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European Journal of Experimental Biology, 2015, 5(11):31-36

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Effect of probiotic bacteria *Bacillus licheniformis* and *Lactobacillus rhamnosus* on growth of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931)

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ABSTRACT

The effects of single and combined use of two different probiotic bacterial species, Bacillus licheniformis and Lactobacillus rhamnosus on growth performance of Pacific white shrimp Litopenaeus vannamei was evaluated. Shrimp were treated with probiotics in entire crop duration with different test diets. Twelve culture ponds were divided into four groups of each three, Control (CP) without probiotic treatment and experimental (PB1: Bacillus licheniformis; PB2: Lactobacillus rhamnosus; PB3: Bacillus licheniformis & Lactobacillus rhamnosus) probiotic treated groups. Test diets were prepared with Bacillus licheniformis for PB1 @ ~10 billion CFU/kg, PB2 prepared with Lactobacillus rhamnosus @~ 8 billion CFU/ kg feed and PB3 was supplemented with both bacterial species in similar concentrations. Shrimp growth parameters, Net Weight Gain (NWG), Specific Growth Rate (SGR), Average Body Weight (ABW), and Average Daily Weight Gain (ADWG) were recorded at different intervals (30, 60, 90 and 120 days) of culture duration in both control and probiotic treated shrimp. Probiotic fed shrimp showed significantly higher (P< 0.01) growth than the control (CP) group. Among the probiotic treated experimental groups, PB3 showed maximum percent increase in all the growth parameters was observed. The results of this study indicated that the multiple probiotic strains have a greater influence to improve the growth of shrimp.

Key words: Probiotics, Growth, *Bacillus sps*, *Lactobacillus sps*, *Litopenaeus vannamei*

INTRODUCTION

In recent years aquaculture is regarded as one of the fastest growing and expanding industries in the world and contributes significantly to the world economy (Das *et al*., 2008). In Indian subcontinent three fourths of aquaculture development occurred in the east coast along the Bay of Bengal. In India while inland waters are largely utilized to support agriculture, aquaculture is primarily brackish water based and a great majority of aqua farms are located at the tail end of rivers and streams. The importance and role of shrimp farming was realized in the early seventies in India. The culture of penaeids has become intensified since 1986 with the cultivation of *Penaeus monodon.* During the last few years, white spot disease has spread worldwide and caused large scale mortalities and severe damage to shrimp culture, particularly in Asia leading to massive economic losses (Lightner, 1996; Flegel, 1997). Due to this disease problem the beginning of this century there has been a marked shift from the farming of indigenous black tiger shrimp, *Penaeus monodon* to the culture of exotic shrimp *Litopenaeus vannamei* in most of the South East Asian countries. Antibiotics have been selected as traditional disease control strategy for decades in aquatic animals. However, long term use of the antibiotics leads to many negative impacts such as drug residues and drug resistance (Jiang *et al*., 2013a; Pandiyan *et al.*, 2013). However, several farmers, of late, are using probiotics to improve quality by balancing bacterial population and reducing pathogenic bacteria load. The use of probiotics in culture of

aquatic organisms is increasing rapidly with the advent of environment friendly aquaculture practices (Gatesoupe, 1999). The present study aims to evaluate the performance of two different probiotic species of *Bacillus licheniformis* and *Lactobacillus rhamnosus* both individual and combined effect on growth of shrimp *Litopenaeus vannamei*.

