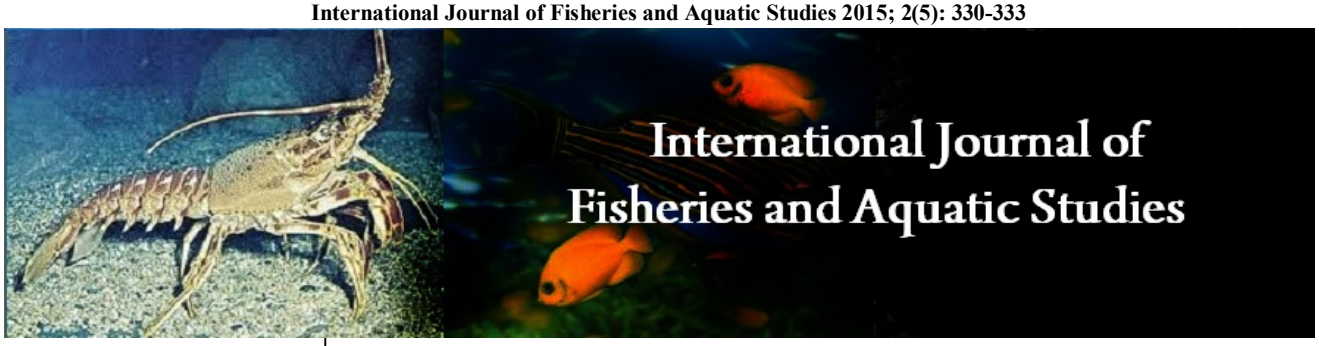

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Isolation, enzymatic and antibacterial activity of intestinal bacteria of yellow molly (*Poecilia latipinna*) and its role on growth

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Abstract
The present study deals with the isolation, enzymatic and antibacterial activity of intestinal bacteria of Yellow molly and its role on growth. The intestinal sample was collected by dissecting the abdomen of the fish. The sample was serially diluted and the appropriate dilutions 10^{-6} were selected for the isolation of bacteria. The serially diluted sample was plated over sterilized nutrient agar medium and incubated at 37°C for 24hrs. The isolated and identified bacteria such as *Pseudomonas sp., Streptococcus sp., Staphylococcus sp., E. coli. sp.,* and *Aeromonas sp.,* were subjected for its efficacy to produce enzymes like amylase, cellulase, lipase and protease. Antibacterial activity of intestinal bacteria was also carried out. Six experimental feeds having different concentration of *Pseudomonas sp.,* such as FI control (without probiotics), F2 (1ml), F3 (2ml), F4 (3ml), F5 (4ml) and F6 (1ml of yeast) were prepared by using fish meal, groundnut oil cake, wheat flour and tapioca. Fish were fed with the feed at a rate of 4% of live body weight and reared for a period of 45 days. Growth parameters such as condition factor, FC, FCE, FCR, Growth, PG, RGR, Assimilation, Metabolism, GGE and NGE were calculated after 45 days and ANOVA was studied for FC, Growth, GGE and NGE. Results showed that growth parameters higher in F5 containing 4ml of *Pseudomonas sp.,* and lower in F1 control.

Keywords: Intestinal bacteria, isolation, enzymatic productivity, antibacterial activity.

1. Introduction
Ornamental fish keeping gives pleasure to the young and old alike. Aquarium creates an excellent opportunity to watch the glittering colours and exuberant, untiring and graceful movements of fishes besides being a rewarding feast to the eyes and relaxation to the mind. In ornamental fishes intestinal micro flora has been reported to aid in the digestion of algal cells the production of amino acids and the secretion of inhibitory substances that prevent colonization by bacterial pathogens (Fong and Mann 1980) [8]. Ornamental fish culture shown a rapid progress during the past few years but some major problems are hindering the progress path and disease being one of them. For successful ornamental fish culture quality feed, good environment and disease free seeds were among the different techniques, the study of digestive enzymes is an essential step towards understanding the mechanism of digestion and the organism adapts to changes in the nutrition environment.
Few reports concerning microbial enzymatic production in the gastrointestinal tract of fish are however, available (Stickney and Shumway 1974 [18] ; Prejs and Blaszcyk 1977 [13] ; Lindsay and Harris 1980 [11] ; Lesel *et al.,* 1986 [10] ; Das and Tripathi 1991 [6] ; Saha and Ray 1998 [15]). These processes can be initially examined by analyzing the activity of digestive enzymes like amylase, cellulase, lipase and protease. The present study was isolation, enzymatic and anti bacterial activity of some intestinal bacteria of ornamental fish yellow molly was totally wanting. Hence the present study was carried out.

