IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Role Of Intestinal Bacteria On The Growth Of Mickey Mouse Molly *Xiphophorus Maculatus*

K. Thamayanthi[1] and M.R.Rajan[2]*

[1]Student, Department of Biology, The Gandhigram Rural Institute- Deemed to be University, Gandhigram-624 302, Tamil Nadu, India

[2] Senior Professor, Department of Biology, The Gandhigram Rural Institute- Deemed to be University, Gandhigram-624 302, Tamil Nadu, India

Abstract

Six distinct colonies were isolated from the intestine of Mickey Mouse molly *Xiphophorus maculatus* through serial dilution, pour plate, spread plate and streak plate methods. Based on the biochemical tests, the isolated bacteria were S1 (*Leuconostoc sp.,)* S2 (*Bacillus sp.,)* S3 (*Lactobacillus sp.,)* S4 (*Paracoccus sp.,)* S5 (*Lactobacillus sp.,) and* S6 (*Bacillus sp.).* Enzymatic productivity (Amylase, Protease and Lipase) of the isolated bacteria were carried out. Mass multiplication of *Leuconostoc sp., Bacillus sp., Lactobacillus sp., Paracoccus sp., Lactobacillus sp., Bacillus sp.,* was done in nutrient broth. Antibacterial activity of the intestinal bacteria of Mickey Mouse Molly was carried out with the help of selective media along with a commercial antibiotic (Streptomycin).Five different feeds having different bacteria such as control (without bacteria) Feed I(1ml of *Bacillus sp),* Feed II (1 ml of each *Bacillus sp.,* and *Lactobacillus sp.,),* Feed III (*Bacillus sp., Lactobacillus sp., Paracoccus sp.,),* Feed IV (1 ml of each *Bacillus sp., Lactobacillus sp.,* and *Paracoccus sp.,)*Feed V (1ml of each *Bacillus sp., Lactobacillus sp., Paracoccus sp.,* and *Leuconastoc sp.,)* were prepared. Feed utilisation parameters of Mickey Mouse Molly were estimated after 21 days. Based on the antibacterial test, the *Bacillus sp., Lactobacillus sp., Paracoccus sp., and Leuconostoc sp.* have higher inhibition. The feed consumption, feed conversion efficiency and growth, assimilation, metabolism Gross and net growth efficiency were higher in Feed V.

Keywords: Intestinal bacteria, biochemical, antibacterial, growth, Micky mouse molly

1.Introduction

Among aquaculture, ornamental fish culture is “Live jewels” which makes the garden pool enjoy fishes' beauty. In the 20th century, the rearing of ornamental fish and the keeping of aquariums gained worldwide attention. India earns foreign exchange by exporting a large quantity of ornamental fish. Sustainability, profitability and well-being of ornamental fish production is decided by feed and feeding management, which is essential for health and reproduction of ornamental fishes(1) . Ornamental fishes are affected by bacteria, fungi, viruses and parasites (2). Control of bacterial diseases is a challenging problem in aquaculture. Various vaccines and antibiotics have been developed recently to control diseases (3) . The excessive use of antibiotics in ornamental fish production causes multidrug resistance. The multitude of virulence factors that are related to ornamental fish pathogenicity and the antimicrobial resistance determinants related to multidrug resistance (4). So there is growing concern about probiotics to reduce these problems(5). In this scenario, probiotics are live microorganisms that control pathogens and also enhance the growth of fish. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Different probiotics, either monospecies or multispecies supplementation, can eventually elevate phagocytic, lysozyme, and complement activity as well as disease resistance, and the gut immune system of fish (6). The work related to the isolation and characterization of intestinal bacteria of Micky Mouse Molly and its role in growth is lacking. Hence, the present study was carried out.

2. Materials and Methods

Fish collection:

For the present study, Mickey Mouse Molly *Xiphophorus maculatus* (2.7±0.05g) was collected from Aqua Gardens, Madurai, Tamil Nadu, India and transported to the laboratory in polythene bags filled with aerated water.

