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RESEARCH ARTICLE

GROWTH POTENTIALS OF PACIFIC SHRIMP *LITOPENAEUS VANNAMEI* UNDER DIFFERENT STOCKING DENSITIES WITH PROBIOTICS APPLICATION

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ABSTRACT

Litopenaeus vannamei is one of the most intensively cultivated shrimp all over the world including Asia and India. In the present investigation an attempt was made to study the effect of stocking densities on the growth potentials and subsequently production rates along with the usage of selected probiotics in the culture operation of *Litopenaeus vannamei* for 120 days. With the usage of different types of probiotics i.e. Soil, Water and Feed Probiotics, the growth potentials were enhanced and production rates were also significantly increased when compared to pond performance results obtained for ponds, which were cultured without probiotics. The growth potentials were more pronounced in the lower stocking densities compared to higher stocking densities.

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INTRODUCTION

Aquaculture, probably the fastest growing food-producing sector, poses the greatest potential to meet demands for aquatic food supply. In large scale production facilities, where aquatic animals exposed to structural conditions, problems related to diseases and differentiation of environmental conditions often occur and result in serious economic losses. In recent years, the disease problems caused by *Vibrio* species have emerged as major constants in Aquaculture production. The application of Antibiotics or other chemicals to culture ponds expendable and deferential. So therefore to overcome this problem certain substances like probiotics are used. Probiotics generally includes Bacteria, Cyanobacteria, Microalgae and Fungi etc. Probiotic bacteria are generally called the Bacteria which can improve the water quality of Aquaculture and inhibit the pathogens in water there by increasing the productivity (Michel, 2014; Balcazar, 2003; Nagendra Prasad & Srinivasulu Reddy, 2016). Many Researchers attempted to use different types of probiotics in Aquaculture to regulate the microflora of Aquaculture water, control pathogenic microorganisms to enhance decomposition of the undesirable organic substances in Aquaculture water and improve ecological environment of Aquaculture (Verchuere, 2000; Soundarapadian, 2008). In addition the use of probiotics can increase the population of food organisms, improve the

nutrition level of Aquaculture candidate species and improve immunity of cultured animals to pathogenic microorganisms (Prasuna Devi, 2015; Naresh, 2014). The present investigation was aimed to understand the impact of different types of probiotics i.e. Soil, Water and Feed supplemented probiotics on the growth patterns and economic analysis of *Litopenaeus vannamei* culture operation natural environs under different stocking densities.

MATERIALS AND METHODS

The present study was carried out in shrimp culture farms located in and around Ramayapatnam (Latitude 15° 02' 55" N; Longitude 80° 02' 50" E) Prakasam Dist, Andhra Pradesh, South India during the summer months from April to July, 2016. The entire farm has an extent area of 15 ha and four ponds were selected for the present study. Water for culture operation was drawn with the help of motors from Buckingham canal, which was very close proximity with the culture farm. The culture ponds selected in the present investigation are approximately with on average size of 1.8 to 1.9 ha, and are rectangular in shape with clay loamy soil suitable for semi intensive type of culture operation. During culture operation the hydrological conditions including salinity (12 ± 1 ppt), pH (7.5 ± 0.2) Transparency (35-55 cm) Dissolved oxygen (3.8 ± 0.2 ppm) and Temperature (30 ± 2° C) were maintained. In all the four ponds selected were maintained a water depth of 1.5 mts. Initially all the selected ponds were allowed to dry and splinter to increase the capacity of oxidation of Hydrogen Sulphide and to eliminate the fish

