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Characterisation of the Bacterial Flora Associated with the Biofilter in Fresh Water Home Aquarium

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The role of filter bed soil bacterial flora on maintenance and improvement of environmental parameters of the fresh water home aquarium has been analysed. The study was carried out as a preliminary step to develop a microbial consortium for use in the biofilters of freshwater home aquaria. The results showed considerable improvement in the water quality, particularly significant improvement in the dissolved oxygen content. There was two log increase in the total heterotrophic bacterial (THB) load of the filter bed soil and the characterisation of the microflora showed selection of gram positive microorganisms, especially of the genera *Bacillus* towards the end of the experimental period.

Keywords : Home aquaria, biofilter, microflora, water quality

Ornamental fish trade and aquarium industry is gaining worldwide popularity and also it beckons greater economic benefit to the people. Aquariums are miniature form of ecosystems; so any change in that environment will affect the fishes growing in it. The deterioration of the water quality could result in stress of the aquarium fish leading to the deterioration of health and further infection by opportunistic pathogens.

For the effective grow up and healthy condition of the fishes good water quality is essential. The water quality parameters such as temperarture, DO, pH, nitrate content, ammonia etc.has a crucial role in the healthy maintenance of aquarium fish. Filters are commonly used for the purification and aeration of water in aquariums. Biofilters in aquaculture are composed of different types of bacteria, which can purify and maintain the water quality and thereby extensively reduce the water exchange frequency in the aquariums, a major problem in the aquarium management.

The present investigation has been taken up as a primary step to find out the effectiveness of biofilter microflora to degrade various organic wastes accumulated on the filter bed and the resultant enhancement in the physico-chemical parameters of the aquarium environment. The microflora associated with the filter bed soil has been characterised up to generic level in order to find out any possible selection of particular genera during the study period of 30 days. The ability of the isolates to degrade various organic wastes has also been analysed.

Materials and Methods

Glass tanks (4mm thickness, float glass) of size 60cm x 30cm x 30cm were used to set up the aquarium. The tanks were filled up to 80% of their capacity with fresh water. In one of the tanks a biofilter has been set up (Fig. 1). Along the 4 sides of the bottom of the tank a rectangular framework of perforated PVC pipes (1.3cm diameter) is provided to allow the filtered water to enter into the tube. On top of the PVC frame a

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sand filter of 5cm thickness was given using washed coarse river sand. With the help of a T-joint another PVC pipe (1.3cm) is fixed perpendicular to the collection pipe at the bottom so as to bring the filtered water to the top. This filtered water is then forced out by inserting the tip of the air pump in this tube. Once the filtered water is forced out through the opening at the top it will create negative pressure in the collection tube at the bottom, which will then receive the filtered water from above, and the cycle will go on and the other tank without biofilter set up has been used as control.

Carassius auratus (gold fish) was used for the study. A batch of 10 healthy fishes of uniform size was introduced into the tank with biofilter set up and another set of 10 was introduced into control tank that received only aeration. The fishes were fed with commercial pelleted feed for ornamental fishes. Water quality parameters in both the tanks were analyzed at the start of the experiment and also at regular intervals of 10 days (10^{th}, 20^{th} and 30^{th} day) for a month.

Temperature and pH were measured *in situ* by using a mercury bulb thermometer and a portable pH scan respectively. Dissolved Oxygen was estimated by modified Winkler's method (Greenberg *et al.*, 1992). Nitrate content was determined by spectrophotometric method (APHA 1998).

In order to determine the role of bacterial flora of the filter bed soil on the water quality parameters, the total heterotrophic bacterial (THB) population associated with it has been estimated before the introduction of the fish and at the end of the experiment. The THB was determined by spread plate method. Appropriate dilutions were spread plated on tryptic soy agar (TSA) and incubated at 30°C as it was close to the water temperature in the aquaria. Thirty colonies each from the samples of initial and final filter bed soil were isolated, restreaked to ensure purity and characterized up to generic level (Buchanan & Gibbons, 1979). The ability of the isolates to degrade various organic wastes was analysed by evaluating their ability to produce various hydrolytic enzymes such as gelatinase, amylase and lipase using plate assay. The ability to split urea and nitrate reducing activity of the isolates were also determined using Christensen's urea agar and nitrate broth respectively.

Results and discussion

Physico-chemical parameters of the water samples from the experimental and control tank are represented in Table 1. Temperature showed similar profile in the control and experimental tank, because it reflects the ambient air temperature. There was considerable improvement in the pH as it increased from an initial value of 4.68 to 5.68 and 6.87 in the water samples of experimental and control tank respectively. Increase in the dissolved oxygen (DO) level of the experimental tank was significant as it went up from an initial value of 7.2 to a final value of 8.2mg/l. There was 2mg/l

Table 1. Physico chemical parameters of the water samples from experimental* and control tank.

Parameter	Initial		After 10 days		After 20 days		After 30 days	
	Control	Expt.*	Control	Expt.*	Control	Expt.*	Control	Expt.*
Temperature (°C)	27	27	31	31	29	29	28	28
pH	4.68	4.68	6.72	5.63	6.68	5.74	6.87	5.68
Dissolved Oxygen (mg/l)	7.2	7.2	6.4	7.2	6.2	8.2	6.2	8.2[a]
Nitrate (mg/l)	0.68	0.68	0.65	0.63	0.64	0.58	0.59	0.54

* Experimental tank - With biofilter
[a] Statistically significant

improvement in the DO content of the water in experimental tank when compared to that of the control tank. The dissolved oxygen content of the water sample from the control tank recorded decline from 7.2mg/l to 6.2 mg/l, in spite of the aeration it received. The improvement in the DO must have resulted partly from the aeration and from the effective holding and breakdown of organic wastes by the biofilter microflora on the filter bed itself.

