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### Short Communication

## Application of *Streptomyces* as a Probiotic in the Laboratory Culture of *Penaeus monodon* (Fabricius)

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### Abstract

Probiotic supplementation of live microorganisms in aquaculture aids in preventing disease, thereby increasing production and decreasing economic loss. In the present study, *Streptomyces* cells were incorporated in *Penaeus monodon* (Fabricius) laboratory culture for 25 days. *Streptomyces* was inoculated into the feed in different concentrations (0, 2.5, 5.0, 7.5, and 10.0 g/kg feed) on days 1, 10, and 20. Growth was monitored on days 5, 15, and 25. Experimental culture tanks provided with *Streptomyces* had better water quality parameters than the control tank. At the cell concentration of 10 g, the pH was 7.9, ammonia 0.00067 ppm, nitrate 0.00285 ppm, phosphate 0.00224 ppm, silicate 0.00836 ppm, total heterotrophic bacteria  $3.279 \times 10^5$  CFU/ml, and total *Vibrio*  $0.2 \times 10^2$  CFU/ml. Growth increased as the *Streptomyces* cell concentration increased. At 10 g concentration, growth in length and weight was 15.79% and 57.97%, respectively, and the interval between molts was 12.5 days.

### Introduction

The black tiger shrimp, *Penaeus monodon*, is the most widely cultured species in India but the rapid development of shrimp aquaculture received a temporary setback due to the outbreak of a disease in the early 1990s (Fast and Menasveta, 2000). This happened because of indiscriminate use of antibiotics and drugs which resulted in the development

of resistance among pathogenic microorganisms (Karunasagar et al., 1994; Lee et al., 1996) and water pollution by ammonia and other toxic substances (Jeyasekaran et al., 2003). Antibiotic residues in shrimps are regarded as hazardous for human health. Hence, the search for a permanent solution led to the study of beneficial microorganisms

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or 'probiotics' to keep the pond environment clean and maintain water parameters within optimal ranges.

Probiotics applied through feed beneficially act upon shrimp growth, ultimately increasing production. Palaniselvam and Kathiresan (1998) found that a cyanobacterium feed enhanced the gross growth efficiency and net growth efficiency, however, it reduced consumption, production, growth rate, assimilation, and metabolism.

Actinomycetes are gram-positive organisms with a high G+C (>55%) content in their DNA. The *Streptomyces* genus is very common in marine environments. Marine actinomycetes, especially *Streptomyces*, have long been considered chemical factories for antibiotic production (Okazaki and Okami, 1972; Ellaiah and Reddy, 1987). However, reports are scanty on the application of actinomycetes as probiotics in aquaculture. Probiotics are being used extensively in shrimp culture but imported formulations are costly. There is a need to find effective and cheaper alternatives. Hence, in the present study the efficacy of *Streptomyces* as a probiotic in the laboratory culture of *P. monodon* (Fabricius) was evaluated.

### Materials and Methods

**Experimental animals.** Post larvae (PL 20) of *P. monodon* were procured from the hatchery and it was ensured that the seeds were free from any disease. Prior to stocking, the post larvae were measured for total length and weight. Twenty-five individuals were stocked per tank in five 40-l plastic tanks containing 35 l filtered estuarine water (salinity 25-30‰) with proper aeration.

**Actinomycetes isolation.** Sediment samples were collected from the Vellar estuary on the southeast coast of India (11°29'N, 79°46'E). After two-fold dilutions, 0.5 ml aliquots of the samples were plated onto starch-casein agar medium (Hi-Media, Mumbai, India) supplemented with nystatin and cyclohexamide at 25 µg/ml and 10 µg/ml, respectively, to minimize contamination with fungi and 10 µg nalidixic acid/ml to minimize bacterial growth (Ravel et al., 1998). The

plates were incubated for one week at 30±0.2°C and actinomycetes colonies were characterized by their distinctive powdery appearance (Kokare et al., 2004).