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eggs, crab larvae and other unwanted predators. The pond bottom was scrapped 3 to 5 cm by using a tractor blade to avoid top soil. Then the pond bottom was ploughed horizontally and vertically a depth of 30 cm to remove the obnoxious gases, increase fertility. The soil pH was recorded in the ponds with the help of one type pH meter. For increasing the availability of nutrients, required amount of lime was applied to neutralize the acid soil, condition of the soil based on the average pH level of the pond. *Litopenaeus vannamei* of 0.65 ± 0.05 g were selected from the local aquafarms and were stocked in different ponds at the rate of 20 pcs/M² and 40 pcs/M² in the culture ponds. Four ponds were selected in the present investigation. Pond-1 stocked with 20 pcs/M² fed with commercial feed and cultured for 120 days, Pond-2 was stocked with 20 pcs/M² and probiotics were used in the culture operation. Similarly Pond-3 was stocked with 40 pcs/M² fed with commercial feed and Pond-4 also with 40 pcs/M² and probiotics were broadcasted. The four ponds selected in the present investigation were ranged from 1.8 to 1.9 ha. Pond-1 & Pond-3 were broadcasted with commercial feed i.e. CP Brand feed. Pond-2 & Pond-4 were along with commercial CP Brand feed, broadcasted with probiotics. In the present investigation soil, water and feed probiotics selected were Biomax, Probio Aqua and Feed Act, respectively. Biomax was broadcasted at the rate 4-5 kg/ha (1 M water depth), Probio Aqua 4 lit/ha (1 M water depth), and Feed Act 10 g/1 kg Feed were applied by following the manufacturer's instructions. The culture period was continued for a period of 121-123 days. Growth performance studies were conducted for 120 days with every fortnight i.e. 15 days and the observations were recorded. Growth parameters including Average body weights, Average daily growth rates, Specific growth rates, Feed conversion ratio, were monitored and tabulated. The growth parameters can be calculated by adopting the following formulas.

$$\text{Feed Conversion Ratio (FCR)} : \frac{\text{Total amount of Feed consumed (kgs)}}{\text{Total Biomass of prawns (kgs)}} \times 100$$

Weight gain:

$$\text{Weight of the Animal (G) at the end of culture period} - \text{Weight of the Animal (G) at the time of Stocking}$$

$$\text{Survival rate} : \frac{\text{Total Number of Live animals}}{\text{Total Number of Seed stocked}} \times 100$$

Feed Efficiency Ratio: 1/Feed Conversion Ratio

$$\text{Protein Efficiency Ratio} : \frac{\text{Weight gain}}{\text{Protein intake}}$$

Average daily growth rates:

$$\frac{\text{Weight of the prawn (G) at the end of culture period} - \text{Weight of the prawn (G) at the time of stocking}}{\text{Total Number of days of culture operation}}$$

Specific growth rates:

$$\frac{\text{Log weight of the prawn at the end of the culture period} - \text{Log weight of the prawn at the time of start of the culture period}}{\text{Total number of days of culture operation}} \times 100$$

$$\text{SGR} = \frac{(\text{Log } W_2 - \text{Log } W_1)}{T} \times 100$$

Where

W₁ : Weight of the prawn at the start of the culture operations

W₂: Weight of the prawn at the end of the culture operation

T : Total number of Culture operation.

Probiotic Feed Preparation

Probiotic supplemented feeds were prepared as described by Naresh (2016). Probiotic bacterial species *Bacillus licheniformis* (MTCC: 1520) and *Lactobacillus rhamnorus* (MTCC: 1408) were procured from microbial type of culture collection and gene Bank, Institute of microbial Technology, Chandigarh, India. The Bacterial culture was maintained in the nutrient broth and were harvested by centrifuging at 10,000 RPM for 10-12 minutes and washed with phosphate buffer and were re-suspended in phosphate Buffer Saline (pH 7.4). This re-suspended bacteria were mixed uniformly to the feed pellets by spraying method. The probiotic blended feed prepared was dried at 40^o C and packed in air tight polythene covers and stored in Refrigerator. The probiotic blended feed with *Lactobacillus rhamnosus* and *Bacillus licheniformis* (@ 10 billion CFU/Kg) feed were prepared once in 10 days.

