International Journal of Research Studies in Science, Engineering and Technology
Volume 5, Issue 12, 2018, PP 9-22
ISSN : 2349-476X

Survival, Growth, Activities of Digestive Enzymes, Concentrations of Basic Biochemical Constituents and Competitive Exclusion of Pathogenic Bacteria in Bacillus Coagulans Supplemented Diet Fed Macrobrachium Rosenbergii Post-Larvae

S. Manjula, P. Saravana Bhavan*, M. Karthik, D. Anitha, R. Kalpana,T. Manjula

Department of Zoology, Bharathiar University, Coimbatore – 641046, Tamil Nadu, India

***Corresponding Author:** P. Saravana Bhavan, Department of Zoology, Bharathiar University, Coimbatore – 641046, Tamil Nadu, India.Email: bhavan@buc.edu.in*

ABSTRACT

This work emphasized the growth of the prawn, Macrobrachium rosenbergii post larvae (PL) supplemented with a probiotic bacterium, Bacillus coagulans at five different serially diluted concentrations (10-1, 10-3, 10-5, 10-7 and 10-9). A 90 days feeding trial with 10-7 (CFU, 2.28x10-7) dilution produced appreciable survival rate, growth and nutritional value in terms of total protein, amino acid, carbohydrate and lipid. This may be due to maintenance of good intestinal health in M. rosenbergii PL, which were evident from the elevated activities of digestive enzymes, protease, amylase and lipase. The analyses of gut microflora revealed the presence of Streptococcus spp., Klebsiella spp., E. coli and Staphylococcus spp., in control PL, and Staphylococcus spp., E. coli, Bacillus spp., and Lactobacillus spp., in the experimental PL fed with B. coagulans supplemented feed. The pathogenic bacteria, Streptococcus spp., and Klebsiella spp., found in the gut of control prawns have competitively been exclude in the gut of experimental prawns.

Keywords: *Probiotics, Prawn, Survival, Growth, Pathogenic bacteria.*

INTRODUCTION

World population is increasing at an exponential rate and consequently the demand for food is also increasing. Aquaculture is the world's fastest growing animal protein production sector for human consumption. The cultivable animal includes fin fish, crustacean, mollusc, amphibian, freshwater turtle, sea cucumber, sea urchin, sea squirt, edible jellyfish etc. The edible crustaceans, such as crab, shrimp, prawn, crayfish and lobster constitute one of the major sources of nutritious food with good quantum of protein. Aquaculture animal can avail their food like, plankton etc., from the natural aquatic environment, but in the case of pond culture, they cannot get the adequate live food. Hence, the cultivable species are offered artificial feeds. The optimum dietary protein levels for the growth of *M.* rosenbergii PL have been reported to be 35-40% [1-5]. The optimum carbohydrate level proposed to be 25-35% [5]. The optimum fat level should be 6-8% [3-5]. The artificial feeds are major concerns for shrimp farmers, representing up to 60% of the total variable production costs [6, 7]. It must fulfil several characteristics, such as properties such as odour, texture, and flavour, and physical properties such as particle size. In addition, the feed should contain all the essential nutrients, such as protein, amino acids, carbohydrate, lipid, fatty acids, fibre, vitamins and minerals at desirable level. The artificial feed should be readily available at low cost, highly digestible with all essential nutrients.

The probiotics as living microbial cells added as dietary supplements, which improve the health of humans and livestock [8]. Consumers need probiotic bacteria in concentrations above 105-106 cells g-1 of food consumed to electively derive health-promoting benefits [9].

The use of beneficial bacteria to displace pathogens through competition is being used in the aquaculture industry as a preferable method instead of administering antibiotics [10, 11]. Several probiotics either as monospecies or multispecies supplements are commercially available for improving growth and disease resistance in aquaculture practices [8, 12-15].

Certainly the use of Bacillus led to increased survival and growth of zoea and mysis phases of white shrimp notably when the probiotic was added only in the water [16]. Bacillus 48 enhanced the survival of larvae, increased food absorption and conferred improved growth on common snook, Centropomus undecimalis [17]. Conversely, there was a lack of success during the 100-day administration in feed of Bacillus S11 as wet or lyophilized cells, or as saline suspensions to penaeids [18]. Mixed cultures with Bacillus improved the performance of the rotifer Brachionus plicatilis in water [19]. Four strains of Bacillus obtained from the digestive tracts of healthy white shrimp were applied to water at a concentration of 1 × 105 CFU ml−1 daily and led to improved health of white leg shrimp larvae [20].

The previous studies have suggested that probiotic supplementation can be reduced disease outbreaks by enhancing the immune system functions in fish, shrimp and prawn [21-27] and can reduce culture costs by improving the growth and feed efficiency. In addition, the application of probiotics can lead to an improvement in water quality [28-32]. In the white shrimp, Litopenaeus vannamei treated with Bacillus P64, Lactobacillus acidophilus RS058, Rhodopseduomonas palustris GH642 and B. coagulans NJ105 as water additives exhibited enhanced growth performance and immune modulatory response [21, 33-35].

B. coagulans is a Gram-positive lactic acid-forming bacterial species within the genus Bacillus. Many factors make B. coagulans a good candidate for probiotic use; it produces organic acids, possesses the capacity to sporulate, and is easily culturable in bulk quantity [36]. It lacks the ability to adhere to the intestinal epithelium, it is completely eliminated in four to five days unless chronic administration is maintained [37]. B. coagulans used as a food ingredient it secretes a bacteriocin, coagulin, which has activity against a broad spectrum of enteric microbes [36]. Mandel et al., [38] reported that B. coagulans used as an adjunct therapy for relieving symptoms of rheumatoid arthritis.

In the view of the above, in the present study, B. coagulans was incorporated at 10-1, 10-3 10-5, 10-7 and 10-9 serially diluted concentrations with formulated diets and fed to M. rosenbergii PL for assessing its ability on survival and growth promotion. Under this aim the following objectives were addressed: (1) To identify an optimum colony formation unit (CFU) of B. coagulans for recommending the aquaculture industry; (2) To evaluate the role of B. coagulans on improvement of nutritional quality of M. rosenbergii, contents of basic biochemical constituents (total protein, amino acid, carbohydrate and lipid) were estimated, and activities of digestive enzymes (protease, amylase and lipase) were assayed; (3) To understand the competitive exclusion ability of B. coagulans over pathogenic bacteria, its colony establishment in the gut of prawns was evaluated, and in-vitro antibacterial activities against Streptococcus spp., and Klebsiella spp., were tested.

