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Effect of Probiotics on *Vibrio* Bacterial Populations in Culture Ponds of *Litopenaeus vannamei* at Vadacheepurupalli, Andhra Pradesh, India

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Abstract : The current study was carried out for 147 days to assess the *Vibrio* count of *Litopenaeus vannamei* culture ponds from Vadacheepurupalli, Visakhapatnam District, Andhra Pradesh, India. Three ponds were selected, one is control and other two were experimental (Pond A and B). The physico-chemical parameters of the culture ponds were recorded by adopting standard methods. During summer crop the control ponds were harvested at 24.0 gm on 107th day and at 31.0 gm on 121st day for the year 2012 and 2013 respectively due to the incidence of *Vibriosis* disease. Where as in winter crop during 2012 the control pond was harvested at 5.5 gm on 47th day because of *Vibriosis* but in 2013 the control pond was harvested normally at 32.5 gm on 145th day, as this pond is free of *Vibriosis.* Experimental ponds were harvested normally in summer season at 30.5 gm on 124th day & 31.0 gm on 127th day during 2012 and 34.0 gm on 127th day & 35.5 gm on 127th day in the year 2013 respectively. Where as in winter season of 2012 the experimental ponds were harvested normally at 28.0 gm on 124th day as well as on 127th day. In the year 2013 winter, the experimental ponds were harvested at 36.0 gm at 146th day and 35.5 gm at 147th day respectively. This study suggests that the probiotics are the key agents which have a great impact on the reduction of total *Vibrio* count in culture ponds of L*. vannamei*.

Key words: *Litopenaeus vannamei*, *Vibrio*, sediment and temperature.

Introduction

The shrimp *Litopenaeus vannamei* has been introduced from past couple of years in Indian aquaculture systems in the interest of commercial production due to major failure of *Penaeus monodon* (black tiger). The shrimp exports play an important role in the economy of India and also create opportunity to reduce the unemployment problems. The commercial production of *L. vannamei* in culture ponds provides a good source of protein in India as well as in other developing countries because of its unique taste and a great demand in International markets.

The usage of antibiotics and toxic chemicals in shrimp culture are almost banned, it is high time to look after the alternatives. The abuse of antimicrobial drugs, pesticides, and disinfectants in aquaculture has caused the evolution of resistant strains of bacteria and concern of the society (Esiobu *et al.,* 2002; Boyd and Massaaut, 1999). Thus, the use of probiotics in the culture of aquatic organisms is increasing with the demand for more environment-friendly aquaculture practices (Gatesoupe, 1999). The probiotics are live microorganisms, which when consumed in adequate amounts; confer a health benefit for the host (FAO/WHO, 2001).

Improved water quality and healthy management of aquaculture pond ecosystems has been associated with the application of probiotics (Srinivas and Manjulatha, 2005; Srinivas *et al*., 2014). It has been reported that use of *Bacillus* sp. improved water quality, survival and growth rates and the health status of juvenile *P. monodon* and reduced the pathogenic *Vibrios* (Dalmin *et al.,* 2001). Recent research also shows that the use of commercial probiotics in *L. vannamei* ponds can reduce concentrations of nitrogen and phosphorus and increase the shrimp yields (Wang *et al.*, 2005). Since the studies on the application of probiotics from Indian aquaculture ponds were very scanty, the present experimentation aimed to establish information on *Vibrio* bacterial reduction before and after application of probiotics in culture ponds with special reference to *L. vannamei.*

Materials and methods

The investigation of the current study was carried out in Vadacheepurupalli farm which is located in Visakhapatnam District, Andhra Pradesh, India. For estimation of Dissolved oxygen (DO) the standard Winkler's method (Grasshoff *et al.,* 1999) was used. Salinity was determined using an Atago Salinometer (Model TSK, accuracy ± 0.001) and the pH of the water was determined by using an ELICO LI 610 pH meter (accuracy ± 0.01). Temperature of the water was recorded by using thermometer. For the enumeration of bacterial growth TCBS agar was used for *Vibrio* species (M870S, Hi-media). The resulted strains were analyzed by following the Bergey's manual of bacteriology (David *et al.,* 1974).

Statistical Interpretation: Statistical interpretation of the data was performed by using Microcal origin pro 8.0 version computer based programme.

