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Efficacy of Probiotics on Survival, Growth, Biochemical Changes and Energy Utilization Performance of *Macrobrachium rosenbergii* (De Man 1879) Post-larvae

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Abstract

The efficacy of combined probiotics, *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* (LS+SC) on survival, growth, biochemical changes and energy utilization of *Macrobrachium rosenbergii* post larvae (PL) was examined. Each probiotic organism was individually tested at four different concentrations (1-4%) separately. The best concentration in each probiotic species was combined and tested for its suitability in aquaculture usage. The basal diet was incorporated with probiotics, LS+SC (4:4) at five different concentrations 0% (control), 1%, 2%, 3% and 4%. These diets were fed to *M. rosenbergii* PL for a period of 90 days. After the feeding trail, 2% LS+SC incorporated diet had significantly (P<0.05) higher survival, WG, SGR, FCE and PER compared with other experimental groups than the control. Whereas, the FCR was significantly (P<0.05) lower in 2% LS+SC incorporated diet fed PL. Similarly the proximate composition of the protein, amino acid, carbohydrate, lipid and ash content were significantly (P<0.05) higher in 2% LS+SC incorporated diet fed PL than the control. The energy utilization parameters were significantly (P<0.05) higher in 2% LS+SC incorporated diet fed PL than the control. This study indicated that combined probiotics, LS+SC incorporated diets were beneficial for M. rosenbergii in terms of increasing growth and enhancing energy utilization performances.

Keywords: M. rosenbergii; Growth; Biochemical composition; Energy utilization; *L. sporogenes; S. cerevisiae.*

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1. Introduction

The giant river prawn, *Macrobrachium rosenbergii* is one among crustaceans, native to Southeast Asia, South Pacific countries, northern Oceania, and Western Pacific islands. *M. rosenbergii* has become the main freshwater prawn species for small-scale and large-scale farming because of its fast growth, large size, better meat quality, omnivorous feeding habit and established domestic and export markets in Asia [1]. A probiotic is

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generally defined as a live microbial food supplement, which improves the balance of the host animals' intestinal flora [2]. The use of probiotics in the aquatic organisms is increasing with the demand for more environment-friendly aquaculture practices [3]. A number of studies have shown that probiotics can improve the survival, growth and biochemical changes of the freshwater prawn *M. rosenbergii* [4-13]. Therefore, the present study was undertaken to determine the effect of feeding combined probiotics, *Lactobacillus sporogenes* and yeast *Saccharomyces cerevisiae* on the survival, growth, biochemical changes and energy utilization performance of the freshwater prawn *M. rosenbergii* PL.

2. 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 commencement of the feeding experiment. The experimental water had these physicochemical parameters: pH, 7.20±0.30; total dissolved solids, 0.98±0.10 g/L^{-1}; dissolved oxygen, 7.30±0.40 mg/L^{-1}; BOD, 4.00±1.60 mg/L^{-1}; COD, 10.00±9.00 mg/L^{-1} and ammonia, 0.068±0.008 mg/L^{-1}.

Table 1. Ingredients and proximate composition of prepared diets.

Ingredients (%)	Control	Experimental diets (*L. sporogenes* + *S. cerevisiae* incorporated)			
		1%	2%	3%	4%
Fish meal	33.84	33.84	33.84	34.84	35.84
Ground nut oil cake	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+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

2.1. *Diet preparation*

The composition of the experimental diets is given in Table 1. The probiotics, *L. sporogenes* (Uni-Sankyo Ltd, Maharashtra, India) and yeast *S. cerevisiae* (Intercare Ltd, Gujarat, India) one gram of lyophilized powders contains $15x10^7$ and $10x10^7$cfu cells respectively. The probiotics, LS+SC (4:4) 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 concentration of LS+SC (4:4) was mixed with the dough and the diets were pelletized separately with a locally made (Kolkata, India) hand pelletizer. 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.

2.2. *Feeding experiment*

M. rosenbergii (PL-30) with the length and weight range of 1.54±0.02cm and 0.22±0.03g respectively were used for feeding experiment. 40 PL for each diet in triplicate were maintained in plastic tanks with 20 L water. The PL was maintained at the stocking density of 2/l. One group served as control, which devoid of probiotics (0%). The experimental groups were fed with the respective concentration of LS+SC (4:4) incorporated diets. The feeding was adjusted to two times a day (6:00 am and 6:00 pm). The daily ration was given at the rate of 10% of the body weight of PL with two equal half throughout the experimental period. The unfed feed, faeces and moult if any, were collected after the respective hours of feeding. The feeding experiment was prolonged for 90 days; mild aeration was given continuously in order to maintain the optimal oxygen level.

