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S. Ramamoorthy
University of Montana

H. Sass
Universität Oldenburg

H. Langner
University of Montana

P. Schumann
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

R. M. Kroppenstedt
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

See next page for additional authors

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S. Ramamoorthy,[1] H. Sass,[2] H. Langner,[3] P. Schumann,[4] R. M. Kroppenstedt,[4] S. Spring,[4] J. Overmann[5] and R. F. Rosenzweig[1]

Correspondence
R. F. Rosenzweig
Frank.Rosenzweig@mso.umt.edu

[1]Division of Biological Sciences, Program in Microbial Ecology, University of Montana, Missoula, MT 59812-4824, USA

[2]Institut für Chemie und Biologie des Meeres, Universität Oldenburg, D-26111 Oldenburg, Germany

[3]Department of Geology, University of Montana, Missoula, MT 59812, USA

[4]DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

[5]Section Microbiology, Department Biology I, Ludwig-Maximilians-Universität München, Maria-Ward-Str. 1a, D-80638 München, Germany

A novel sulfate-reducing bacterium was isolated from pristine sediments of Lake Stechlin, Germany. This strain, STP12^T, was found to contain predominantly *c*-type cytochromes and to reduce sulfate, sulfite and thiosulfate using lactate as an electron donor. Although STP12^T could not utilize elemental sulfur as an electron acceptor, it could support growth by dissimilatory Fe(III) reduction. In a comparison of 16S rRNA gene sequences, STP12^T was 96·7 % similar to *Desulfosporosinus auripigmenti* DSM 13351^T, 96·5 % similar to *Desulfosporosinus meridiei* DSM 13257^T and 96·4 % similar to *Desulfosporosinus orientis* DSM 765^T. DNA–DNA hybridization experiments revealed that strain STP12^T shows only 32 % reassociation with the type strain of the type species of the genus, *D. orientis* DSM 765^T. These data, considered in conjunction with strain-specific differences in heavy metal tolerance, cell-wall chemotaxonomy and riboprint patterns, support recognition of strain STP12^T (=DSM 15449^T=JCM 12239^T) as the type strain of a distinct and novel species within the genus *Desulfosporosinus*, *Desulfosporosinus lacus* sp. nov.

The genus *Desulfosporosinus* currently contains three species with validly published names, *Desulfosporosinus orientis* (Stackebrandt *et al.*, 1997), *Desulfosporosinus auripigmenti* (Stackebrandt *et al.*, 2003) and *Desulfosporosinus meridiei* (Robertson *et al.*, 2001). Isolates from locales separated by thousands of kilometres exist for some of these species (e.g. DSM 8344; Vainshtein *et al.*, 1994). Culture- and non-culture-based studies have revealed the existence of *Desulfosporosinus*-like organisms in environmental settings as diverse as permafrost (Vainshtein *et al.*, 1994), pristine aquifers (Detmers *et al.*, 2004), municipal drinking water (Bade, 2000) and rice plant roots (Scheid *et al.*, 2004).

Multiple lines of evidence suggest that members of the genus also commonly inhabit industrially impacted soils and sediments. *D. auripigmenti* was isolated from an arsenic-contaminated watershed, and is one of a limited number of bacteria known to respire arsenate (Newman *et al.*, 1997). Recently, another arsenic-reducing *Desulfosporosinus* strain was isolated from arsenic-contaminated sediments; this organism has the capacity to metabolize a wide variety of aromatic compounds (Liu *et al.*, 2004). Kusel *et al.* (2001) reported the isolation of *Desulfosporosinus*-like organisms from a coal-mining-impacted lake, while Robertson *et al.* (2000) isolated eight *Desulfosporosinus* strains from a hydrocarbon-contaminated soil. Two of these were later described as *D. meridiei* (Robertson *et al.*, 2001) and their activity was linked to toluene mineralization (Robertson *et al.*, 2000; Franzmann *et al.*, 2002). *Desulfosporosinus*-like strains have repeatedly been seen to dominate subsurface bacterial communities associated with radionuclide-contaminated sediment. This observation is consistent across widely dispersed habitats in the United States that

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain STP12^T is AJ582757.

