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Enrichment of *Artemia* nauplii with *Lactobacillus sporogenes* for Enhancing the Survival, Growth and Levels of Biochemical Constituents in the Post-Larvae of the Freshwater Prawn *Macrobrachium rosenbergii*

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Abstract

In this study, the live feed, *Artemia franciscana* naupli was enriched with probiotic bacterium, *Lactobacillus sporogenes* and fed to the freshwater prawn, *Macrobrachium rosenbergii* post larvae (PL) for attaining good survival, growth and contents of biochemical constituents. *Artemia* was enriched with different concentrations of *L. sporogenes* ($15x10^7$, $30x10^7$, $45x10^7$ and $60x10^7$ cfu cells) and fed to *M. rosenbergii* PL (1,5 cm length and 0.03 g weight) for a period of 45 days. The PL fed with $60x10^7$ cfu cells of *L. sporogenes* enriched *Artemia* produced significantly ($P<0.05$) higher survival and growth when compared to that of other concentrations of *L. sporogenes*, and the control fed with un-enriched *Artemia*. The biomass increase, total weight gain, specific growth rate, condition factor and mean conversion ratio were found to be higher in PL fed with *L. sporogenes* enriched *Artemia* when compared with control. The enhanced growth performance of PL fed with *L. sporogenes* was further confirmed by the lower food conversion ratio recorded. The levels of biochemical constituents, such as total protein, amino acids, carbohydrate and lipid contents were found to significantly higher ($P<0.05$) in PL fed on *L. sporogenes* enriched *Artemia* particularly at $60x10^7$ cfu cells when compared to that of control. Significantly ($P<0.05$) higher ash and lower moisture contents were also observed in PL fed with $60x10^7$ cfu cells of L. *sporogenes* enriched *Artemia*. Therefore, it is evident that $60x10^7$ cfu cells of *L. sporogenes* can be considered as suitable concentration for attaining good survival and growth of *M. rosenbergeii* PL.

Keywords: *Lactobacillus sporogenes*, Survival rate, Growth rate, Protein, Amino acid, Carbohydrate, Lipid, Ash.

Introduction

Aquaculture of the giant freshwater prawn, *Macrobrachium rosenbergii* are widely in practice, especially in Asian countries. Average national production of *M. rosenbergii* from still water ponds has increased from 0.6 tonnes/ha/year in 1974 to 2.2 tonnes/ha/year during 2001–2002 (Tripathi, 2003). It's production was even demonstrated as high as 8–12 tones/ha/year (FAO, 2010). According to FAO statistics, world production of *M. rosenbergii* exceeded 400,000 tons in 2010, and this is 20-fold increases compared to the early 1990's production (FAO, 2010). The farming of *M. rosenbergii* has gained increasing interest in recent years. An annual production of over 30,000 tons has been achieved through the use of monoculture practices (FAO, 2010). *Artemia* is one of the most important live feeds for commercial production of shellfish larvae. Since it is a continuous, nonselective and particulate filter feeder, it feeds upon a wide variety of food particles. Therefore, it is considered as a live feed and a multipurpose vector in aquaculture.

Certain microbes play a very important and critical role in aquaculture, both at hatchery and grow-out levels, because water quality and disease control can directly be regulated by them and such microbes are called probiotics (Kumar *et al*., 2006). Probiotics are live microbial food supplements, which improves the balance of intestinal micro flora of host organisms. Probiotics enhances food absorption, which in turn ultimately increases growth rate (Fuller, 1992). The range of probiotics both Gram-negative and Gram-positive bacteria, yeast and unicellular algae are play successful role in aquaculture. *Bacillus* bacterium has been reported to increase growth and survival in *Fenneropenaeus indicus* (Ziaei-Nejad *et al*., 2006). The competitive exclusion, based on the removal of pathogen by beneficial microbes has been stressed in aquaculture (Gatesoupe, 1999; Gomez-Gill *et al.*, 2000). Further, the immunity in Indian white shrimp, *Penaeus indicus* has been reported to improve and attained higher survival rate due to probiotics (Uma *et al*., 1999). Some studies have attributed to enhancement of animal growth due to the nutritional benefits of probiotic bacteria owing to production of

vitamins, amino acids, minerals, trace elements and digestive enzymes (Holzapfel *et al.*, 1998). Therefore, probiotics can be administered either as a food supplement or as an additive to water.

