BREVIBACTERIUM LEUCINOPHAGUM SPEC. NOV.

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The genus Brevibacterium Breed was established in 1953 (1), and together with the genus Kurthia Trevisan constitutes the family Brevibacteriaceae Breed (2). The members of Brevibacterium are typically short, unbranched, gram-positive, non-sporulating, rod-shaped bacteria. The type species is B. linens (Weigmann) Breed.

During a series of investigations of amino acid metabolism we have isolated from soil a new species of bacterium which possesses characters typical of the genus Brevibacterium. We propose the name Brevibacterium leucinophagum for this species.

A type culture has been deposited with the American Type Culture Collection, Washington, D. C. (ATCC No. 13809)

METHODS

The organism was isolated from a sample of flower bed soil. A few milligrams of soil were introduced directly into a medium containing 1% (w/v) L-leucine and mineral salts. After 24 hrs at 37°C the heavily turbid broth was streaked on Petri plates containing the same medium solidified with 2% agar. Well isolated colonies were picked and maintained on plain nutrient agar slants.

Standard bacteriological techniques were used in the diagnosis of the species (3). Each test was repeated at least three or four times at intervals of approximately two months. Determination of fermentable carbohydrates was made by inoculation of tubes of plain nutrient broth supplemented with 0.5-1.0% of the carbohydrate and containing bromthymol blue and a Durham vial.

RESULTS

Cultural characteristics

Flasks of liquid media show silkiness when swirled after 8-12 hrs at 30°C. Turbidity becomes moderately heavy, and
a thin membrane forms on the surface. This is easily dislodged and settles to the bottom while the broth remains turbid. The slight ring which remains soon gives rise to another membrane.

Colonies on the surface of gelatin or nutrient agar plates are small (1-2 mm in diameter), circular with entire edge, raised, butyrous and glistening. Colonies appear white to buff-colored when viewed by reflected light, iridescent by transmitted light. Agar streaks show moderate, beaded growth.

Gelatin and agar stabs show no growth, either in the presence or absence of fermentable carbohydrate. Similarly, glucose broth tubes overlaid with sterile mineral oil do not support growth.

**Morphological characteristics**

The cells stain readily and uniformly with the usual aniline dyes and are gram positive during the early phases of growth, but lose their gram positive character after 12-16 hrs at 30°. The cells are very short, stubby rods, 0.4-0.7 x 0.7-1.4 microns; young cells from liquid cultures often resemble cocci. At temperatures above 30° long, unbranched filaments and pleomorphic forms often occur. No characteristic cell arrangements are noted. Endospores are not formed. No lipid inclusion bodies are formed in carbohydrate media. The species is not motile.

**Physiological characteristics**

Acid, but no gas, is readily produced in glucose broth; methyl red tests are negative, however, and no acetyl-methylcarbinol is formed. No acid or gas production was noted when fructose, lactose, sucrose, maltose, mannose, raffinose, glycerol, mannitol, sorbitol and salicin were tested. When first isolated, the species produced slight acidity but no gas in the presence of xylose, but this property was lost after several transfers on nutrient agar. A slightly basic reaction was usually noted in a peptone medium (pH 7.5-7.6 by the glass electrode), but no ammonia could be detected with Nessler's reagent.

Nitrates are not attacked, nor is urea. Good growth is obtained in 24-48 hrs at 30° when washed cells are inoculated into media containing ammonium phosphate as sole nitrogen source.
BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

Since B. leucinophagum does not grow anaerobically in stab cultures, gelatin hydrolysis was tested by the Frazier method as modified by Smith (4). Gelatin is not hydrolyzed. Hydrogen sulfide production was not detected when agar plates containing either lead acetate or ferrous sulfate were inoculated with the test organism. Starch is not hydrolyzed.

Indole is not produced in Bacto-tryptone (Difco) broth cultures. Repeated subculture in the synthetic medium of Koser yields heavy growth, indicating that citrate is readily utilized as sole carbon source. Other intermediates in the tricarboxylic acid cycle (α-ketoglutarate, succinate, malate, fumarate and acetate) are rapidly utilized, suggesting operation of this cycle.

When tubes of litmus milk were incubated at 30°, no immediate changes took place. After 4-5 days, however, the litmus in the depths of the tubes was slowly reduced, and after 10-12 days the contents of the tubes became solid. Litmus at the surface remained blue, indicating that the curd which formed was not due to accumulation of acid. No peptonization of the casein was apparent, however, even after 14 days.

No hemolysis occurs on sheep blood agar plates at 30° or 37°.

Cultures of this species remain viable for periods up to 3 months when agar slants are stored at 4°; broth cultures at this temperature do not remain viable longer than 3-4 weeks, however. Cultures grow well, though slowly, at 10°, and show optimum growth at 30°. At 37° many long, unbranched filaments and pleomorphic forms are formed rapidly in liquid media. No growth occurs at 45°.

Manometric experiments with washed, whole cells showed very high rates of oxygen uptake on L-leucine (700-800 microliters per hr per 150 mg wet cells). The mechanism of dissimilation of leucine by this species will be reported elsewhere.

DISCUSSION

The properties of this species indicate that it is properly classified in the family Brevibacteriaceae Breed according to the 7th edition of Bergey's Manual. This family includes two genera, Brevibacterium and Kurthia, which are differentiated primarily by the fact that members of the latter
genus form long, unbranched rods or filaments and do not utilize carbohydrates. Although long, unbranched rods have been observed in cultures of the organism described here, this occurs only at temperatures above the optimum, and has never been observed at lower temperatures. In addition these filamentous forms are accompanied by the presence of distorted, swollen and club-shaped cells which are typical of the pleomorphic forms found in bacterial cultures exposed to degenerative influences. Serial microscopic examination of developing cultures shows no evidence that these filaments are formed first and then divide into coccoid elements, as is described for the genus Kurthia.

Although 23 species of Brevibacterium are listed in the 7th edition of Bergey's Manual, a careful comparison of cultural, morphological and physiological characteristics shows that the organism described here is not identical to any of these. This species is non-motile; therefore it is not B. incertum, B. imperiale, B. lipolyticum, B. acetilicum, B. sulfureum or B. helvolum. It produces no pigments when grown on agar, and is therefore not B. linens, B. erythrogenes, B. fulvum, B. insectiphilium, B. brunneum, B. vitarumen, B. maris or B. fuscum. Other properties differ sufficiently also to show that it is not a pigmentless variety of these species. Nitrites are not produced from nitrates; therefore this species is not B. stationis, B. quale or B. ammoniagenes. B. leucinophagum does not liquefy gelatin, as do B. sociovivum, B. immotum and B. marinopiscosum. Furthermore, sea water or similar saline solutions are not required to initiate growth. The organism described here is not identical to B. minutiferula since the latter produces slight acidity from sucrose and in litmus milk. B. leucinophagum possesses none of the cultural characteristics of B. heali. Finally, it produces acid from glucose only, whereas B. tegumenticola produces acid from glucose, maltose and sucrose.

The evidence thus warrants the proposal of a new species.

**SUMMARY**

A bacterium, isolated from soil with L-leucine as sole carbon and nitrogen source, was found to possess properties typical of the genus Brevibacterium Breed, family Brevibacteriaceae Breed. It is a new species for which the name *Brevibacterium leucinophagum* is proposed.
REFERENCES