Bacterial and strain collection:

Intestinal content of Mickey Mouse Molly was collected by dissecting the abdomen of the fish, serially diluted, and appropriate dilutions 10^{-5}, 10^{-6} were selected for the isolation of bacteria. The serially diluted sample was plated over a sterilized nutrient agar medium and incubated at 37 ℃ for 24 hours.

Media culture:

Nutrient agar was used for the present study, and different incubation temperatures were used in order to obtain a wide range of isolates. The incubation time ranged from 24 hours, depending on the incubation temperature, and colonies were counted and isolated.

Identification of bacteria:

Nutrient agar was used for the study. The predominant colonies on the nutrient agar medium were selected and identified based on the cellular morphology, and microscopic and biochemical characteristics. The tests used for examining the colonies were Indole, Methyl red, Voges-Proskauer, Citrate, Catalase, Gelatin hydrolysis, Strach, and Casein for the Lipase test and identified at the genus level of bacteria.

Enzyme productivity:

The intestinal bacteria of Mickey Mouse Molly were examined for the productivity of digestive enzymes like amylase, protease, and lipase using selective media [7].

Antimicrobial activity of intestinal bacteria

Selected intestinal bacteria were examined for double-layer screening antibacterial activity using selective media [8]. The different pathogens were selected, such as *Enterococcus faecalis and Stenotrophomonas maltophilia.*

Mass culture of isolated bacteria:

The isolated bacterial species were Bacillus, Lactobacillus, Paracoccus, and Leuconostoc, and mass was multiplied by inoculating into the nutrient broth.

Growth studies:

For growth studies, Mickey Mouse Molly (2.7 ± 0.05g) was collected from Aqua Gardens, Madurai, Tamil Nadu, India, and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in glass aquaria for 10 days at 28±2℃ during acclimation, fish were fed with trained feed containing fish meal, ground oil cake, wheat flour, and rice bran in the form of dry pellets.

Selection of feed ingredients:

The raw material is selected based on its ability to supply nutrients such as protein, carbohydrates, and fat at low cost. After knowing the protein content of the major ingredients [(fish meal (58%), ground nut oil cake (44%), wheat flour (11%), and tapioca (3%)] by Micro-Kjeldhal method, one control and four experimental feeds were prepared.

Experimental feed preparation:

The compounds used for feed preparation were dried, powdered, and sieved. The ingredients were weighed and mixed thoroughly with 130-150 ml of distilled water. The mixed feedstuff was put in the autoclave for 15 minutes at 100℃ and cooled. After cooling, fish oil, sunflower oil, suppletive mix, sodium chloride, and sodium benzoate were added. Four different feeds such as control without bacteria (Feed I), 1ml of *Bacillus sp.*, (Feed II)1 ml each of *Bacillus sp., and Lactobacillus sp.*, (Feed III) 1ml each of Bacillus sp., Lactobacillus sp., and *Paracoccus sp.*, (Feed IV) and 1 ml each of Bacillus sp., Lactobacillus sp., Paracoccus sp., and Leuconostoc *sp.*(Feed V*)*, were prepared (Table 1). The feedstuff was mixed well, and then it was extruded with the help of a pelletizer. The pellets were dried at room temperature. This formulated feed was kept in air-tight containers until used to prevent contamination.

Table 1. Composition of different Ingredients in the experimental feed (g/100gm) of Mickey Mouse Molly

Ingredients	Feed I (Control)	Feed II	Feed III	Feed IV	Feed V
Fish meal	33.75	33.75	33.75	33.75	33.75
Ground Nut Oil Cake	33.75	33.75	33.75	33.75	33.75
Wheat flour	11.2	11.2	11.2	11.2	11.2
Tapioca	11.2	11.2	11.2	11.2	11.2
Fish oil	2	2	2	2	2
Sunflower oil	2	2	2	2	2
Supplevite mix	2	2	2	2	2
Sodium chloride	2	2	2	2	2
Sodium benzoate	2	2	2	2	2
Bacteria	0	1 ml of *Bacillus sp.,*	1 ml each *Bacillus & Lactobacillus sp.,*	1 ml each of *Bacillus sp., Lactobacillus sp., & Paracoccus sp.,*	1ml each of *Bacillus sp., Lactobacillus sp., Paracoccus sp., & Leuconastac sp.,*

