

# Efficacy of Probiotics on Growth and Sustainable Production of Black Tiger Shrimp, *Penaeus monodon* Fabricius 1798 in Brackishwater Ponds of West Bengal, India

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## Abstract

Probiotics are bio-friendly agents which have been recently used in sustainable forms of aquaculture to produce shrimp. In the present study, the efficacy of probiotics was assessed by counting both the beneficial (total heterotrophic bacteria; THB) and pathogenic (green *Vibrio* and luminous *Vibrio*) bacteria *vis-à-vis* harvest outcomes (survival, body weight, feed conversion and production) of cultured shrimp, *Penaeus monodon* Fabricius 1798. During the 105-day culture period, soil quality (redox potential, organic matter and organic carbon) and water quality (transparency, NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N) parameters changed significantly ( $p < 0.05$ ) and favoured better environment for growth of shrimp in probiotics treated ponds than in the control. Microbial count of THB was significantly higher ( $p < 0.01$ ) in treated ponds but a reverse result was obtained for both green *Vibrio* and luminous *Vibrio* ( $p < 0.05$ , 0.01) count which were higher in the control ponds. After harvest, a significantly higher growth and production yield ( $p < 0.05$ , 0.01) of shrimp in the treatment ponds suggested that probiotics played a vital role on better growth and sustainable production of black tiger shrimp by modulating the bacterial population and improving the pond environment.

## Introduction

Increasing demand of seafood throughout the world has led to a worldwide expansion of shrimp culture. However, not all shrimp culture techniques are environmentally friendly and sustainable shrimp production requires specific culture practices (Phillips et al. 1993; Primavera 1994). Recent shrimp farming practices are affected by various diseases caused mainly by luminous *Vibrio* and other bacteria (Soundarapandian et al. 2010). Outbreaks of luminous vibriosis caused by *Vibrio harveyi* are major constraints in shrimp production with potentially catastrophic impacts (Ruangpan 1991; Lavilla Pitogo 1995). To combat such diseases, beneficial bacteria (probiotics) have been used for environment-friendly aquaculture practices to displace pathogens by modifying bacterial composition of water and sediment in culture ponds (Moriarty 1997). Probiotics are generally defined as live microbial food supplements which improve the balance of the host animal's intestinal flora (Fuller 1989). Probiotics as live or dead micro-organisms with health benefits to the host have been used in aquaculture for disease control and can be administered either

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as food supplements or as additives to the water (Moriarty 1998). Probiotics were also used to improve shrimp health by minimising disease incidence (Gatesoupe 1999; Senok et al. 2005). In aquaculture, green *Vibrio* and luminous *Vibrio* are considered as pathogenic bacteria causing diseases to shrimp, whereas total heterotrophic bacteria (THB) in general and particularly species of *Bacillus*, *Pseudomonas* and *Lactobacillus* provide beneficial effects (Jaganmohan and Prasad 2010). However, the beneficial effect of using such microbial products in aquaculture is still debatable and controversial as their efficacy is yet unclear. The use of terrestrial bacterial species as probiotics has had limited success, as bacterial strain characteristics are dependent upon the environment in which they thrive (Moriarty 1997) and information on the use of probiotics is very scarce particularly in commercial shrimp farming.

In the present study an attempt was made to culture the black tiger shrimp, *Penaeus monodon* Fabricius 1798 in earthen ponds treated with probiotics. The efficacy of probiotics on the sustainable production of *P. monodon* in a semi-intensive culture system was examined in the light of: i) various soil and water quality parameters; ii) microbial count (THB, green *Vibrio* and luminous *Vibrio*); and iii) production outcome (survival, growth, feed conversion and yield) after harvest.

## Materials and Methods

### *Experimental ponds*

*Penaeus monodon* was cultured during September-December, 2009 in the Kar shrimp farm at Keshabpur, Haldia (West Bengal), India (Lat 21°55' N, Long 88°46' E). Six rectangular earthen ponds (~ 0.5 ha) with both inlet and outlet facilities, were used in the experiment. Three ponds were randomly selected for probiotics treated series with another three ponds used for the control series (without probiotics). The source of water for all the experimental ponds was from the creek of the Haldi River. Water depth of the ponds was maintained between 110 cm and 120 cm throughout the culture period. Twenty-day old post larvae (PL<sub>20</sub>) of *P. monodon* (PCR tested negative for white spot syndrome virus, and are healthy) were procured from a reputed shrimp hatchery and stocked with 20 pcs·m<sup>-2</sup> in experimental ponds after acclimatisation by keeping the polythene bags containing the PLs afloat in the pond water for one h and for an added ½ h after an equal volume of pond water was added into the polythene bags. Water exchange (0-10%) was performed once weekly and there was no exchange during the first 20 days, although water was added to maintain losses due to seepage and evaporation. Aeration was maintained for 4-8 h·day<sup>-1</sup>. The feed ration was divided into portions and fed over 2-4 times a day and the quantity was reduced or increased as per regular check tray observations (Clifford 1992). Shrimp were harvested after a grow-out period of 105 days. Both the treatment and control ponds were prepared and managed following the method described by Hasan et al. (2011).