Lactobacillus are rod shaped, short chained, gram positive, non-sporing, facultatively anaerobic and rarely motile by peritrichous flagella. They are widely distributed in the environment, especially in animal and vegetable food products and inhabit in the gastrointestinal tract of birds and mammals (Holt *et al.*, 1993). The applications of probiotics for developing sustainable aquaculture practices, particularly in feed formulations have received considerable attention (Verschurere *et al*., 2000; Venkat *et al*., 2004; Wang, 2005; Saad *et al*., 2009). The lactic acid bacterium (LAB) has been reported to improve water quality, nutrition and survival as a means to increase population growth and aquaculture output (Shiri Hanceville *et al.*, 1998; Skjermo and Vadstein, 1999; Gatesoupe, 1994; Verschurere *et al*., 2000; Planas *et al*., 2004). LAB has also been used as growth promoters of *Oreochromis niloticus* (Lara-Flores *et al.*, 2003). The most commonly used probiotics in animal nutrition are single or mixed strains of LAB viz., *Lactobacillus bulgaricus, Lactobacillus acidophillus, Lactobacillus sporogenes, Lactobacillus casei, Lactobacillus plantarum* and *Streptococcus thermophillus* (Jacobsen *et al*., 1999). Since probiotics are reported to effectively improve growth, nutrition, immunity and survival of host organisms, in the present work an attempt has been made to identify an optimal concentration of *L. sporogenes* to encapsulate in *Artemia* for attaining good survival, growth and concentration of biochemical constitutes in *M. rosenbergii* PL.

Materials and Methods

The post larvae of freshwater prawn, *M. rosenbergii* (PL-5) were purchased from the Rosen fisheries, Trissur, Kerala, India and were stocked in cement tanks (1000 L) filled with ground water. They were acclimated to ambient laboratory conditions for 10 days (PL-15) and fed with live *Artemia franciscana* nauplii two times a day as well as boiled egg albumin thread once a day. The ground water had these physicochemical parameters: pH, 7; total dissolved solids, 0.9 mg L^{-1}; dissolved oxygen, 7.2 mg L^{-1}; BOD, 30.0 mg L^{-1}; COD, 125.0 mg L^{-1} and ammonia, 0.028 mg L^{-1}. Similarly, the water (hatchery water) in which the PL were maintained in the hatchery had these physicochemical characteristics: pH, 6.8; total dissolved solids, 1.2 g L^{-1}; dissolved oxygen, 6.5 mg L^{-1}; BOD, 42.0 mg L^{-1}; COD, 140.0 mg L^{-1} and ammonia, 1.20 mg L^{-1}.

Cysts of the brine shrimp, *A. franciscana* were allowed to hatch under optimum hatching condition (salinity, 35 ppt; temperature, 27.1º C; pH, 7.5 and 100 W Bulb) in the laboratory. After hatch-out, the *Artemia* naupli were separately reared in 35 ppt artificial saline water for 24 h (up to II-instar stage). The lyophilized powder of *L. sporogenes* (Uni-Sankyo Ltd., Maharashtra, India) was purchased from local medical shop. Each sachet contains not less than 150 million spores of cells. In a pilot study, three concentrations of *L. sporogenes* $25x10^7$, $50x10^7$ and $75x10^7$ cfu/ L^{-1} was tested to check the survival rate of *M. rosenbergii* PL and found that $75x10^7$ cfu/ L^{-1} was produced less survival. Therefore, four different concentrations of *L. sporogenes* viz., $15x10^7$, $30x10^7$, $45x10^7$ and $60x10^7$ cfu/ L^{-1} were selected. II-instar stage *Artemia* nauplii (at a density of 1000 individuals L^{-1}) were transferred to individual enrichment container contained the desired concentration of *L. sporogenes* and maintained at room temperature (27 ± 1° C) for 12 hrs (Immanuel *et al.*, 2001). Strong aeration was provided in order to maintain the oxygen level at >5 ppm. The enriched *Artemia* nauplii were harvested, rinsed with tap water and fed to *M. rosenbergii* PL.

Post-larvae of *M. rosenbergii* (PL-15 (total length,1.5 cm; weight, 0.03 g) used in this study was divided into five groups each contained 120 individuals and housed in three aquaria of 20 L capacity at the stocking density of 2 PL L^{-1} (40 PL/ 20 L). One group served as control and fed with 3 g of un-enriched *Artemia* naupli per day in two equal half (1.5 g x 2 = 3 g; 6:00 am and 6:00 pm). The other groups were fed at the same rate with the respective concentrations of *L. sporogenes* enriched *Artemia* nauplii. The unfed *Artemia* nauplii were collected after the respective hours of feeding. The experiment was prolonged for 45 days (PL 15 to PL 60). Mild aeration was given continuously to maintain the optimal oxygen level. The water medium was renewed daily by siphoning method causing minimum disturbance to PL. The experiment was conducted in triplicates. Sampling was done on day 15, 30 and 45 of feeding schedule. The growth parameters, such as survival rate (S), biomass increase (BI), weight gain (WG), specific growth rate (SGR), condition factor (CF), mean conversion (MC) and food conversion rate (FCR) were determined individually by adopting the following formulae.

