CHARACTERISTICS OF SOME SOIL CYTOPHAGAS

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The soil cytophagas have hitherto been regarded as highly specialized organisms capable of utilizing few if any energy sources other than cellulose. For example Jensen (1940) speaks of the genus Cytophaga in the following terms: "Strictly aerobic organisms of uncertain systematic position. . . . They attack no other carbon compound than cellulose, . . ." Recently Stanier (1942, a) has shown that certain soil forms can utilize glucose and cellulose if these sugars are not heat sterilized. He also demonstrated the existence of halophilic forms of marine origin that exhibited a considerably greater degree of versatility (1941).

During the study of the aerobic mesophilic cellulose organisms of soil, in which water-insoluble cellulose dextrins were employed for isolation and purification (Fuller and Norman, 1942), soil cytophagas were frequently obtained. Several pure cultures were selected for detailed study and certain of these were found to differ considerably in physiological requirements from the classical Spirochaeta cytophaga (emend, Sporocytophaga myxococoides, Stanier, 1942), and Cytophaga hutchinsonii, Winogradsky, which have heretofore been regarded as the dominant soil forms. There has been much controversy as to the nature and systematic position of the cytophagas. This has been comprehensively reviewed by Stanier (1942) who has recently revised the classification of this interesting group of myxobacteria. The three new species described below have been named in accordance with his system, but certain modifications are necessary in the key to the genus Cytophaga in order that they may be included.

CHARACTERISTICS OF THE ORGANISMS

Each of the organisms described below is easily recognizable as being a cytophaga. The vegetative cells are long rods the ends of which may be somewhat pointed in the early stages of growth. In older cultures the cells seem to become shorter and thicker, and to have rounded ends. There is some variability in the dimensions of the cells at all stages. The most characteristic feature is the bent and sinuous form of many of the cells, which lack a rigid cell wall and are capable of flexing and straightening. The motility of these organisms is not flagellar, but caused by the flexing of the cells. Movement of groups of cells takes place only on a solid surface. Of the three cultures, only Sporocytophaga congregata produces spores or microcysts. These are round and larger in diameter than the breadth of the vegetative cells. Development of spores occurs centrally in the cell.

No particular difficulties were encountered in the culturing of these organisms.

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Well-defined but rather restricted colonies are obtained on agar in the presence of a suitable energy source and 0.5 ml of 10 per cent yeast water per liter. Nitrate was the form in which nitrogen was ordinarily supplied. The various carbohydrates were added to give a concentration of 0.1 per cent.

DESCRIPTION OF CULTURES

_Cytophaga albogilva_ n. sp.

(Albogilva is from the Latin, *albus*, meaning white, and the Latin, *gilvus*, meaning pale yellow.)

**Morphology**

*Vegetative cells*: Long, flexuous rods with pointed ends 4.5-7.5 μ long, 0.3-0.5 μ broad, arranged singly.

*Spores*: Absent.

*Motility*: Present, but not by flagella.

*Staining*: Gram negative.

**Cultural characteristics**

*Gelatin stab*: Liquefies gelatin in 7 days.

*Starch agar slant*: Heavy, pale yellow growth in 2 days.

*Starch agar (8 per cent NaCl)*: No growth.

*Litmus milk*: No growth.

*Indole*: Negative.

*Nitrite formation*: Negative.

*Carbohydrates*: Glucose, galactose, lactose, maltose, sucrose, gum arabic, pectin, starch, and hemicellulose are utilized. No acid or gas produced.

*Cellulose*: Very scanty growth appears on filter paper when first isolated from soil. This ability is soon lost. Water-insoluble cellulose and water-soluble cellulose dextrins are utilized, and this property remains stable.

*Nitrogen*: Ammonia, nitrate, and peptone are utilized.

*Oxygen*: Highly aerobic.

*Temperature*: Optimum 22-30°C.

*Habitat*: Soil.

**Colony characteristics**

*Starch agar*: 5 day old culture—*color*, cream to pale yellow; *size*, 2-4 mm.; *shape*, irregularly round; *elevation*, concave; *edge*, entire and irregular.

*Water-insoluble dextrin agar*: 5 day old culture—*color*, milky-white; *size*, 1 mm.; *shape*, round; *elevation*, concave; *edge*, irregular.

_Cytophaga deprimata_ n. sp.

(Deprimata is from the Latin verb, *deprime*, meaning to sink down, to depress.)

**Morphology**

*Vegetative cells*: Long, flexuous rods with pointed ends 5.5-10 μ long, 0.3-0.5 μ broad, arranged singly.

*Spores*: Absent.

*Motility*: Present, but not by flagella.

*Staining*: Gram negative.

**Cultural characteristics**

*Gelatin stab*: Liquefies gelatin in 4 days.

