Reclassification of *Desulfotomaculum auripigmentum* as *Desulfosporosinus auripigmenti* corrig., comb. nov.

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The species *Desulfotomaculum auripigmentum* is reclassified as *Desulfosporosinus auripigmenti* corrig., comb. nov. on the basis of morphological and physiological traits, phylogenetic position and chemotaxonomic properties. Characteristics supplementary to those provided in the original description reveal that the type strain, DSM 13351^T (=ATCC 700205^T), forms oval, subterminal to terminal spores, possesses LL-diaminopimelic acid and contains MK-7 as the predominant menaquinone, while the whole-cell methanolysate contains even-carbon, straight-chain saturated and mono-unsaturated fatty acids and 1,1-dimethylacetals as major components. DNA–DNA reassociation values below 30 % for *Desulfosporosinus orientis* DSM 765^T and *Desulfosporosinus meridiei* DSM 13257^T demonstrate that strain DSM 13351^T shows sufficient genomic differences to maintain its species status. Lack of motility, a smaller cell diameter and the ability to use malate and glycerol as electron donors and fumarate and arsenate as electron acceptors are the main properties that differentiate *Desulfosporosinus auripigmenti* from the other two species of the genus.

The species *Desulfotomaculum auripigmentum*, type strain ATCC 700205^T (=DSM 13351^T) (Newman *et al.*, 1997), was affiliated with *Desulfotomaculum* mainly on the basis of 16S rDNA analysis. This non-motile, sausage-shaped, arsenate- and sulfate-reducing Gram-positively staining bacterium, for which spore formation had not been reported, was placed as a phylogenetic neighbour of *Desulfotomaculum orientis* in the 16S rDNA dendrogram of relationships (96·2 % similarity). However, in the same year in which *Desulfotomaculum auripigmentum* was described, *Desulfotomaculum orientis* was reclassified as the type species of a new genus, namely *Desulfosporosinus*, as *Desulfosporosinus orientis* (Stackebrandt *et al.*, 1997). As the publication processes overlapped each other, none of the two research groups was aware of the other group's work. In 2001, a second species of the genus *Desulfosporosinus*, *Desulfosporosinus meridiei* (type strain DSM 13257^T), was described (Robertson *et al.*, 2001) which branched phylogenetically adjacent to *Desulfotomaculum auripigmentum* DSM 13351^T (97·6 % 16S rRNA gene sequence similarity), while the sequence similarity to *Desulfosporosinus orientis* DSM 765^T was slightly lower (96·7 %). Despite the grouping of a *Desulfotomaculum* species between two *Desulfosporosinus* species, the generic affiliation of *Desulfotomaculum auripigmentum* remained unchallenged.

As the 16S rRNA gene sequence of the type strain of *Desulfotomaculum auripigmentum*, ATCC 700205^T, comprised only 1263 nt (GenBank accession no. U85624), the sequence analysis of strain DSM 13351^T was repeated (AJ493051), using the method described by Rainey *et al.* (1996), and the phylogenetic position was reassessed by applying the treeing algorithm of De Soete (1983). Using the new sequence comprising 1532 bases, *Desulfotomaculum auripigmentum* was found to share 97·4 and 97·9 % similarity with *Desulfosporosinus orientis* DSM 765^T and *Desulfosporosinus meridiei*, respectively. *Desulfosporosinus orientis* DSM 7439, the 16S rRNA gene sequence of which was also determined in this study (GenBank accession no. AJ493052), was highly related to the type strain DSM 765^T (99·5 %), while strain DSM 8344 was significantly less closely related to DSM 765^T (96·2 %) (Stackebrandt *et al.*, 1997; Robertson *et al.*, 2001). Strain DSM 8344 showed 97·6 % similarity to the type strains of *Desulfotomaculum auripigmentum* and *Desulfosporosinus meridiei*. All these strains formed a coherent phylogenetic cluster that formed a sister lineage to the *Desulfitobacterium* lineage (93·1–94·4 % similarity) (Fig. 1). Members of the genus *Desulfotomaculum*, described as being phylogenetically heterogeneous and forming three major clusters (Stackebrandt *et al.*, 1997), were less than 90 % similar to members of the

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Desulfosporosinus auripigmenti* DSM 13351^T and *Desulfosporosinus orientis* DSM 7493 are AJ493051 and AJ493052, respectively.

