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Effects of Probiotics on Survival, Growth and Biochemical Constituents of Freshwater Prawn *Macrobrachium rosenbergii* Post Larvae

C. Seenivasan[1,*], P. Saravana Bhavan[1], S. Radhakrishnan[1], T. Muralisankar[1]

[1] Bharathiar University, Department of Zoology, Crustacean Biology Laboratory, Coimbatore-641046, Tamilnadu, India.

* Corresponding Author: Tel.: +91.94881-75470; Fax: +88.176 4692554;
E-mail: crustaceanseenu@gmail.com

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Abstract

The present study was attempted to examine the combined effects of probiotics, *Lactobacillus sporogenes* (LS), *Bacillus subtilis* (BS) and yeast, *Saccharomyces cerevisiae* (SC) on survival, growth, biochemical changes and energy utilization performance of the freshwater prawn *M. rosenbergii* post larvae. The probiotics, *L. sporogenes* (4), *B. subtilis* (3) and *S. cerevisiae* (4) were taken and mixed. 1%, 2%, 3% and 4% of LS+BS+SC (4+3+4) was incorporated with basal diet. Diet without probiotics served as control. PL-30 of *M. rosenbergii* was fed with LS+BS+SC (4+3+4) incorporated diet for a period of 90 days. The growth parameters, such as survival, weight gain, specific growth rate, feed conversion efficiency and protein efficiency rate were significantly ($P<0.05$) higher in 3% LS+BS+SC incorporated diet fed post larvae (PL) followed by other experimental groups when compared with control. Whereas, the feed conversion ratio was significantly ($P<0.05$) lower in 3% LS+BS+SC incorporated diet fed PL. Similarly the tissues biochemical composition such as protein, amino acid, carbohydrate and lipid content were significantly ($P<0.05$) higher in 3% LS+BS+SC incorporated diet fed PL. However, insignificant difference was recorded in moisture content between control and experimental groups. The energy utilization parameters, such as feeding rate, absorption rate, conversion rate, NH_3 excretory rate and metabolic rate were significantly ($P<0.05$) higher in 3% LS+BS+SC incorporated diet fed PL. Therefore, the probiotics 3% LS+BS+SC incorporated diet fed PL was produced better growth performance.

Keywords: *M. rosenbergii, L. sporogenes, B. subtilis, S. cerevisiae,* growth performance, biochemical constituents, energy utilization.

Introduction

Macrobrachium rosenbergii was fast growing, being able to grow in freshwater and low brackish water conditions. This species has many biological advantages for commercial culture including attaining maturation in captivity, a relatively large size, rapid growth rate. Other advantages include omnivorous feeding habits (Ling, 1969).

The term "probiotic" which literally means "for life" has since been employed to describe these health-promoting bacteria. The World Health Organization has defined probiotic bacteria as "live microorganisms which when administrated in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). The use of probiotics in the culture of aquatic organisms is increasing with demand for more environment friendly aquaculture practices (Gatesoupe, 1999). The microorganisms used as probiotics, including *Lactobacillus*, *Bacillius* and yeasts, have been reported in penaeids and fish (Rengpipat *et al*., 2000; Hong *et al*., 2005; Li *et al*., 2005; El-Haroun *et al*., 2006; Balcázar *et al*., 2007a, 2007b; Bagheri *et al*., 2008; Ghosh *et al*., 2008; Capkin and Altinok, 2009; Al-Dohail *et al*., 2009; Pooramini *et al*., 2009; Boonthai *et al*., 2011). The effects of probiotics for freshwater prawn, *M. rosenbergii* have been reported by Seenivasan *et al*. (2012); Seenivasan *et al*. (2011); Saad *et al*. (2009); Shinde *et al*. (2008); Keysami *et al*. (2007); Venkat *et al*. (2004) and Suralikar and Sahu, (2001). The present study was attempted to examine the effect of combined probiotics, *Lactobacillus sporogenes*, *Bacillus subtilis* and yeast, *Saccharomyces cervisiae* on survival, growth, biochemical changes and energy utilization performance of the freshwater prawn *M. rosenbergii* PL.

Materials and Methods

The post larvae of freshwater prawn, *M. rosenbergii* (PL 15) were purchased from a Happy

Bay Annexe, Kanchipuram, Tamilnadu, India and were stocked in a cement tank (1000 L) filled with freshwater. The PL were acclimatised at ambient laboratory conditions for 15 days (up to PL 30) and starved for 24 h before the beginning of the feeding experiment. The experimental water presented the following physicochemical parameters: pH, 7.10±0.50; total dissolved solids, 0.98±0.10 g/L; dissolved oxygen, 7.30±0.40 mg/L; BOD, 40.00±1.60 mg/L; COD, 120.00±9.00 mg/L and ammonia, 0.068±0.008 mg/L.

