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Research Article

ENZYMATIC PRODUCTIVITY AND MOLECULAR CHARACTERIZATION OF INTESTINAL BACTERIA OF YELLOW MOLLY *(POECILIA LATIPINNA)* IN RELATION TO GROWTH

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ABSTRACT

The present study deals with the enzymatic productivity and molecular characterization of intestinal bacteria of yellow molly. The identified intestinal bacteria was *Escherichia* sp., (YM1), *Pseudomonas* sp., (YM2), *Staphylococcus* sp., (YM3), *Aeromonus* sp., (YM4), *Streptococcus* sp., (YM5). The intestinal bacteria were qualitatively screened on the basis of their extra cellular enzyme producing ability and molecular characterization of intestinal bacteria. The digestive enzyme productivity (amylase, cellulase, lipase and protease) of intestinal bacteria was higher in *Escherichia* sp., (YM1) and other was lower productivity. Based on the isolation and enzymatic productivity the selected bacterium was molecular characterized. The selected bacteria *Eschershia fergusonii* was mass multiplied and preparation of probiotic feed. A 45 day feeding experiment was conducted to measure the effects of yellow molly with intestinal bacteria (*Escherichia* sp.,) brewer's yeast (*Saccharomyces cervisiae)* and control feed. Six experimental feeds such as F1 control (without probiotic), F2 (1 ml of *Escherichia* sp.,) F3 (2ml of *Escherichia* sp.,) F4 (3ml of *Escherichia* sp.,) F5 (4ml of *Escherichia* sp.,) and F6 (1ml of yeast) were prepared and given to yellow molly. From the results, the growth performance like feed consumption, feed conversion efficiency, Growth, percentage growth, relative growth, gross growth efficiency and net growth efficiency was higher in feed V and lower in Feed I (without probiotic feed).

INTRODUCTION

Among aquaculture techniques, ornamental fish keeping is one of the most popular hobbies of the world. The growing interest in aquarium fishes has resulted in steady increase in aquarium fish trade globally. Ornamental fish trade is economically important and profitable (Das and Kavita 2003). The prosperity of the ornamental fish industry has induced the indiscriminate use of antibiotics, which has led to the development of drug - resistant strains of pathogenic microorganisms (Amabile – Cuevas *et al.,* 1995). Fish receive pathogenic bacteria from the aquatic environment through water, food and are populated with bacteria. Being rich in nutrient the environment of digestive tract of fish confers a favorable culture environment for the microorganisms. The enzymes producing intestinal bacteria their, source and significant in fish is scare. The relative amount of amylase, cellulase, lipase and protease producing bacteria in the gastrointestinal tract of fishes, intestinal isolates were evaluated for extra cellular enzyme producing capacities (Nibeditakar and Koushik Gosh 2008).

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Disease outbreak is being increasingly recognized as one of the most important constraints in aquaculture production in many countries including ornamental fish culture (Nicolas et al., 1989). So for conventional approaches such as use of disinfectants and anti microbial drugs, have had limited success in the prevention or cure of ornamental fish diseases. The probiotic microorganisms are beneficial and plays an important role with respect to the well being of fish (Trust and Sparrow 1974). The isolation, identification of intestinal bacteria of yellow molly and its probiotic effect on growth and survival is totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS

For the present study Yellow molly (*Poecilia latipinna*) were collected from Angel Aquarium, Dindigul, Tamil Nadu and transported to the laboratory in polythene bags filled with aerated water. Intestinal contents of the yellow molly was dissected out and gut contents were serial dilution and plated on nutrient agar media. Five bacteria colonies were identified. The enzymatic activity (Amylolytic, Cellulolytic, Lipolytic, and Proteolytic) of intestinal bacteria were identified from the culture plate using selective media.

