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Effect of Dietary Probiotics Isolated from the Gut of Tilapia, *Oreochromis mossambicus* (Peters, 1852) on its Growth Performance and Digestive Enzyme Activities

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Abstract: A 60 days experiment was conducted to determine the effect of different doses of two probiotics on growth parameters and digestive enzyme activities of tilapia, *Oreochromis mossambicus*. Tilapia were fed with probiotics *Lactobacillus plantarum* and *Bacillus coagulans* isolated from the gut of tilapia, at concentrations of 1×10^2 CFU g⁻¹, 1×10^4 CFU g⁻¹, 1×10^6 CFU g⁻¹, 1×10^8 CFU g⁻¹ and the probiotic bacteria fed separately. The growth parameters such as %weight gain, specific growth rate (SGR), feed conversion ratio and feed efficiency and the digestive enzyme assays such as amylase, protease and lipase were measured. During the 60 days experiment all the parameters were measured at 15 days intervals. The results show significant improvement in digestive enzyme activities and growth parameters.

Keywords: Probiotics, *Lactobacillus plantarum*, *Bacillus coagulans*, Growth rate, Digestive enzymes, *Oreochromis mossambicus*, Tilapia

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Introduction

Aquaculture is a rapidly growing sector as it plays an important role to achieve global protein food demand and is the major animal protein source for the world human population. Culture systems are being

intensified to meet the increasing demand and to improve the yield and profitability ratios. In the past decade, probiotics have received a lot of attention as a strategy towards enhancing performance and health

management in aquaculture systems (Ringo and Song, 2016; Sayes *et al.*, 2018). Antimicrobials and feed additives are used to enhance the health status of cultured organisms and to avoid, reduce or treat diseases and disease outbreaks (Rico *et al.*, 2013). Administration of antibiotics has been the most extensively used tool to control bacterial infections in aquaculture but indiscriminate and unscientific use of it has led to a hoard of issues like alteration of gut symbiotic microflora, emergence of drug resistant bacteria, causing damages to aquatic ecosystem and other environmental problems resulting in new regulatory policies and restrictions imposed by regulators on the usage of antibiotics in several countries (FAO, 2003, Ibrahim, 2015).

An alternative and effective approach to antibiotic administration to livestock is the use of probiotics (Modesto *et al.*, 2009), which is outlined as a live, dead or element of a microbial cell that once administered via the feed or the rearing water influences the host by increasing disease resistance, feed optimization, growth performance, tolerance response, and health standards, which are probably achieved via rising the microbial balance of the hosts than the close surroundings (Martínez Cruz *et al.*, 2012). Probiotics gained attention in aquaculture after its successes in human and animal feeding practices. It has beneficial effects in disease control and competes with various environmental stressors as well as promotes the growth of the cultured organisms by improving feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, anti-mutagenic, anti-carcinogenic activity, and increases immune

responses. These probiotics are harmless bacteria that help the host animal and contribute, directly or indirectly to protect the host animal against harmful pathogens (Pandiyan *et al.*, 2013). A wide range of micro-organisms including Gram-negative and Gram-positive bacteria have been used as probiotics in aquaculture such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pseudomonas*, *Enterococcus*, *Carnobacterium*, *Shewanella*, *Bacillus*, *Aeromonas*, *Vibrio*, *Enterobacter*, *Clostridium*, and *Saccharomyces* species (Nayak, 2010). The present study has made an attempt to evaluate the effects of probiotic bacterial strains isolated from the gut of tilapia on the growth performance and the digestive enzyme activities in tilapia, *Oreochromis mossambicus*. Tilapia is the second most farmed fish world-wide and its production has quadrupled over the past decade because of its marketability, suitability for aquaculture, and stable market prices (Wang and Lu, 2016).

Materials and Methods

The fry of tilapia, *Oreochromis mossambicus* was purchased from Aqua fish farm, Kottakkal, Malappuram, Kerala, India. Average length (standard length) and weight of the fishes used for the study was 3.45 ± 0.61 cm and 1.75 ± 0.54 g respectively. The fish were acclimatized for one week before conducting the experiment. During the adaptation period, the fish were fed twice a day with pellet without the addition of probiotics at a dose of 3% body weight. After acclimatization process, the fishes were distributed into aquaria with a capacity of 20 liters. Twenty fish each were kept per aquaria and each experiment had three replications.

