THE BACTERIAL FLORA OF DISTILLED AND STORED
WATER. III. NEW SPECIES OF THE GENERA
CORYNEBACTERIUM, FLAVOBACTERIUM,
SPIRILLUM AND PSEUDOMONAS

Einar Leifson

Department of Microbiology
Stritch School of Medicine and Graduate School,
Loyola University, Chicago, Illinois

SUMMARY. Names are proposed and descriptions given for five new species of bacteria isolated from stored distilled water from various laboratories. Corynebacterium aquaticum n.sp. has many of the characteristics of the motile plant pathogenic bacteria that have been placed in genus Corynebacterium. Other species named are Flavobacterium capsulatum n.sp., Spirillum delicatum n.sp., Pseudomonas lanceolata n.sp., and Pseudomonas huttiensis n.sp. Cultures of the type strain of each new species have been deposited in the American Type Culture Collection.

- - - - -

Corynebacterium aquaticum n.sp.

Ten cultures of this species were isolated from the Chicago, Washington, England and New Zealand distilled water samples. The organisms are small slender rods with long peritrichous flagella and morphologically closely resemble cultures labeled Cellulomonas previously studied by the author. Being negative on cellulose it would seem foolish to attempt to classify the organisms into this rather questionable genus. The similarity of the organisms to the phytopathogenic corynebacteria seems sufficiently close to justify placing them in the genus Corynebacterium as a single species.

Description

Morphology. Small somewhat pleomorphic rods averaging 0.4 by 1-2 μ . Weakly Gram-positive. Motile with peritrichous flagella which are unusually long, 10 μ or more. The number of flagella is usually 1 or 2, occasionally 3 or more. In some strains the flagellar curvature is pure normal, in others pure curly and in still others a mixture of curly and normal. The average normal wavelength is 2.3 μ and the average curly wavelength 1.2 μ . The curly flagella generally have a very uniform curvature but the normal flagella are often quite irregular with a tendency to small amplitude, (Figs. 1, 2, 3).

Cultural characteristics. Colonies on agar when young are colorless, smooth and somewhat opaque. Older colonies have a greenish-yellow pigmentation. In broth the growth is uniformly turbid and without pellicle. The sediment which forms in older cultures is distinctly greenish-yellow due to a water-insoluble pigment. The organisms grow readily on simple peptone or yeast extract media. Oxygen relations, aerobic; temperature relations, most strains mesophilic but two strains were psychrophilic and failed to grow at 37°C; osmotic relations, neither halophobic nor halophilic; pH relations, neutrophilic.

Physiological characteristics. Acid produced aerobically but not anaerobically from glucose, sucrose, maltose, d-mannose and xylose; d-sorbitol variable; raffinose, lactose, starch and cellulose negative. Gelatin negative. Catalase positive. Nitrate negative except for 2 strains from Chicago samples which were positive. All strains grew in vitamin-free Casamino Acids (Difco). The mesophilic strains did not grow or produce acid in the glucose-salts medium but the two psychrophilic strains grew and produced acid. Evidently the mesophilic strains have some accessory growth factor requirement not shared by the psychrophilic strains.

Habitat. Common in distilled water supplies and probably natural fresh water.

Type strain. A culture of strain 150 has been deposited in the American Type Culture Collection, Washington (ATCC 14665), as the type.

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMYFlavobacterium capsulatum n. sp.

Three cultures of this species were isolates from distilled water, one from a Chicago sample and two from a California sample. The organisms are atrichous and hence the classification is somewhat arbitrary. Either Flavobacterium or Xanthomonas would be a suitable genus. The genus Flavobacterium is preferable since this genus has many atrichous species, and the species which are flagellated are peritrichous and generally poorly flagellated at best.

Description

Morphology. Relatively large, straight, Gram-negative rods averaging 0.6 by 2-5 μ . Short and long chains are common. Nonmotile and nonflagellated. A huge capsule is very characteristic and was produced on all media and under all growth conditions studied (Fig. 4).

Cultural characteristics. Colonies on agar grow fairly large, somewhat mucoid and deep yellow. Growth on slants is heavy, somewhat mucoid and yellow. Growth in broth is uniformly turbid with pellicle and yellow pigmentation. The pigment is water insoluble. The organism grows readily on simple peptone media. Oxygen relations, aerobic; temperature relations, mesophilic; osmotic relations, neither halophobic nor halophilic; pH relations, neutrophilic.

Physiological characteristics. Acid produced aerobically but not anaerobically from glucose, sucrose, maltose, d-mannose, xylose, d-sorbitol, raffinose, lactose. Starch and cellulose negative. Gelatin negative. Nitrate reduced to nitrite. Catalase positive. Grows in vitamin-free Casamino Acids (Difco). Grows and produces acid in glucose-salts medium. Accessory growth factors not required.

Habitat. Isolated from distilled water.

