Isolation Of Probiotic Bacteria Of *Cirrhinus Mrigala* Used As Effective Feed For Enhancing Its Growth, Haematological And Biochemical Parameters

Vidhya Bharathi .P[1*], Rajan.M.R[2], Charulatha.C[3]

[1*,2,3]Department of Biology, The Gandhigram Rural Institute(DTBU),Dindigul.

***Corresponding author:** Vidhya Bharathi .P
*Email: bharathividhya932@gmail.com

Abstract
In this work, bacterial colonies from the gut of *Cirrhinus mrigala* were isolated. The colonies were inspected as *Leuconostoc mensenteroids, Lactobacillus pentasus, Lactobacillus amylophilus, Staphylococcus waneri, Lactobacillus paracesei, Lactobacillus gasseri* and *Lactobacillus colini* all bacterial strains by biochemical characterization, 16s rRNA sequencing. Enzyme productivity also examined. The inhibitory activity of gut bacteria was tested against pathogens such as *Staphylococcus aures, Enterococcus faecalis, Stenotrophomonas maltophilia* and *Pseudomonas aeroginosa* compared with commercial antibiotic Streptomycin. Different experimental feeds were prepared such as Feed I (control no bacteria), Feed II (1ml of *Lactobacillus amylophilus*), Feed III (1 ml of each *Lactobacillus amylophilus* and *Leuconostoc mesenteroides*), Feed IV (1ml of each *Lactobacillus amylophilus, Leuconostoc mesenteroides* and *Lactobacillus colinoids*), and Feed V (1 ml of each *Lactobacillus amylophilus, Leuconostoc mesenteroides, Lactobacillus colinoids* and *Lactobacillus gasseri*). The treatment consists of five groups and each group were provided with five fishes. Triplicates were performed, thus totally 75 fishes were used. The experiment last for 28 days. Feed utilization, feed conversion efficiency (FCR) and relative growth rate were checked. Among the various treatment, fish fed with Feed V (1ml of each *Lactobacillus amylophilus, Leuconostoc mesenteroides, Lactobacillus colinoids* and *Lactobacillus gasseri*) have shown higher feed consumption, feed utilization, FCR, growth and net weight gain compared to other groups fed with feed I, II, III,IV. Biochemical and Haematological parameters also greater in Feed V. Based on the results, it is obvious that the gut bacteria of mrigal had the potential to be used in commercial feed preparation for enhancing the fish growth for sustained aquaculture production.

Key words: Gastrointestinal bacteria, *Cirrhinus mrigala*, Aquaculture, Fish growth, Haematological parameters, Probiotic feeds.

1.Introduction:

Aquaculture is a rapidly growing food production sector that has seen significant increases in production activities over the past decade. Freshwater and marine water environments are being utilized worldwide for the production of aquatic species, serving various purposes such as trade, economic development, medicine, and job opportunities. Edible fish, in particular, serves as an effective source of protein and contains vital amino acids, minerals, vitamins, and more. Feed additives are employed to improve growth performance, immune system response, disease resistance, and as an alternative to antibiotics, the use of beneficial feed additives such as prebiotics, probiotics, and synbiotics is becoming increasingly popular in aquaculture (Irianto and Austin,2002). Probiotics, which include bacteria, bacteriophages, microalgae, and yeast, are commonly used as feed supplements (Pankaj kumar *et al.,* 2017). Probiotics promote the host's well-being by enhancing digestion and the immune system, and also by preventing the proliferation of harmful microorganisms (Wang yanbo and Xu zirong, 2006). Additionally, probiotics can boost the function of digestive enzymes, which enhance feed digestibility. (Zokaeifar *et al.,* 2012) Studies have shown that probiotic supplementation can reduce disease outbreaks in fish by enhancing the immune system and modulating hematological parameters and intestinal structure (He *et al.,*2011: Michael *et al.,* 2019). Thus in the current investigation, intestinal bacteria were isolated and identified from *Cirrhinus mrigala* by biochemical test and 16s rRNA sequencing. Further the effect of intestinal bacteria on feed consumption and growth of *Cirrhinus mrigala* was determined.

2. Materials and Methods:

2.1 Fish collection:

For the current research work, *Cirrhinus mrigala* were collected from KVR Fish farm, Palani, Dindigul. Fishes were transported to the laboratory of Biology Department, The Gandhigram Rural Institute, Dindigul, India, using polythene bags which was filled with oxygenated water.

2.2 Strain isolation:
The digestive system of mrigal fish was dissected, and the contents of the intestines were diluted in a series of dilutions up to a 10^{-6} dilution. These dilutions were then plated onto a nutrient agar medium. The agar medium plates were incubated at 37°C for one day. The bacterial strains of mrigal fish were successfully grown on the nutrient agar medium.

2.3 Bacterial identification:
At an appropriate time, colonies were enumerated in nutrient agar medium. The predominant colonies were identified by cell morphology, biochemical characterization.

2.4 Molecular sequencing:
Bacterial strains are identified by 16s rRNA sequencing.

2.5 Enzyme productivity:
Bacterial strains from *Cirrhinus mrigala* was inspected for enzyme production such as amylase, protease and lipase.

2.6 Antibacterial activity:
Four pathogenic bacterial strains such as *Enterococcus faecalis, Staphylococcus aureus, Stenotromonas maltophilia* and *Pseudomonas aeroginosa* were used for antibacterial activity. To prepare test organism, one loop of culture from nutrient agar medium was transferred into nutrient broth and it is incubated at 37^0C for 24 hours. For this antagonistic activity was carried out by well diffusion method. Streptomycin (Commercial antibiotic) was considered as positive control. For antibacterial activity, nutrient agar medium was poured into sterilized petriplates. Fish pathogens are swabbed on nutrient agar medium. Well was created by using well cutter. One well was filled by positive control, second well was filled by probiotic sample, and third well was loaded by distilled water (negative control). Plates are incubated at 37^0C for one day. Triplicates were maintained.

2.7 Mass culture:
The isolated bacterial strains such as *Lactobacillus amylophilus, Leuconostoc mesenteroides, Lactobacillus colinis* and *Lactobacillus gasseri* were mass multiplied based on enzymatic productivity and antagonistic activity.