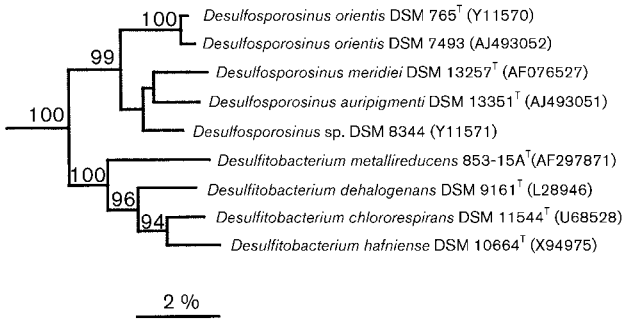

Fig. 1. Dendrogram of 16S rRNA gene sequence relationships (De Soete, 1983), displaying the phylogenetic position of *Desulfotomaculum auripigmentum* DSM 13351^T, reclassified as *Desulfosporosinus auripigmenti* in this study. Numbers at branching points refer to bootstrap values (1000 resamplings). Bar, 2 nucleotide substitutions per 100 sequence positions. The tree was rooted with the 16S rRNA gene sequences of *Desulfotomaculum* species.

two lineages embracing the genera *Desulfosporosinus* and *Desulfitobacterium*.

The phylogenetic clustering of *Desulfotomaculum auripigmentum* with members of the genus *Desulfosporosinus* raises the question of whether this species should be reclassified as a species of the genus *Desulfosporosinus*. The 16S rRNA gene sequences of *Desulfosporosinus* strains and *Desulfotomaculum auripigmentum* contain several exclusive signature nucleotides, which clearly distinguish these organisms from members of the genus *Desulfitobacterium* (Table 1). The respective nucleotides of *Desulfotomaculum* species are not listed because the species do not constitute a phylogenetically coherent taxon. Members of the genus

Table 1. Oligonucleotide signatures distinguishing members of the genus *Desulfosporosinus* and *Desulfotomaculum auripigmentum* from members of the genus *Desulfitobacterium*

16S rRNA gene position*	*Desulfosporosinus* spp., *Desulfotomaculum auripigmentum*	*Desulfitobacterium* spp.
140-223	A-U	U-A
418-425	U-A	C-G
443-491	U-A	C-G
444-490	G-C	A-U
600-638	C-G	U-G
1117-1183	G-U	A-U
1119-1154	A-U	G-C
1310-1327	C-G	U-A
1311-1326	A-U	U-A
1312-1325	G-C	C-G
1313-1324	G-C	U-A

*According to Brosius *et al.* (1978).

Desulfosporosinus, as well as *Desulfotomaculum auripigmentum*, possess LL-diaminopimelic acid as the diagnostic amino acid of peptidoglycan, as determined by the methods of Schleifer & Kandler (1972). However, LL-diaminopimelic acid also occurs in some members of the genus *Desulfotomaculum*, for example, *Desulfotomaculum thermoacetoxidans* DSM 5813^T and *Desulfotomaculum thermobenzoicum* subsp. *thermobenzoicum* DSM 6193^T, and in species of the genus *Desulfitobacterium* [*Desulfitobacterium dehalogenans* DSM 9161^T, *Desulfitobacterium hafniense* DSM 10664^T, as well as in *Desulfitobacterium* sp. strain PCE1 (Gerritse *et al.*, 1992)], whereas *Desulfotomaculum aeronauticum* DSM 10349^T, *Desulfotomaculum geothermicum* DSM 3669^T and *Desulfotomaculum nigrificans* DSM 574^T contained *meso*-diaminopimelic acid (result of this study, except for strain PCE1). Thus, this property is not exclusive taxonomically.

