Publications

Available Online

J. Sci. Res. **5** (2), 363-370 (2013)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Effects of Probiotics on Growth and Survival of Shrimp (*Penaeus monodon*) in Coastal Pond at Khulna, Bangladesh

M. I. Hossain[1], M. M. Kamal[1], M. A. Mannan[2], M. A. B. Bhuyain[3], and M. I. Hossain[4*]

[1]Gazi Fish Culture Ltd. Dacope, Khulna

[2]Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh

[3]Institute of Marine Sciences & Fisheries, University of Chittagong

[4]Department of Fisheries, University of Rajshahi, Rajshahi

Received 18 September 2012, accepted in final revised form 28 February 2013

Abstract

The study was carried out for 138 days to know the growth and survival rate of *P. monodon* by applying probiotics in Gazi Fish Culture Ltd. Dacope, Khulna, Bangladesh. Six experimental ponds (4000 m^2 in size i.e. one acre) were selected of which three were probiotic ponds and three were controlled. After pond preparation, PL_{15} (average weight of each 4.75±0.09 g) was stocked at the rate of 13 per m^2 following polymerase chain reaction (PCR) test. CP NASA shrimp feed was used during the study period. Transparency, salinity, pH, dissolved oxygen (DO), temperature, total Ammonia Nitrogen (TAN) were recorded by standard measurements. The average final body weight of the harvested shrimp is 37.67±1.15 g in probiotics ponds and 27.33±0.58 g in controlled ponds and the difference was significant ($P < 0.01$) between these two productions. The average survival rate was 90.67±1.15 % in probiotic pond and 71.00±3.0 % in controlled pond. The average daily gain (ADG) in weight was 0.27±0.01 g and 0.19±0.01 g in probiotic and controlled ponds, respectively. The result showed that probiotic plays an important role in maintaining water quality parameters, soil quality and health management as well as increases the growth and survival of shrimp.

Key words: Probiotics; *P. monodon;* Water quality parameter; Growth rate; Survival rate.

doi: http://dx.doi.org/10.3329/jsr.v5i2.11815 J. Sci. Res. **5** (2), 363-370 (2013)

1. Introduction

Probiotics are live nonpathogenic microorganisms that provide colonization resistance to the pathogenic microbes and thus are effective in prevention and treatment of some diseases. Fuller [1] defined probiotics as live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance. Probiotics, lactic acid bacteria and Bacillus spp. as 'bio-friendly agents' can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms

* *Corresponding author:* bitanrubd@yahoo.com

[2]. The use of beneficial bacteria (probiotics) to displace pathogens by competitive processes is being used in the animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture [3]. Shrimp (*P. monodon)* play an important role in the economy of Bangladesh. Shrimp is of great importance in earning foreign exchange and also to meet up protein demand and to solve unemployment problem for the increasing population. Among the exportable fish and fisheries items of Bangladesh the contribution of shrimp is 57% [4]. One of the major problems at present in the shrimp aquaculture is the microbial disease caused by self-pond pollution [5]. Probiotic bacteria improve the health of shrimp by controlling pathogens and improving water quality by modifying the microbial community composition of water [6]. The main probiotic bacteria documented in shrimp grow-out are *Bacillus spp.* strains [7-8] such as *Bacillus subtilis* [9-10] or Gram-negative bacteria strains [11-13]. Several reviews [5, 10-11, 14-21] detail the various developments made in the use of probiotics in aquatic cultured species, including shrimp. Based on the previous research on probiotics it is suggested that the use of probiotic bacteria in aquaculture has tremendous scope and the study on application of probiotics in aquaculture has a glorious future [22-23]. The present study, therefore, has been conducted with the objective of supplementing probiotics in the diet of *P. monodon* and assessing their growth performance and survival rate by semi intensive culture in Bangladesh.