And selected bacteria identified at the genus and species level. The isolated *E.coli. Spp.,* (10^6) cells was mass multiplied by inoculating them into the nutrient broth. Yellow molly fingerlings (3 ± 0.20 g) acclimated in glass aquaria (60×45×45) for a period of 10 days at 28 ± 2° C during acclimation fishes were fed with trainee feed containing fish meal, ground nut oil cake , wheat flour and rice bran in the form of dry pellets. One control (without probiotic feed), four experimental feeds by using commercially available probiont (yeast) was prepared according to square method (Ali. 1980). Composition of different ingredients in experimental feeds were given in Table 1.

Table 1. 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 (10^5Cells)	-	1ml	2ml	3ml	4ml	1ml Yeast

GNOC – Groundnut oil cake

Table 2. 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 - Escherichia sp., YM2 - Pseudomonas sp., YM3 - Staphylococcus sp., YM4 - Aeromonus sp., YM4 - Streptococcus sp., (+ - Low, ++ - High).

Table 3. Feed utilization and growth parameters of Yellow molly *Poecilia latipinna* in relation to different concentration of *Escherichia fergusonii* (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 (g/g live wt/30 days)	3.86 ± 1.40	4.0 ± 0.8	3.46 ± 0.47	4.04 ± 1.00	4.46 ± 0.36	3.86 ± 0.67
2	Feed Conversion efficiency	0.17 ± 0.03	0.17 ± 0.08	0.18 ± 0.06	0.11 ± 0.02	0.20 ± 0.07	0.15 ± 0.08
3	Feed conversion ratio	5.78 ± 0.95	6.84 ± 3.33	5.71 ± 2.13	9.43 ± 1.69	5.21 ± 1.56	7.58 ± 3.34
4	Growth (g/g live wt/ 30 days)	0.68 ± 0.25	0.67 ± 0.32	0.64 ± 0.14	0.30 ± 0.31	0.80 ± 0.19	0.59 ± 0.28
5	Percentage growth (%)	35.23 ± 9.00	38.70 ± 20.80	46.32 ± 12.53	16.43 ± 17.16	46.95 ± 5.26	37.54 ± 24.66
6	Relative growth rate	0.26 ± 0.11	0.33 ± 0.16	0.31 ± 0.07	0.15 ± 0.15	0.4 ± 0.09	0.29 ± 0.13
7	Assimilation	3.67 ± 1.46	3.83 ± 0.75	3.30 ± 0.45	4.30 ± 0.37	3.88 ± 1.02	3.69 ± 0.62
8	Metabolism	2.99 ± 1.26	3.16 ± 0.83	2.66 ± 0.57	4.01 ± 0.15	3.07 ± 1.04	3.1 ± 0.78
9	Gross growth efficiency (%)	17.54 ± 3.02	17.17 ± 8..27	18.99 ± 6.42	6.49 ± 6.28	20.63 ± 7.36	15.92 ± 9.38
10	Net growth efficiency (%)	18.67 ± 3.24	17.86 ± 8.5	19.97 ± 6.91	6.71 ± 6.48	21.59 ± 7.96	16.62 ± 9.63

Experimental Feed Preparation

The raw material is selected based on their ability to supply nutrients such as protein, carbohydrates and fat at low cast. After knowing the protein content by Micro-Kjeldahl method (Jeyaraman, 1992). The components used for feed preparation was dried, powdered and sieved through 425 micron sieve. The ingredients were weighed and mixed thoroughly with 130 - 150 ml of distilled water. The mixed feed stuff was put in autoclave for 15 min at 100° C and cooled. After cooling, fish oil, sunflower oil, supplevite - mix, sodium chloride, sodium benzoate and different quantity of bacteria (1, 2, 3, 4 ml and 1ml yeast) were mixed with the feed. And then it was extruded with the help of Pelletizer. The pellets were dried in room temperature. This formulated feed was kept in air tight container at -20° C until used to prevent contamination. Feed utilization parameters were calculated at the end of 45 days. The experimental results of analytical variance (ANOVA) using Microsoft Excel (version 2007). One way ANOVA method was used for the analysis using DMRT (version 2005) according to (Sendecor & Cochran 1961). The data was growth parameters of feed consumption was 0.950 NS, growth was 0.271 NS, gross growth efficiency was 0.265 NS and net growth efficiency was 0.266 NS.

