than those of T_3 (1,130.46 kg/ha) and T_1 (940.3 kg/ha). The findings obtained from the study revealed that higher growth, survival and production of prawn were found from the ponds treated with super biotic probiotics than the other probiotics treated ponds.

Table 4: Growth, survival and production (mean ± SD) of *Macrobrachium rosenbergii* in different treatments during the culture period.

Particulars	Treatments		
	T_1	**T_2**	**T_3**
Stocking density (no./m^2)	2.5	2.5	2.5
Stocking size (g)	3.0±1.65	3.0±1.74	3.0±1.80
Harvesting size (g)	55.4±2.71 ^{c}	70.6±9.52 ^{a}	62.3±12.10 ^{b}
Survival (%)	76.9±2.18 ^{b}	84.0±1.20 ^{a}	81.9±1.26 ^{a}
FCR	3.20	2.71	2.92
Production (kg/ha)	940.3±25.41 ^{b}	1210.4±30.25 ^{a}	1130.46±35.45 ^{a}

Figures with different superscript in the same row differ significantly ($p<0.05$)

In case of *Macrobrachium rosenbergii*, production Bacillus, was able to colonize both the culture water and shrimp digestive tract, Bacillus also was able to replace vibrio spp. In the gut of shrimp, thereby increasing shrimp survival [27]. Bacillus is able to out-compete other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotic [18, 33]. In this study Super PS was applied in T1 where observed significant increment in growth including improved feed conversion ratio (FCR) which in turn contributed to the improved survival rate of shrimp

Discussion

According to the present findings, it is clearly noted that probiotics created positive impact on soil and water quality parameters for keeping congenial farm environment, which agrees with the finding of Sabrina *et al* (2017) [30] and Rubia *et al* (2017) [29]. In the present study, production was higher in all probiotics supplemented ponds than control ponds which similar to the findings of Noh *et al*. (1994) [24]; Bogut *et al*. (1998) [6] and Nikoskelaine *et al*. (2001) [23] Sabrina *et al* (2017) [30] and Rubia *et al* (2017) [29] who obtained better growth response with diets supplemented with probiotics containing bacteria. Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to increase the growth rate and welfare of farmed aquatic animals (Lara-Flores *et al*, 2003 [15]; Bligh and Dyer, 1959 [5]; Macey and Coyne, 2005 [17]; Wang *et al*, 2005 [36]. Despite that, He *et al.* (2009) [13] revealed that growth performances fishes were not significantly influenced by dietary supplementation of yeast (S. cerevisiae) at different levels. In the present study, the highest FCR and the lowest PER was found in control ponds no probiotics was used. These indicate that, fish fed control diet consumed more diet than the other treatments. Evidence is available that indicates gastrointestinal bacteria take part in the decomposition of nutrients, provide the macroorganisms with physiologically active materials (Bairagi *et al*, 2002 [2], 2004 [3]; Ramirez and Dixon, 2003 [26]; Sugita *et al*, 1992 [31], 1997 [32]; Wang and Xu, 2006 [35]; Wang, 2007 [36]; Ai *et al*, 2011 [1]) and thus facilitate feed utilization and digestion. This may account for the enhanced PER by dietary supplementation of probiotics in the present study. The addition of probiotic as live supplements in the diet allows probiotic to survive passage through the intestinal tract (Fuller, 1992) [11]. Microorganisms and their enzymes have an important role in the digestion process (Munilla-Moran *et al*., 1990) [20] by increasing the total enzyme activity of the gut (Ding *et al*., 2004 [8]; Ziaei-Nejad *et al*., 2006 [38]) and stimulating the production of endogenous enzymes (Ochoa-Salano and Olmos-Soto, 2006 [25]; Wang, 2007 [36]) which in turn can increase the food digestibility. In addition, the exogenous enzymes have a broader pH range than endogenous enzymes that prolongs the digestion period and may allow better hydrolysis of substrates. As pointed by several authors the digestive enzymes (amylase, protease and lipase) could be improved by administration of probiotics to the diet (Ziaei-Nejad *et al*., 2006 [38]; Wang, 2007 [36])

Conclusions

Probiotics is important catalyst for increasing survivality, enhancing growth, reducing pathogen; keep the culture environment congenial in the prawn farm. Now a days, bacterial diseases are considered to be a major threat in prawn farming. So, findings of the present study could be applied extensively to the farmer`s level as a mitigation/preventive major of prawn bacterial disease, to increase the production in our country. Thus farmers would get higher production and economic return.

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