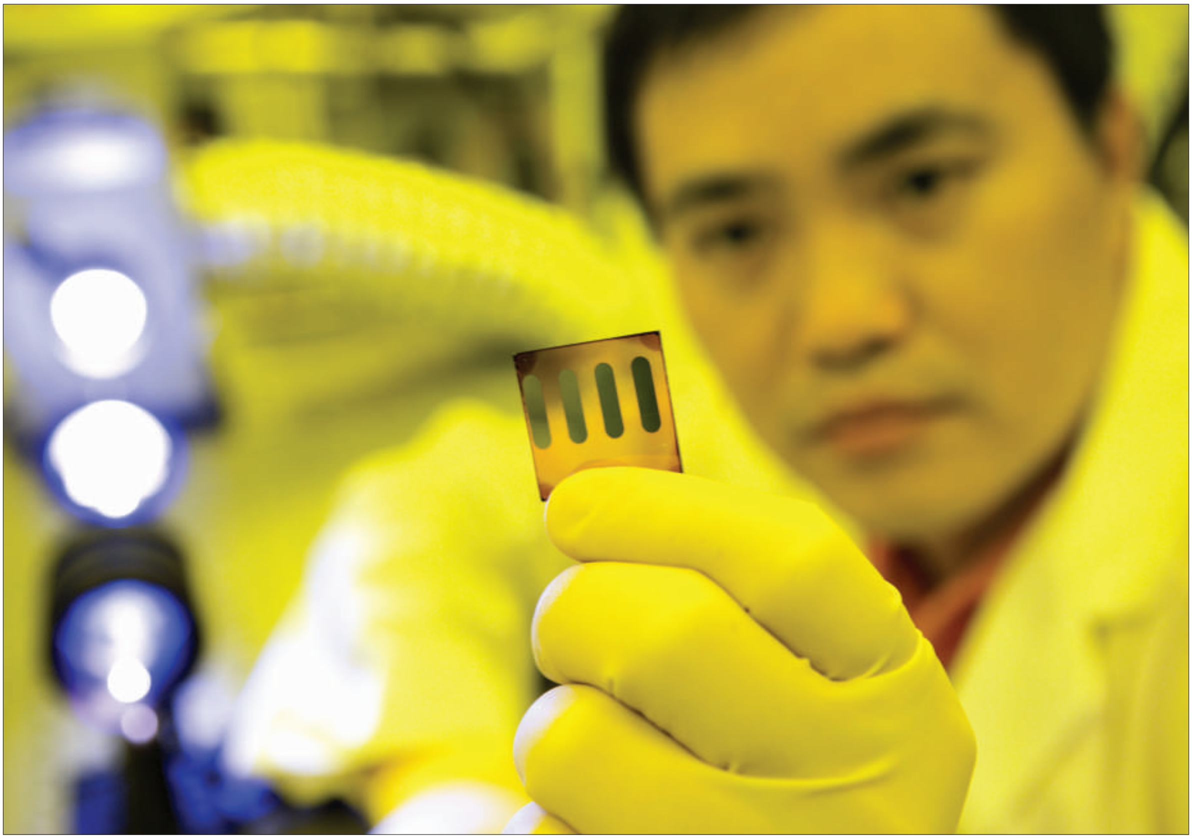

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Research Article
Role of Probiotics for Reduction of *Vibrio* Contamination Load in Tiger Shrimp (*Penaeus monodon*) Cultured Pond

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Abstract

Background and Objective: Shrimps are considered as a major source of food globally. Aquaculture shrimps are consisting of different diseases which are effecting the production of shrimp's culture. The present work was carried out for one culture operation in a semi-intensive shrimp (*Penaeus monodon*) cultured pond of Eastern India Fisheries and Agro product private L.td. **Materials and Methods:** For bacteriological analysis, the water sample of both control and the treated pond was collected in a 500 mL PVC bottle below the surface water of the pond. In similar ways the sediment and shrimp mussel was collected on 44, 67 and 103 days of culture. The pond probiotics (Uni Ecosense) and feed probiotics (Uni Max) of potency 22×10^9 CFU mL^{-1} and 3×10^9 CFU g^{-1} of uniform composition, contain 11 number of *Bacillus* sp. as beneficial bacteria were selected for application in treated pond. **Results:** The *Vibrio* load concentration is more in control pond as compared to treated pond water and sediment and nil in shrimp mussels. The *Bacillus* sp. load was more in probiotics treated pond as a comparison to control pond and it increased with corresponds to days of culture representing the increase of *Bacillus* sp. after each application of probiotics. **Conclusion:** The *Vibrio* green and colonies concentration was lower in treated pond of water, sediment and shrimp mussels and commercial probiotics encapsulated with bacterial strain *Bacillus subtilis*, *Bacillus polyxmya*, *Bacillus megaterium*, *Bacillus lichinofromis* etc., played a momentous role in for maintaining the hygienic condition of pond and act as an effective tool to reduce the microbial contamination.