To verify the species status of the two *Desulfosporosinus* species and of *Desulfotomaculum auripigmentum*, DNA–DNA pairing studies were performed between the type strains. DNA was isolated by chromatography on hydroxylapatite by the procedure of Cashion *et al.* (1977). DNA–DNA hybridization was carried out in 2 × SSC at 65 °C according to De Ley *et al.* (1970), using a Gilford System 2600 spectrophotometer (Gilford Instrument Laboratories) equipped with a Gilford 2527-R thermoprogrammer and plotter. While *Desulfosporosinus orientis* DSM 765^T shared 54 % DNA binding with *Desulfosporosinus meridiei* DSM 13257^T, the DNA similarity of these type strains to *Desulfotomaculum auripigmentum* DSM 13351^T was 30 and 29 %, respectively. DNA–DNA similarity values below 55 % correlated with the moderate 16S rRNA gene sequence similarities and confirmed that each of the three species are genomically distinct taxa. This is also reflected by different *Eco*RI riboprint patterns (Fig. 2) generated by the RiboPrinter (a microbial characterization system; DuPont Qualicon) according to Bruce (1996).

In contrast to the type strains of *Desulfosporosinus orientis* and *Desulfosporosinus meridiei*, *Desulfotomaculum auripigmentum* DSM 13551^T is not motile, and spores had not been observed. In this respect, strain DSM 13551^T also differs from the genus description of *Desulfotomaculum* (Campbell & Singleton, 1986), reported to embrace spore-forming and motile organisms. To determine whether *Desulfotomaculum auripigmentum* does in fact fail to produce spores, strain DSM 13551^T was grown in DSMZ medium no. 641 (Deutsche Sammlung von Mikroorganismen und Zellkulturen *Catalogue of Strains*, 2001, 7th edn), containing 2·5 g lactate l^{-1}, at 28 °C for 4 days. Subterminal to terminal ellipsoid spores were formed in some cells (Fig. 3). The same medium was used for obtaining information on chemotaxonomic properties lacking in the original description of *Desulfotomaculum auripigmentum*, i.e. isoprenoid quinones (Collins *et al.*, 1977; Groth *et al.*, 1996) and cellular fatty acids (Miller, 1982; Sasser, 1990). MK-7 was the principal quinone

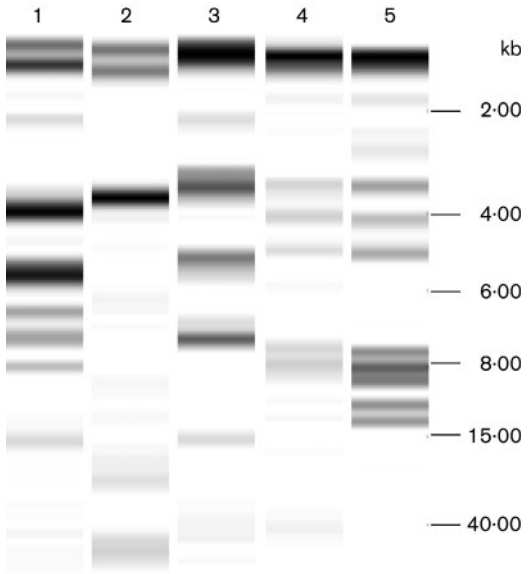

Fig. 2. Riboprint patterns of *Desulfosporosinus* strains and *Desulfosporosinus auripigmenti* DSM 13351^T, generated with *Eco*RI. Lanes: 1, *Desulfosporosinus meridiei* DSM 13257^T; 2, *Desulfosporosinus auripigmenti* DSM 13351^T; 3, *Desulfosporosinus* sp. DSM 8344; 4, *Desulfosporosinus orientis* DSM 765^T; 5, *Desulfosporosinus orientis* DSM 7439.