Probiotics strains and culture conditions

Bacillus coagulans and *Lactobacillus plantarum* used in this study were isolated from gut of *O. mossambicus* and identified based on the phenotypic characteristics and 16S rRNA gene sequencing. They were grown at 37 C for 24 h in MRS broth. The pellets were washed once, and then resuspended in sterile 0.85% NaCl solution. The number of the bacterial cells in the suspension was determined by MRS agar plate count method (Zheng *et al.*, 2018).

Diet preparation

Diet procedure of Jana *et al.* (2012) and Rani and Rani (2016) was used with slight modification to prepare the experimental diets. Groundnut oilcake 30%, rice bran 25%, soya bean 23%, fish meal 20%, tapioca flour 1% and vitamin mineral mix 1% was used for the preparation of basal diet. Primarily, full fat soybean was autoclaved at 121 C at 15 lbs to reduce the level of heat-labile antinutritional factors (Garg *et al.*, 2002) and pelleted. Probiotic supplemented feeds are prepared by the addition of *Bacillus coagulans* and *Lactobacillus plantarum* separately at different levels such as 1×10^2 , 1×10^4 , 1×10^6 , 1×10^8 cfu/g per diet. The growth parameters measured were per cent weight gain, specific growth rate, feed conversion ratio and feed efficiency and digestive enzyme assays such as amylase, protease and lipase. All the parameters were measured for a duration of 15 days (DG 15), 30 days (DG 30), 45 days (DG 45) and 60 days (DG 60) separately.

Estimation of growth performance

At the end of each feeding trial, fish in each aquarium were individually weighed and growth performances were calculated

according to Sevier *et al.*, (2000) and Dawood *et al.* (2016) using the following equations:

$$\text{Per cent weight gain} = \frac{\text{Final weight} - \text{initial weight}}{\text{Final weight}} \times 100$$

$$\text{Specific growth rate} = \frac{\text{Final weight} - \text{initial weight}}{\text{Experimental period in days}} \times 100$$

$$\text{Feed conversion rate} = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}}$$

$$\text{Feed efficiency} = \frac{\text{Weight gain (g)}}{\text{Feed intake (g)}}$$

Digestive enzyme analysis

Amylase enzyme activity was assayed by following the procedure of Bernfeld (1955) where one unit of enzyme activity represents the amount of enzyme required to release one μg of maltose per minute during assay conditions. Protease activity was measured following the procedure of Walter *et al.* (1984), with casein as substrate. Lipase assay was measured following the method described by Bier (1955) with olive oil as substrate.

Data analysis

For statistical analyses, one-way analysis of variance (ANOVA) was used to evaluate the effect of probiotics supplementation. Significance was assigned at the 0.05% level. All statistical analyses were performed using Statistical Package for Social Science (SPSS, Armonk, NY, USA) software. Results were reported as mean $\pm$ standard deviation (SD) and group means were compared.

Results

The effects of probiotic supplement with *B. coagulans* and *L. plantarum* on per cent weight gain, SGR, FCR and feed efficiency of

Table 1: Growth performance parameters of *Oreochromis mossambicus* fed on diets supplemented with *Bacillus coagulans* at different probiotic levels