Survival rate (SR, %) = No. of live animals/ No. of animals initially introduced X 100

Biomass increase (BI, g) = Final weight of the prawn (g) – Initial weight of the prawn (g)

Weight gain (WG, %) = Final weight (g) – initial weight (g)/ Initial weight X 100

Specific growth rate (SGR, %) = Final weight (g) - Initial weight (g)/ Days of experiment X 100

Condition factor (CF, %) = Prawn weight (g)/ Prawn length $(cm)^3$ X 100

Mean conversion (MC, g) = Final live weight of the animal (g) + Exuvial weight (g)

Food conversion rate (FCR, g) = Feed intake (g)/ Weight gain (g)

On each sampling day, 20 prawns in each group were taken for morphometric measurements. The

biochemical constituents, such as total protein (Lowry *et al.*, 1951), amino acid (Moore and Stein, 1948), lipid (Folch *et al.*, 1957), carbohydrate (Roe, 1955), ash and moisture content (APHA, 2005) were determined in each group of prawns. The analyses were made in triplicates. Microbial analyses (APHA, 2005) were performed in the rearing (control) water, control PL gut and experimental PL gut. One way analysis of variance (ANOVA; SPSS, Version-13.0) was used to determine whether significant variations were existed between different experiments, and between control and experiments. Differences between means were determined and compared by DMRT test. Data are presented as means ± standard deviations of three individual observations. All the data showed significance at $P<0.05$.

Result and Discussion

The initial average body length and total body mass of the PL was 1.5 cm and 0.03 g respectively (Table 1). On day 15, 30 and 45 of feeding trial the length and weight of PL were found to be increase gradually in all the experimental as well as in control group (Table 1). However, maximum length (2.40-3.80 cm) and weight (0.24-0.47 g) were attained in PL fed with $60x10^7$ cfu cells of *L. sporogenes* enriched *Artemia* when compared with control (1.62-2.20 cm; 0.09-0.15 g) PL fed with un-enriched *Artemia.* This was followed by the other concentrations of *L. sporogenes* in the following order $45x10^7$ cfu cells (3.5 cm; 0.45 g) > $30x10^7$ cfu cells (2.96 cm; 0.30 g) > $15x10^7$ cfu cells (2.9 cm; 0.29 g). The differences were found to be statistically significant ($P<0.05$).

The survival rate was significantly ($P<0.05$) higher in *M. rosenbergii* PL fed with L. *sporogenes* enriched *Artemia* when compared with control (Table 1). Maximum survival rate of 90% was recorded in the PL fed with $60x10^7$ cfu cells of *L. sporogenes* enriched *Artemia.* In other concentrations ($45x10^7$, $30x10^7$ and $15x10^7$ cfu cells) of *L. sporogenes* enriched *Artemia* fed PL only 72.5% to 77.5% of survival was recorded. In the control group, the survival recorded was only 55%. The survival was gradually decreased from day 15 to day 45 in all the tested groups. However, this was stabilized on days 30 and 45 in the PL fed with $60x10^7$ cfu cells of *L. sporogenes* enriched *Artemia*. Similar improved survival has been reported in *M. rosenbergii* PL fed with Biogen® supplemented diets (Saad *et al.*, 2009) and diets supplemented with different probiotics (Shinde *et al.*, 2008), and in the Indian white shrimp, *F. indicus* after feeding with *Bacillus* (Ziaei-Nejad *et al.*, 2006).

In general, LAB has the ability to attach with the gut epithelium and establish its colony there. In the present study, the higher survival rate observed in *L. sporogenes* enriched *Artemia* fed PL may be due to administration of significant changes in the population of gut micro flora. In the present study, $220x10^{-4}$ cfu cells were recorded in colony establishment of *L. sporogenes* (Figure 1). This may be exerted by the elimination of harmful bacteria due to establishment of the beneficial probiont, *L. sporogenes* in the intestine of *M. rosenbergii* or colonization of *L. sporogenes* may be dominant over harmful bacteria by their large presence, saturate the adhesion receptors and prevent the pathogenic bacteria from attachment and colonization (Vine *et al.*, 2004; Venkat *et al.*, 2004).