Key words: Probiotics, *Vibrio*, tiger, cultured pond, *Penaeus monodon*, contamination

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Globally the infectious disease is a major problem to aquaculture. In farmed aquatic animals various approach has been suggested to mitigate the problematic effect[1]. In a different part of the world, the antimicrobial drugs including antibiotics had been approved for the treatment of bacterial diseases but in the aquaculture system, it exerted a strong selection pressure and adapted to disease resistance[1]. Due to strong uses of antibiotics and its accumulation in shrimp/prawn meat making the consignment unacceptable in the international market, therefore, it is highly essential to find an alternative to antibiotics and the concept of probiotics is one of the promising areas for research and development. The immune stimulants and its immune-modulating properties help in macrophage activation, increases lysozyme, respiratory burst, leukocyte activity and increase resistance against infection[2]. The micro-organisms play a leading role in the circulation of material, energy flow and healthy maintenance of the whole culture system[3]. The bacteria such as *Vibrio*, *Rickettsiella* and *Salmonella* are the important pathogen from bacterioplankton and it studies shows a positive relationship with shrimp diseases and act as a biological indicator to evaluate the occurrence of shrimp disease[4-6]. In coastal ecosystem, *Vibrio* contributing a major role for food and water-borne pathogen and member of this family supply all most 60% of the total bacterial population[7]. It is considered as autochthonous marine and estuarine microflora due to isolation from water, sediment, invertebrate and fishes[8].

Generally, the bacterial performing a duel role corresponds to environmental condition one is beneficial forms where it helps in the recycling of nutrients and degradation of organic matter to clearing the environment[9]. The other form represents pathogenic which cause bad water quality, stress and disease as they act as a primary and secondary pathogen[10-12]. To prevail over this disease problem, the use of antibiotics and disinfectants is a common technique in shrimp cultivation. Due to the overuse of these chemicals, antibiotic-resistant bacteria will be developed and the surrounding environment will be affected[13,14]. So, in this juncture, environmentally friendly treatment as probiotics came to the first option for suggesting as a remedial measure which is ecologically safe and cost-effective to shrimp culturist[9]. In the coastal environment, the most frequent beneficial bacteria are *Bacillus* spp., *Nitrosomonas* spp. and *Nitrobacter* spp. The *Bacillus* spp help in the degradation of organic matter which facilitates nutrient recycling and fights with pathogenic bacteria for food and substrate and secretes an enzyme which contains Gram-negative bacteria.

The aquaculture environment in general and *Vibrio* in particular, more attention is paid to *Vibrio* infection because it creates major disease problem in aquaculture system. Due to this disease problem, the health of shrimp is badly affected as result the shrimp culturist lose their crop[12,15]. Earlier various studies have been conducted regarding various role of probiotic in aquaculture by Verschuere *et al.*[16], Nayak[17], Lazado and Caipang[18,19] and Akhter *et al.*[20]. So, in this regard present study is majorly focus on importance of probiotics in relation to *vibrio* load contamination in tiger shrimp culture recognized as substitutes that can minimize the reliance of aquaculture industries over antibiotics.

MATERIALS AND METHODS

Experimental setup: The present work was carried out for one culture operation in a semi-intensive shrimp (*Penaeus monodon*) cultured pond of Eastern India Fisheries and Agroproduct private L.td. located at central Balasore of Odisha, India, on 21°24′50″North latitude to 86°57′50″ East longitude, 0.8 ha each having a stocking density of 1,20,000. This research project was conducted from July, 2007-January, 2008. Six ponds have been chosen for experiment out of the 3 ponds are control and 3 ponds are treated. Pond no. 1, 2, 3 were taken as controlled pond i.e., C_1, C_2 and C_3 where probiotics were not used and pond no. 4, 5, 6 was taken as treated pond i.e., T_1, T_2 and T_3 where probiotics were used.

