

Reclassification of *Desulfobacterium macestii* as *Desulfomicrobium macestii* comb. nov.

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Phylogenetic, chemotaxonomic and metabolic data obtained for *Desulfobacterium macestii* indicate that this species is not a member of the genus *Desulfobacterium*, 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 *Desulfobacterium macestii* as *Desulfomicrobium macestii* comb. nov.

Desulfobacterium macestii Gogotova and Vainshtein (1989) was described for a motile, Gram-negative, rod-shaped, non-spore-forming, sulfate-reducing strain from the water of a sulfide spring at Matsesta, Russia. The strain used hydrogen, formate, lactate, pyruvate and ethanol for growth and sulfate as an electron donor, and did not contain desulfovirdin. On the basis of these phenotypic properties, this strain (M-9^T) was classified as a member of the genus *Desulfobacterium* Widdel and Bak (1992). This report presents data that are inconsistent with the current classification, but provides evidence for reclassification of strain M-9^T (= VKM B-1598^T = DSM 4194^T) as a member of the genus *Desulfomicrobium*.

The genus *Desulfomicrobium* was described by Rozanova *et al.* (1988, 1994) for rod-shaped, non-spore-forming, Gram-negative, sulfate-reducing bacteria that do not contain desulfovirdin and perform incomplete oxidation of organic compounds to acetate. At the time of writing, the genus includes the species *Desulfomicrobium baculatum* (Rozanova *et al.* 1988, 1994), *Desulfomicrobium apsheronum* (Rozanova *et al.*, 1988, 1994), *Desulfomicrobium escambiense* (Sharak Genthner *et al.*, 1994, 1996), *Desulfomicrobium norvegicum* (Sharak Genthner *et al.*, 1997), *Desulfomicrobium orale* (Langendijk *et al.*, 2001) and '*Desulfomicrobium hypogeium*' (Krumholz *et al.*, 1999); these have

been isolated from various habitats, such as the stratal water of an oil deposit, manganese ore, subsurface sandstone, forest pond, brackish water, fresh water and marine water or sediment, respectively. Phylogenetically, the genus *Desulfomicrobium* groups within the family *Desulfovibrionaceae* (Devereux *et al.*, 1990; Tourova *et al.*, 1998) and is phylogenetically, physiologically and chemotaxonomically distinct (e.g. in its type of oxidation of organic compounds, DNA G+C content, cellular fatty acid pattern and menaquinones) from members of the genus *Desulfobacterium* (Collins & Widdel, 1986; Widdel & Bak, 1992).

Previously, *Desulfobacterium 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 chemoautotroph, and with ethanol, lactate or pyruvate as a chemoorganotroph (Gogotova & Vainshtein, 1989). These results were confirmed in the present study. In addition, it was found that *Desulfobacterium 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 presence 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

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The GenBank accession numbers for the 16S rRNA gene sequences described in this paper are: AJ237604, *Desulfomicrobium macestii* DSM 4194^T; AJ277894, *Desulfomicrobium baculatum* DSM 4028^T; AJ277897, *Desulfomicrobium norvegicum* DSM 1741^T; AJ237606, *Desulfobacula phenolica* DSM 3384^T; AJ237601, *Desulfobacterium anilini* DSM 4660^T.

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 hypogeium*' (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^T and '*Desulfomicrobium hypogeium*' CN-A^T 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^T (GenBank no. AJ277894) by 1.3%. This in the sequence explains the different phylogenetic positions of *Desulfomicrobium 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}ω10c (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}ω7c (6.3%). In particular, iso C_{17:1}ω10c has been regarded as a marker for members of the *Desulfovibrionaceae* (Edlund *et al.*, 1985; Taylor & Parkes, 1985; Tourouva *et al.*, 1998). 10-Methyl fatty acids, claimed to be characteristic of the genera *Desulfobacter* and *Desulfobacterium*

