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Zoology

Performance of Probiotic Bacterium, *Enterococcus gallinarum* Enriched *Artemia franciscana* Nauplii on Survival, Growth and Basic Biochemical Constituents of the Prawn *Macrobrachium rosenbergii* Post Larvae

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Abstract **Original Research Article**

This work was emphasized the growth performance of the prawn, *Macrobrachium rosenbergii* post larvae (PL) fed with a probiotic bacterium, *Enterococcus gallinarum* enriched *Artemia franciscana* nauplii at five different serially diluted concentrations (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-9}). A 45 day, feeding trial, showed significantly ($P<0.05$) increased survival rate, growth rate, contents of total protein, amino acid, carbohydrate and lipid, and activities of digestive enzymes (protease, amylase and lipase) in test prawns, particularly at 847×10^{-7}, CFU. At this concentration, the consortium of the gut microflora of unenriched *Artemia* nauplii fed prawn showed the presence of *E. coli*; *Klebsiella* sp., *Citrobacter* sp., *Acinetobacter* sp., *Streptococcus* sp., *Bacillus* sp., *Staphylococcus* sp., and *Pseudomonas* sp. But in *E. gallinarum* enrich *Artemia* nauplii fed PL showed the presence of *Enterococcus* sp., *Streptococcus* sp., *E. coli*, *Bacillus* sp., and *Klebsiella* sp. Thus, it was found the pathogenic bacteria, *Citrobacter* sp., *Acinetobacter* sp., *Staphylococcus* sp., and *Pseudomonas* sp., were replaced due to the colony establishment of *Enterococcus* sp., in test prawns. Hence, *E. gallinarum* can be used as a probiotic for sustainable culture of *M. rosenbergii.*

Keywords: Prawn, *E. gallinarum*, *Artemia,* growth, survival, protein.

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INTRODUCTION

The production of marine and freshwater crustaceans contributes 17.3% and 4.5%, respectively [1] in world aquaculture. Crustaceans possess physiological and/or behavioral adaptation strategies to survive under a plethora of conditions and these adaptations may be specific for the species, feeding habits, gender, food availability and molting cycle [2]. The giant river prawn, *Macrobrachium rosenbergii*, the largest and fastest growing prawn species, is cultured either under monoculture or polyculture with major carps. Commercially important freshwater prawns in India are *M. rosenbergii, Macrobrachium malcolmsonii* and *Macrobrachium gangeticum*.

The probiotics as living microbial cells added as dietary supplements for aquaculture. These beneficial bacteria preferably displace pathogenic bacteria through competitive [3-5]. *Enterococci* belong to the lactic acid bacteria have been used widely in the food industry as probiotics [6, 7]. Some strains of *Enterococcus faecium* and *Enterococcus faecalis* are used as probiotics and are ingested in high numbers, generally in the form of pharmaceutical preparations. In human, these probiotics are administered to treat diarrhoea, antibiotic-associated diarrhoea or irritable bowel syndrome, to lower cholesterol levels, to improve host immunity etc., [8]. Furthermore, Enterococcus genus is isolated from the intestine of common carp and fresh water prawn *M. rosenbergii* [9]. In the present study, *Artemia franciscana* nauplii was enriched with *Enterococcus gallinarum* and fed to *M. rosenbergii* PL as a live feed for assessing their survival rate, growth performance, content of basic biochemical constituents and activities of digestive enzymes, protease, lipase and amylase.

MATERIALS AND METHODS

Procurement of *Enterococcus gallinarum* (7049) and its sub culture

The lyophilized powder of *E. gallinarum* (MTCC 7049) was purchased from Microbial Type Culture Collection (MTCC), Chandigarh, India. It was subjected to sub-culture with Nutrient broth (Hi-media, India, pH, 6.5 at Temperature, 25 °C), contained peptic digestion of animal tissues (5 g L^{-1}), Beef extract (1.5 g L^{-1}), Sodium chloride (5.0 g L^{-1}), and yeast extract (1.5 g L^{-1}). The culture medium was prepared and treated according to the manufacturer's protocol. The medium (13 g) was

mixed with 1000 mL of distilled water, enclosed in a screw cap container and autoclaved at 121°C for 15 minutes. The probiotic *E. gallinarum* was inoculated into the broth and it was incubated for 24 hours at 37°C in a shaking incubator for its growth activity. The clear broth turned into turbid, which indicates the growth of *E. gallinarum* (Figure 1). After incubation, the *E. gallinarum* cells were harvested by centrifugation (5000 rpm, 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. 30µl of suspension was spread over the agar plate. The appearance of white colonies was observed (Figure. 2). The broth was serially diluted up to 10^{-9} and 20 µl was spread on nutrient agar to enumerate the CFU in each dilution in order to optimize it, which showed presence 2078 colonies at 10^{-1} dilution, 1774 at 10^{-3}, 1061 at 10^{-5}, 847 at 10^{-7}, and 547 at 10^{-9}.

