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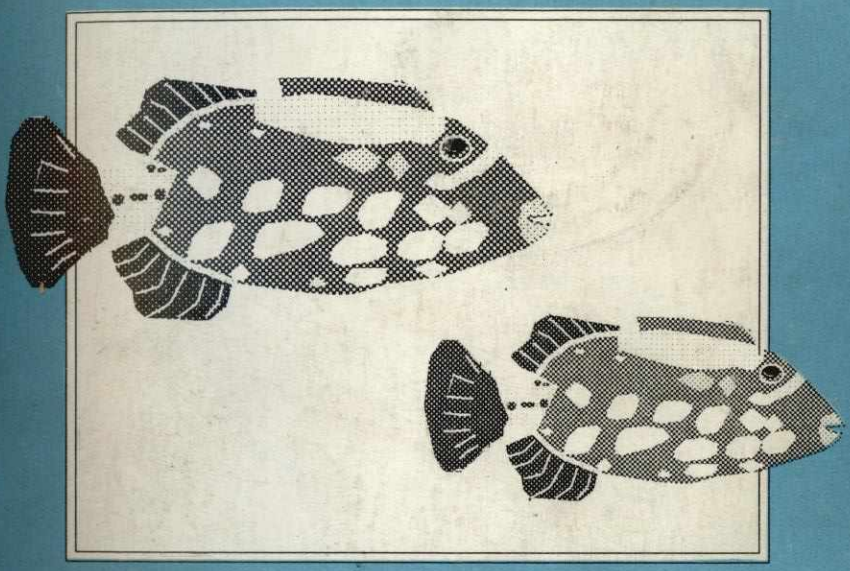

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# Isolation and Identification of *Aeromonas Hydrophila* from Fish with the Use of the API 20E System

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## ABSTRACT

**Five species of freshwater fish and nine marine fishes were studied to determine the presence of *Aeromonas hydrophila*, an etiological agent in fish disease. This was done with the API 20E System (Analytab Products, Plainview, N.Y.) the standardized miniaturized version of conventional procedures for identifying Enterobacteriaceae and other gram-negative bacteria. This is a ready-to-use microtube system designed to perform 23 standard biochemical tests from isolated colonies of bacteria on plating medium.**

**All the five freshwater fishes and two of the nine marine fishes were found positive for *Aeromonas hydrophila.***

**Keywords**: bacteria, Aeromoues hydrophila, diseases.

## INTRODUCTION

*Aeromonas hydrophila* is a common inhabitant of the aquatic environment, classified as a member of the family *Vibrionaceae* and the genus *Aeromonas* (Veron, 1966; Schubert, 1974). It has been identified as etiological agent in the disease of fish (Boulanger et al., 1977; Hazen et al., 1978 and Miller and Chapman, 1976), amphibians (Emerson and Norris, 1905), and reptiles (Marcus, 1971; McCoy and Seidler, 1973; Shotts, et al., 1972). It was originally isolated and described in 1891 by Sanarelli, who demonstrated pathogenicity of the organism for frogs.

An unpublished Indonesian report showed that *A. hydrophila* caused a severe disease outbreak in *Clarias batrachus* in Java. It is also the most common cause of bacterial haemorrhagic septicaemia of *C. batrachus* and *C. macropcephalus* in polyculture ponds in Thailand. The disease occurs in three distinct forms: a) abdominal dropsy, characterized by distension of the visceral cavity with fluid; b) ulcerations, characterized by skin and muscle lesions; and c) generalized bacterial haemorrhagic septicaemia (Kabata, 1985).

Miller and Chapman (1976) reported the mortality of 37,500 fishes in a North Carolina reservoir over a 13-day period due to an epizootic disease caused by *A. hydrophila*. Such reports are getting more common in the southeastern United States.

In the Philippines, during the period covering from January to February 1986, a mysterious fish disease alarmed fish farmers culturing catfish, mudfish and gourami in Laguna Lake. Harvested fishes were found to have ulcerations on the dorsal, ventral and tail portions. Villagers termed the phenomena "Kurikong," a skin disease characterized by itchiness. The fish seemed to have rubbed themselves against hard objects. Their scales were discolored and there was reddening of the underlying skin in the early stage which later developed into ulceration in the advanced stage.

During the disease outbreak, the external appearance of the fish samples (gourami, mudfish and catfish) was observed to resemble infections caused by bacterial haemorrhagic septicaemia. Initial signs showed skin discoloration and appearance of blisters which developed into open sores and ulcerative lesions. These eventually led to exposure of bones in the more advanced stages.

## MATERIALS AND METHOD

The following species of fish (purchased from local markets) were used in the experiment: mudfish (*Ophicephalus striatus*), catfish (*Clarias batrachus*) "tawilis" (*Harengula tawilis*), gourami (*Plasalit*), tilapia (*T. nilotica*), milkfish (*Chanos-chanos*), bisugo (*Nemipterus sp.*), shortbodied mackerel (*Rastrelliger brachysomas*), roundscad (*Decapterus sp.*), skipjack (tulingan) (*Katsowonus pelamis*), caesio (*Caesio sp.*), crevalle (*Caranx sp.*), big-eyed scad (*Selar crumenophthalmus*) and big-eyed tuna (*Thunnus obessus*).