MATERIALS AND METHODS

Procurement of B. Coagulans (MTCC 2302) and its Sub-Culture

Pure culture of the probiotic bacterium, *B. coagulans* was procured from Microbial Type Culture Collection (MTCC 2302), Chandigarh, India, in lyophilized powder form. The culture medium was prepared and treated according to manufacturer's protocol (Table 1). The medium (1.3 g) was mixed with distilled water (100 ml) in a screw cap container and autoclaved at 121 °C for 15 minutes.

The broth was later dispensed into 100 ml sterile conical flask. A loop-full of lyophilized powder of *B. coagulans* was inoculated into the broth. The culture flask was incubated for 12 hours at 37°C in a shaking incubator for their growth activity. The clear broth turned into turbid, which indicates the growth of *B. coagulans* (Fig. 1). After incubation, the *B. coagulans* cells were harvested by centrifugation (5000 rpm, 10 min). Actually, the supernatant was filtered by passing through a 0.25 µM silica gel coated membrane (Himedia, India), 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 (Fig. 2).

The broth was serially diluted up to 10^{-9} and seeded on nutrient agar to see the CFU in each dilution in order to optimise it, which showed presence 5.76 colonies at 10^{-1} dilution, 4.58 at

10^{-3}, 2.98 at 10^{-5}, 2.28 at 10^{-7}, and 1.00 at 10^{-9} (Table 2).

Table 1. *Composition of nutrient broth for mass culture and nutrient agar for sub-culture of B. coagulans*

Composition	g / Litre
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar*	12.00
Final pH (at 25 °C)	7.4±0.2

**for plate culture only*

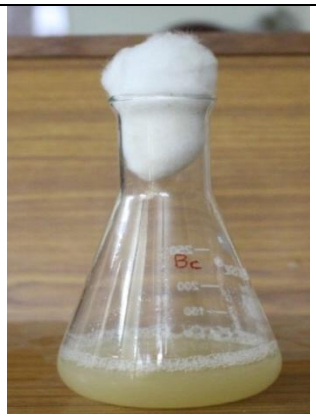

Fig1. *Broth culture morphology of B. coagulans*

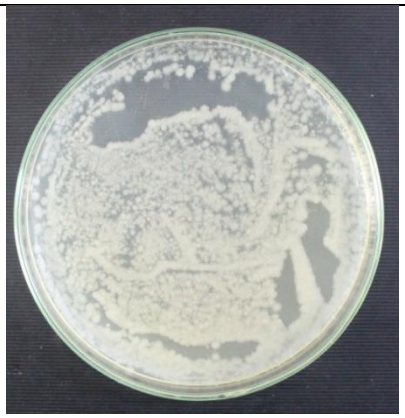

Fig2. *Spread plate culture morphology of B. coagulans on nutrient agar*

Table 2. *CFU of B. coagulans in nutrient agar plate culture at serial dilution*

Serial Dilution	CFU
10^{-1}	$5.76x10^{-1}$
10^{-3}	$4.58x10^{-3}$
10^{-5}	$2.98x10^{-5}$
10^{-7}	$2.28x10^{-7}$
10^{-9}	$1.00x10^{-9}$

CFU, Colony formation unit

Feed Preparation

The branded feed basal ingredients, such as fish meal, groundnut oilcake, soybean meal, wheat bran, tapioca flour, sunflower oil and hen egg were purchased from local merchants at Coimbatore, India. Vitamin B-complex with vitamin-C (Pfizer Ltd., Mumbai, India) was purchased from local medical shop. The micro pulverized and sieved basal ingredients, such as fishmeal (25%), groundnut oil cake (25%), 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 (Table 3).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 (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. Tapioca flour (5%) and Egg albumin (7%), and sunflower oil (2%) were added as binding agents and lipid source respectively. With this, different serially diluted concentrations of *B. coagulans* at 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-9} were incorporated. Dough was prepared with 10% boiled water and

pelletized in a manual pelletizer fixed with 3mm diameter die and pellets were collected in aluminum trays. Then the feed was dried until the moisture content reached less than 10% under room temperature. The feed was physically examined for visual appearance, such as uniformity, color and fragrant. The pellets were with smooth surface. The feed prepared without incorporation of *B. coagulans* was served as control. The prepared feed was subjected to analyses of proximate composition and mineral contents. *B. coagulans* incorporated feeds were prepared once in 15 days in order to maintain its viability.

Table 3. *Proximate compositions and mineral contents in the basal diet formulated*

Proximate compositions (%)	
Crude protein	45.88 [39]
Total Nitrogen-free extract	33.55 [39]
Ether extract (Crude fat)	7.28 [39]
Crude fiber	1.57 [39]
Ash	7.25 [39]
Moisture	11.71 [39]
Gross energy	4395 kcal/kg [40]
Mineral contents (%)	
Sand and silica (Acid insoluble ash)	0.88 [39]
Calcium	0.90 [39]
Phosphorus	0.82 [39]
Iron	175.87 ppm [41]
Copper	25.86 ppm [39]
Salt	0.56 [39]

Viability of B. Coagulans in the Formulated Feeds

B. coagulans incorporated feed (1 g) was taken on day 15 of its formulation and dissolved in autoclaved double distilled water (10 mL). It was serially diluted up to 10^{-7} then 20 µL was spread over nutrient agar medium incubated at 37 °C for 24 h and the colony morphology was observed, which was compared with the original *B. coagulans* sub-culture morphology. The growth of *B. coagulans* was observed in all the culture plates seeded with different feeds prepared by serially diluted *B. coagulans* (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-9}) except control, at 10^{-7} dilution. Therefore, *B. coagulans* was viable in serially diluted feeds even on day-15 after its formulation.

Procurement and Acclimatization of Experimental Animal

The post larvae (PL-18) of the freshwater prawn, *M. rosenbergii* were procured from a prawn culture nursery pond, Singanallur, Coimbatore, 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 (6 × 3 × 3 feet) with ground water for two weeks. The physicochemical parameters of diluents control water are presented in Table 4.

Table 4. *The physico-chemical parameters of the ground water*

Parameter	**Value**	**Method**
Temperature (°C)	23 ± 0.20 °C	Mercury thermometer
pH	7.2 ± 0.20	pH meter
TDS (g/l)	0.97 ± 0.07	[42]
DO_2 (mg/l)	4.10 ± 0.30	[43]
Salinity (mg/l)	0.64 ± 0.01	Water Analysis kit
EC (mS/cm)	1.02± 0.01	Water Analysis kit
Ammonia (mg/l)	0.031 ± 0.007	[44]

TDS, total dissolved solids; DO_2, dissolved oxygen; EC, Electrical conductivity.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, moult and dead prawns were removed by siphoning without disturbing the prawns.