2. Materials and methods
2.1 Collection and isolation of bacteria
The experimental fish Yellow molly (*Poecilia latipinna*) was collected from Angel Aquarium, Dindigul, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. The intestinal sample was collected by dissecting the abdomen of the fish. The sample was serially diluted and the appropriate dilutions 10^{-6} were selected for the isolation of bacteria. The serial diluted sample was plated over sterilized Nutrient agar plates

and incubated at 37°C for 24hrs. After incubation the colonies were enumerated and the predominant colony were selected and identified based on the morphological, microscopic and biochemical characteristics like Gram staining, Indole test, Methyl red test, Voges-proskauer test, Citrate, Starch test and Gelatin hydrolysis test. (John G. Holt *et al.,* 1994) [9]. The biochemical characteristics of intestinal bacteria of yellow molly was given Table 1. The isolated *Pseudomonas sp.,* (10^5 Cells) was mass multiplied by inoculating in nutrient broth. For growth studies, fishes were acclimated in glass aquaria (60×45×45 cm) for a period of 10 days at 28 ± 2^0 C. During acclimation, fishes were fed with trainee feed containing fish meal, groundnut oil cake, wheat flour and rice bran in the form of dry Pellets. One control (without bacteria), four experimental feeds by using different quantity (1,2,3,& 4 ml) of isolated bacteria and one feed by using commercially available probiont (yeast) was prepared according to square method (Ali.1980) [2]. Composition of different ingredients in experimental feeds is given in Table 2.

Table 1: Biochemical characterization of intestinal bacteria of Yellow molly

Tests	YM1	YM2	YM3	YM4	YM5
Simple staining	Rods	Cocci	Cocci	Rods	Rods
Gram's staining	Negative	Positive	Positive	Negative	Negative
Motility	Motile	Non - motile	Non-Motile	Motile	Motile
Indole	Negative	Negative	Negative	Positive	Positive
Methyl Red	Negative	Negative	Negative	Positive	Positive
Voges Prokauser	Negative	Positive	Negative	Positive	Positive
Citrate	Positive	Positive	Negative	Not performed	Positive
Catalase	Positive	Negative	Positive	Positive	Positive
Starch	Positive	Negative	Negative	Negative	Positive
Gelatin hydrolysis	Positive	Positive	Positive	Negative	Positive
Lipid	Positive	Positive	Positive	Positive	Not Performed
Identification result	*Pseudomonas sp.,*	*Streptococcus sp.,*	*Staphylococcus sp.,*	*E. coli. sp.,*	*Aeromonas sp.,*

Table 2: Composition of different ingredients in experimental feeds (g/100gm):

S. No	INGREDIENTS	EXPERIMENTAL FEEDS					
		Feed I control	Feed II	Feed III	Feed IV	Feed V	Feed VI
1	Fishmeal	33.75	33.75	33.75	37.50	33.75	33.75
2	GNOC	33.75	33.75	33.75	33.75	33.75	33.75
3	Wheat flour	11.25	11.25	11.25	11.25	11.25	11.25
4	Topioca	11.25	11.25	11.25	11.25	11.25	11.25
5	Fish oil	2	2	2	2	2	2
6	Sunflower oil	2	2	2	2	2	2
7	Suppelvite mix	4	4	4	4	4	4
8	Sodium chloride	1	1	1	1	1	1
9	Sodium benzoate	1	1	1	1	1	1
10	Microbes (10^5 Cells)	-	1ml	2ml	3ml	4ml	1ml Yeast

GNOC – Groundnut Oil Cake.

2.2 Experimental design for growth studies

For the present study uniform size of Yellow molly (*Poecilia latipinna*) (5 ± 0.30 g) were selected and then the fishes were introduced in the rectangular glass tanks (45 cm L × 22 cm B × 22cm H) having a capacity of 18 liters. Five fishes were introduced in each tank. For each treatment triplicates were maintained. During rearing, the fishes were fed on ad-labitum diet of 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 fishes. The unfed was dried to constant weight. The fecal matter was collected daily before changing the water with least disturbance to the fishes and dried at 95°C. Approximately 70 % of water in the tank was replaced with tap water. The experiment was continued for 45 days. On the 45th day length and weight of the fishes were measured in live condition for calculating condition factor (K) (Weatherley and Gill 1987) [20]. for individual fish before and after the experiment and other feed utilization parameters.

