BACTERIOLOGICAL SURVEY OF FRESHWATER FISHES OF THE TENSAW RIVER, ALABAMA

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INTRODUCTION

The study was undertaken to determine the incidence of systematic bacteria infection which would be present in an assumed healthy natural fish population. Basic knowledge of bacteria infections in healthy fish populations is limited and it is hoped that this work will contribute to a better understanding of the problem.

In North America, several microbiological surveys were made utilizing hatchery fish (Bullock and Snieszko, 1969; Evelyn and McDermott, 1961) and natural populations (in part, Evelyn and McDermott, 1961; Rabb and McDermott, 1962). Two notable European papers, Bisset (1948) and Van der Struik (1965) dealt with characteristic European faunas. Earlier relevant literature was adequately reviewed by these papers, especially the works of Evelyn and McDermott (1961) and Van der Struik (1965).

The present authors sampled fish from apparently healthy fauna of the Tensaw River Drainage, Alabama. This survey differed from others in that it was directed toward healthy non-salmonid fish populations.

METHODS

Beef Heart Infusion Agar\(^2\) (BHI) and Sabouraud Dextrose Agar\(^2\) (SDA) were used for the isolation of bacteria and fungi.

The fish kidney was chosen as the organ for sampling since it was demonstrated in several studies to usually contain bacteria if bacteria are present in other organs (Van der Struik, 1965). In addition, the kidney is readily accessible in dissection with reduced chance of gut contamination.

A 10-ft seine was used in ten of the eleven collections. One collection was made by the use of rotenone and all collections were made during November and December of 1969. Several habitat types were sampled including river, creek, slough, and estuary.

In all collections, fishes were taken directly from the water and placed alive on ice. Care was taken to ensure that the fishes were not exposed to water that collected at the bottom of the ice containers on the way to the laboratory. All fishes were examined in the laboratory.

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\(^2\)Difco Laboratories, Inc., Detroit, Michigan.
with in 10 hr of capture.

Upon examination, an attempt was made to select as many fish species as possible from each collection. Two hundred fishes were examined representing 41 species. Fishes were taken directly from ice and disinfected externally by swabbing with 95% alcohol. The body cavity was opened and a portion of the kidney tissue was removed aseptically and streaked on BHI plates. A portion (1-3 mm³) of tissue was used to lessen the possibility of missing bacteria that might be present in small numbers. In all examinations special precautions were taken to be sure that no cuts in the alimentary canal occurred during dissection.

Twenty-four-hour cultures of the isolates were characterized as follows:

<table>
<thead>
<tr>
<th>Gram stain</th>
<th>Oxidative fermentative glucose</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(Hugh and Leifson, 1953)</td>
</tr>
<tr>
<td>Motility</td>
<td>H2S production</td>
</tr>
<tr>
<td>Morphology of slant</td>
<td>Starch hydrolysis</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>2,3 butanediol (Bullock, 1961)</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>Elaboration of fluorescin</td>
</tr>
<tr>
<td></td>
<td>(Kovacs, 1956)</td>
</tr>
<tr>
<td></td>
<td>(Bacto-Pseudomonas Agar F²)</td>
</tr>
</tbody>
</table>

If the isolate was Gram negative, motile, short rod, and demonstrated a positive cytochrome oxidase test, it was presumed to be a pseudomonad. Furthermore, bacteria that hydrolyzed starch, produced hydrogen sulfide, were oxidative and fermentative in the glucose medium, and produced 2, 3 butanediol were considered *Aeromonas liquefaciens*. Bacteria that did not hydrolyze starch, did not produce hydrogen sulfide, were not fermentative in the glucose medium, and did not produced 2,3 butanediol were designated *Pseudomonas*.

If the isolate exhibited a negative cytochrome oxidase test it was further subjected to the following tests:

| Flagella stain (Novel, 1939)       | Kligler's iron                                                     |
| Gluconate                           | Mannitol fermentation                                              |
| Catalase                            | Sucrose fermentation                                               |
| Nitrate                             | Latose fermentation                                                |
| Urease                              | Maltose fermentation                                               |
| Simmons citrate                     | Glucose fermentation                                               |
| Voges-Proskauer                     | Litmus milk                                                        |
Methyl red     Nutrient gelatin
Indol-Nitrate     Lysine decarboxylase

RESULTS

Of 200 fishes examined, 40 (20%) were positive for bacteria (Table 1). Of the 40 fishes infected, only five were infected with more than one bacterial species with a maximum of three in one of the fishes. No fungi were isolated.

Of the 46 cultures obtained, 30 were initially assigned to *Pseudomonas* or *Aeromonas* by the first set of tests mentioned above (Table 2). Of these, five were presumptively identified as *Aeromonas liquefaciens* (=*Aeromonas liquefaciens*) and although special medium was used for the detection of *Pseudomonas flourescens*, no *P. flourescens* was found.

Table 1. Incidence of bacteria in hosts.

<table>
<thead>
<tr>
<th>Result</th>
<th>Number of hosts</th>
<th>Percent of hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Positive with two or more</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>bacterial species per host</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>160</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 2. Identification of cultures.

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Number of cultures</th>
<th>Percent of cultures(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em></td>
<td>26</td>
<td>57</td>
</tr>
<tr>
<td><em>Aeromonas</em></td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Unidentified</td>
<td>160</td>
<td>32</td>
</tr>
</tbody>
</table>

\(^a\)Nearest whole percent.

After the initial sorting, 13 of the remaining cultures were subjected to the second set of tests outlined above. The cultures of this group were not initially given the identity of pseudomonads because they exhibited a negative cytochrome oxidase test. Despite the fact that the isolates were monotrichous Gram negative rods and possessed
other pseudomonad favored characteristics, the established importance of the oxidase test in characterizing pseudomonads cannot be ignored. These cultures thus remain unidentified.

**DISCUSSION AND CONCLUSIONS**

The data obtained in this study indicate a lower incidence of systemic bacterial infection than other surveys of healthy fishes. The data agree most closely with those of Bullock and Snieszko (1969) in which apparently healthy hatchery fishes were sampled and those of Bisset (1948) in which wild fishes were sampled, but are much lower than incidences found in other similar studies of hatchery and natural populations (Evelyn, 1960; Evelyn and McDermott, 1961; Rabb and McDermott, 1962; and Van der Struik, 1965).

Two common, warmwater fish pathogens (*A. liquefaciens* and *P. flourescens*) were expected to be encountered in this survey. These two species are commonly encountered in Southeastern U.S. fish kills and were reported in high incidence in surveys of Van der Struik (1965) and Bullock and Snieszko (1969). The *Pseudomonas* isolates reported here, however, did not produce fluorescence which is considered a characteristic for *P. flourescens*. *A. liquefaciens* was isolated in relatively few instances (2.5%) when compared to the other published works.

The fact that no fungi were isolated was indicative that apparently healthy fish do not carry fungal infections in the kidney.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


