ORIGINAL SCIENTIFIC PAPER

CODEN RIBAEG ISSN 1330-061X

STUDY OF PROBIOTICS ON THE SEED PRODUCTION OF BLACK TIGER SHRIMP *Penaeus monodon*

Sheikh Aftab Uddin[1*], M. Abdul Kader[1], M. Nurul Azim Sikder[1], M. Abdul Hakim[2], M. Mahbub Alam[3], Ashraful Haque Azad[4], Chowdhury Kamrul Hasan[5]

[1] Institute of Marine Sciences and Fisheries, University of Chittagong, Chittagong-4331, Bangladesh
[2] Department of Microbiology, University of Chittagong, Chittagong-4331, Bangladesh
[3] Marine Fisheries Discipline, Marine Fisheries Academy, Fish harbour, Chittagong-4000, Bangladesh
[4] Bangladesh Fisheries Research Institute, Marine Station, Cox's Bazar, Bangladesh
[5] Department of Environmental Science, School of Environmental Science and Management, Independent University, Bangladesh, Plot 16, Block B, Bashundhara R/A,Dhaka, Bangladesh
* Corresponding Author, E-mail: aftabims@yahoo.com

ARTICLE INFO

Received: 13 March 2013
Received in revised form: 15 June 2013
Accepted: 5 July 2013
Available online: 7 July 2013

Keywords:
Probiotics
Shrimp health
Penaeus monodon
Seed production
Bangladesh

ABSTRACT

Currently, antibiotics are widely used in shrimp hatcheries to control bacterial infections. Appearance of antibiotic resistant pathogens and restriction on the use of antibiotics have led to the development of alternatives to antibiotics in hatchery systems. In light of this, an attempt was undertaken to investigate the effects of probiotics on the larval rearing of *Penaeus monodon*, compared with the control tanks (without probiotics). The results showed that several issues significantly improved with administering probiotics in the experimental tanks compared with the tanks without probiotics. For example, the concentration of ammonia was estimated to be 1.25 mg/L which was less than a half of what was measured in the control tanks. The size variation was observed more in the control tanks than in the experimental tanks. Moreover, the muscle gut ratio of PL_{15} was about 85 to 92% in the probiotic treated tank and 70 to 80% in the control tank during the eight cycles of production. The fouling organisms were more in the control tank compared to the experimental tanks. The average length of PL_{15} was maximum when reared in the experimental tanks compared to the control tanks. The final survival rate of PL_{15} from the control and experimental tank was 35 and 52%, respectively. The present investigation indicated that probiotics played an important role in the growth, survival and health status of *P. monodon* larvae.

INTRODUCTION

The decline of global shrimp production along with the outbreak of different diseases, causing mass mortalities of shrimps in hatcheries (Lavilla-Pitogo et al., 1990; Jiravanichpaisal and Miyazaki, 1994; Karunasagar et al., 1994), has led to the indiscriminate use of antibiotics and a variety of other disinfectants (Aftabuddin and Kader, 2006; Walker and Mohan, 2009; Boonthai et al., 2011). Consequently, the shrimp industry is frequented by multiple drug-resistant bacteria such as *Aeromonas hydrophila, A. salmonicida, Edwardsiella tarda, E. ictaluri, Vibrio anguillarum, V. salmonicida, Pasteurella piscida, Yersinia ruckeri* (De Paola et al., 1995; Weston, 1996; Cabello, 2006) which are becoming increasingly difficult to control and eradicate (Vine et al., 2006). As some of the antimicrobial agents have been banned in some countries (Robert et al., 1995; FAO, 2002) considering the threat to environment and human health

(Samuelsen et al., 1992; Graslund et al., 2002), probiotics, including *Bacillus* spp., have been recognized as an alternative and beneficial method of controlling the microbial environment (Ringø and Gatesoupe, 1998; Gatesoupe, 1999; Ringø and Birkbeck, 1999; Verschuere et al., 2000, Irianto and Austin, 2002).