Collection of water, sediment and shrimp mussel sample: For bacteriological analysis, the water sample of both control and the treated pond was collected in a 500 mL PVC bottle below the surface water of the pond on 44, 49, 61, 67, 82, 88, 103, 109 day of culture during early hours which was thoroughly mixed for 2 min for homogenous mixing and distribution of bacteria. The sediment sample was collected on 44, 67 and 103 days of culture by a vertical core from the central portion of the pond and the collected sample was aseptically shifted into zip lock polyethylene pouch using a sterilized spatula. After that with the help of a sterile spatula, a required 10 g of sediment sample was added by 100 mL of deionized water and the sediment diluents mixture was agitated in a mechanical shaker for 3 min with proper care to avoid heating. The shrimp was collected from both the control and treated pond by using cast net and then packed individually by zip-lock plastic 44, 67 and 103 days of culture. The shrimp sample was cleaned with 50% aged sterile seawater, after cleaning it soaked with a cotton-tipped applicator. The mussels were removed by alcohol-rinsed dried forceps and it homogenized mixture in alcohol-washed pestle and mortar with sterile water blank.

Analytical methods: The collected samples of water, sediment and shrimp were brought to the laboratory immediately by an airtight incubated icebox and the analysis was performed within an hour of collection to avoid possible contamination. The total heterotrophic bacteria analysis was carried out by serial dilution methodology where all the samples were serially diluted by using sterile water blank. The water blanks were prepared by 50% aged seawater which was sterilized by autoclave at 15 lbs in 121°C for 15 min. Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar medium was used for isolating for *Vibrio*. The samples were augmented in alkaline peptone water blank for 1 h and then transferred to TCBS agar medium by spread plate method by serial dilutions of 10^{-4} dilution and incubated for 24 h at 37°C. The developed colonies of both green and yellow were streaked on to brain heart infusion agar (BHAI) and further characterization by following the standard method of food and drug Administration Manual by Kaysner *et al.*[21].

Probiotics application: The probiotics product selected for culture pond belongs to Sanzyme Ltd. Both the pond probiotics (Uni Ecosense) and feed probiotics (Uni Max) were selected for the application having the potency of 22×10^9 CFU mL^{-1}, 3×10^9 CFU g^{-1} having a uniform composition of 11 number of *Bacillus* spp. as beneficial bacteria such as; *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*/Lactic acid, *Bacillus pumilus*, *Bacillus mesentricus*, *Bacillus circulans*, *Bacillus clausii* and *Bacillus altitudinus*.

RESULTS

Pond water-green colonies and yellow colonies: The green colonies concentration varied from 1.00-9.48 CFU mL^{-1} both in the controlled and treated pond. In the controlled pond, the highest green colonies concentration were observed in Days of Culture (DOC)-108 being 3×10^9 CFU mL^{-1} in pond C_1 and the lowest green colonies concentration was observed in DoC-44 1.5 $\times10^3$ CFU mL^{-1} C_1 pond and the mean standard deviation ranged between $3.3\times10^3\pm0.57$ to $3.7\times10^7\pm2.03$ CFU mL^{-1}. Likewise in treated pond, the highest green colonies concentration was observed in DoC-103 being 3.1×10^6 CFU mL^{-1} in pond T_3 and the lowest green colonies concentration was observed in DoC-49 being 1.1×10^1 CFU mL^{-1} in pond T_1 and T_3 and the mean standard deviation ranged between $1.2\times10^1\pm0.68$ to $3\times10^6\pm1.24$ CFU mL^{-1} which are represented Fig. 1(a). Similarly, the yellow colony's concentration varied from 2.2×10^2-9×10^4 CFU mL both in the controlled and treated pond. In the controlled pond, the highest yellow colony's concentration was observed in DoC-61 being 9×10^7 CFU mL^{-1} in pond C_1 and the lowest yellow colonies concentration was observed in DoC-49 being 5×10^3 CFU mL^{-1} in C_2 pond and the mean standard deviation ranged between $3\times10^4\pm0.60$ to $5\times10^6\pm2.82$ CFU mL^{-1}. Likewise in treated pond, the highest yellow colonies concentration was observed in DoC-103 being 2.5×10^7 CFU mL^{-1} in pond T_1 and the lowest yellow colonies concentration was observed in DoC-49 being 2.2×10^2 CFU mL^{-1} in pond T_1 after application of probiotics and the mean standard deviation ranged between $3\times10^3\pm1.21$ to $3\times10^6\pm0.98$ CFU mL^{-1} which are represented Fig. 1a.