Fig-1: Mother culture morphology of *E. gallinarum*

Fig-2: Spread plate culture morphology of *E. gallinarum* on nutrient agar

Feed preparation

The micro pulverized and sieved (0.3mm) basal ingredients, such as fishmeal (25%), groundnut oil cake (25%), and soybean meal (25%) were used as protein sources; wheat bran (10%) was used as carbohydrate source. These ingredients were taken at different ratio based on Pearson's square method to maintain 40% protein level and thoroughly mixed. The mixed feed ingredients were steam cooked for 15 min at 95-100°C and allowed to cool at room temperature. In such a way diets were prepared. Vitamin B complex with vitamin C (1%) was added in the form of BECOSULES CAPSULES (Pfizer Ltd., Mumbai, India). 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. Tapioca flour (5%) and egg albumin (7%) were used as binding agents, and sunflower oil (2%) was added as lipid source. Dough was prepared with 10% boiled water and pelletized in a manual pelletizer fixed with 3mm diameter and pellets were collected in aluminum trays. Then the feed was dried under room temperature until the moisture content reached less than 10%. The feed was physically examined for visual appearance, such as uniformity, color and fragrance. The pellets were with smooth surface. The prepared feed was subjected for the analyses of proximate compositions and mineral contents (Table 1).

Table-1: Proximate compositions and mineral contents in the basal diet formulated

Proximate composition	**(%)**
Crude protein	46.79
Total Nitrogen-free extract	32.21
Ether extract (Crude fat)	6.19
Crude fiber	1.33
Ash	6.81
Moisture	9.84
Gross energy	4443 kcal/kg
Minerals	
Sand and silica (Acid insoluble ash)	0.88
Calcium	0.80
Phosphorus	0.90
Iron	0.11
Copper	0.002
Salt	0.58

Enrichment of *Artemia* nauplii with *E. gallinarum*

The brine shrimp, *A. franciscana* cysts were purchased from Aqua world, Paris Corner, Chennai, India. The cysts (2 g/ 20 L and 15 g kg^{-1} body biomass of the prawns for feeding trial) were taken and hydrated in 1 L^{-1} of purified artificial saltwater (prepared from artificial sea salt powder 35.0 g L^{-1}, pH of 6.5). After 12-15 h, the cysts burst, and the embryo surround by the hatching membrane become visible. After a few hours, the brownish orange colored nauplii came out. The 48-hr old *Artemia* nauplii were filtered and transferred to 1 L capacity glass beaker. Five such groups were enriched with $2078x10^{-1}$, $1774x10^{-3}$, $1061x10^{-5}$, $847x10^{-7}$, and $547x10^{-9}$ concentrations of *E. gallinarum* for 1 hour (Fig.3). Then they were washed with freshwater and fed to *M. rosenbergii* PL.

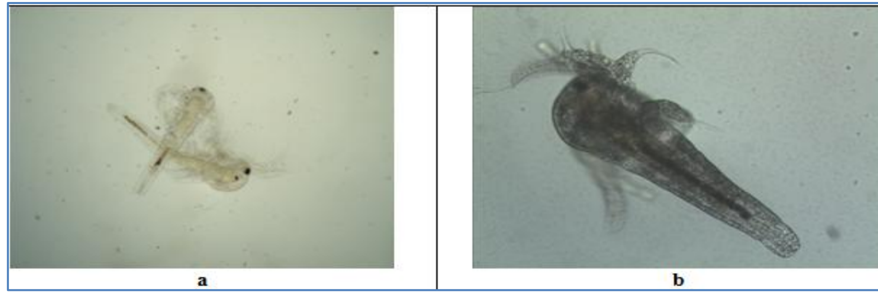

Fig-3: 24 hrs. old *A. franciscana* nauplii. a, Un-enriched; b, Enriched with *E. gallinarum*

Procurement and acclimatization of experimental animal

The post larvae (PL-8) of the freshwater prawn, *M. rosenbergii* were procured from a prawn culture nursery pond, Marakkanam, Chennai, India. They were transported to the laboratory in polythene bags filled with oxygenated water. The prawns were acclimatized to the ambient laboratory condition in cement tanks with ground water for two weeks. The diluent control (ground water) had these physicochemical parameters: Temperature (°C), 28±2.2; pH, 7.4±0.10; TDS (g/L), 0.94±0.05; DO (mg/L), 4.25±0.25; Salinity (mg/L), 0.70±0.02; EC (Ms/cm), 1.01±0.01 and Ammonia (mg/L), 0.028±0.006. During acclimatization the prawns were fed with boiled egg albumin and artificially formulate feed (our laboratory prepared feed). More than 50% of tank water was routinely renewed every day in order to maintain a healthy environment and aeration was provided. This ensures sufficient oxygen supply to the prawns and an environment devoid of accumulated metabolic wastes. The unfed feeds, faecal material, exuvia and moults, and dead prawns if any were removed by siphoning without disturbing the prawns.

Feeding trail

The PL of *M. rosenbergii* ranged from 1.1±0.05cm in length and 0.05±0.003 g of body mass were taken. They were divided into seven groups, PL fed with artificial feed (group 1) PL fed with unenriched *Artemia* nauplii (group 2), and groups 3-7, PL fed with *E. gallinarum* ($2078x10^{-1}$, $1774x10^{-3}$, $1061x10^{-5}$, $847x10^{-7}$, and $547x10^{-9}$) enriched *Artemia* nauplii. Each group consist of 30 individuals filled with 25L of ground water. The water medium was renewed every 24 hrs by siphoning method without severe disturbance to the prawn and aerated adequately. During feeding trial, the feces and moult were removed while renewing aquarium water. The experiment was extended for a period of 45 days. At the end of the feeding trial the final length and weight were measured for calculating nutritional indices and estimating basic biochemical constituents. The water medium was aerated adequately.