2.8 Growth study:
Cirrhinus mrigala (1.9±0.05) was acclimated for 15 days at 28 ± 2^0C. Fishes were fed with dry pellets, which contain wheat flour, groundnut oil cake, and rice bran, which are used as raw materials.

2.8.1 Selection of feed ingredients:
The materials were preferred based on their nutrients, such as proteins, carbohydrates, and fats. One control and four experimental feeds are prepared.

2.8.2 Experimental design:
This study focused on Cirrhinus mrigala fish, which were selected to be of a similar size (1.9±0.05) for uniformity. Five fish were placed in each tank, and their length and weight were recorded. The fish were fed a specialized diet twice a day, and any uneaten food was collected after one hour. Fecal matter was also collected before changing the water in the tanks. The fecal matter was then dried at a temperature of 95°C. In tank, 70% of the water was replaced with ground water. Various parameters related to the utilization of the feed, such as condition factor, feed consumption, feed conversion efficiency, feed conversion ratio, growth, relative growth rate, assimilation, metabolism, gross growth efficiency, and net growth efficiency, were measured and analyzed.

3.Result and DISCUSSION:
The study identified and isolated several types of bacteria from the intestine of *Cirrhinus mrigala* fish, including *Leuconostoc mesenteroids, Lactobacillus pentosus, Lactobacillus amylophilus*, *Staphylococcus waneri, Lactobacillus paracesei, Lactobacillus gasseri*, and *Lactobacillus colini* (Table-1). Intestinal bacteria have been found to play a role in promoting the survival and growth of fish by enhancing the immune system and controlling the growth of pathogenic bacteria (Ai *et al.,* 2011; Bency Thankappan *et al.,* 2015).

Table 1 Biochemical characterization of intestinal bacteria of *Cirrhinus mrigala*

Tests	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**
Simple staining	Cocci	Rod	Rod	Cocci	Rod	Rod	Rod
Gram's staining	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Motility test	Non Motile	Non Motile	Non Motile	Non Motile	Non Motile	Non Motile	Non Motile
Indole test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Methyl red test	Negative	Negative	Negative	Positive	Negative	Negative	Negative
Vogues progauser test	Negative	Negative	Negative	Positive	Negative	Negative	Negative
Citrate test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Catalase test	Negative	Negative	Negative	Positive	Negative	Negative	Negative
Starch test	Positive	Positive	Positive	Negative	Positive	Positive	Negative
Gelatin hydrolysis Test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Oxidase test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Identification result	***Leuconostoc mesenteroids***	***Lactobacillus pentasus***	***Lactobacillus amylophilus***	***Staphylococcus waneri***	***Lactobacillus paracesei***	***Lactobacillus gasseri***	***Lactobacillus colini***

Selective media to investigate the gut bacteria of *Cirrhinus mrigala* for the production of digestive enzymes such as protease, amylase, and lipase. Upon conducting the tests, the specific strains of *Leuconostoc mesenteroids, Lactobacillus amylophilus, Lactobacillus gasseri,* and *Lactobacillus colini* exhibited higher levels of digestive enzyme synthesis (Table-2; Fig 1,2,3). (Sivakumar and Rajan, 2013) reported the enzymatic productivity of *Bacillus* species from the intestine of Gold fish *Carassius auratus.* (Balaji *et al.,* 2012) reported that the gastrointestinal tract of *Cyprinus carpio* (koi carp) contained bacteria that produce potent protease enzymes. (Muge Hekimoglu *et al.,* 2014) reportedthe feeding of the koi carp with *Artemia nauplii* resulted the production of digestive enzymes.

Table 2 Enzyme productivity of *Cirrhinus mrigala*

Intestinal bacteria	Amylase	Protease	Lipase
Leuconostoc mesenteroids (M1)	++	+ + +	+++
Lactobacillus pentasus (M2)	++	+ +	++
Lactobacillus amylophilus (M3)	+ + +	+ + +	+++
Staphylococcus waneri (M4)	+	+	+
Lactobacillus paracesei (M5)	++	+ +	+
Lactobacillus gasseri (M6)	++	+ +	++
Lactobacillus colini (M7)	++	+ + +	+++

+ - Nil or absent, + + - Low productivity (Positive), + + + - High productivity (Positive)

Figure1: Amylase production

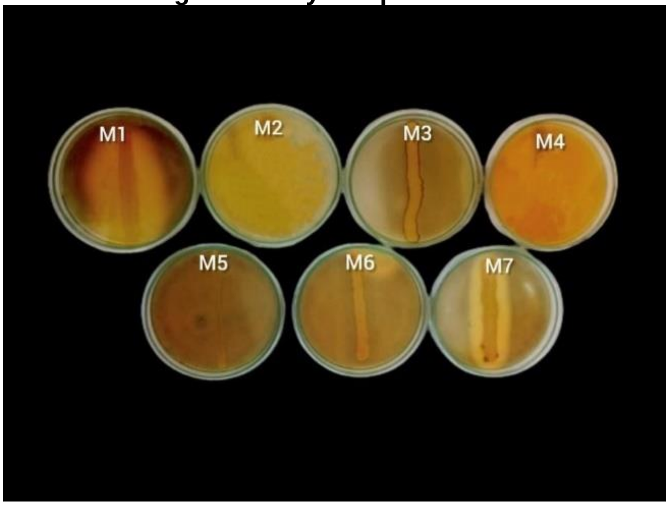


Figure2:Protease production

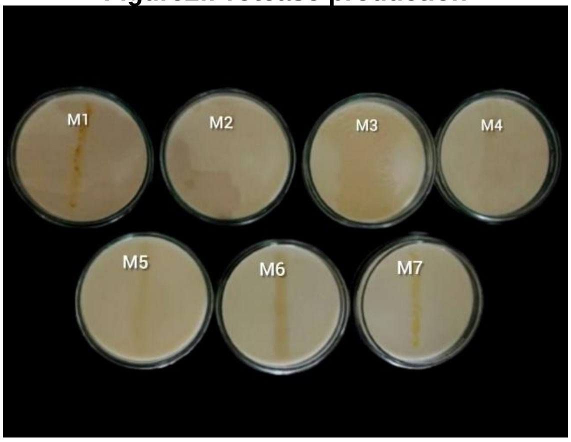
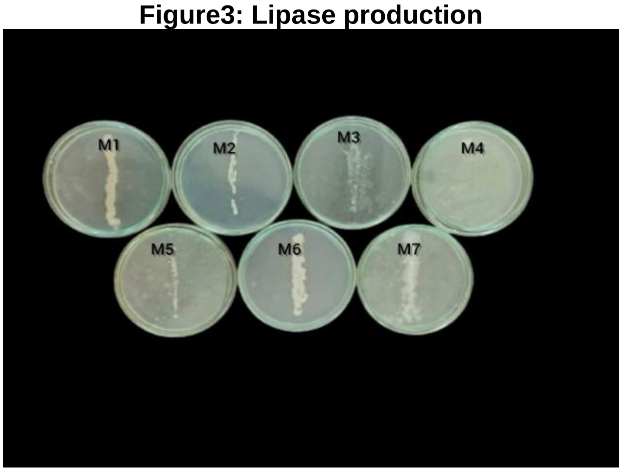


