Certain aquatic bacteria associated with western Montana waters

Richard Gaylord Raymond

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CERTAIN AQUATIC BACTERIA
ASSOCIATED WITH WESTERN MONTANA WATERS

by

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B.A., Montana State University, 1951

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of the requirements for the degree of
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R. G. R.
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INTRODUCTION

Man, in his study of microorganisms, has turned most of his attention to those forms of a pathogenic nature. Little consideration has been given bacterial populations from an ecological standpoint. Ferdinand Cohn (12) in 1872 first clearly expressed the important part played by microorganisms in the transformation of organic and inorganic substances on earth with the result that these may be used over and over again to sustain life of other organisms. It then becomes important to determine the characteristics of these microorganisms, not only as possible etiological agents of diseases, but also as portraying a significant role in the cycle of matter. The ultimate objective should stress the interrelationship of bacteria rather than the isolation and identification of specific species.

Water is the particular environmental situation considered in this study. The existence of aquatic bacteria has been known since 1675 when Antony van Leeuwenhoek observed them in rain water, well water, and sea water (6). The studies of aquatic bacteria up to the present time, however, have been concerned largely with determination of the suitability of water for human consumption. Little emphasis has been placed on the nature of the normal bacterial flora.
The literature is lacking in specific information concerning the normal flora of water. We find, however, that several workers have proposed the existence of certain genera and species. Frobisher (7) states that there is a large group of gram-negative rods, resembling the Enterobacteriaceae morphologically, rarely or never found in the intestinal tract, which live in soil and water as saprophytes. This group includes members of the genera Chromobacterium, Flavobacterium, Achromobacter, Pseudomonas, Agrobacterium, and Alcaligenes. Topley and Wilson (13) divide the water bacteria into three groups: Bacilli including the genera Pseudomonas, Chromobacterium, and Achromobacter; Cocci, with emphasis on the genus Micrococcus; and Sarcina, chiefly the species Sarcina lutea. Buchanan (3) postulates the presence of members of the genus Sarcina as well as other cocci producing pink, red, yellow, and orange pigments plus members of the Bacillus subtilis group, the latter depending largely on drainage. Jordan (8) considers some of the bacteria commonly found in natural water to be Pseudomonas fluorescens variations liquifaciens and non-liquifaciens, Bacillus subtilis, Bacillus mesentericus, Flavobacterium proteus, Aerobacter cloacae, Achromobacter liquifaciens, Chromobacterium violaceum, and many chromogenic and non-chromogenic micrococci. From the above it can be seen that there is common agreement among several workers concerning some of the genera and a few of the species associated with
natural water.

This investigation is involved principally with the gram-negative group of bacteria although several gram-positive cultures were also isolated. Through the determination of the types of bacterial forms isolated it is believed that an indication may be obtained as to a portion of the bacterial population that exists in the waters of Western Montana.

Included in the study was an original series of cultures isolated from several species of trout. The latter were either reared in hatcheries or taken from their natural aquatic habitat. The skin, gill region, body cavity, and kidney region of the fish were sources of many of these bacteria. This portion of the investigation was done in order to ascertain a possible relationship between those cultures isolated from fish and those found to occur in the immediate water.

The waters from which another series of bacteria was isolated included mountain streams, fresh-water lakes, and in one instance an artesian well. All of the sources were located in Western Montana. The areas of collection are shown in Figure I, page 5.

The original series of cultures was obtained from the following hatcheries; Anaconda, Arlee, Big Timber, Creston, Ennis, Great Falls, Lewistown, Somers, and the Harriman-Vaughn Hatchery located at Post Creek. Non-hatchery
sources included in the original series were Flathead Lake and Hebgen Lake. Subsequent series of collections were taken from the Arlee Hatchery, the Harriman-Vaughn Hatchery; and the waters of Post Creek, and McDonald Lake. The cultures were labelled according to their origin.

From the above sources a total of 184 cultures were isolated. It is with these cultures that this report is concerned. A dichotomous type of classification, with the cultural characteristics as a basis, was proposed as a means of determining the relationship between the organisms as well as ascertaining their possible taxonomic position according to described genera and species.
Figure 1.
Map Showing Areas of Collections

-5-

- Collections taken from fish and water.
- Collections taken from water only.
CHAPTER I

TECHNIQUE

Materials

Media used included the following:

1. Tryptone agar as described by Ordal and Rucker (9). This medium was modified to include 0.05% $K_2HPO_4$ and 0.1% yeast extract. The final pH was 7.2.