### Probiotics application

Commercial probiotics (water, soil and gut probiotics) comprising different microbes in combination with mutagen (immuno-stimulant) (Table 1) were applied in treatment ponds using doses recommended by the manufacturer (CP Aqua, Chennai, India) while control ponds were free from probiotics application. Before and 2 days after stocking, pH fixer (mixed with freshwater and sugar) was applied (Table 2). Super biotic (mixed with freshwater and sugar) and super PS (mixed with sand) were broadcasted in the morning hours with water agitation by aerators (Tables 2 and 3). A mixture of super biotic, zymetin (gut probiotics) and mutagen (immune enhancer) were bound with sugar and coated with feed and applied twice daily (Table 4) (Saundarapandian et al. 2010).

**Table 1.** Different probiotics, immunostimulant and their mode of use in treated ponds.

| Probiotics   | Ingredients                                                                                                                   | Mode of use   |
|--------------|-------------------------------------------------------------------------------------------------------------------------------|---------------|
| pH Fixer     | <i>Bacillus</i> sp.                                                                                                           | Water         |
| Super Biotic | <i>Bacillus</i> sp. and <i>Streptococcus</i> sp.                                                                              | Water and gut |
| Super PS     | <i>Rhodobacter</i> sp. and <i>Rhodococcus</i> sp.                                                                             | Soil          |
| Zymetin      | <i>Streptococcus faecalis</i> , <i>Bacillus mesentericus</i> ,<br><i>Clostridium butyricum</i> , Protease, Lipase, Beer yeast | Gut           |
| Mutagen      | Minerals, amino acids, vitamins and immune enhancer                                                                           | Gut           |

**Table 2.** Dosage of probiotics before stocking in treatment ponds (per ha).

| Days of culture  | Water probiotics | Soil probiotics | Water probiotics |
|------------------|------------------|-----------------|------------------|
|                  | (Super biotic)   | (Super PS)      | (pH fixer)       |
| 0 (Pre-stocking) | 3.0 kg           | 20.0 L          | 4.0 kg           |

**Table 3.** Dosage of water and soil probiotics during production period (per ha).

| Days of culture | Water probiotics<br>(Super biotic) | Soil probiotics<br>(Super PS) |
|-----------------|------------------------------------|-------------------------------|
| 45              | 3.0 kg                             | 20.0 L                        |
| 60              | 3.0 kg                             | 20.0 L                        |
| 75              | 3.0 kg                             | 20.0 L                        |
| 90              | 3.0 kg                             | 20.0 L                        |
| 100             | 3.0 kg                             | 20.0 L                        |

**Table 4.** Dosage of gut probiotics during production period (per kg of feed).

| Days of culture | Super biotic | Mutagen | Zymetin |
|-----------------|--------------|---------|---------|
| 41-50           | 10 g         | 10 g    | 10 g    |
| 51-60           | 10 g         | 10 g    | 10 g    |
| 61-70           | 10 g         | 10 g    | 10 g    |
| 71-90           | 10 g         | 10 g    | 10 g    |
| 91-100          | 10 g         | 10 g    | 10 g    |

### **Water and soil parameters analyses**

Different water quality parameters like temperature, dissolved oxygen and pH were monitored twice daily (06-00 and 16-00 h) while salinity and transparency were monitored once a week (11-00 h) *in situ* using the multi-parameter device YSI, MP556 mode and Secchi disc. Other nutrients (NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, PO<sub>4</sub>-P) and total alkalinity were monitored monthly during the whole production period (Boyd and Tucker 1992; APHA 1995). Soil samples from the bottom sediments of the experimental ponds were collected using Ekman's dredger at monthly intervals throughout the culture period. Soil pH, redox potential, organic carbon and organic matter were measured following standard methods (Boyd and Tucker 1992; Boyd 1995).

### **Bacterial population count**

The water and sediment samples of both the treatment and control ponds were collected separately from different parts of the ponds using sterile conical flasks for the estimation of THB, green *Vibrio* and luminous *Vibrio* population at four different culture phases: (i) before stocking (pre-stocking phase; PS); (ii) stocking to day of culture (DOC) 30 (initial phase; IN); (iii) DOC 31-70 (intermediate phase; IM); (iv) DOC 71 to harvest (pre-harvest phase; PH). Each water and soil sample was transferred to another sterile conical flask (200 mL) containing 99 mL of sterile diluents and serial dilution was performed to get 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> suspension samples. For enumeration of THB and *Vibrio* (green and luminous), Zobell marine agar and TCBS medium were used respectively (Jaganmohan and Prasad 2010).

