Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 1 (5): 448-452, 2006
ISSN 1816-4935

Diversity of Bacterial Populations in Recirculating Marine Aquarium with Different Marine Ornamental Fishes

K. Raja, Olivia J. Fernando, R. Thavasi, S. Jayalaksmi and T. Balasubramanian
Marine Research Aquarium, CAS in Marine Biology, Annamalai University
Annamalai Nagar-608 002, India

Abstract: The bacterial population of water from the Marine Research Aquarium was examined qualitatively and quantitatively. Lower concentrations (1.3×10^2) were found in the initial phase of the aquarium tank and increased moderately day by day. A total of 1109 strains were isolated from 30 water samples. The isolates comprised of 16 species 10 genera; 86.65% of these isolates were gram-negative rods. The *Vibrio* isolates comprised 48.9% of the total population. The generic compositions of the bacterial isolates were *Flavobacterium*, *Pseudomonas*, *Salmonella*, *Bacillus*, *Staphylococcus*, *Alcaligenes*, *Aeromonas*, *Enterobacter* and *Cytoplaga*. The results show that the survival rate of the fish species *Pomacanthus imperator*, *P. semicirculatus*, *Chaetodon collare*, *C. vagabundus*, *C. plebius* are poor probably due to abundance of the *Vibrio* population. The higher concentration of other genera may also increases the mortality rate among these ornamental fishes. Majority of the bacterial strains isolated are also considered as human pathogens.

Key words: THB, aquarium, recirculating aquaria, marine ornamental fishes, pathogens

Introduction

Generally, organic matter in recirculating aquariums is derived from uneaten feed or diets, dead bodies, wounded fishes and excreta of fish. These inorganic matters can directly affect total bacterial populations of the aquaria. The water quality of recirculating system is mainly affected by the bacterial flora in the water column and filter materials as well as the different elements of this closed system. Lesser survival can be observed in fishes that are maintained in water with high bacterial pathogens. In the present study, the recycling of water in the aquarium was done mainly through three different facilities i.e., 1) a mechanical filter in the bottom of the aquarium tanks to remove suspended materials 2) a canister filter in individual tanks that contains charcoal and bioballs to transform the toxic soluble matter into non-toxic compounds. 3) A UV sterilizer to control bacterial populations. All these materials provide a substratum for bacterial growth in a closed system. The nitrifying bacteria from the biological filter medium control the stability of water in addition to their nitrifying action.

The low efficiency of biological filter media causes the bloom of opportunistic pathogenic bacteria in the rearing tanks (Blancheton and Canaguier, 1995). The exhibition aquaria and closed aquaculture systems reproduce aquatic environments with high densities of fish and the fishes kept in such intensive systems are very sensitive to the microbial flora of the water (Blanch *et al*., 1999). Moreover, bacterial populations in aquaculture facilities and in exhibition aquaria may differ from those of the natural environment and may affect the nutrition and health of the fish. The influence of associated bacteria in water, food and initial stages of fish larvae, on the survival of fish in captivity has been demonstrated by Nicolas *et al*. (1989) and Westerrdahl *et al*. (1994). In addition, aquaria containing

Corresponding Author: K. Raja, Marine Research Aquarium, CAS in Marine Biology, Parangipettai, Cuddlaore Dist, Tamil Nadu, India, Pin Code: 608 502 Tel: 9894506950

ornamental fish are often found in school classrooms, medical and dental offices, eating establishments, department stores, nursing homes and even in hospital wards. It is apparent that the presence of potentially pathogenic microorganisms and opportunistic pathogenic bacteria in these aquaria would present a risk to the public health. The knowledge about these aquarium pathogenic organisms is important to hobbyist. Thus, the present study was initiated to qualitatively and quantitatively determine the bacterial population in the water supplied with ornamental fish.

Materials and Methods

The ornamental fishes were purchased from Gulf of Mannar southeast coast of India and transported more than 350 km in polyethylene package filled by one third of pure oxygen. These fishes were quarantined in UV sterilized water for three days; only after this initial quarantine treatment, the fishes were introduced into the newly established experimental cum public aquaria. The schematic diagram of the newly established recirculating Marine Research Aquarium system used in this study and the sampling locations are shown in Fig. 1. In the present experimental aquaria, 20% of the water was recycled through the various filters every day. The survival of the fishes were regularly monitored throughout the study period and the total heterotrophic bacterial populations also examined. Total number of fish species introduced and their sizes are shown in Table 1.

