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Effect of probiotics on bacterial population and health status of shrimp in culture pond ecosystem

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The artificially manufactured probiotics having beneficial bacteria, *Bacillus* spp. was applied regularly in a modified extensive shrimp (*Penaeus monodon*) culture pond, located on the bank of Vellar estuary, Parangipettai. The populations of total heterotrophic bacteria (THB), beneficial bacteria (*Bacillus* spp.) and pathogenic bacteria (vibrios) were monitored in water and sediment of the pond. The results were compared with a control pond, situated in the same location having same water spread area, stocking density, species managed with same technologies and optimum environmental parameters in which no probiotic was applied. The populations of THB and *Bacillus* spp. in the experimental pond increased and the vibrios decreased after each application of probiotics. But the result of the control pond showed an increasing trend of the populations of THB, *Bacillus* spp. and vibrios towards days of culture. The control pond had lower levels of THB and *Bacillus* spp. and higher levels of vibrios than the probiotic applied (experimental) pond. Also the probiotics maintained optimum transparency and low organic load in the experimental pond as compared to control. In general, water and sediment had almost equal number of *Bacillus* spp. and vibrios, but sediment had higher THB load than water. The applications of probiotics lesser pathogenic vibrios and enhance beneficial bacilli in the culture leading to improved water quality, promoted growth and survival rates and increased the health status of the shrimp without stress and disease outbreaks. Thus the application of probiotics could lead to disease-free and profitable shrimp culture operations which will be helpful for shrimp farmers as most of them are now-a-days severely affected by microbial diseases.

Shrimp culture has become one of the major industries earning more foreign exchange because of its excellent taste, universal appeal and high market value. Since the culture is carried out artificially with advanced technologies in a confined environment, microorganisms in general and bacteria in particular, make more contribution to successful operation. Generally bacteria play two major roles as beneficial bacteria and pathogenic forms. Beneficial bacteria are helpful in nutrient recycling and organic matter degradation and thus clear the environment[1]. Pathogenic bacteria are the causative agents of bad water quality, stress and diseases as they act as primary and secondary pathogens[2-4]. To overcome this pathogen problem, application of disinfectants and antibiotics is a common practice in shrimp culture. Instead of preventing and arresting the diseases, the unwanted and unwarranted usage of the chemicals damages the environment and develops antibiotic resistant bacteria[5,6]. Hence, eco-friendly treatments came in to existence and among these, probiotic application came first as it is environmentally safe and cost effective also[1]. In coastal ecosystem the most abundant beneficial bacteria are *Bacillus* spp., *Nitrosomonas* spp. and *Nitrobacter* spp. Among these, *Bacillus* degrades organic matter, facilitates nutrients recycling, competes with the pathogenic bacteria for food and substrates and secretes enzymes to contain gram-negative pathogenic bacteria[1]. Among pathogens the priority is given to vibrios as they directly cause diseases and indirectly affect the shrimp health through developing poor water quality and stress[7,4]. The probiotic approach to disease control has not been used very much in aquaculture[8], and only a few reports on probiotics are there[9-11,1]. Hence the present work has been designed to find out the role of probiotic bacteria (*Bacillus* spp.) in improving the water quality and reducing the pathogenic bacteria (vibrios) and disease occurrences in modified extensive shrimp culture pond.

The present work was carried out for one culture operation (Nov. 97 to Mar. 98) in a modified extensive shrimp (*Penaeus monodon*) culture pond, located on the bank of Vellar estuary (lat. 11° 29' N; long. 79° 47' E), India, having 0.8 ha water spread area with stocking density of 10/m^2. The water and sediment samples were

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collected on 1, 20, 40, 60, 90, 120 and 150 Days of culture (DOC). Collections were made on early hours of the day after 24 hr of each application of *Bacillus* spp. based artificially manufactured probiotics (ARDA-Tek, Australia) in which the population of *Bacillus* spp. was 10^9/ml. Totally 0.7 litre of probiotics was applied to the pond. During the collection, samples were also taken from a pond that located on the same location with the same water spread area and similar stocking density.

Water—For bacteriological assessment, water was collected in 500 ml screw-capped PVC bottles just below the water surface. Enough air space was left in the bottles to allow thorough mixing. Necessary precautionary measures were undertaken to minimize the contamination through handling. In order to avoid clustering of bacterial cells, the container with water sample was shaken vigorously for 2 min to ensure complete mixing and homogenous distribution of the bacteria. Transparency was recorded with the help of secchi disc.