METHODS

Experimental design for growth studies:

For the present study, uniform-sized Mickey Mouse Molly (2.7±0.05 g) were selected, and then the fish were introduced into rectangular glass tanks having a capacity of 18 litres. Five fish were introduced into each tank. The length and weight of the fish were taken. During rearing, the fish were fed with the prepared feed twice a day for 1 hour each from 9-10 am and 4-5 pm. The unfed were collected after one hour of feeding without disturbing the fish. The faecal matter was collected daily before changing the water with the least disturbance to the fishes and dried at 95℃. Approximately 70% of the water in the tank was replaced with tap water. The experiment was continued for 21 days. On the 21st day length and weight of the fish were measured in live condition. Feed utilization parameters such as feed consumption, Feed conversion efficiency, Feed conversion Ratio, Growth, Percentage growth, relative growth rate, Assimilation, Metabolism, Gross and Net Growth Efficiency. The experiment results are presented in the form of tables using MS EXCEL (version 2007). Mean and standard deviation and also calculated with the help of the same tool. The one–way **ANOVA** method was used for the analysis using MS EXCEL (version 2007). The data was input manually and computed. The output results obtained from the software indicate whether the difference is between the treatments and days. The sum of square variations (SS), Degree of freedom (DF), Variability of sample means (MS), Critical probability value (F), and probability (prob) were also obtained.

3. Results

Identification of bacterial Isolates:

The organism isolated from the intestinal content was identified using biochemical tests (Table 2) and enzymatic productivity (Table 3). Based on the test, the selected *Bacillus sp., Lactobacillus sp., Paracoccus sp., Leuconstoc sp.*,(S2), (S3), (S4), (S1) found to be producing higher quantity of digestive enzymes.

Tests	S1	S2	S3	S4	S5	S6
Simple Staining	Cocci	Rod	Rod	Cocci	Rod	Rod
Gram Staining	Gram-positive	Gram-positive	Gram-positive	Gram-negative	Gram-positive	Gram-positive
Motility test	Non motile	Motile	Non motile	Non motile	Non motile	Motile
Indole test	Positive	Negative	Negative	Negative	Negative	Positive
Methyl Red test	Positive	Positive	Negative	Negative	Negative	Negative
Vogues Prokauser Test	Positive	Positive	Negative	Negative	Negative	Negative
Citrate test	Positive	Positive	Negative	Positive	Negative	Positive
Catalase test	Negative	Positive	Negative	Positive	Negative	Positive
Strach test	Positive	Positive	Positive	Positive	Positive	Positive
Gelatin test	Negative	Gram-positive	Gram Negative	Positive	Gram Negative	Gram-positive
Oxidase test	Negative	Positive	Negative	Positive	Negative	Positive
Identification	*Leuconastoc sp*	*Bacillus Sp*	*Lactobacillus sp*	*Paracoccus sp*	*Lactobacillus sp*	*Bacillus sp*

Table 2. Biochemical characterization of intestinal bacteria of Mickey Mouse Molly

Table 3. Enzyme productivity of intestinal bacteria of Mickey Mouse Molly

S.No	Intestinal bacteria	Amylase	Lipase	Protease
1.	Leuconstoc sp., (S1)	++	++	+++
2.	Bacillus sp., (S2)	+++	++	+++
3.	Lactobacillus sp., (S3)	+++	++	+++
4.	Paracoccus sp., (S4)	++	+++	++
5.	Lactobacillus sp., (S5)	+	+	++
6.	Bacillus sp., (S6)	+++	++	++

+- Nil (Absent) or (Negative), ++ - Low productivity (Positive), +++ - High productivity (Positive)

Enumeration of intestinal bacteria of Mickey Mouse Molly:

The enumeration of intestinal bacteria of Mickey Mouse Molly is presented in Table 4.