FeedingTrail

M. rosenbergii (≠ PL-35) ranging from 2.86±0.05cm in length and 0.39±0.01g in weight were used in this experiment. The prawns were divided into six experimental groups along with control, each group contained 40 PLs in 35L ground water and the experiment was conduct up to 90 days. In a triplicate experimental setup, five experimental groups were fed with 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-9} serially diluted concentration of *B. coagulans* incorporated diets, respectively. The control group was fed with basal diet formulated without incorporation of *B. coagulans.* The unfed feed, exuvia and moults were siphoned out daily without severe disturbance to the prawn while renewing the water medium. On the 90th day of experiment the survival rate was calculated and the morphometric data, such as the final length and weight were measured for evaluating the nutritional indices. The assays of digestive enzymes activities and estimations of concentrations of basic biochemical constituents were also done.

Calculation of Nutritional Indices

Nutritional indices, such as survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency rate (PER) were determined by following equations [45].

- Survival (%) = Total No. of live animals/Total No. of initial animals × 100
- Weight gain (g) = Final weight (g) – Initial weight (g)
- Specific growth rate, (%) = *log* W_2 – *log* $W_1/t \times 100$
- Where, W_1 & W_2 = Initial and Final weight respectively (g), and t= Total number of experimental days
- Food conversion ratio (g) = Total Feed intake (g)/ Total weight gain of the prawn (g)
- Protein efficiency ratio (g) = Total Weight gain of PL (g)/ Total Protein consumed (g)

Assays of Digestive Enzymes

Activities of digestive enzymes were assayed at '0'day and at 60th day of feeding trial. The digestive tract of three prawns from each replicate were carefully dissected and homogenized in ice-cold distilled water and centrifuged at 9000 g under 4 ºC 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., [46], 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 et al., [47], the specific activity of amylase was calculated as milligrams of maltose liberated per gram of protein per hour (mg/g/h). Lipase activity was assayed by the method of Furne et al., [46], one unit of lipase activity was defined as the amount of free fatty acid released from triacylglycerol per unit time.

Estimations of Basic Biochemical Constituents

The initial and final concentrations of total protein, amino acid and carbohydrate in experimental PL were estimated by adopting the methodology of Lowry et al., [48], Moore & Stein [49] and Roe [50], respectively. The total lipid was extracted by Folch et al., (1957) [51] method, and estimated gravimetrically by Barnes and Blackstock [52] method. The contents of ash and moisture were analysed by following AOAC [39] methodology were estimated.

Gut Microbial Colonization

The gut of control prawns and the gut of experimental prawns fed with the best concentration of *B. coagulans* (10^{-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 homogenate were serially diluted up to 10^{-4} 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 [53]. 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).

Anti-Microbial Activity of B. Coagulans against Pathogenic Bacteria by Diffusion Assay

Two pathogenic bacteria (*Streptococcus* spp., and *Klebsiella* spp.,) were isolated from prawn gut culture and maintained in slant culture. Actually, standardized bacterial suspension was prepared by picking a colony of respective bacteria using sterile wire loop and suspending it in to the 250 ml conical flask with 100 ml of nutrient broths. These pathogenic bacteria were sub cultured on nutrient agar (Himedia-India) plate. The culture media was prepared and treated according to manufacturer's protocol. Agar medium (28 g) was mixed with 100 of distilled water, enclosed in a screw cap container and autoclaved at 121 °C for 15 minutes. The nutrient agar medium (15 ml) was later dispensed into 9.5 cm diameter sterile petri-plates and allowed to solidify. They were seeded with 24 hr culture of bacterial strains *Streptococcus* spp., and *Klebsiella* spp., respectively. Three wells were created with 1 cm diameter and 30µl of prepared *B. coagulans* supernatant was poured in to single well and another well was filled with distilled water as a negative control.

The positive control was placed on each plate with specific disc. The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

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 statistically (95%) significant.

RESULTS AND DISCUSSION

Survival, Growth, Digestive Enzymes and Biochemical Constituents

The SR, WG, SGR and PER were found to be significantly increased (P<0.05) in *B. coagulans* supplemented feeds fed *M. rosenbergii* PL when compared with control. Among different serially diluted concentrations of *B. coagulans*, 10^{-7} (CFU = 2.76 x 10^{-7}) showed the best performance. In the case of FCR was found to be correspondingly decreased in *B. coagulans* supplemented feeds fed PL. Therefore, *B. coagulans* supplemented feed was quality one and produced better growth and survival performances in *M. rosenbergii* PL (Table 5). The activities of digestive enzymes (protease, amylase and lipase) and concentrations of basic biochemical constituents (total protein, amino acid, carbohydrate and lipid) were also found to be improved at 10^{-7} dilution of *B. coagulans* supplemented feed fed *M. rosenbergii* PL (Table 6).

Table 5. *Nutritional indices of M. rosenbergii fed with B. coagulans incorporated feeds*

Parameter	Control	10^{-1}	10^{-3}	10^{-5}	10^{-7}	10^{-9}
SR (%)	78.33±1.44	81.66±1.44	83.33±1.44	85.83±2.88	89.16±3.81	87.50±2.50
Length(cm)	4.03±0.20	4.46±0.05	4.73±0.05	5.33±0.11	5.96±0.23	5.76±0.05
Weight (g)	0.71±0.09	0.98±0.09	1.65±0.04	2.08±0.13	2.84±0.04	2.55±0.04
LG (cm)	1.16±0.23	1.60±0.10	1.86±0.11	2.46±0.15	3.10±0.20	2.90±0.10
WG (g)	0.32±0.10	0.59±0.08	1.26±0.03	1.69±0.12	2.45±0.03	2.16±0.03
SGR (%)	0.30±0.06	0.44±0.03	0.67±0.01	0.77±0.02	0.90±0.01	0.86±0.01
FCR (g)	5.23±0.72	4.15±2.19	2.60±0.06	1.82±0.03	1.06±0.08	1.72±0.02
PER (g)	0.13±0.04	0.91±0.13	1.80±0.04	2.20±0.16	2.72±0.04	2.57±0.04

Initial morphometric data 2.86±0.05 cm length and 0.39±0.05 g weight Each value is mean ± standard deviation 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; FCR, food conversion ratio; PER, protein efficiency ratio.