Pond sediment-green colonies and yellow colonies: The green colony's concentration varied from 1.2×10^2 to 2.1×10^6 CFU mL^{-1} both in the controlled and treated pond. In the controlled pond, the highest green colonies concentration was observed in DoC-103 being 2.1×10^6 CFU mL^{-1} in pond C_2 and the lowest green colonies concentration was observed in DoC-67 being 3.6×10^3 CFU mL^{-1} in C_1 pond and the mean standard deviation ranged between $2\times10^4\pm0.61$ to $3\times10^5\pm1.06$ CFU mL^{-1}. Likewise in treated pond, the highest green colonies concentration was observed in DoC-67 being 2.5×10^5 CFU mL^{-1} in pond T_1 and the lowest green colonies concentration was observed in DoC-44 being 1.2×10^2 CFU mL^{-1} in pond T_3 and the mean standard deviation ranged between $1.9\times10^3\pm1.31$ to $2\times10^5\pm0.69$ CFU mL^{-1}. Which are depleted in Fig. 1b.

The yellow colony's concentration varied from 2.8×10^4 to 5.2×10^7 CFU mL^{-1} both in controlled and treated ponds. In the controlled pond, the highest yellow colonies concentration was observed in DoC-67 being 5.2×10^7 CFU mL^{-1} in pond C_3 and the lowest yellow colonies concentration was observed in DoC-103 being 3.9×10^9 CFU mL^{-1} in C_1 pond and the mean standard deviation ranged between $5.3\times10^4\pm0.45$ to $6.3\times10^6\pm0.96$ CFU mL^{-1}. Likewise in treated pond, the highest yellow colonies concentration was observed in DoC-67 being 6.2×10^4 CFU mL^{-1} in pond