2. Nutrient agar as described by Salle (10). This was enriched with 0.1% yeast extract. Tryptone, 0.4% was substituted for the peptone prescribed. The final pH was 7.2.


4. Infusion agar using Difco Veal Infusion base. This was modified to include 1.0% tryptose, 1.5% agar, and 0.5% glucose. The final pH was 7.3.

5. Carbohydrate media as described in Zinsser and Bayne-Jones (14). Phenol red was used as the indicator.

6. Nutrient broth, adjusted to a pH of 7.2.

7. Potato slants, prepared according to the formula in Zinsser and Bayne-Jones (14).

8. Certain other differential media as described in the Difco Manual (5).

Isolation and Identification Procedures

In the various habitat areas plates containing the modification of Ordal's media were streaked. These plates were incubated at 6°C and were observed until growth appeared,
which was usually between five and seven days.

From these original plates single colonies were streaked on a second series of plates. These were incubated at 25°C. Observations were made at 24 hours and 48 hours. From the second series of plates tryptone yeast extract agar slants were inoculated. These were incubated at 25°C for 24 hours and held at 6°C for future use as stock cultures. Gram stains were made on the bacteria from each type of colony at the time of original isolation and also on the stock cultures prior to use.

A series of physical and biochemical tests were performed. These determinations included; motility, gelatin liquifaction, cell morphology, indole production, nitrate reduction, reaction in litmus milk, fermenting capacity, growth on enriched medium, production of a fluorescent pigment, type of growth on agar slants, growth on potato slants, colony characteristics, chromogenesis on nutrient agar, and the type of growth in nutrient broth. With the exception of the growth on potato and the production of a fluorescent pigment, the determinations were conducted according to the procedures recommended by the Society of American Bacteriologists (4).

Growth on potato was determined by streaking slants prepared from potato strips sterilized in the autoclave. The production of a fluorescent pigment was ascertained by streaking aluminum touch plates containing tryptone yeast extract
agar. These were incubated at 25°C and exposed to ultraviolet light at 24 hour and 48 hour intervals. Figure 2 is a photograph showing production by bacteria of fluorescent pigments.

Each culture was subjected to this series of determinations three times or more.

After several trials within different temperature ranges, the temperature found to be optimum for growth was approximately 25°C. This was used throughout the experimental work.
Figure 2
Photograph illustrating the varying degrees of fluorescence produced by bacteria of the genus Pseudomonas.
### Table I