### **Shrimp growth and production**

The average body weight and survival of the shrimp were recorded fortnightly in all the ponds throughout the culture period. Cast net was used to haul a minimum of 100 shrimp randomly to measure the individual weight of shrimp every fortnightly. Four hauls of animals by cast net were used for estimation of survival on and from 45<sup>th</sup> day to 105<sup>th</sup> DOC. Health of shrimp and abnormality were recorded during each fortnightly sampling. Average body weight, average daily growth, survival rate, feed conversion and production yield of *P. monodon* during culture were estimated following the conventional formulae (Hasan et al. 2011).

Average body weight (ABW) = Total weight of 100 random sampling of shrimp/100

Average daily growth (ADG) = ABW on day of sampling - Initial ABW/ Interval between samplings (DOC)

Feed conversion ratio (FCR) = Total feed consumed (kg) / total yield (kg)

Survival rate = (N/N<sub>0</sub>) X 100 [where, N<sub>0</sub> & N are initial and final number of shrimp, respectively]

Production Yield (kg) = No. of survived shrimp x ABW

### ***Cost calculation***

Production costs, gross revenue, net profit, benefit cost ratio (BCR) and return on investment (ROI) were calculated following the standard method of Hari et al. (2004).

### ***Statistical analysis***

All the data were subjected to statistical analysis (Gomez and Gomez 1984). Single factor analysis of variance (ANOVA) with the help of MS Excel and computer software SPSS (version 7.5) were used at 1% and 5% levels of probability between the treatment and control series.

## **Results**

### ***Water and soil quality parameters***

The range and mean values of the different physico-chemical parameters of water are presented in Table 5. Ambient morning temperature ranged from 21.3-29.4 °C, while pH (7.6-8.2) was alkaline throughout the culture period in all the ponds. Mean dissolved oxygen was  $4.25 \pm 0.5$  mg·L<sup>-1</sup> in probiotics treated ponds, whereas it was  $3.51 \pm 0.7$  mg·L<sup>-1</sup> in the control ponds during the morning. Average transparency and salinity of water in control and treatment ponds ranged from  $66.2 \pm 5$  to  $40.6 \pm 6.2$  cm; and  $11.7 \pm 2.9$  to  $12.3 \pm 2.6$  ppt respectively, indicating significant increase ( $p < 0.05$ ) of transparency in the control ponds. The range of total alkalinity was within optimum levels in both control (95-122 mg·L<sup>-1</sup>) and treatment ponds (108-142 mg·L<sup>-1</sup>). Total alkalinity was significantly higher ( $p < 0.05$ ) in the treatment ponds than the control. On the other hand, the mean values of NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N were significantly higher ( $p < 0.05$ ) in control ponds than the treatment ponds but there was no significant difference in the concentration of PO<sub>4</sub>-P between the treatments and the controls. Soil pH, redox potential (Eh), organic carbon and organic matter ranged from 5.9-6.4, -83 to -264mV, 0.08-0.48% and 0.14-0.82% respectively in control ponds; and were 5.8-6.4, -32 to -110mV, 0.06-0.31% and 0.10-0.53% respectively in treatment ponds indicating that the values of Eh, organic carbon and organic matter significantly increased ( $p < 0.05$ ) in control ponds. The level of Eh was lowest and organic carbon/matter was on the higher side at the last phase of the culture period (PH phase) in control ponds. It was reduced by 52% in treated ponds when compared to the control. However, levels of temperature, salinity, pH, DO and orthophosphate of water and pH of soil did not differ significantly in treatment and control ponds throughout the culture period (Table 5).

**Table 5.** Range and mean ( $\pm$ SE) values of different water and soil quality parameters in treatment and control ponds.