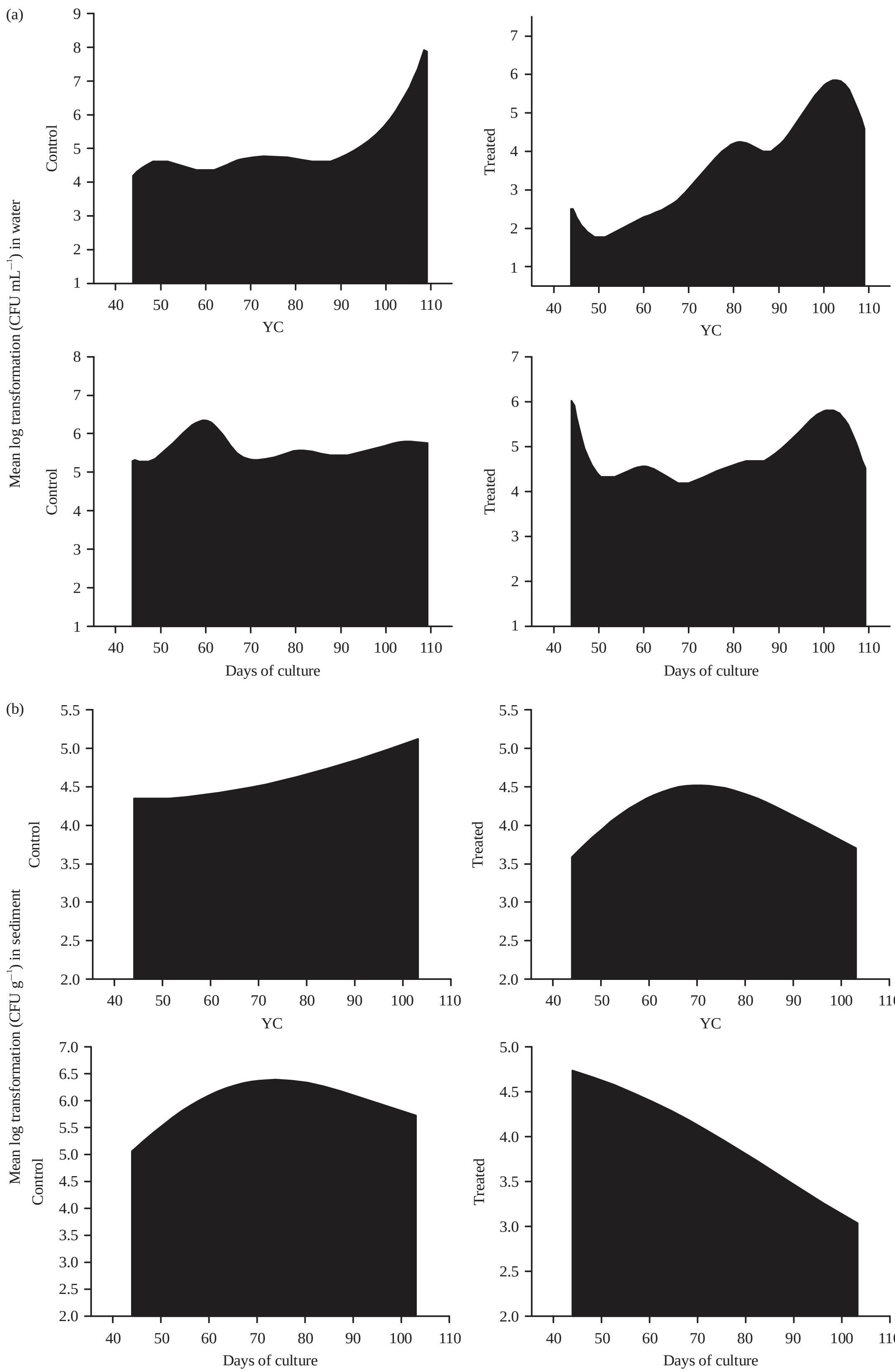

Fig. 1(a-c): Continued

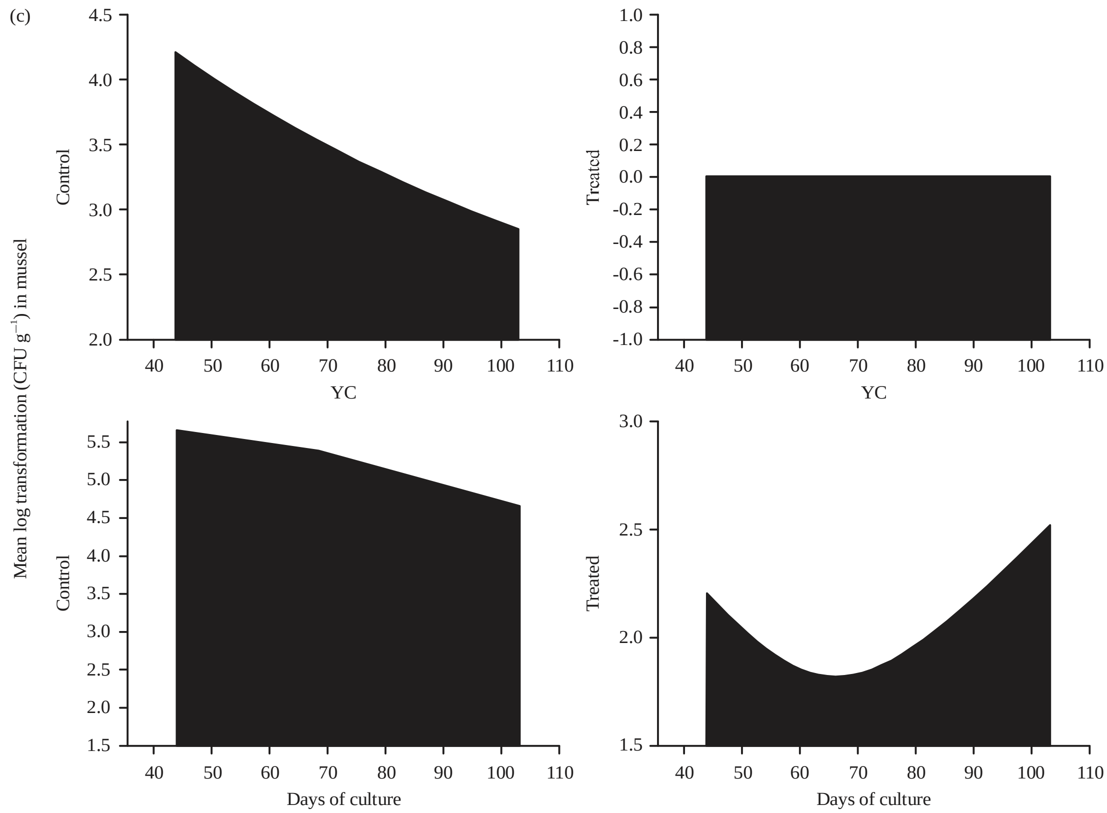

Fig. 1(a-c): *Vibrios* counts in control and treated pond, mean log transformation (CFU mL^{-1}) in (a) Water, (b) Sediment and (c) Mussel