*Starch agar slant*: Heavy, yellow pigment that turns orange yellow with age.
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Starch agar (8 per cent NaCl): No growth.
Litmus milk: No noticeable growth.
Indole: Negative.
Nitrite formation: Negative.
Carbohydrates: Glucose, lactose, maltose, sucrose, pectin, starch, and hemicellulose are utilized. No acid or gas produced.
Cellulose: Very scanty yellow growth appears on filter paper on first isolation from the soil. Cellulose attacking ability is soon lost. Cellulose dextrins are slowly used.
Nitrogen: Ammonium, nitrate, and peptone in addition to yeast are used.
Oxygen: Highly aerobic.
Temperature: Optimum 25-30°C.
Habitat: Soil.

Colony characteristics

Starch agar: 5 day old culture—color, smoky to faint yellow. Older colonies become bright yellow; size, 2-5 mm.; shape, irregular; elevation, concave; edge, irregular. Spreads indistinguishably into the surrounding medium, which develops shallow depressions around the colonies. Small colonies may give an agar plate a characteristic pitted appearance.
Water-insoluble dextrin-agar: 7 day old culture—color, milky white; size, pin point (1-2 mm.); shape, irregularly round; elevation: concave; edge, indistinct. Agar medium develops shallow depressions.

Sporocytophaga congregata n. sp.

(Congregata is from the Latin verb congrego, meaning to assemble.)

Morphology

Vegetative cells: Long, flexuous rods with pointed ends, 5.5-8.0 μ long and 0.5-0.7 μ broad.
Spores: Present, round, 0.7-1.1 in diameter. Killed by heating at 80°C. for 10 minutes.
Motility: Present, but not by flagella.
Staining: Gram negative.

Cultural characteristics

Gelatin stab: liquefies gelatin in 3 days.
Starch agar slant: Yellow growth occurs mostly beneath the surface and finally spreads throughout the agar slant concentrating on the lower surface as an orange slime.
Starch agar (8% NaCl): No growth.
Litmus milk: Growth, but no digestion or curd formation.
Indole: Negative.
Nitrite formation: Negative.
Carbohydrates: Glucose, galactose, lactose, maltose, sucrose, arabinose, calcium gluconate, starch, pectin, and hemicellulose are utilized. No acid or gas is produced.
Nitrogen: Ammonium, nitrate, and peptone are used.
Cellulose: Filter paper is not utilized. Water-insoluble and water-soluble dextrins are utilized.
Oxygen: Highly aerobic.
Temperature: Optimum 25-30°C.
Habitat: Soil.

Colony characteristics

Starch agar: 5 day old culture—color, smoky or hazy, later turning yellow; shape, irregularly round; size, 5-8 mm. in diameter; elevation, slightly concave; colony surface, as the colony becomes older, typical swarming is prominent, but at first more within the colony
than at the periphery. The vegetative cells gather in groups and in these regions a large number of spherical spores are found. Not all colonies show marginal swarming. Edge, smooth and entire at first, later becoming irregular.

*Water-insoluble dextrin agar:* 5 day old culture—*color*, pale and hazy white; *shape*, round; *size*, 1-3 mm.; *elevation*, concave, with hollowing of the agar limited to area of colony growth. After 7 days typical swarming is noticeable. Edge, smooth at first, later becoming irregular.

**DISCUSSION**

According to the classification of the myxobacteria developed by Stanier (1942, b), *Cytophaga albogilva* n. sp. and *Cytophaga deprimata* n. sp. fall in the genus *Cytophaga*, and *Sporocytophaga congregata* n. sp. in the genus *Sporocytophaga* on morphological grounds. The two former give cream to yellow pigmented colonies on starch, and are similar in morphology to *C. hutchinsonii*, which is yellow pigmented on cellulose. *C. albogilva* and *C. deprimata* utilize a substantial number of polysaccharides and sugars whereas *C. hutchinsonii* is apparently limited to cellulose and the closely related sugars, cellobiose and glucose. The cellulose-decomposing ability of *C. albogilva* and *C. deprimata* was feeble on first isolation, and was soon lost when cultured on simpler carbohydrates. In some respects the physiological characteristics of these two organisms are similar to those cytophagas of marine origin (Stanier, 1941) which however are obligately halophilic. The soil forms do not tolerate salt concentrations equivalent to sea water.

The two organisms *C. albogilva* and *C. deprimata* are easily recognizable as being distinct. The average cell length of the former is less than that of the latter, and the pigmentation on starch is different. The colony development of *C. albogilva* is somewhat restricted, but the colonies are clearly defined. Those of *C. deprimata*, on the other hand, are more spreading and merge imperceptibly into the medium. Often the whole plate may be covered with a thin filmy growth. Very characteristic is the fact that agar round the colony is caused to shrink so that a shallow depression is formed. This is apparent sometimes before the colony is clearly visible so that the plate has a pitted appearance (see figure 1). It was at first suspected that the agar was undergoing some attack but no clear evidence in support of this view could be obtained. The organism is incapable of developing on agar alone. The phenomenon is presumably identical with that described by Stanier (1942, b) as “etching” of the agar, and is probably caused by water loss from the gel structure.

