The bacterium *Flavobacterium aquatile* (Frankland and Frankland) Bergey et al. was isolated and described as *Bacillus aquatile* (Frankland and Frankland, 1889). The species was selected by Bergey et al. (1923) as the type for the genus *Flavobacterium*. With the exception of studies by Gary (1950) the genus appears to have received very little systematic investigation. If the type concept of taxonomy is followed, then the type species should play an important role in any arrangement of a genus. The studies which were made by Gary, and those which are reported here, make it apparent that the cultures which are in circulation as *F. aquatile* cannot be accepted as such. These cultures, together with others considered as possible representatives of the type species, form a heterogeneous group. Further, the original description of *B. aquatilis*, which has been emended very little, is of such nature that heterogeneity is more probable than ready recognition.

**Original description of Bacillus aquatilis.** The Franklands (1889) recognized *B. aquatilis* as a slender, rod-shaped bacterium about 2.5 μ long. Filamentous structures 17 μ in length were reported, and endospores were not seen. Differentiation of the species from similar forms found in water was accomplished on the basis of the type of motion shown, the appearance of colonies upon gelatin plates, and the slowness with which the organisms grew. Motility in a hanging drop preparation was not observed; instead the authors described an oscillatory movement for both individual cells and filaments, with many of the latter showing no observable motion. Colonies on gelatin plates developed slowly and were yellow-brown. Some surface colonies gave a characteristic appearance due to the extension of twisted filaments from a reddish granular center. A slow liquefaction of gelatin occurred. Growth upon agar appears to have been filiform and yellow in color. Beyond these characteristics, which were regarded as major ones in the differentiation of *B. aquatilis* from *B. arborescens*, the authors mentioned only a few cultural properties. Thus, a scanty yellow growth occurred upon potato, broth cultures were turbid but without a pellicle, and nitrates disappeared without the formation of nitrites. Other workers have added little to the original description of this species (Breed et al., 1948). It is to be noted that the Franklands’ study was made for the purpose of differentiating cultures of bacteria which they found in water and soil and that descriptive characters were selected on this basis.

Tataroff (1891) reported that *B. aquatilis* formed endospores. In the same publication he described an organism, *B. aquatilis alpha*, which he regarded as similar to *B. aquatilis* except that the surface colonies on nutrient gelatin were lobate and the growth upon an agar slope, mucoid. Frankland and Frankland (1894) accepted *B. aquatilis alpha* as being the same as their *B. aquatilis* but made no mention of Tataroff’s claim that *B. aquatilis* produced endospores. Chester (1897) and Migula (1900) assigned the species to the genus *Bacterium* indicating lack of endospores. Migula also proposed the name *Bacterium pseudoaquatile* for *Bacillus aquatilis alpha* Tataroff. Apparently no alterations in the original description were made, and no reference to reisolation, until the first edition of *Bergey’s Manual* (Bergey et al., 1923). At this time the species was selected as the type for the newly formed genus *Flavobacterium*. The description was emended, and the species characterized as gram negative, nitrate reducing, and peritrichate. This description was continued until *Bergey’s Manual* reached the sixth edition (Breed et al., 1948). Here the species resumed its original characterization. Throughout most of the de-

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1 Published with the approval of the Director, Idaho Agricultural Experiment Station, as Research Paper No. 384.
scriptions *F. aquatile* has been identified by its apparent lack of motility, the nature of the colony upon gelatin, yellow pigmentation, and the slow development and limited growth upon most media.

