International Journal of Fisheries and Aquatic Studies 2015; 2(4): 319-323

ISSN: 2347-5129
IJFAS 2015; 2(4): 319-323

www.fisheriesjournal.com
Received: 22-12-2014
Accepted: 29-01-2015

Subharanjani S
Department of Microbiology and Biotechnology, the Standard Fireworks Rajaratnam College, Sivakasi, Tamilnadu.

Gunarani R
Research Department of Zoology, VHNSN College, Virudhunagar, Tamilnadu.

Prema P
Research Department of Zoology, VHNSN College, Virudhunagar, Tamilnadu.

Immanuel G
Centre for Marine Science and Technology, M.S. University, Rajakkamangalam, Tamilnadu.

Correspondence
Subharanjani S
Department of Microbiology and Biotechnology, the Standard Fireworks Rajaratnam College, Sivakasi, Tamilnadu.

Potential influence of probiotic bacteria on the growth gut microflora of *Carassius auratus*

Subharanjani S, Gunarani R, Prema P, Immanuel G

Abstract
The objectives of the present study were designed to evaluate the probiotic potential of selected probiotic strains on the growth and gut microflora of *Carassius auratus*. Standard methods were followed for analyzing Physico-chemical parameters of experimental water. Four different probionts, such as *Lactobacillus acidophilus*, Lactobacillus plantarum, *Lactobacillus sporogenes* were incorporated with control diet to evaluate their effect on the growth performance in *Carassius auratus*. A maximum weight gain of 0.629 ± 0.003g was recorded in the fishes fed with *L.sporogenes* incorporated diet followed by *L. plantarum* and *L. acidophilus* diets. Study reveals the antagonistic effect of probiotic strains against *V. parahaemolyticus* as well as disease resistance and survival rate of *Carassius auratus.*

Keywords: Specific growth rate, Initial body weight, Final body weight, antagonistic effect etc.

1. Introduction
Aquaculture has become an integral tool for providing and fulfilling the protein requirement across the globe. An increase in demand and rapid industrialization, aquatic animals particularly fishes have become more susceptible and prone to diseases. The susceptibility to diseases has increased due to anthropogenic conditions. The use of Probiotic in aquaculture is targeted at minimizing disease, increasing resistance and enhancing feed quotient. Probiotics also improve water quality and control bacterial infections as they contain mixture of various bacterial species. The commonly used species are *Bacillus* sp., *Lactobacillus sp., Enterococcus sp., Carnobacterium* sp., and even yeast is used in the formulation of Probiotic diet. Probiotics improve the digestibility of nutrients and increase tolerance to stress. Bacterial infections cause more than 9% mortality in various fish hatcheries. Antibiotics and vaccines have been developed and are being used for the treatment of fish disease but they are not completely effective as many bacteria causing infection to fishes develop resistance against specific antibiotic. Probiotic application is more cost effective in aquaculture industry. Amongst probiotic bacteria for aquaculture *Bacillus* sp. *Lactobacillus* spp. and *Streptococcus* sp. are more widely used and have proved to enhance the health of aquatic animals [1]. Crucian carp, *Carassius auratus,* is one of the oldest cultured fish in the world, which is mainly produced in China. Limited investigations on the intestinal microbiota of goldfish (*C. auratus*) indicated that members from Proteobacteria and Firmicutes were dominant, and species of the genera *Aeromonas, Acinetobacter, Bacillus, Cetobacterium, Clostridium, Lactococcus, Pseudomonas, Shewanella, Staphylococcus, Streptococcus*, and *Vibrio* were common [2, 3, 4]. Probiotic bacteria could produce digestive enzymes and essential growth nutrients such as vitamins and amino acids, which are benefit for enhancing the best growth, also they could benefit to their invertebrate host by competitive exclusion against pathogens [5, 6]. However, an in-depth study was still needed to comprehend the effect of probiotic in the intestine of this important species for aquaculture. The present study was designed to study the effect of probiotic mixed diet on the growth and disease resistance of ornamental fish *Carassius auratus.*

2. Materials and Methods
2.1 Experimental fish
The experimental goldfish as *Carassius auratus* were procured from commercial local fish market located at Virudhunagar, Tamilnadu, India. The fishes were allowed to acclimatize to the laboratory conditions and then used for the experimental studies.