Sediment—Sediment samples were collected using an alcohol-rinsed, air-dried, vertical corer. The untouched central portion of the collected sample was aseptically transferred into new polythene bags using a sterile spatula for further bacteriological analysis. The upper layer of sediment was used for the analysis of total organic carbon. Using a sterile spatula, the required quantity of the sediment sample was transferred from the polythene bag to a conical flask containing sterile water as diluent. The sediment diluent mixture was agitated in a mechanical shaker for 3 min with proper care to avoid heating. Total organic carbon content was analysed as per Walkley and Black[12].

All the collected samples were brought to the laboratory immediately and done analysis of total heterotrophic bacteria, *Bacillus* spp. and vibrios, within an hour of collection to avoid contamination.

For THB analysis, all the samples were serially diluted using sterile water blanks. The blanks were prepared with aged seawater (50%) and sterilized in an autoclave at 15 lbs. and 121°C for 15 min. Water blank (99 ml) was used for sediment samples. To enumerate THB population, 1 ml sample from desired dilution was pipette out into a sterile petridish. To this, 15-20 ml melted and cooled Zobell's marine agar medium (Hi-media, Bombay) were poured and mixed thoroughly. Plates in duplicate were incubated in an inverted position at the room temperature (28°± 2°C) for 3-4 days. The plates containing viable bacterial colonies were counted and is expressed as Colony Forming Units (CFU/ ml) for water sample and CFU/g dry weight for other samples[13]. A total of 100 strains were isolated from all samples and identified up to the generic level following the scheme of Oliver[14]. Thus the genus *Bacillus* was identified and quantified.

Estimation of vibrios population—Thiosulphate citrate bile salts sucrose (TCBS) agar medium was used for isolating vibrios. Samples were enriched in alkaline peptone water blank for 1 hr and then transferred to TCBS agar medium poured plates and incubated for 20-24 hr at 35-37°C. Developed colonies (green and yellow) were streaked on to Brain Heart Infusion Agar (BHIA) for further characterization by following biochemical tests outlined in U.S.[15].

The results presented in Fig. 1 clearly demonstrate that *Bacillus* spp. load was more in the probiotic-applied pond than the control pond. The load increased towards days of culture (DOC) indicating the increase of *Bacillus* spp. after each application of probiotics. In contrast, vibrios population declined towards DOC indicating that application of probiotics reduced vibrios population. However, the THB load showed an increasing trend towards DOC both in the experimental and control ponds. Though a reduction of vibrios load was observed due to the application of probiotics in the experimental pond, it did not influence much on the difference of THB populations between the control and experimental ponds. It might have been due to the compensation of more *Bacillus* spp. in the experimental pond.

Generally *Bacillus* spp. contribute around 20% of THB and vibrios contribute around 40% of THB in coastal ecosystem[16,17]. This indicates that the increasing trend of THB obviously refers to the increase of vibrios which leads to the bad water quality and increases the chances of disease

Table 1—Transparency of water and total organic carbon of sediment in the ponds and survival rate and production of the shrimp cultured

	Experiment pond	Control pond
Transparency (m)	0.45 – 0.55	0.25 – 0.40
Total organic carbon (%)	0.35 – 0.40	0.40 – 0.60
Survival rate (%)	83	55
Average growth g/animal)	33	24
Production	2.2	1

outbreaks. Manipulation of the bacterial species composition will lead to keep the pathogenic bacteria like vibrios under control. Several commercial products of viable bacteria are being used to control water quality in ponds. Among these, the priority is given to *Bacillus* spp.[1]. The present investigation dealing with *Bacillus* spp. coincides with improved water quality, survival and growth rates, and health status of the shrimp and reduced the pathogenic vibrios. The number of 10^9 CFU/ml *Bacillus* spp. in the probiotic became 10^3 CFU/ml after application in pond environment because of the effects of autochthonous flora such as *Flavobacterium, Micrococcus* and *Alcaligenes* etc. prevailing in the ecosystem. At the same time the load of vibrios reduced drastically[10].

