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Analysis of microbial biodiversity in intestine of ornamental fishes gut

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Abstract
The study of microbiota of ornamental fishes gives the view on both pathogenic and beneficial strains in fish gut. Total of nine bacterial strains were isolated from four different ornamental fishes namely *Cichla ocellaris*, *Barbonymus schwanenfeldii*, *Parachromis managuensis* and *Cyprinus carpio*. From the results, analyzing the dominance and microbial diversity of four different fish it is shown that *Cyprinus carpio* has highest dominant bacterial load and *Barbonymus schwanenfeldii* has highest microbial diversity. Out of these nine strains, strains like *Vibrio metschnikovii*, *Vibrio cincinnatiensis*, *Aeromonas veronii* and *Micrococcus halobius* indicated the constant evolving aquatic ecosystem surrounding the fishes. Since the probiotic is one of the strains which is present in the gut micro flora, it enhances and helps in the betterment of the growth of the fishes. This work promotes the use of these gut microbial microorganisms as probiotics and to render the fishes a disease free growth.

Keywords: Aquaculture, ornamental fishes, probiotics, gut microbial flora

1. Introduction
Aquaculture is an emerging industry as one of the promising enterprises for providing nutritional and food security to humans and supplying the protein demands. Aquaculture has made significant advances in years in the production of a wide range of aquatic organisms, both for edible and ornamental species [1-2]. Ornamental fishes usually mean attractive colorful fishes of various characteristics, which are kept as pets in confined space of an aquarium or a garden pool for fun and fancy. Ornamental fish breeding provides good employment and income as a non-fishery activity.

The advancements in fish breeding have given rise to several diseases due to infectious and non-infectious agents [3]. The various methods to treat these bacterial diseases are environmental manipulation, proper nutrition, immunological protection and chemotherapy. Ornamental fishes are found in various public places such as hospitals and schools, and the presence of certain pathogenic microbes will pose a risk to the public.

The gut microbiota of fish functions collectively as an extra organ for fish. The microbes colonizing in the fish gut provides protection against pathogens and tolerance to commensal bacteria and harmless antigens [4]. Due to the surrounding environment the fishes are prone to numerous micro-organisms all over their surface. These microbial floras can be found to colonize in various parts such as the skin, gills and the Gastro Intestinal (GI) tract. These micro-organisms can be pathogenic or non-pathogenic. Among these the microbes present in the GI tract are simple in diversity than those found in other parts and help in the growth of these fishes through better absorption of food and in maintaining the intestinal microbial flora.

The growth of the fish is also affected by the presence of this internal microbial flora. These bacteria play an important role in the aquaculture industry. The main disadvantage is that they cause diseases which may rapidly spread through the aquatic hosts. Some microbes are specific for an organism and may be important for its healthy development. The imbalance of these microbes in the aquatic environment may lead to pathogenesis.

The use of antibiotics with farm animals, however, caused tissue residue of the antibiotics and an imbalance of normal intestinal flora as well as a reduction in beneficial intestinal microbial populations and the generation of antibiotic-resistant bacteria [5]. In recent times anti-microbial substances from few beneficial microbes are been isolated and used as probiotic that inhibits the presence of pathogenic bacteria in the aquatic environment.

It is an ecofriendly approach using probiotics to stabilize gut microflora, improve microbial balance leading to improved feed absorption and enhanced disease resistance [6].

Probiotics, defined as "a viable singular or mixed culture of microorganisms which when applied to animals or humans beneficially affects the host by improving the properties of the indigenous microflora" [7], are strongly recommended as an alternative for antibiotics for industrial animals. The supplementation of probiotics through feed is a better method of ensuring the efficiency of the probiotic bacteria in the GI tract of fish.

Thus studying microbial flora of fish helps in understanding the fish microbial diversity, pathogens in fish and also the beneficial bacteria of fish which can be used as probiotics.

2. Materials and methods

2.1 Sample collection

Fishes were procured from the local market in Chennai. The weight of the fish ranged from 34.2 – 140.0g, with average weight of 60g. The fishes were packed in ice boxes and transported to the laboratory within 2 hours for isolation of gut bacteria.