A transmission electron micrograph of cells of strain STP12^T, a graph showing lactate oxidation and growth and a summary of the metal-tolerance profiles of strain STP12^T and related strains are available as supplementary material in IJSEM Online.

include the Midnight Mine, WA (Suzuki *et al.*, 2002), the DOE Field Research Center in Oak Ridge, TN (Shelobolina *et al.*, 2003), and uranium mine tailings in Shiprock, NM (Nevin *et al.*, 2003). Significantly, enrichment of *Desulfosporosinus*-like microbes has been associated with stimulated removal of solution-phase uranium (Nevin *et al.*, 2003). In such contexts, uranium precipitation may occur secondarily to sulfidogenesis or as a primary result of dissimilatory U(VI) reduction (Suzuki *et al.*, 2002, 2003, 2004).

Herein, we report the isolation and characterization of a novel *Desulfosporosinus* strain from a pristine environment, sediments of Lake Stechlin, Germany. Strain STP12^T was found by 16S rRNA gene sequence comparison to have similarity values of 96·4–96·7 % relative to other members of the genus; DNA–DNA hybridization experiments revealed only 32 % reassociation with the type strain of the type species, *D. orientis* DSM 765^T. We describe genetic and phenotypic attributes of STP12^T investigated in parallel with the three *Desulfosporosinus* species with validly published names. We contend that ribosomal gene sequence differences and hybridization data, considered along with strain-specific differences in cytochromes, cell-wall chemotaxonomy and riboprint patterns, support recognition of STP12^T as representing a distinct and novel species within the genus *Desulfosporosinus*.

Strain STP12^T was isolated from the highest positive tubes of a most-probable number (MPN) series inoculated with sediment from Lake Stechlin a pristine, dimictic, oligotrophic freshwater lake located 100 km north of Berlin (Germany) (Sass *et al.*, 1997). The culture medium used was that described by Widdel & Pfennig (1977), modified by Widdel (1980). Medium was reduced by addition of 0·1 ml freshly prepared sodium dithionate stock solution (3 % w/v). Na_2SO_4 (10 mM) and sodium lactate (10 mM) were added separately from sterile anaerobic stocks. Anaerobic techniques were used in all medium preparation and culture manipulations. Physiological attributes of strain STP12^T were evaluated in parallel with those of other members of the genus *Desulfosporosinus*. *D. meridiei* DSM 13257^T (Robertson *et al.*, 2001), *D. orientis* DSM 765^T (Campbell & Postgate, 1965) and *D. auripigmenti* DSM 13351^T (Newman *et al.*, 1997; Stackebrandt *et al.*, 2003) were obtained from the DSMZ. Bacteria were initially cultivated on the appropriate recommended DSMZ medium. Because all strains grew on Widdel & Pfennig (1977) medium as modified by Widdel (1980), this medium was used for all comparative experiments. Unless otherwise indicated, all physiological experiments were performed in triplicate at 30 °C, lasting until cultures reached stationary phase. Uninoculated media and/or autoclaved cells served as negative controls.

Gram staining was performed using the Fisher Gram stain set protocol (Magee *et al.*, 1975). Spore formation was tested by culturing strains in media containing 10 mM sulfate and 10 mM lactate through late-stationary phase and then assessing presence of spores by phase-contrast light microscopy and transmission electron microscopy (TEM). Cultures deemed positive for spore formation were incubated at 85 °C for 30 min and then transferred to fresh medium and monitored for outgrowth.

Samples for negative staining and electron microscopy were pipetted onto a nickel grid coated with 0·25 % Formvar solution in ethylene dichloride and stained for 30–60 s with 2 % uranyl acetate. Thereafter, the grid was mounted on a grid holder and viewed using a 1K/1K AMT camera attached to a Hitachi H-7100 transmission electron microscope. Samples for TEM were prepared by fixing cells with Karnovsky's fixative and washing three times with 0·1 M sodium cacodylate buffer. Samples were pelleted by centrifugation, embedded in 1 % agarose (with 0·01 % azide), post-fixed with 1 % buffered osmium tetroxide and then dehydrated in a graded ethanol series followed by propylene oxide. Bacterial samples were infiltrated in a polybed 812 resin and polymerized at 60 °C for 2 days. Sections (70 nm) were collected on 100-mesh copper–rhodium grids, post-stained with 2 % uranyl acetate followed by Reynold's lead citrate (Bozzola & Russell, 1999) and then viewed using a 1K/1K AMT camera attached to a Hitachi H-7100 transmission electron microscope.

