Bangladesh J. Fish. Res., 13(2), 2009: 185-193

Suitability of commercially available *Bacillus* probiotics on growth, survival and production of black tiger shrimp (*Penaeus monodon* Fab.)

M.L. Islam*, S.K. Pal and M.J. Alam
Brackishwater Station, Bangladesh Fisheries Research Institute
Paikgacha, Khulna 9280, Bangladesh
*Corresponding author

Abstract
Different forms of *Bacillus* probiotics was assessed in the earthen ponds on tiger shrimp (*Penaeus monodon*) culture. The experiment was designed with three different treatments depending on the mode of application (T1= oral probiotics; T2= spreading probiotics and T3= oral+ spreading probiotics). The shrimp was cultured for 120 days with the stocking density of 6-PL/m^2. Oral probiotics in the respective ponds were supplied with feeds. Whereas, spreading probiotics was applied to the pond water during pond preparation at 30, 60 and 90 days of culture period. Results of the experiment revealed that, all forms of *Bacillus* probiotic had effective role to keep the culture environment friendly in terms of mineralization of organic matter, nitrogen and phosphorus content in bottom sediment; holding of water transparency in a congenial state, increasing the density of planktonic biomass and boosting the THB-*Vibrio* ratio in water and sediment with insignificance ($p>0.05$) difference between different treatments. Whilst, spreading form of *Bacillus* probiotic showed higher weight gain (27.58±1.18g), survival rate (70.75±8.54%) and production (1167.66±109.62 kg/ha) and expected lower FCR (1.81±0.06) values with significant difference ($p<0.01$) with others methods of application, indicated its superiority in tiger shrimp culture.

Keywords: Probiotics, *Penaeus monodon*, shrimp culture

Introduction

Shrimp aquaculture particularly the culture of *Penaeus monodon*, has expanded rapidly throughout Asia in recent decades. Bangladesh has a trend of expanding culture areas as it has a high value of sea food products in the international market. One of the critical issues of shrimp culturists today involves the management of water and the volume of organic sludge being created within the pond during rearing periods and later being released into surrounding waterways. The use of probiotics in aquaculture is increasing with the demand for more environment friendly aquaculture practices (Gatesoupe 1999). A growing concern for the high consumption of antibiotics in aquaculture has initiated a search for alternative methods of disease control. In aquaculture, particularly for shrimp, the use of beneficial bacteria such as probiotics is gaining in importance as an alternative to chemicals or antibiotics uses. A probiotics is generally defined as a live microbial support which improves the balance of the environment's micro flora (Fuller 1989). It has shown to have several modes of action in aquaculture: 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 (Thompson *et al.* 1999, Verschuere *et al.* 2000). In shrimp aquaculture, probiotics can be administrated either as a food supplement or as an additive to the bottom sediment via water (Moriarty 1998). *Bacillus* bacteria have the putative activities due to secretion of many exoenzymes (Moriarty 1996, 1998). The present study focused the suitability of different type (oral or spreading) of *Bacillus* probionts on micro flora, water and sediment quality management, growth, survival and production of tiger shrimp under zero water exchanged culture condition.

Materials and methods

Suitability of different forms (oral and spreading) of probiotics (beneficial microbs of *Bacillus spp*) on the culture environment, growth performance and production of tiger shrimp (*Penaeus monodon*) was assessed by a field level experiment. The experiment was designed with three different treatments (T1= oral probiotics; T2= spreading probiotics and T3= oral+spreading probiotics). Each treatment had three replications. The experiment was conducted in the earthen ponds having an average area of 0.1 ha each located in the pond complex at Brackishwater Station of Bangladesh Fisheries Research Institute. The experiment was designed for a culture period of 120 days with the stocking density of 6 PL/m^2, which was a bit higher than that proposed by Islam and Alam (2008) under modified improved culture system.

The ponds were prepared through drying, liming the bottom soil (@250 kg/ha of Ca_2O) and installment of in-pond nursery enclosure by fine nylon mosquito net and bamboo frame (about 10% of culture area in each pond). Ponds were filled up with tidal water to a depth of 90-110 cm and treated with Phostoxin (@1Tablet/20-25 ton of water) to kill all unwanted animals introduced with tidal water. After then fertilization was done with urea @1.0ppm, TSP @2.0ppm, MP @0.5ppm and molasses @3.0ppm to supplement the primary nutrient for primary producers. After 7 days of fertilization, stocking of shrimp post larvae (ABW 0.007g) was done within the prepared nursery enclosure in each pond according to the experimental design.

