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Bacterial Load Associated with Culture of Ornamental Fishes in Glass Aquarium

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Abstract

The study was made to enumerate both the qualitative and quantitative bacterial load in ornamental fish culture systems. The samples for bacterial count were taken at an interval of five days from the water of aquariums in which four ornamental fishes viz. goldfish, guppy, molly and platy were reared and from the gut of cultured fishes. The experiment was conducted for a period of 45 days. The bacterial load in the gut were found to be higher than their culture water. The classification of the bacterial load revealed the abundance of G+ve rods in both culture water and fish gut throughout the culture period. The most remarkable feature evolved was the total absence of G-ve rods in the gut of all fish from about the day 25 of culture with the total dominance of G+ve rods.
Key words **: Bacterial load, Ornamental fish, Aquarium.**

Ornamental fish culture is having vast potential and market demand. The success of industry depends on availability of quality fish. To keep the ornamental fishes healthy, free from stress and disease aquarium management is a necessity. Over feeding as in aquaculture or a poorly managed aquarium accelerates the degradation of the water quality which leads to proliferation of diseases. It has been demonstrated in aquarium environments that bacterial populations change quantitatively and qualitatively both temporally and spatially within a aquarium. The bacterial populations in the aquarium can be divided into heterotrophs that use organic carbon. This can range from proteins and fats to simple sugars. Some produce enzymes to solubilize fats. Others produce enzymes to solubilize proteins. Still others are required to solubilize carbohydrates or cellulose. Many strains do not synthesize enzymes for solubilization of solids; these bacteria grow on products solubilized by others and (b) lithotrophs that use inorganic carbon. This group includes nitrifying bacteria which incorporate bicarbonate and convert it into cellular proteins and fats. The microbes most frequently encountered in aquarium culture water and culture fish belong to the genera *Aeromonas, Pseudomonas, Edwardsiella, Flavobacterium, Yersinia, Flexibacter, Cytophaga, Renibacterium, Mycobacterium, Streptococcus* and *Bacillus*.

The present study was conducted with the objective to enumerate the bacterial load in water of aquarium and gut of aquarium fish both quantitatively and qualitatively.

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Methods

The experiment was conducted for a period of six weeks from 12 April to 22 May, 2004 in the Aquarium Laboratory of Central Institute of Fisheries Education, Kolkata Center, Kolkata.

Experimental Set Up

The experiments were performed in glass aquaria each with a capacity of 50 liters. The glass aquaria were disinfected with bleaching powder, scrubbed with detergent powder, rinsed with fresh water and dried for a day or two. Clean, clear filtered and sterilized water were used to fill the aquarium tanks. Four types of aquarium fishes viz.,

Table 1. Nutrient agar (1). Sterilized by autoclaving at 121 C for 15 minutes.

Ingredients	
Peptic digest of animal tissue	5 g
Sodium chloride	10 g
Beef extract	1.5 g
Yeast extract	1.5 g
Agar	15 g
Distilled water	1,000 ml
pH	7.4±2

goldfish (*Carassius auratus*), guppy (*Poecilia reticulata*), black molly (*Poecilia sphenops*) and platy (*Xiphophorus maculatus*) were brought from a local market and aclimatised in filtered sterilized water for a fortnight before the start of the experiment. The fishes were stocked in the aquarium tanks @ 10 numbers/tank and fed twice daily with protein rich feed (protein 35%) @ 5% of body weight. Artificial aeration (air stone compressor) and filter (undergravel) were provided in all the aquarium tanks to maintain the physico chemical parameters of culture water at optimum level.

Bacteriological Media and Diluents

The bacteriological media used in the present study was nutrient agar (NA), which was procured from Hi-Media, Mumbai (India) (Table 1). One % sodium chloride solution (NaCl) (wt/vol) solution was used as diluent for the enumeration of bacteria.