Single colonies were picked up and purified by successive restreaking and identified as *Streptomyces* sp. by cell wall amino acids and whole cell sugar analyses (Lechevalier and Lechevalier, 1970). The strain was inoculated in 50 ml of starch casein broth in an Erlenmeyer flask and incubated at room temperature. The *Streptomyces* grew as a mat on the surface of the broth (non-motile form). The mat was harvested after one week, oven dried (40±0.5°C for 5 days), ground, and incorporated into the starter feed at different concentrations (2.5, 5.0, 7.5, or 10.0 g per kg feed) using egg albumin as a binder (Gopalakannan et al., 2004). The inoculation was made twice a day (morning and evening) on days 1, 10, and 20 of culture.

**Maintenance.** Every morning, up to 20% of the water was exchanged. Feed was given at 6-9% of the body weight, twice a day, half at dawn (6:00) and half at dusk (18:00). Water temperature was measured daily using a Celsius thermometer. The salinity (ppt) of each tank was recorded daily using a hand refractometer (ATAGO, S/m11, Japan) after changing the water. The pH of the water was measured daily using a pH meter. Dissolved oxygen was estimated by the modified Winkler's method described by Strickland and Parsons (1972) after collecting water samples in a BOD bottle. Ammonia, nitrate, phosphate, and silicate were estimated following the method of Strickland and Parsons (1972).

**Microbial evaluation.** The microbial population in the tank and shrimp growth were evaluated on days 5, 15, and 25. Water samples were serially diluted and spread on Zobell's marine agar medium (Hi-Media, Mumbai, India) for total heterotrophic bacteria count and TCBS medium (Hi-Media, Mumbai, India) for total *Vibrio* count. A few shrimp from each tank were sacrificed on day 25 for examination of their gut microflora.

**Growth.** Growth was determined by the difference between the initial and final length-

weights. Correlation coefficient values were calculated with cell concentrations and water quality parameters in the treated tanks. F values (ANOVA) were calculated to compare the growth (length, weight, and intermolt duration) between the control and each probiotic group and a *t* test was carried out to compare growth.

### Results

Water quality parameters are given in Table 1. The temperature varied 27-31°C and there were no significant differences between tanks. There were no significant differences between tanks in salinity or dissolved oxygen level but the pH varied greatly between the control and the experimental tanks. In the control it ranged 6.9-7, in most of the experimental tanks 7.0-7.9, and in the 10.0 g treatment 7.5-8.5. The pH increased with the increase in cell concentration and the correlation coefficient value between *Streptomyces* cell concentration and pH was highly significant ( $r = 0.9583$ ;  $p < 0.005$ ).

There were differences in ammonia level between the control and the experimental tanks and a significant negative correlation with the probiotics concentration ( $r = -0.9632$ ;  $p < 0.001$ ). In the control tank, the nitrate value decreased from 0.00025 to 0.00019 ppm but in the experimental tanks the average nitrate level increased significantly ( $r = 0.9862$ ;  $p < 0.001$ ) with the increase in probiotic concentration. The phosphate concentration ranged from 0.00043 to 0.00224 in the experimental tanks ( $r = 0.9919$ ;  $p < 0.001$ ) while it increased from 0.00018 to 0.00054 ppm in the control. A similar trend occurred in the silicate level. There was only a trace in the control while it increased from 0.00136 to 0.00836 ppm in the experimental tanks, significantly and positively correlated with the *Streptomyces* cell concentration ( $r = 0.9695$ ;  $p < 0.001$ ).

With time, the total heterotrophic bacteria count dropped in the control tank and rose in the experimental tanks. The correlation between the increase in the experimental tanks and the increase in probiotic concentration was highly significant ( $r = 0.9468$ ;  $p < 0.001$ ). In contrast, the total *Vibrio* count rose in the control tank and dropped in the experimental tanks.

