***Desulfosporosinus meridiei* sp. nov., a spore-forming sulfate-reducing bacterium isolated from gasoline-contaminated groundwater**

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Eight strains of spore-forming, sulfate-reducing bacteria, isolated from groundwater contaminated with motor fuel [mostly benzene, toluene ethylbenzene and xylene (BTEX) compounds] in sandy soil near Perth, Australia, were closely related to *Desulfosporosinus* (previously *Desulfotomaculum*) *orientis* DSM 765^T (95.3–97.3% 16S rDNA sequence similarity). Whole-cell fatty acids were dominated by even-carbon, straight-chain saturated and mono-unsaturated fatty acids, in particular 16:0, 16:1*cis*9, 14:0 and 18:1*cis*11. The strains grew at temperatures between 4 and 42 °C and in medium containing up to 4% NaCl. The eight strains clustered into two main groups based on phylogeny, randomly amplified polymorphic DNA (RAPD)-PCR patterns and nutritional characteristics. Representatives of the two groups, strain S5 (group A) and strain S10^T (group B) had 81% DNA–DNA homology with each other and therefore should be accommodated in the same species. Strain S10^T had less than 38% homology with *Desulfosporosinus orientis* DSM 765^T, the most closely phylogenetically related type strain available. The new strains were distinguished from *Desulfosporosinus orientis* DSM 765^T by different banding patterns in a RAPD-PCR, and phenotypically by their inability to utilize fumarate as a carbon and energy source with sulfate as the electron acceptor and by their lower tolerance to NaCl. The DNA G+C contents were 46.8 and 46.9 mol% for strains S5 and S10^T, respectively (*Desulfosporosinus orientis* DSM 765^T 45.9 mol%). It is proposed that these new strains be placed in a new species of the genus *Desulfosporosinus*. The name *Desulfosporosinus meridiei* is proposed, with strain S10^T as the type strain (= DSM 13257^T = NCIMB 13706^T).

Keywords: *Desulfosporosinus meridiei*, sulfate-reducing bacteria, BTEX, groundwater

INTRODUCTION

The genus *Desulfosporosinus* was proposed in 1997 (Stackebrandt *et al.*, 1997) to accommodate the species previously known as *Desulfotomaculum orientis*. The type strain of *Desulfosporosinus orientis* (DSM 765^T) is a mesophilic, spore-forming, sulfate-reducing bacterium isolated from soil in Singapore (Adams & Postgate, 1959). Vainshtein *et al.* (1995) isolated a mesophilic, spore-forming sulfate-reducer from ancient permafrost in Russia, which they classified as a strain of the species *Desulfosporosinus orientis* (DSM

8344), on the basis of phenotypic characteristics. The placement of these two strains into a new genus was based on the finding that, in a phylogenetic analysis, they formed a separate cluster from other *Desulfotomaculum* spp., being more closely related to *Desulfitobacterium* spp. than to the other *Desulfotomaculum* spp. (Stackebrandt *et al.*, 1997). Members of the genus *Desulfosporosinus* are distinguished phenotypically from *Desulfotomaculum* spp. by the ability of *Desulfosporosinus* spp. to grow autotrophically using hydrogen, carbon dioxide and sulfate (Klemps *et al.*, 1985).

Recently, eight new strains of spore-forming, sulfate-reducing bacteria were isolated from a gasoline-contaminated shallow aquifer in the sandy soils of the Swan Coastal Plain near Perth in the south-west of

Abbreviations: BTEX, benzene, toluene ethylbenzene and xylene; DCE, dichloroethene; ddH₂O, double-distilled H₂O; FID, flame-ionization detection; PCE, perchloroethene; RAPD, randomly amplified polymorphic DNA; SPI, septum-equipped programmable injector; TCE, trichloroethene.

Fig. 1. Phylogenetic tree of sulfate-reducing strains (from a BTEX contamination plume in Western Australia) and their nearest relatives. The tree was constructed using PHYLIP and the Jukes–Cantor measure of distance. Bootstrap values are indicated. Figure from Robertson *et al.* (2000).

Australia (Robertson *et al.*, 2000). These were found, by 16S rDNA sequence comparison, to have 95.3–97.3% sequence similarities with *Desulfosporosinus orientis* 765^T (Fig. 1). This paper reports further characteristics of these strains, their phenotypic attributes and their level of DNA–DNA homology with *Desulfosporosinus orientis* DSM 765^T. We propose that these strains are representatives of a new species within the genus *Desulfosporosinus*, for which we propose the name *Desulfosporosinus meridiei* sp. nov.

METHODS

Bacterial strains. Eight spore-forming, sulfate-reducing bacteria were isolated from groundwater in a sandy Bassendean Association soil at Eden Hill, on the Swan Coastal Plain in Western Australia (Robertson *et al.*, 2000). The groundwater had been previously contaminated with aromatic compounds [(mostly benzene, toluene ethylbenzene and xylene (BTEX)] that originated from a leaking underground fuel-storage tank. *Desulfosporosinus orientis* DSM 765^T and *Desulfosporosinus orientis* DSM 8344 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen. *Bacillus subtilis* (ATCC 6051^T) was obtained from the Australian Collection of Microorganisms.