Calculation of nutritional indices

The survival rate (SR), length gain (LG), weight gain (WG), and specific growth rate (SGR) were individually calculated Tekinay & Davis, [10].

Survival (%) = Total No. of live animals/Total No. of initial animals× 100

Length gain (cm) = Final length (cm) – Initial length (cm)

Weight gain (g) = Final weight (g) – Initial weight (g)

Specific growth rate, (%) = log W2 – log W1/ t ×100

Where, W1 & W2 = Initial and Final weight respectively (g), and t = Total number of experimental days.

Estimations of basic biochemical constituents

The initial and final concentrations of basic biochemical constituents, such as total protein, amino acid and carbohydrate were estimated in test prawns adopting the methodology of Lowry *et al*. [11], Moore & Stein, [12] and Roe, [13], respectively, and the total lipid was extracted gravimetrically following the method of Folch *et al*. [14] and estimated by adopting the method of Barnes & Blackstock, [15]. The contents of ash and moisture were analysed by following AOAC [16] methodology.

Assays of digestive enzymes activities

Activities of digestive enzymes (protease, amylase and lipase) were assayed at 45th day of feeding trial. The digestive tract of three prawns from each replicate were carefully dissected out and homogenized in ice-cold distilled water and centrifuged at 9000 g under 4ºC for 20 min. The supernatant was used as a source of crude enzyme. Total protease activity was determined by casein-hydrolysis method of Furne *et al*. [17], where one unit of enzyme activity represented the amount of enzyme required to liberate 1 μg of tyrosine per minute. Amylase activity was determined according to Bernfeld [18], the specific activity of amylase was calculated as milligrams of maltose liberated per gram of starch per hour (mg/g/h). Lipase activity was assayed by the method of Furne *et al*. [17], one unit of lipase activity was defined as the amount of free fatty acid released from triacylglycerol per unit time.

Gut microbial colonization

The gut of control prawns and the gut of experimental prawns fed with the best concentration of *E. gallinarum* ($847x10^{-7}$) were subjected to bacterial culture. The prawns were deactivated by keeping them in

freezer at 20 ºC for 10 minutes. Then the surface was sterilized with 50 ppm formalin for 30 seconds in order to remove the external flora. Then the digestive tract was dissected out individually and homogenized with phosphate buffered saline (pH, 7.2) under aseptic condition. Afterwards the homogenates were serially diluted up to 10^{-7} dilution individually. From this 0.5 mL of aliquots were taken and mixed with agar nutrient broth for 24 h at 35 ºC. 0.1 ml of broth culture was seeded over the surface of freshly prepared nutrient agar plates and incubated at 37 ºC for 24 h. The different bacterial colonies were identified and they were confirmed through routine bacteriological tests Holt *et al*. [19]. The following tests, such as Gram's staining, motility test, indole test, methyl red test, Voges Proskauer test, citrate utilization test, starch hydrolases, gelatin hydrolases, nitrate reduction test, oxidase test, catalase test and carbohydrate fermentation test were performed. The bacterial colony was enumerated with the formula, Bacteria count (CFU/ g) = Number of colonies × Dilution factor/ Volume of sample (g).

STATISTICAL ANALYSIS

Data between control versus experiments and between experiments were subjected to statistical analysis through one-way ANOVA and subsequent post-hoc multiple comparison with DMRT by adopting SPSS (v20). All the details of statistical analyses were given in respective tables. The *P* values less than 0.05 were considered as statistically (95%) significant.

RESULTS AND DISCUSSION

Survival rate and nutritional indices

The survival rate (SR), and growth performance (LG, WG and SGR) were found to be significantly (P<0.05) higher in serially diluted (2078x10^{-1}, 1774x10^{-3}, 1061x10^{-5}, 847x10^{-7}, and 547x10^{-9}) *E. gallinarum* enriched *Artemia* nauplii fed to prawns when compared with un-enriched *Artemia* and the PL fed with pelletized feed. Among different concentrations of *E. gallinarum,* 847x10^{-7} produced the best growth performance (Table 2; Fig. 2).

Mahmood *et al.* [20] observed that *Artemia* enriched with symbiotic bacteria *Pediococcus acidilactici* and *Fructooligo saccharide* fed Angelfish *(Pterophyllum scalare)* juvenile improves growth performance. Salem Ahmed *et al.* [21] reported in European sea bass (*Dicentrarchus labrax*) that significantly (p<0.05) higher total length gain and survival, growth rate when treated with green water plus marine probiotic bacteria (G+MP) enriched rotifers (*Brachionus plicatilis*) and *Artemia* nauplii. Bhavan *et al.*[22] reported that *spirulina* enrichment *Artemia* has produced better growth in *M. rosenbergii* than that of *Yeast* enriched *Artemia*. Similarly, Seenivasan *et al*. [23, 24] reported in *M. rosenbergii* post larvae fed with bio-encapsulated diet containing *L. sporogenes*, and Binifit™) [23]. When parrot fish fed with the diet containing *B. subtilis* E20 10^8 CFU kg^{-1} resulted in a significantly increased weight gain. This could have been due to the nutrient supplements and exo enzymes provided by *B. subtilis* E20 [25].