In the present study, *L. sporogenes* enriched *Artemia* fed prawns resulted in significant increase ($P<0.05$) of SGR, WG, BI, CF and MC (Table 2). In support to these the FCR was found to decrease ($P<0.05$) in *L. sporogenes* enriched *Artemia* fed prawns (Table 2). Therefore, the overall growth was higher particularly, in $60x10^7$ cfu cells of *L. sporogenes* enriched *Artemia* fed prawns. This indicates the fact that this much quantity of *L. sporogenes* addition was required to attain good growth performance in *M. rosenbergii* PL. Similar results have been reported in *M. rosenbergii* fed with bio-encapsulated *Lactobacillus cremoris* (Suralikar and Sahu, 2001), *L. sporogenes* and *L. acidophilus*

Table 1. Morphometeric data and survival of *M.rosenbergii* PL fed with different concentration of *L. sporogenes* enriched *Artemia*

Days	Parameter	Initial (0 day)	Control	Concentration of *L. sporogenes*				F-value
				$15x10^7$ cfu	$30x10^7$ cfu	$45x10^7$ cfu	$60x10^7$ cfu	
15-days	Length (cm)	1.22 ±0.06	1.62±0.12^c	2.02±0.12^b	2.14±0.24^{ab}	2.20±0.10^{ab}	2.40±0.10^a	11.83
	Weight (g)	0.02±0.007	0.09±0.01^b	0.16±0.03^{ab}	0.18±0.06^{ab}	0.20±0.05^a	0.24±0.06^a	3.88
	Survival (%)	100.0±0.00	80.00±8.00^c	85.00±2.65^{bc}	87.50±1.00^{abc}	90.00±5.00^{ab}	95.00±3.00^a	3.88
30-days	Length (cm)	1.22 ± 0.06	1.80±0.20^b	2.40±0.10^a	2.55±0.26^a	2.48±0.15^a	2.62±0.20^a	8.71
	Weight (g)	0.02±0.007	0.14±0.03^c	0.24±0.04^{bc}	0.26±0.08^{abc}	0.36±0.05^{ab}	0.39±0.10^a	6.17
	Survival (%)	100.00±0.00	65.00±4.36^c	77.50±2.50^b	82.50±4.09^{ab}	85.00±6.24^{ab}	90.00±2.65^a	6.17
45-days	Length (cm)	1.22 ± 0.06	2.20±0.60^c	2.90±0.40^{bc}	2.96±0.40^{abc}	3.50±0.50^{ab}	3.80±0.50^a	5.34
	Weight (g)	0.02±0.007	0.15±0.05^c	0.29±0.08^b	0.30±0.05^b	0.45±0.05^a	0.47±0.03^a	3.88
	Survival (%)	100.00±0.00	55.00±2.00^c	72.50±3.00^b	75.00±4.00^b	77.50±4.00^b	90.00±3.00^a	5.94

Each value is mean ± SD of 3 individual observations. The mean differences are significant at ($P<0.05$).

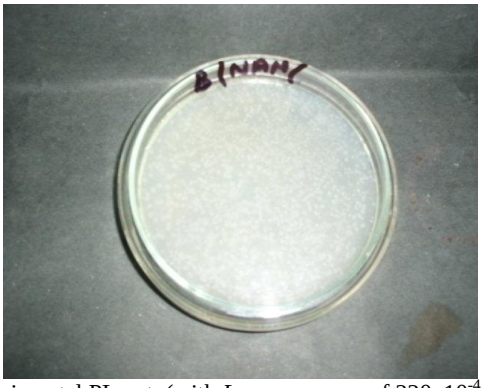

Figure 1. Spread plate culture of experimental PL gut (with *L. sporogenes* of $220x10^{-4}$ cfu cells).

(Venkat *et al.,* 2004) and Biogen® supplemented diets (Saad *et al.,* 2009); in *P. indicus* fed with 'Lactosac' supplemented diet (Uma *et al.,* 1999) and ornamental fishes, *Carassius auratus* and *Xiphophorus helleri* fed with *Lactobacillus spp* (Ashim *et al.,* 2009). According to Shinde *et al.,* (2008), different probiotics supplemented diets have improved the growth of *M. rosenbergii* PL. It has been reported that *Rhodobacter sphaeroides* and *Bacillus coagulans* supplemented diets have improved the weight of the shrimp, *P. monodon* (Wang, 2007). It has also been reported that *B. subtilis* and other species of *Bacillus* have significantly improved the growth of the shrimp, *Penaeus japonicus* when supplemented with feeds (Dakar and Goher, 2004). In the fish, *Cyprinus carpio*, the probiotic, *Streptococcus faecium* has improved the growth and feeding efficiency (Bogut *et al.,* 1998). Similarly, in turbot larvae, the bio-encapsulated LAB and *Bacillus toyoi* have improved the growth (Gatesoupe, 1991).