Diet Preparation

One gram of lyophilized powders of *L. sporogenes* (Uni-Sankyo Ltd., Maharashtra, India), *B. subtilis* (Tablets, India Ltd), and *S. cerevisiae* (Intercare Ltd, Gujarat, India) contains $15x10^7$, $10x10^7$ and $10x10^7$ CFU cells, respectively. The probiotics, LS+BS+SC (4:3:4) were incorporated in to the test diets at five different concentrations: individually 0% (control), 1%, 2%, 3% and 4% respectively. Diet formulation was done basically by ''Pearson's square-method'' using determined values of 40% protein content (Table 1). The proportion of each ingredient required was calculated precisely providing allowance for the premix. The dough was steam cooked and cooled to room temperature. After that different the concentration of LS+BS+SC (4:3:4) was mixed with the dough and the diets were pelletized separately with a locally made hand pelletizer (Kolkata, India). The pellets were dried in a thermostatic oven (M/s Modern Industrial, Mumbai, India) at 40^0 C until it reached constant weight and stored in airtight jars at room temperature. The biochemical constituents of the experimental diets were determined such as total protein (Lowry *et al*., 1951), amino acid (Moore and Stein, 1948), lipid (Folch *et al*., 1957), carbohydrate (Roe, 1955), ash and moisture contents (APHA, 2005). The analyses were made in triplicates. These diets were freshly produced after 30 days to ensure high probiotic viability throughout the duration of feeding trail. In the control diet, no probiotics were added.

Feeding Experiment

During 90 days, *M. rosenbergii* (PL-30) with the length and weight range of 1.54±0.03 cm and 0.24±0.02 g respectively were used for feeding experiment. The PL was maintained in a stocking density of 2/L, in fifteen 20 L plastic tanks (40 PL per tank in triplicate) continuously aerated in order to maintain the optimal oxygen level (by one air stones)**.** One group served as control (0% of probiotics) and the experimental groups were fed with the respective concentration of LS+BS+SC (1, 2, 3 and 4%) incorporated into diets. The feed was provided two times a day ($6:^{00}$ am and $6:^{00}$ pm). The daily amount of feed was calculated according 10% of PL body weight. Unfed feed, feces and moult (if any) were collected after feeding.

Growth Study

After the feeding trial, the growth parameters such as survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), feed conversion efficiency (FCE) and protein efficiency rate (PER) were individually determined by following equations (Tekinay and Davis, 2001).

SR (%) = Total No. of live animals/Total No. of initial animals x 100

WG (g) = Final weight (g) – Initial weight (g)

Table 1. Ingredients and proximate composition of prepared diets with *Lactobacillus sporogenes* (LS), *Bacillus subtilis* (BS) and yeast *Saccharomyces cerevisiae* (SC)

Ingredients (%)	Control diet	Experimental diets (LS+BS+SC incorporated)			
		1%	2%	3%	4%
Fish meal	33.84	33.84	33.84	34.84	35.84
Ground nut oil	25.00	25.00	25.00	25.00	24.00
Soybean meal	24.00	24.00	23.00	21.00	20.00
Corn flour	4.00	3.00	3.00	3.00	3.00
Egg albumin	5.06	5.06	5.06	5.06	5.06
Tapioca flour	5.10	5.10	5.10	5.10	5.10
Cod liver oil	2.00	2.00	2.00	2.00	2.00
Vitamin B-complex mix	1.00	1.00	1.00	1.00	1.00
Probiotics (LS+BS+SC)	0.00	1.00	2.00	3.00	4.00
		Proximate composition			
Protein (%)	40.10	40.00	39.63	39.52	39.40
Carbohydrate (%)	21.76	21.10	20.71	20.01	19.50
Lipid (%)	9.28	9.24	9.17	9.08	8.90
Ash (%)	14.00	13.00	12.00	13.00	14.00
Moisture (%)	9.50	9.90	9.40	9.10	9.10
Digestible energy (k.cal/kg)	3296.86	3262.52	3228.17	3193.83	3159.49

SGR (%) = log w_2 – log w_1/ t x100

where, w_1 & w_2 = Initial and Final weight (g) respectively**,** and t = Total number of experimental days)