The correlation between the decrease in *Vibrio* population and the increase of cell supplement was highly significant ( $r = -0.9898$ ;  $p < 0.001$ ). Actinomycetes colonies were isolated after 25 days from all the experimental tanks but not from the control tank.

Growth in terms of length and weight was greater in the experimental tanks than in the control and it increased with the increase in *Streptomyces* cell concentration (Table 2). The length and weight increased significantly above the control in the 7.5 g treatment (one-way ANOVA;  $F_{\text{Length}} = 6.072$ ,  $F_{\text{Weight}} = 6.060$ ;  $F_{\text{crit}} = 5.987$ ;  $p < 0.05$ ) and 10.0 g treatment ( $F_{\text{Length}} = 7.121$ ,  $F_{\text{Weight}} = 6.876$ ,  $F_{\text{crit}} = 5.987$ ;  $p < 0.05$ ), but not in the 2.5 and 5.0 g treatments. The animals in the experimental tanks molted sooner than those in the control. *t* values showed that growth of animals in the 10 g treatment was significantly greater in length (59.13;  $p < 0.001$ ) and weight (16.35;  $p < 0.001$ ) and that the intermolt duration in animals in the 10 g treatment was significantly shorter (10.21;  $p < 0.001$ ) than in control animals.

### Discussion

Water quality plays an important role in aquaculture production. Water quality deteriorates during culture mainly due to the accumulation of metabolic wastes of living organisms, decomposition of unutilized feed, and decay of biotic materials. Changes in water quality can influence survival of organisms as they become vulnerable to disease. But addition of beneficial bacteria as probiotics helps maintain water quality, thereby improving survival and growth.

Dissolved oxygen in the culture medium is an important factor not only for the respiration of aquatic organisms but also to maintain a favorable chemical and hygienic environment in the water body. Therefore, the tanks were continuously aerated during the study and the oxygen level did not vary significantly between the control and experimental tanks. A similar result was reported by Sharma and Bhukhar (2000) who investigated the impact of a commercial probiotic on common carp.

The pH of the culture medium plays an important role on the organisms. It changes

Table 1. Water quality parameters (means±SD) and total heterotrophic bacteria (THB) and *Vibrio* (TVC) counts (CFU/ml) in tanks with different *Streptomyces* concentrations.

Parameter	Control (no <i>Streptomyces</i> )	<i>Streptomyces</i> culture concentration (g/kg feed)			
		2.5	5.0	7.5	10.0
Temperature (°C)	28.33±0.577	28.87±0.576	28.82±0.493	28.86±0.475	29.33±0.763
Salinity (ppt)	33.16±0.763	33.16±1.040	33.5±0.500	33.0±0.500	32.83±0.763
Dissolved oxygen (ppm)	7.75±0.217	7.89±0.025	7.93±0.028	7.93±0.035	7.93±0.037
pH	6.8±0.098	7.0±0.104	7.1±0.108	7.4±0.200	7.9±0.305
Ammonia (ppm)	0.00584±0.00095	0.00305±0.00083	0.00169±0.00032	0.00111±0.00015	0.00067±0.00026
Nitrate (ppm)	0.00021±0.000025	0.00032±0.000015	0.00088±0.000070	0.00222±0.00038	0.00285±0.00011
Phosphate (ppm)	0.00036±0.00003	0.00043±0.000031	0.00088±0.00002	0.00145±0.000029	0.00224±0.00004
Silicate (ppm)	trace	0.00136±0.000025	0.00290±0.000032	0.00448±0.000041	0.00836±0.000043
Initial THB (x 10 <sup>3</sup> )	11.67	122.3	157.2	273.1	315
Final THB (x 10 <sup>3</sup> )	5.4	164	213.5	356	341.2
Mean THB (x 10 <sup>3</sup> )	7.3±0.23	146.8±5.9	181.4±6.8	316.6±7.0	327.9±7.4
Initial TVC	1200	42	35	30	23
Final TVC	1800	24	2800	1800	1600
Mean TVC	1500±12.5	35±5.1	30±7.0	23±9.8	20±12.4

Table 2. Growth of *Penaeus monodon* with different concentrations of *Streptomyces* (n = 25).