Culture media. For the experiments and analyses reported here (except for the testing of carbon sources and electron acceptors), the Eden Hill strains were cultivated on a medium ('cultivation medium') with the following composition (g l⁻¹): NH₄Cl (1.0), Na₂SO₄ (2.0), Na₂S₂O₃ · 5H₂O

(1.0), MgSO₄ · 7H₂O (1.0), CaCl₂ · 2H₂O (0.1), KH₂PO₄ (0.5), yeast extract (1.0), NaHCO₃ (2.0), sodium pyruvate (2.0), Na₂S · 9H₂O (0.075), trace-element solution (10 ml) and vitamin solution (10 ml) in double-distilled water (ddH₂O). The trace-element solution was from DSMZ medium 320 (*DSM Catalogue of Strains*, 1989) and the vitamin solution was from DSMZ medium 141 (*DSM Catalogue of Strains*, 1989). Trace elements, vitamins, NaHCO₃, sodium pyruvate and Na₂S · 9H₂O were added from sterile, anaerobic stocks to the sterile, anaerobic medium. The final pH of the medium was 7.0–7.5. This medium allowed more rapid growth of the strains than that used previously (Robertson *et al.*, 2000).

For testing of electron acceptors, Na₂SO₄ and Na₂S₂O₃ · 5H₂O were omitted from the medium and MgSO₄ · 7H₂O (1.0 g l⁻¹) was replaced with MgCl₂ · 6H₂O (0.8 g l⁻¹; 'sulfate-free medium'). Lactate (10 mM) was used as the carbon source. When elemental sulfur and nitrate were tested, Na₂S₂O₄ · H₂O (20 mg l⁻¹) was used as the reducing agent instead of Na₂S · 9H₂O. For the testing of various carbon sources, the cultivation medium described above was used, except that the yeast extract was omitted and Na₂S · 9H₂O was replaced with Na₂S₂O₄ · H₂O (20 mg l⁻¹) as the reducing agent ('mineral medium'). Sodium pyruvate was replaced with the appropriate carbon source. Fermentation tests were conducted in sulfate-free mineral medium.

Cells of *Desulfosporosinus orientis* DSM 765^T were grown for DNA extractions [for randomly amplified polymorphic DNA (RAPD)-PCR and for determination of DNA G + C content and DNA–DNA homologies] on medium containing the following (g l⁻¹): K₂HPO₄ (0.5), NH₄Cl (1.0),

Table 1. Electron donors and acceptors tested for use by Eden Hill sulfate-reducing strains and by *Desulfosporosinus orientis* DSM 765^T

Electron acceptors were tested with 10 mM lactate as the electron donor. Electron donors were used in the presence of 18 mM sulfate and 4 mM thiosulfate as the electron acceptors. Concentrations, in mM, are given in parentheses. The following electron acceptors were positive for all strains: sulfite (5), thiosulfate (10), sulfur (2 g l⁻¹), DMSO (10) and Fe(III) (1 g l⁻¹). The following electron acceptors were negative for all strains: Mn(IV) (20) and As(V) (1). Small amounts of trichloroethene and other compounds were produced from perchloroethene by all strains in the presence of pyruvate and sulfate. The following electron donors were positive for all strains: pyruvate (18), lactate (10) and H₂/CO₂ + 1 mM acetate (1). The following electron donors were negative for all strains: acetate (10), propionate (10), malate (10) and benzoate (3). Electron donors: caprate (1) was positive for strains S5, S10^T and *Desulfosporosinus orientis* DSM 765^T; H₂/CO₂ was positive for strains S10^T and *Desulfosporosinus orientis* DSM 765^T; valerate (5), fructose (10) and 3,4,5-trimethoxybenzoic acid (2·5) were negative for strains S5, S10^T and *Desulfosporosinus orientis* DSM 765^T. +, Good growth and sulfide production; (+), weak growth and sulfide production; ND, not determined; S, sulfide production only, and no increase in turbidity observed above substrate-free control.

Electron acceptor/donor	Strain							
	T1	T2	S4	S5	S6	S7	S10 ^T	DSM 765 ^T
Electron acceptor:								
Nitrate (5)	+	+	—	+	—	—	—	—
Electron donor (using sulfate):								
Formate (10)	—	—	—	—	+	+	+	+
Butyrate (5)	ND	ND	ND	+	ND	ND	+	S
Caproate (2)	—	—	—	+	—	—	—	+
Caprylate (2)	(+)	(+)	+	+	—	—	—	+
Methanol (10)	—	—	—	—	(+)	—	+	+
Ethanol (10)	(+)	(+)	(+)	(+)	+	+	+	+
Fumarate (10)	—	—	—	—	—	—	—	+
Syringic acid (2·5)	—	—	—	—	+	+	+	+

Na₂SO₄ (1·0), CaCl₂·2H₂O (0·1), MgSO₄·7H₂O (2·0), sodium lactate (70%, w/v) (2·0), yeast extract (1·0), sodium thioglycollate (0·1) and ascorbic acid (0·1) in ddH₂O. The final pH of the medium was approximately 6·1. For all other experiments, *Desulfosporosinus orientis* DSM 765^T was grown on the same medium as the Eden Hill strains. *Desulfosporosinus orientis* DSM 8344 was grown on the same medium as the Eden Hill strains. *B. subtilis* was grown on Trypstone Soya Broth (Oxoid).