**Key to the Groups and Subgroups of Gram-Negative Bacteria Isolated**

<table>
<thead>
<tr>
<th>Group</th>
<th>Key Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><strong>Gram-negative.</strong></td>
</tr>
<tr>
<td>B</td>
<td>Motile.</td>
</tr>
<tr>
<td>C</td>
<td>Gelatin liquified.</td>
</tr>
<tr>
<td>D</td>
<td>Indole produced.</td>
</tr>
<tr>
<td>E</td>
<td>Nitrates reduced. <strong>Group I</strong></td>
</tr>
<tr>
<td>a</td>
<td>Acid produced in glucose, sucrose, and mannitol. <strong>(Subgroup A)</strong></td>
</tr>
<tr>
<td>b</td>
<td>Acid and gas produced in glucose and mannitol. <strong>(Subgroup B)</strong></td>
</tr>
<tr>
<td>c</td>
<td>Acid and gas produced in glucose, sucrose, and mannitol. <strong>(Subgroup C)</strong></td>
</tr>
<tr>
<td>EE</td>
<td>Nitrates not reduced. <strong>Group II</strong></td>
</tr>
<tr>
<td>a</td>
<td>Acid and gas produced in glucose, sucrose, and mannitol. <strong>(Subgroup A)</strong></td>
</tr>
<tr>
<td>DD</td>
<td>Indole not produced.</td>
</tr>
<tr>
<td>E</td>
<td>Nitrates reduced. <strong>Group III</strong></td>
</tr>
<tr>
<td>a</td>
<td>Those carbohydrates tested were not fermented. <strong>(Subgroup A)</strong></td>
</tr>
<tr>
<td>b</td>
<td>$\text{H}_2\text{S}$ produced. <strong>(Subgroup B)</strong></td>
</tr>
<tr>
<td>c</td>
<td>Acid produced in glucose. <strong>(Subgroup C)</strong></td>
</tr>
<tr>
<td>d</td>
<td>Acid produced in glucose and sucrose. <strong>(Subgroup D)</strong></td>
</tr>
<tr>
<td>EE</td>
<td>Nitrates not reduced. <strong>Group IV</strong></td>
</tr>
<tr>
<td>a</td>
<td>Those carbohydrates tested were not fermented. <strong>(Subgroup A)</strong></td>
</tr>
<tr>
<td>b</td>
<td>Acid produced in glucose. <strong>(Subgroup B)</strong></td>
</tr>
<tr>
<td>c</td>
<td>Acid produced in glucose and sucrose. <strong>(Subgroup C)</strong></td>
</tr>
<tr>
<td>CC</td>
<td>Gelatin not liquified.</td>
</tr>
<tr>
<td>D</td>
<td>Indole produced.</td>
</tr>
<tr>
<td>E</td>
<td>Nitrates reduced. <strong>Group V</strong></td>
</tr>
<tr>
<td>EE</td>
<td>Nitrates not reduced. <strong>Group VI</strong></td>
</tr>
<tr>
<td>DD</td>
<td>Indole not produced.</td>
</tr>
<tr>
<td>E</td>
<td>Nitrates reduced. <strong>Group VII</strong></td>
</tr>
<tr>
<td>a</td>
<td>Those carbohydrates tested were not fermented. <strong>(Subgroup A)</strong></td>
</tr>
<tr>
<td>b</td>
<td>Acid produced in glucose. <strong>(Subgroup B)</strong></td>
</tr>
<tr>
<td>c</td>
<td>Acid and gas produced in glucose and mannitol. <strong>(Subgroup C)</strong></td>
</tr>
<tr>
<td>d</td>
<td>Acid and gas produced in glucose, lactose, and mannitol. <strong>(Subgroup D)</strong></td>
</tr>
<tr>
<td>EE</td>
<td>Nitrates not reduced. <strong>Group VIII</strong></td>
</tr>
</tbody>
</table>
| a | Those carbohydrates tested were not...
fermented. (Subgroup A)
b. Acid produced in glucose. (Subgroup B)

BB. Non-motile.
C. Gelatin liquified.
D. Indole produced.
E. Nitrates reduced..........Group IX
   a. Those carbohydrates tested were not fermented. (Subgroup A)
   b. Acid produced in glucose and lactose. (Subgroup B)
   c. Acid produced in glucose and mannitol.
D. Indole not produced.
E. Nitrates reduced..........Group XI
   a. Acid produced in glucose. (Subgroup A)
   b. Acid produced in glucose and lactose. (Subgroup B)
   c. Acid produced in glucose, sucrose, lactose, and mannitol. (Subgroup C)
   d. Acid and gas produced in glucose, sucrose, and mannitol. (Subgroup D)
E. Nitrates not reduced......Group XII
   a. Those carbohydrates tested were not fermented. (Subgroup A)
   b. Acid produced in glucose. (Subgroup B)
   c. Acid produced in glucose and sucrose. (Subgroup C)
   d. Acid produced in glucose, sucrose, and mannitol. (Subgroup D)
   e. Acid produced in glucose, sucrose, and lactose. (Subgroup E)
   f. Acid produced in glucose, sucrose, lactose, and mannitol. (Subgroup F)
CC. Gelatin not liquified.
D. Indole produced.
E. Nitrates reduced..........Group XIII
   a. Acid produced in glucose and mannitol. (Subgroup A)
E. Nitrates not reduced......Group XIV
DD. Indole not produced.
E. Nitrates reduced..........Group XV
   a. Those carbohydrates tested were not fermented. (Subgroup A)
   b. Acid produced in glucose. (Subgroup B)
   c. Acid produced in glucose and mannitol. (Subgroup C)
   d. Acid produced in glucose, sucrose, lactose, and mannitol. (Subgroup D)
   e. Acid and gas produced in glucose and mannitol. (Subgroup E)
EE. Nitrates not reduced......Group XVI
a. Those carbohydrates tested were not fermented. (Subgroup A)
b. Acid produced in glucose. (Subgroup B)
CHAPTER II

RESULTS

A dichotomous type of approach was used to delineate various major groups. The characteristics utilized in the division of the cultures into these major groups were those which appeared to show the least degree of variation. These included; motility, gelatin liquifaction, indole production, and nitrate reduction. Subgroups were designated then according to the ability of the cultures to ferment sugars. This method resulted in the formation of 16 major groups and 39 subgroups as illustrated by the Table on page 10.

