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Research Article

Presumptive and Definitive Identification of *Aeromonas* from infected *Pangasius hypophthalmus* in Culture Ponds of West Godavari and Krishna Districts of Andhra Pradesh

M. Phani Kumar*, K. Sree Ramulu
Department of Zoology, Andhra University, Visakhapatnam- 530 003, Andhra Pradesh, India.

***Corresponding author**
M. Phani Kumar
Email: phdphani@gmail.com

Abstract: The paper deals with the presumptive identification of *Aeromonas* definitive identification of three species of Aeromonas ie *Aeromonas hydrophila, Aeromonas caviae and Aeromonas sobria* in various organs of *Pangasius hypophthalmus* infected with red disease in culture ponds of West Godavari and Krishna districts of Andhra Pradesh.
Keywords: *Pangasius hypophthalmus,* Red disease, *Aeromonas hydrophila, Aeromonas caviae* and *Aeromonas sobria*

INTRODUCTION

Commercial aquaculture production of catfish, *Pangasius hypophthalmus* has increased rapidly in recent years and has contributed significantly to food security and economic development. It is well adapted to low oxygen water and can survive on a low diet fishmeal. In India, Andhra Pradesh is the leading state with 25,000 ha under *Pangasius hypophthalmus* culture in freshwater sector. The culture of *Pangasius hypophthalmus* can be considered as a unique aquatic farming system in many ways. Production is the fastest growth recorded in any aquaculture sector, ever, based on a single species, superseding the production per unit for any form of primary production [1]. Bacteria always present in the water environment, multiply and invade to fish and spread the disease when reaching the suitable condition [2]. *Aeromonas hydrophila* has been recovered from a wide range of freshwater fish species worldwide [3]. *A. hydrophila* has been associated with tail and fin rot, haemorrhagic septicaemia and epizootic ulcerative syndrome (EUS) [3, 4].

MATERIAL AND METHODS

Diseased *Pangasius* fish samples were collected from culture ponds of Kaikaluru and Bhimavaram of Krishna and west Godavari districts in Andhra Pradesh, India 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 [5], Growth in Nutrient broth, Motility test, Grams staining, Oxidase test, Catalase test.

Definitive identification test**s:** 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

RESULTS

Through presumptive identification, *Aeromonas is* identified and through definitive identification, *Aeromonas Species A. caviae, A. hydrophila, and A. sobria* are identified.

DISCUSSION

This disease often occurs during the change from the dry to rainy season and during the flood season in MRD [6]. In the present findings more number of

infections was recorded in rainy seasons. This is similar with the findings of Khoi et al.[6]. 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. Similar conditions of Red Disease were also reported in the rural carp culture by several authors[7]. 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 [8]. 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. The liver of infected fish showed the same symptoms as discussed by Huizinga et al. [8].

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 fermenators. 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 [9-11]. The study indicates that motile aeromonad septicemias are generally mediated by stress. Elevated water temperature [11] , 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 Walters and Plumb [10]. The monitoring of environmental variables can therefore enable one to forecast stressful situations and possibly avoid problems before they arise. As stated by *Rychlicki and Zarnecki* [9] wherever this red disease occurs pond 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 .

Table: 1 Presumptive Identification of *Aeromonas* in infected *Pangasius hypophthalmus*

Culture plate	GROWTH ON Aeromonas MEDIUM(colony charcteristics)	BROTH CHARACTERS		MOTILITY	STAINING	OXIDISE	CATALASE	RESULT
		Test tube	Small Flask					
1	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
2	Medium green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	- ve	Aeromonas present
3	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
4	Medium green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	- ve	Aeromonas present
5	medium green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
6	irregularl green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	- ve	Aeromonas present
7	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
8	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
9	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
10	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
11	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	Aeromonas present
12	small green colonyAeromonas	+ ve	+ ve	+ ve	- ve	+ ve	- ve	Aeromonas present

Table: 2 Definitive Identification of Aeromonas from the infected *Pangasius hypophthalmus*

Culture plate	Colony picked (Isolates)	DECARBOXYLASE			TSI			SF				ST				MOF	I	MR	VP	NIT	C	SPECIES
		ARG	LYS	ORNI	ACID BULT	ALK SALT	H2S	G	S	L	M	0%	3%	8%	11 %							
1	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	- ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	- ve	- ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.hydrophilla
2	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	- ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	- ve	- ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.hydrophilla
3	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	- ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	- ve	- ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.hydrophilla
4	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	- ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	- ve	- ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.caviae
5	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	+ ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	+ ve	+ ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.sobria
6	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	+ ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	+ ve	+ ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.sobria
7	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	+ ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	+ ve	+ ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.hydrophilla
8	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	+ ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	+ ve	+ ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.sobria
9	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	+ ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	- ve	- ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.caviae
10	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	+ ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	- ve	- ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.hydrophilla
11	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	+ ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	- ve	- ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.hydrophilla
12	Aeromonas	- ve	+ ve	+ ve	+ ve	- ve	+ ve	A+G+	A+G+	A+G+	A+G+	+ ve	+ ve	- ve	- ve	I	+ ve	+ ve	- ve	+ ve	+ ve	A.sobria

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.

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