Unless otherwise stated, cultures were grown under an N₂ atmosphere in glass containers sealed with butyl rubber. Tubes containing DMSO (tested as an electron acceptor) were sealed with Teflon-lined butyl rubber septa (Supelco). An inoculum of between 1 and 4% was used. All cultures were incubated at 28 °C unless stated otherwise.

RAPD-PCR. DNA was extracted from all Eden Hill strains and from *Desulfosporosinus orientis* DSM 8344 by using the guanidine thiocyanate method of Pitcher *et al.* (1989). DNA was extracted from cells of *B. subtilis* (ATCC 6051^T) by using the CTAB technique of Wilson (1998). For both of these techniques, 0·15–0·3 g (wet weight) cells was used for each strain. DNA was extracted from the entire wet cell mass of a 3 l culture of *Desulfosporosinus orientis* DSM 765^T by using a French pressure cell (Aminco) at 1200 kPa. The DNA was then purified using proteinase K and RNase. All DNA was stored at –20 °C.

The RAPD-PCR reaction mix consisted of 2·5 µl 10 × MgCl₂-free PCR buffer (Boehringer Mannheim), 2 µM MgCl₂, 400 µM each dNTP, 0·4 µM 10-mer primer (OPA-5: AGGGGTCTTG) (Operon Technologies), 1 µl crude DNA suspension for strains T1, T2, S5, S8, S10^T and *Desulfo-*

sporosinus orientis DSM 8344 and 0·5 µl crude DNA mix for strains S4, S6, S7 and *Desulfosporosinus orientis* DSM 765^T, 1·25 U *Taq* DNA polymerase (Boehringer Mannheim) and ddH₂O to make a total volume of 25 µl. Three primer concentrations were initially tested (0·2, 0·4 and 0·8 µM). A negative control containing 1 µl ddH₂O instead of DNA solution was used.

Amplification was performed in an MJ Research PTC-200 Peltier Thermal Cycler using the following programme: 94 °C for 1 min, 35 °C for 1 min and 72 °C for 2 min, 40 times, followed by 72 °C for 10 min (based on Williams *et al.*, 1990). Products were observed by using agarose gel electrophoresis with a 1·5% agarose gel.

DNA G + C content and DNA–DNA homologies. DNA was extracted from strains S5 and S10^T and from *Desulfosporosinus orientis* DSM 765^T, using a French pressure cell (see above). The DNA G + C content was determined by means of the thermal denaturation of the DNA, using *Escherichia coli* K-12 as an internal standard (Sly *et al.*, 1986). DNA–DNA reassociation values were determined by using the spectrophotometric renaturation rate kinetic procedure, as adapted by Huß *et al.* (1983).

Morphology. A Gram stain was performed on cells of *Desulfosporosinus orientis* DSM 765^T and strains S5 and S10^T. Transmission electron microscopy of whole cells was performed using negative staining with 2% sodium silicotungstate. Thin sections were made using cells fixed in 2·5% glutaraldehyde. The sections were stained with 0·13 M lead citrate solution (pH 12·0).

Carbon sources and electron acceptors. The carbon sources and electron acceptors tested are indicated in Table 1. In

addition, the following growth substrates were tested in the absence of an added electron acceptor: lactate (10 mM), pyruvate (18 mM), ethanol (10 mM), methanol (10 mM) and H_2/CO_2 (excess). Cells were inoculated (3% inoculum) into the growth media directly from the cultivation medium (the concentration of yeast extract in the test medium was 30 mg l^{-1}), except in the cases of H_2/CO_2 , H_2/CO_2 + acetate and fermentation tests. In these cases, strain S10^T and *Desulfosporosinus orientis* DSM 765^T were inoculated from a culture which had been subcultured on mineral medium several times (the concentration of yeast extract in the test medium was $7 \times 10^{-7}\text{ mg l}^{-1}$). Strain S5 was inoculated directly from the cultivation medium for H_2/CO_2 + acetate and the fermentation treatments, as above, as it did not grow at lower concentrations of yeast extract (therefore autotrophic growth could not be tested for strain S5). Growth by sulfate reduction was determined after 2 weeks by measuring optical density at 600 nm using a Varian DMS 100 UV-visible spectrophotometer, and sulfide production (Cord-Ruwisch, 1985). Nitrate, nitrite and ammonia concentrations were measured in nitrate-reduction treatments, dimethylsulfide was measured in the headspace of DMSO-reduction treatments and acetate production was measured in fermentation treatments (see below). Reduction of Fe(III) to Fe(II) was identified by the formation of a black FeS precipitate.