Parameter	Control (no <i>Streptomyces</i> )	<i>Streptomyces</i> culture concentration (g/kg feed)			
		2.5	5.0	7.5	10.0
Initial avg length (mm)	10.78	10.75	10.72	10.70	10.76
Final avg length (mm)	11.22	11.40	11.63	11.86	12.46
Length gain (mm)	0.44	0.65	0.91	1.16	1.70
Growth in length (%)	4.08	6.04	8.48	10.84	15.79
Initial avg wt (g)	1.013	1.011	1.000	1.001	1.004
Final avg wt (g)	1.345	1.389	1.412	1.497	1.586
Wt gain (g)	0.332	0.378	0.412	0.496	0.582
Growth in wt (%)	32.77	37.39	41.20	49.55	57.97
Intermolt duration, days (mean±SD)	15.5±1.213	15.2±1.024	14.5±0.980	13.7±0.817	12.5±0.834
Survival (%)	72	80	80	88	92

with the accumulation of residual feed, dead shells, and excreta. The toxicity of ammonia is pH-linked. In the present study, the pH increased during the experiment to the required range of 7.5-8.5 in the 10.0 g treatment as probiotics can control acidity (Sambasivam et al., 2003). Thus, *Streptomyces* was helpful in maintaining the pH at the desired level.

Ammonia is the main end product of protein catabolism in aerobic conditions. Nitrate is reduced to ammonia in anaerobic conditions. The ammonia level should be less than 1 ppm in the culture medium. In both control and experimental tanks, the ammonia level was below this mark. However, there was a build-up of ammonia in the control tank as the experiment progressed while the ammonia level dropped in the experimental tanks as the cell concentration increased. Microorganisms can convert ammonia into nitrate by nitrification through the intermediary product nitrite. This was corroborated by the increases in the nitrate level in the experimental tanks during the course of the experiment.

Microorganisms are unavoidable in the culture system. They have beneficial as well as detrimental effects. In aquaculture systems, total heterotrophic bacteria play a significant role through mineralization and decomposition of wastes and provide supplementary feed for shrimp larvae (Sunilkumar, 1996) whereas *Vibrio*, the natural microflora of shrimp (Lightner, 1993), can cause disease and mass mortality. In the present study, total heterotrophic bacteria increased as the probiotic cell concentration increased and decreased in the control tank as the experiment progressed. In contrast, the *Vibrio* load decreased with the increase of cell concentration and increased in the control tank. Prabhu et al. (1999), Dalmin et al. (2001), and Moriarty (1998) reported the same trends in total heterotrophic bacteria and *Vibrio* when a bacterial probiont was applied in shrimp culture ponds.

Reports on controlling *Vibrio* in aquaculture by applying actinomycetes are meager. In the present study, the *Vibrio* count decreased as the *Streptomyces* cell concentration increased, possibly due to antagonism

of *Streptomyces* sp. towards *Vibrio* spp. The marine *Streptomyces* can produce antibiotics *in vitro* which inhibit the growth of the human pathogenic *Vibrio cholerae* (Balagurunathan, 1992; Siva Kumar, 2001) and the finfish and shellfish pathogen *Vibrio* spp. (Dhevendaran and Annie, 1999). From the present study it is postulated that marine *Streptomyces* may produce antibiotics that prevent the shrimp pathogenic *Vibrio* in the culture system.