The recognition of these two new species of the genus *Cytophaga* will make it necessary to broaden the description of the physiological abilities of members of this genus. The genus description of *Cytophaga*, as written by Winogradsky (1929) included the statement “incapable of using carbonaceous materials as food, except cellulose, which is hydrolyzed. Growth in ordinary culture media is feeble.” In his revision Stanier (1942, b) wisely omitted reference to the physiology of the bacteria and defined the genus entirely on morphological characteristics. However, in his proposed key to the species of this genus, Stanier restricted the soil group to a narrow range of physiological characteristics,
defining them as "Specialized cellulose-decomposers with a limited range of carbon sources. Cannot develop in the absence of carbohydrates. Grow well in mineral medium with a suitable carbohydrate and an inorganic nitrogen source." The marine group is stated as having a greater degree of versatility. Although the prime purpose of a key is to indicate groupings of known species and to facilitate their separation, it is desirable that the organization of any key should be such that possible future additions of species differing considerably in properties may be incorporated without substantial revision. For this reason the restrictive definition of the soil group of cytophagas is inexpedient.

Inasmuch as members of the marine group are obligately halophilic and those of the soil group are not, a major separation that takes advantage of this difference has much in its favor. First it provides for the soil cytophagas that do not attack cellulose, and second it provides a place for those bacteria that utilize a wide range of carbohydrates and are not halophilic. The ability to attack starch suffices to separate the starch-utilizing forms, C. albogilva and C. deprimata from the non-starch-attacking cytophagas of Winogradsky. Separations within these groups may then be accomplished on the basis of pigmentation on starch and cellulose respectively.

In arriving at suitable names for the new soil cytophagas described in the text, first consideration was given to the Latin adjectives that clearly described some outstanding characteristic of the organism. C. albogilva was so named because of the formation of a pale yellow pigment on starch and C. deprimata was selected as the name of a species of cytophaga that produces shallow depressions or pits when grown on agar media.
Following the above suggestions the revised key to the species of the genus *Cytophaga* is as follows:

**Key to the species of the genus *Cytophaga***

I. Obligately halophilic
   A. Dark pigment on cellulose
      1. *Cytophaga krzemieniewskae*
   B. No pigment on cellulose
      2. *Cytophaga diffluens*

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**Figs. 2, 3, and 4. Growth of Sporocytophaga congregata on starch agar**

Figure 2 shows the shallow depressions produced by the colonies. Figure 3 shows growth at twelve days with margins still entire, and swarming visible within the colony. Figure 4, taken at nineteen days shows swarming and movement of cells from the periphery of the colony.

II. Not obligately halophilic
   A. Utilizes starch
      (a) Produces yellow to orange pigment on starch
         3. *Cytophaga deprimata*
      (b) Produces cream to pale yellow pigment on starch
         4. *Cytophaga albogilva*
   B. Does not utilize starch
      (a) Produces yellow pigment on cellulose
         5. *Cytophaga hutchinsonii*
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(b) Produces orange pigment on cellulose
   6. *Cytophaga aurantiaca*
(c) Produces pink pigment on cellulose
    7. *Cytophaga rubra*
(d) Produces olive-green pigment on cellulose
   8. *Cytophaga tenuissima*

The identity of *Sporocytophaga congregata* within the genus *Sporocytophaga*, Stanier, as a new species is without question. Of the two species recognized as belonging to this genus, morphologically, *S. congregata* most closely resembles *S. myxococcoides*, Stanier, or *Spirochaeta cytophaga*, Hutchinson and Clayton, since both produce round spores in addition to the long rods. Unlike *S. myxococcoides*, *S. congregata* does not attack filter paper but utilizes many different carbon sources. Moreover this new organism shows typical swarming and grouping or clumping of cells within the colony. Marginal movement does not always occur but is more frequent in old colonies than young (figures 2–4).

The only change necessary in the key to the genus *Sporocytophaga* in order to include this new species is to subdivide the group having spherical microcysts on the basis of the utilization of starch or simple sugars.

I. Microcysts spherical
   A. Utilizes starch
      1. *Sporocytophaga congregata*
   B. Does not utilize starch
      2. *Sporocytophaga myxococcoides*

II. Microcysts oval
   3. *Sporocytophaga ellipsoспора*

SUMMARY

Three new species of soil cytophaga have been isolated from soil and described. Each exhibits a greater degree of physiological versatility than the species hitherto recognized.

Under laboratory conditions none of the organisms attacks cellulose, and all utilize a wide range of carbon sources.

Two of the organisms are recognized as belonging to the genus *Cytophaga* and one, on the basis of spore formation, to the genus *Sporocytophaga*.

Revision of the key to the genus *Cytophaga* is necessary to permit the inclusion of these forms. The addition of the new species of *Sporocytophaga* to that genus involves only a minor change.

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REFERENCES