**CULTURES AND METHODS**

The cultures assembled for this study came from several sources (table 1). Cultures F3, F25, and F35 were separate transplants of the strain which is carried as *F. aquatile* in the American Type Culture Collection. Originally these strains were sent to the American Type Culture Collection from the Department of Bacteriology, Ohio State University, which had obtained them from C. D. Kelly who, in turn, received them from E. W. Taylor, London, as possible strains of *F. aquatile*. Similarly, cultures F9 and F10 were obtained by N. D. Gary, Indiana University, from Dr. Taylor. Culture F36 was a recent isolation by Dr. Taylor who went to the habitat from which the Franklands originally isolated *B. aquatile* to obtain the culture. The strain was received as a viable transplant. The last culture of the collection, F39, was a transplant of the species which Jensen and Petersen (1952) isolated from soil as a decomposer of 2,4-dichlorophenoxyacetic acid (2,4-D) and which they tentatively identified as *F. aquatile*. All of the cultures, except F36 and F39 which were relatively recent isolations, had been in stock culture collections 5 years or more and had varied cultural histories.

Studies of morphology and cultural properties followed accepted procedures. Flagella were stained using the method of Leifson (1951) and the silver impregnation technique of Kingma-Bolites (1948). Specimens were also chromium shadowed and examined for flagella using an RCA electron microscope.

The cultures were maintained both as actively growing stocks and in the lyophilized condition. All cultures except F36 grew well and remained viable 4 months or longer upon a medium designated M1. This contained, in per cent, proteose peptone, 0.5; yeast extract, 0.1; beef extract, 0.2; NaCl, 0.3; and agar, 1.2. Culture F36 did not grow well upon this medium, nor would it remain viable for more than a few days. Taylor (1952, *personal communication*) recommended a sodium caseinate medium for culture F36, and a medium (M5) containing, in per cent, sodium caseinate, 0.2; proteose peptone, 0.1; yeast extract, 0.05; KH2PO4, 0.05; agar, 1.2, proved satisfactory. Litmus milk agar, prepared by adding litmus milk to an equal volume of 3 per cent agar, was used as a standard medium for the visual differentiation of pigmentation. Usually the milk medium was supplemented with 0.1 per cent yeast extract.

Cells for extraction of pigments were grown in liquid media shaken continuously during the 7 day incubation at room temperature. Such cells were harvested using a Foerst continuous-type centrifuge and then dried in vacuo over MgCl2. The pigments were extracted using a 1:1 methanol-petroleum ether mixture. Usually the cells were ground with powdered pyrex glass to complete the extraction. The extracted pigments were dried and then taken up in petroleum ether (Eastman ligroin, bp 35–60 C) and absorption spectra determined using a Beckman Model B spectrophotometer.

Morphological and cultural studies used to establish F39 as a strain of *Sporocytophaga* were patterned after those of Stanier (1940, 1942). Cultures of F39 were propagated upon tryptose agar (Difco) or upon the peptone-yeast extract-sodium acetate agar of Pringsheim and Robinow (1947). The cells were stained using 1 per cent crystal violet for 20–30 min or Giemsa stain for 15 min since gram stained preparations were faint and ill-defined.

**DESCRIPTION OF THE CULTURES**

**Consideration of cultures as possible strains of *F. aquatile*** In addition to the properties which are

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**TABLE 1**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Source</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3, F25</td>
<td>American Type Culture Collection, no. 8375</td>
<td>Ohio State University</td>
</tr>
<tr>
<td>F35</td>
<td>University of Connecticut</td>
<td>American Type Culture Collection, no. 8375</td>
</tr>
<tr>
<td>F9</td>
<td>Indiana University</td>
<td>E. W. Taylor</td>
</tr>
<tr>
<td>F10</td>
<td>Indiana University</td>
<td>E. W. Taylor</td>
</tr>
<tr>
<td>F36</td>
<td>E. W. Taylor</td>
<td>Deep well, Kent, England</td>
</tr>
<tr>
<td>F39</td>
<td>H. L. Jensen</td>
<td>Soil, Denmark</td>
</tr>
</tbody>
</table>
**TABLE 2**