FCR (g) = Total Feed intake (g)/ Total weight gain of the prawn (g)

FCE (%) = Biomass (g)/ Total Feed intake (g) x 100

PER (g) = Total Weight gain of PL (g)/ Total Protein consumed (g)

Energy Utilization

The energy content of whole prawns, diets, moult and feces was measured using Parr 1281 Oxygen Bomb Calorimeter. The energy budget was calculated using the equation (C = (P+E) + R + F +U) derived by Petrusewicz and Macfadyen (1970); where, C is the energy consumed in food; P is the growth; R is the material lost as heat due to metabolism; F is the energy lost in faeces; U is the energy lost in excretion and; E is the energy lost in exuvia.

Feeding Rate = Mean Food Consumption (k.cal/day) / Initial live weight of the prawn (g)

Mean Absorption = Mean Food Consumption (k.cal/day) – Mean Food Excreted as Faeces (k.cal/day)

Absorption Rate = Mean absorption (k.cal/day) / Initial live weight of the prawn (g)

Mean Conversion = Mean weight gain (k.cal/day) + Mean exuvial weight (k.cal/day)

Conversion rate, P = Mean Conversion (k.cal/day) / Initial live weight of the prawn (g) NH_3 Excretion rate, U = Mean NH_3 Excretion (k.cal/day) / Initial live weight of the prawn (g)

Metabolic Rate, R = Absorption rate (k.cal./g/day – Conversion rate (k.cal/g/day) +

NH_3 excretion rate (k.cal/g/day)

Biochemical Constituents of the Experimental Animals

The biochemical constituents, such as total protein (Lowry *et al*., 1951), amino acid (Moore and Stein, 1948), lipid (Folch *et al*., 1957), carbohydrate (Roe, 1955), ash and moisture content (APHA, 2005) were determined in muscle of each group of prawns. Ash and moisture contents (APHA, 2005) were measured in whole prawns according each treatment. The analyses were made in triplicates.

Microbial Study

Microbial analyses (APHA, 2005) and yeast isolation (Bowman and Ahearn, 1975) were performed in the rearing water, control PL gut and experimental PL gut.

Statistical Analyses

One way analysis of variance (ANOVA; SPSS, 13.0) was used to determine whether significant variation between the treatments existed. Differences between means were determined and compared by post hoc multiple comparison test (DMRT). All the tests used a significance level of $P<0.05$. Data are reported as means ± standard deviations.

Results and Discussion

Growth Performance

The combined probiotics diets fed *M. rosenbergii* PL morphometric, survival and growth performance datas were showed in Table 2. The initial average body length and weight of PL was 1.54±0.03 cm and 0.24±0.02 g. After the feeding experiment, the morphometric data (final length and weight), survival and growth performance (WG, SGR, FCE and PER) were significantly ($P<0.05$) higher in 3% LS+BS+SC supplemented diet, followed by the PL fed with 2%, 1% and 4% when compared with control. The FCR was found to be lower in PL fed with 3% LS+BS+SC supplemented diet ($P<0.05$), followed by the PL fed with 2%, 1% and 4% than the control.

Similar results have been reported in *M. rosenbergii* postlarvae fed with bio-encapsulated diet containing *L. sporogenes* (Seenivasan *et al*., 2012), Binifit^{TM}. (Seenivasan *et al*., 2011) and *Bacillus* spp (Ranisha *et al*., 2010). Similarly, reported the growth was increase in *M. rosenbergii* fed with bio-encapsulated diet containing *B. subtilis* (Keysami *et al*., 2007), *L. acidophilus and L. sporogenes* (Venkat *et al*., 2004) and *L. ceremoris* (Suralikar and Sahu, 2001), in *M. amazonicum* juvenile fed with *S. cerevisiae* and yeast derivatives inclusion diet (Hisano *et al*., 2008), and the *P. monodon* fed with *Bacillus* sp supplemented diets had enhanced the growth performance (Boonthai *et al*., 2011). And also, several researcher are reported that probiotics *L. plantarum* and *B. megaterium* supplemented diets had improved the growth performance in *Catla catla* (Parthasarathy and Ravi, 2011); in Koi Carp fed with *L. acidophilus* and yeast *S. cervisiae* (Dhanaraj *et al*., 2010); in *Cyprinus carpio* and *Onchorhynchus mykiss* fed with *Bacillus* spp supplemented diets (Bagheri *et al*., 2008) and the juvenile *Dentex dentex* fed with *B. toyoi* incorporated diet (Hidalgo *et al*., 2006).