To test for the oxidation of lactate coupled with sulfate reduction, mid-exponential phase cells were used to inoculate (5 % inoculum) fresh medium amended with 10 mM lactate and 5 mM sulfate. Negative controls consisted of (i) live cells in fresh medium without lactate, (ii) autoclaved cells added to complete medium (Madigan *et al.*, 1997) and (iii) live cells filtered through a 0·2 μm sterile filter, with the filtrate added to complete medium. Culture tubes were subsampled periodically to estimate cell number and concentrations of H_2S, lactate and acetate. Culture densities were estimated spectrophotometrically at 420 nm. Direct cell counting was used to confirm growth on selenate, manganese oxide and Fe(III). Cells were stained with 10 μg 4′,6-diamidino-2-phenylindole ml^{-1} (DAPI; Sigma) and then cells were counted by epifluorescence microscopy using a Zeiss Axioskop. Growth yield was defined as bacterial dry mass obtained from a given amount of substrate, as described by Cypionka & Pfennig (1986).

Oxygen sensitivity was tested by transferring late-exponential phase cells grown on sodium lactate (10 mM) and sodium sulfate (5 mM) into sterile flasks and shaking them under air at 30 °C for 24 h, whereupon they were reintroduced into anoxic medium containing 10 mM lactate and 5 mM sulfate. Growth was monitored as described below. Production of sulfide was assayed by testing for black FeS precipitate upon addition of 0·05 % $Fe(NH_4)_2(SO_4)_2$.

Temperature and pH optima were estimated by culturing cells in basal medium amended with 10 mM sodium lactate and 5 mM sodium sulfate. To determine temperature optima, cells were grown at pH 7·0 at 4, 10, 15, 21, 28, 30, 32, 37, 42 and 45 °C. To determine pH optima, the

bicarbonate buffer was replaced with 20 mM (each) HEPES, PIPES, MES and Tris base and the initial medium pH was set using 1 M HCl or 1 M NaOH. Population growth at pH 6·0, 6·5, 7·0 and 7·5 was estimated by following the change in optical density; growth rates were calculated using the linear portion of a semi-logarithmic plot of optical density against time.

Desulfosporosinus strains were evaluated for their ability to grow using the electron donors listed in Table 1. Anaerobic stock solutions (1 M) were prepared for each donor and aliquots were added to defined medium to yield the concentrations indicated. Each donor was tested using sulfate (5 mM) as the terminal electron acceptor. Triplicate experiments were initiated by the addition of 10 % mid-exponential-phase inocula to fresh medium. Change in cell density and sulfide production was monitored as described below. Organisms were considered positive for utilization of an electron donor if 2·5 to 3 doublings occurred after the third passage with simultaneous production of sulfide, as indicated by formation of black precipitate when amended with 0·05 % $Fe(NH_4)_2(SO_4)_2$. Inoculated medium lacking substrate served as a negative control.

Bacteria were also tested for their ability to utilize the terminal electron acceptors indicated in Table 1 using sodium lactate (10 mM) as a carbon source and electron donor. Anaerobic stock solutions (1 M) were prepared for each electron acceptor and aliquots were added to defined medium to yield the indicated concentrations. Experiments were initiated by the addition of 10 % mid-exponential-phase inocula to fresh medium. Change in cell density and

Table 1. Morphological and chemotaxonomic features of STP12^T in relation to described members of the genus *Desulfosporosinus*

All strains are Gram-negative, display peritrichous flagella and produce oval endospores. Electron acceptors were tested in the presence of 10 mM lactate as the electron donor. Electron donors were tested in the presence of 5 mM sodium sulfate as the electron acceptor. Concentrations (in mM unless indicated) are given in parentheses. All strains failed to grow in the presence of lactate when provided with either nitrate (5), selenate (10) or Mn(IV) (10). Strains grew equally well in the presence of sulfate when butyrate (5), pyruvate (10), ethanol (10) or yeast extract (1 g l^{-1}) were provided as electron donors. No strain grew on acetate (10), propionate (5), succinate (1), benzoate (1) or glucose (10) in the presence of sulfate. Use of electron acceptors and donors is scored in terms of OD_{420} as: −, no growth; +, <0·250; ++, 0·250–0·500; +++, >0·500. ND, Not determined.