Sterilization

The glasswares were sterilized in an oven at 180 C for I hour. Bacteriological media, diluents and autoclavable labwares were generally sterilized in an autoclave at 121 C (15 lbs) for 15 minutes, unless otherwise specified.

Sampling Procedure

The water samples were collected from each aquarium at regular intervals of five days in sterilized glass bottles of 250 ml capacity. As for the fish gut, the fishes were collected from the aquariums and the gut were taken out aseptically by dissecting the fish using sterilized scalpels and scissors. The gut contents were macerated and homogenized in mortar and pastle and suspended in sterile saline for enumeration.

Bacteriological Analyses

Total Heterotrophic Counts. Spread plate technique was followed for total heterotrophic bacterial enumeration. Water samples collected in sterile bottles were suitably diluted (10^{-4}) using 1% saline. Aliquots of 0.1 ml each of dilutions were spread on the surface of prepoured and dried NA plates. Inoculated plates were incubated at ambient temperature (30±2 C) for 48 hours and the colonies were counted.

The homogenized gut samples were suitably diluted upto 10^{-5} using 1% saline and bacterial enumeration was done by spread plate technique as for water samples.

Bacterial Classification by Gram Staining

The bacterial colonies were picked at random from culture plates and subjected to Gram staining for its identification. In Gram staining, a smear of the bacterial culture was air dried or heat fixed onto clean grease free glass slides. The slides were then flooded with Crystal violet stain (1) reagent for 1 minute. The slides after being washed in tap water were flooded with iodine mordant for 1 minute. The slides again after washing in direct tap water were immersed in decolouriser solution (95% ethanol) for 30 sec with gentle agitation. The smear after being made dry by blotting it in absorbent paper were immersed for 2 minutes in counter stain and then washed in tap water until no color appears in the washing water. The slides were then made dry by blotting it in absorbent paper and examined under microscope.

Statistical Analysis

Two way ANOVA was followed to test the significance of difference between the bacteriological parameters of water and fish gut according to the type of fish and days of culture (2).

Table 2. Total heterotrophic bacterial count/ml of water or/g of gut sample.

Tank		Days of culture								
		5	10	15	20	25	30	35	40	45
Goldfish	Water	2×10^5	4.3×10^5	1.5×10^5	6.7×10^5	2.1×10^6	1.5×10^6	1.5×10^6	4×10^6	6.5×10^6
	Gut	4.8×10^7	6.2×10^7	5×10^7	2.2×10^8	6.3×10^8	2.5×10^9	9.9×10^8	1.2×10^9	3.2×10^9
Black	Water	6.7×10^5	3.1×10^5	6.6×10^5	6.6×10^5	1.1×10^7	2.2×10^6	2.2×10^6	3.4×10^6	9×10^6
molly	Gut	5.4×10^7	6.1×10^6	1.9×10^7	6.9×10^7	1.1×10^8	3.1×10^8	9.8×10^8	5.6×10^8	1.1×10^9
Platy	Water	2.3×10^6	7.5×10^5	1.8×10^6	1.8×10^6	3×10^6	3×10^6	7.8×10^5	5.3×10^6	7.8×10^6
	Gut	4.7×10^6	9.5×10^6	3.2×10^6	4.7×10^7	4.3×10^7	5.2×10^7	9×10^7	7.9×10^7	2×10^8
Guppy	Water	1.3×10^6	1.1×10^7	7.2×10^6	7.2×10^6	3.3×10^6	8.3×10^6	2.2×10^7	5.1×10^7	6.4×10^7
	Gut	3.1×10^7	3.7×10^7	9.1×10^6	4.2×10^7	9.2×10^7	7.7×10^7	3.4×10^8	8.2×10^8	1.6×10^9