2. Materials and Methods

The study was done in Gazi Fish culture Ltd., Dacope, Khulna, Bangladesh. The culture was done for 138 days. Initially ponds were re-excavated and allowed to sun dry to increase the capacity of oxidation of hydrogen sulphide and to eliminate other obnoxious gases. The soil pH was recorded in the pond by pH meter. The average pH was calculated and required amount of lime was applied to maintain the optimum pH. The ponds were fenced by blue net to prevent entering virus carrier species. During the high tidal period the ponds were filling in the water by filtration with small mesh size filter net. The initial water levels in the ponds were maintained at 1.5 m level. After filling the pond, crab net was fitting surrounding the ponds. Ponds were bleached at 60 ppm bleaching, containing 30% chlorine. Four paddle wheel aerators (2 HP each) were set in the four corner position of the pond. For plankton growing the organic compounds and minerals such as rice bran, fish meal, molasses, yeast, dolomite, nutrilake, a-soil and soda mix were applied in the probiotics and controlled ponds.

Water salinity, temperature, transparency, alkalinity, dissolve oxygen, pH, TAN were measured and monitored regularly. The water level was measured by using a hand made wooden scale with cm marking. The water salinity of the pond was measured by using a hand refrectometer (Erma-Japan). The pH, alkalinity, total ammonia nitrogen (TAN) of the pond water was recorded by using pH test kit, alkalinity kit and ammonia test kit respectively (Advance Pharma, Thailand). Water temperature was measured by using a standard centigrade thermometer. The dissolved oxygen (DO) was estimated by D. O. test kit (Advance Pharma, Thailand). Transparency was observed by using a secchi disc.

Ponds were ready for stocking after three weeks. Then PCR tested healthy *P. monodon* seeds (PL_{15}) which were purchased from a commercial hatchery were stocked at a density of $13/m^2$. Before stocking, the seeds were acclimatized to the pond environment. For this the seed bags were allowed to float on the water surface in each pond for 30 minutes in order to adjust the temperature. The bags were opened and the pond water was introduced slowly by sprinkling into the bags for 60 minutes to equalize with pond water parameters. After acclimatization, seeds were released slowly to the ponds water.

During the culture period different types of soil, water and feed probiotics such as Super PS, Super biotic, Pro-w, mutagen, zymatin and Pro-2 were applied for maintaining soil, water quality and feed consumption of shrimp in probiotics ponds. After 138 days of rearing, shrimp was harvested by pumping the pond.

The feeding schedule was based on the feed chart given by the CP Aquaculture (India) Pvt. Ltd. Company. Later the feeding was adjusted based on the check tray observation and body weight sampling. Four check trays were installed in each pond for monitoring feed intake. The required amount of feed were dispensed at certain interval in 24 hours period as follows- 25% in the morning (6.00am), 20% at noon (11.00 pm), 30% at evening (6.00 pm) and 25% at night (10.00pm). The feed was broadcasted by rope method.

Additional water exchange was not done for the first 60 days. After that 12 cm of water was added regularly in every 15 days interval till harvesting begins. After 15 days of stocking, sampling of shrimp was done weekly during early hours of the day with a cast net and weights are recorded. Survival rate and average body weight (ABW) of the shrimp were estimated and condition of shrimp health was observed.

All data were analyzed statistically using GraphPad Prism 5 statistical software (GraphPad Software, Inc., San Diego, CA) after they were checked for normal distribution and homogeneity of variance. Only percent data had to be arcsine transformed before analysis; however, non-transformed data are presented in tables. A student t-test was used to examine treatment effects on weight gain, survival, growth and production. All statistical analyses were considered significant at 5% ($P < 0.05$).

3. Results

The salinity was found between 08-18 ppt during the culture period. The temperature of the water was ranged between 27 to 33°C during entire culture period. Transparency ranged from 25 to 55 cm in probiotics ponds and 20 to 65 cm in controlled ponds respectively during the culture period. The average pH was 7.5 to 8.8 in probiotics pond and 7.2 to 9.5 in controlled ponds during the culture time. The alkalinity was measured in 130/130 to 130/110 and 80/80 to 130/130 in probiotics and controlled ponds, respectively. TAN was 0 to 1.0 mg/l in probiotics ponds and 0 to 4.0 mg/l in controlled ponds in the culture period. The dissolved oxygen was recorded maximum 8.0 ppm and minimum 4.5 ppm in probiotics ponds and maximum 7.0 ppm and minimum 4.0 ppm in controlled ponds (Table 1).