Results

At Vadacheepurupalli during the culture period in the summer crop of year 2012, the parameters of salinity, pH, and temperature in the study ponds ranged between 25-43 , 7.8-8.9, and 30-34ºC respectively. Total *Vibrio* count in the water and sediment of the control pond at 90 days of culture was $30.0\times10^2 \pm 0.47$ cfu ml^{-1} and $0.49\times10^2 \pm 0.24$ cfu mg^{-1} respectively. This control pond harvested at 24.0 g on 107th day with the effect of *Vibriosis*. The total *Vibrio* counts in the experimental pond A & pond B were recorded as $29.0\times10^2 \pm 0.23$ cfu ml^{-1} and $2.63\times10^2 \pm 0.32$ cfu ml^{-1} in the pond water and $25.7\times10^2 \pm 0.29$ cfu mg^{-1} and $3.12\times10^2 \pm 0.25$ cfu mg^{-1} in the sediment at 120 days of culture respectively. The experimental ponds A & B were harvested normally at 30.5 g & 31.0 g on 124th day respectively. (Figure- 1).

Similarly during the winter crop of 2012 the physico-chemical parameters such as salinity, pH, and temperature of the study ponds were ranged between 21-25 ‰, 7.5-8.8, and 25-30ºC respectively. The total *Vibrio* count (TVC) recorded in the control pond is $25.5\times10^2 \pm 0.21$ cfu ml^{-1} and $0.16\times10^2 \pm 0.17$ cfu mg^{-1} respectively in water and sediment of the pond which values are recorded at 30 days of culture. Subsequently this pond is harvested due to the incidence of the *Vibriosis* disease at 5.5 g on 47th day. When considering the total *Vibrio* counts in the experimental ponds, the observed readings at 120 days of culture are recorded as $1.81\times10^2 \pm 0.86$ cfu ml^{-1} and $1.23\times10^2 \pm 0.14$ cfu ml^{-1} in the pond water and $2.23\times10^2 \pm 0.42$ cfu mg^{-1} & $2.07\times10^2 \pm 0.35$ cfu mg^{-1} in the pond sediment respectively in experimental pond A & pond B. These two ponds A & B were harvested normally at 28.0 g on 124th day & 127th day respectively. (Figure-2)

In the same way the parameters like salinity, pH, and temperature of the study ponds ranged between 25-37 , 7.8-8.8, and 28-33ºC respectively in the summer crop of the study year 2013. The readings of total *Vibrio* count (TVC) were $17.2\times10^2 \pm 0.10$ cfu ml^{-1}, $2.25\times10^2 \pm 0.16$ cfu ml^{-1} and $0.41\times10^2 \pm 0.49$ cfu ml^{-1} in the water and $23.5\times10^2 \pm 0.45$ cfu mg^{-1}, $3.97\times10^2 \pm 0.42$ cfu mg^{-1} and $0.64\times10^2 \pm 0.60$ cfu mg^{-1} in the sediment of the ponds in control pond, experimental pond A & pond B respectively. In this study area all the three ponds i.e. control pond, experimental pond A & pond B were harvested normally. The control pond is harvested at 31.0 g on 121st day, and the experimental pond A & pond B were harvested at 34.0 g & 35.5 g on 127th day respectively. (Figure- 3)

At the same time the salinity, pH, and temperature of the study ponds ranged between 18-21 ‰, 7.9-8.9, and 25-29ºC respectively in the winter crop of year 2013. The total *vibrio* count in the pond water was recorded as $2.97\times10^2 \pm 0.38$ cfu ml^{-1}, $0.35\times10^2 \pm 0.73$ cfu ml^{-1} and $0.20\times10^2 \pm$ cfu ml^{-1} in control, experimental pond A & pond B respectively. Whereas in the sediment the readings were noticed as $28.7\times10^2 \pm 0.54$ cfu mg^{-1}, $0.81\times10^2 \pm 0.92$ cfu mg^{-1} and $0.22\times10^2 \pm 0.31$cfu mg^{-1}respectively in control, experimental pond A & pond B at 120 days of culture. All the three ponds are harvested normally at 32.5 g, 36.0 g & 36.5 g on 145th day, 146th day and 147th day respectively. (Figure-4)

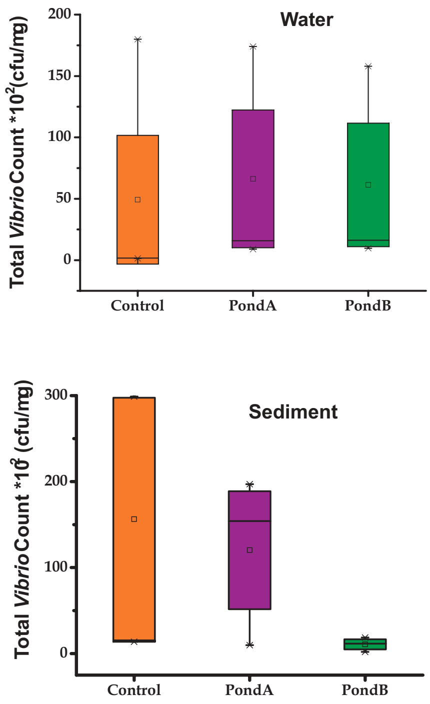

Fig. 1. Total *Vibrio* count of culture ponds at Vadacheepurupalli during summer 2012