**Characteristics of Bacillus aquatilis compared with cultures considered to be the same species**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Motility in Wet Mounts</th>
<th>Colonies on Nutrient Gelatin</th>
<th>Growth on Agar slope</th>
<th>Action on Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. aquatilis*</td>
<td>Stationary to oscillatory</td>
<td>Yellow-brown to reddish, irregular, lobed, rhizoid extensions from center to margin, slow liquefaction</td>
<td>Yellow, mucoid, fimbriated, yellow</td>
<td>Utilized, no ((NO_2)^-), NH_3 present</td>
</tr>
<tr>
<td>F3, F25, F35</td>
<td>Rotatory movement, limited progress</td>
<td>Yellow, entire, liquefaction variable with strain</td>
<td>Yellow, filiform</td>
<td>Reduced to ((NO_2)^-)</td>
</tr>
<tr>
<td>F10</td>
<td>Rotatory movement, limited progress</td>
<td>Yellow, entire or margins raised and irregular, no liquefaction</td>
<td>Yellow, filiform</td>
<td>Abundant, yellow Utilized, no ((NO_2)^-) formed</td>
</tr>
<tr>
<td>F9</td>
<td>Rotatory and progressive, long filaments undulate slowly</td>
<td>Light brown, irregular, translucent, no liquefaction</td>
<td>Light brown, translucent, entire, later spreading and arborescent</td>
<td>None Utilized, no ((NO_2)^-) formed</td>
</tr>
<tr>
<td>F36</td>
<td>Long filaments and single cells stationary</td>
<td>Yellow-brown to red-brown, margin fimbriated or lobed, limited liquefaction</td>
<td>Yellow to yellow-brown, mucoid, filiform</td>
<td>Scant to moderate, light yellow Not utilized, no ((NO_2)^-) formed</td>
</tr>
<tr>
<td>F39</td>
<td>Stationary</td>
<td>Yellow, fimbriate margins, limited liquefaction</td>
<td>Fimbriate, yellow to dark yellow</td>
<td>Abundant, yellow Not utilized, no ((NO_2)^-) formed</td>
</tr>
</tbody>
</table>

* According to Frankland and Frankland (1889).
† According to Tataroff (1891) for B. aquatilis alpha.

applied to the genus *Flavobacterium* (Breed et al., 1948), the type species should agree relatively well with the limited description given for *B. aquatilis* by the Franklands (1889). When selected cultural properties of the strains used here were compared with the original description, it was apparent that differences existed (table 2). On the basis of motility alone cultures F3, F10, F25, and F35 fail in a comparison with the original description while cultures F9, F36, and F39 deserve consideration. Culture F9 only displayed motion that might be termed oscillatory.

**Motility and flagellation.** A small percentage of the cells in cultures F3, F10, F25, and F35 showed slight motion in hanging drop preparations, a motion described as twisting and rotating. For some bacteria motility of this nature has been interpreted to mean polar attachment of flagella (Kluyver and van Niel, 1936; van Niel and Allen, 1952). Cells from these cultures showed...
unquestionable polar arrangements of flagella at all ages examined, 18 to 96 hours, and in one instance in a 10 day old culture. This is illustrated in figures 1 and 2 using cultures F10 and F3. The cells were nearly always polar monotrichous.

Culture F9 formed filamentous structures having lengths of 50 μ or less in broth cultures. Many of these showed a slow twisting motion along with a sideways displacement suggestive of the oscillating motion described by the Franklands (1889) for B. aquatilis. The individual cells were slender, about 0.5 by 3 μ in stained preparations, and showed a leisurely rotation only. Flagellar stains or chromium shadowed preparations showed lophotrichate arrangements (figure 3) resembling that which is said to be typical of the genus Spirillum (Kingma-Boltjes, 1948). An electron micrograph prepared from a suspension of F9 clearly shows a twisted construction for the terminal flagella (figure 4). A twisted and rope-like appendage is seen to have unwound revealing individual components. This pictures the condition described recently by Pijper et al. (1953) for Spirillum serpens, and was said to represent a pathological process. While S. serpens appeared to have but one twisted terminal flagellum, F9 shows at least 3 such structures. If one assumes a minimum of 4 components for each flagellum, as is suggested by the electron micrograph, these could give rise to a polar tuft having 12 or more finer structures capable of staining and appearing as individual flagella. Since the flagellation of F9 indicated that the culture might be related to...
the genus *Spirillum*, attempts were made to demonstrate a spirillar morphology. Agar impression smears were fixed with osmic acid and stained for cell walls using the method of Pringsheim and Robinow (1947), or stained with Loeffler's methylene blue for 30 min. It was found that some of the larger cells showed a sigmoid shape especially in the cell wall preparations. Also the polar tufts of flagella were faintly stained by the cell wall method. This latter finding agrees with that of Pijper et al. (1953) for *S. serpens*. Isolated colonies of F9 were used to inoculate semisolid M1 medium (0.5 per cent agar) in such a way that giant colonies would form. About 25 per cent of the colonies appearing on the semisolid agar plates spread rapidly, e.g., swarmed. Subcultures from the periphery of the swarm gave populations in which the spirillar aspect was readily apparent.