The possible reason for the overall growth response in PL may be because of increased digestibility due to presence of *L. sporogenes*, which ultimately lead to increased absorption as reported by Douillet and Langdon, 1993 and Uma *et al.,* 1999 in the oyster, *Crassostrea gigas* and the shrimp, *P. indicus* respectively. Improved feed utilization by probiotic supplementation have extensively been reported in aquaculture species (Suralikar and Sahu, 2001; Lara-Flores *et al.,* 2003; Ziaei-Nejad *et al.,* 2006; Shinde *et al.,* 2008). Increased growth may also be attributed due to vitamins as lactic acid bacteria are reportedly produced B-complex vitamins in the gut of aquaculture animals (Goldin and Gorbach, 1992; Mondal *et al*., 2003; Abraham *et al*., 2007). Moreover, *L. sporogenes* might have enhanced the palatability of the live feed, *Aremia* nauplii. In this study, presence of *L. sporogenes* could decrease the amount of food energy utilized for general body maintenance in PL. Therefore, major fraction of consumed food energy may be utilized for growth, which was evident from the lower food conversion rate recorded in PL fed with *L. sporogenes* enriched *Artemia* (Tables 1 and 2).

Table 3 represents the data pertaining to contents of biochemical constituents, such as total protein, amino acid, carbohydrate, lipid, ash and moisture in *M. rosenbergii* PL fed with *L. sporogenes* enriched *Artemia*. The levels of these constituents except moisture were found to proportionately higher in PL fed with *L. sporogenes* enriched *Artemia* on all the sampling days. These elevations were greater in the highest concentration ($60x10^{7}$ cfu cells) of *L. sporogenes* enriched *Artemia* fed prawns followed by the other concentrations, such as $45x10^{7}$, $30x10^{7}$ and $15x10^{7}$ cfu cells of *L. sporogenes* when compared with un-enriched *Artemia* fed PL group. These differences were found to be statistically significant ($P<0.05$). In the case of moisture content just the reverse was recorded in all sampling days. The decrease in the content of moisture was also found to statistically significant ($P<0.05$) when compared to that of control group. It has been reported that bio-encapsulated *L. sporogenes* and *L. acidophilus* have significantly enhanced the biochemical proximate composition in *M. rosenbergii* PL (Venkat *et al.,* 2004). Similarly, Saad *et al.* (2009) have reported that the commercial probiotic, Biogen® supplemented diets significantly enhanced the carcasses biochemical proximate composition in *M. rosenbergii* PL. The beneficial effects of *Lactobacillus* sp., and other probiotics in *M. rosenbergii* PL culture have also been supported by Suralikar and Sahu (2001) and Shinde *et al*. (2008).

The qualitative bacterial study showed that the rearing control medium had the following bacteria, such as *Bacillus spp., Bacillus cereus, Pseudomonas spp., E. coli* and *Streptococcus spp.,* (Table 4; Figure 2). In addition to all these bacterial species the control PL gut contained *Klebsiella pneumonia* (Table 5; Figure 3). In the experimental PL, in addition to these bacteria strains except *Pseudomonas*, colony establishment of *L. sporogenes* ($220x10^{-4}$ cfu cells) were observed (Table 6; Figures 1 and 4). All necessary confirmation biochemical tests were performed and the results were presented (Tables 4-7; Figure 2-4). Similar results have been reported in the gut of *M. rosenbergii* PL fed with bio-encapsulated *L. sporogenes* and *L. acidophilus* (Venkat *et al.,* 2004). It has also been reported in fish, *Poecilia recticulata* fed with bio-encapsulated *L. acidophilus* that established in the gut (Sridevi and Ramasubramanian

Table 2. Growth parameters of *M. rosenbergii* PL fed with different concentration of *L. sporogenes* enriched *Artemia*