2.3. *Growth study*

After the feeding trial, the growth parameters such as survival (S), 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 [6].

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

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

SGR (%) = $\log w_2 - \log w_1/t \times 100$ (where, w_1 and w_2 = Initial and final weight, respectively (g)**,** and t = Total number of experimental days)

Feed conversion rate (g) = Total feed intake (g)/ Total weight gain of the prawn (g)

Feed conversion efficiency (%) = Biomass (g)/ Total Feed intake (g) × 100

Protein efficiency rate (g) = Total weight gain of PL (g)/Total protein consumed (g)

2.4. *Energy utilization study*

The energy content of whole prawns, feeds, moult and faeces was measured using Parr 1281 Oxygen Bomb Calorimeter. The energy budget was calculated using the equation ($C = (P_g+E) + R + F + U$) derived by Petrusewicz and Macfadyen [14]; where, C is the energy consumed in food; P_g 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 (FR) = Mean food consumption (kcal/day)/Initial live wt. of the prawn (g)

Mean absorption = Mean food consumption (kcal/day) – Mean food excreted as faeces (kcal/day)

Absorption rate (AR) = Mean absorption (kcal/day)/Initial live wt. of the prawn (g)

Mean conversion = Mean weight gain (kcal/day) + Mean exuvial weight (kcal/day)

Conversion rate (CR) = Mean conversion (kcal/day)/Initial live wt. of the prawn (g)

NH_3 excretion rate (AE) = Mean NH_3 excretion (kcal/day) /Initial live wt. of the prawn (g)

Metabolic Rate (MR) = Absorption rate (kcal/g/day) – Conversion rate (kcal/g/day) + NH_3 excretion rate (kcal/g/day).

2.5. *Biochemical constituents of the experimental animals*

The initial and final day of the experiment, the biochemical constituents of the experimental animals were determined. The biochemical constituents, such as total protein [15], amino acid [16], lipid [17], carbohydrate [18], ash and moisture contents [19] of individual diet fed prawns were measured.

2.6. *Microbial study*

Microbial analyses [19], and yeast isolation [20] were performed in the rearing (control) water, control PL gut and experimental PL gut.

2.7. *Statistical analyses*

The data obtained in the present study were subjected to different statistical interpretations. 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 mean± standard deviations.

3. Results and Discussion

3.1. *Morphometric data*

Table 2 represents the morphometric data of LS+SC (4:4) supplemented diet fed PL group. The initial average body length and weight of PL was 1.54±0.02cm and 0.22±0.03g, respectively. After 90 days of feeding experiment, the final length and weight were found to be higher in PL fed with 2% LS+SC incorporated diet followed by the other experimental groups such as 3% LS+SC, 4% LS+SC and 1% LS+SC diets than the control. These differences were found to be statistically significant ($P < 0.05$). Similar

Table 2. The morphometric data, growth performance, biochemical constituents and energy utilization of *M. rosenbergii* PL fed with *L. sporogenes*+*S. cerevisiae* (4:4) incorporated diets.

