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Presumptive and Definitive Identification of *Aeromonas* from infected Ornamental Gold fish (*Carassius auratus auratus*)

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The paper deals with the presumptive identification of *Aeromonas*, definitive identification of two species of *Aeromonas* i.e. *Aeromonas hydrophila*, *Aeromonas salmonicida* in various organs of Gold fish infected with red disease.

Keyword: Gold fish, Red disease, *Aeromonas hydrophila*, *Aeromonas salmonicida*.

1. Introduction

The **Gold fish** is a freshwater fish. It was one of the earliest fish to be domesticated, and is one of the most commonly kept aquarium fish. Gold fish breeds vary greatly in size, body shape, fin configuration and coloration. *Aeromonas hydrophila* has been recovered from a wide range of freshwater fish species worldwide ^[1]. *A. hydrophila* has been associated with tail and fin rot, haemorrhagic septicaemia and epizootic ulcerative syndrome (EUS) ^[2]. Red disease is often identified by the appearance of red ulcers and lesions on fish. Typically, "red disease" is presumed to be caused by two organisms, *Aeromonas hydrophila*, a bacterium, and *Heteropolaria* sp. (formerly *Epistylis* sp.), a protozoan. Sores caused by *Heteropolaria* species can be characterized by white-grey, cotton-like patches on the body surface or the fins. Due to the irritation, the fish will "flash," or rub, to rid itself of the parasite, causing scale loss and ulceration of the already damaged area. This allows the bacterium *Aeromonas hydrophila* to enter.

2. Materials and Methods

Infectious Gold fish samples were collected from aquariums, after examining the gross clinical signs and external characteristics of fish. The collected fish exhibited red ulcerations/lesions on the body followed by reddening at the tips of paired and unpaired fins. In some of the samples there was loss of scales appearing reddish white patches with deep wound like appearance. The vital organs like kidney and liver were observed for structural changes and later cut for further examination along with body slime on *Aeromonas* selective agar plates. Tests used for identification of *Aeromonas* are categorized as:

Presumptive identification tests: the test includes *Aeromonas* isolation media ^[3], Growth in Nutrient broth, Motility test, Grams staining, Oxidase test, Catalase test.

Definitive identification tests: This involves the identification of *Aeromonas*, up to *Species* level from samples found positive using a set of biochemical tests.

Tests related to carbohydrate Metabolism: Sugar fermentation, Methyl red test, proskauer Voges test, TSI.

Tests related to Aminoacid & protein metabolism: Indole test, Lysine, ornithine decarboxylase & arginine dehydrolase test.

Tests for both carbohydrate & protein metabolism: citrate Utilization

Other tests – Salt tolerance test, Nitrate reduction test.

Table 1: Presumptive Identification of *Aeromonas*- From the Red sore/ulcer of Gold Fish

Culture Plate	Growth On Aeromonal Medium (Colony Characteristics)	Broth Characters		Motility	Staining	Oxidise	Catalase	Result
		T	SF					
1	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
	Medium green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
2	small green colonyAeromonas	+ ve	+ ve	- ve	- ve	+ ve	+ ve	Aeromonas present
	Medium green colonyAeromonas	+ ve	+ ve	- ve	- ve	- ve	+ ve	Aeromonas present
3	medium green colonyAeromonas	+ ve	- ve	+ ve	- ve	- ve	+ ve	Aeromonas present
	irregularl green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
4	small green colonyAeromonas	+ ve	+ ve	- ve	- ve	- ve	- ve	Aeromonas present
	small green colonyAeromonas	+ ve	+ ve	- ve	- ve	+ ve	- ve	Aeromonas present
5	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	- ve	Aeromonas present
	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	- ve	Aeromonas present
6	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	- ve	Aeromonas present
	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	- ve	Aeromonas present

Table 2: Definitive Identification of *Aeromonas*- From the Red sore/ulcer of Gold Fish