Growth is closely associated with molting in crustaceans. Growth is a continuous process for crustaceans, interrupted only by molting periods. In optimum environmental conditions, crustaceans grow and molt quickly. In the present study, the probiotic improved the water quality. Animals molted significantly faster in the experimental tanks than in the control (*t* test;  $p < 0.001$ ). The increased growth in terms of length and weight was also statistically significant (*t* test;  $p < 0.001$ ) and can be attributed to improvement of the water quality by the probiotic, as reported by Rengpipat et al. (2003) with the bacterial probiont *Bacillus*. The significant variation of shrimp growth between the cell concentrations 7.5 g and 10.0 g (ANOVA;  $p < 0.05$ ) supports the efficacy of the *Streptomyces* strain in the present experiment. Beside its antagonism towards *Vibrio*, its growth enhancement factor may be due to the proteolytic enzyme produced by probiont actinomycetes as in the case of the *Bacillus* probiont (Rengpipat et al., 1998). The enzymatic activities of *Streptomyces* were reported by Shyng Yang and Yi Wang (1999), Ranjekar and Sridhar (2002), Ellaiah et al. (2002, 2004). Actinomycetes were present in the gut of animals from all experimental tanks. Thus, the actinomycetes cells applied to the feed reached the intestine of the animals and improved their health.

This study clearly showed that *Streptomyces* supplementation improved the growth, survival, and disease resistance of the black tiger shrimp, *P. monodon*. Above all, it could be effective in controlling the pathogenic *Vibrio* in shrimp culture. All the beneficial influences of probiotics discussed here indicate that *Streptomyces* probionts are going to play an important role in aquaculture, especially in shrimp culture, in the

near future, especially against the backdrop of an increasing number of antibiotic-resistant bacteria, frequent disease outbreaks, and cost effectiveness.

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#### References

- Balagurunathan R., 1992. *Antagonistic Actinomycetes from Indian Shallow Sea Sediments with Reference to  $\alpha$   $\beta$ -unsaturated  $\gamma$ -lactone Type of Antibiotic from Streptomyces griseobrunneus (P-33)*. Ph.D. thesis, Annamalai Univ., India. 83 pp.
- Dalmin G., Kathiresan K. and A. Purushothaman, 2001. Effect of probiotics on bacterial population and health status of shrimp in culture pond ecosystem. *Indian J. Exp. Biol.*, 39:939-942.
- Dhevendaran K. and K. Annie, 1999. Antibiotic and L-asparaginase activity of *Streptomyces* isolated from fish, shellfish and sediment of Veli estuarine lake along Kerala coast. *Indian J. Mar. Sci.*, 28:335-337.
- Ellaiah P. and A.P.C. Reddy, 1987. Isolation of actinomycetes from marine sediments off Visakhapatnam, east coast of India. *Indian J. Mar. Sci.*, 16:134-135.
- Ellaiah P., Adinarayana K., Naveen Babu K., Thaer A., Srinivasulu B. and T. Prabhakar, 2002. Bio-active actinomycetes from marine sediments off Bay of Bengal near Machilipatnam. *Geobios*, 29:97-100.
- Ellaiah P., Ramana T., Bapi Raju K.V.V.S.N., Sujatha P. and A. Uma Sankar, 2004. Investigations on marine actinomycetes from Bay of Bengal near Kakinada coast of Andhra Pradesh. *Asian J. Microbiol. Biotech. Environ. Sci.*, 6:53-56.
- Fast A.W. and P. Menasveta, 2000. Some recent issues and innovations in marine