Parameters	Control diet	Experimental diets				F-Value
		1% LS+SC	2%LS+SC	3%LS+SC	4%LS+SC	
Initial length (cm)	1.54±0.02	1.54±0.02	1.54±0.02	1.54±0.02	1.54±0.02	-
Final length (cm)	4.70^{b}±0.24	5.42^{a}±0.31	5.94^{a}±0.26	5.74^{a}±0.27	5.50^{a}±0.30	8.68
Initial weight (g)	0.22±0.03	0.22±0.03	0.22±0.03	0.22±0.03	0.22±0.03	-
Final weight (g)	1.16^{b}±0.10	1.60^{ab}±0.18	1.93^{a}±0.22	1.85^{a}±0.31	1.68^{a}±0.40	3.91
S (%)	80.00±2.50^{b}	85.00±2.50^{ab}	87.50±2.50^{a}	82.50±3.00^{ab}	80.00±3.00^{b}	4.34
WG (g)	0.94±0.10^{c}	1.38±0.14^{b}	1.71±0.16^{a}	1.63±0.20^{b}	1.46±0.19^{ab}	10.33
SGR (%)	0.802±0.026^{d}	0.957±0.034^{c}	1.047±0.028^{a}	1.027±0.023^{ab}	0.980±0.034^{bc}	32.61
FCR (g)	3.30±0.22^{a}	2.56±0.18^{b}	2.41±0.17^{b}	2.43±0.15^{b}	2.48±0.24^{b}	11.15
FCE (%)	0.94±0.09^{b}	1.34±0.15^{a}	1.46±0.22^{a}	1.53±0.13^{a}	1.39±0.24^{a}	5.20
PER (g)	0.66±0.08^{b}	0.86±0.04^{a}	0.91±0.06^{a}	0.91±0.09^{a}	0.89±0.05^{a}	7.59
Protein (%)	59.40±3.70^{b}	61.80±2.60^{ab}	65.10±2.52^{a}	63.08±2.48^{ab}	62.08±2.68^{ab}	1.60
Amino acid (%)	23.14±2.48^{a}	26.08±2.69^{a}	28.60±3.74^{a}	27.00±3.00^{a}	24.90±2.96^{a}	1.42
Carbohydrate (%)	11.96±1.34^{c}	14.10±1.51^{bc}	18.00±1.28^{a}	16.20±1.92^{ab}	13.61±1.98^{bc}	6.24
Lipid (%)	9.00±1.73^{c}	12.00±1.44^{ab}	13.68±1.58^{a}	12.01±1.24^{ab}	10.60±1.52^{bc}	4.05
Ash (%)	16.00±1.37^{b}	18.00±1.09^{ab}	19.40±1.42^{b}	18.10±1.41^{ab}	17.10±1.26^{a}	2.77
Moisture (%)	76.42±4.05^{a}	76.00±3.38^{a}	75.00±3.43^{a}	75.30±3.19^{a}	76.30±3.00^{a}	<1
FR (k.cal/g/day)	0.396±0.028^{c}	0.452±0.075^{d}	0.520±0.082^{c}	0.493±0.074^{c}	0.459±0.058^{b}	8.67
AR (k.cal/g/day)	0.340±0.066^{b}	0.406±0.051^{c}	0.489±0.049^{b}	0.452±0.010^{bc}	0.422±0.022^{ab}	20.29
CR (k.cal/g/day)	0.212±0.092^{a}	0.253±0.067^{a}	0.325±0.052^{a}	0.293±0.047^{a}	0.274±0.060^{a}	12.06
AE (k.cal/g/day)	0.011±0.007^{ab}	0.013±0.004^{ab}	0.021±0.006^{ab}	0.019±0.008^{a}	0.017±0.013^{ab}	6.14
MR (k.cal/g/day)	0.139±0.022^{b}	0.166±0.031^{bc}	0.185±0.024^{b}	0.178±0.036^{ab}	0.165±0.029^{ab}	2.01

Each value is a mean±SD of three replicate analysis, within each row means with different superscripts letters are statistically significant P<0.05 (one way ANOVA and subsequently *post hoc* multiple comparison with DMRT).
S - survival; WG - weight gain; SGR - specific growth rate; FCR - feed conversion rate; FCE - feed conversion efficiency; PER - protein efficiency rate; FR- feeding rate; AR- absorption rate; CR- conversion rate; AE- NH_3 excretory rate; MR- metabolic rate

improved morphometric data (length and weight) has been reported in *M. rosenbergii* PL fed with *L. sporogenes* bioencapsulated diets [5], *Bacillus* spp KKU02 and *Bacillus* spp KKU03 supplemented diets [11] and in shrimp, *Penaus indicus* after feeding with *L. acidophilus, S. cremoris, L. bulgaricus*-56 and *L. bulgaricus*-57 incorporated diets [21], *Bacillus* sp incorporated diets [22], *Bacillus* spp incorporated diets [23], *B. subtilis* UTM 126 incorporated diets [24].