Shrimp larvae were initially fed with commercial feed of SAUDI-BANGLA shrimp nursery feed and reared for 14 days within the nursery enclosure. Then shrimp juveniles were released from the nursery enclosure by up folding the net to spread over the whole culture pond. After then SAUDI-BANGLA grow out feed was applied twice a day @ 3-4% of body weight for the entire culture period. Oral probiotics in the respective ponds were supplied (@5g/kg of feed) with adding the required feeds. Whereas, spreading probiotics was applied (@1.0ppm) at 30, 60 and 90 days of culture mixing with sand for immediately reaching to the bottom sediment. Ponds were fortnightly treated with 6-10 ppm of dolomite for the entire culture period as a scheduled basis depending on the water pH. Additional liming was also done after every heavy rain fall and on demands. Ponds were fertilized with urea @0.5-1.0ppm, TSP @1.0-1.5ppm and MP @0.3-0.4ppm for the 1st two months of culture depending on the primary productivity.

Total heterotrophic bacteria (THB) and *Vibrio* sp. bacteria in water and sediment sample were monitored bi-weekly intervals following the methods of Barrow and Feltham (1993). During the entire culture period, pond ecological parameters like, temperature, transparency, water depth, pH, Dissolved oxygen and salinity were monitored bi-weekly intervals. Whereas, bottom sediment sample was collected at initial and end of the culture cycle, processed and analyzed. Analysis of water quality and soil sample was done following the methods of (APHA 1985).

After 120 days of rearing, complete harvesting of shrimp was done by dewatering the ponds. Then growth, survival rate, FCR and production were estimated. ANOVA was done with a significance level of $P<0.05$ to observe the differences in growth, survival rate, production and FCR values for different treatments.

Results and discussion

Soil pH of pond sediments reduced a little bit in all the treatments with the progress of culture period (Fig. 1). But organic matter, nitrogen and phosphorus content in sediment were found to a stable state in initial and end samples (Figs. 2-4) in all the treatments without any significant difference ($p>0.05$) among treatments. In case of improved shrimp farming (higher stocking rates and supplemented with feed, fertilizers etc) accumulation of nutrient enriched (N and P) organic matter in bottom sediment occurred (Briggs and Fungi-Smith 1994, 1998; Budford *et al.* 2003b; Islam and Alam 2008) due to uneaten feeds, molted shell and excreta, which increased metabolic toxicity (Briggs and Fungi-Smith 1998; Fast and Menasveta 2000). But in this experiment, we did not found so, might due to the positive effect of probiotics for immediate and subsequent mineralization of organic matters onto the bottom sediment. This observation was supported by Rengpipat *et al.* (1998a) and Rengpipat *et al.* (1998b), who stated that *Bacillus*, used as a probiotics, was able to colonize in both the culture water and sediment.

Water transparency in all the treatments was moderate at initial stage of culture but subsequently decreased with the progress of culture duration (Fig. 5), whereas, phytoplankton concentration increased with the increase in culture duration (Fig. 6), but there was no significance difference ($p>0.05$) among treatments. In intensive shrimp monoculture, wastes derived from feeding often stimulate phytoplankton growth and lead to dense blooms in ponds (Briggs and Fungi-Smith 1998; Fast and Menasveta 2000). Higher stocking density may lead higher in flask of organic matter onto the bottom sediment (Paez-Osuna *et al.* 1997). Bacterial activity on organic matter released available primary nutrient in water, which is preferable source of nutrient to the primary producer (Saha 2000) that stimulated the growth of primary producer in the respective ponds might caused lowered transparency level in the respective treatments. In this study, observation on transparency and phytoplankton dynamics was supported by the above mentioned authors.

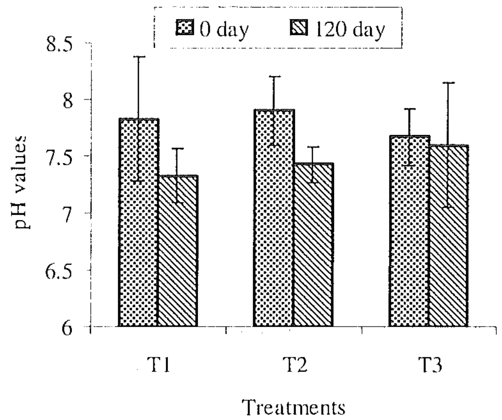

Fig. 1. Status in soil pH under different treatments.