Table 4. Enumeration intestinal bacteria of Mickey mouse molly

S.No	Intestinal Bacteria	Dilution Factor			
		10^{-5}		10^{-6}	
		O	R	O	R
1.	Leuconastoc sp., (S1)	110	99	119	98
2.	Bacillus sp., (S2)	104	101	106	97
3.	Lactobacillus sp., (S3)	111	108	113	91
4.	Paracoccus sp., (S4)	107	98	117	99
5.	Lactobacillus sp., (S5)	119	97	104	101
6.	Bacillus sp., (S6)	110	101	115	98

Antibacterial Activity:

Antibacterial activity of intestinal bacteria of Mickey mouse molly was carried out with the help of selective media along with a commercial antibiotic Streptomycin (Fig.1). Based on the test the *Bacillus sp., Lactobacillus sp., Paracoccus sp., Leuconastoc sp.,* (S2), (S3), (S4), (S1) have shown higher activity.

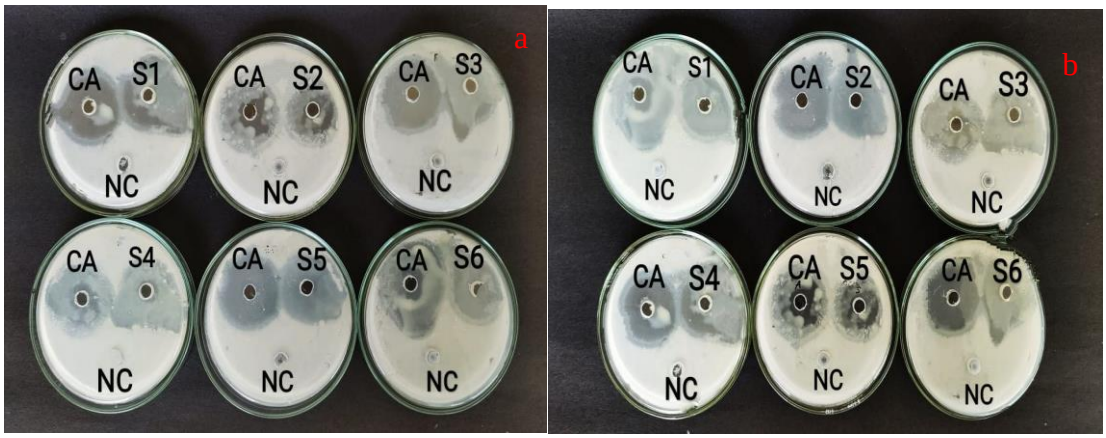

Figure1. Antibacterial activity of *Enterococcus faecalis*(a) and *Stenotrophomonas maltophilia* (b)

Feed Utilization

The different feed utilization and growth parameters are presented in Table 5. ANOVA (Analysis of variance) of growth parameters (Feed consumption, growth, Gross Growth Efficiency, Net growth Efficiency) are presented in Table 6. Feed consumption of Mickey mouse molly was higher in feed V (12.16±0.35) containing each 1ml of *Bacillus sp., Lactobacillus sp., Paracoccus sp., Leuconastoc sp.,* and lower in feed I (Control) (6.16±0.04). The feed conversion efficiency of Mickey mouse molly was higher in feed V (2.56±0.20). In feed I, II, III and IV feed conversion efficiency gradually increased. Feed conversion ratio of Mickey mouse molly was best in feed II (1.56±0.15) in feed V. Growth of Mickey mouse molly is higher in feed V (1.73±0.15). Percentage growth of Mickey mouse molly was higher in feed II (20.3). The relative growth rate of Mickey mouse molly was higher in feed II (1.96±0.15)). Assimilation and metabolism of Mickey mouse molly was higher in feed V (5.33±0.15 and6.53±0.15). Gross and net growth efficiency Mickey mouse molly in higher in feed V.

Table 5. Feed utilization and growth parameters of Mickey mouse molly in relation to different bacteria. Each value is the average (± SD) performance of 5 individuals in triplicates reared for 21 days.