GC: Green colonies, YC: Yellow colonies

T_1 and lowest yellow colonies concentration was observed in DoC-67 being 2.8×10^4 CFU mL^{-1} at pond T_3 after application of probiotics and the mean standard deviation range between $6.4\times10^4\pm0.20$ to $4.3\times10^4\pm1.68$ CFU mL^{-1} which are represented in Fig. 1b.

Shrimp muscle-green and yellow colonies: The green colonies concentration varied from zero to 1.2×10^7 CFU mL^{-1} both in controlled and treated ponds. In the controlled pond, the highest green colonies concentration was observed in DoC-44 being 1.2×10^7 CFU mL^{-1} in pond C_1 and the lowest green colonies concentration was observed in DoC-103 being 1.3×10^2 CFU mL^{-1} in C_2 pond and the mean standard deviation range between $1.5\times10^2\pm1.24$ to $1.6\times10^4\pm1.95$ CFU mL^{-1}. Likewise in the treated pond, the green colonies concentration was found to be zero in all DoC and all the pond which are represented in Fig. 1c.

The yellow colony's concentration varied from 1.5×10^1 to 2.8×10^8 CFU mL^{-1} both in the controlled and treated pond. In the controlled pond, the highest yellow colonies concentration was observed in DoC-44 being 2.8×10^8 CFU mL^{-1} in pond C_1 and the lowest yellow colonies concentration was observed in DoC-44 being 2×10^2 CFU mL^{-1} in C_2 pond and the mean standard deviation ranged between $2.2\times10^4\pm0.48$ to $3.2\times10^5\pm3.06$ CFU mL^{-1}. Likewise in treated pond, the highest yellow colonies concentration was found in DoC 103 being 1.6×10^3 CFU mL^{-1} and the lowest yellow colonies concentration was found in DoC 67 being 1.5×10^1 CFU mL^{-1} in T_1 pond after application of probiotics and the mean standard deviation ranged between $2.1\times10^1\pm0.66$ to $1.1\times10^2\pm0.62$ CFU mL^{-1} which are represented in Fig.1c.

Result depitted in Fig. 2(a-c) *Bacillus* spp load was more in probiotics treated pond as a comparison to control pond and it increased with corresponds to days of culture representing the increase of *Bacillus* spp. after each application of probiotics. In contrast, *Vibriosis* (Fig. 1a-c) indicating a decline condition towards days of culture due to the application of probiotic and reduce *Vibrio* load in the treated pond. However, the THB load demonstrated an increasing trend towards days of culture in the treated and