| Parameter                              | Control     |                     | Treatment   |                                |
|----------------------------------------|-------------|---------------------|-------------|--------------------------------|
|                                        | Range       | Average             | Range       | Average                        |
|                                        |             | (mean $\pm$ SE)     |             | (mean $\pm$ SE)                |
| <b>Water quality</b>                   |             |                     |             |                                |
| Transparency (cm)                      | 40-100      | 66.2 $\pm$ 15       | 30-55       | 40.6 $\pm$ 6.2 <sup>a</sup>    |
| Temperature ( $^{\circ}$ C)            | 21.3--29.4  | 25.7 $\pm$ 2.5      | 21.4-29.4   | 25.9 $\pm$ 2.4                 |
| Salinity (ppt)                         | 7-18        | 11.7 $\pm$ 2.9      | 7-18        | 12.3 $\pm$ 2.6                 |
| pH                                     | 7.6-8.2     | 7.91 $\pm$ 0.12     | 7.8-8.2     | 7.94 $\pm$ 0.10                |
| Total alkalinity (mg l <sup>-1</sup> ) | 95-122      | 93.17 $\pm$ 4.13    | 108-142     | 122.9 $\pm$ 9.14 <sup>a</sup>  |
| Dissolved oxygen (mg l <sup>-1</sup> ) | 2.4-5.1     | 3.51 $\pm$ 0.71     | 3.0-5.6     | 4.2 $\pm$ 0.5                  |
| Ammonium- N (mg l <sup>-1</sup> )      | 0.13-0.38   | 0.18 $\pm$ 0.02     | 0.06-0.14   | 0.09 $\pm$ 0.02 <sup>a</sup>   |
| Nitrite - N (mg l <sup>-1</sup> )      | 0.08-0.22   | 0.12 $\pm$ 0.01     | 0.06-0.12   | 0.07 $\pm$ 0.01 <sup>a</sup>   |
| Nitrate - N (mg l <sup>-1</sup> )      | 0.72-1.44   | 0.61 $\pm$ 0.04     | 0.39-0.85   | 0.43 $\pm$ 0.04 <sup>a</sup>   |
| Orthophosphate (mg l <sup>-1</sup> )   | 0.03-0.16   | 0.09 $\pm$ 0.04     | 0.03-0.12   | 0.07 $\pm$ 0.03                |
| <b>Soil quality</b>                    |             |                     |             |                                |
| pH                                     | 5.9-6.4     | 6.12 $\pm$ 0.16     | 5.8-6.4     | 6.21 $\pm$ 0.20                |
| Redox potential (mV)                   | -83 to -264 | -115.75 $\pm$ 18.78 | -32 to -110 | -55.5 $\pm$ 10.66 <sup>a</sup> |
| Organic carbon (%)                     | 0.08-0.48   | 0.28 $\pm$ 0.02     | 0.06-0.31   | 0.19 $\pm$ 0.03 <sup>a</sup>   |
| Organic matter (%)                     | 0.14-0.82   | 0.49 $\pm$ 0.03     | 0.10-0.53   | 0.33 $\pm$ 0.03 <sup>a</sup>   |

<sup>a</sup> in the same row with superscript is significantly different. *p* values: <sup>a</sup><0.05

