

CONF-770511--2

STRAIN SPECIFICITY OF *AEROMONAS HYDROPHILA*:
AN IMMUNOFLUORESCENCE STUDY

by

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A paper proposed for presentation
at *American Society for Microbiology*
May 8-13, 1977,
New Orleans, Louisiana

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ABSTRACT

Fluorescent antibodies prepared to *Aeromonas hydrophila* were species specific when compared to seven other *Aeromonas* spp. obtained from the American Type Culture Collection (ATCC). The *A. hydrophila* were isolated from water, largemouth bass, and alligators from a warm monomictic lake thermally altered by production reactor effluents. Over 200 isolates from water, bass, alligators, and human samples were characterized serologically, biochemically, and for drug sensitivity. The isolates were then grouped. The specificity of the antibodies was used to establish a relationship between the infective agents of the largemouth bass and the alligators when compared to those

* The information contained in this article was developed during the course of work under Contract No. AT(07-2)-1 with the U.S. Energy Research and Development Administration.

aeromonads found in the water. Groups established from biochemical, drug sensitivity testing, and serological characterization showed similarities. The *A. hydrophila* associated with the infections of the largemouth bass and the alligators do not appear to be the serological strain dominant in the water column nor the strains from ATCC. The *A. hydrophila* strain isolated from both external lesions and internal necrotic areas of the sampled alligators appear similar to those in and on the infected largemouth bass.

INTRODUCTION

Par Pond fish, particularly the largemouth bass, and alligator populations are infected with *Aeromonas hydrophila*, which cause "red sore disease." The bacteria have been shown to survive for extended periods of time in Par Pond,¹ a warm monomictic lake thermally altered by effluents from a Savannah River Plant (SRP) production nuclear reactor, but do not survive as well in Clark Hill, a nearby large reservoir on the Savannah River. The present study was initiated to characterize the various types of *A. hydrophila* in Par Pond and in the alligators and bass populations.

Strain-specific fluorescent antibodies were prepared to various strains of *A. hydrophila* isolated from water, largemouth bass, and alligators from Par Pond. Over 200 isolates from water, bass, alligators, and human samples were characterized serologically, biochemically, and for drug sensitivity. The isolates were then grouped. *A. hydrophila* associated with the infections of the

largemouth bass and the alligators do not appear to be the serological strain dominant in the water column of Par Pond nor in the strains examined from the American Type Culture Collection (ATCC).

MATERIAL AND METHODS

Bacterial Isolates

A. hydrophila were isolated with RS medium² from a variety of habitats and from specimens obtained from ATCC. Colonies characteristic of *A. hydrophila* were subjected to 23 different biochemical tests by using the API system.³ In addition, each of the 255 isolates was characterized as to their sensitivity or resistance to the following antibiotics: chloramphenicol, erythromycin, bacitracin, novobiocin, penicillin, streptomycin, vancomycin, furadantin, tetracycline, chloromycetin, ampicillin, nalidixic acid, and sulfathiazole.

Immunofluorescence Preparations

Cultures of *A. hydrophila* isolated from Par Pond water, infected largemouth bass, and American alligators were checked for purity and grown to a density of 10^8 cells/mL for the preparation of the individual fluorescent antibodies. Cells were harvested by centrifugation, washed, resuspended in phosphate buffered saline (pH 7.2) solution, and injected intravenously into rabbits. After injections were completed, test bleedings were made, and the antibody levels were shown by tube agglutination to be greater

than 1:5120 for each homologous system. Antisera were harvested by cardiac puncture, fractionated, and conjugated with a fluorochrome dye. All procedures were as previously described.⁴

Methods for preparing and staining contact slides and all fluorescence microscopy and photomicrography are detailed.⁵ Gelatin-rhodamine isothiocyanate conjugate was used to suppress nonspecific absorption of the fluorescent antibodies. The techniques for preparing terrestrial and aquatic samples for immunofluorescence examination on membrane filters were as previously reported.⁶

RESULTS AND DISCUSSION

Each of the 255 cultures of *A. hydrophila* was analyzed for similarities in biochemical and nutritional requirements, drug sensitivity, and the degree of immunological similarity. All of the isolates appeared to be similar in their biochemical reactions and their utilization of various nutritional substrates established by the API system. All isolates were resistant to high doses of penicillin, ampicillin, bacitracin, and novobiocin antibiotics. (The drug resistance data are summarized in Table 1.) All the isolates were similar in their response to antibiotics regardless of where the isolates were obtained.

The immunofluorescence data indicate that the isolates obtained from the ATCC are not serologically the same as the isolates obtained from Par Pond samples. Likewise, the isolates from the water are generally not the organisms causing the

infections in the bass nor in the alligators. On the other hand, those isolates from infected bass appear to be serologically similar to isolates taken from internal tissues of the alligators.

Data also suggest that the various strains of *A. hydrophila* are similar in their biochemical, nutritional, and drug sensitivity requirements; but they are dissimilar in their serological reactions.

Thus, given strains of *A. hydrophila* appear to be more optimally adapted to the aquatic habitates, and other strains appear to survive better in association with cold-blooded animals.

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TABLE 1

Percentages of Isolates Resistant to Tested Drugs

Antibiotics (conc., mcg)	Fura- dantin (300)	Strepto- mycin (10)	Tetra- cycline (30)	Penicil- lin (10 units)	Ampicil- lin (10)	Nalidixic Acid (30)	Sulfa- thiazole (150)	Baci- tracin (10)	Chloramp- henicol (30)	Erythrom- ycin (15)	Novobio- cin (30)	Vancomi- cin (30)	Chloromy- cetin (30)
Par Pond:													
Water n=115	14	4	1	100	100	0	45	86	14	48	100	19	0
Bass n=107	15	2	7.6	100	91	1	47	94	6	13	100	6	2
Alligator n=25	25	0	0	100	75	0	50	100	0	29	100	19	0
ATCC:													
14486	N.D.	+	N.D.	+	N.D.	N.D.	N.D.	+	-	+	+	+	N.D.
7966	-	+	-	+	+	-	+	+	-	+	-	-	-
15467				+	+			+	-	+	+		
13137	+	-	-	+	+	-	+	N.D.	-	-	+	-	-
15338	+	+	-	+	+	-	+	+	+	+	+	-	-
14715	-	+	-	+	+	-	+	+	+	+	+	+	-
19570	-	+	-	+	+	-	+	+	-	+	+	+	-
15468				+	+		+	+		+	+	+	

N.D. = Not Determined.