Days	*L. sporogenes* Concentration	BI (g)	WG (%)	SGR (%)	CF (%)	MC (g)	FCR (g)
15-days	Control	0.065 ± 0.018^{d}	350	0.433 ± 0.10^{c}	5.55 ± 1.0^{b}	0.621 ± 0.021^{b}	5.55 ± 0.50^{c}
	$15x10^7$ cfu	0.135 ± 0.013^{c}	700	0.900 ± 0.26^{b}	7.92 ± 1.66^{ab}	0.909 ± 0.104^{ab}	3.12 ± 0.12^{bc}
	$30x10^7$ cfu	0.155 ± 0.032^{bc}	800	1.003 ± 0.003^{b}	8.41 ± 0.41^{ab}	1.053 ± 0.058^{ab}	2.77 ± 0.40^{bc}
	$45x10^7$ cfu	0.175 ± 0.01^{b}	900	1.166 ± 0.172^{ab}	9.09 ± 1.13^{ab}	1.337 ± 0.338^{a}	2.50 ± 0.20^{b}
	$60x10^7$ cfu	0.215 ± 0.005^{a}	1100	1.433 ± 0.208^{a}	10.00 ± 4.35^{a}	1.481 ± 0.592^{a}	2.08 ± 0.08^{a}
	F- value	27.1		13.37	1.73	38.57	39.62
30-days	Control	0.115 ± 0.015^{c}	600	0.383 ± 0.101^{c}	7.18 ± 2.29^{b}	0.621 ± 0.021^{a}	3.57 ± 0.40^{c}
	$15x10^7$ cfu	0.215 ± 0.10^{c}	1100	0.716 ± 0.108^{b}	10.0 ± 2.64^{b}	0.765 ± 0.10^{d}	2.08 ± 0.30^{c}
	$30x10^7$ cfu	0.235 ± 0.037^{bc}	1200	0.783 ± 0.003^{b}	10.19 ± 1.10^{b}	0.909 ± 0.009^{c}	1.92 ± 0.30^{b}
	$45x10^7$ cfu	0.335 ± 0.057^{ab}	1700	1.116 ± 0.016^{a}	12.42 ± 1.58^{a}	1.053 ± 0.058^{b}	1.38 ± 0.10^{b}
	$60x10^7$ cfu	0.365 ± 0.070^{a}	1850	1.216 ± 0.10^{a}	12.85 ± 2.85^{a}	1.137 ± 0.040^{a}	1.28 ± 0.20^{a}
	F- value	7.56		51.46	5.35	38.57	32.40
45-days	Control	0.133 ± 0.02^{c}	650	0.221 ± 0.021^{c}	7.70 ± 0.81^{b}	0.493 ± 0.083^{c}	2.77 ± 0.30^{c}
	$15x10^7$ cfu	0.281 ± 0.081^{b}	1350	0.441 ± 0.03^{b}	10.68 ± 1.46^{ab}	$0.510 \pm 0.05b^{c}$	1.72 ± 0.10^{c}
	$30x10^7$ cfu	0.285 ± 0.075^{b}	1400	0.468 ± 0.048^{b}	10.92 ± 1.75^{ab}	$0.549 \pm .0.049^{abc}$	1.66 ± 0.20^{b}
	$45x10^7$ cfu	0.425 ± 0.025^{a}	2150	0.708 ± 0.002^{a}	14.51 ± 3.51^{a}	0.581 ± 0.07^{ab}	1.11 ± 0.11^{b}
	$60x10^7$ cfu	0.477 ± 0.067^{a}	2250	0.745 ± 0.045^{a}	14.88 ± 2.44^{a}	$0.621 \pm .021^{a}$	1.06 ± 0.06^{a}
	F- value	15.55		113.83	3.55	4.12	46.23

Each value is mean ± SD of 3 individual observations. The mean differences are significant at ($P<0.05$).
BI, Biomass (%); WG, Weight gain (g); SGR, Specific growth rate (%); CF, Condition factor (%);
MC, Mean conversion; FCR, Feed Conversion ratio (g)

Table 3. Proximate composition of muscle tissue in *M. rosenbergii* PL fed with different concentration of *L. sporogenes* enriched *Artemia*