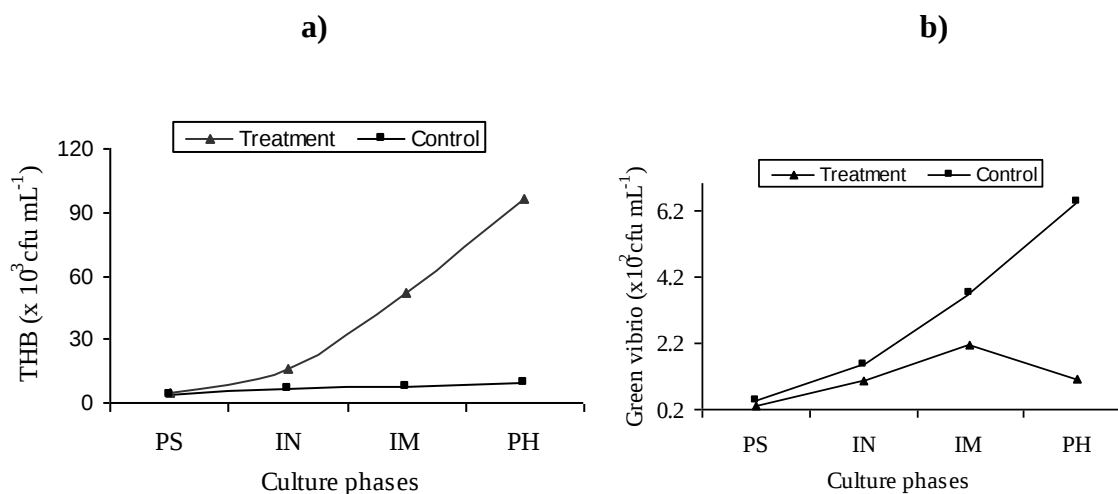
### Bacterial population count

The differential count of bacterial populations in both the treatment and control ponds is presented in Table 6. THB count was significantly higher ( $p < 0.01$ ) at pre-harvest phase in treatment ponds ( $96.6 \pm 32$ )  $\times 10^3$  than the control ( $9.9 \pm 1.2$ )  $\times 10^3$ . Increment of THB in treatment ponds was 89.7% more than control ponds in the pre-harvest phase. On the contrary, significantly lower count ( $p < 0.05$ , 0.01) of green *Vibrio* in the treatment ponds was found during intermediate ( $2.1 \pm 0.2$ )  $\times 10^2$  and pre-harvest phases ( $1.1 \pm 0.3$ )  $\times 10^2$  than the control ( $3.7 \pm 0.4$  and  $6.4 \pm 0.9$ )  $\times 10^2$ . Density of THB increased gradually and reached its peak during pre-harvest phase in both the treatment and control ponds but highest green *Vibrio* population was found in intermediate phase for treatment ponds and pre-harvest phase for control ponds (Figs. 1a and 1b) and reduction of green *Vibrio* was 82.9% in treatment ponds. At pre-harvest phase, *Vibrio* load was highest in control ponds and reduced in probiotics treated ponds (Fig. 1b). However, luminous *Vibrio* level increased at intermediate and pre-harvest phases ( $>100$  cfu mL<sup>-1</sup>) in control ponds which was absent in treatment ponds.

**Table 6.** Average microbial density (cfu mL<sup>-1</sup>) of THB ( $\times 10^3$ ), green *Vibrio* ( $\times 10^2$ ) and luminous *Vibrio* during different phases of culture in treatment and control ponds.

|                    | Bacteria type                         | Pre-stocking<br>(PS) | Initial<br>(IN) | Intermediate<br>(IM)        | Pre-harvest<br>(PH)        |
|--------------------|---------------------------------------|----------------------|-----------------|-----------------------------|----------------------------|
| Treatment<br>ponds | Total heterotrophic bacteria<br>(THB) | 4.3 $\pm$ 1.2        | 16.2 $\pm$ 4.4  | 52.2 $\pm$ 9.1 <sup>b</sup> | 96.6 $\pm$ 32              |
|                    | Green <i>Vibrio</i>                   | 0.3 $\pm$ 0.1        | 1.1 $\pm$ 0.2   | 2.1 $\pm$ 0.3               | 1.1 $\pm$ 0.3              |
|                    | Luminous <i>Vibrio</i>                | <10                  | <10             | nil                         | nil                        |
| Control<br>ponds   | Total heterotrophic bacteria<br>(THB) | 3.7 $\pm$ 1.5        | 6.2 $\pm$ 1.6   | 7.8 $\pm$ 1.3               | 9.9 $\pm$ 1.2              |
|                    | Green <i>Vibrio</i>                   | 0.4 $\pm$ 0.1        | 1.5 $\pm$ 0.3   | 3.7 $\pm$ 0.4 <sup>a</sup>  | 6.4 $\pm$ 0.9 <sup>b</sup> |
|                    | Luminous <i>Vibrio</i>                | <10                  | >10             | >100                        | >100                       |

<sup>a, b</sup> in the same row with different superscripts are significantly different. *p* values: <sup>a</sup><0.05; <sup>b</sup><0.01