The reduction of perchloroethene (PCE) was tested in 120 ml culture bottles, sealed with a Wheaton butyl rubber Teflon-faced plug stopper, containing 50 ml cultivation medium and 9 ml bacterial culture. PCE (5 µl) was injected the following day using a glass syringe (final PCE concentration, 138 mg l^{-1}). Culture bottles were incubated on their sides, to maximize contact between the medium and the headspace, for approximately 2 weeks. The headspace was sampled and analysed for PCE and its reduction products [trichloroethene (TCE), 1,1-dichloroethene (1,1-DCE), 1,2-dichloroethene (1,2-DCE) and vinyl chloride] (see below). The combined mass of PCE and each of its reduction products in the aqueous and gaseous phases was calculated using the following dimensionless Henry's Law constants (which give the ratio of the concentration in the gaseous phase to the concentration in the aqueous phase at 25 °C): PCE, 0.723; TCE, 0.392; 1,1-DCE, 1.069; vinyl chloride, 1.137 (Gossett, 1987). No constant was needed for 1,2-DCE as none was detected in any of the samples. For the calculations, the volume of the headspace was 61 ml.

Temperature and salinity ranges. Strains S5 and S10^T and *Desulfosporosinus orientis* DSM 765^T were grown at 4, 10, 15, 21, 28, 32, 37 and 42 °C. To test salinity ranges, strains were grown in cultivation medium in which $1.0\text{ g l}^{-1}\text{ NH}_4\text{Cl}$ was replaced with $0.62\text{ g l}^{-1}\text{ (NH}_4)_2\text{SO}_4$, and $0.10\text{ g l}^{-1}\text{ CaCl}_2 \cdot 2\text{H}_2\text{O}$ was replaced with $0.12\text{ g l}^{-1}\text{ CaSO}_4 \cdot 2\text{H}_2\text{O}$. Sodium chloride was added to achieve final concentrations of 0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 4.5 and 5%. Temperature and salinity treatments were performed in duplicate. Growth was observed by measuring optical density at 600 nm daily on a Varian DMS 100 UV-visible spectrophotometer.

Analytical techniques. Concentrations of PCE and its reduction products as well as dimethyl sulfide were measured in a 15 µl sample of headspace gas by using a Varian 3400 gas chromatograph (GC) fitted with a Varian 1093 septum-equipped programmable injector (SPI) and a Varian Saturn III mass spectrometer (GC-MS). The GC was equipped with a $30\text{ m} \times 0.22\text{ mm i.d.}$ column coated with a $1\text{-}\mu\text{m}$ -thick film of 5% phenyl polysilphenylene-siloxane (BPX5; SGE). At

least three replicate aliquots of each gas sample were analysed.

Total nitrate + nitrite was measured by reducing nitrate to nitrite using a copper/cadmium reductor column (cadmium washed with 2%, w/v, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 35.5 cm of 0.21-cm-i.d. glass tubing). The concentration of the nitrite ion was determined spectrophotometrically in a Technicon Auto-analyser II continuous-flow analytical instrument, using a sulphanilamide colour reagent. An unreduced sample was also analysed for nitrite by the same spectrophotometric method. Ammonia was analysed using the Berthelot Reaction, in which sodium phenoxide (2.4 M phenol, 0.9 M NaOH) and sodium hypochlorite (a 1:5 dilution of commercial household bleach; $\geq 1.05\%$ available chlorine) solutions were added to an ammonium salt, forming indophenol blue. This was then measured spectrophotometrically at 630 nm.

Acetate in a 1 ml aliquot of the liquid culture was converted to acetic acid by acidification using a 100 µl aliquot of 0.4 M oxalic acid. Each solution was analysed by gas chromatography with flame-ionization detection (GC-FID) using a Hewlett Packard 5890A GC fitted with a model 7673A autosampler, a vaporizing injector and a flame-ionization detector. The GC was equipped with a $25\text{ m} \times 0.32\text{-mm-i.d.}$ column coated with a $0.25\text{-}\mu\text{m}$ -thick film of PEG (BP21; SGE).

RESULTS

Morphology

The cells were curved rods, each having a single lateral flagellum (Fig. 2, top). They had elliptical, subterminal spores which sometimes caused swelling of the cell (Fig. 2, bottom). Cells of strains S5 and S6 were generally $2.3\text{--}4.2\text{ }\mu\text{m}$ in length and $0.7\text{--}1.1\text{ }\mu\text{m}$ in width. Cells of strain S7 ranged from 3.5 to $13\text{ }\mu\text{m}$ in length and were $0.7\text{ }\mu\text{m}$ wide. Cells occurred either singly or in chains of two or more cells. Cells of strains S5 and S10^T were Gram-variable, but they mostly stained Gram-negative (as did *Desulfosporosinus orientis* DSM 765^T). Cells were motile only during the early exponential phase.