Sampling for Bacteriological Examinations

The water samples were taken during three different phase i.e., Phase I (1st day), Phase II (15th day) and Phase III (30th day). Totally 30 samples were taken during the present experiment.

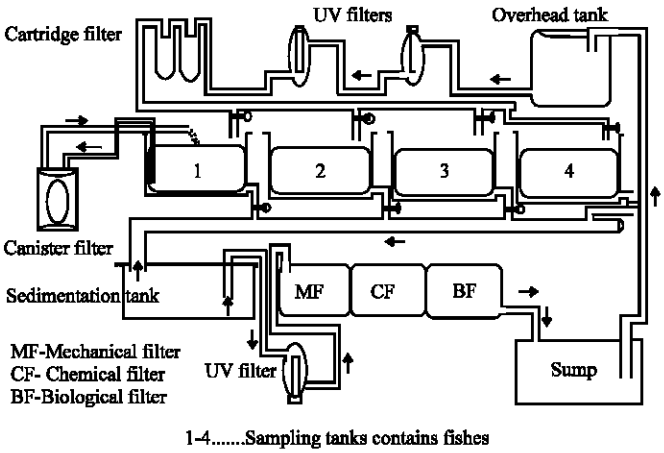

Fig. 1: Technical view of recirculating system in Marine Research Aquarium

Table 1: The fish species characteristics of the sampling aquaria

Sampling tank	Fish species maintained	Total length (cm)	Total No. of fish
1	*Holocentrus diadema*	9-13	13
2	*Arius maculatus*	23-30	3
3	*Chaetodon vagabundus, C. plebius*	8-10, 7-9	13+6
4	*Chaetodon collare*	6-11	29
5	*Pomacanthus imperator, P.semicirculatus*	19-20, 16-18	3+2
6	*Platux orbiculatus*	24-33	2
7	*Zebrasoma veliferum*	13-15	8
8	*Thallosoma lunare*	17-21	15
9	*Pomacentrus coelores*	4-7	65
10	*Amphiprion sebae*, sea anemone	4-11	35+11

Quantitative Bacteriological Examination

Enumeration of Total Heterotrophic Bacteria (THB) was made using the pour plate method on sterile Zobell marine agar medium (Hi-media chemicals Pvt. Ltd, Bombay, India) with the composition of Peptone-5.0 g, Yeast extract 1.0 g, K_2HPO_4-0.5 g, $FeSo_4$-Trace, Agar-15 g, Distilled water-1000 mL and pH-7.2. For plating 1 mL of the serially diluted sample was pipetted out into sterile petri plates. Sterile media was then poured into petri plates aseptically and swivel led for through mixing. After solidification, the plates were incubated in an inverted position for 72 h at 28±1°C. Bacterial colonies developed on Zobell marine agar after the incubation period were counted and their density expressed as Colony Forming Units (CFU) per milliliter.

Qualitative Bacteriological Examination

The different morphological colonies were picked from the Zobell marine agar plates and restreaked in appropriate nutrient agar plates. The tests used to characterize the isolates are described by Edwards and Eving (1972), Skerman (1967) and Smith *et al*. (1972). Identification is facilitated by examination of colonial morphology and pigmentation as well as examination of the shape, arrangement, staining characteristics, flagellar arrangement and motility of the cells. In addition, the ability of the isolates to produce oxidase and catalase, to ferment lactose and to metabolize glucose fermentativety or oxidatively was tested. Gram-negative species were further separated on the basis of carbohydrate utilization, growth in the presence of bile salts, production of urease and the indole, methyl red, Voges-Proskauer and citrate utilization reactions. Other tests used were the orthonitrophenyl, β-D-galactopyranosidase (ONPG) test, the lysine and ornithine decarboxylase tests and the arginine decarboxylase and dihydrolase tests. *Vibrio* species were confirmed by testing the sensitivity of the culture to 2,4 diamino-6,7-diisopropylpteridine. Final identification of the isolates to species level were done with the schemes of Buchanan and Gibbons (1975), Krieg (1984) and Sneath (1986).

Results

Quantitative Bacterial Examination

The results in Table 2 show that the samples from the initial phase contained low concentration (5×10^2-2.3×10^3 CFU mL^{-1}) of total heterotrophic bacteria per mL but it increased in the following phases (1.7×10^3-1.60×10^5 CFU mL^{-1}).