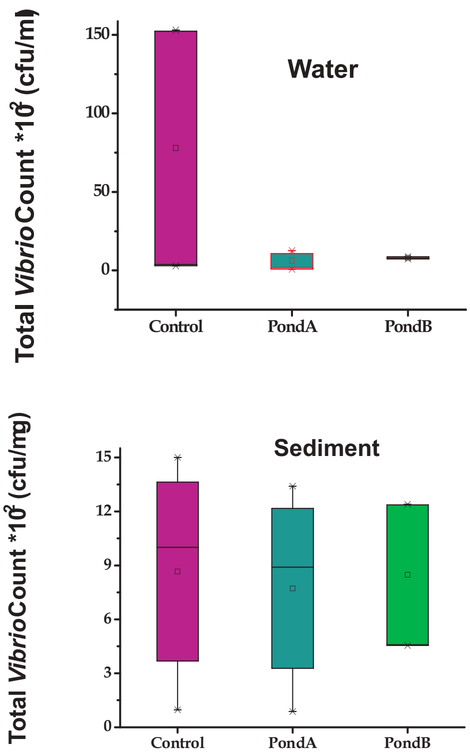

Fig.2. Total *Vibrio* count of culture ponds at Vadacheepurupalli during winter 2012

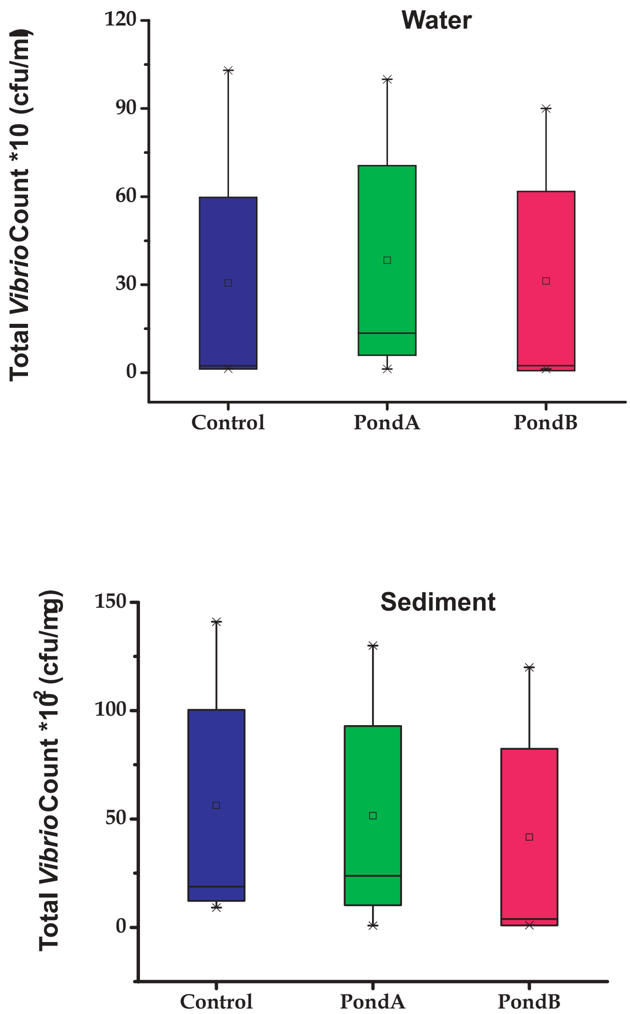

Fig. 3. Total *Vibrio* count of culture ponds at Vadacheepurupalli during summer 2013