Table6. *Concentration of biochemical constituents of M. rosenbergii PL fed with B. coagulans incorporated feeds*

	Parameter	Control	10^{-1}	10^{-3}	10^{-5}	10^{-7}	10^{-9}	f-value
Digestive enzymes (U/ mg protein) (*x10^2 U/mg protein)	Protease	3.66± 1.61^{c}	4.43± 0.14^{bc}	4.57± 0.15^{bc}	4.61± 0.21^{bc}	5.92± 0.20^{a}	4.87± 0.18^{b}	18.18
	Amylase	1.40± 0.18^{e}	2.20± 0.04^{d}	2.46± 0.06^{d}	3.63± 0.12^{c}	4.63± 0.10^{a}	3.98± 0.07^{b}	401.59
	Lipase*	0.40± 0.09^{c}	0.76± 0.08^{b}	0.81± 0.04^{b}	0.91± 0.08^{ab}	1.08± 0.09^{a}	0.94± 0.05^{ab}	29.26
Biochemical constituents (mg/g wet wt.)	Protein	76.92± 2.32^{e}	84.36± 1.96^{e}	88.75± 2.71^{d}	94.40± 1.52^{c}	108.70± 2.43^{a}	102.50± 1.62^{b}	104.50
	Amino acid	27.81± 1.26^{f}	38.60± 0.83^{e}	48.93± 1.50^{d}	57.03± 1.58^{c}	72.35± 1.17^{a}	65.56± 0.79^{b}	186.00
	Carbohydrate	16.68± 0.31^{e}	18.89± 0.50^{e}	24.22± 0.83^{d}	28.14± 0.55^{c}	36.95± 0.93^{a}	32.81± 0.79^{b}	121.56
	Lipid	9.34± 0.24^{f}	13.47± 0.59^{e}	19.75± 0.17^{d}	23.55± 0.33^{c}	37.59± 0.48^{a}	28.38± 0.91^{b}	147.48

Each value is mean ± standard deviation 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).

The improved survival and growth performance, activities of digestive enzymes and contents of basic biochemical constituents were also reported in *M. rosenbergii* fed with probiotics (*Lactobacillus cremoris*, Biogen® (*Bacillus subtilis, B. coagulans, Lactobacillus sporogenes, Lactobacillus acidophilus, Lactobacillus plantarumade*), LactoBacil®plus (*Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Saccharomyces boulardii*), ViBact*, Binifit™ (*B. bifidum, Lactobacillus* sp., *L. acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus*), *Lactobacillus brevis, Lactobacillus fermentum, B. coagulans* and *B. subtilis* [24-27, 54-58]. The elevated activities of digestive enzymes and consequent improvement in survival, growth and nutritional quality have also been reported in *M. rosenbergii* due to *B. coagulans* [59], in *L. vannamei* due to *Clostridium butyricum* [60] and in *Fenneropenaeus indicus* due to *Bacillus* sp., [61]. An increase in the carcasses biochemical proximate composition have been reported in *M. rosenbergii* fed with *L. sporogens* and *L. acidophilus, B. subtilis* and *Saccharomyces cerevisiae* incorporated diets [56, 62-65]. It has also been reported that dietary probiotics, *Bacillus* spp., *Lactobacillus* spp., and *Lactobacillus plantarum* incorporation as a survival, growth promoter and feed utilization of shrimps *F. indicus* and *L. vannamei* [66-69].

Some secondary metabolites of probiotics act as prebiotics, increase the feeding efficiency/ frequency and improve feed conversion ratio [70]. Zheng *et al.*, [71] reported that the supernatant *of L. plantarum*, live bacteria, dead bacteria, and cell-free extract supplementation has improved the growth performance of *L. vannamei*, which was due to elevated digestive enzyme activities. Milad *et al.*, [72] reported that dietary supplementation of *Lactococcus lactis* sub sp. *Lactis* at 10^{-8} CFU g−1 for 8 weeks modulated the growth performance of *L. vannamei*. The increase in intestinal bacterial counts of *Lactobacillus* sp. and *Bacillus* sp. and decrease in that of *Vibrio* spp., of *L. vannamei* fed with *L. lactis* supplemented diets has also been reported [73,74] observed significant increase in growth parameters and decrease in FCR in *M. rosenbergii* fed diets supplemented with different concentrations of *Bacillus licheniformis*. Zokaeifar *et al.*, [75] reported that *Bacillus subtilis* significantly improved the growth and survival in white shrimp. Similarly, Kongnum & Hongpattarakere [69] reported that *L. plantarum* incorporated feed significantly

enhances the growth performance and nutritional utilization in *L. vannamei*. Balcazar *et al.*, [76] reported that the mixed concentration of different probiotics fed to *L. vannamei* showed the best feed conversion ratio and appreciable growth. *L. vannamei* fed with *Bacillus* spp., incorporated diet also reported to produce increased carcase biochemical constituents [67].

Gut Microbial Population

The microflora of control prawn gut revealed presence of *Streptococcus* spp., *Klepsiella* spp., *E. coli*, and *Staphylococcus* spp., (Table 7). The gut of experimental prawns fed with *B. coagulans* incorporated diet showed *Staphylococcus* spp., *E. coli*, *Bacillus* spp., *Lactobacillus* spp., It indicates competitive exclusion of pathogenic bacteria, *Streptococcus* spp., and *Klebsiella* spp., by probiotic *B. coagulans* (Table 7). It suggests that the commonsolistic bacterium, *B. coagulans* might have produced bacteriocins, proteinaceous toxins, which specifically inhibit members of pathogenic bacteria. Thus, the survival and growth of *M. rosenbergii* was found to be improved. Table 8, depicts the confirmative results bacterial species detected in gut of *M. rosenbergii* in the present study.

Table 7. *Microbial load in the gut of M. rosenbergii PL fed with B. coagulans supplemented diet*

Samples	Dilution	Isolated species	Composition (%)	CFU	Total (%)
Control	--	*Streptococcus* spp.,	18.48	34.55×10^{-7}	98.31
		Klebsiella spp.,	18.10	25.20×10^{-7}	
		E. coli	22.62	20.55×10^{-7}	
		Staphylococcus spp.,	26.25	17.20×10^{-7}	
B. coagulans	10^{-7}	*Staphylococcus* spp.,	22.25	27.42×10^{-7}	95.58
		E. coli	19.81	21.45×10^{-7}	
		Bacillus spp.,	29.50	30.25×10^{-7}	
		Lactobacillus spp.,	24.02	29.85×10^{-7}	

Competitive exclusion of pathogenic bacteria, *Pseudomonas aeruginosa*, *Klebsiella. pneumonia*, *Acetonobacter* sp., and *Salmonella* sp., by probiotics, like *L. fermentum*, *L. brevis*, *B. coagulans* and *B. subtilis* supplemented feed fed *M. rosenbergii* have been reported [24-27, 62]. Milad *et al.*, [72] reported that an increase in *Lactobacillus* spp., and *Bacillus* spp., counts and decrease in *Vibrio* spp., in *L. lactis* supplemented feed fed *L. vannamei*, it was an indication of the positive role of this potential probiotic in improving the growth and feed efficiency. Dash *et al.*, (2014) [77] reported that *M. rosenbergii* fed with *Lactobacillus* spp., supplemented feed showed significant decrease of pathogenic bacteria (*Enterobacteriacea*, *Aeromonas* spp., and *Pseudomonas* spp.). It is valid to mention here that probiotics play an important role in welfare of host by maintaining a healthier balance of intestinal microflora which not only provides a defensive barrier against colonization of harmful bacteria but also stimulates the immune system of the host [78-80]. The presence of at least 10 bacterial genera *Escherichia coli*, *Proteus* spp., *Lactococcus* spp., *Enterobacteria* spp., *Lactobacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Klebsiella* spp., *Streptococcus* spp., have been isolated from the gut of *Oreochromis niloticus* and *Clarias gariepinus* [81].