Biochemical Constituents of Experimental Animals

The results on biochemical composition, such as protein, amino acid, carbohydrate, lipid, ash and moisture content of LS+BS+SC supplementation fed *M. rosenbergii* PL group are also provided in Table 2.

Table 2. The morphometric data, growth performance, biochemical constituents and energy utilization of *M. rosenbergii* PL fed with different concentration of LS+BS+SC (4:3:4) supplemented diets

Aspects	Parameters	Control diet	Experimental diets				F-Value
			1% LS+BS+SC	2% LS+BS+SC	3% LS+BS+SC	4% LS+BS+SC	
Morphometry	Initial length (cm)	1.54±0.03	1.54±0.03	1.54±0.03	1.54±0.03	1.54±0.03	-
	Final length (cm)	4.90^{b}±0.26	6.02^{a}±0.35	6.18^{a}±0.31	6.32^{a}±0.18	5.99^{a}±0.23	12.88
	Initial weight (g)	0.24±0.02	0.24±0.02	0.24±0.02	0.24±0.02	0.24±0.02	-
	Final weight (g)	1.18^{b}±0.22	1.69^{a}±0.30	1.88^{a}±0.29	2.10^{a}±0.26	1.60^{ab}±0.25	5.02
Nutritional indices	Survival rate (%)	80.00^{b}±2.50	82.50^{b}±2.50	85.00^{b}±2.50	92.50^{a}±3.00	80.00^{b}±3.00	10.97
	Weight gain (g)	0.94^{c}±0.10	1.45^{b}±0.18	1.64^{ab}±0.20	1.86^{a}±0.24	1.36^{b}±0.13	11.29
	Specific growth rate (%)	0.768^{d}±0.034	0.941^{bc}±0.036	0.993^{ab}±0.039	1.046^{a}±0.031	0.915^{c}±0.025	29.67
	Feed conversion ratio (g)	3.47^{a}±0.17	2.29^{b}±0.17	2.27^{b}±0.23	2.20^{b}±0.19	2.43^{b}±0.21	22.15
	Feed conversion efficiency (%)	0.93^{b}±0.16	1.34^{a}±0.26	1.46^{a}±0.14	1.58^{a}±0.18	1.26^{ab}±0.22	4.71
	Protein efficiency ratio (g)	0.62^{a}±0.07	0.96^{a}±0.11	0.97^{a}±0.09	1.00^{a}±0.06	0.91^{a}±0.05	11.62
Biochemical constituents	Protein (%)	61.30^{c}±3.64	65.10^{bc}±3.00	67.80^{abc}±2.69	69.20^{ab}±2.46	62.30^{a}±2.74	4.04
	Amino acid (%)	29.20^{c} ±3.18	34.60^{abc} ±3.84	36.00^{ab} ±3.19	38.00^{a} ±3.76	31.20^{bc} ±2.62	3.42
	Carbohydrate (%)	10.00^{c} ±1.04	13.80^{ab} ±1.71	14.82^{ab} ±1.80	16.00^{a} ±1.28	12.80^{b} ±1.67	6.69
	Lipid (%)	7.20^{c} ±0.71	11.90^{b} ±1.42	12.60^{ab} ±1.38	14.90^{a} ±1.68	8.90^{c} ±1.59	14.34
	Ash (%)	16.80^{a} ±1.42	17.10^{a} ±1.70	18.20^{a} ±1.62	19.80^{a} ±1.56	17.00^{a} ±1.66	1.92
	Moisture (%)	76.42^{a} ±4.00	76.00^{a} ±3.40	75.30^{a} ±3.20	75.00^{a} ±3.43	76.30^{a} ±3.10	<1
Energy utilization (k.cal/g/day)	Feeding rate	0.359^{d} ±0.051	0.481^{b} ±0.062	0.509^{ab} ±0.042	0.544^{a} ±0.041	0.430^{c} ±0.057	32.86
	Absorption rate	0.306^{d} ±0.038	0.435^{b} ±0.066	0.472^{ab} ±0.037	0.511^{a} ±0.036	0.381^{c} ±0.061	40.96
	Conversion rate	0.152^{d} ±0.044	0.245^{c} ±0.052	0.282^{b} ±0.046	0.326^{a} ±0.021	0.224^{c} ±0.063	31.02
	NH_3 Excretion	0.013^{b} ±0.004	0.017^{b} ±0.009	0.019^{ab} ±0.007	0.025^{a} ±0.010	0.015^{b} ±0.006	5.78
	Metabolic rate	0.167^{b} ±0.064	0.207^{a} ±0.061	0.209^{a} ±0.042	0.210^{a} ±0.036	0.172^{b} ±0.070	5.70

Each value is a mean ± SD of three replicate. Within a row, values with different superscripts are significantly different (P<0.05).

