Emended Description of Strain PS (= OGC 70 = ATCC 33273 = DSM 1537), the Type Strain of *Methanococcus voltae*

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The isolation and characterization of *Methanococcus voltae* PS^T (= OGC 70^T = ATCC 33273^T = DSM 1537^T) (T = type strain) are described.

Balch and Wolfe proposed the name *Methanococcus voltae* for a new species of methanogens because *M. voltae* PS^T (T = type strain) differed from the type strain of *Methanococcus vannielii*, the only other recognized species in the genus *Methanococcus* at that time (1). Comparisons of ribosomal ribonucleic acid catalogs documented the phylogenetic differences between these strains (the S_{AB} value is 0.60), and *M. voltae* requires a higher salt concentration for rapid growth (1). Strain PS^T, which was isolated and described by one of us (Ward, M.S. thesis, University of Florida, Gainesville, 1970), is further described here.

Enrichment and isolation. A sediment sample was obtained near the mouth of the Waccasassa estuary in western Florida by taking a core that was 50 mm in diameter and 0.50 m deep. The sampling site contained a large population of red algae (*Gracilaria* sp.), a sample of which was also obtained. After the samples were returned to the laboratory, the cores were removed from their containers for subsampling; in all subsequent operations, samples were protected from O₂ by flushing vessels with O₂-free N₂. The culture techniques of Hungate (4) were used. The algae were blended in a Waring blender, and 4 g (wet weight) was added to 100 ml of sediment; this preparation was incubated at room temperature until rapid gas production stopped. A portion was removed and serially diluted in O₂-free medium containing (per liter) 12.2 g of MgCl₂ · 6H₂O, 5.3 g of NaCl, 1.7 g of KCl, 1.6 g of MgSO₄ · 7H₂O, 0.6 g of NaHCO₃, 0.5 g of H₃PO₄, 0.4 g of Na₂S · 9H₂O, 0.1 g of L-cysteine hydrochloride, 1 mg of resazurin, and 300 ml of rumen fluid; the medium was maintained under O₂-free N₂, adjusted to pH 7, dispensed into tubes that were sealed with butyl rubber stoppers, and autoclaved in a press. Dilutions were inoculated into roll tube medium at the same composition but solidified with 18 g of purified agar per liter and incubated at 37°C with a gas phase of H₂. After 1 month of incubation, counts of slowly growing colonies which were not present in controls lacking H₂ indicated that there were 50,000 to 90,000 methanogens per ml of sediment. Colonies from the roll tube cultures of the highest dilution were picked, diluted, inoculated into roll tube medium, and incubated. This procedure was repeated until a single colony type remained. The resulting culture, strain PS^T, was maintained by growth in the dilution medium with an H₂ gas phase. No growth resulted when the culture was inoculated into brucella broth, suggesting that no organisms other than methanogens were present; furthermore, a single morphological type was seen by phase-contrast microscopy of liquid cultures.

Characteristics and emended description of the species. Our

characterization of this organism (Ward, M.S. thesis) and data from subsequent studies (3, 5–8) suggest the emended species description given below.

Methanococcus voltae Balch and Wolfe 1979 (in Balch, Fox, Magrum, Woese, and Wolfe 1979) (1) (vol'tae. N.L. gen. n. *voltae*, of Volta, named for the Italian physicist Alessandro Volta, who discovered the combustible nature of gas from anaerobic sediments) cells are irregular cocci that are 1 to 2 µm in diameter and highly motile. Peritrichous flagella are present. Attempts to determine the Gram reaction by the Burke method (2) lead to lysis of cells. Sensitive to lysis by 100 mg of sodium dodecyl sulfate per liter (5).

Surface colonies are clear and convex with smooth edges. Subsurface colonies are cream colored with rough-textured interiors and irregular edges.

Energy-yielding metabolism results in methane formation; the substrates used include H₂-CO₂ and formate but not 2-propanol (9), 2-butanol (9), methanol, ethanol, acetate, propionate, butyrate, or valerate.

Growth occurs at temperatures between 21 and 45°C (most rapidly at 38°C) (6) but not at 49°C (6) or at 15°C. Growth occurs with a medium pH between 6 and 8.0 (6) or 9.0 (most rapidly at pH 7.0 to 7.4). Most rapid growth occurs in the presence of 2 to 4% NaCl (5). NH₄⁺, Ni²⁺, Fe²⁺, Co²⁺, Mg²⁺, Ca²⁺, and possibly SeO₄²⁻ are required. CO₂ (6), acetate (6), either 2-methyl butyrate, propionate, or isoleucine (3, 6), and either isovalerate or leucine (3, 6) are required, and growth is stimulated by pantooylactone (8). Very strictly anaerobic.

Found in marine and estuarine sediments.

The type strain is strain PS (= OGC 70 = ATCC 33273 = DSM 1537), which was isolated from the surficial sediment of an estuary.

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