E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(1): 506-514

www.fisheriesjournal.com
Received: 09-11-2016
Accepted: 10-12-2016

V Narmatha
Department of Zoology
Bharathiar University
Coimbatore, Tamil Nadu, India

Dr. P Saravana Bhavan
Department of Zoology
Bharathiar University
Coimbatore, Tamil Nadu, India

M Karthik
Department of Zoology
Bharathiar University
Coimbatore, Tamil Nadu, India

V Srinivasan
Department of Zoology
Bharathiar University
Coimbatore, Tamil Nadu, India

R Mahendran
Department of Microbial Biotechnology Bharathiar University Coimbatore Tamil Nadu, India

T Satgurunathan
Department of Zoology
Bharathiar University
Coimbatore, Tamil Nadu, India

Correspondence
Dr. P Saravana Bhavan
Professor Department of Zoology
Bharathiar University
Coimbatore, Tamil Nadu, India
E-mail: bhavan@buc.edu.in

Lactobacillus fermentum on ammonia reduction and growth promotion of *Macrobrachium rosenbergii* post-larvae, and *in vitro* competitive exclusions of pathogenic bacteria

V Narmatha, Dr. P Saravana Bhavan, M Karthik, V Srinivasan, R Mahendran and T Satgurunathan

Abstract
The effect of a probiotic bacterium, *Lactobacillus fermentum* on ammonia reduction and growth promotion of *Macrobrachium rosenbergii* post-larvae, and *in vitro* competitive exclusion of certain pathogenic bacteria, *Shigella sonnei, Salmonella bongori, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* were studied. It was also aimed to recommend the ideal quantum of water for maintenance of *M. rosenbergii* PL. *L. fermentum* was viable in the feed even on day 15 after the feed was formulated, and hence, the feed was freshly prepared once in 15 days and fed to experimental PL. Three groups of prawns (0.09±0.02 g) consisted of 15, 30 and 45 PL respectively were maintained with 25 L of ground water irrespective of number of PL. Each group was fed with 0.5, 1.0 and 1.5% of *L. fermentum* incorporated diet for a period of 60 days without renewing the water medium. Control was received the feed without incorporation of *L. fermentum*. Up to 30 days, there was no mortality of PL observed in any of the three experimental as well as control groups. The results revealed that up to 1.8 PL staged prawns can be maintained per liter of water without mortality for 30 days (1.8 PL l^{-1}). The mortality was related with density of PL, ammonia content and concentration of *L. fermentum* in the aquarium. The ammonia concentration was found to be significantly reduced in each experimental group when compared with control. After 30 days, the survival rate was found to be the best in the experimental group maintained with 30 PL (1.2 PL l^{-1}) and fed with 1% of *L. fermentum* incorporated feed, followed by 0.5% and 1.5% when compared with control. In this group, the nutritional indices, such as weight gain, specific growth rate, food conversion ratio and protein efficiency ratio were also found to be the best. The concentrations of total protein, amino acid, carbohydrate and lipid were also found to be significantly (P<0.05) increased in this group. *L. fermentum* colony was established in the gut of *M. rosenbergii* PL. The *in vitro* competitive exclusions of pathogenic bacteria revealed that *P. aeruginosa* was sensitive to Amoxycillin, which was produced 17 mm zone of inhibition. While, the test sample, *L. fermentum* was produced 25 mm zone of inhibition against *P. aeruginosa*. It indicates the fact that *P. aeruginosa* was effectively/competitively excluded by *L. fermentum*. As *L. fermentum* incorporated feed fed *M. rosenbergii* PL produced significantly less ammonia, it can be utilized on aquaculture industry as feed additive.

Keywords: Prawn, mortality, survival, growth, protein, *Pseudomonas aeruginosa*

1. Introduction
Aquaculture has become an important economic activity in many countries, including China, Thailand, Bangladesh, Vietnam, Taiwan, India, Myanmar, Indonesia, Malaysia and USA. It is the world's fastest growing animal protein production sector for human consumption. Among various aquaculture sectors, crustacean aquaculture has become one of the fastest growing animal production sectors in the world. Among several species of freshwater prawns, *Macrobrachium rosenbergii* was the first species, studied extensively and farmed commercially [1]. This species is indigenous to South and Southeast Asia, and the northern oceanic and western Pacific Islands [2]. It has become the main species for small-scale as well as large-scale farming because of its fast growth, large size, good meat quality, omnivorous feeding habit and established domestic and export markets worldwide.
Ammonia is the main excretory product of aquatic organisms including crustaceans [3]. The susceptibility of cultured aquatic species to high concentration of nitrogenous compounds,

such as ammonia, nitrite and nitrate is generally species-specific but high concentrations of these compounds affect prawns and cause high mortality [4, 5]. High ammonia concentration in tanks stocked with high densities of larvae is a potential danger, which may cause death or slow down the prawn growth rate [6, 8].

Probiotics are defined as living microorganisms that upon ingestion in certain numbers exert health effects beyond inherent basic nutrition and preventing diseases [9]. *Lactobacilli* are important probiotics, they have many beneficial health effects on hosts, such as increasing the innate immune response, control intestinal infections through competitive exclusion, influencing cholesterol levels, and possesses antioxidant, anti-carcinogenic and antimicrobial properties [10]. In the present study, the role of a probiotic bacterium, *Lactobacillus fermentum* was studied on ammonia reduction and growth promotion of *M. rosenbergii* post-larvae, and *in vitro* competitive exclusion of certain pathogenic bacteria, *Shigella sonnei, Salmonella bongori, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*. It was also aimed to recommend the ideal quantum of water required for maintenance of *M. rosenbergii* PL.

2. Materials and Methods

2.1. Procurement of *M. rosenbergii* PL and acclimation

The post-larvae of the freshwater prawn, *M. rosenbergii* (PL-5) were procured from the Nursery pond at Singanallur (10.993°N 77.021°E), Coimbatore, India. They were transported to the laboratory in polythene bags filled with oxygenated water and acclimated/ maintained in a cement tank (6×3×3 ft) with ground water [temperature, 22±0.2 °C measured by using a mercury thermometer; pH, 7.1±0.20 measured by using a digital pH meter (µP Based Water and Soil Analysis Kit, Model 1160, Esico, Environmental and Scientific Instruments Company, Hariyana, India); total dissolved solids (TDS), 0.96±0.07 g L^{-1} (APHA, 2005); dissolved oxygen (DO), 6.80±0.30 mg L^{-1} [11]; salinity, 0.63±0.01 mg L^{-1} measured by using a water analysis kit; electrical conductivity (EC), 1.01±0.01 mS cm^{-1} measured by using a water analysis kit; ammonia 0.030±0.007 mg L^{-1} (Phenol hypochloride method of Solorzano [12] to ambient laboratory conditions for two weeks (up to attaining PL 20). They were fed with boiled egg albumin and *Artemia* nauplii each twice a day. Then gradually switched over to artificially formulate feed (our laboratory prepared feed). At least half of the tank water was routinely changed every day and adequately aerated.