RESULTS AND DISCUSSION

Five distinct colonies were isolated from the intestinal content of yellow molly. The isolated colonies are YM1 (*E.coli spp.,*), YM2 (*Streptococci spp.,*), YM3 (*Pseudomonas spp.,*), YM4 (*Staphylococcus spp.,*) and YM5 (*Aeromonous spp.,*). *Escherichia coli* is commonly present in aquatic system and usually these organisms are not pathogens. The other organisms isolated such as *Aeromonous*, *Pseudomonas*, and *Enterococcus* act as a causative agents of bacterial diseases. Lewbert, (1998). The enzymatic productivity of intestinal bacteria was given table 2. The distinct microbial source of the digestive enzymes amylase, cellulase, lipase and protease.

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ORIGIN
        1 gagtggcgga cgggtgagta atgtctggga aactgcctga tggaggggga taactactgg
       61 aaacggtagc taataccgca taacgtctca agaccaaaga gggggacctt cgggcctctt
      121 gccatcggat gtgcccatat gggattagct agtaggtggg gtaacggctc acctaggcga
      181 cgatccctag ctggtctgag aggatgacca gccacactgg aactgagaca cggtccagac
      241 tcctacggga ggcagcagtg gggaatattg cacaatgggc gcaagcctga tgcagccatg
      301 ccgcgtgtat gaagaaggcc ttcgggttgt aaagtacttt cggcggggag gaagggagta
      361 aagttaatac ctttgctcat tgacgttacc cgcagaagaa gcaccggcta actccgtgcc
      421 agcagccgcg gtaatacgga gggtgcaagc gttaatcgga attactgggc gtaaagcgca
      481 cgcaggcggt ttgtaaagtc agatgtgaaa tccccgggct caacctggga actgcatctg
      541 atactgccaa gcttgagtct cgtagagggg ggtagaattc caggtgtagc ggtgaaatgc
      601 gtagagatct ggaggaatac cggtggcgag ggcggccccc tgggacgaag actgacgctc
      661 atgtgcgaaa gcgtggggag caaacaggat tagataccct tggtagtcca cgccgtaaac
      721 gatgtcgact tggaggttgt gccgttgagg cgtggcttcc ggagctaacg tgttaagtcg
      781 accgcctgcg ggagtacggc cgcaaggtta aaactcaaat gaattgacgg gggcccgcac
      841 aagcggtgga gcatgtggtt taattcgatg caacgcgaag aaccttacct ggtcttgaca
      901 tccacagaac tttccagaga tggattggtg ccttcgggaa ctgtgagaca ggtgctgcat
      961 ggctgtcgtc agctcgtgtt gtgaaatgtt gggttaagtc ccgcaacgag cgcaaccctt
     1021 atcctttgtt gccagcggtc cggccgggaa ctcaaaggag actgccagtg ataaactgga
     1081 ggaaggtggg gatgacgtca agtcatcatg gcccttacga ccagggctac acacgtgcta
     1141 caatggcgca tacaaagaga agcgacctcg cgagagcaag cggacctcat aaagtgcgtc
     1201 gtagtccgga ttggagtctg caactcgact ccatgaagtc ggaatcgcta gtaatcgtgg
     1261 atcagaatgc cacggtgaat acgttcccgg gccttgtaca caccgcccgt cacaccattt
     1321 tttgggttgc aaaagaagta ggtagcttaa c
//
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Fig. 1. Genetic Code (Sequence) of (*Escherichia fergusonii*) isolated from intestinal content of yellow molly