Table-2: Survival and growth of *M. rosenbergii* PL fed with pellet feed, un-enriched and *E. gallinarum* enriched *Artemia* nauplii for 45 days.

Parameter	Pelletized feed	PL fed with Un-enriched *Artemia* nauplii	PL fed with *E. gallinarum* enriched *Artemia* nauplii					F-value
			2078x10^{-1} CFU	1774x10^{-3} CFU	1061 x10^{-5} CFU	847x10^{-7} CFU	547x10^{-9} CFU	
SR (%)	71.77±1.92^{f}	76.66±3.37^{e}	83.33±3.33^{d}	86.33±3.33^{c}	90.33±3.33^{ab}	**92.22±1.92^{a}**	91.11±1.92^{ab}	26.46
Length(cm)	1.33±0.06^{f}	1.67±0.06^{e}	1.86±0.11^{d}	2.00±0.10^{c}	2.48±0.20^{b}	**3.28±0.10^{a}**	2.33±0.05^{bc}	101.30
Weight (g)	0.49±0.07^{e}	0.72±0.05^{cd}	0.94±0.05^{c}	1.23±0.11b^{c}	1.39±0.07^{b}	**1.60±0.32^{a}**	1.32±0.04^{bc}	24.34
LG (cm)	0.33±0.11^{f}	0.66±0.11^{e}	0.86±0.15^{cd}	1.00±0.01b^{c}	1.48±0.18^{b}	**2.28±0.19^{a}**	1.33±0.11^{b}	61.76
WG (g)	0.45±0.06^{e}	0.67±0.05^{d}	0.89±0.06^{c}	1.18±0.28^{b}	1.35±0.37^{b}	**1.56±0.31^{a}**	1.27±0.03^{ab}	25.16
SGR (%)	2.65±0.06^{d}	2.81±0.09^{c}	2.93±0.15^{bc}	3.05±0.09^{ab}	3.10±0.13^{ab}	**3.17±0.11^{a}**	3.09±0.11^{ab}	7.62

Initial morphometric data: 1.1±0.05cm length; 0.05±0.006 g weight.
Each value is mean ± SD of three 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).
SR, survival rate; LG, length gain; WG, weight gain, SGR, specific growth rate

Activities of digestive enzymes and contents of basic biochemical constituents

Activities of digestive enzymes, such as protease, amylase and lipase, and the concentrations of basic biochemical constituents, such as total protein, amino acids, carbohydrate, and lipids were found to be significantly (P< 0.05) elevated in *E. gallinarum* enriched *Artemia* nauplii fed prawns when compared with un-enriched *Artemia* and artificial pelletized feed**.** Among the different concentrations, 847x10^{-7} *E. gallinarum* enriched *Artemia* nauplii produced significantly the best performance than that of other concentrations (Table 3).

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Table-3: Concentrations of biochemical constituents and activities of digestive enzymes in *M. rosenbergii* PL fed with pelletized feed, un-enriched and *E. gallinarum* enriched *Artemia* nauplii for 45 days.

Parameter		Pelletized feed	PL fed with Un-enriched *Artemia* nauplii	PL fed with *E. gallinarum* enriched *Artemia* nauplii					F-value
				$2078x10^{-1}$ CFU	$1774x10^{-3}$ CFU	1061 $x10^{-5}$ CFU	$847x10^{-7}$ CFU	$547x10^{-9}$ CFU	
Biochemical constituents (mg/g wet wt.)	Protein	38.64± 0.64^{e}	45.44± 1.15^{e}	60.59± 1.52^{d}	65.89± 9.12^{d}	82.27± 1.29^{b}	**104.86± 1.54^{a}**	67.23± 1.28^{c}	89.77
	Amino acid	21.69± 1.68^{e}	24.00± 1.19^{d}	32.93± 1.04^{c}	44.24± 2.6^{b0}	53.43± 1.60^{b}	**64.61± 1.21^{a}**	51.38± 0.71^{b}	31.58
	Carbo-hydrate	12.28± 0.72^{d}	14.69± 1.50^{e}	17.99± 0.61^{c}	20.60± 1.82^{b}	24.80± 0.98^{ab}	**29.97± 0.99^{a}**	22.32± 0.14^{b}	30.26
	Lipid	5.18± 0.24^{d}	6.92± 0.36^{e}	8.72± 0.23^{c}	10.84± 0.23^{c}	13.20± 0.23^{b}	**15.47± 0.50^{a}**	12.04± 0.22^{c}	11.93
Digestive enzymes (U/ mg protein)	Protease	1.44± 0.04^{f}	1.53± 0.07^{e}	1.84± 0.05^{d}	1.95± 0.1^{c}	2.45± 0.09^{b}	**2.68± 0.12^{a}**	2.27± 0.08^{b}	325.71
	Amylase	0.62± 0.08^{e}	0.74± 0.05^{f}	0.92± 0.1^{d}	1.23± 0.08^{c}	1.38± 0.2^{b}	**1.58± 0.06^{a}**	1.22± 0.11^{c}	58.00
	Lipase*	0.12± 0.03^{f}	0.18± 0.05^{e}	0.28± 0.08^{d}	0.49± 0.07^{d}	0.57± 0.08^{b}	**0.64± 0.07^{a}**	0.51± 0.05^{c}	170.65