RAPD-PCR

The banding patterns produced by the Eden Hill strains were very different from that of *Desulfosporosinus orientis* DSM 765^T, which produced three faint bands at 1400 bp, 990 bp and at a low molecular mass which was beyond the size range of the molecular mass marker (i.e. less than 359 bp). The Eden Hill strains formed two distinct groups, consisting of strains T1, T2, S4 and S5 (group A) and strains S7, S8 and S10^T (group B) (Fig. 3). Strain S6 failed to produce any PCR product in the RAPD protocol used, despite repeated attempts. Group A strains produced three common bands at approximately 2040, 1600 and 1125 bp. Other bands were also present in various group A strains. All group B strains produced a single bright band at 2040 bp. *Desulfosporosinus orientis* DSM 8344 appeared to be similar to the group A strains.

Fig. 2. Transmission electron micrographs of (top) a negatively stained cell of strain S5, showing the single lateral flagellum, and (bottom) a thin section of cells of strain S7, showing an oval-shaped endospore (causing swelling of the cell) and the multilayered cell wall. Bars, 1 µm.

DNA G + C content

The DNA G + C contents of strains S5 and S10^T were 46.8 ± 0.7 and 46.9 ± 0.3 mol %, respectively. The DNA G + C content of *Desulfosporosinus orientis* 765^T, measured at the same time using the same technique, was 45.9 ± 0.5 mol %.

DNA–DNA homologies

The sulfate-reducing strains gave low yields of DNA, so only the most essential hybridizations were performed to determine the level of relatedness between

group A and group B strains and between these strains and the type strain of their nearest phylogenetic relative, *Desulfosporosinus orientis*. DNA from strains S5 (group A) and S10^T (group B) had a DNA–DNA homology of 81.1 %, whereas strain S10^T had 37.7 % homology with *Desulfosporosinus orientis* DSM 765^T.

Carbon sources and electron acceptors

The Eden Hill strains could be distinguished from *Desulfosporosinus orientis* DSM 765^T phenotypically as they were unable to use fumarate as a carbon source and electron donor for sulfate reduction (Table 1). There were several differences between the group A and group B strains in their abilities to use formate, caprylate and syringic acid as carbon sources. Strain S10^T, representative of the group B strains, was autotrophic and all of the Eden Hill strains were shown to be chemolithoheterotrophic (H_2/CO_2 + 1 mM acetate). Strains T1, T2 and S5 were able to reduce nitrate, whereas *Desulfosporosinus orientis* DSM 765^T and the remaining Eden Hill strains were not (Table 1). Those strains which reduced nitrate produced significant quantities of nitrite after 2 weeks, but no ammonia was produced in excess of the uninoculated control (data not shown).

When PCE was added to cultures, small amounts of the products were found. The most important product was TCE, with combined mass values of 4.3–8.4 µg in cultures of strains S6, S7 and S10^T and *Desulfosporosinus orientis* 765^T, and 1.2–1.5 µg in cultures of strains T1, T2, S4 and S5. Trace quantities of 1,1-DCE and vinyl chloride were found in most cultures but no 1,2-DCE was detected in any culture. None of these compounds was detected in the sterile control. Combined mass values for PCE at the end of the incubation period ranged from 2045 to 3440 µg in all cultures, whereas the sterile control contained 3140 µg PCE at the end of the incubation period. These values represent 24–42 % of the amount of PCE originally added to the cultures.

Strains S5 and S10^T and *Desulfosporosinus orientis* DSM 765^T were able to use lactate, pyruvate and ethanol as growth substrates in the absence of an added electron acceptor, producing acetate. Strain S10^T and *Desulfosporosinus orientis* DSM 765^T were able to use methanol as a growth substrate, unlike strain S5. No growth of any of the strains was observed in the presence of excess H_2/CO_2 without an electron acceptor; however, *Desulfosporosinus orientis* DSM 765^T produced a small amount of acetate (1.2 mM).

Temperature and salinity ranges

All three strains tested grew between 10 and 37 °C, but failed to grow at 4 or 42 °C; all showed a decrease in growth in response to increasing salinity. Strains S5 and S10^T were unable to grow at 4 % NaCl whereas *Desulfosporosinus orientis* DSM 765^T grew at 4 and 4.5 % NaCl, but failed to grow at 5 % NaCl.

Fig. 3. Agarose gel electrophoresis (1.5% agarose) of RAPD-PCR fragments of Eden Hill strains and reference strains. Lanes: 1, 13, molecular mass marker VII (Boehringer Mannheim); 2, strain T1; 3, strain T2; 4, strain S4; 5, strain S5; 6, *Desulfosporosinus orientis* DSM 8344; 7, strain S7; 8, strain S8; 9, strain S10^T; 10, *Desulfosporosinus orientis* DSM 765^T; 11, *B. subtilis* (ATCC 6051^T); 12, negative control. Estimated band sizes (bp) are indicated.

