Description of *Bacillus azotoformans* sp. nov.

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The name *Bacillus azotoformans* (ex Pichinoty, de Barjac, Mandel, Greenway, and Garcia 1976) is revived. Strains of this species are motile and produce terminal or subterminal oval endospores in swollen sporangia. Nitrate, nitrite, and nitrous oxide are denitrified with production of nitrogen. The guanine-plus-cytosine content of the deoxyribonucleic acid of this organism is 39.8 ± 1.2 mol% (mean $\pm$ standard deviation). The type strain is ATCC 29788 (= CCM 2849 = CIP R925).

The name *Bacillus azotoformans* Pichinoty, de Barjac, Mandel, Greenway, and Garcia 1976 does not appear on the Approved Lists of Bacterial Names (9), although it was effectively published (6) and a type strain was designated (7). The purpose of this report is to revive the name *Bacillus azotoformans* and to effect its valid publication. Although this species has been described previously (6), we provide a formal description, including formerly unpublished fatty acid and phospholipid compositions.

Fatty acid and phospholipid composition studies were done by using strains 1, 13, and 30 (7). These strains were grown for 24 h at 32°C in yeast extract-salts liquid medium; this medium was made by combining 4 g of yeast extract (Institut Pasteur, Lille, France), 3.575 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.98 g of KH_2PO_4 , 0.03 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g of NH_4Cl , 0.2 ml of a solution containing calcium and various heavy metals chelated with ethylenediaminetetraacetate (8), and distilled water to a volume of 1 liter. Under these conditions, poly- β -hydroxybutyrate is not synthesized. Cells were collected by centrifugation, washed with distilled water, and freeze-dried. The total free lipids were extracted with chloroform-methanol (2:1, vol/vol). After evaporation of the solvents in a vacuum, the residue was extracted with ether. Silica Gel G plates (E. Merck AG, Darmstadt, Germany) were spotted with approximately 10 μg of each sample and then developed in ascending fashion by using a solution containing petroleum ether (bp 50°C) and diethyl ether (4:1, vol/vol). Staining of the developed plates with Rhodamine B revealed no more than traces of triglycerides.

Further development with chloroform-methanol-water (65:25:4, vol/vol) and successive staining were performed with ninhydrin and Dittmer reagents. For fatty acid gas chromatography, the lipids were saponified in 5% (wt/vol) KOH that was dissolved in 33% (vol/vol) aqueous methanol at 100°C for 90 min. Fatty acids were methylated by reacting them with diazomethane in diethyl ether and then injected into a 3-m glass column containing 1% OV-1 on Chromosorb W. A model G-30 gas chromatography (Girdel, Sur-esne, France) was used, and a temperature increase from 130 to 250°C was programmed at 3°C/min. Peaks were identified by adding internal standards and also by comparison with the methyl esters of the fatty acids extracted in a similar manner from *Streptomyces griseus* (1). The bacteriological methods used have been described elsewhere (6, 7). Aminopeptidase was assayed colorimetrically (2).

Description of *Bacillus azotoformans*. *Bacillus azotoformans* (a.zoto.for.mans. French n. *azote* nitrogen; L. part. adj. *formans* forming; *azotoformans* nitrogen forming) (0.9 to 1 by 3 to 10 μm), have rounded ends, and are rapidly motile by means of numerous long peritrichous flagella. Gram reaction negative. Gram type positive. The cells have thick walls, mesosomes, and persistent septa (6). Aminopeptidase is absent (10). The endospores of this organism are oval (1.4 by 2 to 2.5 μm) and make the sporangium bulge; the spores are located terminally or subterminally. Exosporium lacking. Colonies are circular with entire margins, convex, and partially translucent, attaining a diameter of 5 mm on yeast extract-salts agar. Growth is rapid and

produces uniform and dense turbidity in yeast extract-salts liquid medium. Poly- β -hydroxybutyrate is produced when cells are grown in the presence of DL-3-hydroxybutyrate.

Chemoorganotrophic. Growth factors are required. Aerobic metabolism. Glucose is neither fermented nor oxidized. The following respiratory electron acceptors are utilized anaerobically; NO_3^- , NO_2^- , N_2O , $\text{S}_4\text{O}_6^{2-}$, and fumarate. $\text{S}_2\text{O}_3^{2-}$ is not utilized as an electron acceptor. Considerable quantities of N_2 are produced in the course of reduction of NO_3^- , NO_2^- , N_2O . Oxidase reaction positive.