Qualitative Bacteriological Examinations

A total of 1109 isolates were partially characterized from the 30 water samples. The isolates comprised of 16 species from 10 genera of bacteria; 86.65% of these isolates were gram-negative rods (Table 3). The most common species of isolates belonged to the genus *Vibrio*. These *Vibrio* isolates comprised 48.9% of the total isolates and were demonstrated to be present in 27 of the water samples in all the three phases. Of the *Vibrio* isolates 170 were identified as *V. parahaemolyticus*, 155 as *V. valnificus*, 111 as *V. angnillarium* and 106 as *V. cholerae*. Species of *Pseudomonas* comprised 16.22% of the total isolates and were isolated from 22 water samples. This genera is present all the phases except phase III of *Arius maculates*, *P. imperator* and *P. semicirculatus* tanks. In the majority of these *Pseudomonas* isolates were non-pigmented species and were able to grow at 42°C on *Pseudomonas* agar. Typical pyocyanin producing *Pseudomonas aeruginosa* was also isolated in *Pomocentrus coelores* fish tank. In addition, the species *Pseudomonas fluerescens* produce, fluorescein and were isolated from the 6 tanks comprised of 65 isolates. The *Salmonella* is rich (48 isolates) in *P. imperator*, *P. semicirculatus* tanks followed by *A. sebae* (32 isolates), *C. collare* (15 isolates) *C. vagabundus*, *C. plebius* (7 isolates) and *Thallosoma lunare* (5 isolates). Among the other isolates

Table 2: Bacterial concentration in different aquaria samples

	Bacterial concentrations (CFU mL^{-1})		
Samples	1st day (Phase I)	15th day (Phase II)	30th day (Phase III)
1	6.0×10^{2}	1.7×10^{3}	1.7×10^{5}
2	1.3×10^{2}	1.5×10^{5}	1.5×10^{5}
3	1.0×10^{3}	4.0×10^{5}	4.0×10^{4}
4	1.9×10^{3}	8.4×10^{5}	7.1×10^{5}
5	2.3×10^{2}	1.52×10^{5}	1.60×10^{5}
6	5.0×10^{2}	3.4×10^{5}	3.4×10^{5}
7	1.1×10^{3}	1.7×10^{3}	2.1×10^{4}
8	1.5×10^{2}	2.5×10^{3}	1.7×10^{4}
9	1.3×10^{3}	3.8×10^{5}	2.7×10^{5}
10	2.3×10^{3}	8.0×10^{4}	6.7×10^{5}

Table 3: Frequency of isolation of bacterial species from the samples

	Total No. of isolates									
	Samples (each includes 3 phases of the samples)									
Species isolated	1	2	3	4	5	6	7	8	9	10
Vibrio parahaemolyticus	12	-	21	15	83	-	-	-	-	39
V. valnificus	-	22	13	27	65	-	-	-	11	17
V. anguillarum	-	-	6	35	56	-	-	-	-	14
V. choleræ	-	-	31	10	37	22	6	-	-	-
Pseudomonas aeruginosa	-	-	-	21	-	-	11	15	30	-
P. fluerescens	-	10	9	7	19	13	-	7	-	-
P. pseudoalcaligenes	-	-	3	-	-	11	12	6	6	-
Salmonella sp.	-	-	7	15	48	-	-	5	-	32
Bacillus megaterium	13	5	-	6	-	-	6	-	13	28
B. cereus	4	-	-	-	-	12	-	-	-	-
Staphylococcus sp.	3	-	-	-	-	10	5	-	18	-
Alcaligenes fecalis	2	-	-	-	-	-	9	14	-	-
Aeromonas hydrophilla	3	-	-	13	15	-	-	-	-	-
Enterobacter aerogenes	1	-	-	10	-	5	-	-	-	13
Cytoplaga sp.	-	6	-	6	-	-	-	-	-	27
Flavobacterium sp.	2	-	-	9	12	-	-	10	-	-

Bacillus megaterium, B. cereus comprised 7.84% , *Staphylococcus* sp. 3.25%, *Alcaligenes fecalis* 2.25%, *Aeromonas hydrophilla* 2.8%, *Enterobacter aerogenes* 2.61%, *Cytoplaga* sp. 3.52 % and *Flavobacterium* sp. 2.97% and they are isolated from very few of the samples.

