Reclassification of *Desulfo bacterium macestii* as *Desulfomicrobium macestii* comb. nov.

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Phylogenetic, chemotaxonomic and metabolic data obtained for *Desulfo bacterium macestii* indicate that this species is not a member of the genus *Desulfo bacterium*, but of the genus *Desulfomicrobium*. Phylogenetically, it is closely related to *Desulfomicrobium baculatum* and *Desulfomicrobium norvegicum*, but it can be differentiated from these species by its metabolic properties. It is therefore proposed to reclassify *Desulfo bacterium macestii* as *Desulfomicrobium macestii* comb. nov.

Previously, *Desulfo bacterium macestii* had been shown to grow in the absence of organic carbon sources and in the presence of sulfate with hydrogen or formate as a chemoheterotroph, and with ethanol, lactate or pyruvate as a chemoheterotroph (Gogotova & Vainshtein, 1989). These results were confirmed in the present study. In addition, it was found that *Desulfo bacterium macestii* was able to grow on n-butanol and 1,2-propanediol and weakly on n-propanol, but failed to grow on isobutanol, 1,4-butanediol or 2,3-butanediol. In contrast to previous results, fumarate and malate were used as carbon and electron sources in the absence of sulfate. In repeated experiments that used 10–15 mM organic substrate and 10 mM sulfate, cultures grew up to an optical density of 0.260–0.350 and produced 3–5 mM sulfide. In the absence of sulfate, fumarate and pyruvate were fermented, while lactate and malate were not. No growth occurred on lactate with nitrate as the electron acceptor.

Extraction of genomic DNA, PCR-mediated amplification of 16S rDNA and direct sequencing of the purified PCR product were carried out according to Rainey *et al.* (1996). The 16S rDNA sequences were aligned manually with
published sequences obtained from GenBank/EMBL. Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms were reconstructed as described by DeSoete (1983). Bootstrap analysis was used to evaluate the neighbour-joining tree topology by performing 500 resamplings (Felsenstein, 1985).

Species of the genus *Desulfomicrobium* form three lineages: *Desulfomicrobium orale* is the deepest-branching member of the genus and shows <96 % 16S rRNA gene sequence similarity with the other species. The next-deepest-branching member is *Desulfomicrobium escambiense*, which shares between 98·1 and 98·3 % similarity with members of the third lineage; this lineage comprises the closely related species *Desulfomicrobium baculatum*, *Desulfomicrobium norvegicum*, *Desulfomicrobium apsheronum* and *Desulfomicrobium hypogeicum* (99·4–99·8 % similarity). This cluster also contains *Desulfobacterium macestii* DSM 4194\(^T\), which shares 100 % 16S rRNA gene similarity with *Desulfomicrobium norvegicum* DSM 1741\(^T\), 99·8 % similarity with *Desulfomicrobium baculatum* DSM 4028\(^\#\) and *Desulfomicrobium hypogeicum* CN-A\(^\#\) and 99·6 % similarity with *Desulfomicrobium apsheronum* DSM 5918\(^T\). The published sequence of *Desulfomicrobium baculatum* VKM B-1378\(^T\) (GenBank no. AF030438) differs from the newly analysed sequence of *Desulfomicrobium baculatum* DSM 4028\(^\#\) (GenBank no. AJ277894) by 1·3 %. This in the sequence explains the different phylogenetic positions of *Desulfobacterium baculatum*, which branches with *Desulfomicrobium apsheronum* in the study of Langendijk et al. (2001) and with *Desulfomicrobium norvegicum* and *Desulfobacterium macestii* in the present study (Fig. 1).

For analysis of cellular fatty acids, *Desulfobacterium macestii* DSM 4194\(^T\) was grown in a pyruvate- and malate-containing medium with sulfate and thiosulfate as electron acceptors at 35 °C, as described by Vainshtein et al. (1992). Cells were harvested at the end of the exponential-growth phase and washed twice with 1 % (w/v) NaCl. Saponification, methylation of fatty acids, extraction, separation by GC and analysis of the fatty acid methyl esters were done as described previously (Vainshtein et al., 1992; Meier et al., 1993), by using the MIDI system (Microbial ID). The overall fatty acid composition of *Desulfobacterium macestii* was very similar to those of species of the genus *Desulfomicrobium* (Vainshtein et al., 1992; Langendijk et al., 2001) but distinct from those of representatives of the genera *Desulfovibrio* and *Desulfobacterium* (Taylor & Parkes, 1983; Dowling et al., 1986; Vainshtein et al., 1992). The predominant fatty acids in *Desulfobacterium macestii* were the branched-chained, odd-numbered fatty acids iso C\(_{15:0}\) (13·6 %), iso C\(_{17:0}\) (10·1 %) and iso C\(_{17:1\,\omega}10\)c (32·9 %), as well as significant amounts of the unbranched, even-numbered fatty acids C\(_{16:0}\) (4·2 %), C\(_{18:0}\) (5·5 %) and C\(_{18:1\,\omega}7\)c (6·3 %). In particular, iso C\(_{17:1\,\omega}10\)c has been regarded as a marker for members of the family *Desulfovibrionaceae* (Edlund et al., 1985; Taylor & Parkes, 1985; Tourova et al., 1998). 10-Methyl fatty acids, claimed to be characteristic of the genera *Desulfo bacter* and *Desulfobacterium*...
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Desulfomicrobium macestii (ma. ces’ti.i. L. neut. adj. macestii referring to the town Matsesta at Sotschi, Black Sea, Russia, from where the type strain was isolated).

The description is based on the data of Gogotova & Vainshtein (1989) and data from recent studies.

Cells are straight rods, 0·7×1·9–2·0 μm in size and motile by a single polar flagellum. Spores are not formed. Gram-negative. Strictly anaerobic chemo-organotroph or chemo-autotroph. H₂S formate, pyruvate, lactate, ethanol, propanol, butanol, 1,2-propanediol, fumarate and malate are used as electron donors. Incomplete oxidation. Acetate, butyrate, methanol, isobutanol, 1,4-butanediol, 2,3-butanediol, choline, glucose and sucrose are not utilized. Sulfate, sulfite and thiosulfate serve as electron acceptors and are reduced to H₂S. Fermentative growth occurs on pyruvate and fumarate in the absence of sulfate. Optimum temperature for growth is 35 °C, range is 15–40 °C; optimum pH for growth is 7·2, range 0–8·0. Optimum growth occurs in 1·3 % NaCl, range 0–2·5 %. Major fatty acids are branched-chain and odd-numbered: iso C₁₅:₀, iso C₁₇:₀ and iso C₁₇:₁ω₁₀₁₇. Principal menaquinone is MK-6. Cells contain b- and c-type cytochromes and an active hydrogenase. Desulfoviridin is not present. DNA base ratio is 58·0 mol% G + C (by thermal denaturation).

The type strain is M-9 ( =VKM B-1598 = DSM 4194). Isolated from a sulfide spring at Matsesta, Russia.

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References