Cultu re plate	Colony picked (Isolates)	Decarboxylase			TSI			SF			ST				MO F	I	M R	V P	C	Species
		AR G	LY S	ORN I	Acid Bul t	Al k Sal t	H2 S	G	S	M	0 %	3 %	8 %	11 %						
1	Aeromon as	+ ve	d	- ve	+ ve	+ ve	- ve	A+G +	A+G +	A+G +	+ ve	+ ve	- ve	- ve	F	- ve	- ve	- ve	+ ve	A.salmonici da
	Aeromon as	+ ve	d	- ve	+ ve	+ ve	+ ve	A+G +	A+G +	A+G +	+ ve	+ ve	+ ve	- ve	F	+ ve	- ve	+ ve	d	A.hydrophil la
2	Aeromon as	+ ve	d	+ ve	+ ve	+ ve	+ ve	A+G +	A+G +	A+G +	+ ve	+ ve	+ ve	+ ve	F	- ve	- ve	+ ve	d	A.hydrophil la
	Aeromon as	+ ve	+ ve	- ve	+ ve	+ ve	- ve	A-G-	A-G-	A-G-	+ ve	+ ve	+ ve	- ve	F	+ ve	+ ve	- ve	d	A.hydrophil la
3	Aeromon as	+ ve	+ ve	- ve	+ ve	+ ve	- ve	A-G-	A-G-	A-G-	+ ve	+ ve	+ ve	+ ve	F	+ ve	- ve	- ve	d	A.hydrophil la
	Aeromon as	+ ve	+ ve	- ve	+ ve	+ ve	- ve	A-G-	A-G-	A-G-	+ ve	+ ve	+ ve	+ ve	F	- ve	- ve	- ve	- ve	A.hydrophil la
4	Aeromon as	+ ve	+ ve	- ve	+ ve	+ ve	- ve	A-G-	A-G-	A-G-	+ ve	+ ve	+ ve	- ve	F	+ ve	- ve	- ve	- ve	A.hydrophil la
	Aeromon as	+ ve	d	- ve	+ ve	+ ve	- ve	A+G +	A+G +	A+G +	+ ve	+ ve	- ve	- ve	F	- ve	- ve	- ve	+ ve	A.salmonici da
5	Aeromon as	+ ve	d	+ ve	+ ve	+ ve	+ ve	A+G +	A+G +	A+G +	+ ve	+ ve	+ ve	+ ve	F	- ve	- ve	+ ve	d	A.hydrophil la
	Aeromon as	+ ve	+ ve	- ve	+ ve	+ ve	- ve	A-G-	A-G-	A-G-	+ ve	+ ve	+ ve	- ve	F	+ ve	+ ve	- ve	d	A.hydrophil la
6	Aeromon as	+ ve	d	+ ve	+ ve	+ ve	+ ve	A+G +	A+G +	A+G +	+ ve	+ ve	+ ve	+ ve	F	- ve	- ve	+ ve	d	A.hydrophil la
	Aeromon as	+ ve	d	- ve	+ ve	+ ve	- ve	A+G +	A+G +	A+G +	+ ve	+ ve	- ve	- ve	F	- ve	- ve	- ve	+ ve	A.salmonici da

SF: Sugar fermentation, G: Glucose, S: Sucrose, L: Lactose, M: Maltose, A⁺: acid production, G⁺: gas production, MR: Methyl red test, VP: Proskauer Voges test, TSI: Triple sugar iron I: Indole test, LYS and ORNI: Lysine, Ornithine decarboxylase, ARG: arginine dehydrolase test. C: citrate Utilization, MOF: Marine oxidation fermentation (I: inert) i.e. Neither Oxidation nor Reduction, ST: Salt tolerance test, NIT: Nitrate reduction test.

Through presumptive identification, *Aeromonas* is identified and through definitive identification, *Aeromonas* Species like *Aeromonas hydrophila*, *Aeromonas salmonicida* are identified.

3. Discussion

This disease often occurs during the change from the dry to rainy season and during the flood season in MRD [4] in the present findings more number of infections was recorded in rainy seasons. This is similar with the findings of [4]. In the present study, it was observed that the signs of Red disease associated with often fraying and reddening of fins, accompanied by irregular, variably sized areas of de-pigmentation as well as reddish pigmentation that can develop anywhere on the body surface. The skin overlying these

sites is eventually lost, exposing the muscle below. These open sores or ulcers may remain superficial or they can be extensive and invade deeply into muscle, revealing underlying bone in some cases. These ulcers often have ragged white margins bordered by a narrow zone of hemorrhage. Infections can occur in any age fish, but losses are usually most severe in fry and small fingerlings. Similar conditions of Red Disease were also reported in the rural carp culture by several authors [5]. Chronic motile *aeromonad* infections manifest themselves

primarily as ulcerous forms of disease, in which dermal lesions with focal hemorrhage and inflammation are apparent. Both the dermis and epidermis are eroded and the underlying musculature becomes severely necrotic ¹⁶. The liver may become pale or have a greenish coloration while the kidney may become swollen and friable. These organs are apparently attacked by bacterial toxins and lose their structural integrity ¹⁶. The liver of infected fish showed the same symptoms as discussed by ¹⁶.

Samples found positive for *Aeromonas* were selected for species identification and accordingly bio-chemical tests were conducted as per *Bergey's Manual of Determinative Bacteriology*. The putative isolates so selected were short rods, gm negative and were glucose fermentors. Further it is noted that all isolates set for species test were identified as *Aeromonas hydrophila* thus confirming this work done by many researchers in this direction ¹⁷⁻⁹. The study indicates that motile aeromonad septicemias are generally mediated by stress. Elevated water temperature ¹⁸, a decrease in dissolved oxygen concentration, or increases in ammonia and carbon dioxide concentrations have been shown to promote stress in fish and trigger motile aeromonad and *Pseudomonas* infections coinciding the work of ¹⁹. The monitoring of environmental variables can therefore enable one to forecast stressful situations and possibly avoid problems before they arise. As stated by ¹⁷ wherever this red disease occurs fish should not be handled but transferred only after water temperature is high enough for fish to be active and feeding normally. Mortalities can be reduced dramatically (80-90%) when fish, at the time of colder months were given a suitable antibiotic.

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