Table8. *Confirmative results of biochemical tests for microflora present in the gut of M. rosenbergii fed with B. coagulans supplemented diet*

Test	Control				*B. coagulans*			
	St	K	Ec	Sta	St	Ec	Ba	La
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	A	A	A	A	A	A	+	A
Glucose test	A	A	NA	A	A	NA	A	A
Lactose test	A	A	A	A	A	A	A	A
Sucrose test	A	A	A	A	A	A	A	A
Manitol test	A	A	A	A	A	A	A	A
Maltose test	NA	A	Na	NA	NA	Na	A	A

+, Positive; -, Negative; A, Acid production; NA, No acid production ***St****. Streptococcus spp.;* ***K.*** *Klebsiella spp.,;* ***Ec****, Escherichia coli;* ***Sta****, Staphylococcus spp.,;* ***Ba****, Bacillus spp.;* ***La****, Lactobacillus spp.,*

In-Vitro Antibacterial Activity

The results of *in-vitro* antibacterial activity against *Streptococcus* spp., and *Klebsiella* spp., are presented in Figs. 3 and 4; Table 9. The antibiotic, amoxicillin was used as positive control, which produced 15 mm zone of inhibition. Therefore, both *Streptococcus* spp., and *Klebsiella* spp., is sensitive to amoxicillin. The sample of *B. coagulans* showed 14 mm, and 15 mm zone of inhibitions against the pathogenic bacteria, *Streptococcus* spp., and *Klebsiella* spp., respectively. Therefore the probiotic, *B. coagulans* was found to be antagonistic against these pathogenic bacteria, which causes infections in aquatic animals including fishes and prawns. Therefore *B. coagulans* was able to restrict the colonization ability of *Streptococcus* spp., and *Klebsiella* spp., by competitive exclusion in the gut of *M. rosenbergii* PL. No zone of inhibition was seen in negative control, distilled water.

It has been reported that in India, the freshwater prawns are susceptible to diseases due to bacteria, protozoan parasites, fungal pathogens and viruses [82-84]. The complex nature of these disease outbreaks in crustaceans needs an understanding of their infection, control measures and managerial strategies instead of using antibiotics and chemical growth promoters. The nutritional benefits and antimicrobial activity of lactic acid bacteria against pathogenic microorganisms, *E. coli, Salmonella* spp., *Proteus* spp., *P. aeruginosa, Bacillus, Pseudomonas,* and *Listeria* has been reported on several aquatic species [85, 86]. In this way the probiotic *B. coagulans* was studied on *M. rosenbergii* PL, which proved to be anti-pathogenic.

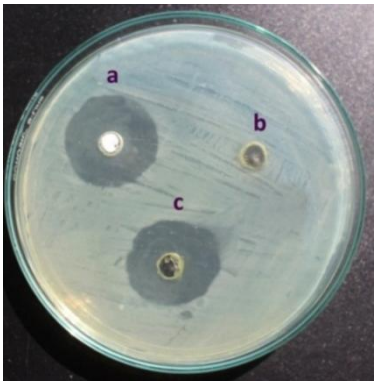

Fig3. *In-vitro antibacterial activity of B. coagulans and antibiotic sensitivity tests against Streptococcus spp.,*

a, amoxicillin; b, distilled water;

c, B. coagulans

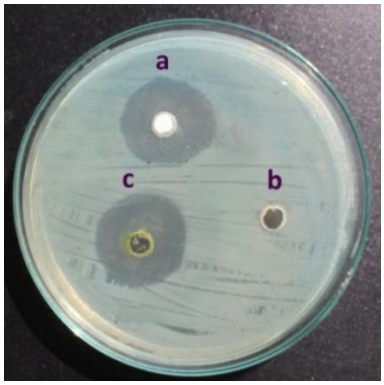

Fig4. *In-vitro antibacterial activity of B. coagulans and antibiotic sensitivity tests against Klebsiella spp.,*

a, amoxicillin; b, distilled water;

c, B. coagulans

Table9. *In-vitro anti-bacterial activities of amoxicillin and B. coagulans against pathogenic bacteria, Streptococcus spp., and Klebsiella spp.,*

Pathogens	**Antibiotic disk**	**Zone of inhibition in positive control (mm)**	**Zone of inhibition in negative control (mm)**	**Zone of inhibition in *B. coagulans* sample (mm)**
Streptococcus	Amoxycillin	15	-	14

spp.,				
Klebsiella spp.,	Amoxycillin	15	-	15

Note: *B. coagulans worked against Streptococcus spp., and Klebsiella spp.,*

CONCLUSION

In this study, *B. coagulans* supplemented feed, particularly at 10^{-7} (CFU 2.28 x 10^{-7}) has produced better survival and growth of *M. rosenbergii* PL, and competitively excluded *Streptococcus* spp., and *Klebsiella* spp. Thus it is suggested that this probiotic possessed immunomodulatory property by improving the general health of PL. Further it is evident that the recorded increase in activity of digestive enzymes should have improved the digestion of protein, carbohydrate and fat, which in turn led to enhanced absorption of nutrients that serves as the reason for better survival, growth and nutritional quality of *M. rosenbergii* PL. Therefore, *B. coagulans* is recommended for sustainable culture of *Macrobrachium*.

REFERENCES

[1] Balazs GH, Ross E, Brooks CC. Preliminary studies on the preparation and feeding of crustacean diets. Aquaculture. 1973; 2:369–377. DOI: 10.1016/0044-8486(73)90168-3.

[2] Millikin MR, Fortner AR, Fair PH, Siek LV. Influence of dietary protein concentration on growth, feed conversion and general metabolism of juvenile prawn *Macrobrachium rosenbergii*. Proc. World Maricult Society. 1980; 114:382–399.

[3] Swamy DN. CIBA, Madras "Training Manual, Short-Term Cource in Biotechnological Approaches in Prawns and Fish Nutrition and Feed Technology". 1995; pp 82–88.

[4] Rangacharyulu PV. Studies on the Nutrition and diet development of the giant freshwater prawn *Macrobrachium rosenbergii*. Ph.D Thesis. University of Kalyani. West Bengal. India, 1999.