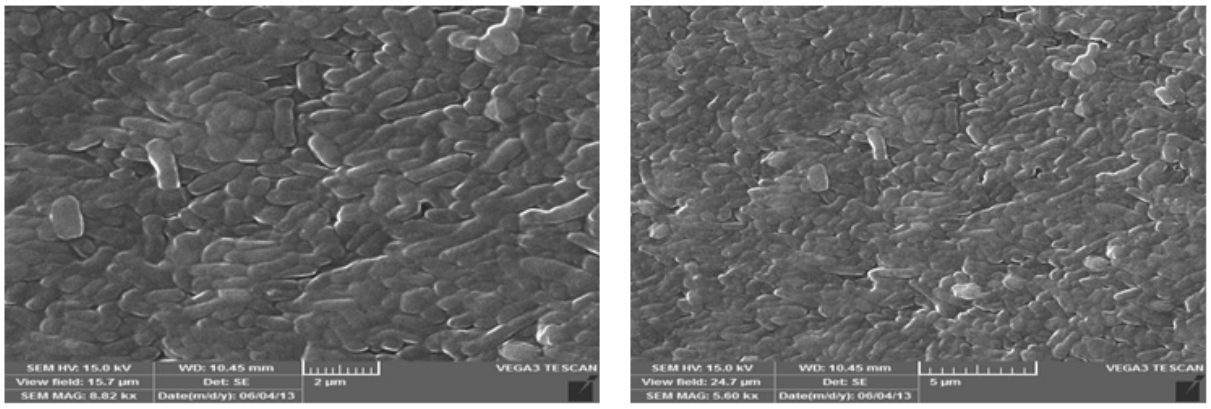

Fig 2. Morphological structure of Escherichia fergusonii isolated from intestinal content of yellow molly using scanning electron micrography

Apart from endogenous sources in fish gut. Abhinanda Bairagi *et al.,* (2002). The partial sequence of the 16s r RNA genetic code for the bacteria isolated from the intestinal content of yellow molly *Poecilia latipinna* given fig.1. Somerita Panda *et al.,* (2013) studied the characterization of pseudomonas aeruginosa isolated from Labeo bata by 16s r RNA gene sequence analysis. The morphological structure of bacteria were examined by the scanning electro micrograph, target bacteria cells of *Shigella sonnei* is given Fig 2.

The average initial condition factor of yellow molly was 1.84 and the final condition factor increased in feed V (2.65) and in all other condition factor decreased. Rajan and Revathi (2011) reported similar condition factor when platy fish was fed with *Bacillus subtitles*. The different feed utilization and growth parameters given table 3. Feed consumption , feed conversion efficiency and growth of yellow molly was higher in feed V containing 4 ml of Shigella sonnei. Gastesoup (1994) recorded improved growth rate of the fish larvae turbot treated with probiotics. Browdy 1998) demonstrated that the probiotic effect of bacterial mixture of feed to the higher growth several authors reported such higher growth in different fishes. Such as catla, Koi carp and Rainbow trout Parthasarthy and Ravi (2011), Dhanraj *et al.,* (2010) and Bagheri *et al.,* 2008). The percentage growth and relative growth rate of yellow molly was higher in feed V. Sreenevasan *et al.,* (2012) reported that the relative growth rate of fresh water prawn was increased when fed with *Bacillus subtilis.* Assimilation and metabolism of yellow molly was higher in feed IV and lower in feed III. Same result was reported Chandra and Rajan (2009) in koi carp. Gross and Net growth efficiency of yellow molly was higher in feed V and lower in feed IV.

REFERENCES

Abhinanda Bairagi, Keka Sarkar Ghosh., Sukanta Kumar Sen and Arun Kumar Ray 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International, 10: 109 – 121.

Ali, S.A. 1980. Feed formulation method. Manual of research methods for fin fish and shell fish nutrition , CMFRI special publication. No. 8: 98.