A number of studies have focused on the use of *Bacillus* spp. in shrimp industry as a useful probiotic (Wang, 2007; Far et al., 2009; Zhou et al., 2009; Sundarapandian and Babu, 2010), which may increase *P. monodon* productivity and improve water quality by decreasing the concentration of ammonia and nitrite (Shariff et al., 2001) and by increasing dissolved oxygen (Wang et al., 2005). Moreover, the *Bacillus* spp. have the ability to potentially inhibit *Vibrio* spp. in shrimp culture facilities (Rengipipat et al., 1998; Moriarty, 1999; Gatesoupe, 1999). Based on the previous study on probiotics, it is evident that probiotics have good ability to stimulate appetite, improve the nutrient absorption and strengthen the host immunity. Therefore, the present study was undertaken to examine the effect of commercial probiotics on the growth, survival and water quality of hatchery reared seed of black tiger shrimp, *P. monodon* in Bangladesh.

MATERIALS AND METHODS

The present investigation was carried out at the Prime Shrimp Hatchery Limited, located on the southeastern coast of Bangladesh (Fig 1) during April 2009-March 2011. Over the last decade, the hatchery, having state-of-the-art facilities, has been producing around 400-500 million of *P. monodon* seeds per year in its 5 production units.

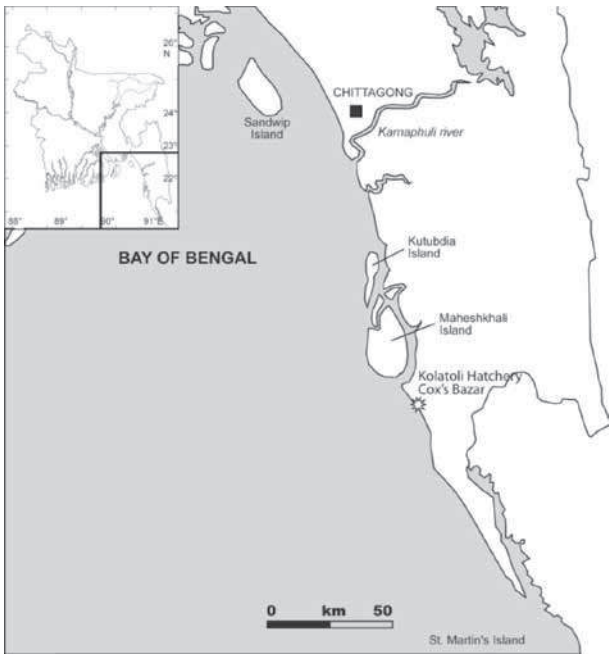

Fig 1. Location of the investigated hatchery at Kolatoly, Cox's Bazar (star in the map)

Larval rearing operation

Five larval rearing tanks (30 ton capacity) were filled with 25 ton UV treated seawater. Three tanks were kept as experimental tanks treated with probiotics and another two were kept as control tanks. Both experimental and control tanks were inoculated with 10 ppm EDTA and 0.05 ppm treflan (fungicide) two hours before stocking. Just before stocking, algae (*Skeletonema sp*.) were added to the tanks at a density of 100,000 cells/ml. The tank water was sufficiently aerated throughout the culture operation. The nauplii (N_5 /N_6) were harvested from the hatching tanks and were released into the larval rearing tanks (LRT) slowly in small quantities at different points of the control tank at 100,000 nauplii/ton. The similar procedure was applied while stocking the experimental tanks, except prior to stocking, they were inoculated with 0.5 ppm probiotics top 10 (containing *Lactobacillus acidophilus*, *Bacillus* spp. and *Saccharomyces cerevisiae*). Each experiment was carried out once in each cycle, continuing up to eight cycles.

Twenty four hours after stocking, the nauplii converted into protozoea I (called zoea I) stage. First feeding was started when the zoea I appeared. The zoeal stages (I to III) were fed with *Skeletonema sp*. thrice daily both in the control and in the experimental tanks. The mysis stages (I to III) were fed with algae at a density of 4 x 10^5 cells/ml both in the control and in the experimental tanks. In addition to algae, mysis stages were fed with commercially available microencapsulated feed like CAR, CD-2 etc. three times a day. These were supplemented with 1 ppm of probiotic mysis stages in the experimental tanks. Newly hatched *Artemia* nauplii were also given as a live feed in mysis III stage. Post larval stages were fed exclusively with freshly hatched live *Artemia* nauplii at 4 individuals per PL (nos. of *Artemia* increased with PL onwards) and artificial feed shrimp flakes three times a day. Probiotics top 10 was also added at 1.5 ppm daily in the experimental tanks with PL.