Feature	STP12^T	*D. orientis* DSM 765^T	*D. meridiei* DSM 13257^T	*D. auripigmenti* DSM 13351^T
Morphology	Curved rods	Curved rods, sometimes paired	Curved rods, single or in short chains	Curved rods, sausage-shaped, sometimes long chains
Cell diameter (μm)	0·6	0·7–1·0	0·7–1·1	0·4
Endospore position	Subterminal	Subterminal	Subterminal	Subterminal to almost terminal
Cytochromes	Cytochrome *c* and traces of *b*-type	Lacks cytochrome *c**	ND	ND
Sulfite reductase	P_{582}	P_{582}	ND	ND
Electron acceptors in presence of lactate				
Sulfite (5)	++	++	+	+++
Thiosulfate (5)	++	++	+	++
Sulfur (10)	−	+	+	+
Arsenate (10)	−	−	−	+
Fe(III) (10)	+	−	−	−
Fumarate (20)	−	+	−	++
Sulfate (10)	++	+	+	+
Electron donors in presence of sulfate				
Formate (10)	+	++	+	+
Lactate (10)	++	++	+	++
Fumarate (10)	−	+	−	−
Malate (1)	−	−	−	+
Methanol (10)	+	+	+	−
Glycerol (5)	+++	−	+	+++
Oxidation of H_2	+ (with CO_2)	+ (with CO_2)	+ (with CO_2)	+ (with acetate)
DNA G+C content (mol%)	42·7	45·9	47	41·6

*Data from Suzuki *et al.* (2004).

sulfide production was monitored as described herein. Organisms were considered positive for utilization of a given electron acceptor if 2·5 to 3 doublings occurred after the third passage. Inoculated medium lacking electron acceptor served as negative control. Reduction of sulfate, sulfite, sulfur and thiosulfate was tested by adding 0·5 % $Fe(NH_4)_2(SO_4)_2$ to culture tubes. If black FeS precipitate formed, cultures were scored positive for reduction of oxidized sulfur compounds. MnO_2 reduction was inferred by a colour change from black MnO_2 to white $MnCO_3$, while formation of red elemental selenium precipitate was indicative of Se(VI) reduction. The change in colour from yellow to white due to the formation of siderite ($FeCO_3$) indicated reduction of ferric pyrophosphate [$Fe_4(P_2O_7)_3$] to ferrous iron (Coleman *et al.*, 1993).

To test for sulfur disproportionation, strains were grown in modified Pfennig medium to mid-exponential phase and then inoculated into fresh medium containing 10 mM thiosulfate, bisulfite or elemental sulfur. Growth and sulfur disproportionation were inferred by measuring the change in optical density and by testing for sulfide through addition of 0·05 % $Fe(NH_4)_2(SO_4)_2$ at the end of each experiment. Oxidase and catalase tests were performed as described by Cappuccino & Sherman (1999).

Metal(loid) tolerance was assayed by inoculating medium containing lactate (10 mM) and Na_2SO_4 (10 mM) along with either $Na_2HAsO_4.7H_2O$, $CdSO_4$, K_2CrO_4 or $ZnSO_4$. The amount of metal added was pre-determined using the geochemical equilibrium-modelling program MINTEQA2 (Allison *et al.*, 1991). Prior to inoculation in metal-amended medium, cells were washed in order to remove all previously produced sulfide. Isolates were considered metal-tolerant if the medium turned black due to formation of FeS precipitate following addition of 0·05 % $Fe(NH_4)_2(SO_4)_2$ and cell density increased by more than threefold.

Sulfide (as hydrogen sulfide, H_2S) concentration was estimated by spectrophotometric assay of methylene blue as described by Cline (1969). Reduced arsenic [as arsenite, As (III)] was quantified spectrophotometrically at 865 nm using the procedure originally described by Johnson & Pilson (1972), later modified by Niggemyer *et al.* (2001). Disappearance of lactate and accumulation of acetate in lactate oxidation measurements was determined using ion chromatography. Culture samples were filtered using 0·2 μm filters before injection onto an ICE-AS6 column connected to a Dionex DX500 chromatography system. Heptafluorobutyric acid (0·4 mM) was the eluent and 5 mM tetrabutyl ammonium hydroxide was used to regenerate the column (Dionex Column manual).

