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Role of Probiotics in Ornamental Fish Platy *Xiphophorus Maculatus*

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The present study deals with the role of probiotics in ornamental fish platy *Xiphophorus maculates.* Two distinct colonies were isolated from the intestine of Platy through serial dilution technique and pour plate method. Two distinct colonies were isolated from the intestine of Platy through serial dilution technique and pour plate method. The isolated colony was identified by using biochemical tests and identified as *Bacillus subtilis.* The identified microbe *Bacillus subtilis* was mass multiplied by using nutrient broth. Five different feeds having different concentration of Bacillus subtilis such as Ex. Feed I (Control), Ex. Feed II (10^1 cells), Ex. Feed III (10^2 cells), Ex. Feed IV (10^3 cells) and Ex. Feed V (10^4 cells) were prepared by using fish meal, ground nut oil cake, wheat flour, tapioca flour, fish oil, sunflower oil, supplevite- mix, sodium chloride and sodium benzoate. Role of microbes on feed utilization parameters such as feed consumption, feed conversion efficiency, feed conversion ratio, growth, percentage growth, relative growth rate, assimilation, metabolism, net and growth efficiency of Platy were studied for a period of 30 days. Feed consumption was higher in Ex. Feed V containing 10^4 cells of *Bacillus subtilis*. The growths, percentage growth, relative growth rate, gross and net growth efficiency was also higher in Ex. Feed V containing higher cells of *Bacillus subtilis*. Assimilation and metabolism were also higher in Ex. Feed V. From the results, it is inferred that the Ex. Feed V was suitable for the growth of platy.

Key words: Probiotics, Ornamental fish, Platy, *Xiphophorus maculates*.

In ornamental fish culture disease outbreaks are being increasingly recognized as a significant constraint on production and trade affecting the economic development of the sector in many countries. Conventional approaches such as the use of disinfectants and antimicrobial drugs, have had limited success in the prevention or cure of aquatic diseases. Further there is a growing concern about the use and particularly, the abuse of antimicrobial drugs not only in human medicine and agriculture but also in aquaculture.

The massive use of antimicrobials for disease control and growth promotion in animals increases the selective presence of excerted on the microbial world and encourages the natural emergence of bacterial resistance. Among diseases, bacterial infections are considered as the major cause of mortality in fish hatcheries. The prophylactic and therapeutic control of the bacterial diseases is based on oral administration of antibiotics. However, such treatment may cause the development of resistant bacteria (Aoki *et al*, 1985), yield residues in fish and introduction of potential hazard to public health and to the environment. Furthermore, the normal microbial

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flora in the digestive tract, which is beneficial to fish, may also be killed or inhibited due to oral chemotherapy(Sugita *et al*, 1996).Vaccines developed and marketed cannot be used as a universal disease control measure in aquaculture. A new method of great interest in the use of probiotic bacteria in aquaculture to improve disease resistance, water quality and or growth of farmed fish (Verschuere *et al*, 2000). Probiotics is a live microbial feed supplement which improves the intestinal microbial balance in favour of the host animal (Fuller, 1989). The work related to the isolation of intestinal micro flora of platy and the probiotic effect on growth and survival of platy is totally wanting. Hence the present study was carried out.

MATERIAL AND METHODS

Sample collection

For the present study red platy *Xiphophorus maculates* were collected from Aqua gardens, Madurai, Tamil Nadu, India and transported to the laboratory in polythene bags filled with water. Intestinal contents from the red platy were serially diluted with sterile water and plated on Nutrient agar. Plates were incubated at 37° C for 24 hours. Different incubation temperatures were used in order to obtain a wide range of isolation. The incubation time ranges from 1 to 3 weeks depending on the incubation temperature, colonies were counted and isolated. After incubation, bacterial colonies were invalid at random from each plate and examined for gram reaction, spore formation, cellular morphology and motility and identified at genus level. The isolation of bacteria was done by using spread plate technique and it was further identified by microscopic and biochemical tests such as indole, methyl red,voges – proskauer, citrate utilization and triplicate sugar iron agar. Finally the identified organisms were further confirmed by streaking them in specific media. The media employed were Nutrient agar medium and Nutrient broth.

Mass culture of isolated bacteria

The isolated bacteria was identified as Bacillus subtilis and mass multiplied by inoculating them in to the nutrient broth.

Selection of feed ingredients

The raw materials are selected based on their ability to supply nutrients such as protein, carbohydrate and fats at low cost. The ingredients used in the present study are fish meal, groundnut oil cake, wheat flour and tapioca. After knowing the protein content by micro- Jeldahl method (Jeyaraman, 1992) the feeds were prepared according to square method (Ali, 1980).