Each value is mean ± standard deviation of three individual observations. *, unit×10^{3}
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

Zokaeifar *et al.* [26] reported that *B. subtilis* strains L10 and G1 have the ability to increase digestive enzyme activities in the gastrointestinal tract of shrimp (*Litopenaeus vannamei*) fed with these *B. subtilis* strains L10 and G1 supplemented diets. Digestive enzymes are among the most important factors that influence the efficiency of feed utilization in fish and hence characterization of these enzymes provides important information regarding the digestive capacity of fish to hydrolyze carbohydrates, proteins and lipids in feed ingredients [27]. Suzer *et al*. [28] suggested that the probiotics can influence digestive processes by enhancing the population of beneficial microorganisms, improving the intestinal microbial balance and microbial enzyme activity, consequently improving the digestibility and absorption of food and feed utilization, which was demonstrated in this study by better growth performance and survival rate in treated groups.

Analysis of gut microbial consortium

In the un-enriched *Artemia* fed prawn gut, the presence of *Escherichia coli*, *Klebsiella* sp., *Citrobacter* sp., *Acetinobacter* sp., *Streptococcus* sp., *Bacillus* sp., *Staphylococcus* sp., and *Pseudomonas* sp., were identified through colony morphology and biochemical tests. In the gut of PL fed with $847x10^{-7}$ concentration of *E. gallinarum* enriched *Artemia* produced the presence of *Enterococcus* sp., *E. coli, Bacillus* sp., *Streptococcus* sp., and *Klebsiella* sp., were identified through colony morphology and biochemical tests. Thus, the study concluded that, C*itrobacter* sp., *Acinetobacter* sp., *Staphylococcus* sp., and *Pseudomonas* sp., were competitively excluded by *Enterococcus* sp., Finally, probiotic bacteria *Enterococcus* sp., was identified and confirmed in the gut of prawn (Table 4-5; Fig. 4-5).

Table-4: Confirmative results of biochemical tests for micro flora present in the gut of *M. rosenbergii* PL fed with 847×10^{-7} CFU of *E. gallinarum* enriched *Artemia* nauplii

Test	*Eg*	Un-enriched *Artemia* nauplii fed PL gut								*E. gallinarum* enriched *Artemia* nauplii fed PL gut				
		Ec	*K* sp.	*C* sp.	*A* sp.	*Ste* sp.	*B* sp.	*P* sp.	*Sta* sp.	*E* sp	*Ec*	*B* sp.	*Sta* sp.	*K* sp.
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	-	-	A	+	+	A	+	+	A	-	-	+	A	A
Glucose test	A	A	A	+	+	A	A	A	A	+	A	A	A	A
Lactose test	A	A	A	+	+	A	A	NA	A	+	A	A	A	A
Sucrose test	A	A	A	+	-	A	A	A	A	-	A	A	A	A
Manitol test	A	A	A	+	-	A	A	A	A	-	A	A	A	A
Maltose test	NA	NA	A	+	-	NA	A	A	NA	+	NA	A	NA	A

+, Positive; -, Negative; A, Acid production; NA, No acid production. *Ec*, *Escherichia coli*; *P*.sp. *Pseudomonas* sp.; *Ste*.sp, *Streptococcus* sp.; *K*.sp, *Klebsiella* sp.; *B*.sp, *Bacillus* sp.; *C*.sp, *Citrobacter* sp,; *A*.sp. *Acetinobacter* sp,; *Sta*.sp, *Staphylococcus* sp,; *E*.sp, *Enterococcus* sp,

The bacterial biomass mixed with feed might help in colonization in the intestine to perform competitive exclusion mechanism in shrimp [29], and colonization with specific microbiota may play a role in balancing the intestinal mucosal immune system, which may contribute towards the induction and maintenance of immunological tolerance or inhibition of the deregulated responses induced by pathogens in the host [3]. *Bacillus*, when used as a probiotic, was able to inhibit pathogens by colonizing both the culture water and *P. mondon* and *P. vannamei*, digestive tract to exclude other harmful bacteria producing an anti-bacterial substance or activating both cellar and humoral immune defences [30,31]. For bacteria to be considered probiotic it must be capable of surviving passage through the gastrointestinal tract. It must then colonize the host digestive system, either by adhering to the mucous membrane surface or the intestinal epithelia. Finally, it must be able to produce inhibitory or antagonist metabolites against undesirable native flora, and reproduce [32-34]. Recently a marine bacterial strain, *Pseudomonas* I2, was isolated from estuarine environmental samples that produced inhibitory compounds against shrimp pathogenic vibrios. This antibacterial compound was shown to be of low molecular weight, heat stable, soluble in chloroform, and resistant to proteolytic enzymes [35]. Such a mechanism may be prevailed in the present results as well.