Type strain. A culture of the type strain 28 has been deposited in the American Type Culture Collection, Washington (ATCC 14666).

Spirillum delicatum n.sp.

This organism differs so much morphologically from typical spirilla that classification in the genus Spirillum is not entirely satisfactory. The genus Vibrio may be more suitable morphologically but not physiologically since the type species of Vibrio ferments carbohydrates which this organism does not. A new genus would be better for the small Gram-negative curved bacteria with predominantly a single polar flagellum, producing either no acid from carbohydrates or showing oxidative metabolism of carbohydrates. Such a new genus will not be proposed at this time and the organism is provisionally placed in the genus Spirillum.

Description

Morphology. Small Gram-negative rods averaging 0.4 by 1-2 μ , with one to several curves. Single individuals are vibrio shaped, multiple individuals are spirillum shaped. Motile with predominantly a single polar flagellum. The flagella are uniform with wavelength averaging 1.3 μ , (Figs. 7, 8).

Cultural characteristics. Colonies on agar are typically small, semitranslucent, smooth and colorless. Growth on slants is thin, smooth and colorless. Growth in broth is uniformly turbid without pellicle or color. Grows readily but slowly in simple peptone or yeast extract media. Oxygen relations, aerobic; temperature relations, mesophilic; osmotic relations, osmophobic or halophobic; pH relations, neutrophilic.

Physiological characteristics. Acid not produced from any carbohydrate tested including glucose, sucrose, maltose, d-mannose, xylose, d-sorbitol, raffinose, lactose. Starch, cellulose, gelatin, catalase negative. Nitrate reduced to nitrite.

Habitat. Isolated from a specimen of distilled water.

Type strain. A culture of the type strain 146 has been deposited in the American Type Culture Collection, Washington (ATCC 14667).

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMYPseudomonadaceae

To this family belongs by far the largest proportion of the bacteria isolated from distilled water. On the basis of pigmentation these bacteria may be divided into three major subgroups: 1) those which produce a greenish water-soluble pigment or are colorless (Pseudomonas), 2) those which produce a yellow water-insoluble pigment (Xanthomonas), 3) those which produce a pink or reddish water-insoluble pigment. For the latter group a distinct genus might be appropriate. Provisionally included in the family are the aggregating bacteria which do not produce definite stalks and may be more closely related to Caulobacter than to any genera of the family Pseudomonadaceae but definite taxonomic proposals for these organisms will not be made until they have been studied further.

Pseudomonas

The largest number of cultures isolated from distilled water belong in this genus. Twenty-nine cultures were typically Pseudomonas aeruginosa: Polar monotrichous flagellation, nitrate reduced to nitrogen gas, gelatin liquefied, oxidative metabolism of glucose, mannose, maltose and xylose but not sucrose, lactose, d-sorbitol or raffinose. Pigmentation variable, some strains producing a definite water-soluble greenish pigment, others not. This type of pigmentation seems to have but minor taxonomic significance. Fourteen cultures had polar multitrichous flagella, green water-soluble pigment, liquefied gelatin, did not reduce nitrate and oxidized a variable number of carbohydrates. Another 16 cultures were gelatin negative, reduced nitrate to nitrite and had somewhat the same carbohydrate metabolism as P. aeruginosa. All these cultures probably could fit into some existing species of Pseudomonas. Twenty cultures with polar monotrichous flagella failed to produce acid from any carbohydrate tested and showed variable reactions on gelatin and nitrate. Some of these were halophobic, some psychrophilic and some both. These are the types which so often are erroneously labeled Alcaligenes. Separation of this latter group from the genus Pseudomonas has some merit and would correspond to the separation of Alcaligenes and Achromobacter among the peritrichous flagellates. Some

rather distinctive pseudomonad types were isolated which seem to merit new species designation and are described below.

Pseudomonas lanceolata n.sp.

From the Vancouver and Japanese samples of distilled water were isolated six cultures with polar monotrichous, faintly staining flagella. The soma was very characteristic, being egg shaped with pointed ends. When first isolated the somata of the Japanese strains were distinctly larger than those of the Vancouver strains. After 3 years in stock, with transfer every 2-3 months, this somatic distinction is less definite. Vancouver strain 195 now shows a mixture of the large and small types, and Japanese strain 141 shows many small types. Correlated with source and original observations on somatic size was requirement of the Japanese (large type) strains for some accessory growth factors. The Vancouver strains showed no accessory growth factor requirements but grew and produced acid in the synthetic glucose-salts medium. In a medium with 0.2% vitamin-free Casamino Acids (Difco) the Japanese strains showed no growth while the Vancouver strains grew quite well. Aside from the variation in somatic size and growth factor requirements the six isolates were very much alike. No changes in cultural or physiological characteristics have been observed after 3 years in stock with many transfers. The group appears sufficiently homogeneous to be classified in a single species. The author has not been able to fit the group into any existing species of Pseudomonas and the specific epithet lanceolata is proposed to describe the characteristic somatic shape.