**Fig. 1.** Variation of microbial count in different phases of culture (a=THB, b= Green *Vibrio*).

### Production performances

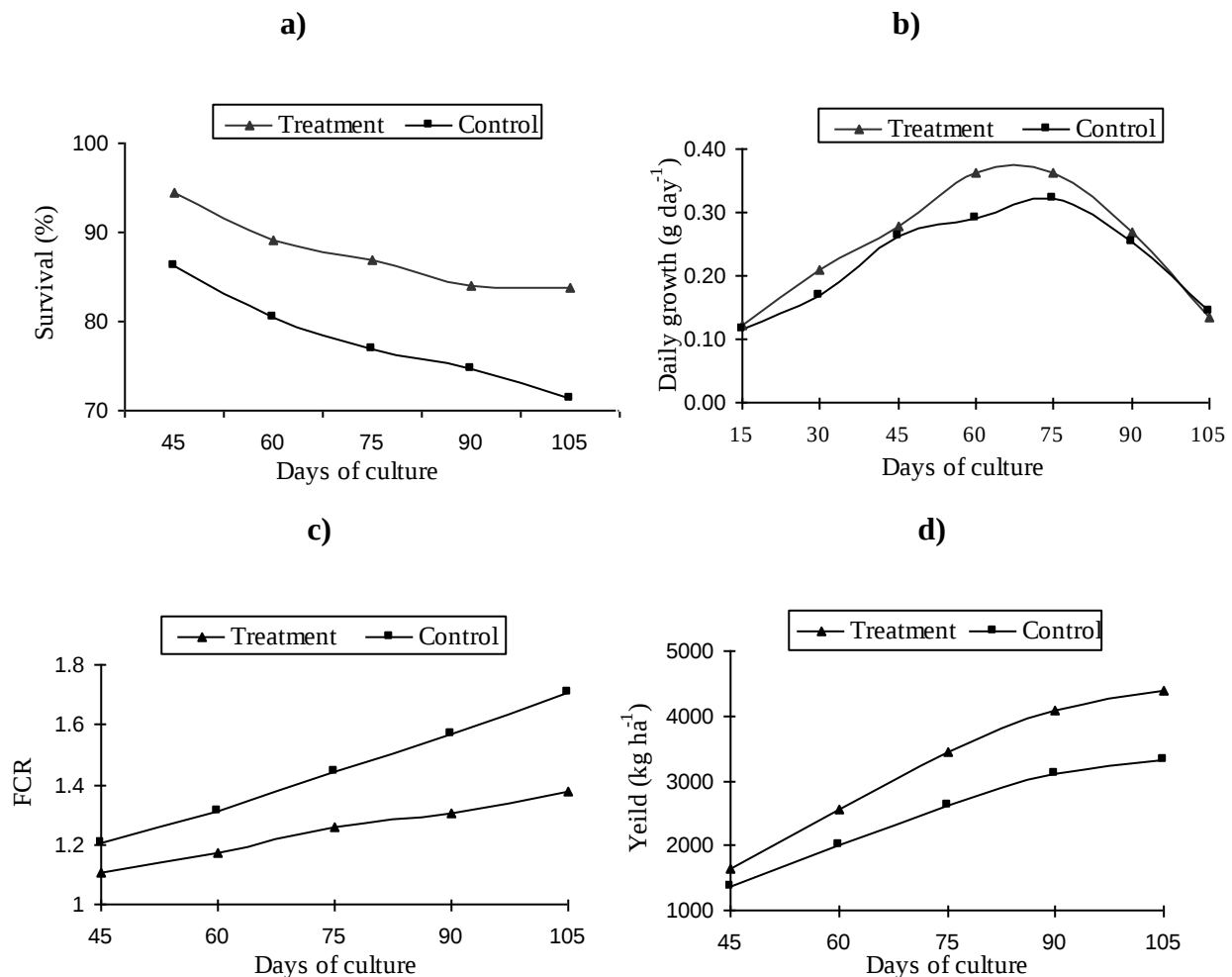
Higher survival rate ( $83.87 \pm 4.42$  %) of shrimp was recorded in the probiotics treated ponds than the control ( $71.4 \pm 7.05$  %) at harvest (Table 7 and Fig. 2a). On the other hand, significant increase of ADG and ABW ( $p < 0.01$ ) of shrimp were recorded in treatment ponds than in the control ponds at harvest. In general, the body weight of shrimp increased with time. ADG exhibited fluctuations of growth rate and it was highest between 60-75 days of culture and the lowest was observed at the last part of culture period when temperature dropped to less than 22.0 °C during the winter period (Fig. 2b). However, feed conversion ratio (FCR) increased slowly as days of culture progressed for both the treatment and control ponds but it was always lower in the treatment ponds (1.38) than in the control (1.71); and the difference (Fig. 2c) was statistically significant ( $p < 0.05$ ).

Finally, significant ( $p < 0.05$ ) increase in average production yield (Fig. 2d) of cultured shrimp was obtained in treatment ponds ( $4381.7 \pm 196$  kg) than the control ponds ( $3331 \pm 320$  kg).

**Table 7.** Growth and production outcome of shrimp at harvest on 105<sup>th</sup> day (n=3 replicates of ponds).

| Production variable                         | Control          | Treatment                     |
|---------------------------------------------|------------------|-------------------------------|
| Final average body weight (g)               | 23.3 $\pm$ 0.40  | 26.13 $\pm$ 0.49 <sup>b</sup> |
| Average daily growth (g day <sup>-1</sup> ) | 0.22 $\pm$ 0.004 | 0.25 $\pm$ 0.004 <sup>b</sup> |
| Final survival (%)                          | 71.4 $\pm$ 7.05  | 83.87 $\pm$ 4.4               |
| Feed conversion ratio                       | 1.71 $\pm$ 0.09  | 1.38 $\pm$ 0.07 <sup>a</sup>  |
| Production yield (kg ha <sup>-1</sup> )     | 3331 $\pm$ 320   | 4381.7 $\pm$ 196 <sup>a</sup> |

<sup>a, b</sup> in the same row with different superscripts are significantly different.  $p$  values: <sup>a</sup><0.05; <sup>b</sup><0.01



**Fig. 2.** Growth and production performance of shrimp as days of culture progressed in treatment and control ponds (a= survival rate, b= average daily growth, c= feed conversion ratio, d=production yield).