2.2 Probiotic Bacterial strain
The Probiotic lactic acid bacteria (LAB) used in the present experiment, *Lactobacillus acidophilus* MTCC 447, *Lactobacillus plantarum* MTCC 2621, *Lactobacillus sporogenes* MTCC 5163 and the target organism *V.parahaemolyticus* MTCC 451 were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India.

2.3 Water quality assessment
The water quality parameters were maintained during the experimental study. The water quality parameters were assessed by standard method [7].

2.4 Fish diet
The fishes were divided randomly into four groups and each group was in three repetitions in separate aquarium. Fresh tap water was stored in rectangular fibreglass tanks for 24 hours under aeration in order to dechlorinated the water. Each aquarium was cleaned daily by siphoning fish faeces and remaining feed with 75% of total water volume, then refilled to fixed volume again. Water temperature range was (29- 32 ºC). Photoperiod was maintained at 12-12 light/dark in both experiments.

2.5 Feeding Experiment
Fishes were divided into four equal groups. So that each 100 litre culture tank had 20 fishes each and all the tanks were provided with aeration. The untreated basal diet served as control and the diet mixed with probiotic strains were considered as treatments (T1-T3). The fishes was fed twice daily at the rate of 3% (average body weight) up to 60 days. The water quality parameters were maintained properly. The fishes in each treatment were counted and weighed on termination of the experiment.

2.6 Determination of nutritional effect
The growth parameters and feed utilization were calculated as follows. Feed conversion ratio (FCR), Specific growth rate (SGR) and Protein efficiency ratio (PER) were calculated using the following formulae:

Feed conversion ratio (FCR) = FI (B2 + B dead – B1) -1

Where FI, B1 and B2 are the feed intake, the biomass at the start and end, respectively, and B dead is the biomass of the dead fish.

Specific growth rate (SGR) = 100 (ln W2-ln W1) T -1

Where, W1 and W2 are the initial and final weight, respectively, and T is the number of days in the feeding period.

Protein efficiency ratio (PER) = [(BWF-BWI)/AP] x 100

Where, BWF is the final mean weight, BWI is the initial mean weight and AP is the amount of protein feed.

2.7 Gut microbial analysis
The gut microbial analysis was carried out in wild fish and one day starved *C.auratus* and also in the experimental fish (10th, 20th and 30th days). For screening the bacteria from the digestive tract, fish was stunned to death and washed several times with sterile distilled water to prevent contamination. The gut was removed by aseptic dissection and divided in to three regions namely foregut (FG), midgut (MG) and hindgut (HG). The three sections were homogenized separately in a mortar and pestle by using sterile distilled water individually and 10% (v/v) homogenates were prepared. Serial dilutions were made using 9 ml sterile distilled water and plated in a triplicate in to the standard nutrient agar. The plates were incubated at room temperature and the CFU.g-1 was counted after incubation.

2.8 *In-vitro* probiotic activity (Well diffusion method)
An *in vitro* antagonistic activity of the probiotic strains were tested against *Vibrio parahaemolyticus* by agar well diffusion assay method. The probiotic strains such as *L. acidophilus*, *L. plantarum* and *L. sporogenes* were grown individually in MRS broth overnight and centrifuged at 10,000 rpm for 10 min and then the resulting supernatant was purified by membrane filtration (0.45 µm pore size). The supernatant fluid was serially diluted up to 1:200. Then 50 µl of two fold diluted sample was transferred in the wells of 5mm diameter in the MRS agar plates which were already inoculated with *Vibrio parahaemolyticus* strain. The plates were incubated at 37 ^{0}C for 24 hours. After incubation, the inhibition zone was measured (mm) around the well.

2.9 Challenge test
After 30 days of feeding, 6 fishes were collected from each treatment were challenged I/P with 0.1 ml fresh culture suspension containing 3x109 cells ml-1 of *V.parahaemolyticus*. The challenged fish were kept under observation for 15 days and the mortality was recorded.

2.10 Statistical analysis
Analysis of Variance (ANOVA) was used to determine the differences between treatments. The mean values were significant at the level of ($P<0.05$). All the statistics were carried out by MS Excel.

3. Results and Discussion
3.1 Water Quality Parameters
The physico-chemical parameters analyzed during the experimental period are given in (Table 1).

Table 1: Physico-chemical parameters during the experiment. Each value (mean±S.D) Is the average for every ten days.