Description

Morphology. Short Gram-negative rods with pointed ends. Small (0.6 by 1.2 μ) and large 0.9 by 1.8 μ) somatic variants have been observed. Organisms usually single or in twos. Motile with polar monotrichous flagella. Flagella uniform with an average wavelength of 1.9 μ . Flagellar variations not observed. Using the Leifson flagella stain (1951) the flagella stained somewhat less readily than the flagella of most bacteria, (Figs. 5, 6).

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

Cultural characteristics. Colonies on agar are colorless, smooth and opaque. Growth on agar slants is smooth and colorless. Growth in broth uniformly turbid, no pellicle. Grows readily on simple peptone or yeast extract media. Oxygen relations, aerobic; temperature relations, good growth at 20 and 30°C but slow growth at 37°C; osmotic relations, osmophobic or halophobic with no growth in media with 1% NaCl; pH relations, neutrophilic.

Physiological characteristics. Slight acid produced from glucose aerobically but not anaerobically. No acid from sucrose, maltose, xylose, lactose, d-sorbitol, d-mannose, raffinose. Starch, cellulose, gelatin, catalase negative. Nitrate reduced to nitrite. Accessory growth factors required by some strains, not by others.

Habitat. Six strains isolated from distilled water from Japan and Vancouver, B.C.

Type strains. A type culture, our laboratory strain 192 (Vancouver), has been deposited in the American Type Culture Collection, Washington (ATCC 14669). At the same place there has been deposited a culture of our laboratory strain 141 (Japan) which is designated as the type culture of an infraspecific form, Pseudomonas lanceolata forma specialis (f. sp.) strain 141 (Japan) (ATCC 14668).

Pseudomonas huttiensis n. sp.

From two of the three distilled water samples from New Zealand, but from no other source, were isolated 8 cultures which were all alike and appeared rather distinctive. Since the 8 cultures were about 20% of the total number isolated from the New Zealand samples this organism must have been one of the major types of bacteria in the water. The cultures have been in stock for about 3 years and the characteristics have not shown any definite changes. The specific epithet huttiensis is proposed in reference to its origin from Lower Hutt, New Zealand.

Description

Morphology. Gram-negative rods with blunt ends averaging 0.4 by 1.8 μ . The soma has a small but distinct curvature. Within the soma are generally visible two to three

PLATE I

Figures 1- 3. Corynebacterium aquaticum.

1. Strain 197. Typical long peritrichous flagella of normal curvature.
2. Strain 292. Typical long peritrichous flagella of curly curvature.
3. Strain 150. Most flagellated individuals of all strains of this species showed only one flagellum, either normal or curly.

Figure 4. Flavobacterium capsulatum. Strain 28. Both short and long forms were seen surrounded by huge capsules. No flagella.

Figures 5, 6. Pseudomonas lanceolata.

5. Strain 141 from a distilled water sample from Japan.
6. Strain 192 from a distilled water sample from Vancouver, B.C. Note the relative size of the soma of the two strains.

Figures 7, 8. Spirillum delicatum. Strain 146.

7. Short form, possibly a single individual.
8. Long or filamentous form found in the same culture as the short form illustrated in Fig. 7.

Figure 9. Pseudomonas huttiensis. Strain 266.

The soma is typically slightly curved. The soma appears to divide and a flagellum is developing from the distal pole of the daughter cell. Note several somatic inclusions. The polar flagella were predominantly multiple and stained with difficulty with Leifson flagella stain.

Plate I. Photomicrographs x 2,000. Leifson flagella stain.

round inclusions about $0.3\ \mu$ in diameter. The organisms are usually single. Motile with polar flagella, frequently bipolar. The number of flagella varies from one to three and the organism may be referred to as multitrichous. Using the Leifson flagella stain, the flagella stain with great difficulty and appear very faint unless special techniques are used. The flagella are of uniform curvature with an average wavelength of $2.1\ \mu$. The distinctive morphological features are the somatic curvature, the round inclusions and the faint staining flagella, (Fig.9).

Cultural characteristics. Colonies on agar are smooth, colorless and opaque. Growth on slants is smooth and colorless. In broth the growth is uniformly turbid without pellicle formation. The organism grows readily on simple peptone or yeast extract media. Oxygen relations, aerobic; temperature relations, mesophilic; osmotic relations, neither halophobic nor halophilic; pH relations, neutrophilic.

Physiological characteristics. Acid produced from glucose and xylose, slight acid from d-mannose and d-sorbitol aerobically but not anaerobically. No acid from sucrose, maltose, lactose, raffinose. Starch, cellulose, gelatin, nitrate, catalase negative. Acid produced from glucose in glucose-salts medium. Accessory growth factors not required.

Habitat. Isolated from distilled water from New Zealand.

Type strain. A culture of our laboratory strain 266 has been deposited in the American Type Culture Collection, Washington (ATCC 14670).