2.2. Procurement of *L. fermentum* and its sub-cultures

The lyophilized powder of *L. fermentum* was procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. It was subjected to sub-culture with De Man, Rogosa and Sharpe (MRS) broth (Hi-media, India, pH at 25 °C, 6.5±0.2) containing Protease peptone (10.0 g L^{-1}), Beef extract (10.0 g L^{-1}), Yeast extract (5.0 g L^{-1}), Dextrose (20.0 g L^{-1}), Polysorbate-80 (1.0 g L^{-1}), Ammonium citrate (2.0 g L^{-1}), Sodium acetate (5.0 g L^{-1}), Magnesium sulphate (0.10 g L^{-1}), Manganese sulphate (0.05 g L^{-1}) and Dipotassium phosphate (2.0 g L^{-1}). The culture medium was prepared and treated according to the manufacturer's protocol. The medium (55.15 g) was mixed with one liter of distilled water, enclosed in a screw cap container and autoclaved at 121 °C for 15 minutes. The broth was later dispensed into 100 mL sterile conical flask then *L. fermentum* was inoculated into the broth. The conical flask was incubated for 12 h at 37 °C in a shaking incubator for their growth activity. After incubation, *L. fermentum* cells were harvested by centrifugation at 5000 rpm for 10 min, washed twice with phosphate-buffered saline (pH 7.2), weighed and re-suspended in the same buffer. It was stored at 4 °C and used for further study.

MRS agar (by including 12.0 g of agar L^{-1} with MRS medium) plate was prepared according to the manufacturer protocol. The agar plates were incubated for 24 h at 37 °C to check the sterility. Then 10 µL broth culture of *L. fermentum* was spread over the agar medium and allowed to 24 h at 37 °C.

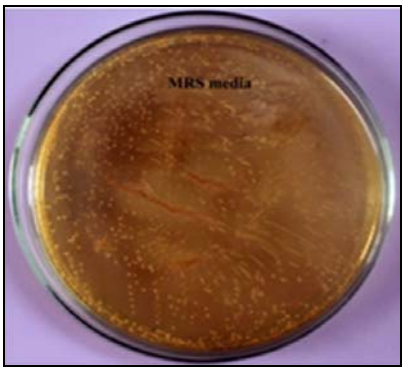

Fig 1: Colony morphology of *L. fermentum* (original material procured from MTCC, Chandigarh, India) on MRS medium.

2.3. Feed preparation

The branded feed basal ingredients (Table 1) were purchased from local merchants in Coimbatore, India. Vitamin B-complex with vitamin-C (Pfizer Ltd., Mumbai, India) was purchased from local Medical shop. First, the basal ingredients powders were sieved (mesh size, 3 mm). Then the fish meal, groundnut oil cake, soybean meal were taken at different ratio based on Pearson's square method to maintain 40% protein level, thoroughly mixed with Wheat bran, steam cooked for 15 min at 95-100 °C and allowed to cool at room temperature then Vitamin B-complex with vitamin C (1%), tapioca flour (5%), egg albumin (7%) and sunflower oil (2%) were added. With this, *L. fermentum* was incorporated at the concentrations of 0.5%, 1.0% and 1.5% levels. Individual dough was prepared with 10% boiled water and pelletized in a manual pelletizer fixed with 3 mm diameter die. Then the feed was dried under room temperature until the moisture content reached less than 10%. The feed prepared without incorporation of *L. fermentum* was served as control.

Table 1: Ingredients used to formulate the basal diet

Basal ingredients (BI)	g/ 100 g
Fish meal	25
Groundnut oil cake	25
Soybean meal	25
Wheat bran	10
Egg albumin	7
Tapioca flour	5
Sunflower oil	2
Vitamin mix*	1
Total	100

*BECOSULES CAPSULES, manufactured by Pfizer. Each capsule contains: Thiamine mononitrate (IP) - 10 mg; Riboflavin (IP) - 10 mg; Pyridoxine hydrochloride (IP) - 3 mg; Vitamin B12 (as tablets 1:100) (IP) – 15 mcg; Niacinamide (IP) - 100 mg; Calcium pantothenate (IP) – 50 mg; Folic acid (IP) - 1.5 mg; Biotin (USP) – 100 mcg; Ascorbic acid (IP) -150 mg.

The prepared feed was subjected to proximate composition analyses [13]. Analysis of total nitrogen was performed after single acid digestion (con. H_2SO_4) using Kjeldahl techniques, titrated against 0.1N HCl and the crude protein content was calculated (N*6.25). The crude fat was extracted with petroleum ether, the extract was dessicated and weighed. For crude fiber, sample was successively digested by boiling the acid and alkali. The extract was converted into ash and the difference was calculated. The sample was ignited in a muffle furnace and the inorganic residue was calculated as total ash. The feed sample was placed in a hot air oven at slightly >100 ºC and the loss of weight calculated as the moisture content. The basal diet formulated contains 45.87% crude protein, 5.66% crude fat, 1.53% crude fibre, 7.68% total ash, 6.71% moisture and 32.55% carbohydrate (total nitrogen free extract) with 4490 k.cal kg^{-1} gross energy (Table 2). In order to maintain the viability of *L. fermentum* in the formulated feeds, they were prepared once in 15-days afresh randomly.

Table 2: Proximate compositions of the basal diet formulated

Proximate composition	Quantity (%)
Crude protein	45.87
Total Nitrogen-free extract	32.55
Ether extract (Crude fat)	5.66
Crude fiber	1.53
Ash	7.68
Moisture	6.71
Gross energy	4490 k.cal/kg

2.4 Viability of *L. fermentum*

The viability of *L. fermentum* in the feeds prepared was analyzed. 1 g of *L. fermentum* incorporated feeds were taken, well dissolved in autoclaved double distilled water (10 mL), it was serially diluted up to 10^{-7} then 20 µL of diluted *L. fermentum* was spread over MRS agar medium, incubated at 37 °C for 24 h, the colony morphology was observed (Figure 2), and the same was compared with the original *L. fermentum* cultured on MRS medium (Figure 1).

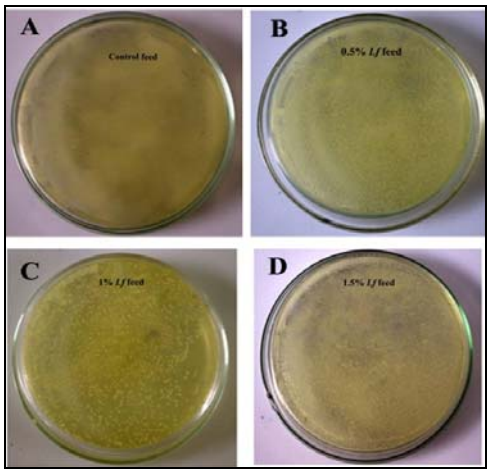

Fig 2: Colony morphology of *L. fermentum* sub-cultured from 15-days old formulated feeds. **A,** Control feed; **B,** 0.5% *L. fermentum* incorporated feed; **C,** 1.0% *L. fermentum* incorporated feed; D, 1.5% *L. fermentum* incorporated feed

2.5 Feeding trial for nutritional indices and biochemical constituents

M. rosenbergii PL of 1.40±0.30 cm in length and 0.09±0.02 g in weight was taken in three groups (Group-I, Group-II and Group-III) each consisted of 15, 30 and 45 individuals respectively in 25 L of ground water. Each experimental group was fed with 0.5, 1.0 and 1.5% *L. fermentum* incorporated diets two times a day (6:00 am and 6:00 pm) at 10% of body weight for a period of 60 days. Control groups were fed with diet prepared with basal ingredients without incorporation of *L. fermentum*. Each group contained a separate control with respective number of prawns in triplicate experimental set-up. The unfed feed, exuvia and moults were removed without severe disturbance to the prawn. The water medium was not renewed, but the level of 25 L was maintained every day (to compensate the daily evaporation and spilling) and aerated adequately. During which the physico-chemical parameters, temperature, pH, salinity, TDS, EC and ammonia were analyzed for every 10 days once by following standard procedures as mentioned elsewhere. Actually, the ammonia level was estimated every day in order to check its concentration.