Continuous aeration was maintained at a level appropriate for each larval stages to provide sufficient oxygen and to keep feed and larvae in suspension. Siphoning was done routinely (daily from mysis stage onwards) to clean the unconsumed food and fecal materials on the tank bottom. Water exchange was done from the mysis III stage on-

wards using mesh size of 0.5 mm and reducing 50% of the water. Once post larvae appeared, salinity was reduced slowly and maintained to 20 ppt. Microbiological analysis was done routinely (using TCBS and/or Marine agar) to assess the evolution of the bacterial populations in each tank.

The water quality parameters of the probiotic and control tanks were regularly monitored. Water quality parameters such as salinity, temperature, pH, dissolved oxygen and ammonia were estimated daily in the morning hours. Water salinity was measured by using a hand refractometer (ATAGO, Japan). The pH of the water was measured by using electronic pen pH meter manufactured by Hanna Instrumental Company (Singapore). Water temperature was measured by using a digital thermometer (DT801, China). Dissolved oxygen (mg/L), alkalinity (ppm) and ammonia (mg/L) were measured by HANNA instruments products, Singapore, Model no. HI 3810, HI 3811 and HI 3826, respectively.

Total length and size variation

To know the size variation, the length of 50 postlarvae were measured individually. The mean length and standard deviation was calculated according to Madhukiran et al. (2009).

Muscle gut ratio (MGR)

A microscopic examination of the relative thickness of ventral abdominal muscle and the gut in the 6th abdominal segment of the tail of the post larvae was conducted to determine the muscle to gut ratio (Madhukiran et al., 2009). To know the MGR at least 20 PL_{15} were examined, indicating the nutritional status of the shrimp. High muscle to gut ratio (4:1) was preferable.

Stress test

A stress test was conducted once they reached PL_{12-15}. Randomly selected sample of about 100 individuals were taken in a beaker containing rearing water salinity with 20 ppm formalin (0.2 ml/L) and allowed for 60 minutes. After 60 minutes, the PL that were still active or which moved when prodded with a needle were counted as a percentage of survival, following the formula: survival (%) = (no. active PL/total PL in beaker) x 100.

Fouling organisms

Randomly selected PL_{12-15} of about 50 individuals were examined by a compound microscope to observe any fouling organisms attached with the PL (Madhukiran et al., 2009). The percentage of fouling was calculated following the formula: fouling (%) = (no. of PL attached with fouling organisms/total no. of PL counted during the observation) x 100.

Monodonbaculo virus (MBV)

Baculoviruses were detected in whole or squashed (stained with malachite green for Monodonbaculovirus) preparations of hepatopancreas or fecal strands from larger-sized larvae, using a high powered light microscope to spot the characteristic viral occlusion bodies (Lightner, 1996).

Test for WSSV

WSSV test was performed by IQ Plus system, which was a new technology based on insulated isothermal PCR that was a compact and portable system. The instrument could detect WSSV within 1 hour. The extraction procedure and others were performed according to the instruction manual of IQ Plus system, GeneReach Biotechnology Corp. Taiwan.

RESULTS AND DISCUSSION

The percentage of survival rate of *P. monodon* larvae of various stages are given in Table 1. The result showed that the survival rate of the nauplii exhibited similar trend both in the control and experimental tanks. The survival rate of Zoea 1 stage in the probiotic treated tanks (PTTs) and control tanks (CTs) was 90% and 80%, respectively. The survival rate of the mysis was maximum (81%) in PTTs and minimum in CTs (66%). The survival rate of all the post larval stages (PL_1- PL_{15}) was higher in PTTs than that of the CTs (Table 1). The survival rate of the PL_{15} stage in PTTs was 52%, while it was only 35% in the control. The findings of present investigation accorded closely with the works of Krishnaprakash et al. (2009) and Soundarapandian and Babu (2010).

Table 1. Percentage of larval survival (average percentage calculated by 8 cycles)

Stages	% survival in control tank	% survival in probiotic treated tank (PTT)
Zoea 1	80	90
Mysis 1	66	81
PL_1	50	72
PL_5	44	64
PL_{10}	40	56
PL_{15}	35	52

Table 2. Health status of *P. monodon* post larvae during the investigation period (April 2009-March 2011, 8 production cycles)