Figure3: Lipase production



Furthermore, the antibacterial activity of the intestinal bacteria of *Cirrhinus mrigala* was evaluated using selective media and a commercial antibiotic (Streptomycin). The results indicated that *Leuconostoc mesenteroids, Lactobacillus amylophilus, Lactobacillus gasseri, and Lactobacillus colini* exhibited significant antibacterial effects against specific pathogens (Table-3; Fig 4,5,6,7). (Karthikeya and Santhosh, 2009) isolated the specific strain of *Lactobacillus plantarum* from digestive system of *Penaeus monodon* (black tiger shrimp) and verified its ability to inhibit the growth of various harmful bacteria. (Anita Bhatnagar and Ritu Lamba, 2015) extracted lactic acid bacteria strains from *Cirrhinus mrigala* (mrigal carp) and investigated their antimicrobial properties against different types of bacteria. Moreover, similar results for *Leuconostoc mesenteroides* were reported (Allameh *et al.,* 2017). The ability to inhibit the growth of harmful bacteria differ according to species of probiotic bacteria.

Table 3 Antibacterial activity of (Double layer screening) intestinal bacteria of *Cirrhinus mrigala*

Intestinal bacteria	Zone of inhibition (mm)											
	P1			P2			P3			P4		
	S	CA	NC	S	CA	NC	S	CA	NC	S	CA	NC
Leuconostoc mesenteroids (M1)	1.8	3.8	-	1.8	3.7	-	1.9	3.9	-	1.9	3.6	-
Lactobacillus pentasus (M2)	1.5	3.7	-	1.6	3.6	-	1.5	3.8	-	1.7	3.8	-
Lactobacillus amylophilus (M3)	1.9	3.8	-	2.7	3.9	-	2.0	3.6	-	2.0	3.6	-
Staphylococcus waneri (M4)	1.6	3.8	-	1.2	3.4	-	1.1	3.5	-	1.0	3.9	-

Lactobacillus paracesei (M5)	1.4	3.6	-	1.6	3.7	-	1.5	3.7	-	1.7	3.6	-
Lactobacillus gasseri (M6)	1.5	3.7	-	1.5	3.6	-	1.6	3.7	-	1.5	3.8	-
Lactobacillus colini (M7)	1.8	3.8	-	1.7	3.7	-	1.7	3.9	-	1.8	3.8	-

Figure4: Antibacterial activity of *Enterococcus faecalis*

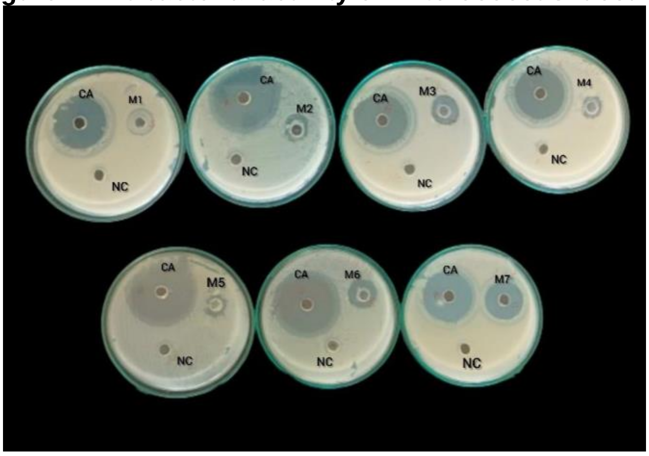


Figure5: Antibacterial activity of *Staphylococcus aureus*

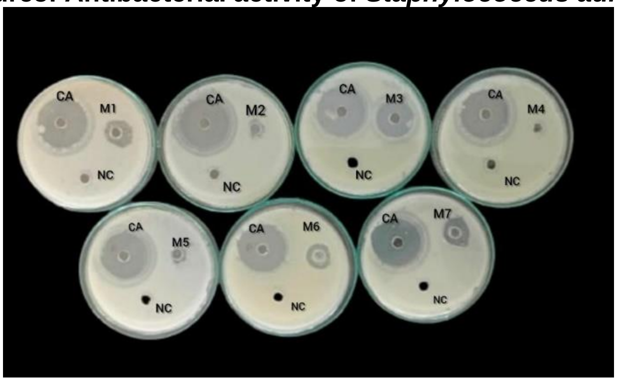


Figure6: Antibacterial activity of *Stenotrophomonas maltophila*

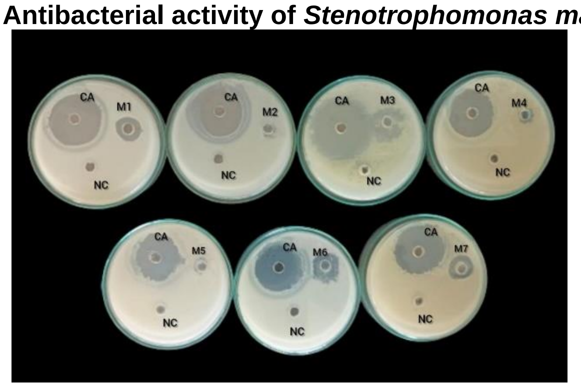
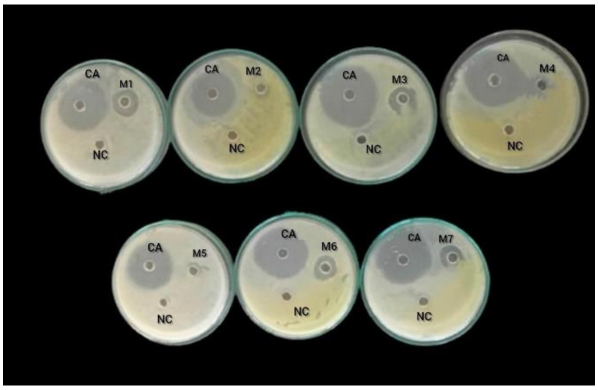