At the end of this experimental period (on 60th day) the final length and weight were measured. At the end of feeding trial, survival rate, nutritional indices, such as weight gain, length gain, specific growth rate, feed conversion ratio and protein efficiency ratio [Survival rate (SR) = final no of prawns / initial no of prawns ×100; Total weight gain (WG) = final weight – initial weight; Total length gain (WG) = final length – initial length; Specific growth rate (SGR) = *log* final wt. – *log* initial wt. / no of exp. days ×100; Feed conversion ratio (FCR) = feed consumed dry wt. / live weight gain (wet wt.); Protein efficiency ratio (PER) = weight gain / protein intake [14]] and concentrations of basic biochemical constitutions, such as total protein [15], amino acid [16], carbohydrate [17] and lipid [18] were analyzed.

2.6 Colonization of *L. fermentum*

The feeding trial revealed that the group maintained with 30 PL and fed with 1% of *L. fermentum* incorporated feed showed the best performance in terms of growth and survival. Experimental PL of this group was taken and deactivated by kept them in freezer at -20 °C for 10 minutes. The surface was sterilized with 50 ppm formalin for 30 seconds to remove the external flora. Then the prawn digestive tract was dissected out individually, homogenized with phosphate buffered saline (pH, 7.2) and serially diluted up to 10^{-5}. From this 0.5 mL of aliquots were taken, mixed with MRS broth and allowed to culture for 24 h at 37 °C. After incubation, 1ml broth culture was spread over the surface of freshly prepared MRS agar plates, incubated at 37 °C for 24 h and the colony morphology was observed (Figure 3), which was compared with the original *L. fermentum* cultured on MRS medium (Figure 1).

2.7 *In Vitro* competitive exclusions of pathogenic bacteria

Nutrient broth (Hi-media India, pH-7.3, containing Peptone (10.0 g L^{-1}), Beef extract (10.0 g L^{-1}) and Sodium chloride (5.0 g L^{-1}) was used for culture of pathogenic bacteria (*S. sonnei, S. bongori, K. pneumoniae, E. coli* and *P. aeruginosa*). The culture medium was prepared and treated according to manufacturer's protocol. The slant cultures of pathogens were procured from PSG Medical College Hospital, Coimbatore, India, and the standardized bacterial suspensions were prepared, incubated for 24 h at 37 °C.

The nutrient agar (Peptic digest of animal tissue, 5.0 g L^{-1}; Sodium chloride, 5.0 g L^{-1}; Beef extract, 1.5 g L^{-1}; Yeast extract, 1.5 g L^{-1}; Agar, 15.0 g L^{-1}; Final pH at 25 °C, 7.4±0.2) plates were prepared for subculture of pathogens, *S. sonnei, S. bongori, K. pneumoniae* and *E. coli*. The culture media was prepared and treated according to the

manufacturer's protocol. Muller-Hinton (Himedia-India) agar (Beef infusion, 300.0 g L^{-1}; Casein acid hydrolysate, 17.5 g L^{-1}; Starch, 1.5 g L^{-1}; Agar, 17.0 g L^{-1}; Final pH at 25 °C, 7.3±0.2) specific medium was used for *P. aeruginosa*. The culture medium (38 g L^{-1}) was prepared and treated according to the manufacturer's protocol. Then individual broth of pathogen was spread over the agar medium at the concentration of 10 µL and allowed to 24 h at 37 °C.

The broth culture of *L. fermentum* was done in MRS medium. After 12 h of incubation the culture was centrifuged at 5000 rpm for 10 min and the supernatant was collected and filtered by passage through a 0.25 µM syringe (Hi-media, India). 30 µL of supernatant was applied on each sample well of the agar plates seeded with target microorganism for positive and negative controls and *L. fermentum* sample. The specific disc (*S. sonnei*: Fluconazole FLC 25 MCG; *P. aeruginosa*: Amoxycillin AMX; *K. pneumoniae*: Amikacin AK; *S. bongori*: Amoxycillin AMX; *E. coli*: Amoxycillin AMX) was used as positive control. The plates were incubated at 37 °C for 24 h. The antibacterial activity was measured (Figure 4).

3. Results and Discussion

The viability analysis revealed that *L. fermentum* was alive in the feeds even on day 15th after they were formulated (Fig. 2). Their colony morphology was compared with original *L. fermentum* supplied by MTCC, Chandigarh, India (Fig. 1) and found that both showed the same morphology. Regarding physicochemical parameters, such as temperature, pH, total dissolved solids, salinity and electrical conductivity, there was no detectable fluctuation found between *L. fermentum* incorporated feed fed PL categories and respective control in each group. However, the levels of these physicochemical parameters were found to be varied from group-I to group-III, due to varied density of the PL. The overall temperature, pH, TDS, salinity and EC was ranged between 19.5-23.7 °C, 6.6-8.1, 0.58-0.83 g L^{-1}, 0.55-0.92 mg L^{-1} and 0.87-1.02 mS cm^{-1} respectively.

Up to 30 days there was no mortality of PL observed in any of the three experimental groups (15, 30 and 45 PLs/ 25 L). Generally, the ammonia concentration was found to be significantly decreased in individual experimental group when compared with respective control up to 30 days (Tables 3-5). However, in each group, among the three *L. fermentum* (0.5, 1.0 and 1.5%) incorporated feed fed PL categories, there was no statistically significant difference observed in ammonia level. Between experimental groups, the ammonia concentration was found to be obviously elevated from group-I to group-III because of the density of PL. The results suggest that up to 1.8 or 2 PL staged prawns per liter of water can be maintained for 30 days (1.8 or 2 PL L^{-1}) without any mortality. When *L. fermentum* incorporated feed was offered, the mortality of PL was reduced particularly, in group-II (30 PL/ 25 L), under which the PL was fed with 1% of *L. fermentum* incorporated feed (Table 4). Thus, the mortality is related with density of the PL and ammonia content in the aquarium. In the present study, the lower quantum of ammonia recorded in the experimental groups when compared with control may be mainly due to the action of *L. fermentum* and other microorganisms associated with the aquarium, which involved in de-nitrification.