Table 1. Water quality parameters of Probiotic treated and control ponds during culture period.

Weekly sampling	Salinity		Temperature		pH		Dissolved oxygen		Total ammonia nitrogen (TAN)	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
	Avg.± SD	Avg.± SD	Avg.± SD	Avg.± SD	Avg.± SD	Avg.± SD	Avg.± SD	Avg.± SD	Avg.± SD	Avg.± SD
1st	17±0.6	17±0.6	30±0.6	30±0.6	8.1±0.5	7.6±0.3	5.5±0.5	5.3±0.6	0.4±0.4	2.7±0.6
2nd	18±0.6	18±0.0	30±1.0	28±1.5	8.6±0.3	8.7±0.2	4.7±1.2	4.8±0.8	0.5±0.2	2.9±0.7
3rd	18±0.6	17±0.0	30±0.6	30±0.6	8.4±0.3	8.6±0.2	5.2±0.3	5.5±0.5	0.5±0.2	2.2±0.5
4th	15±0.6	15±0.6	29±0.6	30±0.6	8.6±0.1	8.3±0.3	6.2±0.3	6.0±0.5	0.60.2±	2.5±0.9
5th	15±1.0	14±0.6	30±1.0	29±1.2	8.7±0.4	8.8±0.2	5.2±0.3	4.7±0.6	0.6±0.1	2.6±0.8
6th	14±0.6	14±0.6	29±1.5	30±0.6	8.3±0.2	8.5±0.2	6.2±0.3	5.7±1.0	0.5±0.3	2.8±0.7
7th	14±0.6	13±0.6	27±0.6	30±1.5	8.4±0.3	8.0±0.1	4.3±0.6	5.0±0.5	0.3±0.2	2.1±0.2
8th	12±1.0	12±0.6	28±0.6	28±1.2	8.1±0.2	8.3±0.2	4.3±0.6	4.8±0.3	0.8±0.2	2.0±0.7
9th	11±0.6	11±1.0	26±1.0	26±1.0	8.0±0.3	8.1±0.3	5.2±0.3	5.0±0.5	0.4±0.2	2.0±0.6
10th	10±0.6	10±0.0	29±1.5	30±1.0	8.1±0.3	8.1±0.3	6.2±0.3	6.0±0.5	0.3±0.2	2.1±0.5
11th	10±0.6	10±0.0	28±0.6	28±0.6	8.8±0.2	8.8±0.2	4.3±0.6	4.8±0.3	0.3±0.5	2.3±0.4
12th	9±1.0	9±0.0	24±0.6	25±1.2	8.8±0.2	8.8±0.2	4.3±0.6	4.7±0.6	0.6±0.4	2.4±0.4
13th	8±0.6	8±0.6	27±1.2	27±1.2	8.5±0.3	8.5±0.3	4.5±0.9	5.2±0.6	0.6±0.3	2.3±0.4
14th	8±0.6	8±0.6	28±1.5	30±0.6	8.6±0.1	8.7±0.1	5.3±0.3	5.2±0.3	0.2±0.3	2.4±0.6

SD: Standard deviation

The average final body weight of the harvested shrimp was 37.67±1.15 g and 27.33±0.58 g in probiotics and controlled ponds, respectively. The average survival rate was 90.67±1.15 % in probiotics ponds and 71.00±3.0 % in controlled ponds. The average daily weight gain was 0.27±0.01 g and 0.19±0.01 g in probiotics and controlled ponds. Average per hectare production was 4385.67±116.10 kg and 2557.67±108.74 kg in probiotics and controlled ponds (Table 2).

Table 2. Growth and survival rate (%) of *P. monodon* in probiotics ponds.