Figure7: Antibacterial activity of *Pseudomonas aeruginosa*



On the basis of biochemical test, enzymatic and antibacterial activity the selected bacteria were *Leuconostoc mesenteroids* (Fig-8), *Lactobacillus amylophilus* (Fig-9), *Lactobacillus gasseri*(Fig-10) and *Lactobacillus colini*(Fig-11) was studied at genus level by genetic sequencing. (Yuniar Mulyani *et al.,* 2018) reported the sequencing of *Bacillus subtilis* isolated from the intestine of common carp. (Suganya *et al.,* 2014) reported the sequence of intestinal bacteria isolated from intestine of Gold fish. (Rajan and Sabitha, 2023) reported the sequence of intestinal bacteria isolated from intestine of Blue morph (*Maylandia lombardoi*).

Figure8 Phylogenetic tree of isolated intestinal bacteria *Leuconostoc mesenteroids* of *Cirrhinus mrigala*

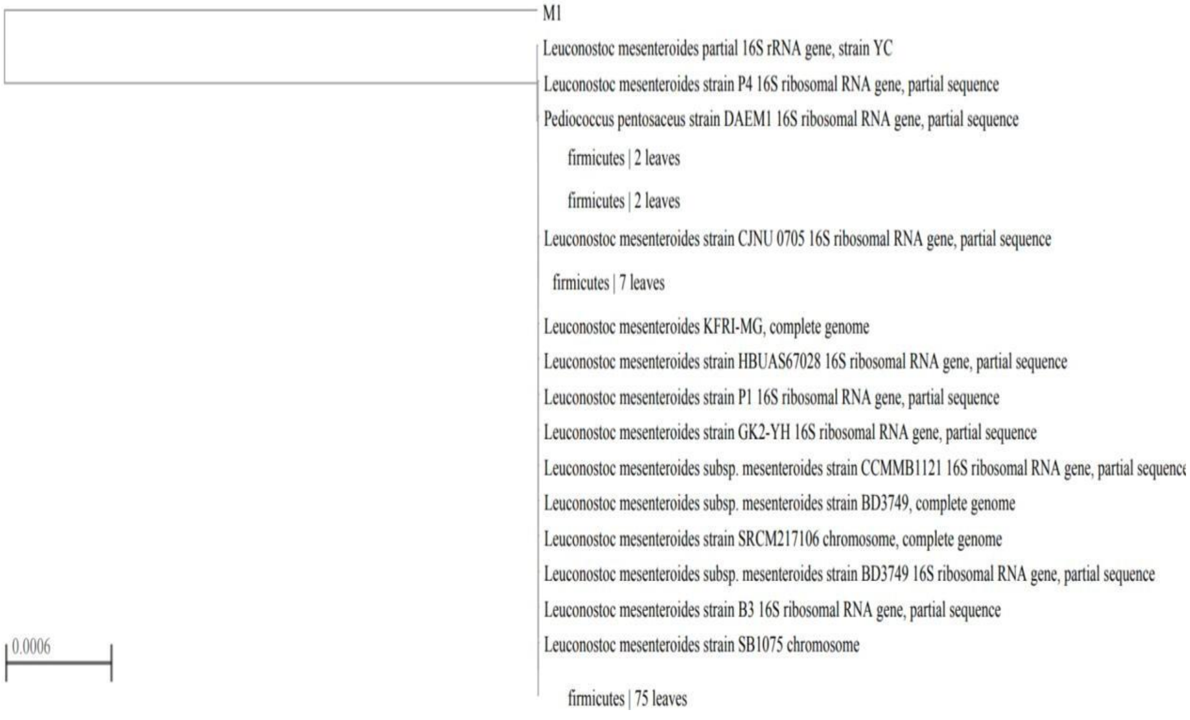


Figure 9 Phylogenetic tree of isolated intestinal bacteria *Lactobacillus amylophilus* of *Cirrhinus mrigala*

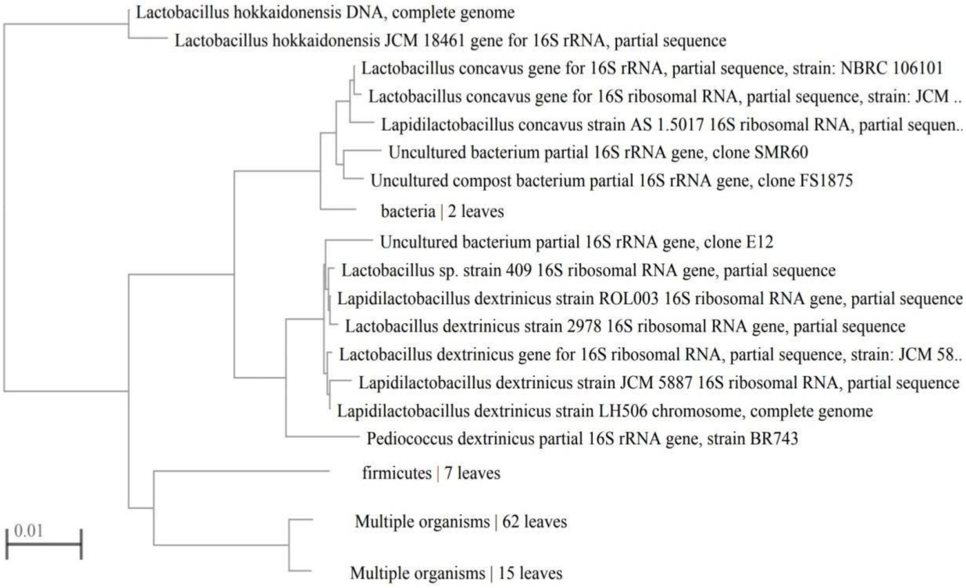


Figure 10 Phylogenetic tree of isolated intestinal bacteria *Lactobacillus gasseri* of *Cirrhinus mrigala*