Parameters	10th day	20th day	30th day
Temperature (ºC)	27.30 ± 0.46	27.30 ± 0.46	28.00±0.63
pH	7.45 ± 0.35	7.55 ± 0.42	7.60±0.30
Chlorinity (ppt)	0.47 ± 0.01	0.47 ± 0.01	0.48±0.01
Salinity (ppt)	0.87 ± 0.02	0.87 ± 0.02	0.88±0.02
Carbonate (mg.L^{-1})	12.40 ± 0.59	12.77 ± 0.86	12.95±0.71
Bicarbonate (mg.L^{-1})	184.92 ± 1.10	185.29 ± 2.05	186.27±2.25
Dissolved oxygen (ml.L^{-1})	4.32 ± 0.33	4.45 ± 0.32	4.50±0.33

It revealed that the recorded value (mean ± S.D) of each parameter was ranged from 27.30 ± 0.46 to 28.00 ± 0.63 ºC (temperature); 7.45 ± 0.35 to 7.60 ± 0.35 (pH); 0.47 ± 0.01 to 0.48 ± 0.01 ppt (chlorinity); 0.87 ± 0.02 to 0.88 ± 0.02 ppt

(salinity); 12.40 ± 0.59 to 12.95 ± 0.71 mg.L^{-1} (carbonate); 184.92 ± 1.10 to 186.27 ± 2.25 mg.L^{-1} (bicarbonate) and 4.32 ± 0.33 to 4.50 ± 0.33 ml.L-1 (dissolved oxygen). The present result revealed that all of the measured parameters were in the acceptable ranges [8, 9].

3.2 Fish growth parameters

Table 2 showed that the final weight, % of weight gain, specific growth rate and protein efficiency ratio of *Carassius auratus* increased significantly when fed a diet containing three different probiotic strains.

Table 2: Final Weight, % of Weight Gain, Specific Growth Rate and Protein Efficiency Ratio of *Carassius auratus*

Treatment	Final Weight	% weight gain	FCR	SGR	PER
C	2.240±0.007	4.543±0.150	1.958±0.009	0.794±0.012	1.83±0.014
T_1	2.719±0.012	26.796±0.193	2.253±0.010	0.791±0.006	1.91±0.007
T_2	2.745±0.006	27.206±0.668	2.336±0.017	0.802±0.017	2.11±0.004
T_3	2.782±0.009	29.195±0.091	2.206±0.010	0.854±0.002	1.98±0.001

The Probiotic strain *L. acidophilus* treated fishes mean weight gain was 0.575 ± 0.002g and *L. plantarum* supplemented diet fed fishes showed 0.587 ± 0.010 (g) weight gain and *L. sporogenes* supplemented diet fed fishes were found 0.629 ± 0.002g weight gain. A mean weight gain of 0.097 ± 0.003 g was observed in the fishes fed with control diet. A maximum weight gain of 0.629 ± 0.003g was recorded in the fishes fed with *L. sporogenes* followed by *L. plantarum* and *L. acidophilus*. Similar observations are also reported in *C.auratus* [10], in carps [11] and in Atlantic cod [12, 13].

Maximum food conversion ratio (2.336 ± 0.017 g) was observed using *L.plantarum* as a probiotic strain. Followed by this, *L. acidophilus* and *L. sporogenes* supplemented diets showed minimum FCR. The data obtained in the present study revealed that the FCR (g) value was more in probiotic supplemented diets than in control diet fed *C.auratus.*

The maximum SGR value (0.854 ± 0.002%/day) was obtained in *L.sporogenes* supplemented diet followed by *L.plantarum* (0.802 ± 0.017%/day) and *L.acidophilus* (0.791 ± 0.006 %/day) respectively. The probiotic supplemented diet had a definite role in enhancing the growth of channel cat fish and turbot larvae [14]. Similarly the present study revealed that probiotic supplemented diet showed enhanced growth of *C.auratus.*

Protein efficiency ratio (PER) was higher than in control. Maximum PER was observed in *L.plantarum* (2.11±0.004), followed by *L.sporogenes* (1.98±0.001) and L. acidophilus is (1.91±0.007) supplemented diets. The similar results that the PER was significantly increase than control [15]. High protein efficiency ratio as well as greater protein values in probiotic treatments may be due to proteins secreted by members of genus Bacillus [16].

3.3 Physiological Analyses

Erythrocyte counts in fish fed diets containing *L.sporogenes* were significantly high (1.059±0.043 million.mm^{-3}), whereas fish fed diet containing *L. acidophilus* and *L. plantarum* (0.626±0.034 and 0.526±0.028 million/mm^3) were low respectively. Hemoglobin content was slightly increased in fish fed diet containing *L.sporogenes* (5.12 g/100 ml) and decreased in fish fed containing *L.acidophilus* and *L.plantarum* (4.11 and 4.57 g/100 ml) respectively.