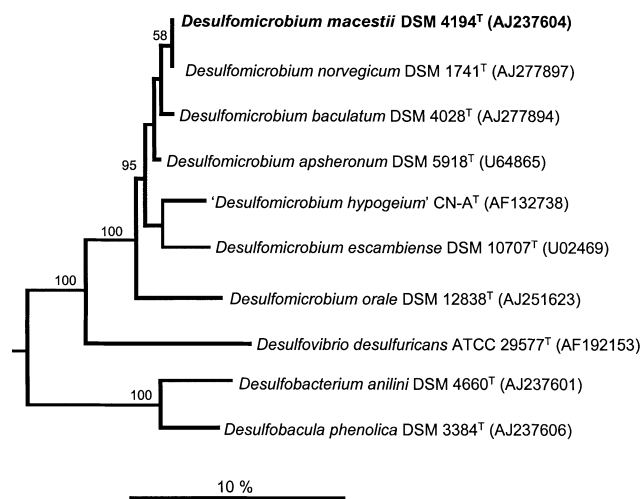


Fig. 1. Dendrogram of 16S rRNA gene sequences (DeSoete, 1983), displaying the phylogenetic position of *Desulfomicrobium macestii* DSM 4194^T. Numbers at branching points refer to bootstrap values (1000 resamplings). The tree was rooted with the 16S rRNA gene sequences of *Myxobacteria* and *Bdellovibrio* species. Bar, 10 nucleotide substitutions per 100 sequence positions.

(Dowling *et al.*, 1986; Vainshtein *et al.*, 1992), were absent from *Desulfobacterium macestii*, as well as from members of the genera *Desulfomicrobium* and *Desulfovibrio*. *Desulfobacterium macestii* differs only slightly from the phylogenetically closely related type strains of *Desulfomicrobium norvegicum* and *Desulfomicrobium baculatum* in quantitative, but not qualitative, fatty acid composition.

Cells for isoprenoid quinone analysis (Kroppenstedt, 1985; Hippe *et al.*, 1997) were grown in the medium described above, modified to contain 10 g NaCl l⁻¹ and 2.2 g Na-lactate l⁻¹ to replace pyruvate and malate. The principal isoprenoid quinone in *Desulfobacterium macestii* was menaquinone with six isoprenoid units (MK-6). This is not in accordance with the menaquinone composition of *Desulfobacterium* species, which contain either MK-7 or MK-7(H₂) as the major menaquinone (Collins & Widdel, 1986; Widdel & Bak, 1992). MK-6 is typically found in members of the family *Desulfovibrionaceae* and has also been detected in *Desulfomicrobium norvegicum* (formerly *Desulfovibrio desulfuricans* Norway 4) (Collins & Widdel, 1986).

Genomic similarities between the closely related species *Desulfomicrobium norvegicum* DSM 1741^T and *Desulfobacterium macestii* DSM 4194^T were analysed by DNA–DNA reassociation, following the renaturation method (Escara & Hutton, 1980; Huß *et al.*, 1983; Jahnke, 1992) in 1 × SSC (saline/sodium citrate) buffer plus 10% DMSO at 65 °C. The mean binary reassociation value of triple determination was 71.7% (range, 69.7–73.5%), which is close to the recommended threshold value for

species delineation (70%; Wayne *et al.*, 1987). The reassociation value for *Desulfomicrobium baculatum* DSM 4028^T and *Desulfomicrobium norvegicum* ATCC 27774^T is only 27.4% (Sharak Genthner *et al.*, 1997), indicating that strain DSM 4028^T and *Desulfobacterium macestii* DSM 4194^T also share significantly less than 70% similarity.

Results of phylogenetic and chemotaxonomic studies clearly identify *Desulfobacterium macestii* as a member of the genus *Desulfomicrobium* within the family *Desulfovibrionaceae*. DNA–DNA hybridization points toward a close relationship with *Desulfomicrobium norvegicum*, but *Desulfobacterium macestii* DSM 4194^T differs from the type strain of *Desulfomicrobium norvegicum*, as well as from *Desulfomicrobium baculatum*, by chemoautotrophic growth on H₂ plus CO₂, lack of growth on malate in the absence of sulfate, and sulfate reduction during growth on fumarate and malate in the presence of sulfate. We therefore propose to reclassify *Desulfobacterium macestii* Gogotova and Vainshtein (1989) as *Desulfomicrobium macestii* comb. nov.

Description of *Desulfomicrobium macestii* comb. nov.

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 chemoautotroph. H₂, 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 is 6.5–8.0. Optimum growth occurs in 1.3% NaCl, range 0–2.5%. Major fatty acids are branched-chain and odd-numbered: iso C_{15:0}, iso C_{17:0} and iso C_{17:1}ω10c. 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^T (=VKM B-1598^T = DSM 4194^T). Isolated from a sulfide spring at Matsesta, Russia.

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