Cells were tested for desulfoviridin using a modification of the method described by Postgate (1959); *Desulfovibrio desulfuricans* ATCC 27774 was used as a positive control and *Escherichia coli* K-12 as a negative control. For determination of cytochromes and sulfite reductases, 2 l freshly grown culture was gassed with CO_2 for 15 min to remove H_2S and then centrifuged and the cell pellet was washed with 150 ml KCl. Cell-free extracts were obtained by treatment of cell pellets with a French press and subsequent centrifugation (12 100 ***g***; 30 min). Cytochromes and sulfite reductases were analysed by recording redox difference and carbon monoxide difference spectra using a Lambda 2S spectrophotometer (Perkin Elmer). Separation and identification of *b*-type and *c*-type cytochromes were performed as described by Widdel (1980).

Cell-wall preparations were obtained by boiling cells in 20 % (w/v) aqueous trichloroacetic acid solution for 20 min. Diaminopimelic acid isomers were detected in cell-wall hydrolysates (4 M HCl, 100 °C, 16 h) by TLC on cellulose sheets (Merck) using the solvent system of Rhuland *et al.* (1955). Isoprenoid quinones were analysed as described previously (Groth *et al.*, 1996). Cellular fatty acid patterns were determined from cells grown to stationary phase in DSMZ medium 641 (http://www.dsmz.de/media). Fatty acid methyl esters were obtained from 40 mg (wet weight) of cells by saponification, methylation and extraction as previously described (Kämpfer & Kroppenstedt, 1996; Kroppenstedt, 1985; Miller, 1982). Fatty acid methyl ester mixtures were separated by an automated gas chromatographic system (model 5890 Series II and 7673 autosampler; Agilent) controlled by MIS software (Microbial ID). Peaks were automatically integrated and fatty acid names and percentages were determined using the Microbial Identification standard software package (Sasser, 1990).

Universal primers (*E. coli* positions 8-27F and 1541R) were used to obtain nearly full-length bacterial 16S rRNA gene fragments from genomic DNA obtained from pure culture isolates of strain STP12^T (as described by Rainey *et al.*, 1996). The resulting PCR product was purified using the Prep-A-gene DNA purification kit (Bio-Rad). Sequencing was done on an Applied Biosystems model 373A automated DNA sequencer using the ABI PRISM dye terminator cycle sequencing kit with AmpliTaq FS (Applied Biosystems) according to the manufacturer's instructions. The almost-complete 16S rRNA gene sequence of the novel strain was aligned with sequences included in the ARB database or those obtained from the EMBL nucleotide sequence database (http://www.ebi.ac.uk) using tools implemented in the ARB package. The resulting alignment was inspected visually and potential errors were corrected manually. Evolutionary distances were calculated based on the algorithms of Jukes & Cantor (1969), using neighbour-joining and parsimony methods of tree reconstruction as implemented in the ARB program package. The databases of 16S rRNA gene sequences used in this study, as well as the phylogenetic programs, are available at http://www.arb-home.de

Genomic DNA for the determination of DNA base composition and DNA–DNA hybridization studies was isolated using a French pressure cell and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). The G + C content was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.*

(1989). DNA–DNA hybridization studies were carried out according to the method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983), using a Gilford System model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992). Ribotyping of cultures was performed as described previously using the Qualicon RiboPrinter system (DuPont) with *Pvu*II or *Eco*RI as restriction enzymes (Bruce, 1996).

Morphology and physiology

Like other representatives of the genera *Desulfosporosinus* and *Desulfotomaculum*, cells of strain STP12^T possessed a Gram-negative cell wall. Cells were curved rods which ranged in length from 2·3 to 3·8 μm and in width from 0·55 to 0·7 μm and which were capable of forming conspicuous endospores (see Supplementary Fig. S1 in IJSEM Online). Flagellation was variable; peritrichous flagella were occasionally observed.

Strain STP12^T coupled oxidation of lactate to reduction of sulfate, conserving energy for growth (data not shown), and belonged to physiological group I of the sulfate-reducing bacteria. Such bacteria do not oxidize acetyl CoA to CO_2, and therefore excrete acetate. All strains tested utilized lactate and sulfate in a 2 : 1 ratio. No increase in cell number, depletion of lactate or reduction of sulfate was detected in autoclaved controls. The ratios determined and shown in Supplementary Fig. S2 (available in IJSEM Online) indicate a lactate to sulfate ratio of 2 : 1 only at the beginning of the experiment. Above sulfide concentrations of 4 mM, sulfate reduction ceased and lactate seems to be exclusively fermented to acetate. This sulfide sensitivity was already reported for *Desulfotomaculum* (including *Desulfosporosinus*) species by Klemps *et al.* (1985).