3.2. *Survival performance*

Table 2 also depicted the survival performance of LS+SC (4:4) incorporated diet fed PL group. After 90 days of feeding trail, the survival performance of *M. rosenbergii* PL was found to be higher in 2% LS+SC incorporated diet. But it was only 80.00% in control diet fed prawn PL. On the other hand, the survival percentage of other experimental diets fed prawns was in the order of 1% LS+SC, 3% LS+SC, 4% LS+SC. These differences were found to be statistically significant (P<0.05). Similar improved survival has been reported in *M. rosenbergii* PL fed with *L. sporogenes* bioencapsulated diets [5], Binifit^{TM} supplemented diets [6], Biogen® supplemented diets [8] and different probiotics supplemented diets [9], and in shrimps, fed with *L. acidophilus, S. cremoris, L. bulgaricus*-56 and *L. bulgaricus*-57 incorporated diets [21], *B. subtilis* incorporated diets [25] and *Bacillus* sp incorporated diets [26].

3.3. *Growth performance*

The results on growth performance of LS+SC (4:4) incorporated diet fed PL group are also displayed in Table 2. At the end of the feeding trail, the weight gain, specific growth rate, feed conversion efficiency and protein efficiency rate were significantly (P<0.05) higher in probiotics LS+SC (4:4) incorporated diet fed groups than the control. The result on weight gain, specific growth rate, feed conversion efficiency and protein efficiency rate were found to be maximum in 2% LS+SC incorporated diet fed prawn PL, followed by 3%, 4% and 1% when compared with control. The differences between control and experimental diets fed prawns were statistically significant (P<0.05). The feed conversion ratio was found to be higher in PL fed with control diet, followed by the PL fed with 1%, 4%, 3% and 2% diets. These differences were found to be statistically significant (P<0.05). Similar results have been reported in *M. rosenbergii* fed with *L. sporogenes* supplemented diets [4], bio-encapsulated *L. sporogenes* [5], Binifit^{TM} incorporated diets [6], *Bacillus* spp supplemented diets [7], Biogen® incorporated diets [8], different probiotics supplemented diets [9], *Bacillus* spp KKU02 and *Bacillus* spp KKU03 incorporated diets [11], bio-encapsulated *B. subtilis* [10], *Saccharomyces cerevisiae* and yeast derivatives supplemented diets [27], bio-encapsulated *L. acidophilus* and *L. sporogenes* [12], bio-encapsulated *L. ceremoris* [13] and in fishes, *L. plantarum* and *B. megaterium* supplemented diets [28] *Lactobacillus acidophilus* and yeast *Saccharomyces cervisiae* incorporated diets [29], *B. subtilis*, *B. licheniformis* and *Enterococcus faecium* incorporated diets [30], bio-encapsulated *L. casei* [31], *Bacillus* spp supplemented diets

[32], *B. subtilis* supplemented diets [33] and *B. toyoi* and *B. cereus* incorporated diets [34].

3.4. *Biochemical constituents of experimental animals*

The results on biochemical constituents, such as protein, amino acid, carbohydrate, lipid, ash and moisture content of LS+SC (4:4) incorporated diet fed PL group are also showed in Table 2. After the feeding trail experiment of 90 days, the protein, amino acid, carbohydrate, lipid and ash contents were found to be maximum in PL fed with 2% LS+SC diet, followed by the PL fed with 3%, 4% and 1% diets when compared with control. These differences were found to be statistically significant ($P<0.05$). In the case of moisture content just the reverse was recorded. A similar result in biochemical composition was previously observed in *M. rosenbergii* PL fed with *L. sporogenes* supplemented diet [4], bio-encapsulated *L. sporogenes* [5], Binifit^{TM} supplemented diets [6], Biogen® incorporated diets [8] and bio-encapsulated *L. sporogenes* and *L. acidophilus* [12] and in fishes *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* supplemented diets [35], *B. subtilis* NIOFSD017 and *L. plantarum* NIOFSD018, and yeast *S. cerevisiae* NIOFSD019 incorporated diets [36], *B. subtilis*, *B. licheniformis* and *Enterococcus faecium* supplemented diets [30], *Bacillus* spp incorporated diets [32], Biogens® incorporated diets [37] and *B. toyoi* and *B. cereus* incorporated diets [34].