Duration of Expt.	Conc. of probiotics	% weight gain	Specific growth rate (SGR)	Feed conversion ratio (FCR)	Feed efficiency (FE)
15 days	Control	6.609±0.17	0.103±0.15	8.478±0.15	7.503±0.26
	10 ²	7.420±0.19	0.193±0.16	7.486±0.13	8.185±0.16
	10 ⁴	11.938±0.16	0.227±0.14	4.426±0.16	10.192±0.14
	10 ⁶	11.955±0.64	0.321±0.17	3.759±0.17	11.231±0.18
	10 ⁸	13.763±0.16	0.355±0.15	3.729±0.15	11.389±0.15
30day	Control	6.523±0.45	0.111±0.24	8.598±0.12	6.912±0.24
	10 ²	8.487±0.63	0.215±0.16	6.470±0.15	8.716±0.37
	10 ⁴	9.728±0.39	0.241±0.41	5.568±0.14	9.996±0.37
	10 ⁶	11.362±0.37	0.359±0.57	4.681±0.13	11.512±0.51
	10 ⁸	13.860±0.19	0.376±0.37	4.419±0.15	11.932±0.64
45 days	Control	7.538±0.69	0.105±0.54	7.359±0.12	7.463±0.75
	10 ²	9.295±0.61	0.230±0.44	5.855±0.15	8.774±0.67
	10 ⁴	13.838±0.63	0.364±0.54	3.736±0.14	10.854±0.45
	10 ⁶	15.812±0.43	0.377±0.53	3.527±0.15	10.718±0.67
	10 ⁸	16.183±0.36	0.397±0.60	3.195±0.14	10.870±0.69
60 days	Control	8.551±0.50	0.064±0.62	6.417±0.15	7.870±0.14
	10 ²	11.213±0.41	0.242±0.53	4.751±0.12	9.7012±0.46
	10 ⁴	13.315±0.62	0.400±0.67	3.906±0.14	13.131±0.46
	10 ⁶	16.971±0.71	0.417±0.49	3.695±0.15	14.562±0.52
	10 ⁸	17.539±0.96	0.450±0.63	3.108±0.12	14.565±0.54

tilapia are provided in Tables 1 and 2, respectively. During feeding periods no death was observed. The highest values of SGR 0.450±0.63 and 1.177±0.12 were observed in fish fed with 1×10^8 CFU g⁻¹ *B. coagulans* and 1×10^8 CFU g⁻¹ *L. plantarum* per diet, respectively. The growth parameters in fish such as per cent weight gain, Specific growth rate and Feed conversion ratio were observed at various probiotics concentrations from 10² to 10⁸ and ranged from 7.486 to 3.729, 8.598 to 4.419, 5.855 to 3.195 and 4.751 to 3.108 for DG15, DG30, DG45 and DG60, respectively. In control feed conservation

ratio observed was 8.478, 8.598, 7.359 and 6.417 for DG15, DG30, DG45 and DG60, respectively. For the group of 15 days, 30 days, 45 days and 60 days administration of *Bacillus coagulans* as feed supplemented probiotics at different concentration, growth parameters such as per cent weight gain (% WG), Specific growth rate (SGR), Feed conversion ratio (FCR) and Feed efficiency increased significantly (P<0.05) (Table 1).

Growth parameters such as per cent weight gain, Specific growth rate, Feed conversion ratio and Feed efficiency were also

Table 2: Growth performance parameters of *Oreochromis mossambicus* fed on diets supplemented with *Lactobacillus plantarum* at different probiotic levels

Duration of Expt.	Conc. of probiotics	% weight gain (%WG)	Specific growth rate (SGR)	Feed conversion ratio (FCR)	Feed efficiency (FE)
15 days	Control	1.539±0.15	0.107±0.18	7.107±0.05	2.606±0.15
	10 ²	2.859±0.16	0.126±0.15	6.071±0.06	4.906±0.16
	10 ⁴	3.352±0.14	0.202±0.17	6.047±0.05	5.780±0.14
	10 ⁶	4.695±0.17	0.240±0.19	5.802±0.04	8.211±0.17
	10 ⁸	5.179±0.15	0.246±0.12	5.068±0.09	9.104±0.15
30 days	Control	3.269±0.16	0.450±0.36	9.379±0.08	5.633±0.19
	10 ²	6.250±0.23	0.591±0.43	8.526±0.05	11.111±0.26
	10 ⁴	6.986±0.42	0.682±0.47	7.989±0.04	12.518±0.36
	10 ⁶	10.207±0.67	0.804±0.34	5.278±0.06	18.946±0.61
	10 ⁸	10.672±0.39	0.849±0.51	5.022±0.05	19.912±0.62
45 days	Control	4.617±0.15	0.523±0.67	9.965±0.08	8.068±0.62
	10 ²	9.820±0.43	0.650±0.46	5.510±0.08	18.148±0.35
	10 ⁴	15.123±0.53	0.993±0.65	3.367±0.06	29.696±0.47
	10 ⁶	15.613±0.49	1.003±0.69	3.243±0.05	30.837±0.55
	10 ⁸	16.377±0.61	1.047±0.15	3.064±0.06	32.640±0.59
60 days	Control	3.771±0.50	0.596±0.12	9.211±0.05	6.532±0.42
	10 ²	13.520±0.51	0.793±0.62	4.433±0.04	26.056±0.64
	10 ⁴	21.358±0.62	0.953±0.51	2.209±0.08	45.265±0.67
	10 ⁶	22.131±0.45	1.147±0.56	2.111±0.07	47.367±0.42
	10 ⁸	23.684±0.52	1.177±0.12	3.838±0.05	51.724±0.69

