MICROCOCCUS DIVERSUS SP. NOV.¹

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ABSTRACT. A flagellated coccus is described which has a DNA base composition (G+C), morphological and physiological characteristics associated with the genus Micrococcus. The organism was isolated from the gut content of a gypsy moth, Porthetria dispers (L.), larva. The characteristics are markedly different from those of previously described species and thus a new species, Micrococcus diversus, is proposed.

According to Leifson (1964), a number of flagellated cocci have been described. Sarcina ureae was reported in 1901 (Beijerinck) and the existence of motile Streptococcus species (Group D) has been observed by Graudal (1952). The first motile coccus described in the literature was Micrococcus agilis isolated by Ali-Cohen (1889). The strain of M. agilis obtained from the American Type Culture Collection (ATCC No. 998) is nonmotile and no other species, with the exception of some marine micrococci including Micrococcus eucinetus (Leifson 1964) have been shown to possess this characteristic. Boháček et al. (1968) suggested that M. eucinetus and the other motile marine micrococci be transferred from this genus to Planococcus since the guanine plus cytosine (G+C) base composition of the DNA of these organisms (41%) is not in the range reported for the genus Micrococcus (65-75%, Hill 1966).

¹ Supported in part by Grant No. 4000, U.S. Dept. of Agriculture, Forest Service.
² Submitted in partial fulfillment of the requirements for the Ph. D. degree, University of Connecticut, Storrs, Conn.
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The new species was found to have a DNA G+C base composition in the range for the genus Micrococcus. This organism can be placed in the group which includes Micrococcus dentrificans (G+C 65-75 per cent, Boháček et al., 1965) and Micrococcus halodentrificans (G+C 64-66, Boháček et al., 1965) because of its Gram-negative staining reaction and other characteristics.

**DESCRIPTION**

**Morphology.** The organism was stained employing the Kopeloff-Beerman modification of the Gram stain and measurement of the soma showed an average diameter of 1.05 μm. \( M. \) diversus cells are Gram-negative. Arrangement of cells as determined in young (18-24 hr) broth cultures (Trypticase Soy Broth, TSB, Baltimore Biological Labs. BBL) is predominately singles and pairs with only occasional grouping of four cells and never eight. Chain or cluster formations were not observed. The shape and arrangement of these cells are clearly shown in Fig. 1. Motility was active with a definite tumbling motion of single and paired cells. The flagellated cells showed a single flagellum. The soma, flagellum and capsule are seen in Fig. 2 (Leifson's stain). Electron microscopy reveals further the long flagellum, capsule and soma as stained with 2 per cent phosphotungstic acid (Fig. 3).

**Cultural characteristics.** Colonies on agar (Trypticase Soy Agar, TSA, BBL) are smooth, moist, glistening, convex, opaque, and circular. The dark pink pigment is insoluble in water. Growth on slants (TSA) is good, moist, glistening and similar to the colonies appearing on the agar. In broth (TSB) growth is faintly turbid in young cultures and a viscid sediment accumulates in older cultures (3 days) leaving a clear supernatant; some surface growth is present but no pellicle is formed.

**Physiological characteristics.** Ability to produce acid from sugars aerobically and/or anaerobically was tested by Hugh and Leifson's method (1953) with the modified medium composition of Baird-Parker (1963). \( M. \) diversus failed to produce acid from the following carbohydrates: arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, raffinose, rhamnose, ribose, salicin, sucrose and xylose. The organism is strongly catalase positive and gives a weak phosphatase reaction.
**Micrococcus diversus n. sp.**

**Figure 1.** M. *diversus* as seen with phase microscopy. 750X.

**Figure 2.** M. *diversus* stained with Leifson's flagella stain. 1200X.

**Figure 3.** M. *diversus* stained with 2% phosphotungstic acid. 9000X.
Tests for detecting the presence of urease, DNase, lecithinase, caseinase, amylase and gelatinase were negative. Indole, acetyl methyl carbinol and H₂S are not produced. Nitrate is reduced and the methyl red test is negative. Hydrolysis of Tween 20, 40, 60 or 80 (Atlas Chemical Industries) as tested by Sierra (1957) was shown to be negative as was the hydrolysis of butter, lard, margarine, olive and Wessen oil. The organism grew well in 2.5 per cent NaCl, faintly in 5 per cent NaCl and failed to grow in 10 per cent NaCl. Optimum pH for growth is 6.5 - 8.0; no growth occurred at pH 5.5 or 9.0. Oxygen relation, aerobic; temperature relations, mesophilic with optimum growth ranging between 20° to 37° C; incubation at 45° C for 3 days is bacteriocidal.

Habitat. Isolated from the gut content of a gypsy moth (Portheretria dispar (Linnaeus), larva.

TAXONOMIC CONSIDERATIONS

The base composition of this organism has been determined (1) from the Tm value (Marmur and Doty, 1962) and (2) by isolation of degraded DNA and estimation of the separated individual bases eluted from a thin layer plate (Wyatt, 1951). The G+C content was found to be 67-69 per cent which placed it within the range of the genus Micrococcus. Since it stains Gram-negatively and is a strict aerobe, the only other possible consideration for spherical types is the genus Neisseria. However, the base composition for the genus Neisseria ranges from 40-50 per cent G+C (Cattin and Cunningham, 1961) and members of the genus ferment a variety of carbohydrates.

M. diversus is similar to M. dentrificans and M. halodenitrificans in G+C content but unlike these species rod-shaped cells are not observed during any phase of the growth cycle. This organism is thusly placed in the genus Micrococcus on the basis of its shape, arrangement, inability to attack glucose fermentatively, pigmentation and DNA base composition.

REFERENCES