The experimental results are presented in the form of tables and graphs using Mat lab 2008a (Version 2007). Mean, Standard deviation and T-test were also calculated with the help of the same tool. One-way ANOVA method was used for the analysis using DMRT (Version 2005) according to (Sendecor and Cochran 1961) [16]. The data was input manually and computed. The output results obtained from the software indicate whether the difference is between the treatments and days. 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 and discussion

From present study, the isolated and identified bacteria from intestine of Yellow molly (*Poecilia latipinna*) were YM1 (*Pseudomonas sp.,*), YM2 *(Streptococcus sp.,)* YM3 (Staphylococcus *sp.,)* YM4 *(E.coli. sp.,)* and YM5 (*Aeromonas sp.,)*. Based on the biochemical tests, enzymatic productivity and antibacterial activity the selected bacteria was *Pseudomonas sp.,* (YM1). Isolated and identified *Aeromonous hydrophilla* and *Pseudomonas aeruginosa* in the intestine of gold fish (Rajan and Akilandeswari 2008) [14]. The study of digestive enzymes is an essential step towards understanding the mechanism of digestion and how organisms adapt to the changing environment. These processes can be initially examined by analyzing the productivity of digestive enzymes like amylase, cellulase, lipase and protease (Abhinanda bairagi *et al.,* 2002) [1]. The enzyme producing microbiota can be beneficially used as probiotic supplement for fish growth. The

enzymatic productivity of Bacillus species from the intestine of gold fish *Carassius auratus* (Sivakumar and Rajan 2013) [17].

The enzymatic productivity were tabulated in Table 3. Antimicrobial ability and growth promoting effects of feed supplemented with probiotic bacterium isolated from gut microflora of *Cirrhinus mrigala* (Anita Bhatnagar and Ritu Lamba 2015) [3]. The isolated strain showed maximum inhibition against the pathogenic strain and selected for further study. The strains that showed halos larger than 2 mm were considered positive. It was found that the inhibitory effects of intestinal bacterial isolates were varied with different target strains (2.8 to 20 mm). Antibacterial activity of intestinal bacteria of yellow molly is presented Table 4. The different feed utilization and growth parameters presented in Table 5. The ANOVA (Analysis of variance) of growth parameters (Feed consumption, growth, Gross Growth efficiency, Net Growth Efficiency) is presented in Table 6. Feed consumption and Feed conversion efficiency of yellow molly was higher in Feed V (8.7±0.60 and 2.14±0.04) containing 4 ml of *Pseudomonas sp.,* and lower in feed 1 (6.1±0.16 and 0.15±0.02). The feed conversion efficiency of *Labeo rohita* was higher in SSf2 (44.09±4.25) and lower in control (35.97±4.06) (Asma chaudhary and Jaed labal Qazi 2007) [4]. Feed conversion Ratio was higher in feed V (12.66±0.65) and lower in feed I (5.78±1.77). reported that the feed conversion ratio was higher in feed T5 (2.75±0.06) and lower in T1 control (2.26± 0.01) (Suganya *et al.,* 2014) [19]. The growth, percentage growth and relative growth of yellow molly was higher in feed V (2.16±0.62, 83.09±0.09 and 3.78±1.25) containing 4 ml of *Pseudomonas sp.,* and lower in control (0.95±0.09, 51.52±0.57 and 1.90±0.18) without probiotics. The higher growth in different fishes such as catla (Parthasarathi and Ravi 2011) [12], koi carp (Dhanaraj *et al.,* 2010) [7] and rainbow trout (Bagheri *et al.,* 2008) [5]. Assimilation and metabolism was higher in feed IV (4.82±0.95 and 4.84±0.76) and lower in feed I (3.10±0.58 and 2.28±0.35). The gross growth efficiency and net growth efficiency was higher in feed V (32.15±0.87 and 29.83±1.15) and lower in feed I (control) (without probiotics).

Table 3: Enzymatic Productivity of Intestinal Bacteria of Yellow molly.

Fish species	Bacteria strain	Amylase	Cellulase	Lipase	Protease
Yellow molly (*Poecilia latipinna*)	YM 1	+++	+++	++	+++
	YM 2	++	++	++	+
	YM 3	+	+	+	+
	YM 4	++	++	+	+
	YM 5	++	+	+	++

YM1-*Pseudomonas sp.,* YM2-*Streptococci sp.,* YM3-*Staphylococci sp.,* YM 4-*E.coli sp.* YM5-*Aeromonas sp.,* (+-Low, ++ -High).

