Cellulomonas cartae sp. nov.

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A cellulolytic, gram-positive organism with a high (76.6 mol%) guanine plus cytosine content in its deoxyribonucleic acid is described as a new species, *Cellulomonas cartae*, because the original description and naming of this organism, as *C. cartalyticum* (sic), were in a patent and hence were neither effectively nor validly published. The taxonomic position of this organism as a member of the genus *Cellulomonas* is confirmed on the basis of biochemical and genetic characteristics. It is further shown that the new species differs from all other species of *Cellulomonas* described to date. The type strain of *C. cartae* is DSM 20106 (= ATCC 21681).

Carta previously described a cellulolytic organism in a patent (G. R. Carta, U.S. Patent no. 3,778,349, 1973) describing the production of single-cell protein material. He named the organism *Cellulomonas cartalyticum* (sic), and a culture was deposited in the American Type Culture Collection (ATCC) under the number 21681. According to Rule 25b of the International Code of Nomenclature of Bacteria (3), however, the inclusion of a new species in a patent does not constitute effective, hence valid, publication. In addition, the organism was originally poorly described, and many characteristics necessary for the proper classification of this organism were not reported in the patent. The results of comprehensive biochemical and physiological studies (7) have shown this organism to share genus-specific characteristics with members of *Cellulomonas* on the one hand and to differ in many characteristics from all well-described members of this genus on the other hand. These results justify the description of this organism as a new species of *Cellulomonas*. The specific epithet proposed by Carta cannot be retained. Because Carta did not give the etymology of the proposed name, it is not clear whether it was formed by a combination of the Greek *lyticus* meaning dissolving and the author's name or the Latin noun *charta*, meaning paper. In the first case, the epithet *cartalyticum* (sic) would be meaningless; in the second case, it would mean paper-dissolving, a property common to all species of the genus. Thus, retention of this epithet would ignore recommendation 12C of the International Code of Nomenclature of Bacteria (3).

MATERIALS AND METHODS

Bacterial strain. DSM 20106 (← ATCC 21681 ← G. R. Carta), from soil, was the only strain studied.

Growth conditions. Cultures were maintained on peptone-yeast extract-glucose (PYG) agar slants (peptone, 1%; yeast extract, 0.5%; glucose, 0.5% Difco agar, 1%) stored at 4°C. Physiological tests were carried out aerobically at 30°C in PYG prepared without the agar. The tests for the utilization of carbon compounds, ammonium production from peptone, and nitrate reduction followed methods described elsewhere (7).

Methods. Colony morphology was observed on PYG incubated at 30°C for 14 days. After fixing the cells from a growing culture with osmium tetroxide (4), treating them with uranyl acetate, and embedding them according to Spurr (5) we prepared thin sections with a LKB-Ultratome III using a glass knife. The sections were viewed with a Siemens Elmiskop Ia electron microscope.

The production of acetic acid and L-lactic acid from glucose was determined with acetate kinase (2) and L-lactate dehydrogenase (1), respectively. The enzymes were purchased from Boehringer, Mannheim. Glucose fermentation was carried out with resting cells in Warburg vessels. CO₂ production was determined manometrically (8). Cellulolytic activity was tested aerobically with 0.5 g of Whatman no. 1 filter paper strips (1 by 0.5 cm) in 30 ml of medium (0.5% peptone and 0.3% yeast extract, pH 7.2) over a period of 10 days. The methods for the determination of the guanine plus cytosine content of the deoxyribonucleic acid (DNA) and the amino acid and sugar composition of the cell wall are described elsewhere (6). The procedures for DNA-DNA hybridization have also been described previously (7).

RESULTS AND DISCUSSION

Cellulomonas cartae sp. nov. (car. ta'. M.L. gen. n. *cartae* of Carta; named for G. R. Carta, the first to isolate this organism).

Gram-positive, nonmotile rods, 0.4 by 0.6 to 1.7 µm (Carta, U.S. Patent no. 3,778,349, 1973).

Cells in the logarithmic growth phase are irregular, curved, and club-shaped or are arranged in V formation (for microphotograph, see reference 7). Approximately 4 days after exhaustion

FIG. 1. Electron micrograph of an ultra-thin section of *Cellulomonas cartae*. Bar, 200 nm.

of the medium, the rods transform into shorter rods or even coccoid cells. On PYG agar, colonies are about 2 to 5 mm in diameter, circular, convex, white, and glistening. Cells in the logarithmic phase of growth are easily decolorized with 96% ethanol when treated for longer than 9 s and therefore appear gram negative. However, *Escherichia coli* cells stain gram negative even after ethanol treatment for as short as 3 s. Therefore, in comparison with *E. coli*, *C. cartae* is definitely gram positive. This observation was confirmed by an electron micrograph (Fig. 1) of an ultra-thin section of *C. cartae*, in which the typical cell wall profile of a gram-positive organism is evident.

The following are utilized as sole carbon sources: glucose, mannose, maltose, sucrose, D-xylose, L-arabinose, lactose, gluconate, mannitol, ribose, L-lactate, acetate, pyruvate, glycerol, and cellobiose. Raffinose, DL-malate, and D-lactate are not used as sole carbon sources.

Acid is produced from glucose, mannose, sucrose, D-xylose, L-arabinose, ribose, gluconate, glycerol, lactose, pyruvate, and cellobiose.

Acetic acid is the main acidic intermediary product of aerobic glucose dissimilation. Maximum acetic acid production (6.5 μmol of acid per ml of PYG medium) is reached in 48 to 72 h. The pH of the medium is 4.8. L-Lactic acid is produced in much smaller quantities (1 μmol /ml of medium). After exhaustion of glucose, the acids are oxidized, resulting in an increase of the pH of the medium (pH 8.8) after 8 days. Resting cells ferment glucose anaerobically to CO_2 , acetic

acid, and L-lactic acid. Cellulose strips are attacked from the edges in liquid medium within the first 48 h. No colonies are visible on the strips. After 48 h, the paper is totally converted to a pulpy mass. The pH after 4 days is 5.5.

Starch is hydrolyzed and gelatin is slowly liquefied (in accord with Carta's results [Carta, U.S. Patent no. 3,778,349, 1973]).

Catalase is produced (Carta, U.S. Patent no. 3,778,349, 1973).

Nitrite is produced from nitrate (in accord with Carta's results [Carta, U.S. Patent no. 3,778,349, 1973]).

Acetylmethylcarbinol is not produced (Carta, U.S. Patent no. 3,778,349, 1973).

Urease and lysine decarboxylase are not produced (Carta, U.S. Patent no. 3,778,349, 1973).

Aerobic to facultatively anaerobic.

DNA-base composition: 76.6 mol% guanine plus cytosine (6).

Cell wall composition, amino acids: DL-alanine, D-glutamic acid, L-lysine, D-serine, and D-aspartic acid; peptidoglycan type: Lys-D-Ser-D-Asp (6); sugars: galactose, fucose, and rhamnose (6). The cell walls do not contain teichoic acid or teichuronic acid (Fiedler, personal communication).

DNA-DNA hybridization experiments revealed a moderate relationship to other strains of *Cellulomonas* (12 to 20%); almost no genetic relationship was found between *C. cartae* and strains of *Arthrobacter* (7).

The type strain is DSM 20106 (= ATCC 21681).

A comparison of the characteristics of *C. cartae* with those of the other species of *Cellulomonas* is published elsewhere (7).

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REPRINT REQUESTS

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