Whole-cell fatty acids

The whole-cell fatty acids were analysed previously (Robertson *et al.*, 2000). The fatty acid profiles of all of the strains were dominated by even-carbon, straight-chain saturated and mono-unsaturated fatty acids, in particular 16:1*cis*9, 16:0, 14:0 and 18:1*cis*11. Small quantities of the cyclopropane fatty acids cyclo17:0 or cyclo19:0 were present in most strains. The branched-chain fatty acids iso-15:0, anteiso-15:0 and anteiso-17:0 were also common in the strains. A detailed description of the fatty acid profile of the strains is given by Robertson *et al.* (2000).

DISCUSSION

There is both genetic and phenotypic evidence that the Eden Hill strains are members of a new species of sulfate-reducing bacteria. The low DNA–DNA homology between strain S10^T and *Desulfosporosinus orientis* DSM 765^T is supported by the very different RAPD-PCR banding patterns of the Eden Hill strains and *Desulfosporosinus orientis* DSM 765^T. The Eden Hill strains could be differentiated from *Desulfosporosinus orientis* DSM 765^T as they did not use fumarate as a carbon and energy source, unlike *Desulfosporosinus orientis* DSM 765^T. Further phenotypic distinctions can be made between the Eden Hill strains and *Desulfosporosinus orientis* DSM 765^T by a combination of characteristics (i.e. the Eden Hill strains either used nitrate as an electron acceptor or did not use formate or caproate as carbon sources, whereas *Desulfosporosinus orientis* DSM 765^T was unable to reduce nitrate and was able to use both formate and caproate as carbon sources). The Eden Hill strains were also less tolerant of increasing salinity than was *Desulfosporosinus orientis* DSM 765^T. They failed to grow at 4% NaCl, whereas *Desulfosporosinus orientis* DSM 765^T was able to grow at NaCl concentrations of less than 5%. In addition, Eden Hill strain S7 was larger (up to 13 µm) than cells of *Desulfo-*

sporosinus orientis DSM 765^T (4.8 × 1.4 µm; Adams & Postgate, 1959). The fatty acid profile of the Eden Hill strains is different from that of *Desulfosporosinus orientis* DSM 765^T. The predominance of even-numbered saturated and unsaturated fatty acids is common to both *Desulfosporosinus orientis* DSM 765^T and the Eden Hill strains; however, *Desulfosporosinus orientis* DSM 765^T lacked branched-chain fatty acids and cyclopropane fatty acids (Stackebrandt *et al.*, 1997). Cells of *Desulfosporosinus orientis* DSM 765^T were observed to have one or two flagella and in some cases to have peritrichous flagella (Adams & Postgate, 1959), whereas electron microscopy of the Eden Hill strains did not show any cells having more than one flagellum.

The Eden Hill strains largely conform to the description of the genus *Desulfosporosinus* (Stackebrandt *et al.*, 1997). They are spore-forming, sulfate-reducing bacteria that mostly produce a negative reaction to the Gram stain, but which phylogenetically belong to the *Clostridium*/*Bacillus* subphylum of the Gram-positive bacteria. They are capable of autotrophic growth, which distinguishes them from members of the genus *Desulfotomaculum*. The Eden Hill strains were also capable of homoacetogenic growth on ethanol. However, strain S5 could not use methanol for homoacetogenic growth, as required by the genus description. Also, the DNA G + C content of S5 and S10^T (46.8 and 46.9 mol%) was greater than the upper limit for this characteristic given in the genus description (45.9 mol%). The fatty acid profile of *Desulfosporosinus orientis* DSM 765^T (which is different from that of the Eden Hill strains) and the presence of peritrichous flagella also form part of the genus description.

These differences between the Eden Hill strains and the description of the genus *Desulfosporosinus* are not sufficiently great to justify the creation of a new genus; however, we propose that the description of the genus be modified to accommodate the Eden Hill strains. An

emended description of the genus is given below. We propose that the Eden Hill strains be placed in a new species of the genus *Desulfosporosinus*, called *Desulfosporosinus meridiei*, and that strain S10^T be the type strain.

In phenotypic and molecular testing, the strains fell into two main groups (based on RAPD-PCR results and nutritional characteristics), designated group A and group B. These groups reflect the phylogenetic relationships among the strains (Robertson *et al.*, 2000). *Desulfotomaculum auripigmentum* is able to reduce As(V) as an electron acceptor in place of sulfate. *Desulfosporosinus meridiei* was unable to do this despite its close relationship with the arsenic reducer. Members of the genus *Desulfitobacterium*, to which *Desulfosporosinus meridiei* is also relatively closely related, dehalogenate halogenated aromatic compounds, e.g. *Desulfitobacterium* sp. strain PCE1 reduces PCE metabolically with lactate (Gerritse *et al.*, 1996). The metabolic reduction of PCE occurs at a rate many orders of magnitude greater than that of its co-metabolic reduction (Beccari *et al.*, 1998). It is not possible to determine whether the reduction of PCE by *Desulfosporosinus meridiei* was metabolic or co-metabolic, as the rate of reduction was not determined. Also, recoveries of PCE were low, so the fate of the remaining PCE is unknown.