The bacilli inhibit pathogenic vibrios by competing for nutrients, and damaging the slime layers of vibrios

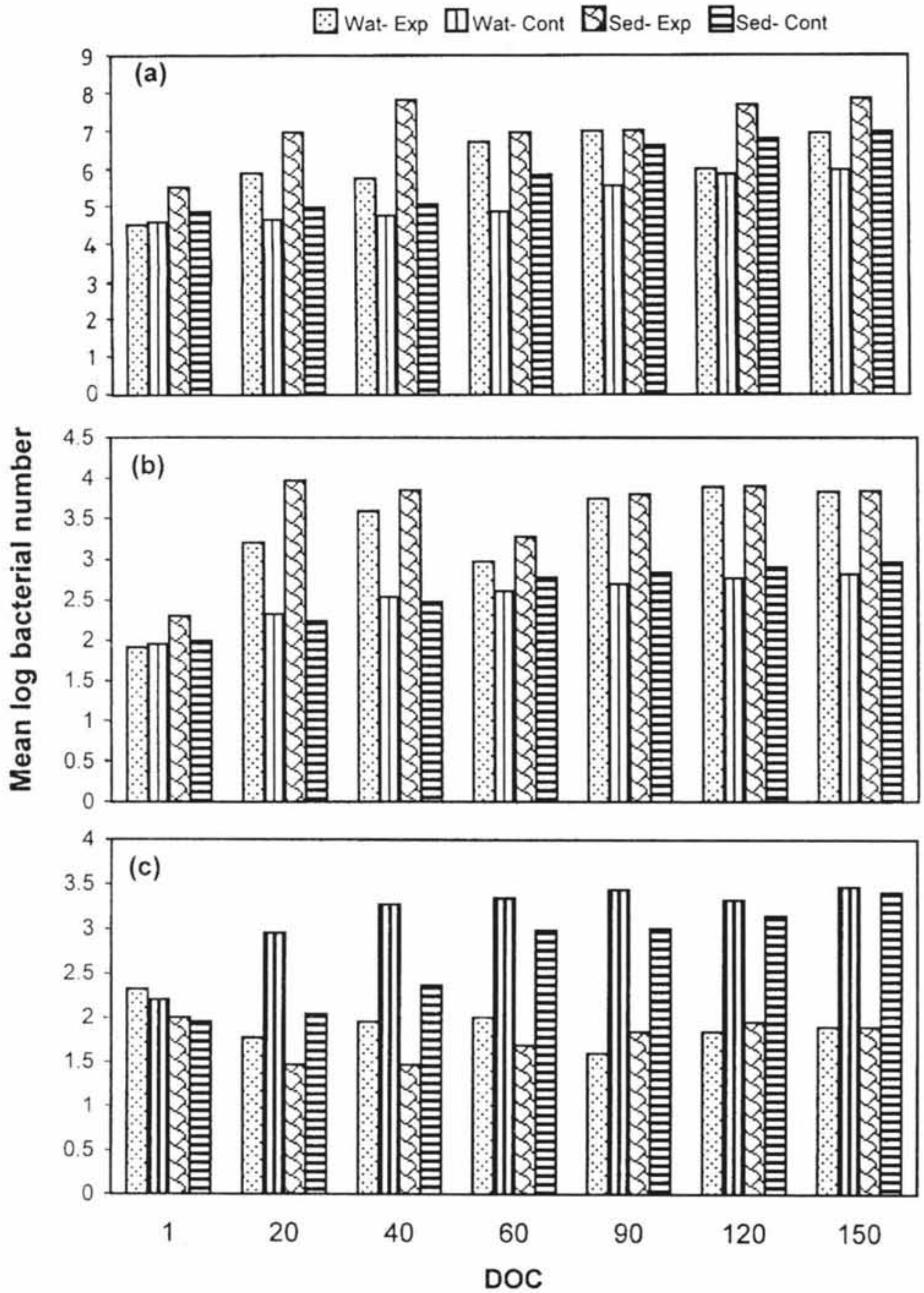

Fig. 1—Counts of THB (a), *Bacillus* species (b), vibrios and (c) in experimental (probiotics treated) and control ponds.

through secreting enzymes that degrade slimes[1]. The bacilli also secrete enzymes that could breakdown the organic flocs and excess nutrients in pond ecosystem. Heavy organic load and nutrients are possible in modified extensive shrimp culture ecosystem because of rapid abundance of digestible organic matter due to over-feeding with pellet feeds, fecal wastes, application of manure and vitamins, and consequential heavy blooms[18,19]. These deteriorate water quality and develop stress, disease and eventual mortality, which are often experienced in shrimp farming practices. In the present investigation, the probiotics applied pond had good water quality as was evident by optimum transparency and less organic matter, good health status of shrimps (without any deformities), low counts of pathogenic vibrios, proper growth and survival rates of the shrimps. The reverse was true with the control pond (Table 1).

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