RESULTS AND DISCUSSION

In the present investigation, growth performance studies were conducted with *Litopenaeus vannamei*. Four culture ponds were selected in the present investigation Pond-1/2 stocked with 20 pcs/M² whereas Pond-3/4 were stocked with 40 pcs/M². Pond-1/3 were treated as control ponds and were fed with commercial brand feed i.e. CP Brand feed. In Pond-2/4 probiotics were broadcasted i.e. Soil, Water and Feed probiotics were used. The culture operation was carried out for a period of 121-123 days. Results pertaining to growth pattern of *Litopenaeus vannamei* was presented in Table. 1 and Figures 1 & 2. Growth patterns were monitored for every 15 days in all the ponds. From the results obtained for growth patterns for *Litopenaeus vannamei*, clearly indicates that probiotic fed prawns are showing relatively more growth rates compared to prawns fed with commercial feed, indicates that probiotics used in the present investigation were significantly inducing the growth patterns in *Litopenaeus vannamei*. Similar kind of observations were also reported in *Litopenaeus vannamei* when fed with different feeds in the presence of water, feed and soil probiotics (Pratap Reddy, 2016) and also in *Macrobrachium rosenbergii* (Rangappa, 2011; Saad, 2009). In Ponds-1 & 2 stocked 20 pcs/M² prawns showed relatively more growth rates compared to Ponds-3 & 4 stocked with 40 pcs/M², indicating that stocking rates are also playing a vital role in the production of final yield in the culture operation. Several authors reported that there was a declined growth and increased yield with increased density in penaeid shrimp culture operation including *Penaeus monodon* and *Litopenaeus vannamei* (Wyban, 1987; Krishna, 2015). Hanson and Goodwin (1977), Maguire and Lee dow (1983) and Allan and Maguire (1992) reported that the growth reduction in shrimp at higher densities attributed to reduction in grazing activity of a pond. Several authors also stated that best economic results were possible at optimum stocking densities and that may depend upon the area of the pond, the required harvesting size of the shrimp and the number of crops per year (Tidwell, 1999). The Length-Weight parameters were recorded during culture of *L. vannamei* in both the stocking densities and presented in Table. 2.

Table 1. Growth patterns Body weights (g) of shrimp *L. vannamei* at different sctocking densities

Days of culture	20/M ²		40/M ²	
	Control	With probiotics	Control	With probiotics
0	0.65	0.65	0.65	0.65
30	4.24 ± 0.18	4.72 ± 0.22	3.85 ± 0.23	4.32 ± 0.22
	PDC	(+11.32)	PDC	(+12.21)
45	7.82 ± 0.28	9.34 ± 0.35	5.12 ± 0.23	7.43 ± 0.24
	PDC	(+19.44)	PDC	(+45.12)
60	12.13 ± 0.32	14.73 ± 0.44	10.18 ± 0.35	10.79 ± 0.32
	PDC	(+21.43)	PDC	(+5.99)
75	17.74 ± 0.44	20.72 ± 0.48	14.42 ± 0.41	17.42 ± 0.39
	PDC	(+16.80)	PDC	(+20.80)
90	23.45 ± 0.49	25.98 ± 0.65	19.35 ± 0.52	21.44 ± 0.49
	PDC	(+10.79)	PDC	(+10.80)
105	29.34 ± 0.65	31.49 ± 0.72	24.13 ± 0.61	26.73 ± 0.45
	PDC	(+7.32)	PDC	(+10.77)
120	31.54 ± 0.72	34.93 ± 0.75	26.19 ± 0.63	28.84 ± 0.61
	PDC	(+10.75)	PDC	(+10.12)
Harvest size 121/123	32.33 ± 0.75	36.39 ± 0.79	28.11 ± 0.73	31.77 ± 0.78
	PDC	(+12.56)	PDC	(+13.02)

All values are Mean ± SD of Six individual observations.

Table 2. Length-Weight Relationship in white shrimp *Litopenaeus vannamei*

Days of culture	Stocking density (20Pcs/M ²)				Stocking density (40 Pcs/M ²)			
	Control		With probiotics		Control		With probiotics	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)
0	1.21	0.65	1.21	0.65	1.21	0.65	1.21	0.65
30	7.77	4.24	7.92	4.72	6.34	3.85	7.54	4.32
	PDC	+552	PDC	+626	PDC	+492	PDC	+564
		PDE		+11.32		PDE		+12.21
45	9.89	7.82	10.21	9.34	8.42	5.12	8.95	7.43
	PDC	1103	PDC	+1337	PDC	+687	PDC	+1043
		PDE		+19.43		PDE		+45.12
60	11.32	11.75	12.41	14.73	10.49	10.18	10.16	10.79
	PDC	+1707	PDC	+2166	PDC	+1466	PDC	+1560
		PDE		+25.36		PDE		+5.99
75	13.79	17.74	14.81	20.72	12.75	14.42	13.74	17.42
	PDC	+2629	PDC	+3088	PDC	+2118	PDC	+2580
		PDE		+16.8		PDE		+20.8
90	15.41	23.45	16.02	25.98	14.72	19.35	14.42	21.44
	PDC	3508	PDC	3877	PDC	2877	PDC	3198
		PDE		+10.79		PDE		+10.8
105	16.28	29.34	17.19	31.49	15.14	24.13	15.18	26.73
	PDC	+4414	PDC	+4745	PDC	+3612	PDC	+4012
		PDE		+7.32		PDE		+10.77
120	17.45	31.54	18.72	34.93	16.05	26.19	16.74	28.84
	PDC	+4752	PDC	+5274	PDC	+3929	PDC	+4337
		PDE		+ 10.74		PDE		+10.12