With this key as a basis, an attempt was made to establish the organisms in known genera. Two methods were used to accomplish this objective. The Mechanical Key for the Generic Identification of Bacteria, as proposed by Skerman (11) was utilized in placing the cultures, by subgroups, into their proper genera. Following this, both the fifth and sixth editions of Bergey's Manual of Determinative Bacteriology (1)(2) were consulted to ascertain a possible correlation between the present bacterial groups and described species.

Group I ..........(12 cultures)

Subgroup A ....(10 cultures)

1. Geographical distribution: Arlee, Harriman-Vaughn Hatchery, Post Creek, and McDonald Lake.
2. Source: Head lesion, gill region, and kidney of rainbow trout, gill lesion of a brook trout, and water.


4. Similarity to described species: \textit{Pseudomonas hydrophila}.

5. Habitat of described species: Water and infected fresh water animals.

\textbf{Subgroup B} \ldots (1 culture)


3. Generic classification: \textit{Achromobacter}.

4. Similarity to described species: This culture differs from the majority of the described species in this genus by virtue of the production of indole; otherwise it manifests like characteristics especially to \textit{Achromobacter delicatulum}.

5. Habitat of described species: Isolated from water and presumably widely distributed in nature.

\textbf{Subgroup C} \ldots (1 culture)


2. Source: Gill lesion of a brook trout.


4. Similarity to described species: With the exception of gelatin liquification this culture resembles \textit{Proteus rettgeri}.
5. Habitat of described species: Fowl typhoid and some cholera-like diseases of birds. It is quite possible that this culture is a representative of the gram-negative group discussed by Frobisher.

Group II ............ (1 culture)

Subgroup A ...... (1 culture)

2. Source: Gill lesion of a brook trout.
4. Similarity to described species: This organism's characteristics would seem to place it as an intermediate between Proteus mirabilis and Proteus rettgeri since it produces $\text{H}_2\text{S}$ as well as acid and gas in mannitol.
5. Habitat of described species: Putrefying materials in the case of Proteus mirabilis and fowl and some cholera-like diseases of birds in the case of Proteus rettgeri.

Group III ............ (12 cultures)

Subgroup A ...... (1 culture)

4. Similarity to described species: Pseudomonas jaegeri.
5. Habitat of described species: Water.
Subgroup B ......(5 cultures)
2. Source: Gill region of a rainbow trout and the skin, gill lesion, and kidney of brook trout.
4. Similarity to described species: One culture was found to resemble Pseudomonas ureae while the others bore resemblance to Xanthomonas panici.
5. Habitat of described species: Pseudomonas ureae is associated with sewage filter beds while Xanthomonas panici is pathogenic on proso millet, Panicum miliaceum. The latter was isolated from water soaked lesions on leaves, sheaths, and culms of millet collected in Wisconsin and in South Dakota. It would not seem unreasonable, therefore, to assume that these forms are associated with water.

Subgroup C ......(4 cultures)
1. Geographical distribution: Lewistown, Post Creek, and McDonald Lake.
2. Source: Gill region of a rainbow trout, body cavity of a brook trout, and water.
3. Generic classification: Although these organisms shared the same group characteristics and fermentative ability they were found to belong to four
different genera. These were; *Pseudomonas*, *Chromobacterium*, *Achromobacter*, and *Cytophaga*. The latter genus belongs to the family *Cytophagaceae* under the order *Myxobacteriales* and will not be treated extensively since this investigation is concerned principally with the order *Eubacteriales*. Members of the genus *Cytophaga* will be mentioned as they were found to occur in the various groups.