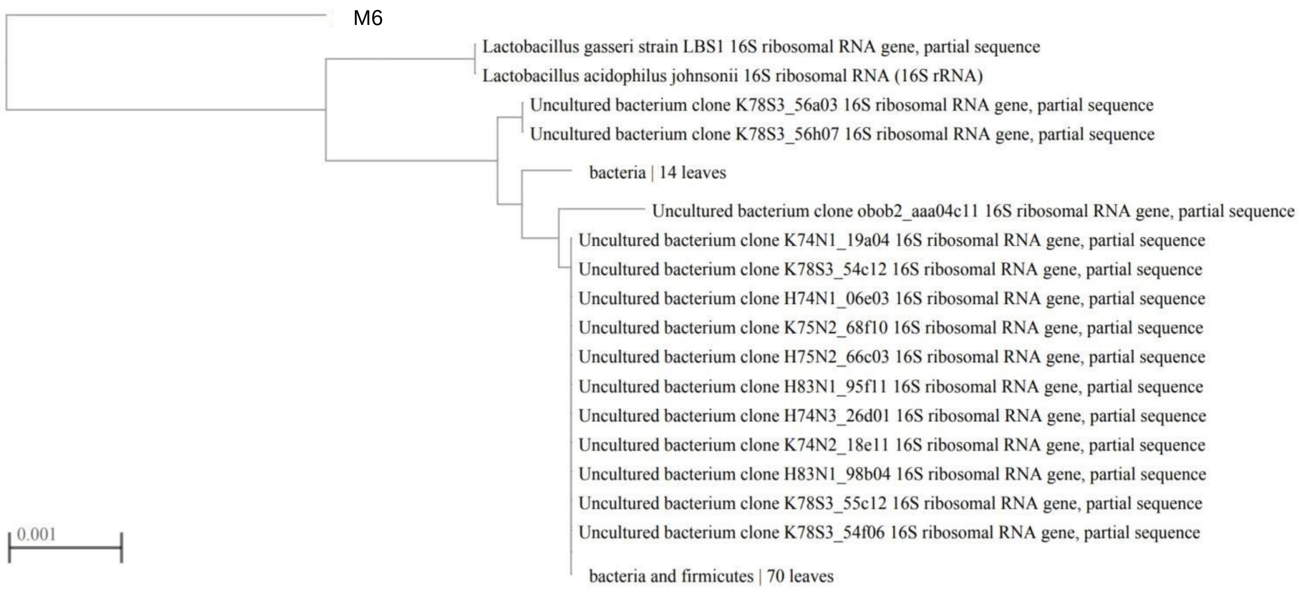
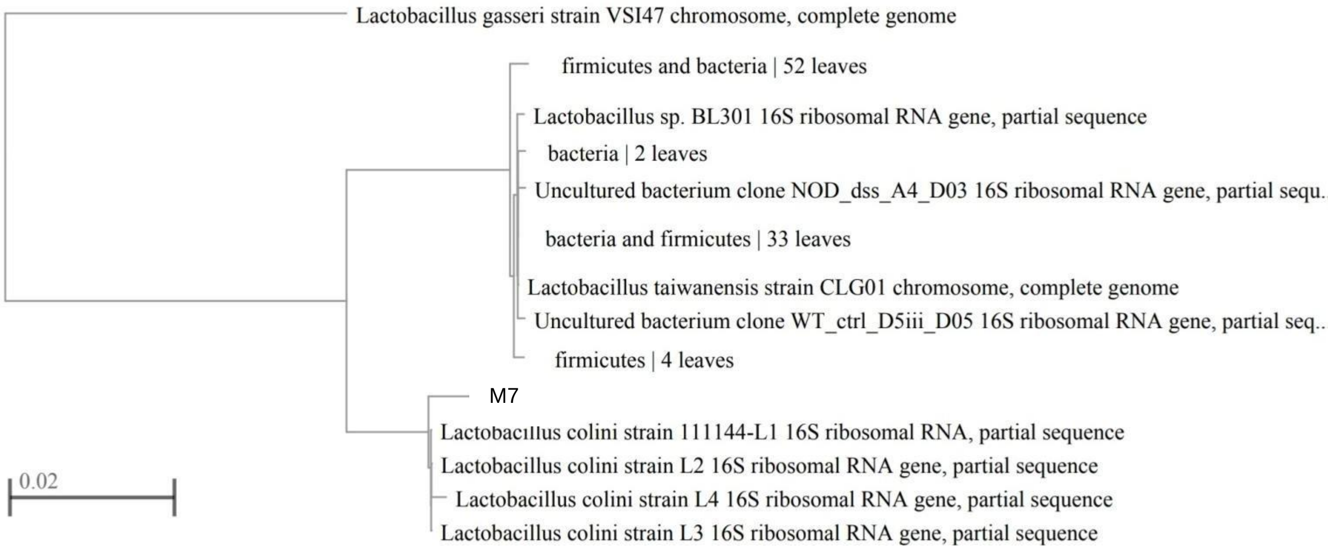


Figure11 Phylogenetic tree of isolated intestinal bacteria *Lactobacillus colini of Cirrhinus mrigala*



To evaluate the quality of the feed, condition factor (K) of *Cirrhinus mrigala* was measured. The initial average condition factor was 3.36±0.05, and it increased in all fish fed with feed V to 4.93±0.65. This increase in condition factor indicates that the fish were able to survive, grow, and reproduce well in the given environment (Table-4). (Sankar and Kulkarni, 2005) observed a similar trend in *Notopterus notopterus* where the condition factor provided information about fish's ability to adapt and thrive. Similarly, (Hoang, 2020) found that the condition factor ranged from 3.13 to 3.18 in Pompano fish, with the highest value observed in fish fed with Diet 2, which was significantly higher compared to fish fed with Diet 1 (P=0.001).

Table 4:Condition factor (K) of *Cirrhinus mrigala*

Feeds	Initial	Final
FeedI(Control)	3.37±0.05	3.70±0.10
FeedII	3.53±0.05	4.37±0.05
FeedIII	3.47±0.05	4.93±0.05
FeedIV	3.50±0.10	5.53±0.05
FeedV	3.47±0.05	6.90±0.10

The feed consumption (FC) of *Cirrhinus mrigala* was higher in feed V, which contained a combination of *Leuconostoc mesenteroids, Lactobacillus amylophilus, Lactobacillus gasseri,* and *Lactobacillus colini,* with an average value of 18.03±0.04. On the other hand, the control feed I had a lower feed consumption of 10±0.08. In feed consumption, similar findings have been reported in other studies on common carp (Bisht *et al.,* 2012) and Blue gourami(Deepika *et al.,* 2019), where specific feed formulation led to higher feed consumption to control diets.