3.4 *In-vitro* antagonistic activity

The antagonistic activity of the beneficial probiotic bacteria namely *L. acidophilus*, *L. plantarum* and *L. sporogenes* against the bacterial pathogen *V. parahaemolyticus* was tested and the zone of inhibition in diameter was provided in Table 3).

Table 3: Antagonistic activity of potential beneficial probiotic strains against pathogenic *V.parahaemolyticus*

Probiotic strain	Zone of inhibition (mm) Mean±S.D
L. acidophilus	24.33±0.47
L. plantarum	26.00±0.82
L. sporogenes	28.67±0.47

Among the three different probiotic strains *L. sporogenes* shows maximum inhibitory zone (28.67±0.47 mm) against *V. parahaemolyticus* followed by *L. plantarum* and *L. sporogenes*. The bacteriocins produced by lactic acid bacteria had inhibitory effect against *V.parahaemolyticus* [16].

3.5 Gut microbial analysis

The total viable count of bacterial strains in the gut region of wild, one day starved and experimental fishes on the 30th day was presented in Table 4.

Table 4: Total viable counts (TVC) in CFU/g of bacterial strains in three different gut regions of wild, one day starved and experimental fishes on 30th day.

Diets	Total viable count (CFU/g)				
	Foregut (FG) (x10^6)	Midgut (MG) (x10^6)	Hindgut (HG) (x10^6)	Total (x10^6)	Mean±S.D (x10^6)
Wild fish	17.67±2.06	11.00±2.16	14.67±2.06	43.34	14.45±2.73
One day starved fish	9.67±2.06	9.33±2.36	13.33±1.25	32.33	10.78±1.81
L.acidophilus (feed A)	12.33±2.06	15.00±2.16	15.67±2.87	43.0	14.33±1.44
L.plantarum (feed B)	13.33±2.06	14.00±1.63	17.97±1.70	45.0	15.00±1.91
L.sporogenes (feed C)	18.33±2.87	13.67±1.25	16.67±1.25	48.67	16.22±1.55
Control (feed D)	12.00±1.63	10.00±2.16	12.67±2.06	34.67	11.56±1.13

The total bacterial population was maximum (48.67 x 10^6 CFU/g) in the gut sample of *C.auratus* fish fed with *L.sporogenes* supplemented diet and was minimum (43.0 x10^6 CFU/g) in the diet containing *L. acidophilus*. Among the three different gut regions, TVC was more in the hind gut region of one day starved fish but in the wild fish it is higher in the foregut region. Two way ANOVA test revealed that the variation in the TVC between different diets and various gut regions were not statistically significant (F=2.028 and 3.633; P>0.05; (Table 5). The total viable count of bacterial strains in the gut regions of

experimental fishes on the 30th day is depicted in (Table 6).

Table 5: Two way analysis of variance for the data on total viable count (TVC) in the gut of *C.auratus* fed with different Probiotic supplemented diet on 30th day

Source of variation	Sum of squares	Degrees of freedom	Mean square	'F'
Between gut	12.967	2	6.484	2.028**
Between feed	35.144	3	11.715	3.663**
Error	19.189	6	3.198	-
Total	67.3	11	-	-

Legend: ** Statistically not significant

Table 6: Total viable counts (TVC) in CFU/g of bacterial strains in three different gut regions of experimental fishes on 30th day.

Diets	Total viable count (CFU/g)				
	Foregut (FG) (x10^6)	Midgut (MG) (x10^6)	Hindgut (HG) (x10^6)	Total (x10^6)	Mean±S.D (x10^6)
L.acidophilus (feed A)	16.67±1.25	15.00±2.45	20.67±1.70	52.34	17.45±2.38
L.plantarum (feed B)	19.00±2.45	16.33±1.25	19.67±2.06	55.00	18.33±1.40
L.sporogenes (feed C)	24.00±1.63	17.00±1.63	27.00±1.41	68.00	22.67±4.19
Control (feed D)	13.67±2.63	11.33±1.70	15.67±2.63	40.67	13.56±1.77

The total bacterial population was more (68.0 x 10^6 CFU/g) in the gut sample of *C. auratus* fed with *L. sporogenes* supplemented diet and was less (52.33 x10^6 CFU/g) in the diet containing *L. acidophilus*. TVC was more in the hind gut region for all the experimental fishes when compared to the other gut regions. TVC in the digestive tract of *C. auratus* treated with different probiotic supplemented diet was much higher than the control group. Two way ANOVA test revealed that the variation in the TVC between different diets and different gut regions were statistically significant (F=12.470 and 15.221; P<0.05; Table 7).