4. Similarity to described species: *Pseudomonas fluorescens*, *Chromobacterium violaceum*, and *Achromobacter iophagum*. The latter liquified gelatin only slightly and might possibly belong in the genus *Pseudomonas* where it resembled *Pseudomonas desmolyticum*. The member of *Cytophaga* exhibited the type of growth characteristic of that genus.

5. Habitat of described species: Water and Soil.

**Subgroup D** .... (1 culture)

2. Source: Skin of a brook trout.
4. Similarity to described species: *Achromobacter iophagum*.
5. Habitat of described species: Soil.

**Subgroup E** .... (1 culture)

2. Source: Body cavity of a cutthroat trout.

4. Similarity to described species: Other than the production of a fluorescent pigment which is characteristic of the genus *Pseudomonas* this culture does not resemble any of the described species of either *Pseudomonas* or related genera. It is possible, therefore, that this is either a new species or a variant of some species such as *Pseudomonas hydrophila* as discussed under Group I, Subgroup A.

Group IV .......... (28 cultures)

Subgroup A .... (5 cultures)


2. Source: Skin and gill region of a rainbow trout, skin of a brook trout, and water.

3. Generic classification: *Pseudomonas* and *Achromobacter*.

4. Similarity to described species: *Pseudomonas jaegeri* and *Achromobacter liquifaciens*.

5. Habitat of described species: Water.

Subgroup B .... (22 cultures)


2. Source: Gill region of a brook trout.


4. Similarity to described species: *Pseudomonas viscosa* and *Achromobacter liquifaciens*. 
5. Habitat of described species: Water.

It should be noted that twenty of the twenty-two cultures in this subgroup produced fluorescent pigments ranging from blue to yellow-green. Such pigments are an outstanding characteristic of the genus *Pseudomonas*. It is in this subgroup that most of the fluorescent forms were located.

**Subgroup C ...... (1 culture)**

2. Source: Gill region of a brook trout.
4. Similarity to described species: *Pseudomonas septata*.
5. Habitat of described species: Pathogenic for sugar beets. This study would seem to determine it as a water form.

**Groups V and VI ....** There were no cultures that possessed the characteristics of these two groups.

**Group VII ........ (18 cultures)**

**Subgroup A ...... (10 cultures)**

2. Source: Skin of rainbow trout, skin of cutthroat trout, gill region and body cavity of brook trout, and water.
3. Generic classification: *Chromobacterium, Pseudo-*
monas, and Achromobacter.

4. Similarity to described species: Chromobacterium violaceum, Pseudomonas putida, and Achromobacter cycloclastes. The Chromobacterium varies from the described species in the liquefaction of gelatin and the production of acid in glucose. The production of a violet pigment, however, is strongly indicative of the genus Chromobacterium.

5. Habitat of described species: Water and soil.

Subgroup B ....(4 cultures)

2. Source: Kidney of a rainbow trout and water.
4. Similarity to described species: There is little similarity to the species in the genus Achromobacter but a resemblance does exist to Pseudomonas desmolyticum. Since Skerman's key does not provide for the non-fluorescent Pseudomonads it is quite probable that some of the cultures which would be placed under the genus Achromobacter should actually belong in the genus Pseudomonas.

5. Habitat of described species: Soil.

Subgroup C ....(3 cultures)

2. Source: Skin of a rainbow trout and the skin and body cavity of cutthroat trout.
4. Similarity to described species: *Salmonella paratyphi A*.
5. Habitat of described species: A natural pathogen of man causing enteric fever. While the cultures in this subgroup were found to resemble *Salmonella paratyphi A* it is more probable that they comprise a portion of the gram-negative group which, while it bears a similarity to the family *Enterobacteriaceae*, is composed of saprophytic forms as suggested by Frobisher.

**Subgroup D** ....(1 culture)

2. Source: Kidney of a brook trout.
4. Similarity to described species: *Escherichia intermedium*.
5. Habitat of described species: Water.

**Group VIII** ........(27 cultures)

**Subgroup A** ....(17 cultures)

2. Source: Skin and gill region of rainbow trout, skin of brook trout, body cavity of cutthroat trout, gill region of salmon, and water.

4. Similarity to described species: Pseudomonas convexa, Flavobacterium invisible, and Achromobacter superficiale.

5. Habitat of described species: Water in the case of Pseudomonas convexa and Flavobacterium invisible, wide distribution in nature in the case of Achromobacter superficiale.