Cultures F36 and F39 showed no independent motion in hanging drop preparations, but F39 showed a remarkable ability to swarm upon semisolid agar plates whereas F36 did not. The preparations to demonstrate flagella, which were made routinely irrespective of manifestations of independent motility, produced apparently contradictory results. Populations of F36 treated with the silver impregnation technique showed faintly stained, thin appendages for both long filaments and single cells (figure 5). Leifson's procedure failed to show these structures. Chromium shadowed specimens of F36 showed the nature of the flagellar-like structures in detail (figure 6). It seems certain that these are not flagella but rather are polysaccharide-like structures similar to the pseudoflagella described by Thjotta and Kass (1946). Electron micrographs and the staining procedures failed to reveal flagella for culture F39. The ability of this culture to swarm could not be associated with flagella.

**Pigmentation and appearance on litmus milk agar.** The results from studies of motility and flagellation permit an arrangement of the cultures used here into 4 groups (table 3). Differences and similarities of the cultures were sharply defined

**Table 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cultures</th>
<th>Flagellation</th>
<th>Appearance on litmus milk agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>F3, F10</td>
<td>Predominantly polar</td>
<td>Abundant, opaque, glistening, margins raised and ridged at infrequent intervals. Pigmentation lemon-yellow. Litmus neutral to alkaline. Casein digested.</td>
</tr>
<tr>
<td></td>
<td>F25, F35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>F39</td>
<td>Nonflagellated</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 6. Culture F36, 2 days old. Pseudoflagella shown by silver impregnation staining.](http://jb.asm.org/)
again when the organisms were grown upon litmus milk agar. The pigmentation shown when these organisms are grown on other media (table 2) also complements the arrangement into 4 groups.

Large cell crops of cultures F3, F10, F35, F9, F36, and F39 were treated to remove the pigments. In each instance absorption spectra of the unresolved mixtures were determined at 5 μμ intervals for the range from 300 to 600 μμμμ. The extinction curves which were prepared are not shown since the data revealed only minor differences. Such differences were impossible to interpret for the unknown and unresolved pigment mixtures which were studied.

Probable identity of the cultures in Groups I and II. The polar flagellation characteristic of the organisms in Group I (table 3) makes their inclusion in the genus Flavobacterium undesirable and their acceptance as the type species impossible. Cultures of Group I form a homogeneous collection, and this is to be expected when their common origin is considered. In one existing taxonomical scheme cultures such as these belong in the Xanthomonas-Pseudomonas assemblage (Breel et al., 1948). The cultures resemble Pseudomonas xanthe Zettnow.

Culture F9 also may be placed in the family Pseudomonadaceae on the basis of morphology and flagellation. The distinctive spirillar tendency appears to have been largely suppressed during propagation on conventional agar media. Growth in liquid cultures or upon 0.5 per cent agar media favors the appearance of the spirillar forms. Tentative placement of the culture in the genus Spirillum seems proper, but specific designation is impracticable.