The following enzymes and electron carriers are present: cytochromes *b* and *c*; oxidase *a* + *a*₃; nitrate reductase *A*; nitrite ($\text{NO}_2^- \rightarrow \text{NO}$), nitric oxide ($\text{NO} \rightarrow \text{N}_2\text{O}$), nitrous oxide ($\text{N}_2\text{O} \rightarrow \text{N}_2$), tetrathionate ($\text{S}_4\text{O}_6^{2-} \rightarrow \text{S}_2\text{O}_3^{2-}$), and fumarate reductases; and L-glutamate dehydrogenase. The following enzymes are not detected: catalase, thiosulfate reductase, arginine dihydrolase, L-phenylalanine deaminase, urease, and β -galactosidase.

The following tests are negative: hydrolysis of gelatin, starch, and Tween 80; growth at pH 5.6; growth in the presence of 5% NaCl, 8% KNO_3 , 0.02% NaN_3 , and 0.001% lysozyme; Voges-Proskauer reaction; indole production; decomposition of tyrosine; production of brown pigment in tyrosine-containing media; decomposition of hippurate with production of benzoate; and conversion of glycerol to dihydroxyacetone.

The following organic acids are degraded: acetate, succinate, DL-lactate, pyruvate, L-malate, citrate, L-glutamate, and L-aspartate. Propionate, glycolate, malonate, glutarate, tartrate, and glycine are not degraded.

The maximum growth temperature is between 42 and 46°C; no growth occurs at 4°C.

Lipids comprise 5.7 to 7.4% of total cell dry weight. Phosphatidylethanolamine is the major phospholipid; cardiolipin and phosphatidylglycerol are also present. The fatty acid composition is as follows: iso- C_{14} , 15 to 30 mol%; *n*- C_{14} , 2 to 5 mol%; iso- C_{15} , 28 to 52 mol%; anteiso- C_{15} , 16 to 20 mol%; *n*- C_{15} , 0 to 1 mol% iso- C_{16} , 5 to 8 mol%; *n*- C_{16} , 2 to 6 mol%.

Guanine-plus-cytosine content of deoxyribonucleic acid: 39.8 ± 1.2 mol% (as determined by buoyant density in CsCl).

Habitat: soils.

Selective isolation can be performed by enrichment culture of pasteurized (10 min, 80°C) soil samples in media containing 1% Bacto-Peptone (Difco Laboratories, Detroit, Mich.) incubated at 32°C in an atmosphere of pure N_2O .

Type strain: strain 1 (= ATCC 29788 = CCM 2849 = CIP R925).

Description of the type strain. The type strain

has all of the characteristics given above for the species. Additional characteristics have been described elsewhere (6, 7).

Characteristics distinguishing *B. azotoformans* from related bacteria. *B. azotoformans* differs from *Clostridium* species by the type of metabolism (aerobic) and the cytochrome content. At present, aerobic, endospore-forming, rod-shaped bacteria are classified in the genus *Bacillus*. A total of 33 species of *Bacillus* are on the Approved Lists of Bacterial Names (9), but only 10 of these are morphologically similar to *B. azotoformans*; i.e., only 10 species produce oval, subterminal to terminal endospores which distend the sporangium (*Bacillus alvei*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus larvae*, *Bacillus laterosporus*, *Bacillus lentimorbus*, *Bacillus macerans*, *Bacillus macquariensis*, *Bacillus polymyxa*, and *Bacillus popilliae*). The following nine species grow anaerobically on sugars, produce acid from glucose under aerobic conditions, and therefore differ from *B. azotoformans*: *B. alvei*, *B. circulans*, *B. larvae*, *B. laterosporus*, *B. lentimorbus*, *B. macerans*, *B. macquariensis*, *B. polymyxa*, and *B. popilliae* (3–5). Moreover, *B. macquariensis* is a psychrophile that grows only at temperature between 0 and 25°C (5). The species most like *B. azotoformans* is *B. brevis*, a strict aerobe that possesses cytochromes *b* and *c*. However, the neotype strain of *B. brevis*, strain 52.86 (= ATCC 8246), produces acid from glucose and mannitol under aerobic conditions, hydrolyzes gelatin and tyrosine (4), produces catalase and arginine dihydrolase but not oxidase *a* + *a*₃ or L-glutamate dehydrogenase, and cannot grow anaerobically in the presence of NO_3^- , NO_2^- , N_2O , $\text{S}_4\text{O}_6^{2-}$, or fumarate; in addition, the guanine-plus-cytosine content of *B. brevis* deoxyribonucleic acid is 48 mol% (6, 7).

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