[5] Mitra G, Mukopadhyay PK, Chattopadhyay DN. Nutrition and feeding in freshwater prawn (*Macrobrachium rosenbergii*) farming. Aquatic Feeds: Formulation and Beyond 2005; 2:17–19.

[6] Akiyama DM, Dominy WG, Lawrence AL. Penaeid shrimp nutrition. In: Fast, A.W., Lester, L.J.Ž. Eds., and Marine Shrimp Culture: Principles and Practices. Elsevier, Amsterdam. 1992; pp. 535–568.

[7] Sarac Z, Thaggard H, Saunders J, Gravel M, Neill A, Cowan RT. Observations on the chemical composition of some commercial prawn feeds and associated growth responses in *Penaeus monodon*. Aquaculture. 1993; 115:97–110. https://doi.org/10.1016/0044-8486(93)90361-2 [Elsevier].

[8] Gatesoupe FJ. The use of probiotics in aquaculture. Aquaculture. 1999; 180:147–165. [Elsevier].

[9] Speck ML. Enumeration of viable *Lactobacillus acidophilus* organisms in dairy products. Journal of Food Protection. 1978; 41(2):135–137. https://doi.org/10.4315/0362-028X-41.2.135

[10] Moriarty DJW. Disease control in shrimp aquaculture with probiotic bacteria. In: CR. Bell M, Brylinsky P. Johnson-Green (Eds.) Microbial biosystems: New frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada. 1999.

[11] Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S, Lilius E. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish & Shellfish Immunology. 2003; 15:443–452. https://doi.org/10.1016/S1050-4648 (03)00023-8 [Elsevier].

[12] Ghosh S, Sinha A, Sahu C. "Probiotic supplementation on growth and Health of live–bearing ornamental fishes". Aquaculture nutrition. 2008; 14(4): 289–299. https://doi.org/10.1111/j.1365-2109.2007.01696.x [Scopus].

[13] Moriarty DJW. Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture. 1998; 164:351–358. DOI: 10.1016/s0044-8486(98)00199-9 [Elsevier].

[14] Das S, Ward LR, Burke C. "Prospects of using marine actinobacteria as probiotics in aquaculture," Applied Microbiology and Biotechnology. 2008; 81(3): pp. 419–429. doi: 10.1007/s00253-008-1731-8 [Springer]

[15] Mehrim AI. Effect of dietary supplementation of Biogen® (Commercial probiotic) on mono-sex Nile tilapia *Oreochromis niloticus* under different stocking densities. Journal of Fisher and Aquatic Science. 2009; 4:261–273. DOI: 10.3923/jfas.2009.261.273

[16] Silva YJ, Costa L, Pereira C, Cunha A, Calado R, Gomes NC, Almeida A. Influence of environmental variables in the efficiency of phage therapy in aquaculture. Microbial Biotechnology. 2014; 7:401–413. doi: 10.1111/1751-7915.12090 [Pub Med].

[17] Kennedy SB, Tucker JW, Neidig CL, Vermeer GK, Cooper VR, Jarrell JL, Sennett DG.

Bacterial Management Strategies for Stock Enhancement of Warm water Marine Fish: A Case Study with Common Snook (*Centropomus Undecimalis*). Bulletin of Marine Science. 1998; 62 (2): 573–588.

[18] Rengpipat S, Phianpak W, Piyatiratitivorakul S, Menasveta P. Effect of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture.1998; 167:301–313. DOI: 10.3354/dao055169 [Pub Med].

[19] Hirata H, Murata O, Yamada S, Ishitani H. Probiotic culture of the rotifer *Brachionus plicatilis*, Hydrobiologia. 1998; 387:495–498.

[20] Luis-Villaseñor IE, Macias-Rodriguez ME, Gomez-Gil B, Ascencio-Valle F, Campa Cordova AI. Beneficial effects of four *Bacillus* strains on the larval cultivation of *Litopenaeus vannamei*. Aquaculture. 2011. doi: 10.1016/j.aquaculture.2011.08.036. [Elsevier].

[21] Kim DH, Austin B. Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. Fish & Shellfish Immunology. 2006; 21:513–524. https://www.ncbi.nlm.nih.gov/pubmed/16631379 [Pub Med].

[22] Mohideen MM, Kader A, Mohan TS, Mohamed SP, Hussain MIZ. Effect of Probiotic Bacteria on the Growth rate of Fresh Water Fish, *Catla catla*. International Journal of Biological Technology. 2010; 1(2):113–117.

[23] Wang Y, Gu Q. Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response Marine Biology Research. 2010; 6: 327-332. http://dx.doi.org/10.1080/17451000903300893

[24] Narmatha V, Saravana Bhavan P, Karthik M, Srinivasan V, Mahendran R, Satgurunathan T. *Lactobacillus fermentum* on ammonia reduction and growth promotion of *Macrobrachium rosenbergii* post larvae, and in vitro competitive exclusions of pathogenic bacteria. International Journal of Fisheries and Aquatic Studies. 2017; 5(1): 506–514.

[25] Karthik M, Saravana Bhavan P. Supplementation of Lactobacillus *brevis* for Growth Promotion of the Freshwater Prawn *Macrobrachium rosenbergii* Post Larvae and identification of Gut Microflora through 16s R dna. Research Journal of Biotechnology. (2018a); 13(1):34–50.

[26] Karthik M, Saravana Bhavan P, Manjula T. Growth Promoting Potential and Colonization Ability of Probiotics (*Bacillus coagulans* and *Bacillus subtilis*) on the Freshwater Prawn *Macrobrachium rosenbergii* Post-Larvae. Insights Biol Med. (2018b); 2:007–018. https://doi.org/10.29328/journal.ibm.1001011.

[27] Karthika M, Saravana Bhavana P, Seenivasana V, Asaikkuttia A, Muralisankara T. Mahendranb Dietary Supplementation of *Lactobacillus fermentum* for Improving the Survival, Growth and Nutritional Profiles of the Prawn *Macrobrachium rosenbergii*, and16S rDNA based Identification of its Establishment. Scholar Reports. (2018c); V3-I1-06:38–62. doi:10.1016/j.anifeedsci.2005.09.003

[28] Wang Yanbo, Xu Zirong. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Animal Feed Science and Technology. 2006; 127:283–292. DOI: 10.1016/j.anifeedsci.2005.09.003[Elsevier].

[29] Osman HAM, Ibrahim TB, Soliman W, Aboud O. Improvement growth and immune status using a potential probiotic bacteria *Micrococcus* species among culured *Oreochromis niloticus*. New York Science Journal. 2010; 3(10):5–11.

[30] Faramarzi M, Kiaalvandi S, Iranshahi F. The Effect of Probiotics on Growth Performance and Body Composition of Common Carp (*Cyprinus carpio*), Journal of Animal and Veterinary Advances. 2011; 10(18):2408–2413. DOI: 10.3923/javaa.2011.2408.2413.