Cultures F36 and F39. Cultures F39 and F36, though very different one from the other, present cultural characteristics which are superficially similar to those of the Franklands’ B. aquatilis. It will be shown, however, that F39 is not a true bacterium and that of the organisms used in this study only F36 is acceptable as the type species of the genus Flavobacterium.

Identity of culture F39. Organism F39 presented characteristics which suggested that it was not a true bacterium. When the culture was received, well defined colonies were not obtained upon agar plates; instead a thin diffused growth appeared. Hanging drop preparations showed no motility, nor could flagella be demonstrated. Additional tests for motility, behavior upon 0.5 per cent agar plates, indicated that the cells were motile since the growth swarmed rapidly, within 6 hr, from the point of inoculation. Microscopic study of the swarming growth in phase contrast showed the cells to be somewhat flexible and to move forwards or backwards in a deliberate, almost purposeful manner. These properties suggested myxobacteria, particularly cytophaga (Stanier, 1942). Stanier (1940) and Fuller and Norman (1943) have shown that certain cytophaga grow well upon ordinary laboratory media, and the latter publication reports a widespread distribution and common occurrence of such forms in the soil.

Cultures of F39 contained cells which measured about 0.5 by 3 to 8 μμ. These measurements were made using cell suspensions from 24 hr, or younger, cultures grown on tryptose agar slopes or in peptone-yeast extract-sodium acetate broth. The smears were heat-fixed and stained with crystal violet. Usually the cell ends were well rounded which is not characteristic of cytophaga subjected to the harsh procedures used in staining true bacteria (Stanier, 1942). The stained preparations usually showed a few cells measuring 0.5 by 1.5 μμ. It was impossible to know whether the shorter elements represented microcysts or shortened vegetative cells. Microcysts of Sporocytophaga are said to be greater in diameter than the vegetative cells and to stain more uniformly and intensely, characteristics not observed for F39. The cultural studies which were being made concomitantly revealed a different aspect of the morphology.

Investigations of cellulolytic ability of F39 included culturing in a mineral base medium to which sterile filter paper strips were added. It was necessary to supplement the mineral solution with 0.05 per cent yeast extract to obtain growth. In some instances the mineral base was solidified with 1.2 per cent agar and the filter paper laid on the surface. Pronounced cellulolytic activity was not observed. The filter paper strips on the mineral base agar showed growth and light yellow pigmentation at the point of inoculation, as well as a thin, gray, diffuse growth upon the agar. The filter paper became translucent where growth occurred. Phase microscopic examination of the filter paper showed round-ended cells, about 0.5 by 2.5 μμ, displaying a definite flexing with the same deliberate motion seen in the growth from
semisolid agar. Some spherical forms, about 1 μ in diameter, were seen. The liquid medium showed only slight turbidity in 5 weeks at 25 C, and no growth was apparent upon the immersed strip of filter paper. After about 3 weeks of incubation, however, sediment began accumulating in the bottom of the tube. Stained preparations of the sediment showed long, tapered cells, about 0.5 by 4 μ, and masses of micrococcal-like cells approximately 1 μ in diameter. Streak-plating revealed only one type of colony. It was concluded that F39 had assumed a more typical Sporocytophaga-like morphology during its growth in the mineral base-cellulose medium, and that the spherical structures represented microcysts. Transplants from the sediment resulted in a luxuriant yellow growth upon tryptose agar. This was more abundant than that of the parent cultures, and the distinctive fringed margins were not always conspicuous. This substrain was designated F39.2D. Phase contrast examination of the sediment showed only round-ended cells and spherical microcysts. Usually masses of the latter were found where vegetative cells were clustered. This behavior is said to characterize Sporocytophaga congregata (Fuller and Norman, 1943). Organisms F39 would not grow upon mineral base starch agar, but the substrain, F39.2D, grew rapidly and luxuriantly. This is of interest since Fuller and Norman state that S. congregata will utilize starch but that S. myxococoides will not, and they have separated these species on such a basis. Microcyst formation occurred readily on the starch agar. Tests of heat resistance showed that cell suspensions containing microcysts withstanded 80 C for 10 min but not 15 min. Additional studies of cultural properties showed general agreement with those described for S. congregata except that the pigment did not assume an orange coloration upon aging. It is concluded that culture F39 is a strain of S. congregata.