Subgroup B .... (10 cultures)

1. Geographical distribution: Arlee, Post Creek, and McDonald Lake.

2. Source: Gill region of rainbow trout, skin and kidney of brook trout, body cavity of cutthroat trout, and water.


4. Similarity to described species: Pseudomonas ovalis and Achromobacter superficiale. The latter also resembles Flavobacterium lactis but is more accurately placed in the genus Achromobacter.

5. Habitat of described species: Pseudomonas ovalis is found in soil while Achromobacter superficiale is found in water.

Group IX ...............There were no cultures that possessed the characteristics of this group.
Group Z ...........(4 cultures)

Subgroup A ....(1 culture)
2. Source: Skin of a rainbow trout.
3. Generic classification: Flavobacterium or possibly Xanthomonas.
4. Similarity to described species: The production of indole, in which respect this culture is positive, is not common in the genus Flavobacterium although its other characteristics are indicative of the genus in general. It should be noted, however, that yellow pigmented forms are also found in the genus Xanthomonas where indole production is not uncommon.
5. Habitat of described species: Soil and water. This is the common habitat of the genus Flavobacterium.

Subgroup B ....(2 cultures)
2. Source: Skin and gill region of rainbow trout.
3. Generic classification: Flavobacterium or Xanthomonas.
4. Similarity to described species: Here, as in Subgroup A, the cultures belong, according to Skerman, in the genus Flavobacterium which the formation of indole deems questionable. It seems probable that the genus Xanthomonas provides a more logical generic disposition.
5. Habitat of described species: Soil and water as stated for Subgroup A.

Subgroup C ....(1 culture)
2. Source: Gill region of rainbow trout.
3. Generic classification: Flavobacterium or Xanthomonas.
4. Similarity to described species: With the exception of the possible formation of acid in lactose, this culture is closely allied with the members of Subgroup B.

5. Habitat of described species: Soil and water as stated for Subgroup A. Because the members of this group were isolated from essentially the same geographical area it would seem reasonable that they may represent variant strains of a single type differing only slightly in their action on carbohydrates.

Group XI ............(8 cultures)

Subgroup A ....(2 cultures)
1. Geographical distribution: Arlee and McDonald Lake.
4. Similarity to described species: These cultures exhibited the yellow, granular, spreading-type growth as well as the cell morphology which is character-
istic in the genus Cytophaga.

5. Habitat of described species: Soil is the common habitat of the genus.

Subgroup B ...(2 cultures)
1. Geographical distribution: Arlee and McDonald Lake.
2. Source: Gill region of a rainbow trout and water.
4. Similarity to described species: Flavobacterium lutescens is representative of one of the cultures while the other, which, according to Skerman, belongs in the genus Achromobacter shows no species similarity but does exhibit characteristics typical of the genus. It may be assumed that it is an undescribed species of Achromobacter.

5. Habitat of described species: Water and soil.

Subgroup C ...(2 cultures)
2. Source: Skin and gill region of rainbow trout and water.
4. Similarity to described species: Micrococcus conglomeratus resembles one of the cultures while the other exhibited the previously mentioned characteristic growth of the genus Cytophaga.
5. Habitat of described species: Water and soil.

Subgroup D ....(2 cultures)


2. Source: Gill region of a rainbow trout and the kidney of a brook trout.


4. Similarity to described species: It would seem that these cultures probably belong to the gram-negative group which resembles the members of the family Enterobacteriaceae and which have not been specifically described.

Group XII ..........(25 cultures)

Subgroup A ....(7 cultures)

1. Geographical distribution: Great Falls, Big Timber, Arlee, and Post Creek.

2. Source: Gill region and kidney of a rainbow trout, skin and body cavity of a brook trout, and the skin and gill region of a cutthroat trout.


4. Similarity to described species: The production of a fluorescent pigment on the part of one of the cultures is indicative of the genus Pseudomonas although its other characteristics do not bear out its relationship to this genus. There is, however, some resemblance to members of the genus Flavo-
bacterium, described as water forms, e.g., *Flavobacterium helvolum*. The latter as well as *Flavobacterium desidiosum* are similar to several other members of this subgroup. One culture resembles the characteristics ascribed to the genus *Achromobacter* but none of the species. The remainder of this subgroup exhibit the type of growth and cell morphology characteristic of the genus *Cytophaga*.