Production cycles	% size variation	% MGR	% Stress test	% fouling organisms	Presence of LB	Presence of MBV	Presence of WSSV
Cycle 1							
Control tank	17	72	80	25	-	-	-
Experimental tank	11	85	90	12	-	-	-
Cycle 2							
Control tank	20	70	80	30	+	-	-
Experimental tank	11	85	95	15	-	-	-
Cycle 3							
Control tank	15	75	82	20	-	-	-
Experimental tank	11	85	95	10	-	-	-
Cycle 4							
Control tank	16	80	80	25	-	-	-
Experimental tank	12.5	90	95	12	-	-	-
Cycle 5							
Control tank	20	78	75	30	+	-	-
Experimental tank	10	92	96	15	-	-	-
Cycle 6							
Control tank	25	75	73	30	+	-	-
Experimental tank	15	88	90	15	+	-	-
Cycle 7							
Control tank	14	76	78	28	-	-	-
Experimental tank	10	90	96	10	-	-	-
Cycle 8							
Control tank	20	80	73	20	-	-	-
Experimental tank	12	92	90	15	-	-	-

Table 3. The average length of *P. monodon* post larvae reared in both control and experimental tanks

Stages	Length (mm)	
	Control tank (n=20)	Probiotic treated tanks(n=20)
Cycle 1		
PL_1	4.78±1.2	5.38±0.5
PL_5	5.68±0.8	6.83±0.5
PL_{10}	7.98±0.4	8.84±0.2
PL_{15}	9.84±0.5	11.80±0.2
Cycle 2		
PL_1	4.68±1.0	5.48±0.5
PL_5	5.78±0.6	6.90±0.6
PL_{10}	7.70±0.3	8.75±0.2
PL_{15}	9.40±0.4	11.40±0.4
Cycle 3		
PL_1	5.00±0.8	5.38±0.2
PL_5	5.80±0.6	7.83±0.4
PL_{10}	8.0±0.5	9.84±0.2
PL_{15}	10.02±0.4	11.81±0.5
Cycle 4		
PL_1	5.10±0.6	5.38±0.3
PL_5	5.90±0.6	7.85±0.4
PL_{10}	7.90±0.5	9.85±0.5
PL_{15}	9.82±0.4	11.48±0.6
Cycle 5		
PL_1	4.90±0.5	5.28±0.4
PL_5	5.70±0.6	7.76±0.3
PL_{10}	7.75±0.6	9.72±0.5
PL_{15}	9.58±0.5	11.35±0.6
Cycle 6		
PL_1	4.82±0.3	5.25±0.4
PL_5	5.60±0.4	7.65±0.3
PL_{10}	7.63±0.4	9.60±0.4
PL_{15}	9.45±0.4	11.25±0.3
Cycle 7		
PL_1	4.98±0.4	5.48±0.4
PL_5	5.80±0.5	7.80±0.2
PL_{10}	7.85±0.4	9.82±0.3
PL_{15}	9.88±0.5	11.65±0.3
Cycle 8		
PL_1	4.92±0.5	5.38±0.4
PL_5	5.78±0.6	7.72±0.3
PL_{10}	7.75±0.6	9.75±0.5
PL_{15}	9.68±0.5	11.38±0.4

Table 2 demonstrates the various health check assessment of *P. monodon* post larvae. The percentage of size variation was low in PTTs, compared to CTs. In PL_{15}, the muscle gut ratio was about 85 to 92% in PTTs and 70 to 80% in CTs during the eight cycles of production. This implied that the intake of feed in CTs was lower than that of the PTTs. This was due to high stress, which was substantiated by the works of Krishnaprakash et al. (2009), stating that in PL_{16}, the muscle gut ratio was 98% in the experimental tanks (with probiotics containing *Bacillus* sp. and *Streptococcus* spp.) and 75% in the control tanks.

The fouling organisms were more in the control tanks than in the experimental tanks. Fouling organisms, like filamentous bacteria and *Zoothamium* spp., might be commonly seen in the hatcheries, causing less harm to the shrimps than other diseas-

es. The minimum damage to the shrimps due to the presence of fouling organisms might be attributed to the moulting process. In some cases, they spread all over the tanks, subjected to the chemical treatment with 10-25 ppm Formalin. The percentage of fouling organisms in the probiotic treated tanks ranged from 10-12, whereas in the control tanks, it fluctuated from 20-30 during all production cycles.