2.2 Isolation of bacteria from fish gut

The fish surfaces were washed in running tap water, weighed and aseptically eviscerated. Gut samples were surface washed with sterile physiological saline to remove extraneous matter. The weight of the gut ranged from 1.34 to 7.08g. Depending on the weight, the gut samples (with digesta) were mixed with 80-110 ml of sterile saline solution and were homogenized for about 3-5 min, until the gut tissues appeared visibly macerated. Homogenized gut tissues were transferred into 1% peptone broth containing 0.5% NaCl and were kept for enrichment for 24 hours. The enriched broth media were serially diluted to 10^{-1} -10^{-9} dilutions and were plated onto nutrient agar and incubated at 31-37 °C for 24 h. Colonies of 2-3 mm diameter with round margin were obtained suspended within the agar mass. Colonies were picked and streaked on nutrient agar slants until purity for storage at room temperature of 31-37 °C. Further analysis was carried out from the stored cultures.

2.3 Identification of the bacterial colonies:

From the streaked plates three colonies from each of the four fishes were chosen and identified using biochemical characterization. Using the Berge's manual the bacterial strains were identified accordingly.

2.4 Statistical analysis:

The ecological indices such as index of dominance [7] index of diversity [8] and index of evenness [9] were performed.

Index of dominance $c = \Sigma (ni/N)^2$ where ni = number of individual for each species

N = total number of individuals

Shannon index of general diversity

$H = -\Sigma (ni/N) \log_e (ni/N)$ where ni = number of individual for each species

N = total number of individuals Evenness index $e = H/\log_e S$ where H = Shannon index

S = number of species

The significant differences between microbial counts were assessed by using a one way analysis of variance (ANOVA) table. All significance levels were determined at $P< 0.05$.

3. Results and discussion

Four different fishes were collected and identified as *Cichla ocellaris*, *Barbonymus schwanenfeldii*, *Parachromis managuensis* and *Cyprinus carpio*. The bacterial organisms identified from these fish's gut such as *Serratia liquefaciens, Vibrio metschnikovii, Staphylococcus saprophyticus, Vibrio cincinnatiensis, Aeromonas schubertii, Micrococcus halobius, Aureobacterium barkeri, Aeromonas veronii, Micrococcus lylae, Serratia liquefaciens, Aeromonas veronii* and *Micrococcus halobius* (Table 1).

Table 1: Identification of bacteria isolated from ornamental fishes

Fish name	Organism
Cichla ocellaris	*Serratia liquefaciens*
	Vibrio metschnikovii
	Staphylococcus saprophyticus
Barbonymus schwanenfeldii	*Vibrio cincinnatiensis*
	Aeromonas schubertii
	Micrococcus halobius
Parachromis managuensis	*Aureobacterium barkeri*
	Aeromonas veronii
	Micrococcus lylae
Cyprinus carpio	*Serratia liquefaciens*
	Aeromonas veronii
	Micrococcus halobius

The presence of *Aureobacterium barkeri* in *Parachromis managuensis* has not yet been reported. The presence of several strains like *Vibrio metschnikovii*, *Vibrio cincinnatiensis*, *Aeromonas veronii* and *Micrococcus halobius* indicate the constant evolving aquatic ecosystem surrounding the fishes. Hence it is very important to keep an eye on the microbial flora present along with the ornamental fishes. Since these fishes come in contact with humans day to day, we need to take care of the microbial flora since they can adversely affect the humans when in contact.