Experimental feed preparation

The components used for feed preparation are 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 minutes at 100ºC and cooled. After cooling, cultured Bacillus subtilis species in different cells such as 10^1, 10^2, 10^3 and 10^4 are 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 20º C until used to prevent contamination (Table 1).

METHODS

Experimental design for growth studies

For the present study Red Platy Xiphophorus maculates were collected from Angel Aqua Gardens, Madurai, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in plastic round aquaria(60 dia.) for 15 days. 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. After 15 days uniform size of red platy were selected. The length and weight of the fish were taken. Then the fishes were introduced in rectangular glass tanks (45 cm L X 22cm B x 22 cm H) having a capacity of 18 liters. During rearing the fishes were fed ad- libitum diet of prepared feed twice a day for 1 hour 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 faecal matter was collected daily before changing the water with least disturbance to the fishes and dried at 95º C. Approximately 70% of the water in the tank was replaced with tap water. The experiment was continued for 30 days and on the 39th day the fishes were weighed in live condition. Feed utilization

parameters such as condition factor, feed consumption, feed conversion efficiency, feed conversion ratio, growth, percentage growth rate, relative growth rate, assimilation, metabolism and gross and net growth efficiency were calculated. Statistical significance was tested at P> 0.05 probability level.

RESULTS AND DISCUSSION

The experimental work indicates that the isolated bacteria are identified as Bacillus subtilis. The fresh water fish harbour human pathogenic bacteria, including *Aeromonas* spp., *Staphylococcus faecalis*, *Salmonella* spp., and *Streptococcus* spp. in their intestine. Many workers have isolated bacteria from marine source, fish with probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (Gildberg *et al*, 1995). Condition factor (K) of platy *Xiphophorus maculates* was estimated for comparative purposes to assess the feed. The average initial and final condition factor is 2.26 and 2.55 respectively. The final condition factor is increased in all feeds. Suganthi (2009) reported that the final condition factor was increased in feed III containing 2 ml of bacteria in the feed while in other feeds the condition factor was decreased. Similar condition factor was reported when koi carp was fed with *Lactobacillus* to a level of 2 ml in the feed (Chandra & Rajan 2009).(Table 2).The feed utilization and growth parameters of platy were presented in Table 3. Feed consumption of Platy *Xiphophorus maculatus* is higher in Ex. Feed V containing 10^4 cells of *Bacillus subtilis*. The feed consumption of platy in Ex. Feed 1 (control), II, III, IV and V was 1.5, 1.53, 1.6, 1.7 and 1.73 respectively. Similar result was reported in Gold fish containing higher cells of *Pseudomonas* spp. (Selvi, 2005).Chandra & Rajan (2009) reported that the feed consumption of koi carp was higher in Ex. Feed V containing 4 ml of *Lactobacillus*. Feed Conversion Efficiency of platy was higher in Ex. Feed V. The feed conversion efficiency is gradually increased. But in the case of gold fish, feed conversion efficiency gradually decreased from lower to higher concentration of *Pseudomonas* spp. (Selvi, 2005). In black molly the feed conversion efficiency was higher in feed III containing 2 ml of *Staphylococcus* spp. (Suganthi, 2009). Feed Conversion Ratio of platy was best in Ex. Feed V. Same result was reported in gold fish, koi carp and black molly (Selvi, 2005., Chandra & Rajan, 2009 and Suganthi, 2009). Growth of platy was higher in Ex. Feed V (1.65). Same result was reported in gold fish (Selvi, 2005) But in the case of black molly, the growth was higher in control without any bacteria. Browdy (1998) demonstrated that the probiotic effect of bacterial mixture of feed leads to higher growth. Like growth, the percentage growth of platy was increased in Ex. Feed V. But the percentage growth was higher in koi carp fed with 1 ml of Lactobacillus spp (Chandra & Rajan, 2009).The relative growth rate of platy was increased in Ex. Feed V. The relative growth rate of black molly was decreased with increase in the level of *Staphylococcus* spp. in the feed (Suganthi, 2009). Assimilation and metabolism of platy was

Table 1. Composition of different ingredients in Experimental feeds (g /100g)