Table 3: Mortality of *M. rosenbergii* PL and concentration of ammonia in aquarium water during the feeding trial (group-I, 15 PL)

Days	Control			Experimental diets								
	(BI)			BI+LF 0.5%			BI+LF 1.0%			BI+LF 1.5%		
	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)
10	0	3.75± 0.03^{a}	0.25± 0.002	0	1.69± 0.03^{d}	0.11± 0.002	0	1.80± 0.02^{c}	0.12± 0.001	0	1.91± 0.02^{b}	0.12± 0.001
20	0	7.60± 0.14^{a}	0.50± 0.009	0	4.96± 0.08^{b}	0.33± 0.005	0	5.03± 0.07^{b}	0.33± 0.004	0	5.10± 0.11^{b}	0.34± 0.007
30	0	11.61± 0.07^{a}	0.77± 0.004	0	8.90± 0.14^{b}	0.59± 0.009	0	9.01± 0.12^{b}	0.606± 0.008	0	9.12± 0.12^{b}	0.608± 0.008
40	9.0±1.0	13.19± 0.08^{a}	2.19± 0.01	0	12.41± 0.01^{b}	0.82± 0.01	0	12.51± 0.13^{b}	0.83± 0.009	0	11.0± 0.07^{c}	0.73± 0.005
50	1.0±0.0	11.13± 0.05^{c}	2.22± 0.01	6.0±1.0	12.03± 0.07^{b}	1.33± 0.07	0	16.05± 0.11^{a}	1.07± 0.007	0	8.96± 0.01^{d}	0.64± 0.001
60	0	8.71± 0.23^{c}	1.74± 0.05	3.6±0.5	10.37± 0.09^{b}	2.07± 0.01	8.0±1.0	14.85± 0.04^{a}	2.12± 0.006	9.3±0.5	7.96± 0.02^{d}	1.32± 0.009
Total Mortality and its %	10.0 66.66%	--	--	9.6 64.00%	--	--	8.0 53.33%	--	--	9.3 62.00%	--	--

Each mortality value is mean ± SD of three individual observations.

Each ammonia value is mean ± SD of 15 individual observations.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at $P<0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.

BI, basal ingredients; LF, *L. fermentum*; MR, mortality rate; TA, total ammonia; IA/PL, individual ammonia per PL.

Table 4: Mortality of *M. rosenbergii* PL and concentration of ammonia in aquarium water during the feeding trial (group-II, 30 PL)

Days	Control (BI)			Experimental diets: BI+ LF 0.5%			BI+ LF 1.0%			BI+ LF 1.5%		
	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)
10	0	4.50± 0.08^{a}	0.15± 0.002	0	3.63± 0.06^{c}	0.12± 0.002	0	3.74± 0.06^{bc}	0.124± 0.002	0	3.84± 0.10^{b}	0.12± 0.003
20	0	8.99± 0.10^{a}	0.29± 0.003	0	7.18± 0.05^{c}	0.23± 0.001	0	7.32± 0.05^{bc}	0.11± 0.001	0	7.43± 0.12^{b}	0.24± 0.004
30	0	13.66± 0.13^{a}	0.45± 0.004	0	10.56± 0.07^{b}	0.35± 0.002	0	10.70± 0.07^{b}	0.356± 0.002	0	10.77± 0.05^{b}	0.359± 0.001
40	15.0±1.0	12.84± 0.05^{b}	0.85± 0.003	0	14.14± 0.13^{a}	0.47± 0.004	0	14.28± 0.13^{a}	0.47± 0.004	5.0±1.0	14.40± 0.08^{a}	0.57± 0.003
50	11.0±1.0	10.91± 0.01^{c}	1.21± 0.004	4.0±1.0	17.73± 0.13^{a}	0.68± 0.005	0	17.87± 0.13^{a}	0.59± 0.004	14.0±1.0	12.95± 0.07^{b}	1.17± 0.006
60	0	10.05± 0.02^{d}	2.51± 0.007	18.0±1.0	15.85± 0.05^{b}	1.98± 0.007	15.0±1.0	18.40± 0.05^{a}	1.22± 0.003	5.0±1.0	10.52± 0.07^{c}	1.75± 0.01
Total Mortality and its %	26.0 86.66%	--	--	22.0 73.33%	--	--	15.0 50.00%	--	--	24.0 80.00%	--	--

Each mortality value is mean ± SD of three individual observations.
Each ammonia value is mean ± SD of 30 individual observations.
Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P<0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.
BI, basal ingredients; LF, *L. fermentum*; MR, mortality rate; TA, total ammonia; IA/PL, individual ammonia per PL.

Table 5: Mortality of *M. rosenbergii* PL and concentration of ammonia in aquarium water during the feeding trial (group-III, 45 PL)

Days	Control (BI)			Experimental diets: BI+ LF 0.5%			BI+ LF 1.0%			BI+ LF 1.5%		
	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)	MR (%)	TA (mg/l)	IA/PL (mg/l)
10	0	12.02± 0.14^{a}	0.26± 0.003	0	7.33± 0.31^{b}	0.16± 0.007	0	7.48± 0.31^{b}	0.16± 0.006	0	7.58± 0.38^{b}	0.168± 0.008
20	0	22.71± 0.08^{a}	0.50± 0.001	0	14.35± 0.24^{b}	0.31± 0.005	0	14.50± 0.24^{b}	0.32± 0.005	0	14.58± 0.23^{b}	0.324± 0.005
30	0	23.38± 0.03^{a}	0.51± 0.00	0	21.29± 0.09^{c}	0.47± 0.002	0	21.43± 0.09^{bc}	0.473± 0.002	0	21.54± 0.11^{b}	0.478± 0.002
40	12.0±1.0	21.55± 0.05^{c}	0.65± 0.001	17.0±1.0	22.93± 0.05^{b}	0.81± 0.002	11.0±1.0	23.07± 0.05^{a}	0.67± 0.001	11.0±1.0	23.18± 0.07^{a}	0.68± 0.002
50	18.0±1.0	19.07± 0.05^{b}	1.27± 0.004	16.0±1.0	20.58± 0.09^{a}	1.71± 0.007	16.0±1.0	20.73± 0.09^{a}	0.06± 0.004	18.0±1.0	20.73± 0.11^{a}	1.29± 0.008
60	10.0±1.0	15.55± 0.03^{d}	3.11± 0.008	5.0±1.0	17.64± 0.04^{c}	2.52± 0.006	7.0±1.0	17.78± 0.04^{b}	1.61± 0.004	10.0±1.0	17.88± 0.05^{a}	2.98± 0.01
Total Mortality and its %	40.0 88.88%	--	--	38.0 84.44%	--	--	34.0 75.55%	--	--	39.0 86.66%	--	--

Each mortality value is mean ± SD of three individual observations.
Each ammonia value is mean ± SD of 45 individual observations.
Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P<0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.
BI, basal ingredients; LF, *L. fermentum*; MR, mortality rate; TA, total ammonia; IA/PL, individual ammonia per PL.

The survival rate (SR) was found to be 100% up to 30 days in *L. fermentum* incorporated feeds fed PL categories and respective control. After 30 days, the SR was reported to be the best in group-II (30 PL/ 25 L) fed with 1% of *L. fermentum* incorporated feed. The growth parameters, such as length gain (LG), weight gain (WG), specific growth rate (SGR) and protein efficiency ratio (PER) were found to be significantly increased ($P< 0.05$) in *L. fermentum* incorporated feed fed PL categories of each group when compared with respective control. Among the three groups, group-II (30 PL) showed the best performance followed by group-I and group-III (15 and 45 PL/ 25 L). Among the concentrations, 1% of *L. fermentum* incorporated feed fed PL showed the maximum growth performance in all three groups followed by 0.5% and 1.5%. The recorded value for food conversion ratio (FCR) was just in reverse trend, which itself indicates the fact that 1% *L. fermentum* incorporated feed was produced the best growth performance in *M. rosenbergii* PL (Table 6).