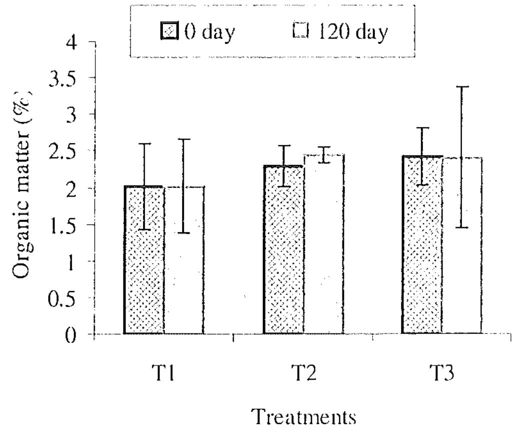

Fig. 2. Status of organic matter in bottom sediment.

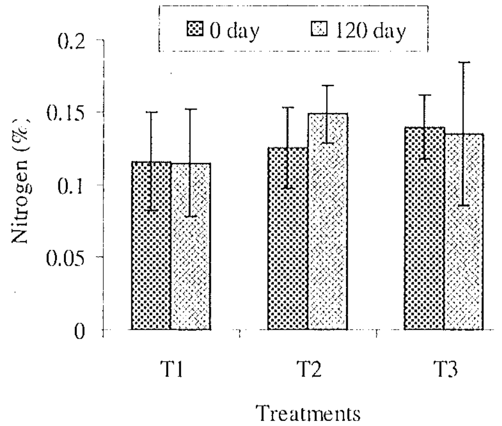

Fig. 3. Status of nitrogen in bottom sediment under different treatments

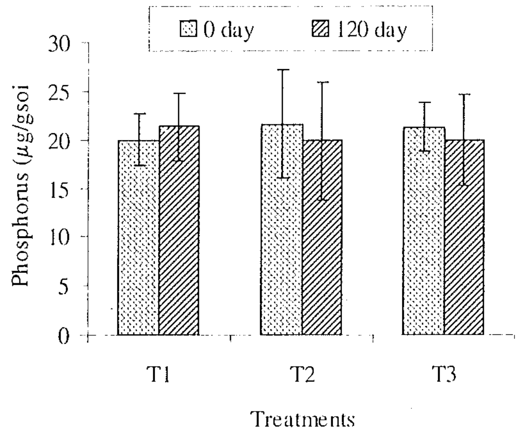

Fig. 4. Status of phosphorus in bottom sediment under different treatments

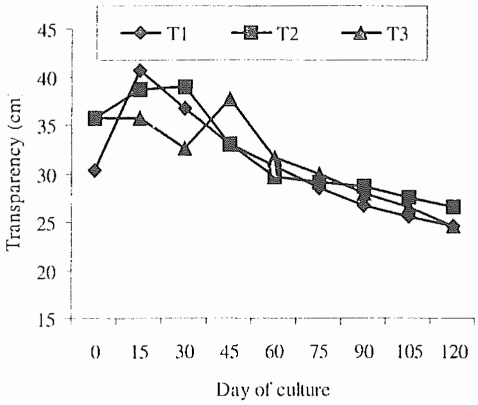

Fig. 5. Trend in transparency under different treatments

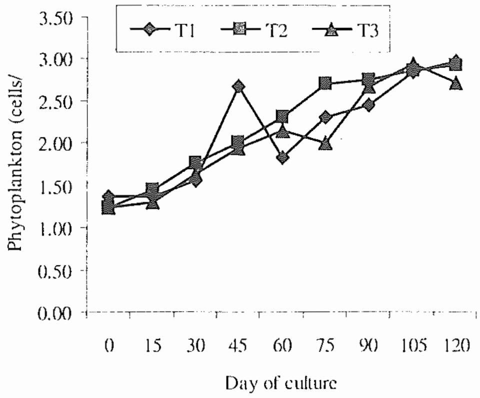

Fig. 6. Trend of phytoplankton concentration under different treatments

Other water quality variables (Table 1) were stable in condition during the culture period without any significant difference ($p>0.05$) among different treatments and were within the acceptable range of brackishwater aquaculture (Wahab *et al.* 2001; Wahab *et al.* 2003; Islam *et al.* 2004; Islam *et al.* 2005; Islam and Alam 2008).