Nitrate content of the water from the control and experimental tank showed decline during the experimental period suggesting reduction of some of the initial nitrate content. It declined from an initial concentration of 0.68mg/l to a final value of 0.54mg/l and 0.59mg/l in the water samples from experimental and control tank respectively. However, no signs of nitrate toxicity have been noticed among the fishes.

The total heterotrophic bacterial (THB) load and the different genera of bacteria associated with the initial and final filter bed soil is given in Table 2. There was 2 log increase in the THB population towards the end of the experiment (after one month), without causing any deleterious effect on the fish. The fishes were quite active and healthy and the water remained very clear. The increase in the bacterial population of the filter bed soil is expected from the utilisation of the waste generated from the fish as well as the feed remnants, which are held on the filter bed. The qualitative change in the initial and final THB population of the filter bed soil is also given in Table 2. There were significant changes in the composition of the microflora of the filter bed soil towards the end of the experiment showing definite selection of certain genera. The most noticeable change was in the percentage population of *Moraxella*, which formed the dominant genera in the initial filter bed soil, and has been completely absent in the final filter bed soil. *Bacillus* has almost doubled its share in the final filter bed soil. It is reported that gram-positive *Bacillus* spp. are generally more efficient in converting organic matter back to CO_2 than are gram-negative bacteria, which would convert a greater percentage of organic carbon to bacterial biomass or slime (Stanier *et al.*, 1963).

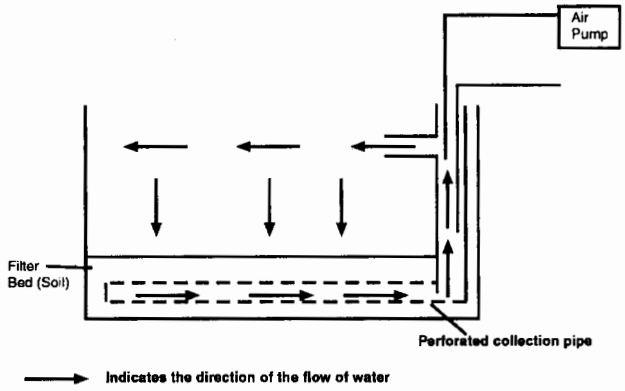

Fig. 1. Schematic representation of the bio-filter assembly

Table 2. Total heterotrophic bacterial (THB) load and percentage incidence of various genera associated with the initial and final filter bed soil.

Parameter	Initial filter bed soil	Final filter bed soil
THB* load	9.8×10^4 cfu/g	1.9×10^6 cfu/g
Generic Composition		
Acinetobacter	20%	9%
Alcaligenes	6.6%	12%
Aeromonas	-	3%
Bacillus	3.3%	6.1%
Corynebacterium	10%	15.6%
Cytophaga/Flavobacterium	-	6.1%
Kurthia	3.3%	12%
Micrococcus	6.6%	-
Moraxella	46.6%	-
Pseudomonas	3.3%	-
Vibrio	-	24.2%
Unknown	-	11%

Other remarkable increase was in the population of *Vibrio*. The other genera which was encountered in the initial and final filter bed soil, though at varying degrees, included *Acinetobacter, Alcaligenes, Corynebacterium* and *Kurthia*. The improvements in the *Bacillus* and *Vibrio* populations were particularly encouraging as their role as probiotic strains were well documented (Gatesoupe, 1997; Ringo & Vadstein, 1998). Apart from the above genera, *Aeromonas* also

showed considerable increase in the final generic composition of the biofilter. The incidence of the genera *Aeromonas* in the biofilter sample is expected from the fish intestine as they form the autochthonous microflora of the fresh water fish intestine (Hatha *et al.*, 2000; Hatha, 2002). *Aeromonas* has also got a ubiquitous distribution in various aquatic bodies.

The variations in the physiological groups in the initial and final filter bed microflora revealed an increase in proteolytic forms. However, amylolytic and lipolytic forms recorded a decrease in the final filter bed soil. This may be due to the selection of the proteolytic forms resulting from the accumulation of proteinaceous waste on the filter bed soil. Nitrate reducing forms and ureolytic forms recorded an increase in the final filter bed soil microflora. Though nitrate reducers are of concern due to their ability to increase nitrite and ammonia concentration, which are toxic to fish, such toxic level build up was not noticed as reflected by the healthy and active nature of the fish maintained in the experimental tank. However, specific estimation of nitrite and ammonia was not attempted in this study.

The present study was carried out as a preliminary step to develop a microbial consortium for use in the biofilters of freshwater home aquaria. Inoculation with specific strains was not attempted in this study and the biofilter was allowed to develop from the indigenous microflora of the washed coarse river sand, which is used as the filter bed. However, we have a very good collection of heterotrophic bacteria in the laboratory, which are identified, to various levels (genera, species and strains). The physiological characterization revealed that many of them, especially from the genera *Bacillus* and *Aeromonas*, have got very good potential for use in biofilters, which could significantly improve the water quality and reduce the frequency of water exchange in home aquaria.

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