Strain STP12^T coupled growth to sulfate respiration over the range 4–32 °C, with μ_{max} of 0·08 h^{-1} at 30 °C. All temperature experiments were conducted at pH 7·0. pH optima were determined at 30 °C as STP12^T attained its μ_{max} at that temperature. Sulfidogenic growth of STP12^T was confined to the pH range 6·5–7·5, although in other experiments using different media this strain has been observed to grow above pH 8·0 (J. Overmann, unpublished results).

Heat-treated cultures of STP12^T incubated in fresh medium increased in cell density and accumulated sulfide. Bacteria were also capable of growth after 24 h of exposure to atmospheric oxygen. These observations can be attributed to the ability of the cells to form high-temperature- and oxygen-resistant endospores.

Electron acceptor and donor preferences for all four strains are summarized in Table 1. All *Desulfosporosinus* strains tested utilized oxidized inorganic sulfur compounds. Yield of strain STP12^T was similar on all three oxidized inorganic sulfur compounds (sulfate, sulfite and thiosulfate) ($OD_{420} \approx$ 0·28). However, unlike certain other *Desulfosporosinus* strains, STP12^T was incapable of using elemental sulfur, fumarate, nitrate or arsenate as electron acceptors. Strain STP12^T and *D. meridiei* DSM 13257^T had similar electron donor utilization profiles; STP12^T was distinct in that it was able to couple growth to sulfate respiration using glycerol as both electron donor and carbon source. All four *Desulfosporosinus* strains tested grew on formate, butyrate, pyruvate, lactate, ethanol and yeast extract.

Yield of strain STP12^T was 5·5 g dry mass per mol lactate when grown at 25 °C. STP12^T also grew autotrophically as a homoacetogen with H_2 and CO_2. Cell density did not increase and sulfide did not accumulate when cells were cultured on acetate, propionate, fumarate, succinate, malate, glucose or benzoate. STP12^T grew fermentatively on either lactate or pyruvate in the absence of sulfate.

The novel isolate failed to disproportionate sulfur presented in the form of thiosulfate, bisulfite or elemental sulfur. Lack of growth and sulfide production were inferred by the absence of change in OD_{420} and the absence of black FeS precipitate following addition of 0·05 % $Fe(NH_4)_2(SO_4)_2$ at the end of each experiment. The novel sulfate reducer STP12^T was both catalase- and oxidase-negative.

Under circumneutral, low E_h conditions, the production of H_2S by sulfate reducers immobilizes solution-phase metal(loid)s as insoluble precipitates. Sulfate reducers have therefore been proposed as agents for accelerated bioremediation of metal-contaminated soils and sediments. With a view towards evaluating their potential in these contexts, we tested the tolerance of STP12^T and its congeners to arsenic, cadmium, zinc and chromium. Results for the four strains are summarized in Supplementary Table S1 available in IJSEM Online. Activity and growth were inferred from the presence of sulfide and change over time in DAPI cell counts. No bacteria tested grew in the presence of Cr, even at the lowest concentration tested (0·4 mM). On the other hand, no strain tested failed to grow in the presence of 2 mM As(V). Among *Desulfosporosinus* strains, only strain STP12^T failed to grow in the presence of 10 mM As(V). Electron acceptor assays, however, clearly demonstrated that only *D. auripigmenti* DSM 13351^T had the capacity for dissimilatory As(V) reduction (data not shown). The highest concentration tested for cadmium (10 μM) inhibited growth of all strains except *D. auripigmenti* DSM 13351^T. Of all cultures tested, only STP12^T failed to grow in the presence of 50 μM zinc, the highest concentration assayed. STP12^T was inhibited even in the presence of 10 μM zinc.

Chemotaxonomy

Cellular fatty acid composition profiles for the novel strain and its three congeners are displayed in Table 2. Fatty acid 16 : 1*cis*9 (26·3 %) predominated in STP12^T. The diagnostic amino acid of STP12^T peptidoglycan was LL-diaminopimelic acid. Isoprenoid quinones MK-7 and MK-5 were present in the ratio of 64 : 36 (ratio of peak areas) in strain STP12^T. STP12^T demonstrated P_{582}-type sulfite reductases having

Table 2. Fatty acid composition of novel strain STP12^T and related *Desulfosporosinus* type strains

Reference strains: 1, *D. auripigmenti* DSM 13351^T (data from Stackebrandt *et al.*, 2003); 2, *D. meridiei* DSM 13257^T (data from this study); 3, *D. orientis* DSM 765^T (H. Hippe and others, personal communication). dma, Dimethylacetal; ald, aldehyde; cyc, cyclopropane. Values are percentages of total fatty acids; values over 10 % are in bold.