CONCLUSION

In this study, *E. gallinarum* has get colonized in the gut of *M. rosenbergii,* eliminated some pathogenic bacteria and enhanced the survival and growth performance. Therefore, it can be used as a probiotic bacterium for sustainable culture and development of *Macrobrachium.*

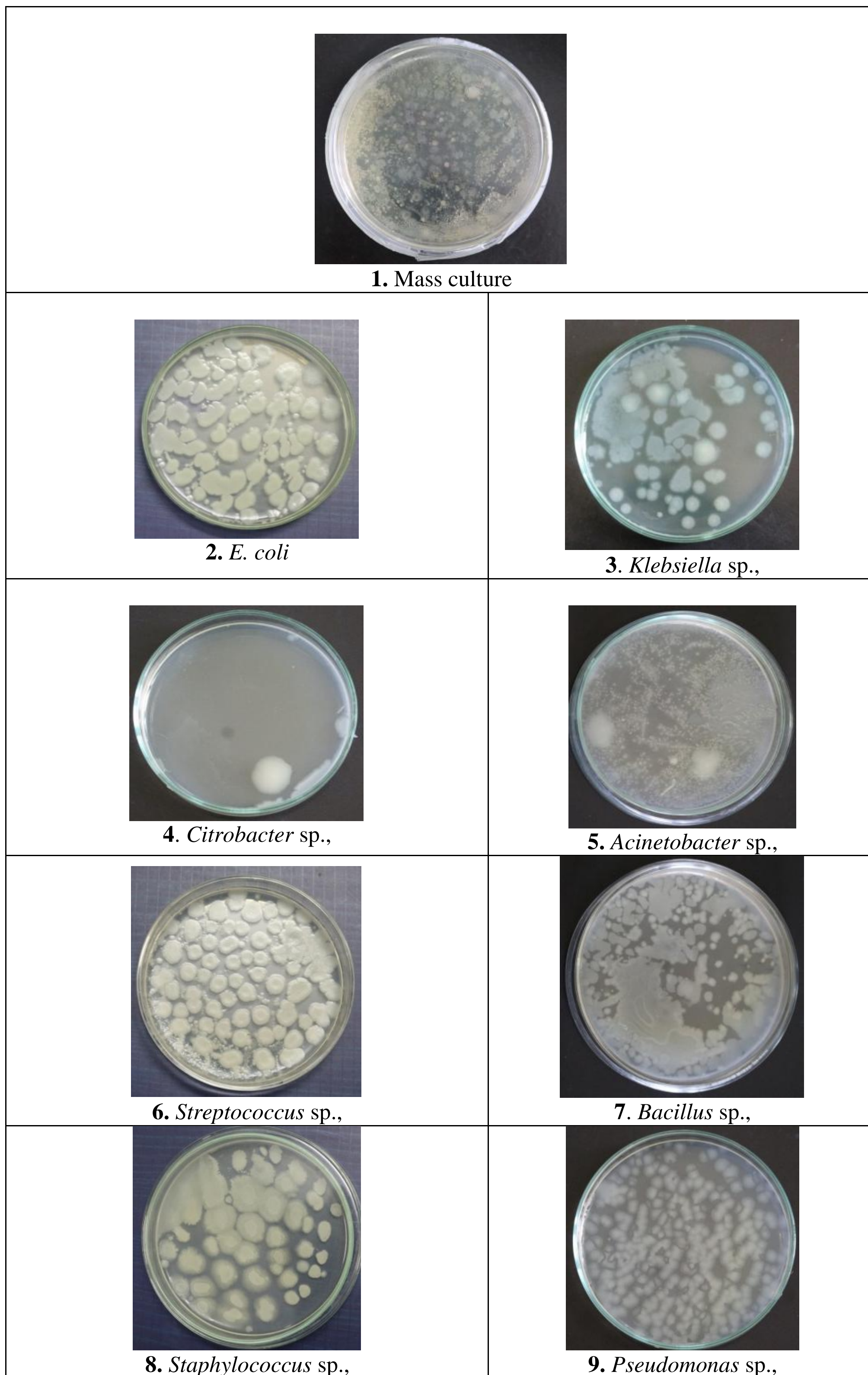
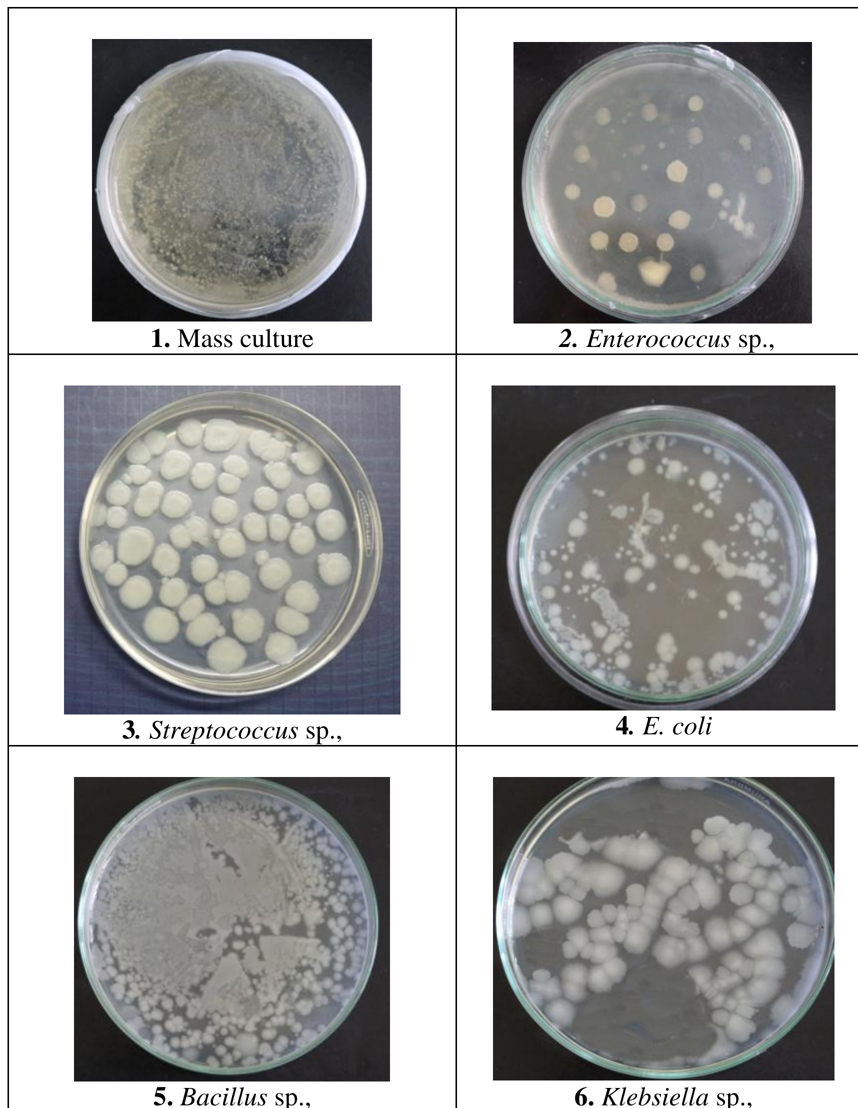