S. No.	Ingredients	Experimental Feeds				
		I (Control)	II	III	IV	V
1.	Fish meal	34.15	34.15	34.15	34.15	34.15
2.	Groundnut oil cake	34.14	34.14	34.14	34.14	34.14
3.	Wheat flour	10.85	10.85	10.85	10.85	10.85
4.	Tapioca	10.85	10.85	10.85	10.85	10.85
5.	Fish oil	2	2	2	2	2
6.	Sunflower oil	2	2	2	2	2
7.	Supplevite – mix	4	4	4	4	4
8.	Sodium chloride	1	1	1	1	1
9.	Sodium benzoate	1	1	1	1	1
10.	Microbes(cells)	-	10^1	10^2	10^3	10^4

Table 2. Condition factor (K)

S.No.	Feeds	Initial	Final
1.	Ex. Feed I (Control)	2.13 ± 0.195	2.33 ± 0.34
2.	Ex. Feed II (10^1 Cells)	2.15 ± 0.35	2.41 ± 0.40
3.	Ex. Feed III (10^2 Cells)	2.30 ± 0.40	2.50 ± 0.46
4.	Ex. Feed IV (10^3Cells)	2.30 ± 0.57	2.70 ± 0.64
5.	Ex. Feed V (10^4 Cells)	2.43 ± 0.59	2.83 ± 0.72

Table 3. Feed utilization parameters of Platy Xiphophorus maculatus in relation to different concentration of Bacillus subtilis. Each value is the average (± S.D) performance of 5 individuals in triplicates reared for 30 days

S. No.	Parameters	Experimental Feeds				
		I(Control)	II	III	IV	V
1.	Feed Consumption (FC)(g/g live wt/ 30 days)	1.50± 0.03 [a]	1.53 ± 0.05[b]	1.6 ± 0.06[c]	1.7 ± 0.07 [d]	1.73 ±0.08[e]
2.	Feed Conversion Efficiency (FCE)	0.14 ± 0.05	0.18 ± 0.07	0.20 ± 0.09	0.21 ± 0.10	0.24 ± 0.13
3.	Feed Conversion Ratio(FCR)	3.63 ± 0.04	4.13 ± 0.07	5.05 ± 0.25	5.72 ± 0.30	7.12 ± 0.80
4.	Growth(G)(g / g live wt/30 days	0.84 ± 0.01[a]	1.05 ± 0.02[b]	1.19 ± 0.04[c]	1.45 ± 0.06[d]	1.65 ± 0.07[e]
5.	Percentage Growth (PG)	22.10 ± 0.05	27.82 ± 0.05	31.49 ± 0.18	38.51 ± 1.03	39.69 ± 1.68
6.	Relative Growth Rate(RGR)	0.42 ± 0.02	0.52 ± 0.03	0.59 ± 0.05	0.72 ± 0.07	0.83 ± 0.08
7.	Assimilation (A)	0.85 ± 0.001	1.06 ± 0.04	1.20 ± 0.09	1.46 ± 0.23	1.66 ± 0.33
8.	Metabolism (M)	0.01 ± 0.001	0.012 ±0.005	0.014 ± 0.006	0.018 ±0.006	0.019 ± 0.006
9.	Gross Growth Efficiency(GGE) (%)	13.92 ± 0.13[a]	17.50 ±0.16[b]	18.52 ± 0.25[c]	21.32 ± 0.59[d]	24.26 ± 1.015[e]
10.	Net Growth Efficiency (NGE) (%)	98.81 ± 0.03[a]	99.05 ± 0.05[b]	99.16 ± 0.077[c]	99.30 ± 0.15[d]	99.39 ± 0.195[e]

Feed Consumption	Growth	Gross Growth Efficiency	Net Growth Efficiency
a Vs b (P > 0.05) S	a Vs b (P > 0.05) S	a Vs b (P > 0.05) S	a Vs b (P > 0.05) S
a Vs c (P > 0.05) S	a Vs c (P > 0.05) S	a Vs c (P > 0.05) S	a Vs c (P > 0.05) S
a Vs e (P > 0.05) S	a Vs d (P > 0.05) S	a Vs d (P > 0.05) S	a Vs d (P > 0.05) S
a Vs d (P > 0.05) S	a Vs e (P > 0.05) S	a Vs e (P > 0.05) S	a Vs e (P > 0.05) S

higher in Ex. Feed V (10^4 cells) and lower in Ex. Feed I (control). Same result was reported in koi carp (Chandra & Rajan, 2009). The gross and net growth efficiency was higher in Ex. Feed V and I. The gross and net growth efficiency gradually increased from Ex. Feed I to Ex. Feed V. Similar result was reported in black molly. From the results, it is inferred that the feed utilization parameters such as growth, percentage growth rate, relative growth rate, gross and net growth efficiency of platy was higher in Ex. Feed V containing higher cells of *Bacillus subtilis* (10^4 cells).

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