(57 %), while MK-5 (40 %) and MK-6 (3 %) were the minor quinones. The major fatty acids (>5 % of the total) were even-carbon, straight-chain saturated and mono-unsaturated fatty acids. 1,1-Dimethylacetals and traces of aldehydes, branched-chain fatty acids and cyclopropane fatty acids occur as well (see species description for the percentage of total values). The fatty acid composition differs from that described for *Desulfosporosinus meridiei*

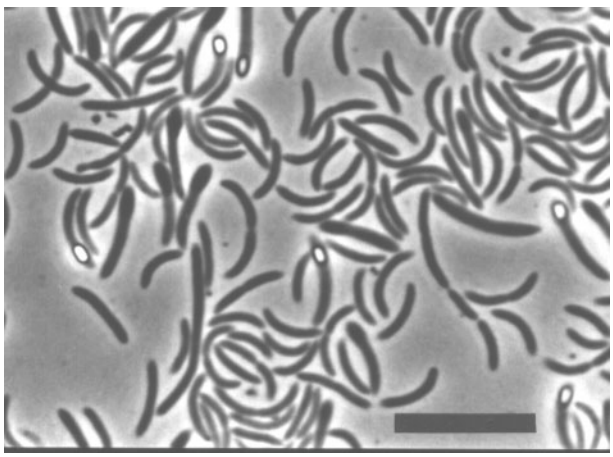

Fig. 3. Phase-contrast micrograph of spore-forming and non-spore-forming cells of *Desulfosporosinus auripigmenti*. Bar, 10 μm.

(Robertson *et al.*, 2000) in the lack of substantial amounts of iso- and anteiso-branched-chain fatty acids (2·5 versus 29 %) but confirms the fatty acid composition given in the emended genus description, in which it is stated that members of the genus *Desulfosporosinus* contain minor amounts of branched-chain fatty acids (Robertson *et al.*, 2001) or even no branched-chain fatty acid (Stackebrandt *et al.*, 1997). We therefore propose, on the basis of the phylogenetic position, common chemotaxonomic properties, sulfate reduction and incomplete oxidation of organic compounds (Table 2), to reclassify *Desulfotomaculum auripigmentum* as *Desulfosporosinus auripigmenti* corrig., comb. nov. (*auripigmentum*, a noun in apposition, has been changed to *auripigmenti*, the genitive noun). Lack of motility, a smaller cell diameter and the ability to use malate and glycerol as electron donors and fumarate and arsenate as electron acceptors differentiate this species from the other two species of the genus *Desulfosporosinus*. This transfer demands the emendation of the genus description of *Desulfosporosinus*.

Emended description of the genus *Desulfosporosinus* (Stackebrandt *et al.* 1997, Robertson *et al.* 2001, emend.)

Desulfosporosinus [De.sul.fo.spo.ro.si′nus. L. pref. *de* from; L. n. *sulfur* sulfur; M.L. n. *spora* spore; L. n. *sinus* bend; N.L. masc. n. *Desulfosporosinus* a spore-forming curved (organism) that reduces sulfur compounds].

Gram-negative rods that have a multi-layered cell wall structure. Endospores present, oval and subterminal to (almost) terminal, causing the cells to swell slightly. Non-motile or motile with lateral or peritrichous flagella. Strictly anaerobic. If determined, desulfoviridin and cytochrome c_3 absent and bisulfite reductase P_{582} present. Sulfate and thiosulfate are reduced to sulfide in the presence of lactate but not in the presence of acetate or fructose. Incomplete oxidation of organic compounds to acetate. Acetate is the fermentation end-product; capable of homoacetogenic growth. Grows autotrophically with hydrogen plus sulfate. LL-Diaminopimelic acid is the diagnostic diamino acid of peptidoglycan. Contains menaquinone with a side-chain with seven isoprene units (MK-7 type). Predominant fatty acids are even-numbered, saturated and unsaturated fatty acids; significant amounts of 1,1-dimethylacetals have been found in *Desulfosporosinus auripigmenti*; traces of iso- and anteiso-branched-chain fatty acids and cyclopropane fatty acids may occur. The G+C content of the DNA is 41·6–45·9 mol%. Phylogenetically, a member of the *Clostridium–Bacillus* subphylum of Gram-positive bacteria.