MATERIALS AND METHODS

The present work was carried out in shrimp culture ponds of Kudithipalem coastal Village (14°.2′E; 80°.5′N) of Nellore District, Andhra Pradesh, India during the summer crop. Modified extensive shrimp culture ponds (~1 ha) were adopted for this work. Culture ponds adopted for this study were uniformly prepared, following usual pre-stocking management methods. The ponds were filled with filtered, chlorinated (20 ppm) and de-chlorinated sea water up to 1.2 m depth. This was followed by manuring and fertilization and water quality variables were maintained at optimum levels. After one week of preparation and maintenance all culture ponds were simultaneously stocked @ 35 / m^2 with *Litopenaeus vannamei* post larvae (PL14) obtained from Bluepark Shrimp Hatchery, Ponnapudi kothur Village near Nellore of Andhra Pradesh, India after PCR screening for White Spot Syndrome Virus (WSSV). Twelve shrimp culture ponds were divided into four groups of each three, control (CP) and probiotic treated (PB1: *Bacillus licheniformis* ; PB2: *Lactobacillus rhamnosus*; PB3:*Bacillus licheniformis & Lactobacillus rhamnosus*) culture ponds. Probiotic bacterial species *Bacillus licheniformis* (MTCC: 1520) and *Lactobacillus rhamnosus* (MTCC: 1408) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial technology (IMTECH), Chandigarh, India.

Probiotic bacterial feed preparations: Probiotic supplemented feed prepared as followed by the method according to Aditya Kumar *et* al., (2014). The 24hrs old bacterial culture was maintained in the nutrient broth, the bacterial species were harvested by centrifuge at 10,000 rpm for 10 min. these harvested bacteria were washed thrice with phosphate buffer saline (PBS, pH 7.4) and the bacterial cells were re-suspended in PBS. This re- suspended bacteria was mixed uniformly to the feed pellets by using sprayer. The prepared probiotic blended feed was then dried at 40^{o}C and packed in air tight polythene bags stored in 4^{o}C. Test diets PB1 supplemented with *Bacillus licheniformis* @ ~10 billion CFU/kg and PB2 prepared with *Lactobacillus rhamnosus* @~ 8 billion CFU/ kg feed, test diets were prepared once in every 15 days.

Feeding of shrimp: After stocking *L. vannamei* post larvae (PL) were fed with CP shrimp feed (CP Aquaculture India Ltd., Chennai, India). Feeding for the first 30 days is dependent on survival in hapas installed and maintained in the culture ponds and regular observation of feed consumption and movement of shrimp in culture ponds. Generally 1-1.5 kg feed is applied on day one to a pond with stocking density of one lakh and increased @ 400-500 g/d for the same density till 30 days. Feed quantity from then on would be calculated depending upon the survival rate and average body weight (ABW). After 30 day period feed consumption is regularly monitored through check tray observation and depending on this the feeding rate can be adjusted at regular intervals. The body weight of shrimp is measured every 7-10 days by random sampling.

Growth indices: Growth parameters were recorded on 30, 60, 90 and 120 days of culture. *L. vannamei* were randomly collected from grow-out ponds in the forenoon (10.00 AM) using cast net. However sampling was avoided during moulting period and during cloudy and rainy conditions for reducing experimental errors. The variations in net weight gain were calculated by the method of Sambhu and Jayaprakas (2001), Specific growth rate was determined by the method of Ravi *et al.*, (1998), Average body weight and average daily weight gain of shrimp was calculated as described by Mustafa and Ridzwan (2000).

Statistical analysis:
Data were statistically analyzed and comparison among different treatments was done by one way analysis of variance (ANOVA) to find out any significant differences among the experimental groups and the comparison between treatments was done using Duncan's Multiple Range Test (DMRT) at $P<0.05$ (Snedecor and Cochran, 1968) (SPSS; 14.0 version).