DOC: Days of Culture.

PDC: Percent deviation over Zero day control.

PDE: Percent deviation over Respective Experimental values.

All PDC values are statistically significant at

P<0.001.

Table 3. Pond performance details of *Litopenaeus vannamei* at different stocking densities

S.No.	Parameter	Stocking density (20/M ²)		Stocking density (40/M ²)	
		Control	with Probiotic	Control	with Probiotic
1	Area (Ha)	1.8	1.8	1.9	1.9
2	Days of culture (days)	121	121	123	123
3	Initial weight (g)	0.65 ± 0.05	0.65 ± 0.05	0.65 ± 0.05	0.65 ± 0.05
4	Final weight (g)	32.33 ± 0.75	36.39 ± 0.79	28.11 ± 0.73	31.77 ± 0.78
5	Weight gain (g)	31.73	35.74	27.46	31.12
6	Weight gain (%)	4874	5498	4225	4788
7	Harvest size (g)	32.33	36.39	28.11	31.77
8	Shrimp count (Nos/kg)	32-33	27-28	34-35	31-32
9	Shrimp harvest (kgs)	5080	5895	7908	8896
10	Percent survival (%)	79	81	72	70
11	Total Feed used (kgs)	2024	2904	2746	3851
12	Feed Conversion Ratio (FCR)	2.51	2.03	2.88	2.31
13	Daily Growth Rate (DGR)	0.264	0.297	0.229	0.253
14	Specific Growth Rate (SGR)	1.39	1.45	1.33	1.36
15	Feed Efficiency Ratio (FER)	0.398	0.492	0.347	0.433
16	Protein Efficiency Ratio (PER)	6.52	7.34	5.64	6.39

Table 4. Profit Analysis of *L. vannamei* culture at different stocking densities in control and probiotic Treated ponds

Parameter	Stocking density (20/M ²)		Stocking density (40/M ²)	
	Control	with Probiotic	Control	with Probiotic
Preparation cost/kg	Rs. 212-00	Rs. 234-00	Rs. 212-00	Rs. 234-00
Prawn production cost/kg	Rs. 350-00	Rs. 350-00	Rs. 350-00	Rs. 350-00
Profit/kg	Rs. 138-00	Rs. 116-00	Rs. 138-00	Rs. 116-00
Profit/culture (Rs.)	Rs. 7,01,040-00	Rs. 6,83,820-00	Rs. 10,91,304-00	Rs. 10,31,936-00