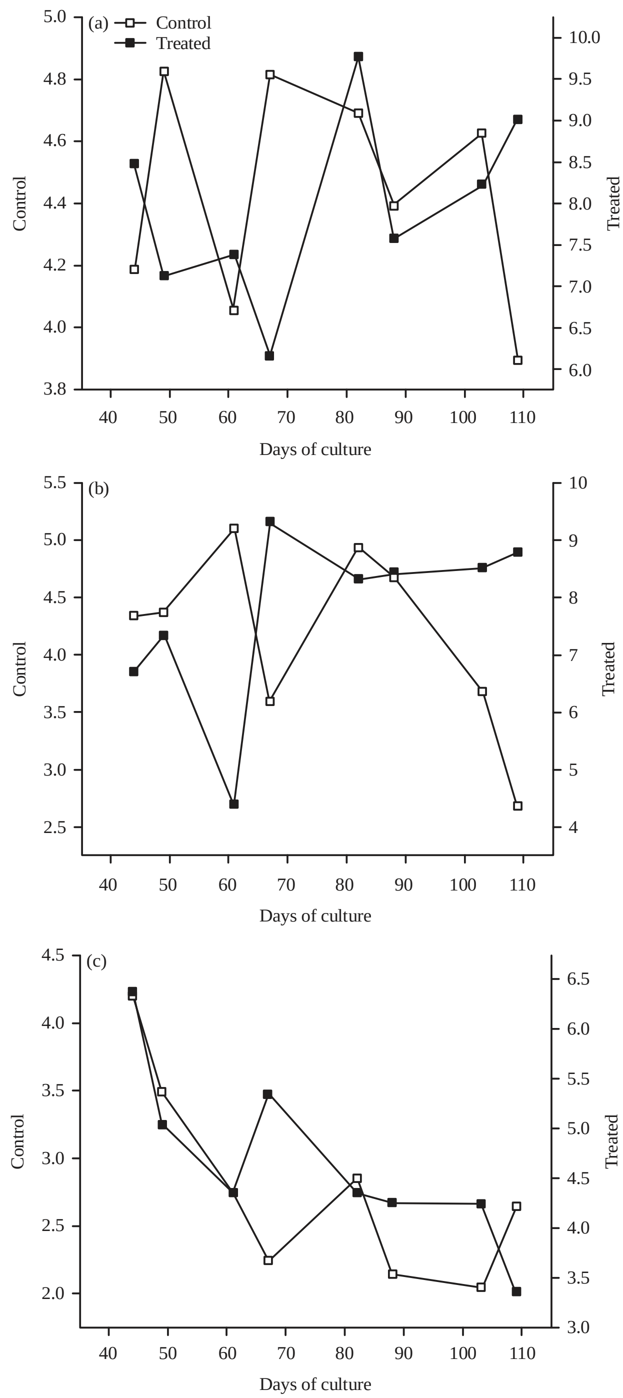

Fig. 2(a-c): *Bacillus* species in control and treated pond, *Bacillus* in (a) Water, (b) Sediment and (c) Mussel

control ponds. Though, a decline of *Vibrio* load was observed due to the application of probiotics in the treated pond, it did not pressure much on the difference of THB population between control and treated pond. It might have been a result of the compensation of more *Bacillus* species in the treated pond.

DISCUSSION

In general *Bacillus* spp. add approximately 20% off THB and vibrios supply around 40% off THB in a coastal environment[23,24]. It specifies that the increasing trend of THB clearly refers to the increase in the vibrios which guide to the bad water quality of pond and increase the possibility of a disease outbreak. Inoculation of bacterial composition will lead to control of the pathogenic bacteria like vibrion under control. Various commercial of viable inoculated bacteria are being used to control water quality in shrimp culture pond. Among this importance is given to *Bacillus* spp. because of high enzymatic activity *Bacillus* species is able to degrade organic sludge in shrimp culture pond[16,25]. As a result of the treated pond having low bacterial colonies of both green and yellow in water, sediment and mussels in comparison to the control pond as cited in Fig. 2(a-c). The number of 22×10^9 CFU mL^{-1} and 3×10^9 CFU g of *Bacillus* species in probiotics becomes 3×10^9 CFU mL^{-1} after application of pond water environment due to the effect of autochthonous flora[22]. In addition to this, the *Bacillus* strain have ability to recover water quality, trigger up immune response and condense epidemic of the pathogen in shrimp culture environment[16,26,27]. In a modified extensive shrimp culture pond there is chance of occurrence of digestible organic matter which may due to overfeeding with pellet feeds, fecal wastes and bloom crash in middle of the culture[10,28]. Due to these stressed environment sometimes disease and eventual mortality noticed in shrimp culture activity. In the present study, the pond treated with probiotics possesses good water quality and low concentration of vibrion as in comparison to the control pond.

CONCLUSION

It can be concluded that the commercial probiotics encapsulated with bacterial strain *Bacillus subtilis*, *Bacillus polyxmya*, *Bacillus megaterium*, *Bacillus lichinofromis* etc., played a momentous role in treated pond for maintaining the hygienic condition of pond and as an effective tool to reduce the microbial contamination.

SIGNIFICANCE STATEMENT

The study discovers significant role of probiotics in reduction of microbial contamination in tiger shrimp (*Penaeus monodon*) culture pond against antibiotics application in shrimp culture. The commercial probiotics like composition *Bacillus* species composition plays a critical role for reduction of microbial load in culture pond. Thus, this concept of probiotics application can help to shrimp culturist to maintain their shrimp culture in sustainable manner for getting more profit and successes.

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