Characterization of F. aquatile strain Taylor. Culture F36 corresponds generally in its properties to those given for F. aquatile (Breed et al., 1948). There are certain deviations. The colonies on nutrient gelatin are not composed of radiate bundles of threads, and the cells show no movements that can be called oscillatory. These two properties have been given differential importance, perhaps more than is merited.

In their publication the Franklands (1889, p. 381) said that colonies developing on nutrient gelatin plates were at first "little characteristic". Only later did the colony margins become more irregular. The characteristic colony appeared after liquefaction commenced, and liquefaction commenced slowly. Also the Franklands (1894) acknowledged Tataroff's Bacillus aquatilis alpha, which produced lobate colonies on a nutrient gelatin, as being a strain of their B. aquatilis. Colonies growing on nutrient agar were described by both the Franklands (1889) and Tataroff (1891) as yellow, smooth, and entire. Growth on slope cultures was said to extend only a little beyond the line of inoculation. It is concluded that while the colonies on nutrient gelatin may be unique, this property is a variable one and primarily of ancillary taxonomic value. The pigmentation ascribed to B. aquatilis growing on nutrient gelatin (table 2) is thought to have greater differential significance than the colonial form. Culture F36 has a similar yellow to orange pigmentation on nutrient gelatin, but many of the nonmotile species of the genus Flavobacterium from which F. aquatile must be separated produce a yellow pigmentation only. Species such as F. fucatum, F. balustinum, F. ferrugineum, and F. esteromericum are of this nature (Breed et al., 1948).

It is believed that the word oscillate should be discarded as a descriptive character for F. aquatile. The Franklands (1889) in describing bacterial motion which they saw in hanging drop preparations used the word oscillate to characterize the behavior of several of their species of bacteria, viz., B. aquatilis, B. arborescens, B. diffusus, and B. ramosus. They recognized also absence of motion, active movements, and rotation upon the long axis of the cell. In the instance of B. diffusus they described both a rotatory and an oscillatory motion. It is not possible to know what the Franklands intended the word oscillate to describe, and it seems proper to propose that the term be discarded since its meaning is equivocal. This has already been done in the present descriptions of F. arborescens, F. diffusum, and Bacillus cereus (Breed et al., 1948) as synonyms of B. arborescens, B. diffusus, and B. ramosus (Frankland and Frankland, 1889). If, then, F. aquatile is regarded as nonmotile, culture F36 is acceptable as a strain of the species. On this basis culture F36 is designated F. aquatile strain.
Taylor, and a description of its morphological and cultural properties is given here.

Flavobacterium aquatile strain Taylor (Frankland and Frankland) Bergey et al. (Synonymy: Bacillus aquatilis Frankland and Frankland, 1889; Bacillus aquatilis alpha Tataroff, 1891; Bacterium aquatile Chester, 1897; Bacterium aquatile Migula, 1900; Bacterium pseudoaquatile Migula, 1900. Bacillus aquatilis Tataroff, 1891, and Bacillus aquatilis Migula, 1900, are not synonyms.)

Morphology. Gram stained cells are 0.5 to 0.7 by 1 to 3 µ, approaching cocccobacillary form in young cultures. Populations in liquid or on solid media show filaments 10 to 40 µ long. Gram negative. No endospores. No motility in hanging drops or semisolid agar media. No flagella observed.

Nutrient agar. Growth on slopes develops slowly and is moderate, mucoid, glistening, transparent, and filiform. Populations do not survive without frequent transfer. Colonies are 1 to 3 mm, entire, circular, raised, smooth, glistening, transparent. Pigmentation light yellow becoming brownish-yellow. Growth becomes more mucoid without increase in pigmentation when sucrose is added.