The average length of all post larvae (PL_1-PL_{15}) was maximum when reared in the experimental tanks compared to the control tanks (Table 3). At PL_{15}, the total length of the larvae ranged from 12.12 to 11.22 mm in the antibiotic treated tank (ATT) and 11.81 to 11.25 mm in PTT; whereas in the control tank, it ranged from 10.02 to 9.40 mm. Krishnaprakash et al. (2009) found that during PL_{12}, PL_{14} and PL_{16} stages, total lengths of the larvae in the experimental tanks were 11.68 mm, 11.98 mm and 12.17 mm, respectively, whereas in the control tank, the growth rates were 11.05 mm, 11.38 mm and 11.79 mm, respectively, which was in close agreement with the present investigation. All tanks were free from Monodon Baculu Virus and White Spot Syndrome Virus disease.

Water quality parameters

Water quality parameters, namely temperature (°C), salinity (ppt), pH, dissolved oxygen (mg/L), ammonia (mg/L) and alkalinity (ppm), are shown in Table 4.

Water temperature was probably the most important environmental variable for larval rearing of *P. monodon* because it directly affected metabolism, oxygen consumption, growth, moulting and survival. The optimum range of temperature for the black tiger shrimp larval rearing is between 28 to 32 °C (Kannupandi et al., 2002), which coincided with the present investigation showing a range from 29 °C - 30 °C. There was no marked difference in temperature between the control and experimental tanks.

P. monodon can tolerate a wide range of salinity. For a shrimp hatchery, the recommended salinity range is 28-35 ppt (Kannupandi et al., 2002). In the present study, salinity was maintained at 29-31 ppt for both control and experimental tanks. Krishnaprakash (2007) also reported almost similar salinity (31 ppt) for the larval rearing of *P. monodon.*

In the present study, the pH level was lower in the control tank (6.4-6.8) which was in congruence with the Law (1988), demonstrating that *P. monodon* could tolerate pH down to 6.0 to 6.5. On the other hand, in the experimental tanks, pH was considerably high (7.8-8.3), which conformed with the findings of Kannupandi et al. (2002) and Krishnaprakash (2007), stating that the suitable pH for the shrimp larval culture ranged from 8.2-8.5. The results indicated that probiotics maintained a desired level of pH in the experimental tanks.

In the present study, the maximum dissolved oxygen (DO) content in the experimental tank was 4.8 mg/L and in the control tank it was 4.2 mg/L, which was in close agreement with the works of Liao and Murai (1986), Krishnaprakash (2007), Krishnaprakash et al. (2009). Surfacing response was first observed when DO reached 1.5-2.1 mg/L. Oxygen levels in the culture tanks were maintained in the desired range by aeration. Continuous aeration was done during the present investigation and therefore the oxygen level did not vary significantly between the control and experimental tanks.

Ammonia exists in water in both ionized (NH_4+) and unionized (NH_3) forms. Unionized ammonia is considered a more toxic form of ammonia due to its ability to diffuse readily across the cell membrane (Fromm and Gillette, 1968; Emerson et al., 1975). In the present study the higher concentration of ammonia (2.5 mg/L) was observed in the control tank and in the probiotic tank it was 1.25 mg/L, which agreed with the results (0-2.1 ppm) of Krishnaprakash (2007) and disagreed with that of

Table 4. Recorded water quality parameters for both control and probiotic treated tanks

	Temp (°C)		Salinity (ppt)		pH		DO (mg/L)		Alkalinity (ppm)		NH_4(ppm)	
Cycle	**CT**	**PTT**	**CT**	**PTT**	**CT**	**PTT**	**CT**	**PTT**	**CT**	**PTT**	**CT**	**PTT**
1	29	29.5	30	30.5	6.5	8.1	4	3.9	135	150	1.8	1
2	30	29.5	31	30	6.8	8	3.9	4	132	155	1.45	0.9
3	29.5	30	29	30	6.55	7.9	4.2	4.8	125	150	1.65	0.95
4	29	29.5	30	30.5	6.5	8	4	4.02	135	140	1.55	1.1
5	29.5	29	30.5	30.5	6.4	7.9	4.1	4.5	128	150	1.9	1.2
6	29	29.5	31	30.5	6.6	7.8	3.9	4.3	125	145	2.5	1.25
7	30	29.5	29	30	6.7	8.2	4.1	4	135	150	1.7	1
8	30	30	29.5	30	6.5	8.3	3.9	3.8	128	155	2.1	0.95

CT=control tank, PTT=probiotic treated tank

Das et al. (1996). The alkalinity of both control and experimental tanks ranged from 125-135 ppm and 140-155 ppm, respectively, which was approximated to the values of 140-160 ppm in the larval rearing tank, reported by Krishnaprakash (2007).