The Feed Conversion Efficiency (FCE) of *Cirrhinus mrigala* was greater in feed V (2.7±0.09). Furthermore, the Feed Conversion Ratio (FCR) of *Cirrhinus mrigala* was found to be the best in feed V compared to other feeds. In feed conversion efficiency of Yellow molly, feed V containing *Pseudomonas sp.,* resulted higher feed conversion efficiency (8.7±0.60 and 2.14±0.04) while feed1 had lower values (6.1±0.16 and 0.15±0.02) (Sivakumar and Rajan, 2015).

The growth of *Cirrhinus mrigala* was significantly higher in feed V, which contained a combination of specific strains of *Leuconostoc mesenteroids, Lactobacillus amylophilus*, *Lactobacillus gasseri,* and *Lactobacillus colini,* compared to the control feed I. The growth is consistent with similar studies on other fish species such as koi carp, Nile tilapia and Catla where specific feed formulations resulted in higher growth rates compared to control diets (Dhanaraj *et al.,* 2010; Gohila *et al.,* 2013; Ramous *et al.,* 2017).

The percentage growth of *Cirrhinus mrigala* was also greater in feed V, indicating that the fish experienced a greater increase in body weight compared to control. The relative growth rate of *Cirrhinus mrigala* was significantly higher in feed V and lower in the control feed I. This suggests that the fish achieved a higher rate of growth when fed with the specific probiotic supplement compared to the control diet. Percentage growth findings have been reported for Nile tilapia and Catla (Tamana Tabassum *et al.,* 2021; Priyanka Arya *et al.,* 2019). The relative growth rate suggests that the fish achieved a higher rate of growth when fed with the specific probiotic supplement compared to control diet. Similar findings have been reported for Bocourti catfish and zebra fish (Pornthep Niamphithak*et al.,* 2017).

The assimilation and metabolism of *Cirrhinus mrigala* were higher in feed V compared to the control feed I. This indicates that the fish were able to process and utilize the nutrients in feed V more efficiently, leading to better assimilation and overall metabolic performance. Assimilation and metabolism indicates the fish were able to process and utilize nutrients in feed. This similar results have been observed in clown fish and gold fish(Sunil kumar and Vishnu, 2011).

The gross and net growth efficiency of *Cirrhinus mrigala* were significantly higher in feed V compared to the control feed I. This suggests that the fish were able to convert the nutrients in feed V into body weight more efficiently, resulting in higher growth efficiency. The gross and net growth efficiency were discussed for Platy by (Pushparaj *et al.,* 2012).

The supplementation of specific probiotic strains in the feed of *Cirrhinus mrigala* led to improved growth, percentage growth, relative growth rate, assimilation, and metabolism, as well as higher gross and net growth efficiency, compared to the control feed without probiotics (Table-5).

Table 5:Feed utilization and growth parameters of *Cirrhinus mrigala* in relation todifferent feeds. Each value is the average (±SD) performance of 5 individuals in triplicates reared in 28 days.

Parameter	Experimentalfeed				
	FeedI (con)	FeedII	FeedIII	FeedIV	FeedV
Feed consumption (FC)(g/glivewt/28days)	10±0.08	12±0.08	14±0.08	16±0.08	18.03±0.04
Feed Conversion Efficiency(FCE)	1.03±0.05	1.56±0.05	2.5±0.09	2.53±0.05	2.7±0.09
FeedConversionRatio(FCR)	19.96±0.05	12±0.09	9.2±0.09	7.96±0.05	7.16±0.05
WeightGain	0.46±0.057	1±0.1	1.46±0.057	2±0.09	2.46±0.057
PercentageGrowth(PG)%	2.42±0.05	5.26±0.05	7.36±0.05	10.5±0.1	12.9±0.1
RelativeGrowthRate(RGR)	0.25±0.01	0.49±0.005	0.74±0.005	1±0.10	1.24±0.005
Assimilation(A)(g/glivewt/28days)	9.86±0.057	11.86±0.057	13.86±0.057	15.86±0.0	17.86±0.057
Metabolism(M)(g/glivewt/28days)	9.36±0.057	10.9±0.09	12.36±0.057	13.86±0.05	15.43±0.057
GrossGrowth Efficiency(GGE) (%)	5±0.10	8.03±0.057	10.03±0.057	11.96±0.057	12.96±0.057
NetGrowth Efficiency(NGE) (%)	5.04±0.005	8.39±0.005	10.78±0.005	12.56±0.005	13.95±0.005

Biochemical parameters such as carbohydrate, protein and lipid in muscle, gill and liver of*Cirrhinus mrigala* are higher in feed V (Table-6). Biochemical parameters such as carbohydrate, protein and lipid of vaccinated rainbow trout and Rohu were observed (Ali kane *et al.,* 2016; Subramani *et al.,* 2017).

Table 6 Biochemical characteristics

	Organs	**Feed I**	**Feed II**	**Feed III**	**Feed IV**	**Feed V**
Protein	Muscle	0.12	0.91	1.69	1.93	3.58
	Gill	0.225	0.35	0.48	0.68	2.47
	Liver	0.27	0.445	0.695	0.84	2.505
Carbohydrate	Muscle	0.08	0.925	0.94	0.96	1.225
	Gill	0.295	0.55	0.63	0.675	1.715
	Liver	0.515	0.79	1.03	1.23	1.645
Lipid	Muscle	0.09	0.9	1.12	1.32	2.56
	Gill	0.217	0.32	0.45	0.57	1.21
	Liver	0.25	0.38	0.55	0.76	1.75

Haematological characteristics of *Cirrhinus mrigala* (Table-8) were significantly influenced by the different levels of probiotics in the feed. Feed V had the highest values for RBC count and Hb%, indicating an increase in red blood cells and hemoglobin levels compared to the control feed I and other feeds. The greatest Hb% was recorded in *Cirrhinus mrigala* fed with feed V for 28 days (3.2), while the lowest was observed in those fed with the control feed I (1.8). Similarly, the Hct% was highest in Cirrhinus mrigala fed with feed V for 28 days (10.6), while the control feed I had the lowest value (4.1).