### Economic analysis

Economic analysis of shrimp production and value in Indian Rupees (INR.) is summarised in Table 8. The application of probiotics had a significant effect ( $p < 0.05$ ) on the gross revenue (INR. 1,139,242) of the harvested shrimp from treatment ponds when compared to the control (INR. 816,095). Net profit and benefit cost ratio (BCR) among the treatments changed significantly ( $p < 0.05$ ) as in the case of gross revenue. Net profit (INR. 385,418) was significantly higher ( $p < 0.05$ ) in probiotics ponds as compared to control (INR. 174,386). However, significantly higher ( $p < 0.05$ ) BCR (0.51) in probiotics ponds as compared to control (0.27) amply speaks for effective net profit (INR. 385,418) and return on investment (ROI) of 51% after harvest (Table 8). Variable cost was found to be higher (INR. 636,910) in treatment ponds than the control for the additional cost incurred for probiotics, other related inputs and extra labour. Net profit and ROI were higher and more attractive in probiotics treated ponds than the control.

**Table 8.** Cost and economic analysis of semi-intensive culture of *P. monodon* treated with probiotics (per hectare).

| Particulars                                        | Control | Treatment            |
|----------------------------------------------------|---------|----------------------|
| <b><u>Variable costs</u></b>                       |         |                      |
| Pond preparation                                   | 11450   | 11450                |
| Fertilisers                                        | 6200    | 2200                 |
| Probiotics and mutagen                             | 0       | 100200               |
| Post larvae (fry)                                  | 124000  | 124000               |
| Feed                                               | 353760  | 362760               |
| Miscellaneous                                      | 33400   | 36300                |
| <b><u>Total variable cost</u></b>                  | 528810  | 636910               |
| Interest for 4 months@13%                          | 19642   | 23657                |
| <b><u>Fixed cost</u></b> (depreciation + interest) | 93257   | 93257                |
| <b><u>Production</u></b>                           |         |                      |
| Total shrimp yield (kg ha <sup>-1</sup> )          | 3331    | 4381.7 <sup>a</sup>  |
| Price of shrimp (INR. kg <sup>-1</sup> )           | 245     | 260                  |
| <b><u>Economic analysis</u></b>                    |         |                      |
| Total production cost (INR.)                       | 641709  | 753824               |
| Gross revenue (INR.)                               | 816095  | 1139242 <sup>a</sup> |
| Net profit (INR.)                                  | 174386  | 385418 <sup>a</sup>  |
| Benefit cost ratio (BCR)                           | 0.27    | 0.51 <sup>a</sup>    |
| Return of investment (ROI)                         | 27%     | 51%                  |

<sup>a</sup> in the same row with different superscript is significantly different.  $p$  values: <sup>a</sup> $< 0.05$ ;

\*US\$ 1 = ₹ 47.1 Indian Rupees (INR.)

### Discussion

The result of the present study shows that a combination of different probiotics and immuno-stimulant plays a vital role on growth, survival and cost-effective sustainable production of *P.*

*monodon* by improving and maintaining good soil and water quality of the culture ponds. Previous studies showed that different water and soil quality parameters have an influence on the sustainable production of shrimp (Ramanathan et al. 2005; Soundarapandian and Gunalan 2008). It was found that among different water and soil quality parameters, transparency, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, redox potential, organic matter and organic carbon increased significantly with time in control ponds than in the treatment ponds. In spite of aeration, lower DO concentration and higher transparency during the last phase of production cycle indicated unstable plankton population and less natural food in control ponds (Abesamis 1989; Soundarapandian and Gunalan 2008). However, optimum level of total alkalinity and low ammonium nitrogen in treatment ponds indicated higher carbon source, buffering capacity and decay of organic matter respectively (Fast and Lester 1992). It was clear from the data that the levels of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  were reduced by 50%, 41.6% and 29.5% respectively in probiotics treatment ponds when compared to the control ponds (Table 5). Lakshmanan and Soundarapandian (2008) found that the concentrations of ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen were higher in the control ponds than the probiotics treated ponds. Hence their findings are in agreement with our present finding. In the present study, higher nitrite nitrogen concentration in control ponds amply speaks for the less aerobic condition caused by organic enrichment from the uneaten feed or feed wastes (Fast and Lester 1992). Some of the benefits of using bacterial products include reduction of nitrate, nitrite, ammonium nitrogen and phosphate levels, increasing dissolved oxygen concentrations and promotion of organic matter decomposition (Boyd 1995). Pond bottom conditions are critical for shrimp culture as *P. monodon* spend most of the time on the soil bottom. High organic matter in the control ponds leads to anaerobic conditions (Boyd 1989); this is also indicated in our study. Redox potential (Eh) value fluctuated greatly in the control ponds possibly due to the varying organic load and the demand for oxygen. Sharp reduction of Eh at the pre-harvest phase (PH) in control ponds indicated deterioration of soil characteristics and the accumulation of more black soil which eventually leads to black gills disease. It was found that ponds treated with probiotics enhanced mineralisation of organic matter, got rid of undesirable waste compounds and created congenial environmental conditions for growth of shrimp. It is apparent from this study that the use of the combination of probiotics improved or changed the population density of various bacteria (total heterotrophic bacteria, green *Vibrio* and luminous *Vibrio*) in the treatment ponds. Farzanfar (2006) reported that beneficial bacteria help the pond system to eliminate organic and inorganic nutrients to a non toxic level suitable for shrimp. This is in agreement with our study where higher counts of THB in treatment ponds (due to the application of probiotics) maintained the aerobic condition of the ponds which is beneficial for shrimp growth (Fig. 1a). In our study a very low abundance or absence of luminous *Vibrio* was also observed in the treatment ponds. It was reported that abundance and virulence of luminous *Vibrio* strains decreased where probiotic strains of *Bacillus* species were added (Moriarty 1998). Ruangpan (1991) reported that the abundance of luminescent *Vibrio* is consistent with occurrence of disease and resulted in very poor harvest as found in our study. Unlike control ponds, no disease incidence (swollen gut and vibriosis) appeared in ponds treated with probiotics (Soundarapandian et al. 2010) as mutagen might have improved the