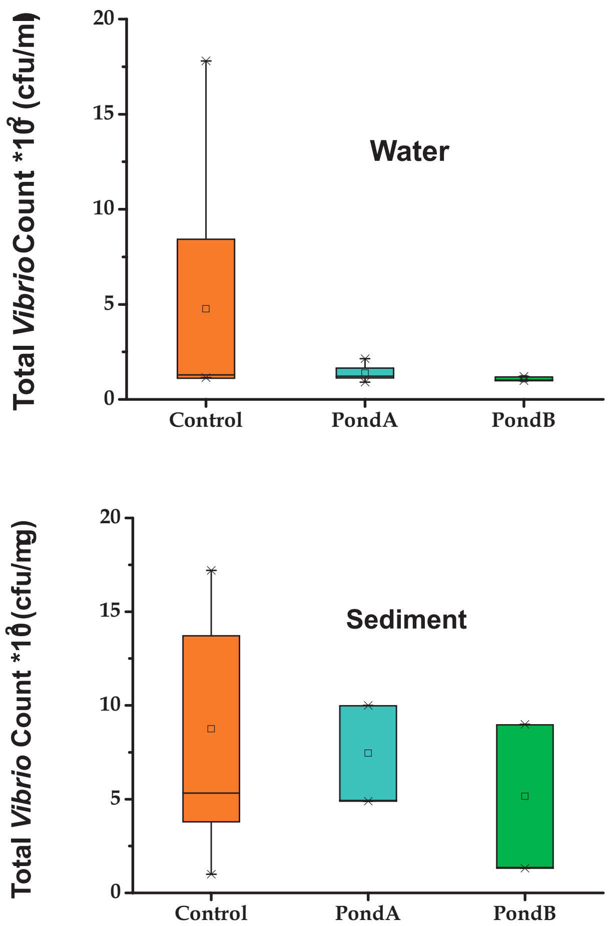

Fig.4 : Total *Vibrio* count of culture ponds at Vadacheepurupalli during winter 2013

Discussion

Information related to probiotics usage on the reduction of total *Vibrio* count in penaeid species culture ponds, with special reference to *Litopenaeus vannamei* is not adequate and thus this study was undertaken to observe the efficiency of probiotics in the culture ponds on the total *Vibrio* count of L. *vannamei* from the culture ponds of Andhra Pradesh, India. The physcio-chemical parameters of these ponds were carefully monitored during the study period because these factors will determine the fate of the cultured species.

The maintenance of good water quality is essential for optimum growth and survival of shrimps. The optimum levels of physical, chemical and biological parameters control the quality of pond waters as well as pond sediment. The level of metabolites in pond water can have an adverse effect on the growth. Good water quality is characterized by adequate oxygen and limited level of metabolites. Excess feed, faecal matter and metabolites will exert tremendous influence on the incidence of bad water quality of the shrimp ponds. Hence critical water quality parameters are to be monitored carefully as adverse conditions may be disastrous to the growing shrimps (Ramanathan *et al*., 2005; Soundarapandian and Gunalan, 2008).

The salinity of the present study was maintained 25-43 ‰, 21-25 ‰, 25-37 ‰, 18-21 ‰, during summer and winter seasons for two consecutive years in all ponds. Muthu (1980), Soundarapandian and Gunalan (2008) and Karthikeyan (1994) recommended a salinity range of 10-35 ppt, ideal for *P. monodon* culture. According to Soundarapandian *et al*., (2010) salinity is an atmost important parameter which determines the growth and health status of the shrimp. The optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production Soundarapandian *et al*., (2010) In the present study pH was maintained between 7.8-8.9 ‰, 7.5-8.8 ‰, 7.8-8.8 ‰, 7.9-8.9 ‰ during summer and winter for the two consecutive years respectively.

Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. Low-level of oxygen hampers metabolic performances in shrimp and can reduce growth and moulting as well cause mortality (Gilles, 2001). The dissolved oxygen in all the culture ponds in the present study was maintained between 3.2 to 4.2 ppm.

Water temperature is probably the most important environmental variables in shrimp cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival. In general, a sudden change of temperature effects the shrimp immune system. The optimum range of temperature for the black tiger shrimp is between 28 to 30ºC (Ramanathan *et al*., 2005). In the current study, temperatures of 30-34ºC, 28-33ºC, 25-30ºC, 25-29ºC, were maintained during summer and winter for the years 2012 and 2013 respectively.

It has been reported that use of *Bacillus* sp. improved water quality, survival and growth rates and increased the health status of juvenile *P. monodon* and reduced the pathogenic *Vibrios* (Dalmin *et al.,* 2001). Balca´zar (2003) demonstrated that the administration of a mixture of bacterial strains (*Bacillus* and *Vibrios sp*.) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens *Vibrio harvei* and white spot syndrome virus.

Bacterial numbers were highly correlated with organic carbon and total nitrogen in the sediment, suggesting that these were limiting factors to bacterial growth. (Burford *et al.,* 1998). Srinivas *et al.,* (2013) reported that there was reduction of the proliferation of the bacteria in all the ponds where the application of probiotics is followed and also the toxic gases of the pond bottom were reduced and oxidation of the organic matter is observed.

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