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Some Studies on Aeromonas Hydrophila Infection in Some Common Ornamental Fishes

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ABSTRACT

The present paper deals with the aeromonas hydrohila infection in some common aquarium fishes. Media employed for isolation of Aeromonas hydrophila were reviewed and choice of Rimbler - Shtts agar was made in the initial phase of the study. It is beyond doubt that in the ulcerative disease of fish the organism may play an important role not only in necrotizing the muscle tissue but also damaging the internal liver and spleen besides the peritoneum.

KEYWORDS : Aquarium fish; disease diagnosis; bacterial agents; histological techniques

1. Introduction

Aeromonas hydrophila is a ubiquitous and opportunistic bacterium, which is a part of the normal microbial flora of fish as well as other aquatic animals. The bacterium can cause diseases under stress conditions in fish, which has been reported extensively from freshwater environments [1-4] reported that Aeromonas hydrophila caused wide range of mortality in gold fish (carassius auratus) in India. Gopalkrishnan [5] reported many instances of entire population of Indian major carps being wiped out by epidemics of Aeromonas hydrophila.

In India Aeromonas hydrophila is a prime causative agent for pathogenicity in fishes and responsible for hemorrhagic septicemia, infections dropsy, rubller, red-mouth, red-pest and freshwater eel diseases. Aeromonas hydrophila is also suspected to be the principal causative agent of ulcerative disease noted in cultured fish in Indo Pacific region, South East Asian countries and Thailand [6]. The microorganism can be isolated from water within a wide range of physiochemical limits (pH 5.2 - 9.8; temperature < 10 – 45 °C).

The present paper based on isolation and identification of Aeromonas hydrophila from disesed aquarium fishes, Gold fish, Angel fish, and Betta of freshwater aquariums of Latur District (Latitude and Longitude of this area is 18 °. 22″ N and 76 °. 35″ E) Maharashtra, India showing various symptoms.

2. Materials and Methods:

Common aquarium fishes such as Gold fish (carracius auratus), Angel fish (Pterophyllum scalare) and Betta (Betta splendens) showing gross pathological lesions were collected from various aquariums of the Latur District of Maharashtra, India.

Materials were collected aseptically from different organs like skin, gill, kidney, liver, eye and heart in sterile container with liquid medium for primary culture of bacteria.

Materials collected aseptically with the help of a platinum loop from different organs were transferred to nutrient broth and incubated at 30 °C. Growth of bacteria, if any were observed after 24 – 28 hours by noting the turbidity in the broth. Primary cultures were made from the turbid broth by streaking in nutrient agar plates. The colony characters such as shape, size and colour were noted using binocular microscope and selected colonies were picked up for slant culture. The agar slants were incubated at 30 °C for 24 – 48 hours. Isolates in nutrient agar slants were maintained in a laboratory at 4 °C through the study period.

Preliminary identification was made on the basis of the colony characteristics. In addition to this, gram staining and cytochrome oxidase tests were done along with comparing the responses to the various biochemical tests.

Identification tests were confirmed by using an Electrobacteriaceae Computer kit (Microsoft Corporation, Redmonas, WA 98073) and Multitan (340/MMC). On the basis of the various biochemical properties and different stains were demarketed with different code numbers.

Results and discussion

Gross Pathology :

Gold fish (carassius auratus) weighing about 49.00 – 52.16 gm and of length 5 - 6 cm showed severe ulceration with haemorrhagic spots around pelvic fin and snout. Scales appeared raised at the inflamed parts on the lateral side of the body. The entire region was disintegrated and severe ulcers were noticed exposing the muscles, which appeared lacerated. The ulcerative areas varied in sizes from 1 to 2 mm in diameter and 0.5 mm deep with dirty grey slimy deposits along with bluish film on head.

Angel fish (Pterophyllum Scalare) weighing around 55 - 65 g and of length about 6 to 7 cm showed eye lesions characterized by periorbital oedema and corneal opacity. Clinically there was bilateral exophthalmia with ground glass like appearance of the eyeballs. On necropsy on other lesions could be seen in any other organs.

