PSEUDOMONAS SPINOSA N. SP.

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ABSTRACT. A new species of Pseudomonas is described which in its native river-water habitat characteristically has a curved soma with many straight spine-like structures, but no flagella. In pure culture the organism has a very long polar flagellum, a fairly straight soma with several round inclusions. It is psychrophilic and rather halophobic and shows a slight oxidative metabolism of some carbohydrates.

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Flagella stains made directly of the bacteria in the water of the DuPage River in Illinois showed at various times many relatively large rods, often curved in the shape of a semi-circle and covered with a number of spine-like structures. No flagella could be seen and the bacterial nature of these organisms appeared doubtful. By enriching water samples with small amounts (0.01-0.1%) of various nutrients such as acetate, ammonium salts and yeast extract, mixed cultures were obtained in which were seen a relatively large rod-shaped organism with an unusually long polar flagellum. In one such enriched sample some of the large polar flagellate rods also showed several straight spines and a variable degree of somatic curvature. The identity of the spined, nonflagellated organism seen in the river water and the polar flagellated rod seen in culture seemed certain. The enriched sample was plated on salt-free nutrient agar and eventually pure cultures of the organism were obtained. The organism has been in stock for over three years but spines have never been observed on the organisms in the pure cultures regardless of the media used. The cause of the spine formation is still unknown. It is proposed that this organism be named Pseudomonas spinosa from its appearance in river water.

METHODS

All media were made with a minimum of nutrients and salts. The basic nutrient broth consisted of peptone (Casitone, Difco) 0.2%, yeast extract 0.1%, K_2HPO_4 0.1%, pH 6.9-7.1. For slants and for plating 1.5% agar was added to the nutrient broth. The Leifson flagella stain was used. Carbohydrate metabolism was determined by the O-F medium of Hugh and Leifson (1953) modified by omission of the NaCl. The nitrate and gelatin media were made by adding respectively to nutrient broth 0.2% sodium nitrate and 8% gelatin, and the starch medium by adding 0.2% soluble starch and 1.5% agar. A strip of filter paper was placed in a tube of the nutrient broth for the cellulose medium. The casamino acids medium was made by adding 0.2% vitamin-free casamino acids (Difco) to distilled water, pH 6.9-7.1. The mineral salts medium consisted of NH_4Cl 0.1%, Na_2SO_4 0.02%, K_2HPO_4 0.02%, $MgCl_2 \cdot 6H_2O$ 0.02%, $CaCl_2$ 0.005%, pH 6.9-7.1. To this was added aseptically a sterile (autoclaved) 10% solution of glucose to a final concentration of 0.5%. Brom thymol blue indicator was added to the cultures after 2-5-day incubation at 30°C. The gelatin tubes were stab inoculated and incubated at 20°C for one week. Catalase was determined by adding H_2O_2 to cultures 3-5 days old. The nitrate medium was tubed with inverted vials to trap any gas produced. Tests for nitrate reduction and starch hydrolysis were routine.

DESCRIPTION

Morphology. Relatively large Gram-negative rods 0.6-0.8 μ in width and 4-6 μ in length. In ordinary nutrient media the rods are fairly straight. In highly dilute media; under conditions somewhat unfavorable as to pH, temperature and salt concentration; or in mixed culture, the rods may show considerable curvature. Under such conditions even branching forms have been seen. The spined forms have not been observed in pure cultures. The soma characteristically shows several large round inclusions about 0.4 μ in diameter. Short chains are frequently observed. The organisms are motile with polar flagella, predominantly single but frequently two flagella and occasionally three.

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

Figure 1. Pseudomonas spinosa showing the polar monotrichous flagellation characteristic of the organism in ordinary nutrient media. Leifson flagella stain, $\times 2,000$.

The flagella are extraordinarily long, up to $15\ \mu$. The wavelength is quite uniform averaging $1.4\ \mu$. The large soma with the round inclusions and the long flagella of relatively short wavelength make a very characteristic picture. Photomicrographs of several morphologic types are illustrated in Leifson's Atlas of Bacterial Flagellation as figures 68a, b, c, d, e and f. (1960).

Figure 1 shows the characteristic flagellation in pure culture.

Cultural characteristics. Colonies on agar tend to be small, colorless, smooth and semitranslucent. Growth on slants is thin, smooth and colorless. In broth the growth is uniform, colorless and without pellicle. Oxygen relations, aerobic; temperature relations, psychrophilic with no growth at 37°C; osmotic relations, rather halophobic or osmophobic with only scanty and very slow growth in media with 1% NaCl or equivalent osmotic pressure; pH relations, neutrophilic.

Physiological characteristics. Weak acid production from glucose, sucrose and maltose aerobically but not anaerobically. No acid from d-mannose, d-sorbitol, xylose, raffinose or lactose. Nitrate reduced to nitrite. All other reactions negative including cellulose, starch, gelatin and catalase. No growth in vitamin-free casamino acids and no growth or acid production in the mineral salts medium with glucose.

Habitat. Isolated from river water, but probably fresh water in general.

Type culture. A culture has been deposited in the American Type Culture Collection, Washington, D.C. (ATCC No. 14606).

REFERENCES

- Hugh, R. and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. *Jour. Bacteriol.* 66:24-26.
- Leifson, E. 1960. *Atlas of Bacterial Flagellation.* Academic Press, New York; London.