APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1988, p. 1061–1062
0099-2240/88/041061-02$02.00/0

Vol. 54, No. 4

NOTES

Isolation of a New Polysaccharide-Digesting Bacterium from a Salt Marsh

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Received 19 October 1987/Accepted 26 January 1988

A new marine bacterium that digested a variety of storage and structural polysaccharides, including agar, was isolated. Strain 2-40 is a nonfermentative gram-negative, polarly flagellated rod that sometimes grew as a filamentous helix and secreted a melaninlike pigment. Its characteristics conform to those of no previously described species.

In the course of attempts to isolate bacteria involved in the decomposition of the salt marsh grass *Spartina alterniflora*, we discovered a unique agar-digesting species that we designated strain 2-40. Besides agar, strain 2-40 degraded a number of other polysaccharides of plant, algal, fungal, and animal origin. This bacterium may provide additional information about the role of bacteria in nutrient cycling and degradation of recalcitrant macromolecules in salt marsh ecosystems (1, 4, 7). Furthermore, it is a new source of polysaccharases for possible commercial applications and for analysis of plant, fungal, and algal cell walls.

The initial culture of strain 2-40 was obtained from 1% peptone–half-strength-seawater agar plates that had been pressed into partially decomposed *S. alterniflora* at a Chesapeake Bay salt marsh in Matthews County, Va. The organism was a gram-negative, pleomorphic rod with a single unsheathed, polar flagellum. Cells averaged 0.5 μm in width and 1.5 to 3.0 μm in length. In stressed cultures, filaments and coils of up to 20 μm were formed. Spheroplasts, which appeared in old cultures, subsequently underwent autolysis.

Growth and standard biochemical tests (8) were carried out by making appropriate additions to Difco marine broth 2216. Sugar oxidation-fermentation tests were performed by a modification of the Leifson method for marine microbes (3). The organism was catalase and peroxidase positive and nonfermentative. Growth required sea salts and carbohydrate. The temperature range for growth was 5 to 40°C, and the most rapid growth occurred at 37°C. The optimum pH was 7.5. Although strain 2-40 grew well in the mineral medium of Niven (5), growth was more rapid with organic nitrogen than with NH_4^+ as the nitrogen source. When tyrosine or peptone was present, a brown, melaninlike pigment was produced. An ethanol-insoluble, anthrone-positive polymer accumulated when glucose was the carbon and energy source.

Strain 2-40 was capable of lipolysis (with Tween 20 and Tween 80) and caused alpha-hemolysis of sheep and human erythrocytes. Acid was produced on arabinose, cellobiose, dulcitol, fructose, galactose, galacturonic acid, glucose, glucuronic acid, maltose, mannose, ribose, salicin, sorbose, sucrose, and xylose.

To assess polysaccharide digestion, cultures were grown on a gyratory shaker at 200 rpm at 25°C in a mineral medium supplemented with polysaccharide as the sole carbon and energy source. Log-phase culture supernatant (0.3 ml) was added to 0.7 ml of 0.5% polysaccharide in 0.01 M potassium phosphate buffer at pH 7.0. The reaction mixtures were incubated for 3 h at 35°C. Hydrolysis of polysaccharides was determined by measuring the release of reducing sugars colorimetrically with dinitrosalicylic acid reagent (9), with glucose as the standard. Chitin breakdown was also measured by *N*-acetylglucosamine release (6). Agar, agarose, alginic acid, carboxymethylcellulase, chitin, glycogen, laminarin, pullulan, sodium polygalacturonate, starch, and xylan were digested. Carrageenan, cellulose, dextran, inulin, pectin, and polygalacturonic acid were not digested.

Comparison of strain 2-40 with organisms with similar morphological and physiological traits suggested that it is not a member of any known species. Its G+C content (45.66 molo/o, as determined by the American Type Culture Collection, Rockville, Md.) was within the range of G+C content of only *Oceanospirillum*, *Vibrio*, and *Alteromonas* spp. (2). Unlike strain 2-40, *Oceanospirillum* spp. do not metabolize sugars and have bipolar flagellation and permanently helical cells. *Vibrio* spp. differ in the fermentation of sugars and possession of sheathed flagella. Strain 2-40 cannot be identified as an existing species of *Alteromonas*, but it could conceivably be classified within this heterogeneous and complex genus, which has been described as follows (2): "Straight or curved rods, 0.7–1.5 μm in diameter and 1.8–3.0 μm in length. Do not accumulate poly-β-hydroxybutyrate (PHB) as an intracellular reserve product. Microcysts or endospores are not formed. Gram-negative. Motile by means of single polar flagella. Chemoorganotrophs capable of respiratory but not fermentative metabolism. Molecular oxygen is a universal electron receptor; do not denitrify . . . require a seawater base for growth . . . grow at 20°C. Common inhabitants of coastal waters . . . The mol% G +C content of the DNA is 38–50 mol% (T_m, Bd)."

Strain 2-40 had the same G+C content as strain LST, a non-polysaccharide-digesting, melanin-producing marine bacterium recently isolated by Weiner et al. (10). However, these workers report no DNA homology between the two organisms (personal communication).

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Additional homology studies are being undertaken to establish the taxonomy of strain 2-40. Further characterization of the polysaccharases of strain 2-40 is also under way. The organism has been deposited in the American Type Culture Collection, Rockville, Md.

This work was supported by George Mason University research grant 2-10144.

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