3.5. *Energy utilization performance*

The energy utilization performance of LS+SC (4:4) incorporated diet fed group of prawn PL is also paved in Table 2. The feeding rate, absorption rate, conversion rate, NH_3 execratory rate and metabolic rate were found to be higher in PL fed with 2% LS+SC diet, followed by the PL fed with 3%, 4% and 1% diets when compared with control. These differences were found to be statistically significant ($P<0.05$). Similarly, Seenivasan et al. [6] showed that Binifit^{TM} supplemented diets had improved the energy utilization performance of freshwater prawn, *M. rosenbergii* PL. Dhanaraj et al. [29] noted that *Lactobacillus acidophilus* and yeast *S. cerevisiae* supplemented diets had improved the energy budget of Koi Carp, *Cyprinus carpio*. It has been reported in pearl spot, *Etroplus suratensis* fed with *Lactobacillus* and yeast supplemented diets had significantly improved the feed energy utilization performance [38]. It has also been reported in Nile tilapia of the nutrient energy utilization performance was higher in *Saccharomyces cerevisiae* supplemented diets [39]. EL-Haroun et al. [37] showed that Biogen® incorporated diets have improved the growth and feed energy utilization performance of Nile tilapia, *Oreochromis niloticus.*

3.6. *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., E. coli, Streptococcus sp.,* and *Klebsiella pneumonia* in the Table 3 and 4. In the experimental PL, the presences of *Klebsiella pneumonia* were replaced by establishment of *L. sporogenes* ($140x10^{-4}$ cfu cells) and *S. cerevisiae* ($90x10^{-4}$ cfu cells) colonies (Table 5). All necessary confirmation biochemical tests were performed and the results are presented (Tables 3 to 6).

Table 3. Biochemical characterization of isolates in control water.

Tests	*Bacillus* sp	*Pseudomonas* sp	*E. coli*	*Streptococcus* sp	*Klebsiella pneumonia*	*L. sporogenes*
Gram's Staining	+	-	-	+	-	-
Motility test	+	+	+	+	-	-
Indole test	-	-	+	-	-	-
Methyl red test	-	-	+	-	-	-
VP test	-	+	-	+	+	-
Citrate utilization test	+	+	-	+	+	-
Starch hydrolases	+	-	+	+	+	-
Gelatin hydrolases	+	+	+	+	+	-
Nitrate reduction test	+	-	+	+	+	-
Oxidase test	-	+	+	-	+	-
Catalase test	+	+	-	-	+	-
Glucose test	A	A	A	A	A	-
Lactose test	A	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 .

Similar results have been reported in the gut of *M. rosenbergii* PL fed with bio-encapsulated *L. sporogenes* [5]. It has been reported in prawn, *M. rosenbergii* fed with bio-encapsulated *L. sporogenes* and *L. acidophilus* that established in the gut [12]. It has also been reported that the probiotic bacterial colonies established in the intestine of the

shrimp, *P. monodon* fed with *Bacillus* S11 supplemented diets [40]. Colony establishment in the gut has also been reported in fishes, *B. subtilis*, *L. lactis* and *S. cerevisiae in Labeo rohita* [41], *B. subtilis* in the Indian major carps [42], *L. acidophilus* in *Poecilia recticulata* [43], *Bacillus* spp in *Onchorhynchus mykiss* [32], *Lactobacillus* spp in the sea bream, *Sparus aurata* [44], Lactobacil, sporolac, and yeast in Juvenile Goldfish, *Carassius auratus* [45] and *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* in *Etroplus suratensis* [38].

Table 4. Biochemical characterization of isolates in control PL gut.

Tests	*Bacillus* sp	*Pseudomonas* sp	*E. coli*	*Streptococcus* sp	*Klebsiella pneumonia*	*L. sporogenes*
Gram's sstaining	+	-	-	+	-	-
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.

The present study concluded that the selected probiotics, LS+SC (4:4) from at optimized concentrations was found to enhance the survival, growth, tissue biochemical constituents and energy utilization of reared prawn *M. rosenbergii*. Further research on the diets produced with optimized concentration of the chosen probiotics organisms may be evaluated under field condition in the candidate species *M. rosenbergii*.

Table 5. Biochemical characterization of isolates in experimental PL gut.

Tests	*Bacillus* sp	*Pseudomonas* sp	*E. coli*	*Streptococcus* sp	*Klebsiella pneumonia*	*L. sporaoenes*
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 6. Overall result of microbial load in control water, control PL and experimental PL.

Isolate Name	Control water (10^{-5})	Control PL gut	Experimental PL gut (2% LS+SC)
Bacillus sp	P	P	P
Pseudomonas sp	P	P	P
E. coli	P	P	P
Streptococcus sp	P	P	P
Klebsiella pneumoniae	P	P	A
L. sporogenes	A	A	P (140×10^{-4} cfu cells)
S. cerevisiae	A	A	P (90×10^{-4} cfu cells)

P = present; A = absent.