Particulars	Probiotics ponds				Controlled ponds			
	1	2	3	Avg.± SD	1	2	3	Avg.± SD
Stocking (number./m^2)	13	13	13	13±0.0	13	13	13	13±0.0
Initial weight (mg)	4.68	4.85	4.72	4.75±0.09	4.78	4.82	4.64	4.75±0.09
DOC	135	137	137	136.33±1.15	138	135	136	136.33±1.53
Survival (%)	92	90	90	90.67±1.15	71	68	74	71.00±3.00
Final weight (gm)	37	39	37	37.67±1.15	27	28	27	27.33±0.58
ADG (gm)	0.27	0.28	0.27	0.27±0.01	0.19	0.2	0.19	0.19±0.01
Harvest (kg/ha)	4372	4508	4277	4385.67±116.10	2662	2445	2566	2557.67±108.74

DOC: Days of culture; ADG: Average daily growth; SD: Standard deviation

The average body weight (gm) of the shrimps is significantly higher in the ponds with probiotics than that of control ponds (Student *t*-test, Two tailed, degree of freedom (df) = 13, $P < 0.01$). It increases when the culture period extends and this increase is higher in probiotics treated ponds rather than control ponds (Table 3).

Table 3. Weekly Growth of *P. monodon* in the culture ponds*.

Weekly growth	Probiotics ponds				Controlled ponds			
	1	2	3	Avg.± SD	1	2	3	Avg.± SD
1st	4	5	4	4.33±0.58	3	3	2.5	2.83±0.29
2nd	7	7	6.5	6.83±0.29	4	4.5	4	4.17±0.29
3rd	9	9	8.5	8.83±0.29	6	6	6	6.00±0.00
4th	12	12	11	11.67±0.58	7.5	8	9	8.17±0.76
5th	14	15	14	14.33±0.58	9.5	11	11	10.50±0.87
6th	16	19	17	17.33±1.53	12	12	10.5	11.50±0.87
7th	19	21	20	20.00±1.00	14	15	12	13.67±1.53
8th	22	23.5	22.5	22.67±0.76	15.5	17	14	15.50±1.50
9th	25	26	25	25.33±0.58	18	19	16	17.67±1.53
10th	27	29	27	27.67±1.15	21	20	18.5	19.83±1.26
11th	30	32	29.5	30.50±1.32	22	23	20	21.67±1.53
12th	32	34	31.5	32.50±1.32	24	25	23	24.00±1.00
13th	35	36	34	35.00±1.00	26	26	25	25.67±0.58
14th	37	39	37	37.67±1.15	27	28	27	27.33±0.58

*First samplings were done after 30 days of culture period

4. Discussion

Information on the efficiency of probiotics on the growth and survival of the cultivable shrimp species, *P. monodon* is not adequate and this study was conducted to observe the efficiency of probiotics (pH Fixer, Super Biotic, Super P S, Mutagen, Zymetin, Pro-w and Pro-2) on the growth and survival of the cultivable shrimp species, *P. monodon* and also to monitor its influence on important water quality parameters. Salinity, temperature, dissolved oxygen, pH and total ammonia nitrogen (TAN) are important water quality parameters considered during the study. Water quality parameters were found more suitable in probiotics ponds than in controlled ponds those matches with the report of Jiravanichpaisal *et al.* [24]. Maintenance of good water quality is essential for optimum growth and survival of shrimps [21].

In the present study the salinity of probiotic ponds ranged from 8-18 ppt. According to Soundarapandian *et al*. [21] salinity is an important parameter in maintaining optimum growth and survival of shrimps. Even though, *P. monodon* is euryhaline aquatic species; it is comfortable when exposed to optimum salinity. At high salinity the shrimp grows slowly but remains healthy and resistant to diseases. If the salinity is low, the shell becomes weak and prone to diseases. Muthu [25], Soundarapandian and Gunalan [26] and Karthikeyan [27] recommended an ideal salinity range of 10-35 ppt for *P. monodon* culture. While