Fig. 1: Amylase activities of *O. mossambicus* fed on diets supplemented with *B. coagulans* at different probiotic levels. Asterisk indicates significance as compared with control.

Fig. 2: Protease activities of *O. mossambicus* fed on diets supplemented with *B. coagulans* at different probiotic levels. Asterisk indicates significance as compared with control.

Fig. 3: Lipase activities of *O. mossambicus* fed on diets supplemented with *B. coagulans* at different probiotic levels. Asterisk indicates significance as compared with control.

Fig. 4: Amylase activities of *O. mossambicus* fed on diets supplemented with *L. plantarum* at different probiotic levels. Asterisk indicates significance as compared with control.

Fig. 5: Protease activities of *O. mossambicus* fed on diets supplemented with *L. plantarum* at different probiotic levels. Asterisk indicates significance as compared with control.

Fig. 6: Lipase activities of *O. mossambicus* fed on diets supplemented with *L. plantarum* at different probiotic levels. Asterisk indicates significance as compared with control.

increased significantly ($P < 0.05$) when compared to the control after administration of *Lactobacillus plantarum* as feed supplemented probiotics at different concentrations (Table 2). All the growth parameters increased proportionate to the concentration of probiotics per diet and duration of administration of probiotics. After the fish were fed with *L. plantarum* for DG60, the high per cent weight gain (%WG), SGR, FCR and FE were observed in fish at the concentration 10^8 cfu/ml with values 23.684 ± 0.52 , 1.177 ± 0.12 , 3.838 ± 0.05 , 51.724 ± 0.69 , whereas fish fed with *B. coagulans* at the concentration 10^8 cfu/ml for

DG60 produced highest values of (%WG), SGR, FCR and FE depicting 17.539 ± 0.96 , 0.450 ± 0.63 , 3.108 ± 0.12 , 14.565 ± 0.54 , respectively. The feed conversion ratio and Feed efficiency were found to increase significantly in fish fed with probiotic supplemented diet when compared to the control.

The digestive enzyme activities of amylase, protease and lipase in control fish and fed with probiotic supplemented diets such as *B. coagulans* and *L. plantarum* for the duration of 15 days, 30 days, 45 days and 60 days were provided in Figures 1-6. Highest activity of amylase, protease and lipase was observed in

fish fed with probiotic supplemented diet when compared to the control. Both probiotics, *B. coagulans* and *L. plantarum* could enhance the activity of digestive enzyme after 60 days of feeding experiment.

Discussion

Probiotics have been extensively researched, and their effects on the growth and health of aquatic animals have been verified in recent years (Chauhan and Singh, 2019) and the application of bacteria for their potential benefit as probiotics in aquaculture is increasing (Balcázar *et al.*, 2006; Gatesoupe, 2007). The present study shows significant increase in growth parameters in fish fed with *Bacillus coagulans* (1×10^8 cfu/g) and *Lactobacillus plantarum* (1×10^8 cfu/g) as probiotic feed supplement. The growth parameters in experimental fishes are influenced by the concentration of probiotics in feed and duration of probiotic administration. Highest values of SGR were observed in fishes fed with high concentration of probiotics per feed and after 60 days duration of feeding experiment. The observations of the present study are in agreement with several authors. Aly *et al.* (2008) evaluated the potential benefit of *Bacillus pumilus* as a probiotic in the culture of tilapia (*O. niloticus*) and showed that a low dose of *Bacillus pumilus* (10^6 g⁻¹ diet fed) induced significant increases in weight gain when used for 2 months. Wang and Xu (2006) found that the addition of probiotics (1 g/kg photosynthetic bacteria and 1 g/kg *Bacillus* sp. isolated from the pond of common carp *Cyprinus carpio*) in carp basal diets improved growth performances, feed conversion ratio and digestive enzyme activities. A similar finding was obtained by Taoka *et al.* (2006), who investigated the effect