After the feeding trail experiment of 90 days, the total protein, amino acid, carbohydrate, lipid and ash contents were found to be maximum in PL fed with 3% LS+BS+SC diet, followed by the PL fed with 2%, 1% and 4% when compared with control. The statistical analysis (DMRT) made on the biochemical constituents between control and experimental diets revealed that the variation between them was significant at (P<0.05).

Similarly, significantly improved basic carcass biochemical constituents was previously observed in *M. rosenbergii* PL fed with bio-encapsulation of *L. sporogenes* (Seenivasan *et al*., 2012) and Biogen® (Saad *et al*., 2009). The probiotics *Streptococcus faecium* and *L. acidophilus*, and the yeast *S. cerevisiae* (Lara-Flores *et al*., 2010), *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, and the yeast *S. cerevisiae* NIOFSD019 (Essa *et al*., 2010) and Biogens® (El-Haroun *et al*., 2006) incorporated diets enhanced the carcass biochemical composition in *O. niloticus*; in *O. mykiss* fed with *B. subtilis*, *B. licheniformis* and *Enterococcus faecium* (Merrifield *et al*., 2009) and *Bacillus* spp (Bagheri *et al*., 2008).

Energy Utilization Performance

The energy utilization performance of LS+BS+SC supplementation fed group of prawn PL is also given in Table 2. The feeding rate, absorption rate, conversion rate, NH_3 execratory rate and metabolic rate were found to be maximum in PL fed with 3% LS+BS+SC diet, followed by the PL fed with 2%, 1% and 4% when compared with control. The statistical analysis (DMRT) made on the energy utilization performance between control and experimental diets revealed that the variation between them was significant (P<0.05).

Similar result was observed in *M. rosenbergii* PL fed with Binifit^{TM} supplemented diets Seenivasan *et al*. (2011). It has been reported that the probioitics *Lactobacillus* and yeast supplemented diets had significantly increased the energy utilization performance and feed utilization in pearl spot, *Etroplus suratensis* (Immanuel *et al*., 2003) in *O. niloticus* fed with *B. subtilis* and *L. plantarum*, and the yeast *S. cerevisiae* (Essa *et al*., 2010). Also, Abdel-Tawwab *et al*. (2008) reported that the growth and feed energy utilization was increase in *S. cerevisiae* supplemented diet fed Nile tilapia, *O. niloticus*. EL-Haroun (2007) reported that probiotics Biogen® supplemented diets had significantly enhance the growth and feed utilization performance in African Catfish, *Clarias gariepinus*. Bomba *et al*., (2002) have been suggested that the probiotics influence digestive processes by enhancing beneficial gut microfloral populations, this intern enhance and absorption of food and feed utilization.

Microbial Study

The qualitative microbial study showed presence of the rearing control medium and control PL gut following bacteria, such as *Bacillus* sp., *Pseudomonas* sp., *Escherichia coli*, *Streptococcus* sp., and *Klebsiella pneumonia* in the Table 3 and 4. In the experimental PL, the presence of *Pseudomonas* sp., and *Klebsiella pneumonia* were replaced by establishment of *L. sporogenes* ($100x10^{-4}$ cfu cells),

B. subtilis ($140x10^{-4}$ CFU) and *S. cerevisiae* ($80x10^{-4}$ CFU) colonies (Table 5). All necessary confirmation biochemical tests were performed and the results are presented (Tables 3-6). Colony establishment of the probiotic *L. sporogenes* has previously been observed by Seenivasan *et al.* (2012). Colony establishment in the gut has also been reported in this species of prawn when fed with bio-encapsulated *L. sporogenes* and *L. acidophilus* (Venkat *et al*., 2004). Colony establishments like *B. subtilis*, *L. lactis* and *S. cerevisiae* in *Labeo rohita* (Mohapatra *et al*., 2011), *B. subtilis* in the Indian major carps (Nayak and Mukherjee, 2011), *Bacillus* spp in rainbow trout, *O. mykiss* (Bagheri *et al.* 2008), *Lactobacillus* spp in the sea bream, *Sparus aurata* (Suzer *et al*., 2008), Lactobacil, sporolac, and yeast in Juvenile Goldfish, *Carassius auratus* (Ahilan *et al*., 2004), *L. acidophilus* and *S. cervisiae* in pearl spot, *E. suratensis* (Immanuel *et al*., 2003) and *Bacillus* S11 at 10^6 cfu g^{-1} in *Penaeus monodon* (Rengpipat *et al*., 2000) has been reported. Therefore, in the present study establishment of LS+BS+SC colony improved the intestinal health, thereby increases feed utilization and nutritional profiles of PL. Therefore, 3% LS+BS+SC can be incorporated in feed formulation for healthy maintenance of *M. rosenbergii* in sustainable developing of its aquaculture.