Nutrient broth. Faint turbidity only.

Potato. White to faint yellow, scant to moderate growth at 20 to 25 C. Potato darkens, pigment becomes bright orange at 10 C.

Limon milk. Reduction and yellow surface ring, no acid 7 days, 20 to 25 C. Casein slowly digested, 14 to 20 days.

Gelatin. Giant colonies irregular to mucoid, uniformly raised, conspicuous center. Yellow becoming orange. Limited liquefaction.

Pigmentation. Varies from light yellow to orange-yellow, depending upon cultural conditions. Red components are most conspicuous at 10 C or when abundant populations are produced. Growth on 2 per cent proteose peptone agar is gray-yellow and orange on litmus milk agar.

Nutritional requirements. No growth with glucose and ammonium chloride as the only source of carbon and nitrogen. Moderate growth, and populations survive infrequent transfer, on media containing sodium caseinate or enzymic digests of casein.

Carbon utilization. Glycerol, xylose, arabinose, glucose, fructose, galactose, mannose, cellobiose, sucrose, maltose, lactose, or raffinose will support growth as principal carbon sources under aerobic conditions. Ethanol, sodium citrate, dextrin, starch, dulcitol, mannitol, and salicin are not used under similar conditions.

Acid production. Acid reactions are not detectable with indicators when tests are conducted in peptone-beef extract media. Semisynthetic media, agar-sloped or liquid, show acid production from glucose, galactose, mannose, sucrose, and maltose. Final pH in unbuffered 1 per cent glucose broth is pH 6.0 to 6.5 in the absence of peptone-beef extract and pH 6.5 to 7.0 if these compounds are present.


Distinguishing characteristics. Diffs from other nonmotile species of the genus Flavobacterium by a number of cultural properties which, when combined, serve to differentiate the species. These are: yellow pigmentation becoming yellow-brown or orange; slow growth and poor survival on peptone-meat extract agar; inability to reduce nitrate to nitrite; failure to grow at 37 C and under anaerobic conditions.

Definition of the genus Flavobacterium. The original definition of Flavobacterium (Bergey et al., 1923) has been retained with little change during the revisions of Bergey's Manual. Peritrichous flagellation was specified in a recent edition (Bergey et al., 1939). Gary (1956, p. 29) proposed to emend the generic description in the following manner. "Gram-negative rods forming a yellow-orange pigment. Motile by peritrichous flagellation or non-motile. Characterised by their active, strictly oxidative attack on carbohydrates. Growing equally well in media with inorganic NH₄ or simple organic nitrogen compounds (glutamic acid, asparagine, etc.) as a source of nitrogen." Gary's description of carbohydrate dissimilation was based upon manometric studies, and no attempt was made to establish metabolic sequences. Also the organisms with which he worked included polar flagellates among which was the culture reported here to be Pseudomonas zanthae. It seems premature to limit the genus to bacteria having an aerobic metabolism. Harrison (1929) has described several facultative anaerobic...
species. The studies of Gary and of Harrison, together with the investigation reported here for *P. aquatile*, do permit a more complete characterization of the genus *Flavobacterium*. The following extended description is offered.

*Flavobacterium* Bergey *et al.*, *pateri*. Gram negative, rod shaped bacteria not forming endospores. Motile with peritrichous flagella or non-motile. Characteristically producing yellow, orange, red, or yellow-brown pigmentation, the hue often depending upon the nutrient medium. Some strains produce only a grey-yellow pigmentation on peptone-meat extract agar, but these have a more pronounced pigmentation on other media, e.g., nutrient gelatin, potato, or litmus milk agar. Pigments are not soluble in the medium, and those which have been studied are carotenoid in nature. Commonly proteolytic. Fermentative metabolism is usually not conspicuous; acid reactions commonly do not develop from carbohydrates when available nitrogen containing organic compounds are in the medium. Gas is not produced from carbohydrates according to the usual cultural tests. Nutritional requirements usually are not complex. Aerobic to facultative anaerobic. Occur in water and soil. Some species are pathogenic to laboratory animals.