RESULTS AND DISCUSSION

The growth performance of shrimp *Litopenaeus vannamei* Net Weight Gain (NWG), Specific Growth Rate (SGR), Average Body Weight (ABW), Average Daily Weight Gain (ADWG) were recorded at different intervals (30, 60, 90 and 120 days) of culture duration in both control and probiotic treated (PB1: *Bacillus licheniformis*; PB2: *Lactobacillus rhamnosus* ; PB3: *Bacillus licheniformis* & *Lactobacillus rhamnosus*) culture ponds during the summer crop. The corresponding percent changes were showed in figures 1 to 4. It is evident from the results that the Net Weight Gain (NWG), Specific Growth Rate (SGR), Average Body Weight (ABW) and Average Daily

Weight Gain (ADWG) were significantly (p<0.05; DMRT) (Table – 1) higher in the probiotic treated (PB1, PB2 and PB3) *L. vannamei* than the controls at different intervals (30, 60, 90, 120) of culture duration. Although the above mentioned growth parameters were increased significantly (Two-way ANOVA); p<0.01 (Table-2) with increase in culture duration in both control (without probiotic) and probiotic treated *L. vannamei*. The magnitude of increase was more pronounced in probiotic (PB1, PB2 and PB3) treated groups than in control group. The maximum percent increase in all the growth parameters were observed in synergistic effect of both *Bacillus licheniformis* & *Lactobacillus rhamnosus*) (PB3 group) treated shrimp followed by PB2 and PB1 groups (Fig: 1 to 4).

Table: 1 Group-wise Mean and Standard Error (Mean ±SE) of growth indices of shrimp

Crop	Group	Specific Growth Rate (SGR)	Average Body Weight (ABW)	Net Weight Gain (NWG)	Average Daily Weight Gain (ADWG)
Summer Crop	CP	4.182±0.07 ^{c}	11.977±0.041^{a}	11.974±0.032^{a}	0.141±0.001^{d}
	PB1	4.217±0.02 ^{b}	12.683±0.041 ^{b}	12.736±0.032 ^{b}	0.150±0.001 ^{b}
	PB2	4.269±0.07 ^{c}	13.250±0.041 ^{c}	13.291±0.032 ^{c}	0.158±0.001 ^{c}
	PB2	4.323±0.07 ^{d}	13.763±0.041 ^{d}	13.753±0.032 ^{d}	0.165±0.001 ^{d}

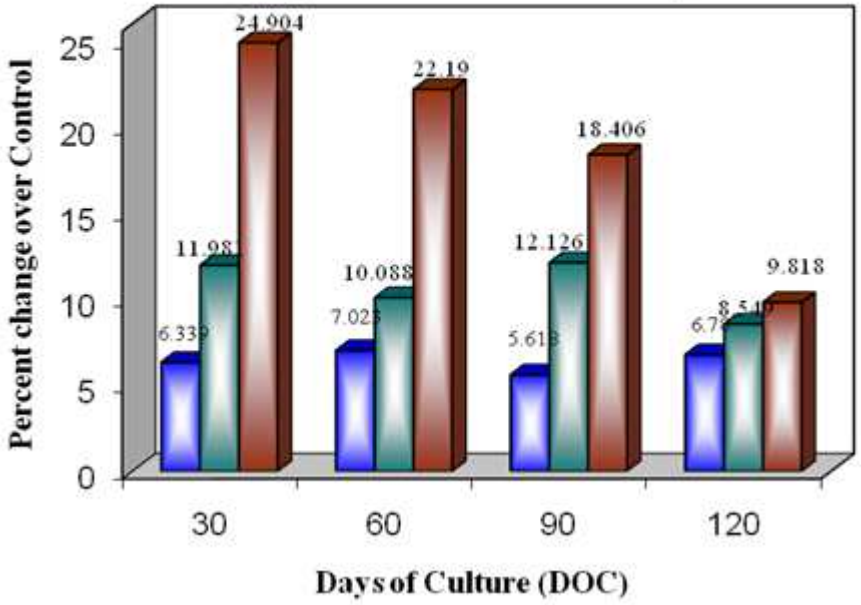
Means having the same superscript in each column do not differ significantly (P<0.05) amongst themselves - DMRT (Duncan's multiple range test)

Table: 2: Two Way ANOVA

Crop	F-value	Specific Growth Rate (SGR)	Average Body Weight (ABW)	Net Weight Gain (NWG)	Average Daily Weight Gain (ADWG)
Summer Crop	F_{Group}	71.750*	345.798*	588.798*	410.406*
	F_{Dur}	68182.018*	52289.179*	60387.302*	6188.922*