immunity against diseases. Microbial count (Table 6) indicated that *Vibrio* sp. was dominant only in the control ponds similar to the findings of Lakshmanan and Soundarapandian (2008). Application of microbial supplement in the treatment ponds hindered the growth of green *Vibrio* and luminous *Vibrio* due to the colonisation of the beneficial microbes like *Bacillus* sp., *Pseudomonas* sp., and *Lactobacillus* sp. in the shrimp gut (Jaganmohan and Prasad 2010). Our study showed that when these bacteria were administered as probiotics in *P. monodon* culture system, growth and survival rate were improved and immunity was enhanced (Rengpipat et al. 2000).

An average survival of 70 to 80% is quite possible if the ideal condition is maintained for *P. monodon* culture (Reddy 2000). In the present study, maximum survival (88.9%) achieved in one of the treatment ponds and minimum survival (63.3%) was found in one of the control ponds (Table 7 and Fig. 2a). Control ponds were dominated by green colonies (causative agents for vibriosis) which lead to lower survival of shrimp when compared to the survival in the treatment ponds. Maximum daily growth rate ( $0.36 \text{ g day}^{-1}$ ) was recorded in treatment and  $0.32 \text{ g day}^{-1}$  in control ponds on DOC 75 when temperature and salinity were congenial for shrimp growth as observed in our previous study (Hasan et al. 2011). Growth and survival rate of the ponds applied with probiotics were higher than control ponds as found in earlier observation (Soundarapandian et al. 2010). Average growth rate of shrimp depends mainly on pond environment and effective management of feeding (Pushparajan and Soundarapandian 2010). Similar findings were observed elsewhere (Shariff et al. 2001; Matias et al. 2002) that certain commercial microbial products enhanced shrimp growth and production. Lower FCR and higher yield with bigger sized shrimp in treatment ponds fetched better return on investment even though additional expenses were incurred for the application of probiotics (Figs. 2c, 2d and Table 8). Sustainability and profitability depend on good feed management as it is the major cost input followed by fry and probiotics costs. In semi-intensive farming, supplementary feed is reported to be the initial source of physiological waste and accounts for around 50% of operational costs (Chanratchakool et al. 1994). Shrimp farming is regarded as the best economic and high pay-off activity in terms of returns to investment in some shrimp farms in India (Krishnan et al. 2001; Hari et al. 2004) and this is in agreement with our present study. The economic analysis performed in the on-farm trial showed probiotics treatment substantially increased net profit, BCR and return on investment when compared to ponds free of probiotics. Furthermore, the combined effect of higher shrimp yield, bigger size and better market price of shrimp from treatment ponds significantly increased the gross revenue and net profit. The adoption of better farm management practices by using probiotics can also increase considerably the harvests and profits as well as promoting sustainable shrimp farming systems (Gatesoupe 1999; Moriarty 1997).

## Conclusion

Use of probiotics in shrimp ponds improves the water quality and production performance as well as socio-economic conditions of farmers which are the key aspects of sustainable aquaculture.

Probiotics play a positive role to the neighbouring aquaculture and agriculture farms which usually receive low saline water from the same creek or water source. Development and usage of site specific gram positive beneficial bacteria is indeed necessary and useful to maintain ecological sustainability in shrimp farming.

### Acknowledgements

The authors are grateful to the owner of Kar shrimp farm (Keshabpur), West Bengal, India for providing the ponds, laboratory facility and required inputs for the present study. We are thankful to the farm technicians for collecting samples and data recording. The authors are also thankful to the anonymous reviewers for critical evaluation and constructive comments to ensure the quality of the manuscript and are also indebted to the editorial team, Asian Fisheries Science, for their efforts in improving the clarity and quality of the manuscript.

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*Received 22/06/2012; Accepted 09/10/2012 (MS12-39)*