5. **Habitat of described species**: Water and soil.

**Subgroup B** (5 cultures)

1. **Geographical distribution**: Somers, Arlee, and McDonald Lake.
2. **Source**: Gill region of rainbow and cutthroat trout and water.
3. **Generic classification**: *Flavobacterium*.
4. **Similarity to described species**: *Flavobacterium arborescens*.
5. **Habitat of described species**: Water.

**Subgroup C** (2 cultures)

1. **Geographical distribution**: Harriman-Vaughn Hatchery and Arlee.
2. **Source**: Skin and gill region of a rainbow trout and water.
3. **Generic classification**: *Flavobacterium* and *Cytophaga*.
4. **Similarity to described species**: *Flavobacterium*
dormitator or Flavobacterium balustinum in the case of one culture. The other culture, like those previously discussed, would be logically placed in the genus Cytophaga.

5. Habitat of described species: Skin of fish, water, and soil.

Subgroup D ....(3 cultures)
2. Source: Gill region of rainbow and cutthroat trout and water.
4. Similarity to described species: Flavobacterium arborescens. There is a variance between these cultures and Subgroup B in fermenting capacity. With the exception of glucose, the fermentation of the other sugars was not definite, but rather suggestive.
5. Habitat of described species: Water.

Subgroup E ....(1 culture)
2. Source: Gill region of rainbow trout.
4. Similarity to described species: Flavobacterium arborescens.
5. Habitat of described species: Water.
Subgroup F  ....(7 cultures)
2. Source: Skin, gill region, and body cavity of cutthroat trout and water.
4. Similarity to described species: Flavobacterium arborescens resembles two of the cultures while the other five exhibited the type of growth typical of the genus Cytophaga.
5. Habitat of described species: Soil and water.
   Again there is a definite similarity between this subgroup and Subgroup B with the exception of fermenting capacity.

Group XIII ..........(1 culture)

Subgroup A ......(1 culture)
4. Similarity to described species: Pasteurella multocida.
5. Habitat of described species: The cause of hemmorrhagic septicemia in birds and mammals.

Group XIV ..........There were no cultures that possessed the characteristics of this group.
Group XV ...........(15 cultures)

Subgroup A .......(8 cultures)
2. Source: Gill region and body cavity of cutthroat trout and water.
4. Similarity to described species: Flavobacterium maris and Flavobacterium flavotennae.
5. Habitat of described species: Flavobacterium maris was isolated from living halibut obtained at 30 to 50 fathoms, Pacific ocean, while Flavobacterium flavotennae is associated with wound infections in frogs. The habitat is unknown for both.

Subgroup B .......(3 cultures)
2. Source: Gill region and body cavity of a brook trout and water.
4. Similarity to described species: Flavobacterium maris resembles one of the cultures while the remainder exhibit characteristics typical of the genus Achromobacter with no emphasis on a specific species. The correlation here is clearer than in the previous subgroup because of the production of
acid in glucose which is described by Bergey as being faint.

5. Habitat of described species: The same as Subgroup A with respect to Flavobacterium maris and salt to fresh water as well as soil in the case of Achromobacter.

Subgroup C ....(1 culture)
4. Similarity to described species: Achromobacter ubiquitum.
5. Habitat of described species: Water.

Subgroup D ....(1 culture)
2. Source: Gill region of a brook trout.
4. Similarity to described species: Achromobacter delmarvae.
5. Habitat of described species: Unknown.

Subgroup E ....(2 cultures)
2. Source: Kidney of a brook trout and water.
4. Similarity to described species: With the exception
of motility there is a resemblance to *Salmonella cholerasuis*. It is probable that these two cultures also belong to the gram-negative group which is similar to the family *Enterobacteriaceae* and that they are saprophytic inhabitants of water.

5. Habitat of described species: This species is associated with hog cholera as a secondary invader and occasionally gives rise to acute gastro-enteritis in man.