The Red Cell Indices, including MCV, MCH, and MCHC, were also measured. The minimum MCV value was observed in *Cirrhinus mrigala* fed with the control feed I for 28 days (143), while the maximum value was recorded in those fed with feed V (204.7). The maximum MCH value was observed in *Cirrhinus mrigala* fed with feed V (61), and the minimum value was found in those fed with the control feed I (48). The maximum MCHC value was recorded in *Cirrhinus mrigala* fed with feed V (50), while the minimum value was observed in those fed with the control feed I (37). These findings suggest that the incorporation of probiotics in the fish's diet, particularly at the higher level found in feed V, positively affected their haematological parameters, promoting better red blood cell count, hemoglobin levels, and red cell indices. The effect of probiotics on haematological parameters in *Cirrhinus mrigala* was reported (Parvati Sharma *et al.,* 2013). Similar report was observed in *Labeo rohita* (Rajikkanu *et al.,*2015).

Table 7 Haematological parameters

Haemotological parameters	Feed I	Feed II	Feed III	Feed IV	Feed V
WBC count (cells/mm^3)	18,900	22,900	24,600	25,000	26,300
Haemoglobin (gm/dl)	1.8	2.2	2.4	2.8	3.2
RBC count (millions/mm^3)	0.2	0.3	0.4	0.5	0.6
Haemotocrit (PCV)(%)	4.1	6.0	6.5	6.8	10.6
Mean Corpuscular volume (MCV) (fi)	143	151	152	159	204.7
Mean Corpuscular Haemoglobin (MCH) (pg)	48	50	53	58	61
Mean Corpuscular Haemoglobin concentration (MCHC)	37	43	45	48	50
Platelets	16,000	19,000	26,000	28,000	32,000

4.CONCLUSION:

Based on the findings, it was determined that several feed utilization parameters, including Feed Conversion Ratio,Feed Consumption, Feed Conversion Efficiency, Relative Growth Rate,Growth, Net Growth Efficiency,Gross Growth Efficiency, Biochemical parameters, and Haematological parameters, were higher in feed V, which contained 1ml each of *Leuconostoc mesenteroids, Lactobacillus amylophilus, Lactobacillus gasseri* and *Lactobacillus colini.* These organisms in the fish's gut flora improve its probiotic properties and contribute to its nutritional advantages.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

5. REFERENCE:

1. **Ai, Q.H., Mai, K.S., Xu and Wang, J (2011)** Effect of dietary supplementation of *Bacillus subtilis* and Fructo oligosaccharide on growth performance, survival rate, non- specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthyscrocea*. Aquaculture, 317: 155-16.
2. **Ali Kane., Mehdi Soltani., Hossein Ali., Ebrahimzahe-Mousavi and Komeil Pakzad (2016)** Influence of probiotic, *Lactobacillus plantarum* on serum biochemical and immune parameters in vaccinated rainbow trout (*Oncorhynchus mykiss*) against streptococcosis/lactococosis. International Journal of Aquatic Biology, 4 (4): 285-294.
3. **Allameh, S.K., Vahid Noaman and Reza Nahavandi (2017)** Effects of probiotic bacteria on fish performance. Advanced Techniques in Clinical Microbiology, 1 (2): 11.
4. **Anita Bhatnagar and Ritu Lamba (2015)** Antimicrobial ability and growth promoting effects of feed supplemented with probiotic bacterium isolated from gut microflora of *Cirrhinusmrigala*. Journal of Integrative Agriculture, 14 (3): 583-592
5. **Balaji, N., Rajasekaran, K.M., Kanipandian, N., Vignesh, V and Thirumurugan, R., (2012)** Isolation and screening of proteolytic bacteria from freshwater fish *Cyprinus carpio*. International Multidisciplinary Research Journal, 2 (6): 8-12.
6. **Bency Thankappan., Dharmaraj Ramesh., Srinivasagan Ramkumar., Kalimuthusamy., Natarajaseenivasan and Kumarasamy Anbarasu (2015)** Characterization of *Bacillus spp.* from the

gastrointestinal tract of *Labeorohita* towards to identify novel probiotics against fish pathogens. Applied Biochemistry and Biotechnology, 175: 340-353.