Fig-4: Agar plate morphology of different bacterial culture from the gut of *M. rosenbergii* PL fed with un-enriched *Artemia* nauplii



1. Mass culture | **2.** *Enterococcus* sp.,
3. *Streptococcus* sp., | **4.** *E. coli*
5. *Bacillus* sp., | **6.** *Klebsiella* sp.,

Fig-5: Agar plate morphology of different bacterial culture from the gut of *M. rosenbergii* PL fed with *E. gallinarum* (CFU $847 \mathrm{x} 10^{-7}$) enriched *Artemia* nauplii

Table-5: Bacterial consortium in the gut of *M. rosenbergii* PL fed with un-enriched and $847 \mathrm{x} 10^{-7}$ CFU of *E. gallinarum* enriched *Artemia* nauplii

Samples	Identified species	Composition (%)
Un-enriched *Artemia* nauplii fed PL gut	*E. coli*	10
	Klebsiella sp.,	8
	Citrobacter sp.,	15
	Acinetobacter sp.,	12
	Streptococcus sp.,	11
	Bacillus sp.,	18
	Staphylococcus sp.,	10
	Pseudomonas sp.,	11
	Total	**93**
E. gallinarum enriched *Artemia* nauplii fed PL gut	*Enterococcus* sp.,	34
	Streptococcus sp.,	10
	E. coli	21
	Bacillus sp.,	21
	Klebsiella sp.,	12
	Total	**97**