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References

1. M. B. New, Aquacul. Res. **26**, 54 (1995). http://dx.doi.org/10.1111/j.1365-2109.1995.tb00859.x
2. R. Fuller, J. Appl. Bacteriology **66,** 365(1989). http://dx.doi.org/10.1111/j.1365-2672.1989.tb05105.x
3. F. J. Gatesoupe. Aquaculture **180,** 147 (1999). http://dx.doi.org/10.1016/S0044-8486(99)00187-8
4. L. Prasad, B. B. Nayak, M. P. S. Kohli, A. K. Reddy, and P. P. Srivastava, Israeli J. Aquaculture **64,** 676 (2012).
5. C. Seenivasan, P. S. Bhavan, S. Radhakrishnan, and R. Shanthi, Turki. J . Fis. Sci. **12,** 23 (2012).
6. C. Seenivasan, P. Saravana Bhavan, and S. Radhakrishnan, Elixir Aquaculture **41,** 5919 (2011).
7. K. Rinisha, M. K. M. Rahiman, R. M. Beevi, A. P. Thomas, and A. A. M. Hatha, Fishery Technology **47,**173 (2010).
8. S. A. Saad, M. M. Habashy, and M. K. Sharshar, World Appl. Sci. J. **6,** 550 (2009).
9. A. N. Shinde, V. B. Mulye, N. D. Chogale, V. R. Bhatkar, R. D. Bondre, and A. S. Mohite, Aquaculture **9,** 7 (2008).
10. M. A. Keysami, C. R. Saad, K. Sijam, H. M. Daud, and A. R. Alimon, Aqua. Nutr. **13,** 131 (2007). http://dx.doi.org/10.1111/j.1365-2095.2007.00463.x
11. S. Deeseenthum, V. Leelavatcharams, and D. J. Brook, Pak. J. Biol. Sci. **10,** 1481 (2007). http://dx.doi.org/10.3923/pjbs.2007.1481.1485
12. H. K. Venkat, P. S. Narottam and K. J. Kamal., Aqua. Res. **35,** 501 (2004). http://dx.doi.org/10.1111/j.1365-2109.2004.01045.x
13. V. Suralikar and N. P. Sahu, J. Appl. Anim. Res. **20,** 117 (2001). http://dx.doi.org/10.1080/09712119.2001.9706744
14. K. Petrusewicz and A. Macfadyen. Productivity of Terrestrial Animals: Principles and Methods, IBP Handbook No. 13 (Blackwell, Oxford, 1970).
15. O. H. Lowry, W. J. Rosenbrough, A. L. Fair, and R. J. Randall. J. Biol. Chem. **193,** 265 (1951). PMid:14907713
16. W. Moore and H. Stein. *In*: Methods in enzymol (eds: S. P. Olowick and N.D. Kaplan) (Academic press, New York, 1948) p. 468.
17. J. Folch, M. Lees, and G. H. Bloane-Stanley, J. Biol. Chem. **266,** 497 (1957).
18. J. H. Roe, J. Biol. Chem. **212,** 335 (1955). PMid:13233235
19. APHA. Standard Methods for the Examination of Water and Wastewater, 19th edn. American Public Health Association, New York (2005).
20. P. I. Bowman and D. G. Ahearn, J. Clin. Microbiol. **2,** 354 (1975). PMid:1102563 PMCid:362809
21. R. Fernandez, M. Sridhar and N. Sridhar, Res. J. Microbiol. **6,** 466 (2011).
22. R. Gomez, D. Geovanny, and M. A. Shen. J. Ocean University of China **7,** 215 (2008). http://dx.doi.org/10.1007/s11802-008-0215-x
23. T. Boonthai, V. Vuthhiphandchai, and S. Nimrat, Aquacult. Nutr. **17,** 634 (2011). http://dx.doi.org/10.1111/j.1365-2095.2011.00865.x
24. J. L. Balcazar, I. De Blas, I. Ruiz-Zarzuela, D. Vendrell, A. C. Calvo, I. Márquez, O. Gironés, and G. L. Muzquiz. Br. J. Nutr. **97,** 522 (2007). http://dx.doi.org/10.1017/S0007114507432986
25. W. Y. Shen, L. L. Fu, W. F. Li, and Y. R Zhu. Aqua. Res. **41,** 1691 (2010). http://dx.doi.org/10.1111/j.1365-2109.2010.02554.x
26. S. Ziaei-Nejad, M. H. Rezaei, G. A. Takami, D. L. Lovett, A. R. Mirvaghefi, and M. Shakouri.