7. **Bisht, A., Singh, U.P and Pandey, N.N (2012)** Probiotic potential of *Bacillus subtilis* for enhancing growth in finger lings of common carp (*Cyprinus carpio Linnaeus*). Indian Journal of Fisheries, 59(3): 103 – 107.
8. **Deepika, G., Sivakumar, P and Rajan, M.R (2019)** Isolation and characterization of bacteria from the gut of Blue Gourami (*Trichogastertricopters*) and its role on growth. Journal of Pure and Applied Microbiology, 13(4): 2479-2487.
9. **Dhanraj, M., Hannifin, SV., Arun Singh., Jesu Arockiaraj, A., Muthu Ramakrishnan, C., Seetharaman, S and Arthimanju, R (2010)** Effect of probiotics on growth Performance of Koi carp (*Cyprinus carpio*). Journal of Applied Aquaculture, 22: 202-209.
10. **Gohila, B., Damodaran, R., Bharathidasan, V and Vinoth, M (2013)** Comparative studies on growth performance of probiotic supplemented Rohu (*Labeorohita*) fingerlings. International Journal of Pharmaceutical and Biological Archives, 4 (1): 84-88.
11. **He SuXu ., Liu WenShu Liu WenShu., Zhou ZhiGang Zhou ZhiGang., Mao Wei Mao Wei., Ren PengFei Ren PengFei., Marubashi, T and Ringo, E (2011)** Evaluation of probiotic strain *Bacillus subtilis* C-3102 as a feed supplement for koi carp (*Cyprinus carpio*). Journal of Aquaculture Research and Development, 2: 5-35.
12. **Hoang, D (2020)** Influence of dietary β-glucan on length-weight relationship, condition factor and relative weight of pompano fish (*Trachinotusovatus*). International Journal of Fisheries and Aquatic Studies, 8 (2): 85-91.
13. **Irianto, A and Austin, B (2002)** Probiotics in aquaculture. Journal of Fish Diseases, 25 (11): 633-642.
14. **Karthikeya, V and Santosh, S.W (2009)** Isolation and partial characterization of Bacteriocin produced from *Lactobacillus plantarum*. African Journal of Microbiological Research, 3: 233–239.
15. **Michael, S,E., Emmanuel Delwin Abarike and Jia Cai (2019)** A review on the probiotic effects on haematological parameters in fish, Journal of Fisheries Sciences, Communication, 13 (3): 25- 31.
16. **Muge Aliye Hekimoglu., Cuneyt Suzer., Sahin Saka and Kursat Fırat (2014)** Enzymatic characteristics and growth parameters of ornamental koi carp (*Cyprinus carpio var. koi*) larvae fed by *Artemia nauplii* and cysts. Turkish Journal of Fisheries and Aquatic Sciences, 14 (1): 125-133.
17. **Pankaj Kumar., Jain, K.K., Sardar, P., Sahu, N.P and Gupta (2017)** Dietary supplementation of acidifier: effect on growth performance and haemato-biochemical parameters in the diet of *Cirrhinusmrigala* juvenile. Aquaculture International, 25: 2101-2116.
18. **Parthasarathy, R and Ravi, D (2011)** Probiotic bacteria as growth promoter and biocontrol agent against *Aeromonas hydrophila* in *Catlacatla*. Indian Journal of Fisheries, 58(3): 87-93.
19. **Parvati Sharma., Ram Chander Sihag and Suresh Kumar Gahlawat (2013)** Effect of probiotic on haematogicalparamaters of diseased fish (*Cirrihinusmrigala*). Journel of Fisheries Science, 7 (4): 323-328.
20. **Pornthep Niamphithak., Siripavee Chareon Wattanasak and Sompong Doolgindachbaporn (2017)** Effect Of Dietary supplement of probiotic (*Lactobacillus Plantarum*) on growth performance, feed utilization and survival rate in Bocourti Catfish. Journel of Pure Applied Microbiology, 11(2): 803-809.
21. **Priyanka Arya., Deepshikha Chandra., Akansha Khati and Chauhan, R.S (2019)** Effect of probiotics supplemented diet on growth performance of *Catlacatla* fingerlings. Journel of Entomology and Zoology studies, 7(6): 202-206.
22. **Pushparaj, A., Ramesh, U and Ambika, P (2012)** Effect of probionts on the growth and food utilization of clown fish (*Amphiprionsebae*). International Journal of Applied Biology and Pharmaceutical Technology, 3(1):309-314.
23. **Rachmawati, D and Samidjan, I (2018)** Performance efficiency of feed utilization, Relative growth rate, survival rate of common carp (*Cyprinus carpio*) through the Addition of phytase in the feed. Earth and Environmental Science, DOI: 10.1088/1755- 13/5/137/1/012027.
24. **Rajan, M.R and Sabitha, A (2023)** Isolation, identification, enzyme productivity and antibacterial Activity of intestinal bacteria of Blue morph *Maylandia Lombardoi* and its role on growth. Sustainability, Agriculture, Food and Environmental Research, 12 (1), http://dx.doi.org/10.7770.
25. **Rajikkannu, M., Nirmala Natarajan., Santhanam, P., Deivasigamani, B., Ilamathi, J and Janani, S (2015)** Effect of probiotics on the haematological parameters of Indian major carp (*Labeorohita*). International Journal of Fisheries and Aquatic Studies, 2 (5): 105-109.
26. **Ramos, M.A., Batista, S., Pires, M.A., Silva, A.P., Pereira, L.F., Saavedra, M.J and Rema, P (2017)** Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. Animal, 11 (8): 1259-1269.

27. **Sankar, D.S and Kulkarni, R.S (2005)** Somatic condition of the fish *Notopterusnotopterus* during different phases of the reproductive cycle. Journal of Environmental Biology, 26(1): 49-53.
28. **Sivakumar, P and Rajan, M.R (2013)** Isolation and Enzymatic productivity of bacillus species from intestine of Gold fish (*Carassius auratus)*. Indian Journal of Applied Microbiology, 16(2): 92-96.
29. **Sivakumar, P and Rajan, M.R (2015)** Enzymatic productivity and Molecular characterization of intestinal bacteria of Yellow molly (*Poecilialatipinna*) in relation to Growth. International Journal of Information Research and Review, 3(6): 2552-2555.
30. **Subramani Munirasu., Vankatachalam Ramasubramanian and Palanisamy Arunkumar (2017)** Effect of Probiotics diet on growth and biochemical performance of freshwater fish *Labeorohita* fingerlings. Journal of Entomology and Zoology Studies, 5 (3): 1374-1379.
31. **Suganya, D., Rajan, M.R and Siva Kumar, P (2014)** Isolation, identification, enzyme and molecular characterization of intestinal bacteria of goldfish (*Carassius auratus*) and its role on growth. Indian Journal of Applied Research, 4 (7): 9-11.
32. **Sunil Kumar, P and Vishnu, P.S (2011)** Effect of *Lactobacillus Acidophilus* on growth of *Etroplussuratensis*. Fishing Chimestry, 31(7): 54-55.
33. **Tamanna Tabassum., AGM Sofi Uddin Mahamud., Tusher Kanti Acharjee., Rashidul Hassan., Tasnim Akter Snigdha., Tazrian Islam., RejowanaAlam., Md UmorKhoiam., Fahmida Akter., Md Redwan Azad., Md Abdullah Al Mahamud., Gias Uddin Ahmed and Tanvir Rahman (2021)** Probiotic supplementations improve growth, water quality, hematology, gut microbiota and intestinal morphology of Nile tilapia. Aquaculture Reports, 21: 100-972.
34. **Wang Yanbo and Xu Zirong (2006)** Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Animal Feed Science and Technology, 127 (3-4): 283-292.
35. **YuniarMulyani, I., Nyoman, P., Aryantha and Sony Suhandono (2018)** Intestinal bacteria of common carp (*Cyprinus carpio L*.) as a biological control agent for Aeromonas. Journal of Pure and Applied Microbiology, 12 (2): 3-15.
36. **Zokaeifar, H., Jose Luis Balcazar., Che Roos Saad., Mohd Salleh Kamarudin., Kamaruzaman Sijam., Aziz Arshad and Naghmeh Nejat (2012)** Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp *(Litopenaeusvannamei*). Fish and Shellfish Immunology, 33 (4): 683-689.