Table 1. Water quality variables under different treatments

Parameters	T1	T2	T3
Temperature (°C)	32.04±1.42	31.94±1.36	31.87±1.39
Transparency (cm)	30.76±5.31	31.98±4.79	31.34±4.51
Depth (cm)	103.48±5.59	107.33±4.62	103.30±4.55
pH	9.02±0.16	8.99±0.26	9.02±0.19
Salinity (ppt)	10.91±1.21	11.13±1.14	10.69±1.29
DO (mg/l)	5.88±0.44	5.77±0.57	6.03±0.37

Status of bacterial load (THB-Vibrio ratio) in pond water and bottom sediment has been presented in Fig. 7 and 8, respectively. In the initial stages, the ratio of THB-Vibrio was very low and it increased subsequently with the progress in culture period in all treatments but the difference between treatments was insignificant ($p>0.05$). This might happened due to repeated application of probiotics and their continuous multiplication receiving the nutrients from the bottom sediment and that was the expectation for keeping the culture environment friendly.

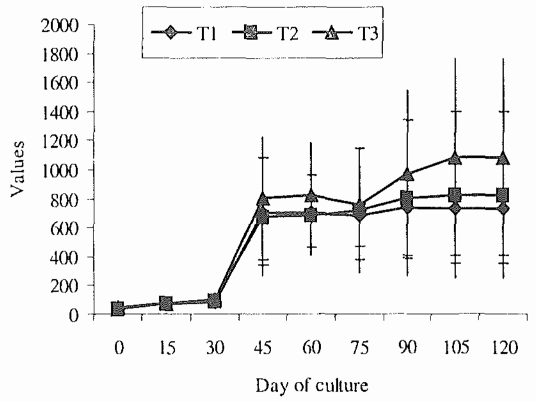

Fig. 7. Bacterial load (THB-Vibrio ration) in pond water under different treatments.

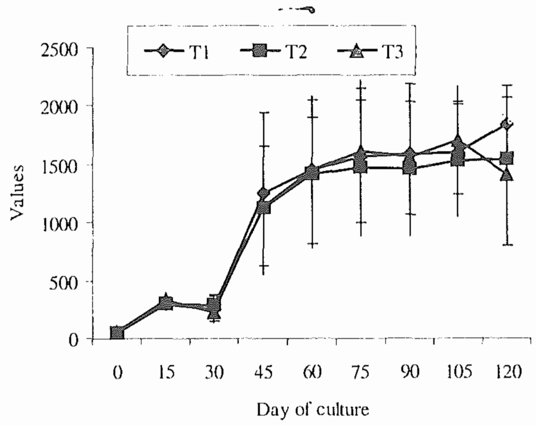

Fig. 8. Bacterial load (THB-Vibrio ration) in pond sediment under different treatments.

Bacillus bacteria in the environment, exclusion of other bacteria (especially harmful bacteria) by the probiont. The *Bacillus* also was able to replace *Vibrio* spp. in the gut of the shrimp (Rengpipat *et al.* 1998a). *Bacillus* bacteria are able to out-compete to other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotics (Moriarty 1998; Verschuere *et al.* 2000). Many different antibiotic compounds are produced naturally by a range of *Bacillus* species, and it appears that other bacteria would be unlikely to have resistant genes to all of the antibiotics produced by the *Bacillus* probionts, especially if they had not been exposed to the *Bacillus* previously (Moriarty 1998).