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References

Abdel-Tawwab, M., Abdel-Rahman, A.M. and Ismael, N.E.M. 2008. Evaluation of commercial live baker's yeast, *Saccharomyces cerevisiae* as a growth and

Table 3. Biochemical characterization of isolates in control water

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

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

Table 4. Biochemical characterization of isolates in control PL gut

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

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

Table 5. Biochemical characterization of isolates in experimental PL gut

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

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

Table 6. Overall result of microbial load in control water, control PL and experimental PL

Isolate Name	Control water (10^{-5})	Control PL gut (10^{-5})	Experimental PL gut (3% LS+BS+SC)
Bacillus sp	P	P	P
Pseudomonas sp	P	P	A
E. coli	P	P	P
Streptococcus sp	P	P	P
Klebsiella pneumoniae	P	P	A
L. sporogenes	A	A	P ($100x10^4$ CFU)
B. subtilis	A	A	P ($140 x10^4$ CFU)
S. cerevisiae	A	A	P ($80 x10^4$ CFU)

P, present; A, absent.

immunity promoter for fry Nile tilapia, *Oreochromis niloticus* (L.) challenged *in situ* with *Aeromonas hydrophila.* Aquaculture, 280: 185–189. doi: 10.1016/j.aquaculture.2008.03.055

Ahilan, B., Shine, G. and Santhanam, R. 2004. Influence of Probiotics on the Growth and Gut Microbial Load of Juvenile Goldfish (*Carassius auratus*). Asian Fisheries Science, 17: 271-278.

Al-Dohail, A.M., Hashim, R. and Aliyu-Paiko, M. 2009. Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. Aquacult Res., 40: 1642-1652. doi: 10.1111/j.1365-2109.2009.02265.x

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 19th Edition, American Public Health Association, New York.

Bagheri, T., Hedayati, S., Yavari, V., Alizade, M. and Farzanfar, A. 2008. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turk. J. Fish. Aquat. Sci., 8: 43-48.

Balcazar, J.L., De Blas, I., Ruiz-Zarzuela, I., Vendrell, D., Calvo, A.C., Márquez, I., Gironés, O. and Muzquiz, G.L. 2007a. Changes in intestinal microbiota and humoral immune response following probiotic administration in brown trout (*Salmo trutta*). Br. J. Nutr., 97: 522–527. doi: 10.1017/S0007114507432986

Balcazar, J.L., De Blas, I., Ruiz-Zarzuela, I., Vendrell, D., Girones, O. and Muzquiz, G.L. 2007b. Enhancement of the immune response and protection induced by probiotic lactic acid bacteria against furunculosis in rainbow trout (*Oncorhynchus mykiss*). FEMS Immunol. Med. Microbiol., 51: 185–193. doi: 10.1111/j.1574-695X.2007.00294.x

Bomba, A., Nemcoal, R., Gancarc koval, S., Herich, R., Guba, P. and Mudron oval, D. 2002. Improvement of the probiotic effect of microorganisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. Brit J. Nutr., 88: 95-99. doi: 10.1079/BJN2002634

Boonthai, T., Vuthhiphandchai, V. and Nimrat, S. 2011. Probiotic bacteria effects on growth and bacterial composition of black tiger shrimp (*Penaus monodon*). Aquacult. Nutr., 17(6): 634-644. doi: 10.1111/j.1365-2095.2011.00865.x

Bowman, P.I. and Ahearn, D.G. 1975. Evaluation of the Uni-Yeast-Tek kit for the identification of medically important yeasts. J. Clin. Microbiol., 2: 354-358.