Table 6: Nutritional indices of *M. rosenbergii* PL (initial length and weight, 1.40±0.30 cm and 0.09±0.02 g respectively) fed with *L. fermentum* incorporated feeds

Parameter		Group-I (15 PL)				Group-II (30 PL)				Group-III (45 PL)			
		Control (BI)	*L. fermentum* incorporation (%)			Control (BI)	*L. fermentum* incorporation (%)			Control (BI)	*L. fermentum* incorporation (%)		
			0.5	1.0	1.5		0.5	1.0	1.5		0.5	1.0	1.5
SR (%)	30th day	100± 0.00	100± 0.00	100± 0.00	100± 0.00	100± 0.00	100± 0.00	100± 0.00	100± 0.00	100± 0.00	100± 0.00	100± 0.00	100± 0.00
	60th day	33.33± 6.66^{b}	35.55± 3.84^{b}	46.66± 6.66^{a}	37.77± 3.84^{ab}	13.33± 5.77^{b}	26.66± 1.24^{b}	50.00± 3.33^{a}	20.00± 10.00^{b}	11.11± 6.66^{a}	15.55± 6.66^{a}	24.44± 6.66^{a}	13.33± 6.66^{a}
FL (cm)		2.03± 0.05^{c}	2.50± 0.26^{b}	3.20± 0.10^{a}	2.23± 0.05^{bc}	2.16± 0.05^{d}	3.00± 0.01^{b}	3.63± 0.15^{a}	2.40± 0.10^{c}	1.73± 0.20^{b}	2.66± 0.20^{a}	2.80± 0.20^{a}	1.96± 0.15^{b}
LG (cm)		0.60± 0.26^{b}	1.06± 0.32^{b}	1.76± 0.20^{a}	0.80± 0.30^{b}	0.70± 0.25^{c}	1.56± 0.40^{b}	2.20± 0.17^{a}	0.96± 0.28^{c}	0.30± 0.10^{b}	1.23± 0.37^{a}	1.36± 0.23^{a}	0.53± 0.15^{b}
FW (g)		0.23± 0.01^{d}	0.32± 0.007^{b}	036± 0.005^{a}	0.274± 0.01^{c}	0.26± 0.01^{d}	0.36± 0.008^{b}	0.39± 0.01^{a}	0.316± 0.005^{c}	0.24± 0.005^{d}	0.30± 0.005^{b}	0.34± 0.004^{a}	0.26± 0.03^{c}
WG (g)		0.14 ± 0.015^{c}	0.22± 0.02^{a}	0.26± 0.015^{a}	0.17± 0.032^{b}	0.16± 0.01^{c}	0.27± 0.01^{a}	0.29± 0.02^{a}	0.22± 0.017^{b}	0.15± 0.015^{c}	0.20± 0.01^{ab}	0.24± 0.02^{a}	0.17± 0.03^{b}
SGR (%)		1.07± 0.13^{a}	1.21± 0.15^{a}	1.25± 0.14^{a}	1.14± 0.16^{a}	1.11± 0.13^{a}	1.26± 0.14^{a}	1.29± 0.14^{a}	1.20± 0.14^{a}	1.09± 0.13^{a}	1.18± 0.14^{a}	1.23± 0.15^{a}	1.12± 0.13^{a}
FCR (g)		3.98± 0.25^{a}	3.02± 0.38^{b}	2.92± 0.08^{b}	4.50± 1.06^{a}	4.02± 0.36^{a}	2.61± 0.16^{c}	2.53± 0.33^{c}	3.41± 0.28^{b}	3.71± 0.10^{a}	3.28± 0.34^{b}	3.14± 0.47^{b}	4.65± 0.77^{ab}
PER (g)		0.55± 0.02^{b}	0.73± 0.09^{a}	0.75± 0.01^{a}	0.50± 0.10^{b}	0.54± 0.04^{b}	0.84± 0.05^{a}	0.87± 0.10^{a}	0.64± 0.05^{b}	0.59± 0.025^{b}	0.66± 0.06^{a}	0.70± 0.09^{a}	0.47± 0.08^{b}

Each value is mean ± SD of three individual observations. Initial length and weight were 1.40±0.30 cm and 0.09±0.02 respectively.
Mean values within the same row in each group sharing different alphabetical letter superscripts are statistically significant at P<0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT (Group wise).
BI, basal ingredients; SR, survival rate; FL, final length; LG, length gain; FW, final weight; WG, weight gain, SGR, specific growth rate; FCR, food conversion ratio; PER, protein efficiency ratio.

The improved results in SR, WG, SGR, FCR and PER have also been reported in *M. rosenbergii* fed with *Bacillus* spp., *Vibrio* spp., Biogen®, Binifit™, LactoBacil® plus, ViBact*, *Bacillus subtilis*, *Lactobacillus sporogenes*, *Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactococcus ceremoris* and yeast, *Saccharomyces cerevisiae* incorporated diets [19, 39]. The improved water quality, survival, growth and health status in juvenile shrimp, *Penaeus monodon* due to *Bacillus* spp., has been reported [40, 41]. The probiotic bacteria such as *Bacillus* spp., *Lactobacillus thermophilus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus salivarius, Lactobacillus rhamnosus* and *Streptococcus lactis* have the potential to induce growth enhancement in cultivable aquatic animals [42, 44].

Similar to that of the survival and growth performance, the concentrations of basic biochemical constituents, such as total protein, amino acid, carbohydrate and lipid were also found to be significantly (P<0.05) increased in *L. fermentum* incorporated feeds fed PL categories of each group when compared with respective control (Table 7). On the whole, among the three concentrations, 1% of *L. fermentum* incorporated feeds fed PL was found to be produced the best result followed by 0.5% and 1.5% when compared with respective control group. Among the three groups, group-II maintained with 30 PL/ 25 L was the best performer, since it effectively reduced the ammonia level in the aquarium and thus produced the best survival, growth and nutritional profile (Table 7).

Table 7: Concentrations of the basic biochemical constituents of *M. rosenbergii* PL fed with *L. fermentum* incorporated feeds

Basic Biochemical Constituents (mg/g)	Initial	Final (on 60th day)											
		Group-I (15 PL)				Group-II (30 PL)				Group-III (45 PL)			
		Control (BI)	% of *L. fermentum*			Control (BI)	% of *L. fermentum*			Control (BI)	% of *L. fermentum*		
			0.5	1.0	1.5		0.5	1.0	1.5		0.5	1.0	1.5
Total Protein	37.45± 1.32	68.66± 3.15^{d}	88.37± 3.98^{b}	113.90± 2.76^{a}	81.14± 3.91^{c}	74.08± 3.06^{d}	92.98± 2.56^{b}	127.34± 3.94^{a}	84.05± 3.36^{c}	63.61± 2.57^{d}	83.53± 3.87^{b}	102.66± 4.12^{a}	74.30± 3.06^{c}
Total Amino Acid	24.31± 2.12	41.07± 1.68^{c}	64.91± 2.18^{a}	69.25± 3.51^{a}	57.56± 2.76^{b}	45.07± 2.19^{d}	65.91± 3.01^{b}	78.93± 2.77^{a}	59.92± 3.48^{c}	40.26± 3.01^{c}	59.65± 2.42^{b}	65.03± 3.29^{a}	55.71± 2.55^{b}
Total Carbohydrate	18.75± 2.56	27.48± 1.89^{c}	32.61± 3.31^{b}	40.74± 2.44^{a}	29.06± 2.09^{bc}	30.22± 3.14^{c}	39.47± 1.98^{b}	45.22± 2.59^{a}	35.75± 2.63^{ab}	26.86± 2.92^{b}	30.22± 2.08^{b}	39.47± 2.75^{a}	27.68± 3.08^{b}
Total Lipid	7.56± 1.58	10.60± 1.59^{b}	12.92± 1.97^{ab}	15.15± 2.03^{a}	14.39± 2.54^{ab}	13.72± 1.85^{b}	14.39± 2.05^{b}	19.85± 2.10^{a}	16.43± 1.75^{ab}	10.19± 1.85^{a}	11.55± 2.13^{a}	13.68± 1.92^{a}	14.06± 2.41^{a}