Results and Discussion

Total Heterotrophic Counts

The total heterotrophic counts of aquarium culture water and fish gut are presented in Table 2. The total heterotrophic bacterial counts of aquarium water were highest in guppy tank with a peak of 6.36×10^7 cfu/ml at the end of culture period. The lowest count of 1.5×10^5 cfu/ml was recorded in Goldfish tank at the day 15 of culture. The total bacterial counts in the water are in close agreement to the reports of Del Rio-Rodriguez and Turnbull (3) who stated their levels to be 4×10^5 to 3×10^8 in water containing ornamental fish. They studied the aerobic bacterial flora associated with tropical ornamental fish over a six-month period and reported that the most prevalent organism were *Bacillus* spp., *Pseudomonas* spp., *Alcaligenes faecalis* and motile *Aeromonas* spp., although the relative prevalence varied between samples. There existed significant differences ($P<0.05$) in the total heterotrophic counts of aquarium water between the different fish tanks. The differences in heterotrophic counts in all the fish tanks between days of culture were however statistically insignificant.

The total heterotrophic counts of fish gut were higher than their respective cultured water with a peak of 3.15×10^9 cfu/g in goldfish gut at the end of the culture period. Platy gut recorded the lowest of 3.15×10^6 cfu/g at the day 15 of culture. The total heterotrophic count of bacteria in the fish gut are in total conformity to the results of Voveriene et al. (4) who observed similar levels in the intestinal tract of four fish from the Curonian lagoon. Voveriene et al. (4) showed the abundance of bacteria in the intestinal bacteriocenosis of different trophic fish groups investigated to vary with different fish species and include bacteria belonging to the genera *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Bacillus*, *Corynebacterium* and *Micrococcus*. Abraham et al. (5) investigated the bacterial flora associated with ornamental fish and reported on the frequent occurrence of *Aeromonas* spp., *Pseudomonas* spp. and G+ve rods from *Carassius auratus* and

Table 3. Percent frequency of G-ve Rods in water/gut sample.

Tank		Days of culture								
		5	10	15	20	25	30	35	40	45
Goldfish	Water	35	30	30	35	45	30	25	25	30
	Gut	15	5	5	10	0	0	0	5	0
Black	Water	55	50	30	35	45	30	25	25	20
molly	Gut	20	5	10	5	0	0	0	0	0
Platy	Water	40	45	55	50	35	40	25	25	20
	Gut	15	15	5	5	0	0	0	0	0
Guppy	Water	65	55	65	50	50	30	30	25	20
	Gut	10	10	10	0	0	0	0	0	0

Xiphophorus helleri. The total heterotrophic counts of the gut of different fish differed significantly ($P<0.05$), as also their total counts with respect to the days of culture.

Bacterial Classification

The bacterial colonies were classified on the basis of Gram staining into three basic types :
(1) G-ve rods
(2) G+ve cocci and
(3) G+ve rods.

G-ve rods. The percent frequency of G-ve rods in aquarium water and fish gut sample are presented in Table 3. The highest occurrence of G-ve rods (65%) was observed in culture water of guppy tank at the start of culture while the lowest occurrence (20%) were observed in all the culture waters at the end of culture. Del Rio-Rodriguez and Turnbull (3) found the prevalence of *Aeromonas* and *Pseudomonas* in fish samples ranging from 3.3 to 43.3% and from 3.3 to 30% respectively which further substantiates our results. Pseudomonads and *Alcaligenes faecalis* dominated the culture water but aeromonads (motile) were found more prevalent in fish tissues. The Freshwater Fisheries Research Center Annual Report 1996, Malaysia showed the presence of *Aeromonas* spp. on most of the cultured ornamental fish and their waters and of which 60% were *A. hydrophila* and the rest were *A. sobriae* and *Aeromonas* spp. (6). The percent frequency of G-ve rods varied significantly ($P<0.05$) between the different fish tanks as also between the days of culture.