Betta (Betta splendens) weighing about 40 - 50 g and of 6.8 - 7.2 cm showed haemorrhagic spots at the base of the dorsal fin. There were whitish nodular cysts mainly confined to tail region. All the internal organs were highly haemorrhagic with marked congestion of the blood vessel.

Isolation and Identification:

In Gold fish, Aeromonas hydrophila was isolated from skin, body fluid, kidney and liver. Eye, heart and skin lesions of Angel fish and kidney, liver, skin lesions of Betta, showed presence of bacterial organisms. (Table 1). Some other bacterial pathogens, which were isolated from the diseased fishes, have not been taken into account in the present study.

Morphology :

The colonies of Aeronomas hydrophila, which were isolated, appeared around, convex and flattened in all solid media [see plate 1 and 2]. The colonies were semitranslucent in nature. In Aeronomas isolation medium it appeared pinhead sized round and golden yellow in colour[see plate 3] and were gram negative, coccobacilli, measuring about 2-2.5 times longer than width (0.3-1.0 µm in diameter and 1.0-3.5 µm in length). The bacterial isolates obtained from different organs of various affected species were subjected to identification using enterobacteriaceae kit, cytochrome oxidase test and biochemical characteristics [see plate 4, 5 and 6]. The detailed results are represented in Table 2.

Table 1 : Species source of diseased fish and organs from which isolations were made

Strain Name	Fish	Organs	Area of Collection
Aeronomas hydrophila	Gold Fish	Liver	Latur Aquariums
		Skin	Chakur
		Kidney	Ausa
		Eye	Nilanga
	Angel Fish	Skin	Ausa
		Body Fluid	Latur
		Skin	Latur
		Skin	Ahmadpur
		Liver	Ahmadpur
	Betta	Eye	Latur
		Heart	Latur
		Skin	Ausa
		Kidney	Chakur
		Skin	Chakur

Table 2 : Identification of *Aeronomas hydrophila* using biochemical test. (Manuallt and Enterobacteriaceae Kit)

Biochemical Test	AHS	AH1	AH2	AH3
Morphology				
Capsule formation	+	+	+	+
Mortality	+	+	+	+
Flagella	+	+	+	+
Physiology				
Catalase production	+	+	+	+
Growth in KCN	+	+	+	+
Growth in Methyl red	-	-	-	-
Growth in Voges proskaur	+	+	+	+
Growth in 2.5 % NaCl	+	+	+	+
Growth in 6.5 % NaCl	-	-	-	-
Carbohydrate Matabolism				
Production of Acid in Glucose	+	+	+	+
L – arabinose	+	+	+	+
D – xylose	-	-	-	-
L – rhamnose	-	-	-	-
D – lactose	-	-	-	-
D – mannose	+	+	+	+
Sorbose	+	+	+	+
D – maltose	+	+	+	+
D – sucrose	+	+	+	+
D – mannitol	+	+	+	+
D – dulcitol	-	-	-	-
D – sorbitol	-	-	-	-
Salicine	-	-	-	-
Esculine hydrolysis	-	-	+	-
Malonate	+	+	+	+
Metabolism in – Nitrogenous Compound				
Urease	-	-	-	-
Lysine decarboxylase	-	-	+	-
Ornithine decarboxykase	-	-	-	-
Hydrogen sulphide	-	-	-	-
Indole	+	+	+	+
Starch hydrolysis	+	+	+	+
Gelatin	+	+	+	+
DNAse hydrolysis	+	+	+	+
ONPG	+	+	+	+
Tween 80 esterase	+	+	+	+
O / 129 vibriostat				
Citrate utilization	+	+	+	+
Nitrate reduction	+	+	+	+
Adonitol	-	-	-	-



Plate 1 : Growth of *Aeromonas hydrophilla* on blood agar isolated from gold fish