Each value is mean ± standard deviation of three individual observations.
Mean values within the same row in each group sharing different alphabetical letter superscripts are statistically significant at P<0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT (Group wise). PL, post larvae; BI, basal ingredients; LF, *L. fermentum.*

The elevations in basic biochemical constituents have also been reported in *M. rosenbergii* fed with probiotics products, Biogen® (Allicin, *B. subtilis* and High Unit Hydrolytic Enzyme), Binifit™ (*Bifidobacterium bifidum, Lactobacillus* sp., *L. acidophilus, L. bulgaricus* and *Streptococcus thermophilus*), LactoBacil® plus (*Bifidobacterium longum, B. bifidum, L. acidophilus, L. rhamnosus* and *Saccharomyces boulardii*), ViBact* (*Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus* and *L. sporogenes*), and individual probiotics, *L. sporogenes, L. acidophilus*, *B. subtilis* and yeast, *S. cerevisiae* [20, 24, 27-31, 33, 38], in the marine prawn, *Litopenaeus vannamei* fed with *Bacillus* spp., [45] and in *Penaeus indicus* fed with Lactic acid bacteria [46] incorporated feeds.

In this study, the colony of *L. fermentum* was established in the gut of *M. rosenbergii* PL (Fig. 3). The colony morphology of *L. fermentum* observed from the feed formulated (Fig. 2) and the gut of *M. rosenbergii* PL (Fig. 3) were similar to that of the original colony of *L. fermentum* observed from the mother culture supplied by MTCC, Chandigarh, India (Fig. 1).

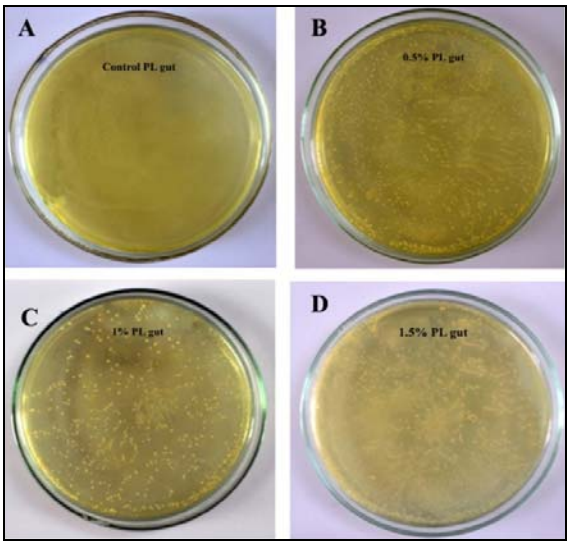

Fig 3: Colony morphology of *L. fermentum* cultured from the gut of *M. rosenbergii* (30 PL/ 25 L) maintained with *L. fermentum* incorporated feeds. A, The gut of control PL; B, The gut of PL fed with 0.5% *L. fermentum* incorporated feed; C, The gut of PL fed with 1.0% *L. fermentum* incorporated feed; D, The gut of PL fed with 1.5% *L. fermentum* incorporated feed

The establishment of the probiotic bacteria, *L. sporogenes, B. subtilis, S. cerevisiae* and *L. acidophilus* in the gut of *M. rosenbergii* PL have also been reported [20, 28-31, 33, 34]. Similarly, the colony establishments of *Bacillus* S11 in *P. monodon* [47], *L. acidophilus* and *S. cerevisiae* in the pearl spot, *Etroplus suratensis* [48], *Lactobacil sporlac* and yeast in the juvenile Goldfish, *Carassius auratus* [49], *Bacillus* spp., in the rainbow trout, *Oncorhynchus mykiss* [50], *Lactobacillus* spp., in the sea bream, *Sparus aurata* [51], *B. subtilis* in *Catla catla* [52] and *B. subtilis, Lactococcus lactis* and *S. cerevisiae* in *Labeo rohita* [53] have been reported.

Regarding the *in vitro* competitive exclusion of pathogenic bacteria, *S. sonnei*, Fluconazole was used as positive control. No zone of inhibition was recorded in both positive control, negative control and the test sample. For *P. aeruginosa*, Amoxycillin was used as positive control, which produced 17 mm zone of inhibition, whereas, the test sample, *L. fermentum* was produced 25 mm zone of inhibition. It indicates the fact that *P. aeruginosa* is sensitive to Amoxycillin as well as *L. fermentum*. Therefore *P. aeruginosa* was competitively excluded by *L. fermentum* (*in vitro*). Amikacin, and Amoxycillin sensitivity were detected against *K. pneumonia, and S. bongori* and *E. coli* respectively. The test sample, *L. fermentum* was found to produce no visible zone of inhibition against *K. pneumonia, S. bongori, E. coli* and *S. sonnei* (Figure 4, Table 8).

In *M. rosenbergii, Pseudomonas* causes black-spot, brown-spot and shell diseases [54]. In the present study, *P. aeruginosa* was effectively/competitively excluded by *L. fermentum* (*in vitro*). Therefore, *L. fermentum* can definitely be recommended as a probiotic for use in aquaculture.

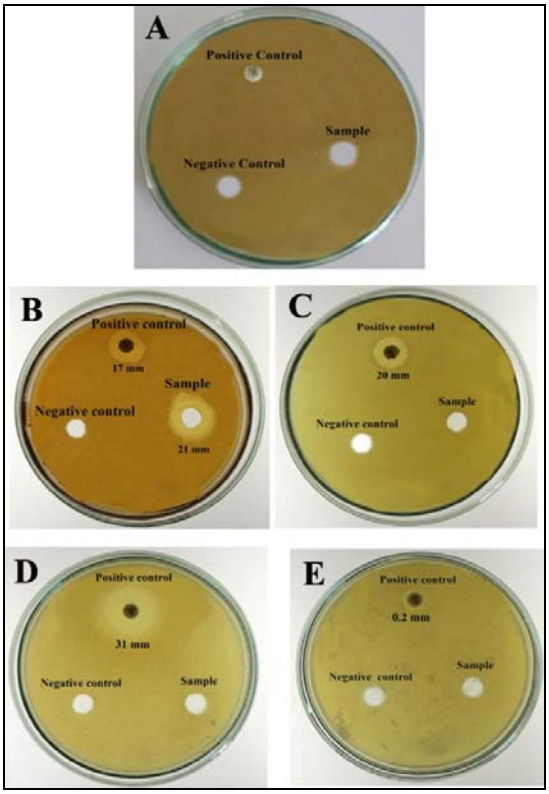

Fig 4: *In-vitro* antibacterial activity of *L. fermentum* and antibiotic sensitivity tests against some pathogenic bacteria. A, Against *S. sonnei*; B, Against *P. aeruginosa*; C, Against *K. pneumoniae*; D, Against *S. bongori*; E, Against *E. coli*