**1% level of Significant (P<0.01); F_{Group} : F-value due to Groups; F_{Dur} : F-value due to duration.*

Fig: 1; Percent change in Net Weight Gain (NWG) of *Litopenaeus vannamei* treated with probiotics (PB1: *Bacillus licheniformis*; PB2: *Lactobacillus rhamnosus*; PB3: *Bacillus licheniformis* & *Lactobacillus rhamnosus*) from successive summer crop



The life cycle of a typical decapod crustacean alternates between a relatively long intermoult period during which it feeds actively and a relatively short moult period during which it sheds the old exoskeleton and increases in size. The moult cycle is closely linked to the process of growth, as ecdysis is the only means by which a crustacean can grow. Very few reports are available on the role of probiotic feed supplementation in the promotion of growth and digestion of farmed aquatic animals, which unfortunately confine only to laboratory studies but not to field studies. Thus the present work has been designed to study the effects of two probiotic bacterial species on growth of *L. vannamei* reared in culture ponds in natural field conditions. Further it has been observed that very few studies have been carried out on the effects of probiotics on survival and growth of *P. monodon* in the field conditions (Dalmin *et al*., 2001; Balakrishnan *et al*., 2003). The results obtained clearly suggests that there were significant increase in net weight gain, specific growth rate, average body weight and average daily weight gain of *L. vannamei* (DMRT;

P<0.05; Table :1) treated with probiotics at different time intervals of culture suggesting probiotics played a positive role in enhancing growth and growth related indices in *L. vannamei*.

Fig:2; Percent change in Specific Growth Rate (SGR) of *Litopenaeus vannamei* treated with probiotics (PB1: *Bacillus licheniformis*; PB2: *Lactobacillus rhamnosus*; *PB3: Bacillus licheniformis* & *Lactobacillus rhamnosus*) from successive summer crop

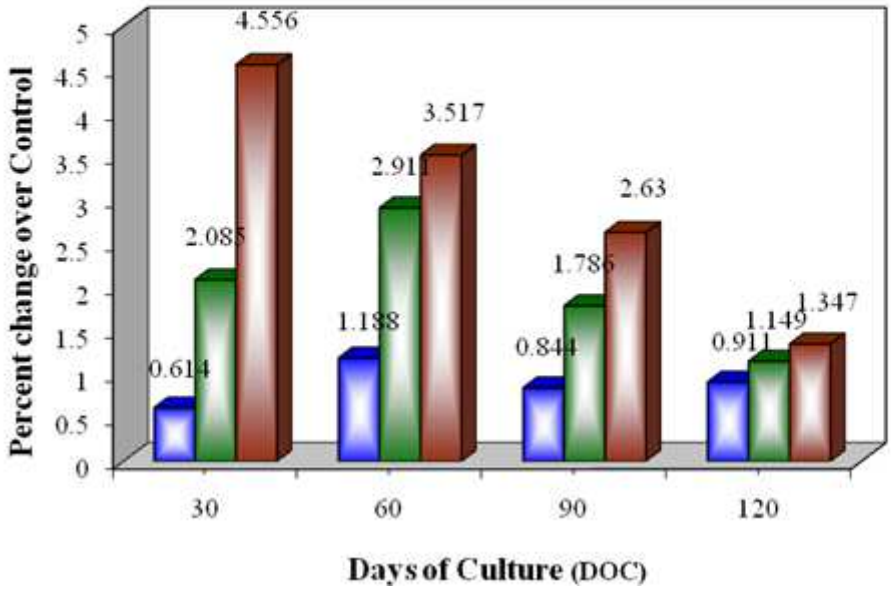
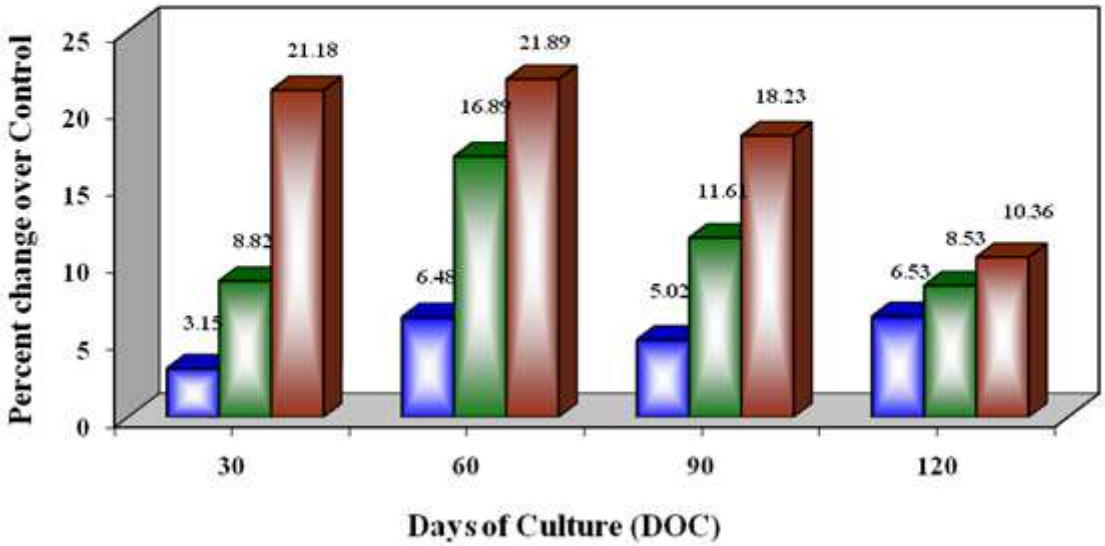