Table 2: Bacterial Load in Intestinal Tract of Fishes (cfu/ml)

Organism	Ornamental Fishes			
	Cichla ocellaris	***Barbonymus schwanenfeldii***	***Parachromis managuensis***	***Cyprinus carpio***
Serratia liquefaciens	$2.03x10^8 \pm 1.58x10^{7\,(a)}$	–	–	$4.96x10^6 \pm 2.51x10^{5\,(a)}$
Vibrio metschnikovii	$5.93x10^{8} \pm 2.08X10^{8(a)}$	–	–	–
Vibrio cincinnatiensis	–	$5.66x10^3 \pm 3.5x102^{(a)}$	–	–
Staphylococcus saprophyticus	$2.33x10^8 \pm 2.08x10^{7(b)}$	–	–	–
Aeromonas schubertii	–	$3.60x10^3 \pm 2.66x10^{2(b)}$	–	–
Aeromonas veronii	–	–	$1.96x10^6 \pm 3.51x10^{5\,(a)}$	$2.96x10^3 \pm 1.55x10^{2(b)}$
Aureobacterium barkeri	–	–	$3.33x10^4 \pm 2.5x10^{3(b)}$	
Micrococcus halobius	–	$1.33x10^3 \pm 3.18x10^{2\,(c)}$	–	$3.9x10^3 \pm 1.71x10^{2(b)}$
Micrococcus lylae	–	–	$1.1x10^3 \pm 3.55x10^{2\,(b)}$	–

Different super scripts in parenthesis show significant difference at $P<0.05$ level.

Anova followed by DMRT's test

The diversity of the microbes in the gut indicates the change of the micro-organisms within the water thereby resulting in more microbial load in the organism. Result in the variation of microbial load which plays an important role in the maintenance of a static gut microbial flora because of environmental factor.

Table 2, shows the mean value of nine bacterial samples present in the intestinal tract of fishes. *Vibrio metschnikovii* in *Cichla ocellaris* has high density bacterial load of $5.93x10^{8}\pm 2.08X10^{8}$ (cfu/ml), whereas, *Micrococcus lylae* in *Parachromis managuensis* has low density bacterial load of $1.1x10^{3} \pm 3.55x10^{2}$ (cfu/ml). ANOVA for bacterial load in intestinal tract of different fishes shows that significant difference at 5% level (Table 3).

Table 3: ANOVA for bacterial load in intestinal tract of fishes

Name of fishes	Degree of Freedom		F	P value
	Between Variables	Within Variables		
Cichla ocellaris	2	6	2226.948	0.000*
Barbonymus schwanenfeldii	2	6	139.726	0.000*
Parachromis managuensis	2	6	92.594	0.000*
Cyprinus carpio	2	6	1160.555	0.000*

*-Significant different at P<0.05 level

The fish *Cyprinus carpio* has highest dominant bacterial load of 0.870. All the samples contained similar bacterial stains. Whereas the *Barbonymus schwanenfeldii* has highest microbial diversity of 0.861, containing various strains in all the different samples processed (Table 4).

Table 4: Ecological indices of micro flora in fishes

	Cichla ocellaris	***Barbonymus schwanenfeldii***	***Parachromis managuensis***	***Cyprinus carpio***
Index of Diversity	0.271	0.861	0.076	0.039
Index of Dominance	0.870	0.419	0.969	0.987
Evenness	0.247	0.784	0.069	0.035

On the other hand the presence of these bacterial strains may have a chance of causing pathogenicity to the fish itself. Thereby by using these organisms as probiotic will antagonise the pathogen in fish gut and can help the fishes to grow healthy without adversely affecting its natural microbial flora. These bacterial strains when converted into probiotic may serve useful purpose to the fish and ensuring the maintenance of natural gut micro flora.

4. Conclusion

From this study it is evident that the microbial flora in the gut of ornamental fish varies greatly depending on the surrounding environment. The effect of these microbial strains adversely affects the growth of the fish. The presence of *Aureobacterium barkeri* in *Parachromis managuensis* has not yet been reported in the above strains of ornamental fishes. The presence of several pathogenic strains is a major study area for aquaculture researchers, which help them to analyse the disease causing pathogens and the methods for treating them. The conversion of the microbial flora present in the gut into a probiotic serves good for the fishes and has no side effects. The fishes fed with probiotics will antagonise the disease causing microorganisms in gut of fish. Currently more emphasis is being given to application of probiotics in larvae culture and live food organisms.

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