Parameters	Experimental feed				
	Feed I (Control)	Feed II	Feed III	Feed IV	Feed V
Feed consumption (g/g live wt/21 days)	6.16±1.04	7.33±0.760.	8.33±0.87	10.33±1.31	12.16 ±0.35
Feed Conversion Efficiency	1.03±0.057	1.13±0.15	1.73±0.20	2.16±0.15	2.56±0.20
Feed Conversion Ratio	1.73 ±0.20	1.56±0.15	1.33±0.20	0.98±0.20	0.73±0.15
Growth (g/g live wt/21 days)	0.53±0.11	1.03±0.20	1.23±0.15	1.53±0.11	1.73±015
Percentage Growth (%)	2.13±0.32	2.03±0.20	1.83±0.20	1.73±0.20	1.76±0.20
Relative Growth Rate	2.06±0.37	1.96±0.15	1.73±0.66	1.53±0.15	1.83±0.90
Assimilation (g/g live wt/21 days)	5.33±0.15	5.53±0.20	6.23±0.11	6.53±0.15	6.73 ±0.15
Metabolism (g/g live wt/21 days)	5.23±0.15	5.46±0.15	5.93±0.32	6.23±0.20	6.53±0.15
Gross Growth Efficiency (%)	7.03±0.15	7.53±0.20	8.23±0.20	9.63±0.20	9.88±0.12
Net Growth Efficiency (%)	7.03±0.20	7.53±0.20	8.23±0.20	9.63±0.20	9.88±0.12

Table 6. ANOVA (Analysis of Variance) of growth parameters (Feed consumption, Growth, Gross growth efficiency, Net growth efficiency) of Mickey Mouse Molly

Parameter	Source	SS	DF	MS	F	Significance
Feed Consumption	Between groups	2.095	3	0.698	5.005	0.030
	Within groups	1.116	8	0.140		**S**
	Total	3.212	11			
Growth	Between groups	0.076	3	0.025	25.027	0.005
	Within groups	0.008	8	0.001		**S**
	Total	0.084	11			
Gross Growth Efficiency	Between groups	1802.120	3	600.707	95.561	0.005
	Within groups	50.289	8	6.286		**S**
	Total	1852.409	11			
Net Growth Efficiency	Between groups	11.744	3	3.915		0.005
	Within groups	0.832	8	0.104	37.620	**S**
	Total	12.576	11			

4. Discussion

Bacillus sp., Lactobacillus sp., Paracoccus sp., and Leuconastoc sp., are Gram-positive, used commercially in probiotic products because its beneficial effects have been documented and the pathogen potential is generally described as low or absent[9] . The presence of these bacteria in the present study confirms that there was no stress on animals due to use of probiotics. Digestive enzymes like amylase, protease and lipase in intestinal bacteria of Mickey mouse molly was carried out with the help of selective media. Based on the test the selected *Bacillus sp., Lactobacillus sp., Paracoccus sp., Leuconstoc sp.,*(S2), (S3), (S4), (S1) found to be producing higher quantity of digestive enzymes. Abhinanda Bairagi *et al.,*(2002)[10] reported that production and characterization of digestive enzymes in Catla. Sivakumar *et al.* (2016)[11] reported the of digestive enzymes in Yellow molly. Antibacterial activity of intestinal bacteria of Mickey mouse molly was carried out with the help of selective media along with a commercial antibiotic (Streptomycin). Based on the test the *Bacillus sp., Lactobacillus sp., Paracoccus sp., Leuconastoc sp.,* (S2), (S3), (S4), (S1) have shown higher activity. Zapata (2013)[12] reported that antimicrobial activities of lactic acid from Nile tilapia. Sivakumar and Rajan (2015)[13] reported that antibacterial activities of intestinal bacteria from Yellow molly.Feed consumption of Mickey mouse molly was higher in feed V (12.16±0.35) containing each 1ml of *Bacillus sp., Lactobacillus sp., Paracoccus sp., Leuconastoc sp.,* and lower in feed I(Control) (6.16±0.04). Rajan and Sabitha (2023)[14] reported that the feed consumption of Blue morph was higher in feed V (4.55± 3.21) containing each 1ml of Bacillus sp, Enterobacter sp, Escherichia sp, Pseudomonas sp,The feed conversion efficiency of Mickey mouse molly was higher in feed V. Rajan and Revathi (2011)[15]reported that the feed conversion efficiency of Platy was higher in experimental feeds (0.24±0.13) and low in control (0.14±0.05).Feed conversion ratio of Mickey mouse molly was best in feed II (1.56±0.15) Deepika *et al.,* (2019)[16] reported that the feed conversion ratio (FCR) of blue gourami was best in feed IV (4.46±0.53). Parathasarathy and Ravi (2011)[17] reported that the feed conversion ratio of *Catla catla* was higher in T1 control (4.76) and lower in T4 (1.45),Growth of Mickey mouse molly is higher in feed V. Dhanraj *et al.,* (2010)[18] reported that the growth of koi carp was higher in diet 3 (0.32±0.07) and lower in control (0.19±0.02). Percentage growth of Mickey mouse molly was higher in feed II. The percentage growth was higher in Ornamental fish Swordtail [19]. Sivakumar *et al., (*2014)[20] reported that the percentage growth of common carp was higher in feed IV.The relative growth rate of Mickey mouse molly was higher in feed II. Asma Chaudhary *et al., (*2021)[21] reported that the relative growth rate of rohu increased when fed with *Bacillus subtilis.* Seenivasan *et al., (*2012)[22] reported that the relative growth rate of freshwater prawn increased when fed with *Bacillus subtilis.* Assimilation and metabolism of Mickey mouse molly was higher in feed V. Similar assimilation is reported in blue gourami Deepika *et al., (*2019)[16]. Rajan and Jeya Christina Arockiaselvi (2013)[23] reported the assimilation of goldfish. Similar assimilation is reported in blue morph[14]. Similar metabolism was also reported by Sivakumar *et al.,* (2014)([20] in Common carp. Gross and net growth efficiency Mickey mouse molly in higher in feed V. Immanuel *et al., (*2003)[24] reported that a similar result when fish fed with Lactobacillus. Pushparaj *et al., (*2012)[25] reported a similar result when fish was fed with Lactobacillus.