Chanratchkool *et al*. [28] maintained the salinity in between 10-30 ppt. Soundarapandian *et al.* [21] stated in their study that pH is one of the vital environmental characteristics, which affects the metabolism and other physiological process of shrimps. In their study pH range was 7.6 to 8.2 for the probiotics treated and control ponds. In the present study pH range was 8-8.8 in probiotic ponds and 7.6-8.8 in controlled ponds. Ramanathan *et al*. [29] said that the optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production. Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation and low levels of dissolved oxygen can cause damages in oxidation state of substances from the oxidized to the reduced form. In the present study dissolved oxygen was found 4.3-6.2 ppm and 4.7-6 ppm in probiotic and controlled ponds respectively. Soundarapandian *et al*. [21] found 3.2 to 4.2 ppm dissolved oxygen in all their culture ponds that is close to the present study. Low-level of oxygen hampers metabolic performances in shrimp and can reduce growth and moulting and also causes mortality [30]. Water temperature is the most important environmental variables in shrimp cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival [21]. The optimum range of temperature for *P. monodon* culture is between 26 to 33ºC [31, 26]. In this study temperature range was found 24-30^0 C and 25-30^0 C in probiotic and controlled pond respectively which was maintained within reference ranges. So it was observed that probiotic does not alter water temperature or it does not have any beneficial effect on it. Ammonia builds up in the water of the fish pond when nitrogen-containing substances decay. The two main nitrogen sources in culturable fish ponds are the waste excretions from the fish and uneaten food. One of the breakdown products of both these substances is ammonia. At farm level, Ammonia level should be less than 1 ppm [21]. In the present study total ammonia nitrogen was 0.2-0.8 and 2-2.9 in probiotic and controlled ponds respectively. Thus maintaining the ammonia level probiotic helps in maintaining good water quality and thereby keeps the shrimp disease free.

The average body weight of the harvested shrimp was 37.67±1.15 g and 27.33±0.58 g in probiotics and controlled ponds, respectively and the difference was significant between these two productions (Student t-test, Two tailed, df = 13, $P < 0.01$). The average daily growth (ADG) was 1.42 times better in probiotic pond than in the control pond whereas per hectare total production was 1.72 times better in probiotic than control pond. Result showed that all probiotic- supplemented diets resulted in higher growth in prawn than the control diet though the amount of feeding was same in both the ponds. This result is very inspiring in shrimp culture with probiotics as size of shrimp is directly related to better foreign exchange earnings. Maeda and Nagami [32] observed that bacterial strains possessing vibrio static activity improved the growth of prawn and crab larvae. Zhenguo *et al*. [33] found that photosynthetic bacteria used in prawn food or culture water improved the growth of the prawn. More or less same result was found by Saad *et al*. [34].

In the present study the average survival rate was 90.67±1.15 % in probiotics ponds and 71.00±3.0 % in controlled ponds and the difference was significant between these two productions (Student t-test, Two tailed, df = 13, $P < 0.01$). In probiotic ponds survival rate was 19.67% more than control ponds. Here in this study with the application of probiotics, survival rate of shrimp has been found more compared to the controlled ponds which is similar to the report of Garriques and Arevalo [35]. According to these authors the use of *V. alginolyticus* as a probiotic agent increases survival and growth in *P. vannamei* postlarvae by competitive

elimination of potential pathogenic bacteria, and also effectively reduces the need for antibiotic prophylaxis in intensive larvae culture system. Maeda and Liao [36] also found higher survival and molt rates of prawn larvae of *P. monodon* by treating the pond with soil extract and the bacterial strain. A farm on Negros, in the Philippines, which had been devastated by luminous Vibrio disease while using heavy doses of antibiotic in feed, achieved survival of 80-100% of shrimp in all ponds treated with probiotics [66].

5. Conclusion

The general conclusion obtained from the present study is that the probiotic plays a vital role in growth, survival and disease resistance of the aquatic animal by maintaining good water quality parameters throughout the culture period. Probiotic treatment offers a promising alternative to the antibiotics for fish and shrimp aquaculture system. In Bangladesh sustainable shrimp culture with probiotics is increasing day by day and unemployment can be mitigated through this sector. Further research is still needed to detect the mode of action of probiotic on *P. monodon* digestibility and its effect on immune response and stress resistance.