- shrimp pond culture. *Rev. Fish. Sci.*, 8:151-233.
- Gopalakannan A., Atulkumar and V. Arul**, 2004. Effect of gut probiotic lactic acid bacteria isolated from marine fish on *Penaeus monodon* post larvae to control the *Vibrio anguillarum*. pp. 169-176. In: S.A.H. Abidi, M. Ravindran, R. Venkatesan, R. Vijayakumaran (eds.). *Proc. Natl. Seminar New Frontiers in Marine Biosci. Res.*, Natl. Inst. Ocean Technol., Chennai, India.
- Jeyasekaran G., Selvaraj S., Shakila R.J. and K. Jayanth**, 2003. Putative probiotic marine bacteria for aquaculture systems. *Fish Farmer*, 26:46-48.
- Karunasagar I., Pai R., Malathi G.R. and I. Karunasagar**, 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. *Aquaculture*, 128:203-209.
- Kokare C.R., Mahadik K.R., Kadam S.S. and B.A. Chopade**, 2004. Isolation of bioactive marine actinomycetes from sediments isolated from Goa and Maharashtra coastlines (west coast of India). *Indian J. Mar. Sci.*, 33:248-256.
- Lechevalier M.P. and H. Lechevalier**, 1970. Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int. J. Syst. Bacteriol.*, 20:435-443.
- Lee K.K., Yu S.R., Chen F.R., Yang T.I. and L.P. Chung**, 1996. Virulence of *Vibrio alginolyticus* isolated from diseased tiger prawn *Penaeus monodon*. *Curr. Microbiol.*, 32:229-231.
- Lightner D.V.**, 1993. Diseases of cultured penaeid shrimp. pp. 393-486. In: J.P. McVey (ed.). *CRC Handbook of Mariculture*, 2<sup>nd</sup> ed., vol. 1. *Crustacean Aquaculture*. CRC Press, Boca Raton, FL.
- Moriarty D.J.W.**, 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, 164:351-358.
- Okazaki T. and Y. Okami**, 1972. Studies on marine microorganisms. II. Actinomycetes in Sagami Bay and their antibiotic substances. *J. Antibiot.*, 25:461-466.
- Palaniselvam V. and K. Kathiresan**, 1998. Potential of a marine cyanobacterium *Phormidium tenue* (Menegh.) Gomont as a shrimp feed supplement. *Seaweed Res. Utilisation*, 20:75-78.
- Prabhu N.M., Nazar A.R., Rajagopal S. and S. Ajmal Khan**, 1999. Use of probiotics in water quality management during shrimp culture. *J. Aquacult. Trop.*, 14:227-236.
- Ranjekar M.K. and K.R. Sridhar** 2002. Occurrence and extracellular enzyme potential of actinomycetes of a thermal spring, southern India. *Asian J. Microbiol. Biotech. Environ. Sci.*, 4:59-64.
- Ravel J., Amoroso M.J., Colwell R.R. and R.T. Hill**, 1998. Mercury-resistant actinomycetes from the Chesapeake Bay. *FEMS Microbiol. Letter*, 162:177-184.
- Rengpipat S., Phianphak W., Piyatirativorakul S. and P. Menasveta**, 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture*, 167:301-313.
- Rengpipat S., Tunyanun A., Fast A.W., Piyatirativorakul S. and P. Menasveta**, 2003. Enhanced growth and resistance to *Vibrio* challenge in pond-reared black tiger shrimp *Penaeus monodon* fed a *Bacillus* probiotic. *Dis. Aquat. Org.*, 55:169-173.
- Sambasivam S., Chandran R. and S. Ajmal Khan**, 2003. Role of probiotics on the environment of shrimp pond. *J. Environ. Biol.*, 24:103-106.
- Sharma O.P. and S.K.S. Bhukhar**, 2000. Effect of Aquazyn-TM-1000, a probiotic on the water quality and growth of *Cyprinus carpio* var. *communis* (L.). *Indian J. Fish.*, 47:209-213.
- Shyng Yang S. and J. Yi Wang**, 1999. Protease and amylase production of *Streptomyces rimosus* in submerged and solid state cultivations. *Bot. Bull. Acad. Sin.*, 40:259-265.
- Siva Kumar K.**, 2001. *Actinomycetes of an Indian Mangrove (Pitchavaram) Environment: An Inventory*. Ph.D. thesis, Annamalai Univ., India. 91 pp.
- Strickland J.D.H. and T.R. Parsons**, 1972. *A Practical Handbook of Sea Water Analysis*. Bull. Fish. Res. Bd., Canada, 167. 310 pp.
- Sunilkumar M.K.**, 1996. Heterotrophic marine bacteria as supplementary feed for larval *Penaeus monodon*. *NAGA the ICLARM Q.*:23-26.