5.Conclusion

The present study conclude that the feed V is suitable for the feed utilization and growth Micky Mouse Molly.

Acknowledgement

The are grateful to the Department of Biology, The Gandhigram Rural Institute- Deemed to be University, Gandhigram, for carry out the study.

References

1. Harsha Haridas, Saravanan K, Praveenraj J, Ravindra Sontakke Y, Gladston SM, Alina. Training manual on freshwater ornamental fish breeding and aquascaping techniques. The Director, ICAR-CIARI, Port Blair. 2019:1-39.
2. Krishna Prema K, Kasturi Jayaraman. Effect of Lactobacillus sp. And Saccharomyces cerevisiae on Goldfish. Indian Journal of Applied Microbiology.2018; 16(2):17-32.
3. Julia W Pridgeon, Philip H. Klesius. Fish vaccines in aquaculture. Major bacterial disease in aquaculture. The Global Aquaculture.2012; 5.
4. Hossian, G-J Heo. Ornamental fish: a potential source of pathogenic and multidrug resistant motile *Aeromonas spp.* Letters in Applied Microbiology.2012; 72 (1): 2-21.
5. Mandira Saha, Subarna Ghosh, Probir Kumar Bandyopadhyay. Description of protozoan parasites parasitizing gold fishes and their possible control by herbal extracts. Proceedings of the National Academy of Sciences, Biological Sciences.2015;90(56): DOI:10.1007⁄ s40011-019-01083-z.
6. Sukanta Kumar Nayak. Probiotics and Immunity: a Fish Perspective. Fish Shell fish Immunology. 2010;29(1):2-14.
7. Muge Aliye Hekimoglu, Cuneyt Suzer, Sahin Saka, Kursat Fırat. 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.2014;14 (1): 125-133.
8. Jawahar Abraham, Suman Mondal Surendra Babu. Effect of commercial aquaculture probiotic and fish gut antagonisitic bacteria flora on the growth and disease resistance of ornamental fishes *Carassius auratus* and *Xiphophorus helleri.* Journal of Fisheries and Aquatic Sciences. 2008;25 (1): 27-30
9. Kim, Austin. Characterization of probiotic bacteria isolated from rainbow trout (*Oncorhynchus mykiss)* intestine. Letters in Applied Microbiology.2006;47(3):141-147.
10. Abhinanda Bairagi, Keka Sarkar Ghosh, Sukanta Kumar Sen, Arun Kumar Ray. Enzyme producing bacteria flora isolated from fish digestive tracts. Aquaculture International. 2002;10: 109-121.
11. Sivakumar P, Rajan MR, Balakrishnan R. Enzymatic productivity and molecular characterization of intestinal bacteria of yellow molly (*Poecilla latipinna)* in relation to growth. International Journal of Information Research and Review. 2016; 03:2552-2555.
12. Zapta, Maurilio Lara- Flores. Antimicrobial activities of lactic acid bacteria strains isolated from *Nile tilapia* intestine. Journal of Biology and Life Sciences.2013; 4(1): 164-171.
13. Sivakumar P, Rajan MR. Isolation, enzymatic and antibacterial activity of intestinal bacteria of yellow molly (*Poecilia latipinna)* and its role on growth. International Journal of Fisheries and Aquatic Sciences. 2015;2(5): 330-333.
14. Rajan MR, Sabitha A. Isolation, Identification, Enzyme productivity and antibacterial activity of intestinal bacteria of blue morph *Mayalandia lombardoi* and its role on growth. Agriculture, Food and Environment Research. 2023;,12 http://dx.doi.org/10.7770/safer-V12N1-art2586.
15. Rajan MR, Revathi V. Isolation, Identification, Enzymatic and molecular characterization of intestinal bacteria of goldfish (*Carassius auratus)* and its role on growth.Journal of Pure and Applied Microbiology. 2011;5(2): 819-823.
16. Deepika G, Sivakumar P, Rajan MR. Isolation and characterization of bacteria from the gut of blue gourami (*Trichogaster tricopters*) and its role on growth. Journal Pure Applied Microbiology. 2019;13(4):2479-2487.
17. Parathasarathy R, Divahar Ravi. Probiotic bacteria as growth promoter and biocontrol agent against aeromonas hydrophila *Catlacatla.* Indian Journal of Fisheries. 2011;58(3).
18. Dhanaraj M, Haniffa MA,Arun Singh SV. Effect of probiotics on growth performance of Koi carp (*Cyprinus carpio).*Journal of Applied Aquaculture.2010;22:202-209.