Table 8: *In-vitro* antibacterial activities of *L. fermentum* against various pathogens and antibiotics sensitivity tests

Pathogens	Antibiotic disk	Zone inhibition (mm)		
		Positive control	Negative control	*L. fermentum* sample
S. sonnei	Fluconazole FLC 25 MCG	-	-	-
P. aeruginosa	Amoxycillin AMX	17	-	21
K. pneumonia	Amikacin AK	20	-	-
S. bongori	Amoxycillin AMX	31	-	-
E. coli	Amoxycillin AMX	0.2	-	-

Note: *L. fermentum* worked against *P. aeruginosa*

4. Conclusion

This study concluded that *L. fermentum* can be used as a probiotic in *M. rosenbergii* culture as it has ammonia reduction capacity. Since it has the colony establishment capacity in the gut, it enhanced the survival, growth and basic biochemical constituents. This study recommends that without renewal of water up to 1.8 or 2 PL staged prawns per liter of water can be maintained for 30 days without mortality. Therefore, *L. fermentum* is recommended as a feed additive for sustainable production of *Macrobrachium*.

5. Acknowledgements

The authors express their deep sense of gratitude to Dr. J.

Angayarkanni, Associate professor, Cancer Therapeutic Laboratory, Department of Microbial Biotechnology, Bharathiar University, Coimbatore, India, for permitting us to conduct the microbiological work, and also gratefully acknowledged the Research Scholars of Dr. J. Angayarkanni, for their comprehensive support to complete this work.

6. References

1. Radhakrishnan EV, Deshmukh VD, Maheswarudu G, Josileen J, Dineshbabu AP, Philipose KK *et al.* Prawn fauna (Crustacea: Decapoda) of India - an annotated checklist of the Penaeoid, Sergestoid, Stenopodid and Caridean prawns. J Mar Biol Ass India. 2012; 54:50-72.
2. Nandlal S, Pickering T. Freshwater prawn *Macrobrachium rosenbergii* farming in Pacific Island countries. Hatchery operation. Noumea, New Caledonia: Secretariat of the Pacific Community. 2005; 1:2.
3. Cavalli RO, Berghe EV, Lavens P, Thuy NTT, Wille M, Sorgeloos P. Ammonia toxicity as a criterion for the evaluation of larval quality in the prawn *Macrobrachium rosenbergii*. Comp Biochem Phys. 2000; 125C:333-343.
4. Krishnaprakash R. Studies on the production of high quality shrimp (*Penaeus monodon* Fabricius) seed in a commercial hatchery. M. Phil thesis, CAS in Marine Biology, Annamalai University, India. 2007, 1-45.
5. Tuan TN, Duc PM, Hatai K. Overview of the use of probiotics in aquaculture. Int J Res Fish Aquacult. 2013; 3:89-97.
6. Daniels WH, D'Abramo LR, Parseval L. Design and management of a closed, recirculating "clearwater" hatchery system for freshwater prawns, *Macrobrachum rosenbergii* De Man, 1879. J Shellfish Res. 1992; 11:65-73.
7. Ostrensky A, Wasielesky Jr W. Acute toxicity of ammonia to various life stages of the sao paulo shrimp, *Penaeus paulensis* Perez-Farfante, 1967. Aquacult. 1995; 132:339-347.
8. Miranda-Filho KC, Leaes Pinho GL, Wasielesy Jr W, Bianchini A. Long term ammonia toxicity to the pink-shrimp *Farfantepenaeus paulensis*. Comp Biochem Physiol. 2009; 150C:377-382.
9. Guarner F, Schaafsma G. Probiotics. Int J Food Microbiol. 1998; 39:237-238.
10. Kalalou I, Zerdani I, Faid M. Antagonistic action of biopreservative *Lactobacillus plantarum* strain on pathogenic *E. coli* 0157: H7 in fresh camel meat stored at 10C. J Dairy Food Sci. 2010; 5:7-13.
11. Winkler LW. Die Best immung des in Wasser gelosten Sauerstoffen. Ber Dtsch Chem Ges. 1888; 21:2843-2855.
12. Solorzano L. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr. 1969; 14:799-801.
13. AOAC. Official Methods of Analysis. 16th ed. AOAC International Publishers, Arlington VA. 1995.
14. Tekinay AA, Davies SJ. Dietary carbohydrate level influencing feed intake, nutrient utilization and plasma glucose concentration in the rainbow trout, *Oncorhynchus mykiss*. Turkish J Vet Anim Sci. 2001; 25:657-666.
15. Lowry OH, Rosenbrough WJ, Fair AL, Randall RJ. Protein measurement with the Folinphenol reagent. J Biol Chem. 1951; 193:265-275.
16. Moore S, Stein WH. Photometric ninhydrinmethod for use in the chromatography of amino acid. J Biol Chem. 1948; 176:367-388.
17. Roe JH. The determination of sugar and blood and spinal fluid with anthrone reagent. J Biol Chem. 1955; 212:335-343.
18. Folch J, Lees M, Bloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957; 266:497-509.
19. Suralikar V, Sahu NP. Effect of feeding probiotic (*Lactobacillus cremoris*) on growth and survival of *Macrobrachium rosenbergii* post larvae. J Appl Animal Res. 2001; 20:117-124.
20. Venkat HK, Sahu NP, Jain KK. Effect of feeding *Lactobacillus* based probiotics on the gut micro-flora, growth and survival of post larvae of *Macrobrachium rosenbergii* (de Man). Aquacult Res. 2004; 35:501-507.
21. Keysami MA, Saad CR, Sijam K, Daud HM, Alimon AR. Effect of *Bacillus subtilis* on growth development and survival of post larvae *Macrobrachium rosenbergii* (De Man). Aquacult Nutr. 2007; 13:131-136.
22. Deeseenthum S, Leelavatcharams V, Brook DJ. Effects of feeding *Baciilus* spp. as probiotic bacteria on growth of giant freshwater prawn (*Macrobrachium rosenbergii*, De Man). Pakistan J Biol Sci. 2007; 10:1481-1485.
23. Shinde AN, Mulye VB, Chogale ND, Bhatkar VR, Bondre RD, Mohite AS. Effect of different probiotics on *Macrobrachium rosenbergii* (De Man) post larvae. Aquacult. 2008; 9:7-12.
24. Saad AS, Habashy MM, Khadiga SM. Growth response of the freshwater prawn, *Macrobrachium rosenbergii* (De Man) to diets having different levels of Biogen. World Appl Sci J. 2009; 6:550-556.
25. Rinisha K, Mujeeb Rahiman KM, Razia Beevi M, Thomas AP, Mohamed Hatha AA. Probiotic Effects of *Bacillus* spp. on the growth and survival of post larvae of *Macrobrachium rosenbergii*. Fish Tech. 2010; 47:173-178.
26. Mujeeb Rahiman KM, Jesmi Y, Thomas AP, Mohamed Hatha AA. Probiotic effect of *Bacillus* NL110 and *Vibrio* NE17 on the survival, growth performance and immune response of *Macrobrachium rosenbergii* (De Man). Aquacult Res. 2010; 41:120-134.
27. Seenivasan C, Bhavan PS, Radhakrishnan S. Effect of probiotics (BinifitTM) on survival, growth, biochemical constituents and energy budget of the freshwater prawn *Macrobrachium rosenbergii* post larvae. Elixir Aquacult. 2011; 41:5919-5927.
28. Seenivasan C, Bhavan PS, Radhakrishnan S, Shanthi R. 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*. Turkish J Fish Aquat Sci. 2012a; 12:23-31.
29. Seenivasan C, Bhavan PS, Radhakrishnan S, Muralisankar T. Effects of probiotics on survival, growth and biochemical constituents of freshwater prawn *Macrobrachium rosenbergii* post larvae. Turkish J Fish Aquat Sci. 2012b; 12:331-338.
30. Seenivasan C, Radhakrishnan S, Muralisankar T, Bhavan PS. Influence of combined probiotics *Lactobacillus sporogenes* and *Bacillus subtilis* on survival, growth, biochemical changes and energy utilization performance of *Macrobrachium rosenbergii* (De Man 1879) post larvae. J Ecobiotechnol. 2012c; 4:29-34.
31. Seenivasan C, Radhakrishnan S, Muralisankar T, Bhavan