References

1. R. Fuller, Probiotics: The Scientific basis (Chapman and Hall, London, UK, 1992) p. 398.
2. A. Farzanfar, FEMS Immunol Med Microbiol. **48**(2), 149 (2006). http://dx.doi.org/10.1111/j.1574-695X.2006.00116.x PMid:17064272
3. R. Havenaar, B. T. Brink, and J. H. J. Huis in't Veld, *Selection of strains for probiotic use. In*: R. Fuller (ed), Probiotics: the scientific basis (Chapman and Hall, London, 1992) pp. 209-224.
4. DoF (Department of Fisheries), Unnata projuktite bagda chingri chas sohayeka (Matshabhabon, Ramna, Dhaka, Bangladesh, 2012).
5. A. K. Jha, J. Bang. Acad. Sci. **35** (2), 237 (2011).
6. D. J. W. Moriarty, O. Decamp, and P. Lavens, Probiotics in Aquaculture (Aquaculture Asia Pacific Magazine, 2005) pp. 14-16.
7. D. J. W. Moriarty, Aquaculture **164,** 351 (1998). http://dx.doi.org/10.1016/S0044-8486(98)00199-9
8. S. Ziaei-Nejad, M. H. Rezaei, G. A. Takami, D. L. Lovett, A. Mirvaghefi, and M. Shakouri, Aquaculture **252**, 516 (2006). http://dx.doi.org/10.1016/j.aquaculture.2005.07.021
9. B. Vaseeharan and P. Ramasamy, Lett. Appl. Microbiol. **36**, 83 (2003). http://dx.doi.org/10.1046/j.1472-765X.2003.01255.x PMid:12535126
10. D. J. W. Moriarty, Disease control in shrimp aquaculture with probiotic bacteria- *Proceedings of the 8th International Symposium on Microbial Ecology, Atlantic Canada Society for Microbial Ecology* (Halifax, Canada, 1999) pp. 237–243.
11. D. Garriques and G. Arevalo, An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of Penaeus vannamei postlarvae in Ecuador. In: C. L. Browdy and J. S. Hopkins (ed.), Swimming through troubled water. *Proceedings of the Special Session on Shrimp Farming, Aquaculture* (World Aquaculture Society, Baton Rouge, La, 1995) pp. 53–59.
12. S. V. Alavandi, K. K. Vijayan, T. C. Santiago, M. Poornima, K. P. Jithendran, S. A. Ali, and J. J. S. Rajan, Fish and Shellfish Immunology **17**, 115 (2004). http://dx.doi.org/10.1016/j.fsi.2003.11.007 PMid:15212731
13. K. K. Vijayan, I. S. B. Singh, N. S. Jayaprakash, S. V. Alavandi, S. S. Pai, R. Preetha, J. J. S. Rajan, and T. C. Santiago, Aquaculture **251**, 192 (2006). http://dx.doi.org/10.1016/j.aquaculture.2005.10.010
14. F. J. Gatesoupe, Aquaculture **180**, 147 (1999). http://dx.doi.org/10.1016/S0044-8486(99)00187-8