Results on production performance of shrimp have been presented in Table 2. Final weight gain of shrimp (27.58±1.18g) was significantly higher ($p<0.01$) in T2 (spreading probiotics) followed by T3 (23.31±0.75g) and T1 (20.56±1.65g). Survival rate of shrimp was statistically insignificant ($p>0.01$) between T2 (70.75±8.54%) and T3 (52.97±4.06%), also between T3 and T1 (41.63±2.17%). But it was significantly different ($p<0.01$) between T2 and T1. Production of shrimp was significantly different ($p<0.01$) among the treatments. Highest level of production was obtained from T2 (1167.66±109.62 kg/ha) where probiotics was applied as spreading method followed by T3 (739.62±33.67 kg/ha) and T1 (512.79±38.47). FCR value was significantly lower ($p<0.01$) in T2 (1.81±0.06) followed by T1 (2.33±0.14) and T3 (2.34±0.11), but it was statistically insignificant ($p>0.01$) between T1 and T3. However, highest production of shrimp in T2 might due to higher body weight gain and also due to higher survival rate. Spreading probiotics might have effective activity to provide congenial environment for shrimp than that of oral ones by rapidly enhancing the proportion of *Bacillus* bacteria in the environment. Increased survival by shrimp might be due to exclusion of other bacteria (especially harmful bacteria) by the probiont, where the *Bacillus* bacteria were dominant. The observed increase in survival in spreading probiotic treatments also might be due to enhanced digestion and increased absorption of food, which in turn contributed to the higher body weight gain and lower FCR values. Administering probiotics significantly improve survival but not growth (Ziaei-Nejad *et al.* 2005). *Bacillus* administration also has been shown to increase shrimp survival by enhancing resistance to pathogens by activating both cellular and humoral immune defenses in shrimp (Rengpipat *et al.* 2000). *Bacillus* surface antigens or their metabolites act as immunogens for shrimp by stimulating phagocytic activity of granulocytes (Itami *et al.* 1998). Whereas, Shariff *et al* (2001) and Mcintosh *et al.* (2000) stated that *P. monodon* and *Litopenaeus vannamei* treated with *Bacillus* probiotics did not significantly increase ($p>0.05$) either survival or growth.

Table 2. Details of production performance of shrimp under different treatments

Parameters	T1 (oral probiotics)	T2 (spreading probiotics)	T3 (oral+spreading probiotics)
Final body weight (g)	20.56 ± 1.65^{cb}	27.58 ± 1.18^{a}	23.31 ± 0.75^{b}
Survival rate (%)	41.63 ± 2.17^{cb}	70.75 ± 8.54^{a}	52.97 ± 4.06^{ba}
Production (kg/ha)	512.79 ± 38.47^{c}	1167.66 ± 109.62^{a}	739.62 ± 33.67^{b}
FCR	2.33 ± 0.14^{ba}	1.81 ± 0.06^{c}	2.34 ± 0.11^{a}

* Different superscript in the same row indicated significantly different ($p<0.01$)

It could be seen (Table 2) that, body weight gain, survival and production of shrimp under spreading probiotics was much more higher than that of Apud *et al.* (1984), who reported an average yield of 340 kg/ha/crop at stocking rate of 4–5/m^2 in monoculture with supplemental feed and improved water management. Body weight gain, survival rate and production of shrimp in this trial was also higher than the observation of Islam and Alam (2008), who reported highest body weight gain of 26.33g, survival rate of 57.76 % and total augmentation of shrimp production of 759.14 kg/ha with a stocking density 5/m^2 under modified improved culture system in a 120 days of rearing period.

However, findings of the present experiment focused that, among the tested forms of bacillus probiotics, spreading one (T2) showed the better performance of shrimp growth, survival rate and production. So, spreading type of probiotics seemed to be superior to the others and might be considered as a biotechnical tool for keeping the culture environment congenial under improved shrimp culture practices. Probiotics could be the best and alternative option as a preventive measure of shrimp disease instead of control by antibiotics for producing biological shrimp.

Acknowledgement

Authors would like to express their sincere thanks to the Bangladesh Fisheries Research Institute (BFRI) authority for financial grants through the Core Research Project.

References

APHA, AWWA and WPCF, 1985. Standard Methods for the Examination of Water and Wastewater, Sixteenth Edition. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC, 1,268 pp.

Apud, F.D., K. Gonzales and N. Deatras, 1984. Survival, growth and production of *Penaeus monodon* fabricus at different stocking densities in earthen ponds with flow-through systems and supplemental feeding. Fish. Res. J. Philippines, 6(2): 1-9.

Barrow and Feltham, 1993. The cowan and steel's manual for the identification of medical bacteria (3rd ed). Cambridge university press. p.325.

Briggs, M. R. P. and S. J. Funge-Smith, 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. *Aquacult. Fish. Manage.*, **25**: 789-811.

Briggs, M. R. P. and S. J. Funge-Smith, 1998. Nutrient budgets in intensive shrimp ponds: implications for sustainability. *Aquaculture*, **164**: 117-133.

Burford M. A., P. .J. Thompson, R. P. McIntosh, R. H. Bauman and D. C. Pearson, 2003b. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, **219**: 393-411.

Fast, A.W. and P. Menasveta, 2000. Some recent issues and innovations in marine shrimp pond culture. *Reviews in Fisheries Science*, **8**(3):151–233.

Fuller, R., 1989. Probiotics in man and animal. *J.Appl. Bacterioal*, **66**: 365-378.

