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Isolation and Identification of Potential Bacteria from the Common Aquarium

KEYWORDS: Aquarium fish;. Antibiotics, bactericides, sidrophores

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ABSTRACT *The present work reports on the antibacterial effects. The antibacterial effects of bacteria are generally due to any of the following factors, either singly or in combination: production of antibiotics, bacteriocines, sidrophores, lysozymes or protease and alternation of pH values by the organic acid production*

Introduction

Freshwater ornamental fishery has become an important component of Indian fisheries contributing 75% of the total aquaculture production and ornamental cichlids fishes contribute about 68% to the total aquaculture production as well as play major role in ornamental fish trade.

But, as with many other industries, this rapid growth of aquaculture has brought with it the problem of disease out breaks. In India *Aeromonas hydrophila* is a prime causative agent for pathogenecity in fishes and responsible for hemorrhagic septicemia, infectious dropsy, rubller, red-mouth, red-pest and freshwater eel diseases (Bandyopadhyay *et al.*, 2003). *Aeromonas hydrophila* is also suspected to be the principal causative agent of ulcerative disease noted in cultured fish in Indo-Pacific region (Tonguthai, 1985), South East Asian countries (Roberts, 1986) and Thailand (Llobrera and Gacuttan, 1987).

A substantial increase in the numbers of opportunistic bacteria such as we should find in systems with other infected fish and / or high level of decomposing organic matter. This is typically the situation when the disease starts to spread and several fish are affected. Under these conditions, especially if fish are still stressed it seems that some opportunistic bacteria can become primary pathogens through weight of numbers. Also in fishes not only pathogenic bacteria found but also beneficial microorganisms present, they are known as probiotic bacteria which help to combact with disease. The purpose of present study was 'How do opportunistic bacteria become pathogenic.

Materials and Methods

The experiment was conducted at Deptt. of Zoology & Aquaculture, R. S. M., Latur, India. Several colonies of the chichlid fishes isolate were inoculated into 1 liter of brain-heart infusion broth (BHIB, Difco) and grown at 25 °C for 48 h on a rotary shaker (200 rpm). A 50 ml portion of the broth was then added to 450 ml of sterile phosphate-buffered saline (PBS, pH 7.4) and each group of fish was netted out and placed in a separate freshly prepared bacterial suspension (3×10^7 CFU ml^{-1}) for 5 min. Following exposure, both bath-challenged and control (uninoculated BHIB) groups were rinsed in 500 ml of sterile PBS for 20 s before being returned to their respective aquaria.

Isolation and identification of bacterial strains:

A freshwater aquarium near Latur Town selling different ornamental fishes was selected for sample collection. Ornamental fishes of medium size (60 ± 28 g) were taken for analysis assuming that they might have a established pattern of intestinal microflora. Fishes were transferred to water collected from pond and brought into the laboratory in live condition. Upon reaching the laboratory, analysis of the intestinal microbial flora was done on samples consisting of excised and cut open with a pair of sterile scissors. Gut content were removed by scrapping, and the intestines were washed three times with sterile saline solution to remove non-adherent microflora. The sample were then homogenized with 10 ml distilled water in Stomacher bags (Stomacher, Lab – Blender 400). Dilution series were prepared from the homogenates and isolation of bacteria was determined through spread plate technique on nutrient agar (Hi – media, India).

Morphologically five different colonies; A-1, A-2, A-3, A-4 and A-5 from (Table – 6.1) were picked up from the plates and followed the streak plate method to ensure purity. The streak plate of different bacterial strains; A-1, A-2, A-3, A-4 and A-5 are presented in plate 4, 5, 6, 7 and 8 respectively. Further these bacterial strains were characterised and identified by different cultural, morphological, physiological and biochemical characteristics.

Table 1 - Isolation of different bacterial strains from the common aquarium fish species

Fishes	Bacterial strains
Cichlids	A-1, A-2
Cyprinidae	A-3, A-4
Anabantedae	A-5

Results

Morphological characteristics of different bacterial strains A-1, A-2, A-3, A-4 and A-5 isolated from ornamental fish species were presented in table 2, 3, 4, 5 and 6 respectively. All the bacterial strains were round shaped colony with entire margin and convex elevation. No pigmentation was found in the colony of all the strains. A-1 and A-5 showed gram-positive reaction while others were gram-negative. All the strains were motile and only strain A-3 and A-4 showed fluorescency. (Typically morphological characteristics of the strain A-1 and A-3 are shown here)

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Table 2 – Morphological characteristics of the strain A-1

Tests		Results
Colony Morphology	Configuration	Round
	Margin	Entire
	Elevation	Convex
	Surface	Rough
	Density	Translucent
	Pigments	-ve
Gram's Reaction		+ve
	Shape	Rod
	Size	Long
	Arrangement	Single
Spore	Endospore	+
	Position	T and ST
	Shape	Oval
Motility		+
Florescence		-

Table 3 - Morphological characteristics of the strain A-3

Tests		Results
Colony Morphology	Configuration	Round
	Margin	Entire
	Elevation	Convex
	Surface	Smooth
	Density	Translucent
	Pigments	-ve
Gram's Reaction		-ve
	Shape	Rod
	Size	Short
	Arrangement	Single
Spore	Endospore	-
Motility		+
Florescence		+

Discussion

The aim of this study was to screen out the Beneficial and harmful bacteria of some common aquarium fishes that could be applied to combat fish pathogens under aquaculture conditions. In my present study, it was clearly noticed that the bacterial species of *Bacillus, Pseudomonas* and *Aeromonas* have the ability to inhibit the pathogenic *Aeromonas hydrophila*. In the earlier report it was found that the *Bacillus sp.* has the ability to inhibit the pathogenic strains (Ozawa *et al.,* 1981; Ogle and Inborr, 1987; Spriet *et al.,* 1987). Recently Rengpipat *et al.* (1998) reported that the *Bacillus sp.* S11 reduce the disease caused by *Aeromonas salmonicida*. In another investigation Gibson et al. (1998) reported that *Aeromonas media* has the ability to prevent death of Pacific oyster *C gigas* when challenged with *V. tubiashii*. Smith and Davey (1993) reported that *Pseudomonas fluorescens* reduce the disease caused by *Aeromonas salmonicida*, Gram *et al.* (2001) also observed the similar phenomenon in Atlantic salmon.

Our present study it was clearly observed that the two intestinal bacteria with antibacterial properties might inhibit the growth of invading bacteria in the intestine of common aquarium fishes in India *Aeromonas hydrophila* is the prime causative agent for pathogenecity in fishes (Bandyopadhyay *et al.,* 2003).

The antibacterial effects of bacteria are generally due to any of the following factors, either singly or in combination: production of antibiotics, bacteriocines, sidrophores, lysozymes or protease and alternation of pH values by the organic acid production (Sugita *et al.*, 1996).

Conclusions:

Our present study it was clearly observed that the two intestinal bacteria with antibacterial properties might inhibit the growth of invading bacteria in the intestine of common aquarium fishes in India *Aeromonas hydrophila* is the prime causative agent for pathogenecity in fishes

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