Table 7: Two way analysis of variance for the data on total viable count (TVC) in the gut of *C.auratus* fed with different Probiotic supplemented diet on 30th day

Source of variation	Sum of squares	Degrees of freedom	Mean square	'F'
Between gut	68.731	2	34.366	12.470*
Between feed	125.847	3	41.949	15.221*
Error	16.537	6	2.756	-
Total	211.115	11	-	-

Legend:* Statistically significant

The total viable count of bacterial strains in the gut region of control and experimental diets fed fishes on the 30th day is given in (Table 8).

Table 8: Total viable counts (TVC) in CFU/g of bacterial strains in three different gut regions of experimental fishes on 30th day.

Diets	Total viable count (CFU/g)				
	Foregut (FG) (x10^6)	Midgut (MG) (x10^6)	Hindgut (HG) (x10^6)	Total (x10^6)	Mean±S.D (x10^6)
L.acidophilus (feed A)	20.67±1.89	16.67±1.70	22.67±1.70	60.00	20.00±2.49
L.plantarum (feed B)	25.00±1.41	18.00±1.63	27.33±1.70	70.33	23.44±3.97
L.sporogenes (feed C)	31.00±2.99	19.67±2.06	36.67±2.06	87.33	29.11±7.07
Control (feed D)	15.67±2.49	14.33±2.06	20.33±2.06	50.33	16.78±2.57

The bacterial population was maximum (87.33 x 10^6 CFU/g) in the gut sample of *C. auratus* fed with *L. sporogenes* supplemented diet. But was minimum (60.0 x10^6 CFU/g) in the diet containing *L. acidophilus*. Among the three different gut region, TVC was more in the hind gut regions of probiotic supplemented diet than the other regions. TVC in the digestive tract of *C.auratus* treated with different probiotic supplemented diet was much higher than the control group. Two way ANOVA test revealed that the data on the TVC of different gut region with different probiotic supplemented diets fed with *C.auratus* were statistically significant (F=11.544 and 10.310; P<0.05; Table 9).

Table 9: Two way analysis of variance for the data on total viable count (TVC) in the gut of *C.auratus* fed with different probiotic supplemented diets on 30th day

Source of variation	Sum of squares	Degrees of freedom	Mean square	'F'
Between gut	186.947	2	93.474	11.544*
Between feed	250.445	3	83.482	10.310*
Error	48.581	6	8.097	-
Total	485.973	11	-	-

Legend: * Statistically significant

The microflora density in fishes usually varies quantitatively and qualitatively in the organs such as skin, gill and intestine. In the intestine itself the diversity may vary in foregut, midgut and hindgut regions. The quantitative variation between the microbes was in the following order gut>gill>skin in pond reared milk fish Chanos chanos [17].

3.6 Challenge test

The mortality of *C. auratus* fed with probiotic supplemented diets is presented in (Table 10).

Table 10: Mortality rate of *C. auratus* fed diet containing probiotic bacteria and challenged with *Vibrio* parahaemolyticus

Treatment	*L.acidophilus* (Feed A)	*L.plantarum* (Feed B)	*L.sporogenes* (Feed C)	Control (Feed D)
Number of injected fish	6	6	6	6
Dose of Bacteria	0.1ml of $3x10^9$ cells/ml	0.1ml of $3x10^9$ cells/ml	0.1ml of $3x10^9$ cells/ml	0.1ml of $3x10^9$ cells/ml
No. of fish survive	3	5	4	2
No. of fish dead	3	1	2	4
Survival rate (%)	50.0	83.33	66.66	33.33

It showed that the survival percentage was 83.33% of *C.auratus* fed on the diet containing *L. plantarum* after 30 days and challenged by pathogenic *V. parahaemolyticus* (0.1 ml of $3x10^9$ cells /ml). The survival percentage of *C.auratus* which fed on the diet containing *L. sporogenes* was 66.66% and *L.acidophilus* was 50%. But the control diet fed fishes showed less (33.33%) survival than the experimental diet fed *C.auratus.*

4. Conclusion

Lactobacilli do offer ample scope as Probionts showing antagonism towards *Vibrio* pathogens. Results from the present study clearly demonstrated that the Probionts were able to suppress *V.parahaemolyticus* growth, it can be hypothesized that they have the ability to colonize the gastrointestinal tract of goldfish, which however, merits further confirmation. Consequently, they may prove to be suitable candidates for oral administration to goldfish, in commercial ventures to improve health and to protect them from *Vibrio* infections.