Days	Treatments	Protein (mg/ g)	Carbohydrate (mg/ g)	Lipid (mg/ g)	Amino acid (mg/ g)	Ash (%)	Moisture (%)
15-days	Initial	40.70 ± 1.53	6.50 ± 0.66	2.36 ± 0.57	32.47 ±0.66	2.00 ± 0.56	82.30 ±3.10
	Control	47.90 ± 3.53^{c}	7.45 ± 1.28^{b}	4.48 ± 1.30^{a}	38.47 ± 2.00^{c}	2.20 ± 0.50^{b}	81.25 ± 3.05^{a}
	$15x10^7$ cfu	52.50 ± 3.04^{c}	8.04 ± 1.02^{b}	5.00 ± 1.72^{a}	40.62 ± 4.06^{c}	2.80 ± 0.80^{b}	80.70 ± 2.70^{a}
	$30x10^7$ cfu	57.70 ± 2.36^{b}	9.50 ± 1.55^{b}	5.70 ± 1.57^{a}	44.00 ± 2.64^{bc}	3.00 ± 0.30^{b}	80.50 ± 3.20^{a}
	$45x10^7$ cfu	58.90 ± 2.10^{b}	10.80 ± 1.15^{ab}	5.90 ± 1.11^{a}	46.70 ± 4.09^{ab}	4.30 ± 0.40^{a}	80.40 ± 2.75^{a}
	$60x10^7$ cfu	63.84 ± 1.24^{a}	12.99 ± 3.58^{a}	6.20 ± 1.66^{a}	50.74 ± 1.72^{a}	4.60 ± 0.60^{a}	79.90 ± 3.10^{a}
	F- value	16.94	3.86	<1	7.51	11.78	<1
30-days	Control	52.40 ± 3.32^{d}	8.90 ± 3.02^{b}	5.24 ± 1.17^{a}	42.60 ± 3.99^{c}	2.10 ± 0.26^{c}	80.00 ± 3.50^{a}
	$15x10^7$ cfu	58.34 ± 3.52^{cd}	10.10 ± 3.10^{b}	6.10 ± 1.05^{a}	43.94 ± 2.65^{c}	3.10 ± 1.15^{bc}	79.20 ± 3.10^{a}
	$30x10^7$ cfu	66.90 ± 2.57^{bc}	11.60 ± 1.96^{ab}	7.40 ± 1.50^{a}	51.34 ± 2.34^{b}	3.90 ± 0.90^{abc}	78.80 ± 3.40^{a}
	$45x10^7$ cfu	69.44 ± 4.04^{ab}	12.89 ± 1.73^{ab}	7.80 ± 1.83^{a}	54.90 ± 3.53^{b}	4.80 ± 1.10^{ab}	78.20 ± 3.10^{a}
	$60x10^7$ cfu	76.79 ± 3.92^{a}	15.20 ± 2.00^{a}	8.63 ± 1.64^{a}	61.80 ± 3.80^{a}	5.10 ± 1.15^{a}	78.10 ± 3.05^{a}
	F- value	10.87	3.04	1.13	17.08	4.80	<1
45-days	Control	58.92 ± 3.00^{d}	10.21 ± 1.55^{c}	6.33 ± 0.42^{d}	46.13 ± 2.74^{c}	2.20 ± 0.03^{d}	79.30 ± 3.15^{a}
	$15x10^7$ cfu	64.36 ± 2.47^{c}	11.86 ± 1.14^{bc}	7.31 ± 0.31^{c}	50.83 ± 1.58^{d}	3.40 ± 0.40^{c}	78.40 ± 3.20^{a}
	$30x10^7$ cfu	74.58 ± 3.18^{b}	13.80 ± 1.20^{b}	8.12 ± 0.21^{bc}	55.30 ± 2.30^{c}	4.40 ± 0.50^{b}	76.90 ± 3.40^{a}
	$45x10^7$ cfu	76.26 ± 2.52^{b}	14.94 ± 1.96^{b}	8.56 ± 0.44^{b}	59.55 ± 1.77^{b}	5.40 ± 0.40^{a}	76.40 ± 3.30^{a}
	$60x10^7$ cfu	86.42 ± 3.25^{a}	17.91 ± 2.05^{a}	9.93 ± 0.93^{a}	67.93 ± 2.37^{a}	5.80 ± 0.20^{a}	76.20 ± 3.20^{a}
	F- value	41.05	9.89	19.82	43.71	46.46	<1

Each value is mean ± SD of 3 individual observations. The mean difference is significant at ($P<0.05$).

2010).

In this study, a significant improvement in survival, growth and biochemical constituents were achieved when *L. sporogenes* bio-encapsulated *Artemia* were fed to PL of *M. rosenbergii* at the concentration of $60x10^7$ cfu cells. Therefore, it is recommended that *L. sporogenes* can be used as a feed additive either as enrichment material of live feeds or as supplementary material in formulated feeds for enhancing the growth and production of *M. rosenbergeii* PL. Thus, aquaculture of *Macrobrachium* can be promoted in a sustainable manner at nursery level.

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Table 4. Biochemical characterization of isolates in control water

Tests	*Bacillus subtilis*	*Bacillus cereus*	*Pseudomonas sp*	*E. coli*	*Streptococcus sp*	*Klebsiella pneumonia*	*Lactobacillus sporogenes*
Gram's Staining	+	+	-	-	+	-	-
Motility test	+	+	+	+	+	-	-
Indole Test	-	-	-	+	-	-	-
Methyl red Test	-	-	-	+	-	-	-
VP Test	-	-	+	-	+	-	-
Citrate Utilization Test	+	+	+	-	+	-	-
Starch hydrolases	+	+	-	+	+	-	-
Gelatin Hydrolases	+	+	+	+	+	-	-
Nitrate reduction Test	+	+	-	+	+	-	-
Oxidase Test	-	-	+	+	-	-	-
Catalase Test	+	+	+	-	-	-	-
Glucose Test	A	A	A	A	A	-	-
Lactose Test	A	A	NA	A	A	-	-
Sucrose Test	A	A	A	A	A	-	-
Manitol Test	A	A	A	A	A	-	-

+, Positive; - Negative; A, Acid production; NA, No acid production

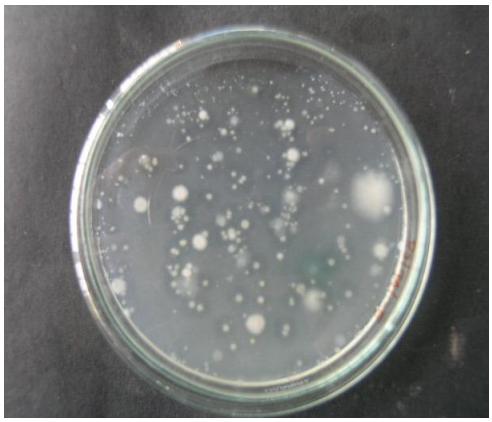

Figure 2. Spread plate culture of control water.