Amabile – Cuevas, C.F., M.Gardnenas – Garcia and M.Ludgear, 1995. Antibiotic resistance. Am.Sci., 83: 320 – 329.

Bagheri, T., Hedayati, S., Yavari, V., Alizade in and farzanfar, A. 2008 Growth , survival and gut microbial load of rainbow trout fry given diet supplement with probiotics. *J.Fish Aquat. Sci*., 8: 43 – 48.

Browdy, C. 1998. The Probiotic effect of bacterial mixture in Rainbow trout, Aquaculture, 164: 3 – 21.

Chandra, R. and Rajan, M.R. 2009. Proiotics effect of industrial bacteria of koi carp *Cyprinus carpio* var. koi. *J. of Pure and Applied Microbiology*, 3 (1) : 363 – 366.

Das, S.K. and Kalita, N. 2003. Captive Breeding of Peacock Eel, *Macrognathus aculeatus* and *Labeo rohita. (Hamilton)*, Aquaculture, Asia, July – Sep Vol.VII,No.3:17.

Dhanaraj, M., Haniffaa, M.A., Arun Singha, S.V. Jesu., Arockiaraj B.A., Muthu Ramakrishnan,C., Seethramana,S., and Arthimanju, R. 2010. Effect of Probiotics on growth performance of Koi carp *(Cyprinus Carpio) J. Appl. Aquaculture*, 22: 202 – 208.

Gatesoupe, F.J. 1994. Lactic acid bacteria the resistance of Turbot larvae, *Scophthalmus maximus*, against Pathogenic vibrio, Aquat., Living Resour.,7: 277- 282.

Jayaraman, J. 1992. Laboratory manual in biochemistry ,Wiley eastern Ltd., New Delhi , Fourth reprint : pp 75 -78.

Lewbert, G.A. 1998. Self - Assessment colour review of ornamental fish. Lowa State University Press. pp: 192.

NibeditaKar and Koushik Ghosh 2008 Enzyme producing bacteria in the gastrointestinal tracts of *Labeo rohita (Hamilton)* and *Channa punctatus* (Bloch).Turkish Journal of Fisheries and Aquatic Science, 8 : 115 - 120.

Nicolas, J.L., Robic, E.and Ansquer, D. 1989. Bacterial Flora associate with a tropic chain consisting of micro – algae, rotifers and turbot larvae influence of bacteria on larval survival. Aquaculture, 83: 237 – 248.

Parthasarathy, R. and Ravi D. 2011. Probiotic bacteria as growth promoter and biocontol agent against *Aeromonas hydrophila* in *Catla catla* .Indian *J.Fish*., 58 : 87 – 93.

Rajan, M.R. and U. Revathi., 2011 Role of Probiotics in Ornamental fish Platy *Xiphophorus maculates*. Journal of pure and Applied Microbiology, 5 (2) : 819 – 823.

Sendecor G.W, and Cochran, G. 1961. Statistical methods Oxford and IBH Publishing New Delhi, India, pp. 593.

Somerita Panda, P.K. Bandyopadhyay and S.N. Chatterjee 2013. Chacteriazation of Pseudomonas aeruginosa PB112 (JN996498) isolated from infected Labeo bata (Hamilton) by 16S rRNA gene sequence analysis and fattiacid methyl ester (FAME) analysis. African journal of Biotechnology Vol.12 (40) pp: 400 – 405.

Srinivasan, C., Saravana Bhavan, P., Radhakrishnan, S. and Muralisankar, T. 2012. Effects of Probiotics on survival, growth and biochemical constituents of fresh water prawn *Macrobcterium resenbergi* post larvae. *Turkish J.of Fish and Aquatic sci.,* 12: 331 – 336.

Trust, T.J. and Sparrow R.A.H. 1974. The bacterial flora in the alimentary tract of freshwater Salmonid fishes. *Canadian journal of Microbiology*, 20: 1219 -1228.

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