Discussion

The present findings show that marine aquarium water in the recycling system contains significant number of a wide variety of bacteria. Position of various filtration system in the present aquarium clearly shows that the experimental tank contains nearly pure water without any bacterial cells. The bacteria were introduced into the water from uneaten feed or diets, dead bodies, wounded fishes and excreta of fish and these paths differed based on the species. For example the fish species *P. imperator* and *C. collare* were underfeeding in the initial phases and results in increase of uneaten feed within the tank. It directly influenced the bacterial population which in turn may affect the health of the fish. The food ingested microbial composition has been reported through the estimation of intestinal micro-flora by various authors (Tanasomwang and Muroga, 1988; Blanch *et al*., 1999). The presence of high concentration of *Vibrio* in the tanks 3, 4, 5 also resulted in lesser survival rate of the fishes maintained in these tanks. The influence of the associated bacteria, *V. anguillarum* in water, food and initial stages of fish on the survival of fish in captivity has been demonstrated by Westerdahl *et al*. (1994). Blanch *et al*. (2001) also monitored the bacteria of the

aquarium water, particularly the *vibrio* populations and determined a higher composition of *Vibrio* populations from several exhibition aquaria with a shared water supply. *P. aeruginosa*, *V. cholerae*, *V. parahaemolyticus* isolated in the present samples are able to survive and multiply in the gut, mucus and tissues of fish and they are also potential pathogens of man (Janssen, 1970; Trust and Money, 1972; Weistreich and Lechtman, 1973). In recirculating aquaria microbial populations multiplies day by day. This is directly affect the fish health that are maintained in high concentrations of harmful microorganisms. Since bacteria present in an aquarium and they could also readily be transferred to humans, besides affecting the fishes the presence of potential pathogens in the aquarium could become a hazard to human health.

Acknowledgments

We thank the authorities of Annamalai University for allowing use of Marine Research Aquarium in the present study.

References

Blanch, A.R., P. Van den Sande, P. Bulto and C. Hispano, 1999. Health management in exhibition aquaria. Bull. Eur. Assoc. Fish Pathol., 6: 282-283.

Blanch, A.R., C.M. Cuellar and C. Hispano, 2001. Diversity of *Vibrio* sp. Populations in several exhibition aquaria with a shared water supply. Lett. Applied Microbiol., 33: 137-143.

Blancheton, J.P. and B. Canaguier, 1995. Bacteria and particulate materials in recirculating seabass (*Dicentrarchus labrax*) production system. Aquaculture, 133: 215-224.

Buchanan, R.E and N.E. Gibbons, 1975. Bergey's Manual of Determinative Bacteriology. 8th Edn., The Williams and Wilkins, Baltimore, pp: 1268.

Edwards, P. R. and W. H. Ewing, 1972. Identification of Enterobacteriaceae. 3rd Edn., Burgess Publishing Co., Minneapolis.

Janssen, W.A., 1970. Fish as Potential Vectors of Human Bacterial Diseases. In: A symposium on diseases of fish and shellfishes. Snieszko, S. F. (Ed.), Am. Fish. Soc. Spec. Publ., 5: 284-290.

Krieg, N.R., 1984. Bergey's Manual of Systematic Bacteriology (Ed. Krieg), Vol. 1, The Williams and Wilkins, Baltimore, 1: 964.

Nicolas, J.L., E. Robic and D. Ansquer, 1989. Bacterial flora associated with a trophic chain consisting of microalgae, rotifers and turbot larvae: Influence of bacteria on larval survival. Aquaculture, 83: 237-248.

Skerman, V.B.D., 1967. A Guide to the Identification of the Genera of Bacteria. 2nd Edn., The Williams and Wilkins Co., Baltimore.

Smith, D.B., K.M. Tomfohrde, D.L. Rhoden and A. Balows, 1972. API system: A multitube micromethod for identification of Enterobacteriaceae. Applied Microbiol., 24: 449-452.

Sneath, P.H.A., 1986. Bergey's Manual of Systematic Bacteriology. Vol. 2, Williams and Wilkins, Baltimore, pp: 965-1599.

Tanasomwang, V. and K. Muroga, 1988. Intestinal microflora of larval and juvenile stages in Japanese flounder (*Paralichthys olivaceus*). Fish Pathol., 23: 77-83.

Trust, T.J. and V.G. Money, 1972. Bacterial population of diets for aquarium fishes. J. Fish. Res. Bd. Can., 29: 429-433.

Weistreich, G.A. and M.D. Lechtman, 1973. Microbiology and Human Diseases. Glencoe Press, New York.

Westerdahl, A., J.C. Olsson, P.L. Conway and S. Kjelleberg, 1994. Charecterization of turbot (*Scophthalmus maximus*) associated bacteria with inhibitory effects against the fish pathogen *Vibrio anguillarum*. Acta Microbiologica et Immunologica Hungarica, 41: 403-409.