Aquaculture. **252,** 516 (2006). http://dx.doi.org/10.1016/j.aquaculture.2005.07.021
27. H. Hisano, R. D. Falcon, M. Maria Barrose, and E. L. Pezzato, Ciencia Animal Brasileira **9**, 657 (2008)
28. R. Parthasarathy and D. Ravi, Indian J. Fish. **58**, 87 (2011)
29. M. Dhanaraja, M. A. Haniffaa, S. V. Arun Singha, A. Jesu Arockiarajb, C. Muthu Ramakrishanana, S. Seetharamana, and R. Arthimanjua, J. Appl. Aquacult. **22**, 202 (2010). http://dx.doi.org/10.1080/10454438.2010.497739
30. D. L. Merrifield, G. Bradley, R. T. M. Baker and S. J. Davies, Aquacult. Nutr. **16,** 496 (2010). http://dx.doi.org/10.1111/j.1365-2095.2009.00688.x
31. H. H. L. Hernandez, J. C. Barrera Mejia, G. C. Mejia, M. Del Carmen, M. Dosta, R. De Lara Andrade, and J. A. M. Sotres, Aquacult Nutri. **16**, 407 (2010). http://dx.doi.org/10.1111/j.1365-2095.2009.00679.x
32. T. Bagheri, S. Hedayati, V. Yavari, M. Alizade, and A. Farzanfar, Turk. J. Fish. Aqua. Sci. **8**, 43 (2008).
33. S. Ghosh, A. Sinha, and C. Sahu, Aqua. Nutri. **14,** 289 (2008). http://dx.doi.org/10.1111/j.1365-2095.2007.00529.x
34. M. C. Hidalgo, A. Skalli, N. E. Abella, M. Arizcun, and G. Cardenete, Aqua. Nutr. **12,** 256 (2006). http://dx.doi.org/10.1111/j.1365-2095.2006.00408.x
35. M. Lara-Flores, L. Olivera-Castillo, and M. A. Olvera-Novoa, Int. J. Fisheries Aqua. **2**, 93 (2010).
36. M. A. Essa, S. S. EL-Serafy, M. M. El-Ezabi, S. M. Daboor, N. A. Esmael, and S. P. Lall, J. Arabian Aqua. Soc. **5,** 2 (2010).
37. E. R. EL-Haroun, A. M. A. S. Goda, and M. A. K. Chowdhury, Aqua. Res. **37**, 1473 (2006).
38. G. Immanuel, V. Menethira, S. Beena and A. Palavesam, Indian J. Fish. **50**, 273 (2003).
39. M. Abdel-Tawwab, A. M. Abdel-Rahman, and N. E. M. Ismael, Aquaculture **280**, 185 (2008). http://dx.doi.org/10.1016/j.aquaculture.2008.03.055
40. S. Rengpipat, S. Runkpratanporn, S. Piyatiratitivorakul, and P. Menasaveta, Aquaculture **191**, 271 (2000). http://dx.doi.org/10.1016/S0044-8486(00)00440-3
41. S. Mohapatra, T. Chakraborty, A. K. Prusty, P. Das, A. K. Paniprasad, and K. N. Mohanta, Aqua. Nutr. **18,** 1 (2012). http://dx.doi.org/10.1111/j.1365-2095.2011.00866.x
42. S. K. Nayak and S. C. Mukherjee, Aqua. Res. **42**, 1034 (2011). http://dx.doi.org/10.1111/j.1365-2109.2010.02686.x
43. P. R. Sreedevi and V. Ramasubramanian, Aqua. Int. **19**, 789 (2011).
44. C. Suzer, D. Coban, O. H. Kamaci, S. Saka, K. Firat, O. Otgucuoglu, and H. Kucuksari. Aquaculture **280,** 140 (2008). http://dx.doi.org/10.1016/j.aquaculture.2008.04.020
45. B. Ahilan, G. Shine, and R. Santhanam, Asian Fisheries Sci. **17,** 271 (2004).