Group XVI (16 cultures)

Subgroup A (14 cultures)


2. Source: Skin and gill region of rainbow, cutthroat, and brook trout as well as water.

3. Generic classification: *Flavobacterium*, *Achromobacter*, and *Cytophaga*.

4. Similarity to described species: Five cultures bore resemblance to *Flavobacterium aquatile*, seven were found to be similar to *Achromobacter candidans* while the remaining two exhibited the characteristic type of growth and cell morphology of the genus *Cytophaga*.

5. Habitat of described species: Water and soil.

Subgroup B (2 cultures)

1. Geographical distribution: Harriman-Vaughn Hatchery
and McDonald Lake.

2. Source: Gill region of a rainbow trout and water.


4. Similarity to described species: *Flavobacterium ovale*.

5. Habitat of described species: Water.

In addition to the above, fourteen gram-positive organisms were isolated during this study and while some might possibly be associated with water or fish, others very probably constituted contamination, either in the field or in the laboratory. In conjunction with Buchanan's supposition, it may be assumed that those cultures belonging in the genus *Sarcina* were the only ones associated with water.

Three yeast cultures were also isolated although no attempt was made toward their identification.
CHAPTER III

DISCUSSION

The gram-negative group comprised approximately 90% of the total cultures investigated and was found to be composed of several genera. Most common were the genera Achromobacter and Flavobacterium. These were found to be associated with fifteen groups each. In the order of their frequency of occurrence members of the following genera also were found to be represented: Pseudomonas, Cytophaga, Salmonella, Chromobacterium, Proteus, Xanthomonas, Escherichia, Micrococcus, and Pasteurella. It is quite possible that some discrepancy might exist due to the fact that the key proposed by Skerman does not acknowledge the non-fluorescent members of the genus Pseudomonas which could result in the erroneous placement of some cultures in the genus Achromobacter. All of the organisms placed, according to the key, in the genus Achromobacter were, however, compared with the described non-fluorescent species of Pseudomonas. Barring the existence of undescribed species in the latter genus, the consideration of both genera in the case of doubtful organisms should have compensated for the incompleteness of Skerman's key.

A similar situation exists between certain representatives of the Flavobacterium and certain of the myxobacteria. According to Jeffers (Unpublished) members of the myxobacteria have been found to exhibit marked variation.
which under certain conditions results in a type of growth not dissimilar to that shown by members of the genus *Flavobacterium*. It would seem reasonable to assume, therefore, that some cultures, placed by the author in the genus *Flavobacterium*, might be members of the genus *Cytophaga*. A specific case in which this is true is the striking similarity between *Flavobacterium arborescens* and certain members of the genus *Cytophaga*. It is interesting to note that those organisms belonging to the genus *Cytophaga* were often inhibited on enriched veal infusion media while the growth of the *Flavobacterium* was enhanced.

It would seem that the most common bacterial forms associated with water are gram-negative rods displaying a rather wide range of variation with respect to length and width but sharing a common morphological diplo-form. Approximately 50% of the organisms were found to be motile. Also, the majority of the cultures were negative with respect to indole production and reduction of nitrates. Nitrate reduction was found to be more common than indole production. This follows the generic descriptions given by Bergey. The production of fluorescent pigments was not as prevalent as was expected considering the number of *Pseudomonas* species isolated. The fermentation of carbohydrates, especially glucose, was quite common. The production of gas, however, was limited largely to the motile organisms.

The isolation of a culture resembling *Pasteurella*
multocida was of interest because of work done by Fishel in 1952 (Unpublished) and Jeffers in 1953 (Unpublished) in this laboratory. Both workers found this organism to be associated with disease in animals located in the area surrounding the source of the culture isolated during this study.

With the exception of the Salmonella-like organisms and the culture resembling Xanthomonas panici, the cultures seemed to occur with about equal frequency from fish and water indicating that in many respects, the normal bacterial flora associated with fish and water is similar.
SUMMARY AND CONCLUSIONS

1. One hundred eighty-four cultures were isolated from sources including both fish and water. Of these organisms, 90% were gram-negative, 53% were motile, 10% produced indole, 36% reduced nitrates, 50% liquified gelatin, and 20% produced fluorescent pigments.

2. The gram-negative group was classified according to described genera and species. The genus *Achromobacter* and the genus *Flavobacterium* were most common with the genus *Pseudomonas* next in frequency of occurrence. Several other genera were also represented.

3. In the area studied there was a correlation between the organisms associated with fish and those associated with water.
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