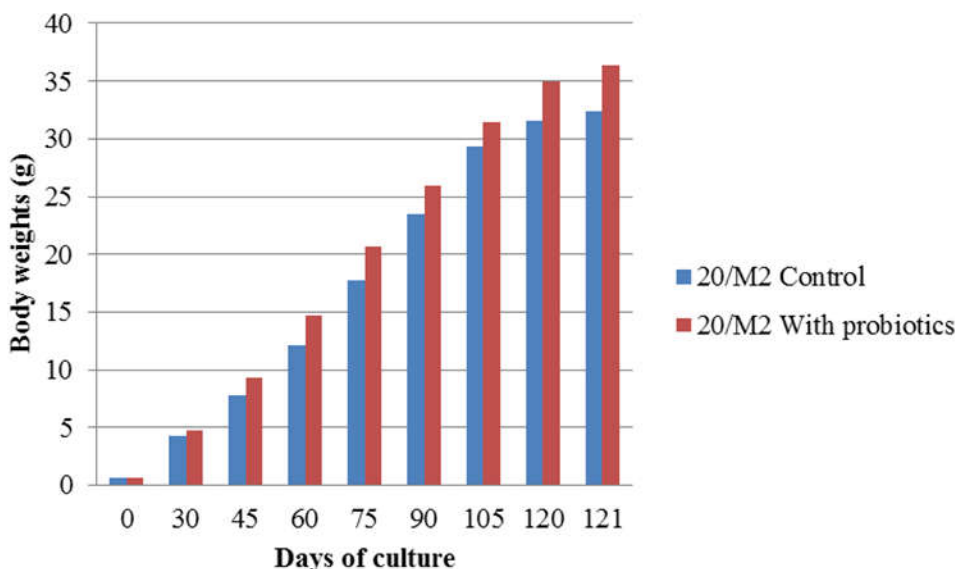


Figure 1. Body weights of *L. vannamei* during culture operation at a stocking densities of 20 pcs/M²

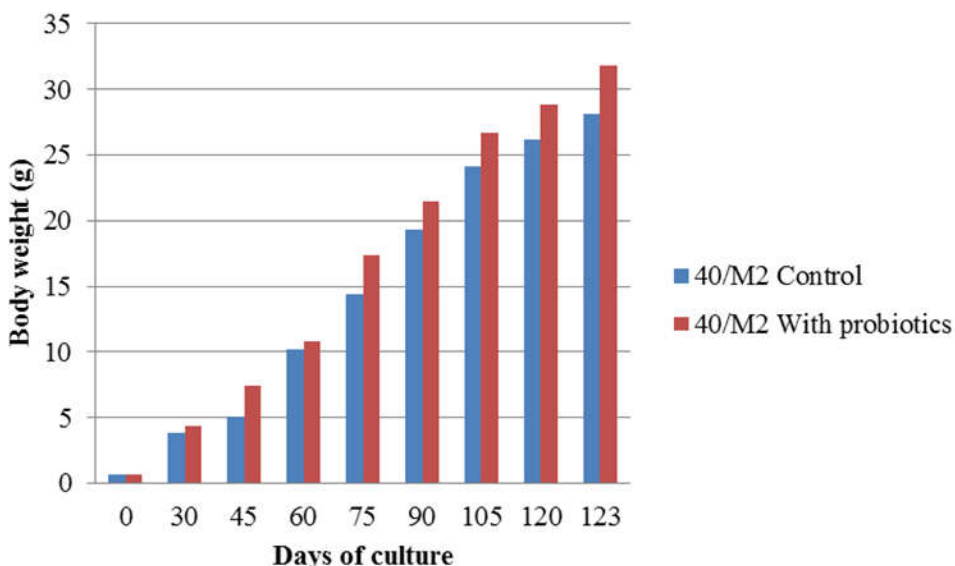


Figure 2. Body weights of *L. vannamei* during culture operation at a stocking densities of 40 pcs/M²

Both Length and weight patterns are significantly increased as the DOC advances. Mathematical models of prawn growth after an objective and practical method for describing patterns of growth data and estimating prawn weight at times between sampling intervals. Accurate estimations of standing biomass and therefore of the amount of feed that must be provided, are Vital to aquaculture management. An accurate Length-Weight Relationship equation allows for conversion of growth-in-

Length to growth-in-weight in stock assessment models, as well as estimation of biomass from the length frequency distribution, condition and morphological characteristics of prawn population, the relationship equation is very important. Shrimp body weight and length were commonly recorded during culture operation and generally useful for culture management purposes for the estimation of growth rates, feed conversion ratio, harvest weights, productivity rates and the