The API 20E System was used to detect and identify the *Aeromonas hydrophila* in these fishes. This system consists of a series of biochemical tests in miniaturized compartments. Each compartment was inoculated with a suspension of bacterial cells in 0.85% sodium chloride solution.





A series of dilutions of each of the fish samples was made with 0.1% peptone water as diluent. These were subsequently subjected to a plate count agar to determine the total bacterial count, and to starch-ampicillin agar, a selective medium for *Aeromona hydrophila.*

Incubation lasted for 24 hours, at 28°C for the plate with the selective medium and at 37°C for the plates containing the plate count agar. After incubation, the colonies in the selective medium were flooded with 5 ml lugol iodine solution. Amylase-positive colonies appeared with a clear zone surrounding the colonies, indicating the presence of *A. hydrophila*. When measured, the colonies were typically 3-5 mm in diameter and were yellow to honey in color. Confirmatory tests for *A. hydrophila* were made with the use of API 20E strips, the oxidase test and the catalase test.

## RESULTS

Results showed that samples for freshwater fish like mudfish, tawilis, catfish, tilapia and gourami with ulcerations taken from Laguna Lake during the fish disease (Kurikong) outbreak were positive for *Aeromonas hydrophila* and had a higher bacterial load as compared to uninfected samples shown in Table 1 and 2. Two of the marine fish samples bisugo (*Nemipterid* sp.) and tuna (*Thunnus abessus*) were also positive for *A. hydrophila.*

This might be due to contamination brought about by pollution in the area where the fish were taken.

Biochemical tests using API 20E System on isolates suspected for *A. hydrophila* are shown in Table 3.

However, total bacterial count for the marine species were still on the acceptable limit for normal fresh fish compared to the infected samples which were too numerous to count at the $10^4$ dilution.

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Table 1. **Presumptive *Aeromonas hydrophila* count and total plate count at 28°C and 37°C for freshwater fishes**

| | *A. hydrophila* 28°C | | TPC 37°C | |
|---|---|---|---|---|
| **SAMPLE** | **Infected** | **Uninfected** | **Infected** | **Uninfected** |
| Tawilis | TNTC x $10^3$ | No sample | TNTC x $10^4$ | No sample |
| Mudfish | TNTC x $10^3$ | 3.0 x $10^3$ | TNTC x $10^4$ | 6.2 x $10^4$ |
| Catfish | TNTC x $10^3$ | 7.0 x $10^2$ | TNTC x $10^4$ | 9.1 x $10^4$ |
| Gourami | 2.8 x $10^4$ | 2.0 x $10^3$ | TNTC x $10^4$ | No sample |
| Tilapia | 4.0 x $10^3$ | 1.9 x $10^3$ | 1.9 x $10^4$ | 4.2 x $10^4$ |

Table 2. ***A. hydrophila*** **and total plate count at 28°C and 37°C for marine fishes**

| SAMPLE | *A. hydrophila* 28°C | TPC 37°C |
|---|---|---|
| Tulingan | ( - ) | $3.8 \times 10^3$ |
| Big-eyed scad | ( - ) | $1.0 \times 10^4$ |
| Roundscad | ( - ) | $8.4 \times 10^4$ |
| Milkfish | ( - ) | $1.3 \times 10^4$ |
| Bisugo | $3.2 \times 10^3$ | $5.5 \times 10^5$ |
| Tuna | $2.6 \times 10^4$ | $8.0 \times 10^5$ |
| Caesio | ( - ) | $6.3 \times 10^4$ |
| Crevalle | ( - ) | $5.2 \times 10^4$ |
| Short-bodied (Mackerel) | ( - ) | $6.5 \times 10^5$ |

Table 3. **The API 20E biochemical test for freshwater fish and marine fish revealed the positive presence of *Aeromonas hydrophila***

| Biochemical test | Freshwater fish | | | | Marine fish | | | |
|---|---|---|---|---|---|---|---|---|
| | Standard Reaction | Mudfish | Tawilis | Catfish | Tilapia | Gourami | Bisugo | Tuna |
| ONPG | + | + | - | + | + | - | + | + |
| Arginine dihydrolase | + | + | + | + | + | + | + | + |
| Lysine decarboxylase | + | + | + | + | + | + | - | + |
| Ornithine decarboxylase | - | - | - | - | - | - | - | - |
| Citrate | - | - | - | - | - | - | - | - |
| H2S | - | - | - | - | - | - | - | - |
| Urease | - | - | - | - | - | - | - | - |
| Tryptophane deaminase | - | - | - | - | - | - | - | - |
| Indole | + | + | + | + | - | + | + | + |
| Voges proskauer | + | + | - | + | + | + | + | + |
| Gelatinase | + | + | + | + | + | + | + | + |
| Glucose | + | + | + | + | + | + | + | + |
| Mannitol | + | + | + | + | + | + | + | + |
| Inositol | - | - | - | - | - | - | - | - |
| Sorbitol | - | - | - | - | - | - | - | - |
| Rhamnose | - | - | - | - | - | - | + | - |
| Saccharose | + | + | + | + | - | + | - | - |
| Melibiose | - | - | - | - | - | - | - | - |
| Amygdalin | - | - | - | - | - | - | - | + |
| Arabinose | - | + | + | - | - | - | + | + |
| Oxidase | + | + | + | + | + | + | + | + |