5. References

1. Gomez R, Geovann D, Shen MA. Influence of probiotics on the growth and digestive enzyme activity of White Pacific shrimp, Litopenaeus vannamei. Journal of Ocean University of China 2008; 7:215- 218.
2. Asfie M, Yoshijima T, Sugita H. Characterization of the goldfish fecal microflora by the fluorescent in situ hybridization method. Fish Sci 2003; 69:21–26.
3. Feng X, Wu ZX, Zhu DM, Wang Y, Pang SF, Yu YM, *et al* Study on digestive enzyme-producing bacteria from the digestive tract of Ctenopharyngodon idellus and *Carassius auratus* gibelio. Freshw Fish 2008; 38:51–57.
4. Silva FC, Nicoli JR, Zambonino-Infante JL, Kaushik S, Gatesoupe FJ. Influence of the diet on microbial diversity of faecal and gastrointestinal contents in gilthead sea bream (Sparus aurata) and intestinal contents in goldfish (*Carassius auratus*). FEMS Microbiol Ecol 2011; 78:285–296.
5. Austin B, Stuckey LE, Robertson PAW, Effendi I, Griffith DRW, A Probiotic strain of *Vibrio* alginolyticus effective in reducing disease caused by *Aeromonas salmonicida*, *Vibrio* ordalli. J Fish Dis 1995; 18:93-96
6. Gomez gil B, Roque A, Turnbull JF. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture 2000; 191:259-270.
7. APHA (American Public Health Association, American Water Works Association, and Water Pollution Control Federation). Standard Methods for the Examination of Water and Wastewater. 17: APHA. Washington, DC, 1989.
8. Armstrong DA, Stephenson MJ, Knight KW. Acute toxicity of nitrite to larvae of the giant Malaysian prawn, Macrobrachium rosenbergii. Aquaculture 1976; 9:39-46.
9. Hseieh CH, Chao NH, Oliviala D, Gomes LA, Liao IC. Culture practices and status of the giant fresh water prawn *Macrobrachium rosenbergii* (de Man), in Taiwan. In proceedings of the 3rd Brazilian shrimp framing congress Joao Pessoa PB. Brazil, 1990, 15-20.
10. Ahilan B, Shine G, Santhanam. Influence of probiotics on the growth and gut microflora load of juvenile goldfish (*Carassius auratus*). Asian Fisheries Science 2004; 17:271-278.
11. Noh SHY, Han K, Won TH, Choi YJ. Effects of antibiotics, enzymes, yeast culture and probiotics on the growth performance of Israeli carp. Korean Journal of Animal Sciences, 1994; 36:480-486.
12. Gildberg A, Johansen, A, Bogwald, J. Growth and survival of Atlantic salmon (*Salmo salar*) fry given diets supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with Aeromonas salmonicida. Aquaculture 1995; 138:23-24.
13. Gildberg A, Mikkeisen H, Sandaker E, Ringo E. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic god (*Gadus morhua*). Hydrobiologia 1997; 352:279-285.
14. Querioz, J.F., Boyd, C.E. Effects of bacterial inoculums in channel catfish ponds. Journal of World Aquaculture Society 1998; 29(1):67-73.
15. Tahere Bagheri1, Seyed Aliakbar Hedayati, Vahid Yavari, Morteza Alizade, Ali Farzanfar. Growth, Survival and Gut Microbial Load of Rainbow Trout (Onchorhynchus mykiss) Fry Given Diet Supplemented with Probiotic during the Two Months of First Feeding. Turkish Journal of Fisheries and Aquatic Sciences 2008; 8:43-48
16. Lewis, CB, Kaiser A, Montville, TJ. Inhibition of food borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl Environ Microbiol 1991; 57:1683-1688.
17. Venugopalan, VK, Nandakumar R, Ramesh A. Microflora of pond cultured milkfish. *Chanos chanos* (Forskal). In proceedings of the symposium on harvest and post-harvest Technology, 1985, 680-688