Sažetak

PROBIOTICI U PROIZVODNJI POTOMSTVA TIGRASTE KOZICE (*Penaeus monodon*)

U mrijestilištima kozica trenutno je vrlo rasprostranjena upotreba antibiotika za sprečavanje bakterijskih infekcija. Pojavom otpornih patogenih svojstva antibiotika i ograničenja njihove upotrebe, razvili su se drugi načini sprečavanja infekcija patogenima u mrijestilištima. Sukladno tome je provedeno istraživanje učinkovitosti probiotika u uzgoju ličinki *Penaeus monodon* te su dobiveni rezultati uspoređeni s onima iz kontrolnih spremnika bez probiotika. Rezultati su pokazali znatno unapređenje nekih odrednica uzgoja u eksperimentalnim spremnicima s dodatkom probiotika (u odnosu na spremnike bez probiotika). Primjerice, koncentracija amonijaka procijenjena je na 1,25 mg/L, što je manje od polovice izmjerene vrijednosti u kontrolnim spremnicima. Veća varijacija veličine zabilježena je u kontrolnim, a manja u eksperimentalnim spremnicima. Štoviše, tijekom osam faza proizvodnje, omjer crijevnih mišića PL_{15} bio je otprilike 85-92% u spremnicima s probioticima, a 70-80% u kontrolnim spremnicima. Obraštajnih organizama bilo je više u kontrolnim nego u eksperimentalnim spremnicima. Prosječna dužina PL_{15} bila je maksimalna za jedinke koje su mriještene u eksperimentalnim spremnicima (u odnosu na kontrolne). Konačna stopa preživljavanja PL_{15} je 35% u kontrolnim i 52% u eksperimentalnim spremnicima. Ovim istraživanjem dokazana je važna uloga probiotika u rastu, preživljavanju i održavanju zdravlja ličinki *P. monodon*.

Ključne riječi: probiotici, zdravlje kozica, *Penaeus monodon*, proizvodnja potomstva, Bangladeš

REFERENCES

Aftabuddin, S., Kader, M. A. (2006): The use of antibiotics in shrimp hatcheries in Bangladesh. Journal of Fisheries and Aquatic Sciences, 1, 1, 64-67.

Boonthai, T. Vuthiphandchai, V., Nimrat, S. (2011): Probiotic bacteria effects on growth and bacterial composition of black tiger shrimp (*Penaeus monodon*), Aquaculture Nutrition, 17, 634-644.

Cabello, F. C. (2006): Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental Microbiology, 8, 1137-1144.

Das, N. G., Sarwar, S. M. M., Parvez, M. M. (1996): Observation on the water quality management of larval and post larval rearing tanks of *P. monodon* hatchery at Cox's Bazar; Bangladesh. Indian Journal of Fisheries, 43, 3, 255-265.

De Paola, A., Peeler, J. T., Rodrick, G. (1995): Effect of oxytetracycline-medicated feed on antibiotic resistance of Gram-negative bacteria in catfish ponds. Applied and Environmental Microbiology, 61, 2335-2340.

Emerson, K., Russo, R. C., Thurston, R. V. (1975): Aqueous ammonia equilibrium calculations: Effect of pH and temperature. Journal of Fisheries Research Board Canada, 33, 2, 379-388.

FAO (2002): The State of World Fisheries and Aquaculture (SOFIA).FAO, Rome, 150 pp.

Far, H. Z., Saad, C. R. B., Daud, H. M., Harmin, S. A., Shakibazadeh, S. (2009): Effect of *Bacillus subtilis* on the growth and survival rate of shrimp (*Litopenaeus vannamei*). African Journal of Biotechnology, 8, 3369-3376.

Fromm, P. O., Gillette, J. R. (1968): Effect of ammonia on blood and nitrogen excretion of rainbow trout (*Salmogairdneri*). Comparative Biochemistry Physiology, 26, 887-896.

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

Graslund, S., Karin, K., Wongtavatchai, J. (2002): Responsible use of antibiotic in shrimp farming. Aquaculture Asia, 7, 17.

Irianto, A., Austin, B. (2002): Probiotics in aquaculture. Journal of Fish Diseases, 25, 633-642.