Table 5. Biochemical characterization of isolates in control PL gut

Tests	*Bacillus subtilis*	*Bacillus cereus*	*Pseudomonas sp*	*E. coli*	*Streptococcus sp*	*Klebsiella pneumonia*	*Lactobacillus sporogenes*
Gram's Staining	+	+	-	-	+	-	-
Motility test	+	+	+	+	+	-	-
Indole Test	-	-	-	+	-	-	-
Methyl red Test	-	-	-	+	-	-	-
VP Test	-	-	+	-	+	+	-
Citrate Utilization Test	+	+	+	-	+	+	-
Starch hydrolases	+	+	-	+	+	+	-
Gelatin Hydrolases	+	+	+	+	+	+	-
Nitrate reduction Test	+	+	-	+	+	+	-
Oxidase Test	-	-	+	+	-	+	-
Catalase Test	+	+	+	-	-	+	-
Glucose Test	A	A	A	A	A	A	-
Lactose Test	A	A	NA	A	A	A	-
Sucrose Test	A	A	A	A	A	A	-
Manitol Test	A	A	A	A	A	A	-

+, Positive; - Negative; A, Acid production; NA, No acid production

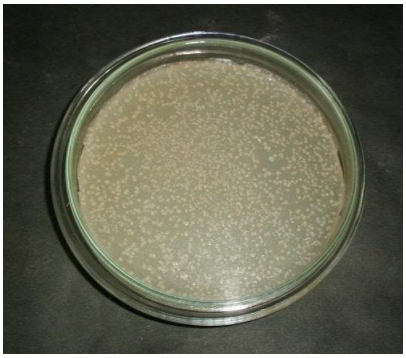

Figure.3. Spread plate culture of control PL gut

Table 6. Biochemical characterization of isolates in Experimental PL gut

Tests	*Bacillus subtilis*	*Bacillus cereus*	*Pseudomonas sp*	*E. coli*	*Streptococcus sp*	*Klebsiella pneumonia*	*Lactobacillus sporogenes*
Gram's Staining	+	+	-	-	+	-	+
Motility test	+	+	-	+	+	-	+
Indole Test	-	-	-	+	-	-	-
Methyl red Test	-	-	-	+	-	-	+
VP Test	-	-	-	-	+	+	+
Citrate Utilization Test	+	+	-	-	+	+	+
Starch hydrolases	+	+	-	+	+	+	+
Gelatin Hydrolases	+	+	-	+	+	+	+
Nitrate reduction Test	+	+	-	+	+	+	-
Oxidase Test	-	-	-	+	-	+	-
Catalase Test	+	+	-	-	-	+	-
Glucose Test	A	A	-	A	A	A	A
Lactose Test	A	A	-	A	A	A	A
Sucrose Test	A	A	-	A	A	A	A
Manitol Test	A	A	-	A	A	A	A

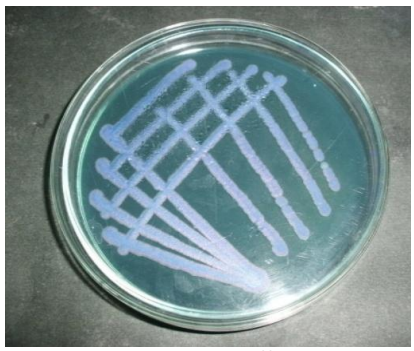

Figure 4. Confirmation test of *L. sporogenes* in Lactic Bacteria Differential Agar medium

Table 7. Overall result of microbial load in control water, control PL and experimental PL

Sl. No.	Isolate Name	Control water (10^{-5})	Control PL gut	Experimental PL gut ($60x10^{7}$ cfu cells)
1.	*Bacillus subtilis*	P	P	P
2.	*Bacillus cereus*	P	P	P
3.	*Pseudomonas sp*	P	P	A
4.	*E.coli*	P	P	P
5.	*Streptococcus sp*	P	P	P
6.	*Klebsiella pneumoniae*	A	P	P
7.	*Lactobacillus sporogenes*	A	A	P (220x10-4 cfu cells)

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