application of morphometric relationships could be a simple alternative to estimate body weight from length measurements. Therefore, the use of morphometric measurements and mathematical models in aquaculture is highly encouraged because that is the most precise and complete way of analysing growth data (Ricker, 1973; Fulton, 1904; Shahina Banu, 2016). In the present investigation growth parameters like, weight gain (g & %), Daily growth rates, Specific growth rates etc were monitored and presented in Table.3. Similarly pond performance details of all the four ponds selected in the present investigation were also presented in Table.3 which includes percent survival rates, Total feed used (Kgs), Total shrimp harvest (Kgs), shrimp count (Nos/Kg), shrimp harvest size (g) etc were presented. The final harvested sizes recorded for prawns are 32.33 ± 0.75 g, 36.39 ± 0.79 for ponds stocked with 20 pcs/M² for control and probiotics treated ponds, respectively, where as 28.11 ± 0.73 , 31.77 ± 0.78 for ponds stocked with 40 pcs/M² for control and probiotics treated ponds, respectively. The present survival rates recorded are 79, 81, 72 & 70 for Ponds-1, 2, 3 & 4, respectively. The results obtained for growth parameters including Average daily growth rates, Specific growth rates, Feed conversion ratio, production yields and Total feed consumed in the present investigation clearly demonstrate that, the commercial feed selected in the present investigation i.e. CP Brand feed and also probiotic supplemented feed and usage of soil and water probiotics significantly enhancing the production yields. The FCR values obtained in the present investigation are also ranging from 2.03 to 2.88 indicating the superior quality of feed nature. The daily growth rates and Specific growth rates obtained also demonstrating the good performance of growth rates of prawns during culture operation. The cost Analysis of *Litopenaeus vannamei* operation in the present investigation was presented in Table. 4. The results obtained also clearly indicate that the culture operation of *Litopenaeus vannamei* is viable and more profitable. The present study was undertaken to ascertain the efficiency of probiotics on the growth patterns of most important cultivable prawn species *Litopenaeus vannamei*.

To date the probiotics can be considered to be a valid alternative to the use of Antibiotics in Aquaculture and in particular in prawn culture activity, to prevent high mortality and to improve yield by enhancing the growth rates. In the last two decades, many studies reported promising results using a beneficial bacterial strain on the culture of finfish sps (Avella, 2010a; Swapna, 2015). The present investigation clearly demonstrates that the growth patterns of *Litopenaeus vannamei* was shown to increase significantly when fed with probiotic incorporated diets. Probiotics that have been studied for use in Aquaculture of crustaceans, particularly prawns includes Bacteria, yeast and microalgae. Lactic acid bacteria, *Bacillus* species were employed to improve the aquatic environment in aquaculture (Farzanfar, 2006). *Lactobacillus* species have yielded strong antimicrobial activity against the pathogenic micro organisms (Rosslund, 2003; Sanni, 1999; Far, 2009). In aquaculture, probiotics can be administered either as food supplements or as additives in the water improved the yields (Rangappa, 2011; Bhavani, 2015). 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 supplemented digestive enzymes (Praveen Kumar, 2012; Zhou, 2009; Ziaei-Nejad, 2006). *Litopenaeus vannamei*, a candidate species for culture has been introduced recently, after devastation of *Penaeus monodon* culture by WSSV in India and also in Andhra Pradesh. Information pertaining to *Litopenaeus vannamei* culture operation, and use of probiotics were relatively scanty in tropical climates including in Andhra Pradesh. Hence present investigation is aimed to probe into the effect of stocking density and use of probiotics in natural field conditions or farm level. In the present investigation two probiotic bacterial species were selected *Bacillus licheniformis* and *Lactobacillus rhamnosus* were incorporated in to the commercial feed and were used as feed probiotics, where as Biomax and Probio Aqua were selected as soil and water probiotics to keep the culture environment hygiene during the *Litopenaeus vannamei* culture operation. Few studies were conducted on the effects of probiotics on the survival and growth of *Penaeus monodon* in the farm operation (Dalmin, 2001). The results obtained for *Litopenaeus vannamei* pertaining to growth rates including Average body weight, Specific growth rate, and Daily growth rates suggests that probiotics use in culture operation clearly enhances the production rates and therefore the probiotics are playing a positive role in enhancing the growth and growth related indices in *Litopenaeus vannamei*.

Conclusion

The Present investigation may be concluded that, the combined effect of Feed supplemented bacteria, soil and water probiotic use induced a significant increase in all the growth indices of shrimp *Litopenaeus vannamei* culture in natural environs. The results obtained clearly suggests that, the use of feed, soil and water probiotics are likely to induce synergistic effect and could promote the growth potentials in significant manner. This both *Bacillus* and *Lactobacillus* sps of bacterial along with selected soil and water probiotics might be playing a promising role as probiotics and substantially increasing the productivity and this type of probiotic usage will pave way for Sustainable Aquaculture activity.

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