Capkin, E. and Altinok I. 2009. Effects of dietary probiotic supplementations on prevention ⁄ treatment of yersiniosis disease. J. Applied Microbiol., 106: 1147–1153. doi: 10.1111/j.1365-2672.2008.04080.x

Dhanaraja, M., Haniffaa, M.A., Arun Singha, S.V., Jesu Arockiarajb, A., Muthu Ramakrishanana, C.,

Seetharamana, S. and Arthimanjua, R. 2010. Effect of probiotics on growth performance of koi carp (*Cyprinus carpio*). J. Appl. Aquaculture, 22: 202–20. doi: 10.1080/10454438.2010.497739

El-Haroun, E.R., Goda, A.M.A.S. and Chowdhury, M.A.K. 2006. Effect of dietary probiotic Biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus.* Aquacul. Res., 37: 1473-1480. doi: 10.1111/j.1365-2109.2006.01584.x

El-Haroun, E.R. 2007. Improved growth rate and feed utilization in farmed African catfish *Clarias gariepinus* (Burchell 1822) through a growth promoter Biogen® supplementation. Journal of Fisheries and Aquatic Sciences, 2: 319-327. doi: 10.3923/jfas.2007.319.327

Essa, M.A., EL-Serafy, S.S., El-Ezabi, M.M., Daboor, S.M., Esmael, N.A. and Lall, S.P. 2010. Effect of different dietary probiotics on growth, feed utilization and digestive enzymes activities of Nile Tilapia, *Oreochromis niloticus.* Journal of the Arabian Aquaculture Society, 5(2): 51-56.

FAO/WHO, 2001. Report on Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. 1–4 October Cordoba, Argentina. Available at: ftp:// ftp.fao.org/ es/esn/food/probio_report_en.pdf (Accessed 23 August 2007).

Folch, J., Lees, M. and Bloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 266: 497-509.

Gatesoupe, F.J. 1999. The use of probiotics in aquaculture, Aquaculture, 180: 147-165. doi: 10.1016/S0044-8486(99)00187-8

Ghosh, S., Sinha, A. and Sahu, C. 2008. Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. Aquacult. Nutri., 14: 289-299. doi: 10.1111/j.1365-2095.2007.00529.x

Hidalgo, M.C., Skalli, A., Abella, N.E., Arizcun, M. and Cardenete, G. 2006. Dietary intake of probiotics and maslinic acid in juvenile dentex (*Dentex dentex* L.): effects on growth performance, survival and liver proteolytic activities. Aquacult. Nutr., 12: 256-266. doi: 10.1111/j.1365-2095.2006.00408.x

Hisano, H., Falcon, R.D., Maria Barrose, M. and Pezzato, E.L. 2008. Influence of yeast and yeast derivatives on growth performance and survival of Juvenile Prawn *Macrobrachium amazonicum.* Ciência Animal Brasileira, 9: 657-662

Hong, H.A., Duc, L.H. and Cutting, S.M. 2005. Cutting SM. The use of bacterial spore former as probiotics. *FEMS* Microbiol. Rev., 29: 813-835. doi: 10.1016/j.femsre.2004.12.001

Immanuel, G., Menethira, V., Beena, S. and Palavesam, A. 2003. Effect of probionts on the growth, food utilization and biochemical changes in pearl spot *Etroplus suratensis* (Bloch). Indian J. Fish., 50: 273-278.

Immanuel, G., Menenthira, V., Prema, P. and Palavesam, A. 2003. Influence of probionts on the gut microbial diversity in pearl spot, *Etroplus suratensis* (Bloch). Indian J. Fish., 50: 499-508.

Keysami, M.A., Saad, C.R., Sijam, K., Daud, H.M. and Alimon, A.R. 2007. Effect of *Bacillus subtilis* on growth development and survival of postlarvae *Macrobrachium rosenbergii* (de Man). Aquac Nutr., 13: 131-136. doi: 10.1111/j.1365-2095.2007.00463.x

Lara-Flores, M., Olivera-Castillo, L. and Olvera-Novoa, M.A. 2010. Effect of the inclusion of a bacterial mix (*Streptococcus faecium and Lactobacillus acidophilus*), and the yeast (*Saccharomyces cerevisiae*) on growth, feed utilization and intestinal enzymatic activity of Nile tilapia (*Oreochromis niloticus*). International Journal of Fisheries and Aquaculture, 2: 93-101.