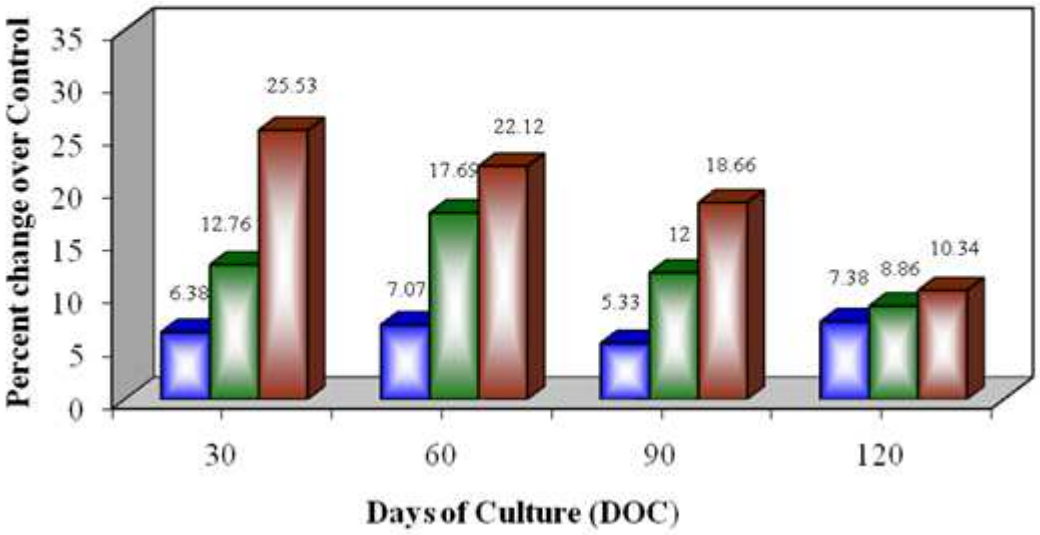
Fig:3; Percent change in Average Body Weight (ABW) of *Litopenaeus vannamei* treated with probiotics (PB1: *Bacillus licheniformis*; PB2: *Lactobacillus rhamnosus*; PB3: *Bacillus licheniformis* & *Lactobacillus rhamnosus*) from successive summer crop.