Table 4: Antibacterial activity of intestinal bacteria of Yellow molly

S. No	INTESTINAL BACTERIA	Zone of Inhibition in mm									
		P1	CA	P2	CA	P3	CA	P4	CA	P5	CA
1	YM1 *(Pseudomonas sp.,,)*	11	04	13	06	12	05	11	05	10	04
2	YM 2 *(Streptococci sp.,)*	08	04	04	03	05	02	07	05	06	02
3	YM 3 *(Staphylococci sp.)*	09	05	07	03	06	02	09	03	05	04
4	YM 4 *(E.coli sp.)*	06	04	09	03	07	04	08	04	06	02
5	YM 5 *(Aeromonas sp.,)*	07	04	08	03	07	03	06	02	07	03

CA – Commercial Antibiotic (Zendamycin), P1-*Staphylococcus aureus*, P2-*Shigella sonnei,* P3-*Enterococcus faecalis*, P4-*Pseudomonas aeruginosa,* P5-*Klebsilla pneumoni*

Table 5: Feed utilization and growth parameters of Yellow molly in relation to different concentration of *Pseudomonas. sp*., (cells) Each value is the average (± sd) performance of 5 individuals in triplicates reared for 45 days.

S.NO	PARAMETERS	EXPERIMENTAL FEEDS					
		FEED I (CONTROL)	FEED II (1 ml)	FEED III (2ml)	FEED IV (3ml)	FEED V (4ml)	FEED VI (1ml Yeast)
1	Feed Consumption(FC) (g/g live wt/45days)	6.1 ± 0.16	6.3 ± 0.80	6.9 ± 0.41	7.4 ± 0.21	8.7 ± 0.60	6.8 ± 0.75
2	Feed Conversion Efficiency (FCE)	0.15 ± 0.02	0.17 ± 0.06	0.16 ± 0.05	1.10 ± 0.01	2.14 ± 0.04	1.15 ± 0.05
3	Feed Conversion Ratio (FCR)	5.78 ±1.77	9.13 ± 1.08	6.63 ± 1.09	7.78 ± 0.56	8.66 ± 0.65	7.52 ± 2.80
4	Growth (G) (g/g live wt/ 45 days)	0.95 ± 0.09	1.16 ± 0.49	1.15 ± 0.35	1.98 ± 0.06	2.16 ± 0.62	1.28 ± 0.42
5	Percentage Growth (PG) (%)	51.52 ± 0.57	52.28 ± 0.19	63.09 ± 0.94	69.15 ± 0.73	83.09 ± 0.09	79.41 ± 0.86
6	Relative Growth Rate (RGR)	1.90 ± 0.18	2.32 ± 0.98	2.28 ± 0.69	2.17 ± 0.11	3.78 ± 1.25	2.54 ± 0.83
7	Assimilation (A)	3.10 ± 0.58	3.13 ± 0.53	3.82 ± 0.91	3.91 ± 0.34	4.82 ± 0.95	3.96 ± 0.75
8	Metabolism (M)	2.28 ± 0.35	2.61 ± 0.74	3.52 ± 0.68	3.61 ±0.31	4.84 ± 0.76	2.69 ± 0.13
9	Gross Growth Efficiency (GGE) (%)	10.71 ± 1.66	18.39 ± 0.23	24.45 ± 0.25	28.94 ± 0.93	32.15 ± 0.87	25.83 ± 0.25
10	Net Growth Efficiency (NGE) (%)	18.96 ± 0.99	20.28 ± 0.90	23.93 ± 0.98	25.49 ± 1.07	29.83 ± 1.15	24.31 ± 0.37

Table 6: ANOVA (Analysis of variance) of growth parameters (FC, Growth, GGE, NGE) of Yellow molly

S. No	Parameters	Source	SS	Df	MS	F	Prob>F
1	Feed Consumption	Columns Error Total	8.7761 20.08 28.8561	5 12 17	1.75522 1.67333	1.05	0.04338 0.1 (S)
2	Growth	Columns Error Total	0.17483 1.8948 2.06963	5 12 17	0.03497 0.1579	0.22	0.9463 0.1 (S)
3	Gross Growth Efficiency	Columns Error Total	70.005 348.552 418.557	5 12 17	14.001 29.046	0.48	0.7833 0.1 (S)
4	Net Growth Efficiency	Columns Error Total	1927.72 1945.53 3873.24	5 12 17	385.544 162.127	2.38	0.1016 0.1 (S)

4. Conclusion

The present study concludes that the isolation, identification, enzymatic and antibacterial activity of intestinal bacteria (*Pseudomonas sp., Streptococcus sp., Staphylococcus sp., E.coli. sp.,* and *Aeromonas sp.,)* indicates that there is a distinct microbial source of digestive enzymes (Amylase, cellulase, lipase, and protease) apart from the endogenous sources in fish gastrointestinal tracts. Antimicrobial ability and growth promoting effects of feed supplemented with probiotic bacterium isolated from intestinal bacteria of yellow molly. Based on the results the higher productivity of digestive enzymes and higher inhibition of antibacterial activity of intestinal bacteria of *Pseudomonas sp.,* was selected for preparation of probiotic feed. So the presence of these organisms in the intestinal flora of the fish enhance the probiotic nature and helps in the nutritional benefits to the fish.

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