Plate 2. : Growth of *Aeromonas hydrophilla* on blood agar isolated from angel fish

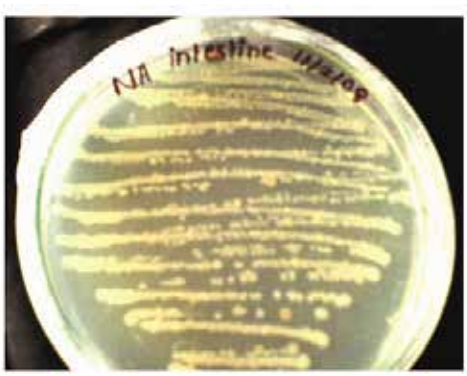


Plate : 3 : Growth of *Aeromonas hydrophilla* on agar plate. isolated from intestine of gold fish

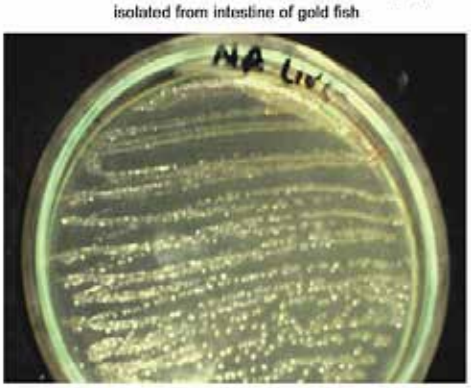


Plate : 4 : Growth of *Aeromonas spp* on agar plate. isolated from liver of angel fish



Plate 5 : Biochemical Test (Indole Test) for identification of unknown bacteria



Plate : 6 : Biochemical Test (Methyl Red Test) for identification of unknown bacteria

Discussion:

The isolation study revealed presence of Aeromonas hydrophila in skin, body fluid, heart, eye, kidney and liver of various species of fish collected from different geographical location. The high percentage incidence of Aeronomas hydrophila in diseased fishes from various parts of Latur district, Maharashtra strongly suggested the ubiquitous nature of the bacterium similar observations were recorded by Llobrera and Gacuttan, [6]; sheriff et al., [7] Nayak et al., [8]. Das and Shotts [9-10] isolated the same bacteria from lesions of ulcers and other visceral organs of different species of fish examined in the Orissa state and Bandyopadhyay et.al. [11] from an ornamental fish (Caraccium Auratus). The bacterium is frequently associated with infections as secondary invader as reported by Roberts [12] and has been branded as a facultative pathogen. It is thought that this organism invade only when the host resistance is lowered by environmental stress factors such as high organic load, over crowdung and sublethal oxygen levels in water. They concluded that higher temperature favoured rapid proliferation of the bacteria and subsequent host mortality. In the present study the isolation of Aeromonas hydraphila was abtly done from the ulcerated skin lesions, kidney, liver, body fluid, eye, heart of Gold fish, Angel fish, Betta showing various clinical manifestations. However, no correlations could be made with the environmental temperature as the disease occurred throughout the year with varying atmospheric temperature.

The balance of ingredients in this medium provided a nutrient base and chemophysical stability. Shotts and Rimbler [10] remarked that the yellow colonies indicated the presence of Aeronomas hydrophila. However it was also pointed out that all the yellow colonies having black centers should be subsequently tested for oxidase activities to eliminate the possibilities of citrobacter sp. and / or other organisms. Similar methods were followed in the present study, which proved to be useful.

Conclusion

Until now the role of Aeromonas hydrophila in various fish diseases was not very clear. The present study has elucidated the role of this organism in dropsy, fin and tail rot and more elaborately in Epizootic Ulcerative Syndrome (EUS). It is beyond doubt that in the ulcerative disease of fish the organism may play an important role not only in necrotizing the muscle tissue but also damaging the internal liver and spleen besides the peritoneum. It is on this basis that prophylactic measures could be formulated in order to contain various diseases caused by this organism.

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