Li, P., Delbert, M. and Gatlin, I.I.I.T. 2005. Evaluation of the prebiotic GroBioticR-A and brewers yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops_M. saxatilis*) challenged *in situ* with *Mycobacterium marinum*. Aquaculture, 248: 197-205. doi: 10.1016/j.aquaculture.2005.03.005

Lowry, O.H., Rosenbrough, W.J., Fair, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Merrifield, D.L., Bradley, G., Baker, R.T.M. and Davies, S.J. 2009. Probiotic application for rainbow trout (*Oncorhynchus mykiss* walbaum) II. Effects on growth performance, feed utilization, intestinal microflora and related health criteria post antibiotic treatment. Aquacult. Nutr., 16(5): 496-503. doi: 10.1111/j.1365-2095.2009.00688.x

Mohapatra, S., Chakraborty, T., Prusty, A.K., Das, P., Paniprasad, A.K. and Mohanta, K.N. 2011. 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. Aquaculture Nutrition, 18(1):1-11. doi: 10.1111/j.1365-2095.2011.00866.x

Moore, W. and Stein, H. 1948. In: S.P. Olowick and N.D. Kaplan (Eds.), Methods in enzymol. Academic press, New York, 468 pp.

Nayak, S.K. and Mukherjee, S.C. 2011. Screening of gastrointestinal bacteria of Indian major carps for a candidate probiotic species for aquaculture practices. Aquacul.Res., 42: 1034-1041. doi: 10.1111/j.1365-2109.2010.02686.x

Parthasarathy, R. and Ravi, D. 2011. Probiotic bacteria as growth promoter and biocontrol agent against *Aeromonas hydrophila* in *Catla catla* (Hamilton, 1822). Indian J. Fish., 58: 87-93.

Petrusewicz, K. and Macfadyen, A. 1970. Productivity of Terrestrial Animals: Principles and Methods, (IBP Handbook No. 13). Blackwell, Oxford.

Pooramini, M., Kamali, A., Hajimoradloo, A., Alizadeh, M. and Ghorbani, R. 2009. Effect of using yeast (*Saccharomyces cerevisiae*) as probiotic on growth parameters, survival and carcass quality in rainbow trout *Oncorhynchus mykiss* fry. Int. Aquat. Res., 1: 39-44.

Rengpipat, S., Runkpratanporn, S., Piyatiratitivorakul, S. and Menasaveta, P. 2000. Immunity enhancement on black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). Aquaculture, 191: 271-288. doi: 10.1016/S0044-8486(00)00440-3

Roe, J.H. 1955. The determination of sugar and blood and spinal fluid with anthrone reagent. J.Biol.Chem., 212: 335-343.

Saad, S.A., Habashy, M.M. and Sharshar, M.K. 2009. Growth response of the freshwater prawn, *Macrobrachium rosenbergii* (De Man), to diets having different levels of Biogen®. World Applied Sciences Journal, 6: 550-556.

Seenivasan, C., Saravana Bhavan, P. and Radhakrishnan, S. 2011. Effect of probiotics (Binifit^{TM}) on survival, growth, biochemical constituents and energy budget of the freshwater prawn *Macrobrachium rosenbergii* post larvae. Elixir Aquaculture, 41: 5919-5927.

Seenivasan, C., Bhavan, P.S. Radhakrishnan, S. and Shanthi,R. 2012. 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*. Turki. J .Fis Sci., 12: 23-31. doi: 10.1111/j.1365-2109.2010.02554.x

Shinde, A.N., Mulye, V.B., Chogale, N.D., Bhatkar, V.R., Bondre, R.D. and Mohite, A.S. 2008. Effect of different probiotics *Macrobrachium Rosenbergii* (De-Man) post larvae. Aquacult., 9: 7-12

Suralikar, V. and Sahu, N.P. 2001. Effect of feeding probiotic (*Lactobacillus cremoris*) on growth and survival of *Macrobrachium rosenbergii* postlarvae. J. Appl. Anim. Res., 20: 117-124. doi: 10.1080/09712119.2001.9706744

Suzer, C., Coban, D., Kamaci, O.H., Saka, S., Firat, K., Otgucuoglu, O. and Kucuksari, H. 2008. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: Effects on growth performance and digestive enzyme activities. Aquaculture, 280: 140-145. doi: 10.1016/j.aquaculture.2008.04.020

Tekinay, A.A. and Davies, S.J. 2001. Dietary carbohydrate level influencing feed intake, nutrient utilisation and plasma glucose concentration in the rainbow trout, *Oncorhynchus mykiss*. Turkish J. Veter. Ani. Sci., 25: 657–666.

Venkat, H.K., Narottam, P.S. and Kamal, K.J. 2004. Effect of feeding *Lactobacillus*-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (De Man). Aquacul. Res., 35: 501-507. doi: 10.1111/j.1365-2109.2004.01045.x