Jiravanichpaisal, P., Miyazaki, T. (1994): Histopathology, biochemistry and pathogenicity of Vibrio harveyi infecting black tiger shrimp Penaeus monodon. Journal of Aquatic Animal Health, 6, 27-35.

Kannupandi, T., Soundarapandian, P., Anantharaman, P. (2002): Hatchery operation manual for *Penaeus monodon* Fabricius. Annmalai University, CAS in Marine Biology Parangipettai, 1-99.

Karunasagar, I., Pai, R., Malathi, G. R., Karunasagar, I. (1994): Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. Aquaculture, 128, 203-209.

Krishnaprakash, R. (2007): Studies on the production of high quality shrimp (*Penaeus monodon*

Fabricius) seed in a commercial hatchery. M. Phil thesis, CAS in Marine Biology, Annamalai University, India, 1-45.

Krishnaprakash, R., Saravanan, R., Murugesan, P., Rajagopal, S. (2009): Usefulness of Probiotics in the Production of High Quality Shrimp (*Penaeus monodon*) Seeds in Hatcheries. World Journal of Zoology, 4, 2, 144-147.

Lavilla-Pitogo, C. R., Baticados, M. C. L., Cruz-Lacierda, E. R., de la Pena, L. D. (1990): Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture, 91, 1-13.

Law, A. T. (1988): Water quality requirements for *Penaeus monodon* culture. Proceedings of the Seminar on Marine Prawn Farming in Malaysia. Malaysia Fisheries Society, Serdang, Malaysia, 53-65.

Liao, I. C., Murai, T. (1986): Effects of dissolved oxygen, temperature and salinity on the oxygen consumption of grass shrimp, *Penaeus monodon*. In: Maclean, J. L., Dizon, L. B., Hosillos, L. V., (Eds.), The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, 641-646.

Lightner, D. V. (1996): A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge, LA, USA, 305 pp.

Madhukiran, N., Soundarapandian, P., John Samuel, N., Dinakaran, G. K. (2009): Recent Technology for the Seed Quality Management of Commercially Important Shrimp *Penaeus monodon* (Fabricius). Current Research Journal of Biological Sciences, 1, 3, 144-149.

Moriarty, D. J. W. (1999): Disease control in shrimp aquaculture with probiotic bacteria. In: Bell, C.R., Brylinsky, M., Johnson-Green, P. (Eds.), Microbial Biosystems: New Frontiers: Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.

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

Ringø, E., Gatesoupe, F. J. (1998): Lactic acid bacteria in fish: a review. Aquaculture, 160, 177-203.

Ringø, E., Birkbeck, T. H. (1999): Intestinal micro flora of fish larvae and fry. Aquaculture Research, 30, 73-93.

Robert, R., Nicolas, J. L., Miner, P. (1995): Mortality control of Pecten maximus larvae in the hatchery. Abstracts. 10th International Pectinid Workshop (Burnell G, ed), Cork, Ireland, 51-52.

Samuelsen, O. B., Torsvik, V., Ervik, A. (1992): Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. Science of the Total Environment, 114, 25–36.

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

Soundarapandian, P., Babu, R. (2010): Effect of Probiotics on the Hatchery Seed Production of Black Tiger Shrimp, *Penaeus monodon* (Fabricius). International Journal of Animal and Veterinary Advances, 2, 1, 9-15.

Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W., (2000): Probiotic bacteria as biological control agents in aquaculture. Microbiol Molecular Biology Review, 64, 655-671.

Vine, N. G., Leukes, W. D., Kaiser, H. (2006): Probiotics in marine larviculture. FEMS Microbiol Review, 30, 404-427.

Wang, Y. B., Xu, Z. R., Xia, M. S. (2005): The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. Fisheries Science, 71, 1036-1041.

Wang, Y. B. (2007): Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture, 269, 259-264.

Walker, P. J., Mohan, C. V. (2009): Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies. Reviews in Aquaculture, 1, 125-154.

Weston, D. P. (1996): Environmental Considerations in the Use of Antibacterial Drugs in Aquaculture. In: Baird, D., Beveridge, M. V. M., Kelly, L. A., Muir, J. F. (Eds.), Aquaculture and Water Resource Management. Blackwell, Oxford, 140-165.

Zhou, X. X., Wang, Y. B., Li, W. F. (2009): Effect of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. Aquaculture, 287, 349-353.