15. S. Rengpipat, S. Rukpratanporn, S. Piyatiratitivorakul, and P. Menasveta, *Probiotics in Aquaculture: A Case Study of Probiotics for Larvae of the Black Tiger Shrimp* (*Penaeus monodon*). In: Flegel TW(ed) Advances in shrimp biotechnology (National Center for Genetic Engineering and Biotechnology, Bangkok, 1998) pp. 177-181.
16. L. Vershuere, G. Rombaut, P. Sorgeloos, and W. Verstraete, Microbiol. Mol. Biol. Rev. **64**, 655 (2000). http://dx.doi.org/10.1128/MMBR.64.4.655-671.2000
17. B. Gomez-Gil, A. Roque, and J. Turnbull, Aquaculture **191,** 259 (2000). http://dx.doi.org/10.1016/S0044-8486(00)00431-2
18. A. Irianto and B. Austin, J. Fish Dis. **25**, 633 (2002). http://dx.doi.org/10.1046/j.1365-2761.2002.00422.x
19. N. G. Vine, W. D. Leukes, and H. Kaiser, FEMS Microbiol. Rev. **30**, 404 (2006). http://dx.doi.org/10.1111/j.1574-6976.2006.00017.x PMid:16594964
20. P. Soundarapandian and S. Sankar, Int. J. Zool. Res. **4**(1), 35 (2008). http://dx.doi.org/10.3923/ijzr.2008.35.41
21. P. Soundarapandian, V. Ramanan, and G. K. Dinakaran, Current Res. J. Soc. Sci. **2** (2), 51 (2010).
22. D. J. W. Moriarty, Aquaculture **151,** 333 (1997). http://dx.doi.org/10.1016/S0044-8486(96)01487-1
23. S. N. Chen, S. L. Huang, and G. H. Kou, Studies on the epizootiology and pathogenicity of bacterial infections in cultured giant tiger prawns, Penaeus monodon in Taiwan. Paper presented at the proceedings of the Workshop, Diseases of cultured Penaeid Shrimp in Asia and the United States, Hawai (1992) p.195.
24. P. Jiravanichpaisal, P. Chuaychuwong, and P. Menasveta, The use of *Lactobacillus* sp. as the probiotic bacteria in the giant tiger shrimp (*Penaeus monodon* Fabricius), Poster session of the 2nd Asia-Pacific marine biotechnology conference and 3rd Asia-pacific conference on algal biotechnology, Phuket Thailand (May, 1997) p. 16.
25. M. S. Muthu, Site selection and type of farms for coastal aquaculture of prawns, *Proceedings of the Symposium on shrimp farming* (Bombay, Marine Products Export Development Authority, 1980) pp. 97-106.
26. P. Soundarapandian and B. Gunalan, Int. J. Zool. Res. **4**(1), 21 (2008). http://dx.doi.org/10.3923/ijzr.2008.21.27
27. J. Karthikeyan, Aquaculture (shrimp farming) its influence on environment, Technical paper submitted to the seminar 'Our Environment-its challenges to development projects', American Civil Engineers, Calcutta, India (September 1994).
28. P. Chanratchkool, J. F. Turunbull, and C. Limsunean, Health management in Shrimp ponds, Aquatic Health Research Institute, Department of Fisheries, Kasetasart University, Bankok (1994) p. 91.
29. N. Ramanathan, P. Padmavathy, T. Francis, S. Athithian, and N. Selvaranjitham, Manual on polyculture of tiger shrimp and carps in freshwater, Tamil Nadu Veterinary and Animal Sciences University, Fisheries College and Research Institute, Thothukudi (2005) pp. 1-161.
30. L. M. Gilles, Environmental factors affect immune response and resistance in Crustaceans, the Advocate (2001) p. 18.
31. C. E. Boyd, Fisheries World **3**, 33 (1995).
32. M. Maeda and K. Nogami, Some aspects of the biocontrolling method in aquaculture, Current topics in marine biotechnology (Japan. Soc. Mar. Biotechnol, Tokyo 1989) pp. 395-397.
33. Q. Zhenguo, T. Ruiying, and H. Ningyu, Marine Sci. **2**, 4 (1992).
34. A. S. Saad, M. M. Habashy, and K. M. Sharshar, World Appl. Sci. J. **6** (4), 550 (2009).
35. D. Garriques and G. Arevalo, An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of Penaeus vannzamei postlarvae in Ecuador, Eds. C. L. Browdy and J. S. Hopkins, Swimming through troubled water, *proceedings of the special session on shrimp farming* (World Aquaculture Society, 1995) pp. 53-59.
36. M. Maeda and I. C. Liao, Aquaculture **21**, 25 (1992).