The type species is *Desulfosporosinus orientis*.

Description of *Desulfosporosinus auripigmenti* corrig., comb. nov.

Basonym *Desulfotomaculum auripigmentum* (Newman *et al.* 1997). *Desulfosporosinus auripigmenti* [au.ri.pig.men′ti. L.

Table 2. Some biochemical and chemotaxonomic characteristics of species of the genus *Desulfosporosinus*, including the reclassified species *Desulfosporosinus auripigmenti*

ND, Not determined. All of the species share the following properties: LL-diaminopimelic acid is present in the peptidoglycan; oxidation of hydrogen with carbon dioxide; sulfate, thiosulfate and sulfite are used as electron acceptors in the presence of lactate; incomplete oxidation.

Property	*Desulfosporosinus orientis* DSM 765^T	*Desulfosporosinus meridiei* DSM 13257^T	*Desulfosporosinus auripigmenti* DSM 13351^T
Gram staining	Negative	Negative/variable	Negative
Morphology	Curved rods, sometimes paired	Curved rods, single or in short chains	Curved rods, sausage-shaped; sometimes long chains
Cell diameter (μm)	0·7–1·0	0·7–1·1	0·4
Motility	+	+	−
Flagella	Peritrichous	Single lateral	−
Endospores	Oval, subterminal	Oval, subterminal	Oval, subterminal to almost terminal
Electron acceptors in the presence of lactate:			
Sulfur	+*	+	−
Nitrate	−	+†	−
Arsenate	−	−	+
DMSO, Fe(III)	+*	+	−
Fumarate	−	−	+
Electron donors:			
Fumarate	+*	−	−
Malate	−	−	+
Glycerol	ND	ND	+
Oxidation of H_2	+, with CO_2	+, with CO_2	+, with acetate
DNA G+C content (mol%)	45 (buoyant density), 45·9 (T_m)	47 (T_m)	41·6 (HPLC)

*As reported by Robertson *et al.* (2001).

†Variable, three out of seven strains were reported as positive (Robertson *et al.*, 2001).

neut. n. *aurum* gold; L. neut. n. *pigmentum* colour, pigment; N.L. gen. n. *auripigmenti* of golden pigment, referring to the colour of precipitate (arsenosulfide, As_2S_3) which is formed after reduction of arsenate and sulfate].

Phylogenetic and chemotaxonomic data indicate that *Desulfotomaculum auripigmentum* is more closely related to species of *Desulfosporosinus* than to any species of *Desulfotomaculum*. The cultural, morphological and physiological description of the species, given by Newman *et al.* (1997), is unchanged. In addition to the original description, cells occasionally form oval, subterminal to terminal spores. The diagnostic amino acid of peptidoglycan is LL-diaminopimelic acid; MK-7 is the predominant isoprenoid quinone; MK-5 and MK-6 are minor components. The major fatty acids (>5 %) are (as percentages of the total) 16 : 1*cis*9 (31·6 %), 16 : 0 (14·2 %), 16 : 0-dimethylacetal (13·4 %), 18 : 1*cis*9 (7·5 %) and 18 : 1*cis*9-dimethylacetal (7·7 %). Smaller amounts (1–5 %) of the following fatty acids are present (percentages of the total are shown in parentheses): 18 : 0 (2·8 %), 14 : 0 (2·2 %), 16 : 0-aldehyde (1·9 %), 16 : 1*cis*7 (1·5 %), 16 : 1 *cis*9-dimethylacetal (1·7 %), 17 : 1*cis*8 (1·3 %), 17 : 1*cis*9 (1·1 %), 18 : 1*cis*11 (2·5 %), 18 : 1*cis*13 (1·0 %), 18 : 0-dimethylacetal (2·0 %) and 18 : 1*cis*11-dimethylacetal (4·4 %). Minor amounts of cyclo 17 : 0 (0·6 %) are present. The G+C content of the DNA is 41·6 mol%.

The type strain is DSM 13351^T (=ATCC 700205^T).

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