Pigmentation is a conspicuous character for genera in at least four families of true bacteria, *Pseudomonadaceae*, *Rhizobiaceae*, *Achromobacteriaceae*, and *Enterobacteriaceae* (Breed *et al.*, 1948). For the genus *Pseudomonas*, *Chromobacterium*, or *Serratia* a chemically distinctive pigment can aid in establishing the taxon. There remains a heterogeneous collection of species, some of which are known to produce carotenoid pigments (Sobin and Stahly, 1942). It may be assumed that these pigments are characteristic of the group as a whole. The pigmentation of these species is a valid taxonomic criterion for the genus to which such species are assigned. This pigmentation, however, has little differential value at the generic level, for example, in the separation of *Xanthomonas* from *Flavobacterium*, although it may prove useful for the separation of species of a given genus. It is desirable to be able to separate *Flavobacterium* from similar organisms such as *Xanthomonas*. These genera are most readily differentiated on the basis of source and flagellation. No nonflagellated *Xanthomonas* are listed by Breed *et al.* (1948), and of the *Pseudomonas* in Appendix I of *Xanthomonas* only one species, *P. tricusciens*, is described as non-motile. It is the custom to classify nonmotile bacteria with motile forms to which they appear related physiologically (Kuyper and van Niel, 1936). In this instance both the genera *Flavobacterium* and *Xanthomonas* appear to be similar physiologically. It seems certain, unless the source indicated differently, that a nonmotile, culturally typical bacterium would be arranged with *Flavobacterium* rather than *Xanthomonas*. For the present this taxonomic expedient may be the most sensible.

Patterns of flagellations are not easily established especially when a bacterium is small. Gary (1950) had this difficulty with many of his stained preparations and consequently arranged his cultures as monotrichous, polytrichous, or nonmotile organisms. Starr and Williams (1952) have shown that electron micrographs often are of little assistance in such a dilemma. Further Leifson and Hugh (1953) have shown that variation in a characteristic mode of flagellation may occur. One of the cultures which these workers used admittedly could have belonged either to the genus *Xanthomonas* or the genus *Flavobacterium* since both patterns of flagellar attachment were shown by variants of the culture. Resolution of taxonomic problems such as this is not possible now. The suggestion of Leifson and Hugh that polar flagellation may be essentially unicellular while peritrichate forms may be multicellular is worthy of some attention.

Rational classification of the pigmented, rod-shaped, gram negative, true bacteria might require that these forms be brought together in a single form-taxon in the sense of Cohn (1872). The tribe *Chromobacteriae* proposed by the Society of American Bacteriologists Committee, 1917, served such a purpose (Bergey *et al.*, 1923)

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**SUMMARY**

Seven cultures which various investigators had tentatively identified as *Flavobacterium aquatile* were compared in their properties to the original
description for this type species. The collection was found to be heterogeneous and could be arranged into four groups on the basis of morphology and pigmentation. These groups were established as I, cultures similar to *Pseudomonas xantho*; II, *Spirillum* sp.; III, *Sporocytophaga congregata*; and IV, a culture resembling the original description sufficiently well to be designated *F. aquatile* strain Taylor. A cultural description of this species is presented.

The genus *Flavobacterium* is described in an extended form, and the problem of distinguishing such organisms from similar gram negative bacteria is considered.

Culture F35, listed in this paper as a transplant of *Flavobacterium aquatile* American Type Culture Collection no. 8375, has proven to be *F. aquatile* 700 of the Midwest Culture Service. Apparently cultures F3, F25, and F35 are strains of an organism used by E.W. Taylor and R.S. Breed in some of their unpublished studies on the genus *Flavobacterium*. These studies were conducted about 1941.

*F. aquatile* strain Taylor has been deposited with the American Type Culture Collection as no. 11947.

REFERENCES