PS. Efficacy of probiotics on survival, growth, biochemical changes and energy utilization performance of *Macrobrachium rosenbergii* (De Man 1879) post larvae. J Sci Res. 2012d; 4:729-740.
32. Seenivasan C, Bhavan PS, Radhakrishnan S, Muralisankar T, Immanuel G, Srinivasan V *et al.* Effect of *Saccharomyces cerevisiae* on survival, growth, biochemical constituents and energy utilization in the prawn *Macrobrachium rosenbergii*. Int J Appl Biol Pharma Tech. 2013; 4:39-47.
33. Seenivasan C, Radhakrishnan S, Shanthi R, Muralisankar T, Bhavan PS. Influence of probiotics on survival, growth, biochemical changes and energy utilization performance of *Macrobrachium rosenbergii* post-larvae. Proc Zool Soc DO. 2014a. 10.1007/s12595-014-0097-4.
34. Seenivasan C, Radhakrishnan S, Shanthi R, Muralisankar T, Bhavan PS. Effect of *Lactobacillus sporogenes* on survival, growth, biochemical constituents and energy utilization of freshwater prawn *Macrobrachium rosenbergii* post larvae. J Basic Appl. Zool. 2014b. DOI: http://dx.doi.org/10.1016 /j.jobaz.2013.12.002.
35. Seenivasan C, Radhakrishnan S, Muralisankar T, Bhavan PS. Effects of probiotics on survival, growth and digestive enzymes activities in freshwater prawn *Macrobrachium rosenbergii* (De Man 1879). Proc Zool Soc. 2014c. DOI: 10.1007/s12595-014-0123-6.
36. Zarif BR, Azin M. Identifying probiotics of *Macrobrachium rosenbergii's* intestine micro flora and their effect in prawn's growth and survival. Adv Life Sci. 2014; 4:123-130.
37. Jayanthi L, Bhavan PS, Srinivasan V, Muralisankar T, Manickam N. Probiotics product (LactoBacil®$_{plus}$) on improvement of survival, growth, digestive enzymes activity, nutritional status and gut microflora of the prawn *Macrobrachium rosenbergii*. Int J Curr Res. 2015a; 7:11440-11453.
38. Jayanthi L, Bhavan PS, Srinivasan V, Muralisankar T, Manickam N. Dietary supplementation of probiotics product (ViBact*) on the survival, growth, biochemical constituents and gut microflora of the giant freshwater prawn *Macrobrachium rosenbergii* post-larvae. Asian J Biochem Pharma. 2015b; 2:67-88.
39. Prasuna Devi DV, Hareesh K, Srinivasulu Reddy M. Studies on the proximate composition of tropical freshwater prawn *Macrobrachium rosenbergii*. Int J Fish Aquat Stud. 2014; 3:329-336.
40. Dalmin G, Kathiresan K, Purushothaman A. Effect of probiotics on bacterial population and health status of shrimp in culture pond ecosystem. Indian J Exp Biol. 2001; 39:939-942.
41. Ngan PTT, Phu TQ. Effects of *Bacilllu*s bacteria (B8, B37, B38) on water quality of black tiger shrimp (*Penaeus monodon*) cultured tanks. Proceedings of the 4th aquaculture and fisheries conference. 2011, 28-41.
42. Salim SS, Vijula K, Andrews SR. Fishery, Probiotics - Boon for Aquaculture. Natural Product Radiance. 2005; 4:53.
43. Decamp O, Moriarty DJW, Laven P. Probiotics for shrimp larviculture: review of field data from Asia and Latin America. Aquacult Resv. 2008; 39:334-338.
44. Talpur AD, Memon AJ, Khan MI, Ikhwanuddin M, Danish Daniel MM, Abol-Munafi AB. Inhibition of pathogens by lactic acid bacteria and application as water additive multi isolates in early stages larviculture of *P. pelagicus* (Linnaeus, 1758). J Anim Plant Sci. 2012; 22:54-64.
45. Yu MC, Li ZJ, Lin HZ, Wen GL, Ma S. Effects of dietary medicinal herbs and *Bacillus* on survival, growth, body composition, and digestive enzyme activity of the white shrimp *Litopenaeus vannamei*. Aquacult Int. 2009; 17:377-384.
46. Fernandez R, Sridhar M, Sridhar N. Effect of Lactic acid bacteria administered orally on growth performance of *Penaeus indicus* (H. Milne Edwards) juveniles. Res. J. Microbiol. 2011; 6:466-479.
47. Rengpipat S, Rukpratanporn S, Piyatiratitivorakul S, Menasaveta P. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). Aquacult. 2011; 191:271-288.
48. Immanuel G, Menethira V, Beena S, Palavesam A. Effect of probionts on the growth, food utilization and biochemical changes in pearl spot *Etroplus suratensis* (Bloch). Indian J Fish. 2003; 50:273-278.
49. Ahilan B, Shine G, Santhanam R. Influence of probiotics on the growth and gut microbial load of juvenile goldfish (*Carassius auratus*). Asian Fish Sci. 2004; 17:271-278.
50. Bagheri T, Hedayati S, Yavari V, Alizade M, Farzanfar A. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turkish J Fish Aquat. 2008; 8:43-48.
51. Suzer C, Coban D, Kamaci OH, Saka S, Firat K, Otgucuoglu O *et al. Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: Effects on growth performance and digestive enzyme activities. Aquacult. 2008; 280:140-145.
52. Nayak SK, Mukherjee SC. Screening of gastrointestinal bacteria of Indian major carps for a candidate probiotic species for aquaculture practices. Aquacult Res. 2011; 42:1034-1041.
53. Mohapatra S, Chakraborty T, Prusty AK, Das P, Paniprasad AK, Mohanta KN. Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquacult. Nutr. 2011; 18:1-11.
54. FAO. State of world aquaculture 2006. FAO fisheries technical paper. Rome. 2006, 500.