[31] Mohapatr S, Chakraborty T, Prusty AK, Das P, Paniprasad K, Mohanta KN. Use of probiotics in the diet of rohu, *Labeo rohita* fingerlings: effects on growth nutrient digestibility, retention digestive enzyme activities and intestinal microflra. Aquaculture Nutrition. 2012; 18:1–11. doi:10.1371/journal.pone.0100929 [Pub Med].

[32] Peterson BC, Booth NJ, Manning BB. 2012. Replacement of fish meal in juvenile channel catfish, *Ictalurus punctatus*, diets using a yeast-derived protein source: the effects on weight gain, food conversion ratio, body composition and survival of catfish challenged with *Edwardsiella ictaluri*. Aquaculture Nutrition, 18:132–137. https://doi.org/10.1111/j.1365-2095.2011.00878.x

[33] Irianto A, Austin B. Probiotics in aquaculture (Review). Journal of Fisheries Diseases. 2002; 633–642. https://doi.org/10.1046/j.1365-2761.2002.00422.x

[34] Panigrahi A, Kiron V, Kobayashi T, Puangkaew J, Satoh S, Sugita H. Immune responses in rainbow trout *Oncorhynchus mykiss* induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. Veterinary Immunology and Immunopathology. 2004; 102(4):379–388. DOI: 10.1016/j.vetimm.2004.08.006[Pub Med].

[35] Gullian M, Thompson F, Rodriguez J. Selection of probiotic bacteria and study of

their immunostimulatory effect in *Penaeus vannamei*. Aquaculture. 2004; 233:1-14. doi:10.1016/j.aquaculture.2003.09.013 [Elsevier].

[36] Hyronimus B, Marrec CL, Maria C, Coagulin U. A bacteriocin-like inhibitory substance produced by *Bacillus coagulans* I4 Journal of Applied Microbiology. 1998; 85(1):42–50. DOI: 10.1046/j.1365-2672.1998.00466.x. [PubMed].

[37] Donskey CJ. Effect of oral *Bacillus coagulans* administration on the density of vancomycin-resistant enterococci in the stool of colonized mice. Lett. Applied Microbiology. 2001; 33:84–88. https://doi.org/10.1046/j.1472-765X.2001.00948.x [PubMed].

[38] Mandel DR, Eichas K, Judith Holmes, *Bacillus coagulans*: A viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial, BMC Complement Alternative Medicine. 2010; 10:1. doi: 10.1186/1472-6882-10-1.

[39] CIFRI. Seed production- Need of the hour. Sixth workshop on Air breading fish culture. 1982; Vol.2.

[40] ICMR. Indian Council of Medical Research Nutrient Requirements and Recommended Dietary Allowances: A Report of the Expert Group of the ICMR. 1999.

[41] APHA D. Standard Methods for the Examination of Water and Waste Water. Method 2540. 21st Edition, 2005.

[42] Winkler LW. Die Bestimmung des in Wasser gelösten Sauerstoffen. Berichte der Deutschen Chemischen Gesellschaft. 1888; 21:2843–2855.

[43] Solorzano L. Determination of Ammonia in Natural Waters by the Phenolhypochlorite Method. Limnology and Oceanography. 1969; 14:799–801.

[44] Tekinay AA. Davies SJ. Dietary carbohydrate level influencing feed intake, nutrient utilisation and plasma glucose concentration in the rainbow trout, *Oncorhynchus mykiss*. Turkish Journal of Veterinary and Animal Sciences. 2001; 25:657–666.

[45] Furne M, Hidalgo MC, Lopez A, Garcia-Gallego M, Morales AE, Domezain A, Domezaine J, Sanz A. Digestive enzyme activities in Adriatic sturgeon *Acipencer naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. Aquaculture. 2005.

[46] Bernfeld P. Amylases, alpha and beta. In: Colowick, S. P. and Kaplan, N. O (Eds.). Methods in enzymology. New York: Academic Press. 1955; 1:149–158. http://dx.doi.org/10.1016/0076-6879 (55)01021-5. [PubMed].

[47] Lowry OH, Rosenbrough WJ, Fair AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry.1951; 193:265–275.

[48] Moore S, Stein WH. Photometris Ninhydrin Method for Use in the Chromatography of Amino Acids Journal of Biological Chemistry.1948; 176:367–388.

[49] Roe SH. The determination of sugar in blood and spinal fluid with anthrone reagent. Journal of Biological Chemistry.1955; 212:334–343.

[50] Folch J, Lees M, Bloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 1957; 266:497–509.

[51] Barnes H, Blackstock J. Estimation of lipids in marine animals and tissues. Detail investigation of the sulpho-phosphovanillin method for total lipids. J Exp Mar Bio Ecol 1973; 12:103–118.

[52] AOAC. Official Methods of Analysis. 18th edn. Association of Official Analytical Chemists; Arlington, VA, USA. 2005.

[53] Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determination Bacteriology, 9th Edition. Baltimore, M.D. Williams' and Wilkins. 1996; pp. 787.

[54] Suralikar V, Sahu NP. Efffect of Feeding Probiotic (*Lactobacillus cremoris*) on Growth and Survival of *Macrobrachium rosenbergii* Post Larvae. Journal of Applied Animal Research. 2001; 20 (1):17–124. https://doi.org/10.1080/09712119.2001.9706744.

[55] Saad AS, Habashy MM, Sharshar KM. Growth Response of the freshwater prawn, *Macrobrachium rosenbergii* (De Man), to diets having different levels of Biogen®. World Applied Sciences Journal. 2009; 6:550–556.

[56] 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 Aquaculture. 2011; 41:5919–5927.

[57] 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*. International Journal of Current Research. 2015a; 7:11440–11453.

[58] Jayanthi L, Bhavan PS, Srinivasan V, Muralisankar T, Manickam N. Dietary

supplementation of probiotics product (ViBact*) on the survival, growth, biochemical constituents and gut microfl ora of the giant freshwater prawn *Macrobrachium rosenbergii* post-larvae. Asian Journal of Biochemical and Pharmaceutical Research. 2015b; 2: 67–88.

[59] Gupta A, Verma G, Gupta P. Growth performance, feed utilization, digestive enzyme activity, innate immunity and protection against *Vibrio harveyi* of freshwater prawn, *Macrobrachium rosenbergii* fed diets supplemented with *Bacillus coagulans*. Aquaculture International. 2016; 24(5). DOI: 10.1007/s10499-016-9996-x.

[60] Nimrat S, Vuthiphandchai V. In vitro evaluation of commercial probiotic products used for marine shrimp cultivation in Thailand. African Journal of Biotechnology. 2011; 10(22):pp. 4643–4650. DOI: 10.5897/AJB10.2404.