Black molly gut exhibited the highest frequency of G-ve rods (20%) at the start of culture. The association of *Flavobacterium columnare* strains of high and low virulence with gill tissue of black mollies (*Poecilia sphenops*) was thoroughly studied by Decostere et al. (7). There was a complete lack of G-ve rods in all the fish gut after about 25 days of culture. This competitive exclusion of G-ve rods from fish gut could be attributed to the antagonistic effect exhibited by G+ve rods on the G-ve bacteria (8, 9). There existed significant differences ($P<0.05$) in the frequency of G-ve rods in all fish gut between the days of culture. The results presented in the paper are in sharp contrary to Shotts and Gratzek (10), Dixon and Issvoran (11), Kuo and Chung (12, 13), Srivibool (14), Siegel et al. (15) and Asfie et al. (16), who reported the presence of aeromonads and pseudomonads in the internal organs of ornamental fish.

G+Cocci. The percent frequency of G+ve cocci in aquarium water and fish gut sample are presented in Table 4. The highest frequency of G+ve cocci (35%) were observed in culture water of black molly and goldfish tank at the end of culture while the lowest occurrence (5%) were observed in the Guppy culture waters in the initial days of culture. Significant differences ($P<0.05$) were observed in the percent frequency of G+ve cocci between the different fish tanks as also between the days of culture.

All the fish gut analysed revealed the highest

Table 4. Percent frequency of G+ve Cocci in water/gut sample.

Tank		Days of culture								
		5	10	15	20	25	30	35	40	45
Goldfish	Water	10	10	15	15	25	25	30	35	30
	Gut	5	5	15	20	10	25	30	20	30
Black	Water	15	15	15	15	15	20	20	30	35
molly	Gut	0	0	10	20	25	20	30	25	30
Platy	Water	15	15	10	15	20	25	30	30	25
	Gut	5	0	20	15	25	25	25	30	20
Guppy	Water	5	5	15	15	10	25	25	30	30
	Gut	5	10	15	15	25	20	30	30	25

Table 5. Percent frequency of G+ve Rods in water/gut sample.

Tank		Days of culture								
		5	10	15	20	25	30	35	40	45
Goldfish	Water	55	60	55	50	30	45	45	40	40
	Gut	80	90	80	70	90	75	70	75	70
Black	Water	30	35	55	50	40	50	55	45	45
molly	Gut	80	95	80	75	75	80	70	75	70
Platy	Water	45	40	35	35	45	35	45	45	55
	Gut	80	85	75	80	75	75	75	70	80
Guppy	Water	30	40	20	35	40	45	45	45	50
	Gut	85	80	75	85	75	80	70	70	75

percent frequency of G+ve cocci at 30 during the later stages of culture of about after a month. The lowest frequency of G+ve cocci (5%) were observed in all the fish gut at the start of culture. Del Rio-Rodriguez and Turnbull (3) found the prevalence of Micrococcus in fish internal organs of ornamental fish cultured in Singapore to range from 0 to 46 % which are in agreement to our results. The percent frequency of G+ve cocci varied significantly ($P<0.05$) between their days of culture. The results agree with Park et al. (17) who isolated strains of *Staphylococcus epidermidis* from the cultured mud loach (*Misgurnus mizolepis*).

G+rods. The percent frequency of G+ve rods in aquarium water and fish gut sample are presented in Table 5. The microbiological analyses of aquarium water and fish gut samples revealed a high percent frequency of G+ve rods throughout the experimental duration of 45 days with a peak of 60 and 95% after the tenth day of culture in goldfish culture water and black molly gut respectively. The results agree with Conroy and Conroy (18), who reported on the occurrence of *Mycobacteria* spp., diagnosed in common and fancy varieties of goldfish (*Carassius auratus*) and guppies (*Lebistes reticulatus*) reared on fish farms in Aragua, Carabobo and Miranda States, Venezuela, and in a population of three-spot gouramies (Trichogaster trichopterus) produced on a fish farm in Colombia and imported to Venezuela for sale as pet fish. The relative abundance of *Bacillus* spp, as a component of normal gut microflora in fishes, by their ability to adhere to the intestinal wall have been well established by various workers (19–21). The percent frequency of G +ve rods between the different culture water, fish gut and days of culture varied insignificantly ($P<0.05$).

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