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REFERENCES

1. FAO. The State of World Fisheries and Aquaculture (SOFIA): Contributing to food security and nutrition for all, Rome: Food and Agriculture Organization. 2016: 200.
2. Sanchez-Paz A, Garcia-Carreno LF, Muhlia-Almazan A, Hernandez-Saavedra N, Yepiz-Plascencia G. Differential expression of trypsin mRNA in the white shrimp (*Penaeus vannamei*) midgut gland under starvation conditions. Journal of Experimental Marine Biology and Ecology. 2006; 292:1–7
3. Gatesoupe FJ. The use of probiotics in aquaculture. Aquaculture. 1999; 180:147–165.
4. Moriarty DJW. Disease control in shrimp aquaculture with probiotic bacteria. In: Bell CR, Brylinsky M, and Johnson-Green P. (Eds.), Microbial Biosystems: New Frontiers: Proceedings of the 8th International Sympo-sium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.
5. Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S, Lilius EM. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) bypotential probiotic bacteria (*Lactobacillus rhamnosus*). Fish and Shellfish Immunology. 2003; 15:443–452.
6. Fisher K, Phillips C. The ecology, epidemiology and virulence of *Enterococcus*. Microbiology. 2009; 155:1749–1757.
7. Foulquie Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L. The role and application of *Enterococci* in food and health. International Journal of Food Microbiology. 2006; 106:1–24.
8. Franz CM, Huch M, Abriouel H, Holzapfel W, Gálvez A. *Enterococci* as probiotics and their implications in food safety. 2011.
9. Cai YM, Suyanandana P, Saman P, Benno Y. Classification and characterization of lactic acid bacteria isolated from the intestine of common carp and freshwater prawns, Journal of Genetics and Applied Microbiology. 1999; 45:177–184.
10. Tekinay AA, Davies SJ. Dietary carbohydrate level influencing feed intake, nutrient utilisation and plasma glucose concentration in the rainbow trout, (*Oncorhynchus mykiss* Walbaum, 1792. Turkish Journal of Veterinary and Animal Sciences. 2001; 25:657–666.
11. Lowry OH, Rosebrough WJ, Fair AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 1951; 193:265–275.
12. Moore S, Stein WH. Methods in enzymol (Eds: Olowick, Spand Kalpan, ND) Academic press New York.pp. 1984; 468.
13. Roe JH. The determination of sugar and blood and spinal fluid with anthrone reagent. Journal of Biological Chemistry. 1995; 212:335–343.
14. Folch J, Lees M, Bloane-Stanely GH. A Simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 1957; 266:497–509.
15. Barnes H, Blackstock J. Estimation of lipids in marine animals and tissues. Detail investigation of the sulpho phosphovanillin method for total lipids. Journal of Experimental Marine Biology and Ecology. 1973; 12:103–118.
16. AOAC. 1995. Official methods of analysis of Association of Analytical Communities international. 2 vol. 16 th Edn. Arlington, VA, USA.
17. Furne M, Hidalgo MC, Lopez A, Garcia Gal lego M, Morales AE, Domezain A. Digestive enzyme activities in adriaticsturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss* . A comparative study. Aquaculture. 2005; 250:391–398.
18. Bernfeld P. Amylases, in: Colowick S.P., Kaplan N.O., (Eds.), Methods in Enzymology. Academic Press, New York. 1955:149–158.
19. Holt JG, Krie NR, Sneath PHA, Stately JT, Williams ST. Bergey's Manual of Determinative Bacteriology, 9 th Edn., Baltimore, Williams and Wilkins. 1996; 787.
20. Mahmood A, Saeed M, Nasrollah A, Seyed HH. The effects of feeding with synbiotic (*Pediococcus acidilactici* and *fructooligosac charide*) enriched adult *Artemia* on skin mucus immune responses, stress resistance, intestinal microbiota and performance of angelfish (*Pterophyllum scalare*). Fish & Shellfish Immunology. 2016; 54:516–522.
21. Salem Ahmed Md, Nour AM, Srour TM, Assem SS, Ibrahim HA, El-Sayed HS. Greenwater, Marine *Bacillus subtilis HS1* Probiotic and Synbiotic Enriched *Artemia* and Rotifers Improved European Seabass *Dicentrarchus labrax* Larvae Early Weaning Length Growth, Survival, Water and Bacteriology quality. American Journal of Life Sciences. 2015; 3(6-1):45–52.
22. Bhavan PS. Devi VG. Shanthi R. Radhakrishnan S. Poongodi R. Basic biochemical constituents and profiles of amino acids in the post larvae of *Macrobrachium rosenbergii* Fed with *Spirulina* and Yeast enriched *Artemia*. International Journal of Science and Research. 2010; 2(3):539–547.
23. Seenivasan C, Saravana Bhavan P, Radhakrishnan S. Effect of probiotics (*Binifit*^{TM}) on survival, growth, biochemical constituents and energy budget of the freshwater prawn *Macrobrachium rosenbergii* post larvae. Elixir Aquaculture. 2011; 41:5919–5927.

24. Seenivasan C, Saravana Bhavan P, Radhakrishnan S, Shanthi R. Enrichment of *Artemia* nauplii with *Lactobacillus sporogenes* for enhancing the survival, growth and levels of biochemical of constituents in the post-larvae of freshwater prawn *Macrobrachium rosenbergii.* Turkish Journal of Fisheries and Aquatic Sciences. 2012a; 12:23–31.
25. Liu CH, Chiu CH, Wang SW. Cheng Dietary administration of the probiotic, *Bacillus subtilis* E20, enhances the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*, Fish & Shellfish Immunology. 2012; 33:699–706.
26. Zokaeifar H, Balcazar JL, Saad CR, Kamarudin MS, Sijam K, Arshad A, Nejat N. Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei.* Fish & Shellfish Immunology. 2012; 33:683–689.
27. Lemieux H, Blier P, Dutil JD. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadusmorhua*). Fish Physiology and Biochemistry. 1999; 20:293–303.
28. Suzer C, Çoban D, Okan Kamaci H, Saka Ş, Firat K, Otgucuoğlu O, Küçüksari H. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: Effects on growth performance and digestive enzyme activities, Aquaculture. 2008; 280:140–145.
29. Rengpipat S, Rukpratanporn S, Piyatiratitivorakul S, Menasaveta P. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium *(Bacillus S11*). Aquaculture. 2000; 191:271–288.
30. Rengpipat S, Phianphak W, Piyatiratitivorakul S, Menasveta P. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture. 1998; 167:301–313.
31. Mariel G, Fabiano T, Jenny R. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. Aquaculture. 2004; 233:1–14.
32. Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional gastrointestinal disorders. Gut. 1999; 45:1143–1147.
33. Verschuere L, Rombaut G, Huys G, Dhont J, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture, review. 2000; 64:655– 671.
34. Ziaei-Nejad S, Rezaei MH, Takami GA, Lovett DL, Mirvaghefi AR, Shakouri M. The effect of *Bacillus spp.* bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus.* Aquaculture. 2006; 252:516–524.
35. Chythanya R, Karunasagar I, Karunasagar I. Inhibition of shrimp pathogenic vibrios by a marine Pseudomonas I 2 strain. Aquaculture. 2002; 208:1–10.