A NEW BACTERIUM, ACHROMOBACTER NEMATOPHILUS
SP. NOV. (ACHROMOBACTERIACEAE: EUBACTERIALES)
ASSOCIATED WITH A NEMATODE

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ABSTRACT. Achromobacter nematophilus sp. nov., isolated from the intestinal lumen of a species of Neoaplectana (Steinernematidae: Nematoda), is described. It can be distinguished from other species of Achromobacter by its larger cell size and action on gelatin and litmus milk.

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While studying the biology of an undescribed species of Neoaplectana (Steinernematidae: Nematoda) designated as DD-156, the authors discovered a bacterium in the intestinal lumen of this nematode.

In an effort to identify the organism, cultures were sent to the following authorities; 1) the State of California Department of Public Health, Berkeley, California; 2) the U.S. Public Health Communicable Disease Center, Atlanta, Georgia; and 3) Dr. M.J. Pickett, Department of Bacteriology, University of California, Los Angeles, California. We are deeply indebted to the above for their time and help in characterizing this bacterium.

Since the organism was unable to be classified, we are describing it as a new species in the genus Achromobacter. A more detailed account of its relationship with the DD-136 nematode will be presented in a later paper.

ACHROMOBACTER NEMATOPHILUS SP. NOV.

Location: in the intestinal lumen of a species of Neoaplectana (Steinernematidae: Nematoda).

Type: cultures sent to the American Type Culture Collection in Rockville, Maryland were assigned ATCC accession number 19061.

MORPHOLOGY (30°C, AC MEDIUM)¹

Large, thick, Gram-negative, nonsporeforming rods, measuring 3.5 to 10.4 μ in length by 0.9 to 1.7 μ in width ($x = 5.4\mu \times 1.1\mu$; $n = 50$) occurring singly, in pairs, and rarely forming longer chains. In older culture, spherical cells are formed, averaging 2.4 μ (1.9-3.12 μ ; $n = 25$) in diameter. Nonmotile, or motile by means of peritrichous flagella.

CULTURAL CHARACTERISTICS

Nutrient Agar: Colonies in 24 hours are greyish-white, convex, circular with slightly irregular margins, about 1 mm in diameter, and showing a slightly rough granular appearance.

Nutrient Agar Slant (30°C): Growth is filiform, and somewhat slow at first.

Tergitol-7 Agar with TTC (30°C):² Colonies are initially blue, gradually becoming darker and developing deep red centers. The reaction is alkaline, turning the background blue. In older cultures, both the brom thymol blue and TTC (triphenyltetrazolium chloride) are absorbed by the cells, leaving the agar clear.

AC Medium (30°C): Growth is aerobic, being restricted to the upper 1/2 inch of the culture tube, and forming a soft pellicle after one week.

Gelatin Stab: Good growth at 20°C and 26°C with liquefaction in five days. No liquefaction was obtained at 35°C and 37°C.

Chitin Agar: No growth at 20°C or 30°C.

Litmus Milk (37°C): Reduction and peptonization in 3 to 7 days.

Brom Cresol Purple Milk (26°C): Slight clot and slightly alkaline after one month.

Loeffler's Blood Serum (37°C): Proteolysis in 3 to 7 days, and slight brown pigmentation in 7 days.

Pseudomonas Agar (37°C): No growth in 7 days.

Urea Agar (37°C): No growth in 7 days.

¹ A rich growth-promoting medium. Difco Manual p. 201.

² A selective medium for *E. coli* and members of the coliform group. Difco Manual p. 175.

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

Mac Conkey Agar: No growth at room temperature, and no or very light growth at 37°C in 3 to 7 days.

Shigella Salmonella (SS) Agar: No growth at 37°C in 14 days.

Triple Sugar Iron Agar (20°C): Butt - Yellow, Slant - No change, H₂S negative.

H₂S (Kligler's Medium): Negative.

Potato: No growth at 30°C.

Motility Agar: Positive in 2 days at 37°C.

Blood Agar, Infusion Agar, and Brilliant Green Agar: All supported growth at room temperature.

BIOLOGICAL CHARACTERISTICS

Catalase: Tests for this enzyme in our laboratory, in Dr. Pickett's Laboratory, and by the State of California Department of Public Health, were negative (grown on nutrient agar and heart infusion agar). However, the U.S. Public Health Service Communicable Disease Center reported weakly positive results.

Oxidase: Communicable Disease Center and Pickett's Laboratory gave negative results. However, the California Department of Public Health reported weakly positive results with the use of a more definitive test; (i. e., colonies grown on heart infusion agar were flooded with M,N-dimethyl-p-phenylenediamine-oxalate).

All sources reported negative results for cytochrome oxidase, pectinase, lysine decarboxylase, Simmons' citrate, phenylalanine, indole³, indole pyruvic acid⁴, nitrate reduction, and the Voges-Proskauer test.

Fermentations: Growth in glucose with the production of acid, but no gas in both open and closed tubes, with a base of nutrient, beef extract, or oxidation-fermentation media⁵. Negative results using these three media were obtained for lactose, xylose, maltose, sucrose, and mannitol. Dr. Pickett's laboratory, using weakly pH buffered sugar solutions for quick results to avoid deamination, obtained positive results with xylose, maltose, fructose, trehalose, and arabinose. Additional negative results were reported by Dr.

³ Difco Manual p. 53.

⁴ See Singer and Volcani 1955.

⁵ Difco Manual supplement p. 201.

Pickett's laboratory for glycerol, inositol, rhamnose, salicin, sorbitol, dulcitol, adonitol, and gluconate.

REQUIREMENTS FOR GROWTH

Oxygen Requirements: aerobic facultative.

Temperature Relations (NA agar): Best growth was obtained between 25°C and 35°C, poor growth at 37°C, and no growth at 42°C.

pH Tolerance (NA agar): No growth at pH 5.0, 5.5, 6.0, 9.0 and 10.0. Good growth at 7.4 and 8.0.

Sodium Chloride Tolerance (NA agar): No growth at 0.0%. Best growth at 0.5%, 1.0%, and 2.0%. Good growth was also obtained at 3.0%. No growth above 4.0%.

DIAGNOSIS

A large rod-shaped, Gram-negative, peritrichously flagellated bacterium; does not form trichomes; will not reduce nitrates; is catalase and oxidase negative; nonchromogenic; produces acid from glucose.

Separated from the existing species of *Achromobacter* by its large size, its ability to liquefy gelatin and its action on litmus milk.

A. nematophilus forms blue colonies on a light blue background on Tergitol-6 medium. No other bacterium in our laboratory produced this reaction.

Thus far, found only inside the intestine of certain nematodes belonging to the genus Neoaplectana and in the body cavity of insects attacked by these nematodes.

REFERENCES

- Breed, R.S., E.G.D. Murray and N.R. Smith. 1957. *Bergey's Manual of Determinative Bacteriology*. 7th ed. Williams and Wilkins Company, Baltimore, 1094 pp.
- Difco Manual. 1963. 9th ed. Difco Laboratories, Inc., Detroit, Michigan, 350 pp.
- _____. 1952. *Supplementary Literature*. Ibid., 316 pp.
- Singer, J. and B.E. Volcani. 1955. An improved ferric chloride test for differentiating Proteus-Providencia group from other Enterobacteriaceae. *Jour. Bact.* 69:303-306.
- Skerman, V.B.D. 1959. *Genera of Bacteria*. Williams and Wilkins Company, Baltimore, 217 pp.