The ability to use nitrate as electron acceptor has been observed in a few species of sulfate-reducing bacteria from several genera (e.g. Widdel & Pfennig, 1982; Keith & Herbert, 1983; Tasaki *et al.*, 1991). However, these strains generally reduced the nitrate to ammonia, with little or no accumulation of nitrite (e.g. Keith & Herbert, 1983; Seitz & Cypionka, 1986). The limiting amounts of the carbon source (Keith *et al.*, 1982), or the lack of a nitrite reductase enzyme (Moura *et al.*, 1997), are possible reasons for the accumulation of nitrite in nitrate-reducing cultures of *Desulfosporosinus meridiei*. Nitrite toxicity is unlikely to have been a problem at the low concentration of nitrate used here (5 mM). *Desulfosporosinus orientis* DSM 765^T was shown to lack both nitrate and nitrite-reducing ability (Mitchell *et al.*, 1986).

Desulfosporosinus meridiei was isolated from a water-saturated soil environment, as were both strains of *Desulfosporosinus orientis*. The ability of *Desulfosporosinus meridiei* to form endospores and to use a range of substrates doubtless allow it to survive in a fluctuating environment such as a shallow, sandy aquifer. It was isolated from groundwater contaminated with aromatic hydrocarbons and yet it does not appear to have adapted to this environment by developing an ability to utilize aromatic compounds as carbon sources. On the other hand, it is able to grow successfully in this environment, apparently by participating in a communal degradation of toluene under sulfate-reducing conditions whereby it possibly serves as a 'hydrogen scavenger' for fermenters or acetogens (Robertson *et al.*, 2000).

Description of *Desulfosporosinus meridiei* sp. nov.

Desulfosporosinus meridiei (me.ri'die.i. M.L. n. *meridies* south; L. fem. gen. n. *meridiei* of the south, referring to its isolation in the Southern Hemisphere).

Gram-negative/Gram-variable curved rods with multilayered cell wall. Cells are 0.7–1.1 µm in width and generally 2.3–4.2 µm in length, cells of some strains reaching up to 13 µm in length. Cells occur singly or in chains of two or more cells, are motile and have a single lateral flagellum. Endospores are produced; they are oval and subterminal and sometimes cause the cells to swell. Sulfate, sulfite, thiosulfate, elemental sulfur, DMSO and Fe(III) serve as electron acceptors in the presence of lactate. Nitrate reduction is variable (3 out of 7 strains, type strain negative). Manganese (IV) and arsenic (V) are not used as electron acceptors. In the presence of sulfate and thiosulfate, the following compounds are used as carbon sources and electron donors: H₂/CO₂ + 1 mM acetate, pyruvate, lactate, ethanol, formate (3 out of 7 strains, type strain positive), caproate (1 out of 7 strains, type strain negative), caprylate (4 out of 7 strains, type strain negative), methanol (2 out of 7 strains, type strain positive), syringic acid (3 out of 7 strains, type strain positive), H₂/CO₂ (only type strain tested), butyrate (2 out of 2 strains, including type strain), caprate (2 out of 2 strains, including type strain) and laurate (2 out of 2 strains, including type strain). The following compounds are not used as carbon sources and electron donors in the presence of sulfate and thiosulfate: acetate, propionate, fumarate, malate, benzoate, valerate (2 strains tested, including type strain), fructose (2 strains tested, including type strain) and 3,4,5-trimethylbenzoic acid (2 strains tested, including type strain). The following compounds are used as carbon and energy sources in the absence of an electron acceptor (2 strains tested, including type strain): lactate, pyruvate, ethanol, methanol (1 out of 2 strains, type strain positive). Perchloroethene is reduced in the presence of sulfate and pyruvate. The temperature range for growth is between 10 and 37 °C. The upper limit of salt tolerance is 4% NaCl. The predominant whole-cell fatty acids are 16:1*cis*9, 16:0, 14:0 and 18:1*cis*11. Both iso- and anteiso-branched-chain fatty acids are present, as well as trace amounts of cyclopropane fatty acids in most strains (cyclo17:0, 6 out of 7 strains, type strain positive; cyclo19:0, 3 out of 7 strains, type strain negative). The G + C content of the DNA is 46.8–46.9 mol%. The type strain is strain S10^T, and it has been deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen (accession no. DSM 13257^T) and the National Collection of Industrial and Marine Bacteria (accession no. NCIMB 13706^T).