19. Rajan MR, Brindha G. Evaluation of dissimilar intestinal bacteria incorporated feeds on growth of ornamental fish swordtail Xiphophorus *helleri.* Letters in Applied Microbiology.2022;75(2). 243-248.
20. Sivakumar P, Rajan Muthuswami Ruby, Ramachandran Pomman. Effect of probiotics on growth performance of common carp Cyprinus carpio var *communis.* International Journal of pharma and Bio Sciences.2014;5(1): 835-839.
21. Asma Chaudhary, Zawar Hussain, Afia Muhammad Akram, Mohammed Alorabi, Nareen Sarwar, Rahat Abdul Rehman, Naveen Ahmad Khan, Muhammad Faiz Khan, Qandeel Minahal, Hesham Ali El Enshasy, Solleh Ramli, Ali Tan Kee Zuan, Alaa Alhazmi, Syed Mohammed Basheeeruddin Asdaq, Samina Qamer, Mohamed Alkafafy. Impact of *Bacillus subtilis* supplemented fed on growth and biochemical constituents in *Labeo rohita* fingerlings. Journal of King Saud University of Science .2021;33: 101668.
22. Seenivasan Chandrasekar, Radhakrishnan Subramanian, Thirunavukkaras Muralisankar, Saravana Bhavan P. *Bacillus subtilis* on survival, growth, biochemical constituents and energy utilization of the freshwater prawn *Macrobrachium rosenbergii* post larvae. The Egyptian Journal of Aquatic Research. 2013;38(3).
23. Rajan MR, Jeyachristina Arockiaselvi J. Probiotic effect of intestinal microflora of Gold fish *Carassius auratus*. International Journal of Current Microbiology and Applied Sciences.2014;3(9): 685-688.
24. Immanuel, Prathvi Rani, Rajan Kumar. Ornamental fish exports India. International Journal of Fisheries and Aquatic Studies. 2003;1(4): 85-92
25. Pushparaj A, Ramesh U, Ambika P. Effect of probiotics on the growth and food utilization of clown fish. International Journal of Applied Biology and Pharamaceutical Technology. 2012; 3(1) : 309-314.