Similar results have been reported by Ravi *et al*. (1998) and Uma *et al*., (1999) in *P. indicus*, Rengpipat *et al.* (1998) in *P. monodon*, Suralikar and Sahu (2001) and Venkat *et al.*, (2004) in *M. rosenbergii*, Aditya Kumar *et* al., (2014) and Kai Hao *et* al., (2014) in *Litopenaeus vannamei* and Saeed Ziaei-Nejad *et al*. (2006) in *Fenneropenaeus indicus* treated with probiotics. Similar results have also been obtained in the fish, *Cyprinus carpio*, treated with probiotics (Wang Yanbo and Xuzirong, 2006). It is probable that probiotics have improved water quality (Moriarty, 1999) and stimulated appetite and improved nutrition by the production of vitamins and breakdown of indigestible compounds in the diet (Irianto and Austin, 2002). Improved feed consumption, complete digestion of the feed

ingested and favorable water quality variables might have enhanced growth and growth related indices in the shrimp.

Fig: 4; Percent Change in Average Daily Weight Gain (ADWG) of *Litopenaeus vannamei* treated with probiotic bacteria (PB1:*Bacillus licheniformis*;PB2:*Lactobacillus rhamnosus*;PB3:*Bacillus licheniformis* & *Lactobacillus rhamnosus*) from successive summer crop



Apart from this growth in crustaceans is closely associated with moulting and the frequency of moulting is positively influenced by the maintenance of favorable environmental conditions (Ravi *et al*., 1998). It is also probable that the maintenance of optimal water quality variables in probiotic treated ponds has enhanced the frequency of moulting thus contributing to faster growth. The results obtained also suggest that there was a probiotic specific growth promoting effect as revealed by percent changes (Figs. 1 to 4).

In almost all the cases PB3 induced a highly significant ($P<0.01$; Table:2. Two way ANOVA) increase in all the growth related indices followed by PB2 and PB1. In general, probiotics maintain the water quality variables at optimal levels and these probiotics administered through feed, beneficially affect the host by improving its intestinal microbial balance which, in turn, results in better digestion (Moriarty, 1999; Gatesoupe, 1999). As such, by implication, the multiple probiotic species, when applied combined should have a more pronouncing effect on shrimp growth than either they applied separately. The percent changes obtained on NWG, SGR, ABW and ADWG of shrimp demonstrate that PB3 is more effective in enhancing growth than either PB1 or PB2.

In aquaculture, probiotics can be administered either as food supplements or as additives to the water improved the yields (Moriarty, 1998). Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Thompson *et al*., 1999; Verschuere *et al.*, 2000). Studies in *P. monodon* with Bacillus bacteria have shown that growth and survival were improved and immunity was enhanced (Rengpipat *et al.*, 2000) and also in *L. vannamei* supplemented with Bacillus species, *B. megaterium* and *B. licheniformis* improved growth, immunity and digestive enzyme activities (Aditya Kumar *et* al., 2014). The findings of present study also suggests that the combination of probiotic bacteria with multiple strains which boosted the growth of shrimp in natural field condition.

CONCLUSION

The present investigation clearly indicate that, interestingly the combined effect of probiotic bacteria treatment induced a highly significant increase in all the growth indices of white shrimp *Litopenaeus vannamei* in culture pond environment. It suggests that, the multiple probiotic bacteria with synergistic effect could promote growth of the shrimp. Thus, *Bacillus licheniformis* and *Lactobacillus rhamnosus* might have a promising role used as probiotics in shrimp culture. This kind of approach can be a better option for the development of sustainable aquaculture.

Acknowledgements
The authors are thankful to the University Grants Commission (UGC), New Delhi, for the financial support received from RGNF research fellowship grant.

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