Desulfosporosinus (Stackebrandt *et al.* 1997) 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, curved rods that have a multilayered cell wall structure. Endospores are produced; they are oval and subterminal and cause the cells to swell slightly. Motile, with lateral or peritrichous flagella. Strictly anaerobic. Growth occurs between 10 and 37 °C. Desulfovibrin and cytochrome c_3 are absent; bisulfite reductase P_{582} is present. Sulfate and thio-sulfate are reduced to sulfide in the presence of lactate, pyruvate and other carbon sources and electron donors. Incomplete oxidation of organic compounds to acetate occurs. Fumarate is sometimes used as a carbon and energy source for sulfate reduction. Nitrate is sometimes reduced to nitrite. Autotrophic growth occurs with hydrogen plus sulfate. Homoacetogenic growth occurs with ethanol and sometimes with methanol. Contains a menaquinone with a side-chain having seven isoprene units (MK-7 type). The predominant fatty acids are even-numbered, saturated and unsaturated fatty acids, and minor amounts of branched chain and cyclopropane fatty acids may occur. The G+C content of the DNA is 44.7–46.9 mol%. Phylogenetically, it is a member of the *Clostridium*–*Bacillus* subphylum of the Gram-positive bacteria. The type species is *Desulfosporosinus orientis*.

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REFERENCES

- Adams, M. & Postgate, J. R. (1959). A new sulfate-reducing vibrio. *J Gen Microbiol* **20**, 252–257.
- Beccari, M., Majone, M., Piemontese, G., Tandoi, V. & Tomei, M. C. (1998). Reductive dechlorination of tetrachloroethene by an anaerobic microbial consortium. *RICHMAG Mag Jan/Feb*, 63–80.
- Cord-Ruwisch, R. (1985). A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbiol Methods* **4**, 33–36.
- Gerritse, J., Renard, V., Pedro Gomes, T. M., Lawson, P. A., Collins, M. D. & Gottschal, J. C. (1996). *Desulfitobacterium* sp. strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or ortho-chlorinated phenols. *Arch Microbiol* **165**, 132–140.
- Gossett, J. M. (1987). Measurement of Henry's Law constants for C_1 and C_2 chlorinated hydrocarbons. *Environ Sci Technol* **21**, 202–208.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Keith, S. M. & Herbert, R. A. (1983). Dissimilatory nitrate reduction by a strain of *Desulfovibrio desulfuricans*. *FEMS Microbiol Lett* **18**, 55–59.
- Keith, S. M., MacFarlane, G. T. & Herbert, R. A. (1982). Dissimilatory nitrate reduction by a strain of *Clostridium butyricum* isolated from estuarine sediments. *Arch Microbiol* **132**, 62–66.
- Klemps, R., Cypionka, H., Widdel, F. & Pfennig, N. (1985). Growth with hydrogen, and further physiological characteristics of *Desulfotomaculum* species. *Arch Microbiol* **143**, 203–208.
- Mitchell, G. J., Gwynfryn Jones, J. & Cole, J. A. (1986). Distribution and regulation of nitrate and nitrite reduction by *Desulfovibrio* and *Desulfotomaculum* species. *Arch Microbiol* **144**, 35–40.
- Moura, I., Bursakov, S., Costa, C. & Moura, J. J. G. (1997). Nitrate and nitrite utilization in sulfate-reducing bacteria. *Anaerobe* **3**, 279–290.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Robertson, W. J., Franzmann, P. D. & Mee, B. J. (2000). Spore-forming, *Desulfosporosinus*-like sulfate-reducing bacteria from a shallow aquifer contaminated with gasoline. *J Appl Microbiol* **88**, 248–259.
- Seitz, H.-J. & Cypionka, H. (1986). Chemolithotrophic growth of *Desulfovibrio desulfuricans* with hydrogen coupled to ammonification of nitrate or nitrite. *Arch Microbiol* **146**, 63–67.
- Sly, L. T., Blackall, L. L., Kraat, P. C., Tian-shen, T. & Sangkhobol, V. (1986). The use of second derivative plots for the determination of mol% guanine plus cytosine of DNA by the thermal denaturation method. *J Microbiol Methods* **5**, 139–156.
- Stackebrandt, E., Sproer, C., Rainey, F. A., Burghardt, J., Puker, O. & Hippe, H. (1997). Phylogenetic analysis of the genus *Desulfotomaculum*: evidence for the misclassification of *Desulfotomaculum guttoideum* and description of *Desulfotomaculum orientis* as *Desulfosporosinus orientis* gen. nov., comb. nov. *Int J Syst Bacteriol* **47**, 1134–1139.
- Tasaki, M., Kamagata, Y., Nakamura, K. & Mikami, E. (1991). Isolation and characterization of a thermophilic benzoate-degrading, sulfate-reducing bacterium, *Desulfotomaculum thermobenzoicum* sp. nov. *Arch Microbiol* **155**, 348–352.
- Vainshtein, M. B., Gogotova, G. I. & Hippe, H. (1995). A sulfate-reducing bacterium from permafrost. *Microbiology* (English translation of *Mikrobiologiya*) **64**, 514–518.
- Widdel, F. & Pfennig, N. (1982). Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids II. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov., sp. nov. *Arch Microbiol* **131**, 360–365.
- Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingney, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* **18**, 6531–6535.
- Wilson, K. (1998). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.5. Edited by F. A. Ausubel and others. New York: Wiley.