Gatesoupe, F. J., 1999. The use of probiotics in aquaculture. *Aquaculture*, **180**: 147-165.

Islam, M. L. and M. J. Alam, 2008. Optimization of stocking rates of tiger shrimp under modified improved culture system. *J. Bangladesh Agril. Univ.*, **6**(2): 341-348.

Islam, M. L., M. J. Alam, S. Rheman, S.U. Ahmed and M.A. Mazid, 2004. Water quality, nutrient dynamics and sediment profile in shrimp farms of the Sundarbans mangrove forest, Bangladesh. *Indian Journal of Marine Sciences*, **33**(2): 170-176.

Islam, M. S., A. Milstein, M. A. Wahab, A. H. M. Kamal and S. Dewan, 2005. Production and economic return of shrimp aquaculture in coastal ponds of different sizes and with different management regimes. *Aquacult. Inter.*, **13**: 489-500.

Itami, T., M. Asano, K. Tokushige, K. Kubono, A. Nakagawa, N. Takeno, H. Nishimura, M. Maeda, M. Kondo and Y. Takahashi, 1998. Enhancement of disease resistance of Kuruma shrimp, *Penaeus japonicus*, after oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. *Aquaculture*, **164**: 277-288.

Mcintosh, D., T. M. Samocha, E. R. Jones, A. L. Lawrence, D. A. McKee, S. Horowitz and A. Horowitz, 2000. The effect of a commercial bacterial supplement on the high-density culturing of *Litopenaeus vannamei* with a low-protein diet in an outdoor tank system and no water exchange. *Aquac. Eng.*, **21**: 215-227.

Moriarty, D. J. W., 1996. Microbial biotechnology: a key ingredient for sustainable aquaculture. *Infofish Int.*, **4**: 29-33.

Moriarty, D. J. W., 1998. Control of luminous *Vibrio* species in paneid aquaculture ponds. *Aquaculture*, **164**: 351-358.

Paez-Osuna, F., S. R. Guerreo-Galval, A. C. Ruiz-Fernandez and R. Espinoza-Angulo, 1997. Fluxes and mass balances of nutrients in a semi-intensive shrimp farm in North-western Mexico. *Mar. Poll. Bull. No.*, **34**: 290-297.

Rengpipat, S., W. Phianphak, S. Piyatiratitivorakul and P. Menasveta, 1998a. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture*, **167**: 301-313.

Rengpipat, S., S. Rukpratanpom, S. Piyatiratitivorakul and P. Menasveta, 1998b. Probiotics in aquaculture: a case study of probiotics for larvae of the black tiger shrimp (*Penaeus monodon*). In: Flegel, T.W. (Ed.), Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok.

Rengpipat, S., S. Rukpratanpom, S. Piyatiratitivorakul and P. Menasveta, 2000. Immunity enhancement on black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* sp). *Aquaculture*, **191**: 271—288.

Saha, S.B. 2000. Oxygen consumption pattern of some common manures used in pond fertilization. *Bangladesh J. Agril.,* **25**(1&2): 97-102.

Shariff, M., F. M. Yusoff, T. N. Devaraja and S. P. Srinivasa Rao, 2001. The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* (Fabricius), ponds. *Aquac. Res.*, **32**: 181—187.

Thompson, F. L., P. C. Abreu and R. Cavalli, 1999. The use of microorganisms as food source for *Penaeus paulensis* larvae. *Aquaculture*, **174**: 139-153.

Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete, 2000. Probiotic bacteria as biological control agent in aquaculture. *Microbiol. Mol. Biol. Rev.*, **64**: 655-671.

Wahab, M. A., A. Bergheim, B. Braaten, M. S. Islam and M. M. Rahman, 2001. Observation on some of the environmental parameters of selected shrimp farms in Khulna, Bangladesh. *Bangladesh J. Fish. Res.*, **5**(1): 75-84.

Wahab, M. A., A. Bergheim and B. Braaten, 2003. Water quality and partial mass budget in extensive shrimp ponds in Bangladesh. *Aquaculture*, **218**: 413-423.

Ziaei-Nejad, S., Mehran Habibi Rezaei, Ghobad Azari Takami, Donald L. Lovett, Ali Reza Mirvaghefi and Mehdi Shakouri, 2005. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. M.Sc Thesis. Tehran University. 100 pp.

(Manuscript received 22 November 2009)