[61] Ziaei-Nejad S. The effects of *Bacillus* spp. Bacteria as a probiotic on growth, survival and digestive activity of Indian white shrimp, *Fenneropenaeus indicus*, larvae and post (M.Sc. thesis).Tehran University. 2004; pp. 100.

[62] Venkat HK, Sahu NP, Jain KK. Effect of feeding Lactobacillus based probiotics on the gut microflora, growth and survival of post larvae of *Macrobranchium rosenbergii* (de Man). Aquaculture Research. 2004; 35:501–507.

[63] 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 Journal of Fisheries and Aquatic Science 2012; 12: 3–31. DOI: 10.4194/1303-2712-v12_1_04

[64] Seenivasan C, Bhavan PS, Radhakrishnan S, Muralisankar T, Immanuel G, Srinivasan V, Manickam N. Effect of *Saccharomyces cerevisiae* on survival, growth, biochemical constituents and energy utilization in the prawn *Macrobrachium rosenbergii*. International Journal of Applied Biology and Pharmaceutical Technology. 2013; 4:39–47.

[65] 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. Proceeding of the Zoological Society. 2014; 68(1):74–83. DOI: 10.1007/s12595-014-0097-4.

[66] 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. doi: 10.1016/j.aquaculture.2005.07.021 [Elsevier].

[67] 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*. Aquaculture International. 2009; 17:377–384.

[68] Fernandez R, Sridhar M, Sridhar N. Effect of Lactic acid bacteria administered orally on growth performance of *Penaeus indicus* (H. Milne Edwards) juveniles. Research Journal of Microbiology. 2011; 6:466–479.

[69] Kongnum K, Hongpattarakere T. Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. Fish and Shellfish Immunology. 2012; 32: 170–177. doi:10.1016/j.fsi.2011.11.008.

[70] Goddard S. Feed management in intensive aquaculture. London, Chapman & Hall. 1996; pp. 198.

[71] Zheng X, Duan Y, Dong H, Zhang J. Effects of Dietary *Lactobacillus plantarum* on Growth Performance, Digestive Enzymes and Gut Morphology of *Litopenaeus vannamei*. 2017; 10(3):504–510. doi: 10.1007/s12602-017-9300-z.

[72] Milad A, Cabacang LC, Reza S, Sakine Y, Mohammad JaliZ. Aqualase a yeast-based in-feed probiotic, modulates intestinal microbiota, immunity and growth of rainbow trout *Oncorhynchus mykiss*. In: Aquaculture Research. 2017; 48(4):1815–1826.

[73] Kumar NR, Raman RP, Jadhao SB, Brahmchari RK, Kumar K, Dash G. Effect of dietary supplementation of *Bacillus licheniformis* on gut microbiota, growth and immune response in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). Aquaculture International 2013; 21(2):387–403. DOI 10.1007/s10499-012-9567-8, 23 [springer].

[74] Adel M, El-Sayed AM, Yeganeh S, Dadar M, Giri SS. Effect of Potential Probiotic *Lactococcus lactis* Subsp. *lactis* on Growth Performance, Intestinal Microbiota, Digestive Enzyme Activities, and Disease Resistance of *Litopenaeus vannamei*. Probiotics Antimicrob Proteins. 2017; 9(2):150–156. doi: 10.1007/s12602-016-9235-9.

[75] Zokaeifar H, Balcazar JL, Saad CR, Kamarudin MS, Sijam K, Arshad A and Nejat N. (2012) Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene

expression and disease resistance of white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol.2012; 33:683–689.

[76] Balcazar J, Rojaas-Luna T. Inhibitory activity of probiotic *Bacillus subtilis* UTM 126 against Vibrio species confers protection against vibriosis in juvenile shrimp (*Litopenaeus vannamei*). Current Microbiology. 2007; 55:409–412.

[77] Dash G, Prakash Raman R, Pani Prasad K, , Makesh M, Pradeep MA, Sen S. Evaluation of *Lactobacillus plantarum* as a water additive on host associated microflora, growth, feed efficiency and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). Aquaculture. 2014; 432:225–236.

[78] Gomez R, Geovanny D, Shen MA. Influence of probiotics on the growth and digestive enzyme activity of white Pacific shrimp (*Litopenaeus vannamei).* Oceanic and Coastal Sea Research. 2008; 7:215–218.

[79] Cain k, swan c. barrier function and immunology. In: the multifunctional gut of fish (ed. by M. Grosell, A. P. Farrell & C. J. Brauner). Academic press, Amsterdam. 2010; pp. 111–134.

[80] Gaggia F, Mattarelli P, Biavati B. Probiotics and prebiotics in animal feeding for safe food production. The International Journal of Food Microbiology. 2010; 15–28.

[81] Charles DK, Mark BM, Samuel M, Andrew T, Nathan LM, Asuman S. Isolation and Identification of Potential Probiotics Bacteria from the Gut of *Oreochromis niloticus* and *Clariasgarie pinus* in Uganda. British Microbiology Research Journal. 2016; 17(5):1-8.

[82] Kamonporn T. Diseases of the freshwater prawn *Macrobrachium rosenbergii* in Thailand. Proceedings of the first symposium on diseases in Asian aquaculture. Asian Fisheries Society.1992; pp. 89–95. DOI: 10.9734/BMRJ/2016/29721

[83] Shariff M, Subasinghe RP, Arthus JR. Diseases in Asian Aquaculture I, p. 513. Asian Fisheries Society, Manila, Philippines. 1992.

[84] Soundara pandian P, Kannupandi T. Disease of freshwater prawn larvae. Aquaculture International. 1998; 47:18–19.

[85] Lonkar P, Harne SD, Kalorey DR, Kurkure NV. Isolation, in vitro antibacterial activity, bacterial sensitivity and plasmid profile of *Lactobacilli*. Asian-Australasian Journal of Animal Sciences. 2005; 18:1336–1342.

[86] Cizeikiene D, Juodeikiene G, Algimantas Paskevicius A, Bartkiene E. Antibacterial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. Food Control. 2013; 31:539–545. DOI: 10.1016/j.foodcont.2012.12.004

Citation: *S. Manjula, P. Saravana Bhavan, M. Karthik, D. Anitha, R. Kalpana,T. Manjula.," Survival, Growth, Activities of Digestive Enzymes, Concentrations of Basic Biochemical Constituents and Competitive Exclusion of Pathogenic Bacteria in Bacillus Coagulans Supplemented Diet Fed Macrobrachium Rosenbergii Post-Larvae", International Journal of Research Studies in Science, Engineering and Technology, vol. 5, no. 12, pp. 9-22, 2018.*