of commercial probiotics (containing *Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum*, and *Saccharomyces cerevisiae*) on growth of Japanese flounder (*Paralichthys olivaceus*) and showed that addition of probiotics to diet or rearing water resulted in enhanced growth of the flounder. He *et al.* (2013) evaluated the effects of low doses of dietary *Bacillus subtilis* C-3102 on the production and the results showed that the administration of low-level (105 CFU/g) *Bacillus subtilis* C-3102 for at least 28 days ensured the expression of beneficial host genes and healthy intestinal microbiota. The beneficial effects of *Lactobacillus* sp on growth response have been observed in Nile tilapia by Lara-Flores *et al.* (2003). According to Balcázar *et al.* (2006), probiotic microorganisms are able to colonize gastrointestinal tract when administered over a long period of time because they have a higher multiplication rate than the rate of expulsion, so that probiotics are constantly added to fish cultures, they adhere to the intestinal mucosa of fish, developing and exercising their multiple benefits.

Balcázar *et al.* (2006) has suggested that the probiotic strains synthesize extracellular enzymes such as proteases, amylases, and lipases as well as provide growth factors such as vitamins, fatty acids, and amino acids. Haroun *et al.* (2006) observed that nutrients are absorbed more efficiently when the feed is supplemented with probiotics. The present study also observed increased enzyme activities of amylase, protease and lipase in fishes fed with the probiotic supplemented diet. The enzyme activities in experimental groups fed with different concentrations of probiotics (1×10^2 CFU g⁻¹, 1×10^4 CFU g⁻¹, 1×10^6 CFU g⁻¹ and 1×10^8 CFU g⁻¹), for the

duration of 60 days of feeding shows significant increase in activities when compared to the control ($P < 0.05$). According to Moriarty (1996), *Bacillus* secretes a wide range of exoenzymes that complement the activities of fish and increases enzymatic digestion. In fact, the bacteria isolated from the digestive tract of aquatic animals have shown chitinases, proteases, cellulases, lipases, and trypsin (Vine, 2006). In guppies (*Poecilia reticulata*, *Poecilia sphenops*) and swordtail (*Xiphophorus helleri*, *Xiphophorus maculatus*), evaluation of the effect of incorporating *Bacillus subtilis* isolated from the intestine of *Cirrhinus mrigala* into their diet showed an increase in the length and weight of the ornamental fishes as well as the specific activity of proteases and amylases in the digestive tract (Ghosh *et al.*, 2008). The administration of *Bacillus* sp. in fish culture increased lipase, protease and amylase activity of fish after the colonization in the gastro-intestinal tract (Buruaina *et al.*, 2014). The improvement of feed utilization for fish fed diet supplemented with *L. plantarum* NIOFSD018 could be due to the improvement in intestinal microbial flora balance which in turn may be leading to a better nutrient digestibility, higher-absorption quality, increased enzyme activities (Tovar-Ramirez *et al.*, 2002; Lara-Flores *et al.*, 2003; Waché *et al.*, 2006; Al-Dohail *et al.*, 2009) and also more degradation of higher molecular weight protein to lower molecular weight peptides and amino acids (De Schrijver and Ollevier, 2000). There is a significant difference in the growth parameters induced by *Bacillus coagulans* and *Lactobacillus plantarum* which is an indication that the kind of probiotic used is one of the factors promoting growth performance of tilapia.

Conclusion

Dietary supplement of probiotic bacteria stimulated the digestive enzyme synthesis in the gastrointestinal tract of the tilapia, especially with administration of high concentration of bacterial cells, suggesting that the concentration and duration of administration are key-factors. An enhanced activity of lipolytic enzymes has been found in *Bacillus* fed groups. Majority of probiotics are capable of secreting lipase, which triggers production and assimilation of essential fatty acids resulting in higher growth and immunity in fish, *Oreochromis mossambicus*.

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