ECL	Fatty acid	STP12^T	1	2	3
14·00	14 : 0	3·7	2·2	3·7	4·0
14·63	iso-15 : 0	0·3			
14·77	15 : 2	0·4		2·8	
14·86	15 : 1*cis*9			1·0	
14·95	16 : 0 ald	0·3	1·9	2·6	2·8
15·63	iso-16 : 0	0·5			
15·77	16 : 1*cis*7	6·0	1·5	3·5	3·0
15·81	16 : 1*cis*9	**26·3**	**31·6**	**33·4**	**25·5**
15·90	16 : 1*cis*11	2·1		1·1	0·9
16·00	16 : 0	**15·1**	**14·2**	**5·8**	**17·4**
16·24	16 : 1*cis*7 dma			1·9	
16·28	16 : 1*cis*9 dma	1·5	1·7	5·6	1·8
16·47	16 : 0 dma	2·5	**13·4**	**13·5**	8·7
16·76	Unknown			1·3	
16·77	17 : 1*cis*8		1·3	0·8	
16·79	17 : 1*cis*9	0·6	1·1		
16·89	17 : 0 cyc		0·6		
17·25	18 : 1 dma?			1·1	
17·47	17 : 0 dma			1·3	
17·76	18 : 1*cis*9	9·3	7·5	6·1	7·5
17·82	18 : 1*cis*11	**16·7**	2·5	3·3	5·6
17·92	18 : 1*cis*13	3·9	1·0	1·0	1·2
18·00	18 : 0	3·6	2·8	2·2	4·3
18·22	18 : 1*cis*9 dma	0·5	7·7	3·9	1·5
18·28	18 : 1*cis*11 dma	1·7	4·4	4·3	1·9
18·47	18 : 0 dma		2·0		1·1
19·36	19 : 0 cyc dma	0·6			
19·77	20 : 1*cis*11	1·7			
19·83	20 : 1*cis*13	0·7			

absorption maxima at 592 nm, and *c*-type cytochromes (λ_{max} 553 nm), although traces of *b*-type cytochromes were also observed in cell-free extracts.

Phylogeny and comparative genomics

Ribotype patterns were derived from restriction endonuclease digestion of chromosomal DNA followed by hybridization to probes for sequences that encode the 5S–16S–23S rRNA operon (Barney *et al.*, 2001). These patterns have been shown to discriminate among closely related bacterial strains (Scott *et al.*, 2003). The ribotype pattern of strain STP12^T was distinct from the type strains of the other described *Desulfosporosinus* species (data not shown).

The DNA G+C content of strain STP12^T was 42·7 mol%.

Fig. 1 depicts phylogenetic relationships for the novel strain. Sequence similarity values based on the comparison of almost-complete 16S rRNA gene sequences indicate that strain STP12^T is 96·5 % similar to *D. meridiei* DSM 13257^T, 96·4 % similar to *D. orientis* DSM 765^T and 96·7 % similar to *D. auripigmenti* DSM 13351^T.

Current thinking holds to the view that, when DNA–DNA hybridization values between two strains are less than 70 %, the strains warrant recognition as members of separate species (Stackebrandt & Goebel, 1994). DNA–DNA hybridization experiments reveal that strain STP12^T shows only 32 % reassociation with *D. orientis* DSM 765^T. STP12^T is also distinguished phenotypically from other members of the genus by its unique fatty acid profile, its inability to use S^0 as an electron acceptor and its ability to reduce iron. Altogether, these data support recognition of STP12^T as representing a novel species.

Description of *Desulfosporosinus lacus* sp. nov.

Desulfosporosinus lacus (la′cus. L. n. *lacus -us* a lake; L. gen. n. *lacus* of a lake).

Cells are rod-shaped, stain Gram-negative and have peritrichous flagella. Individual cell dimensions vary from 0·55 to 0·7 μm in width and 2·3 to 3·8 μm in length. They produce heat-resistant endospores that are oval and subterminal and which sometimes cause the cells to swell. Utilizes sulfate, sulfite, thiosulfate and Fe(III) as terminal electron acceptors in the presence of lactate. Arsenate, fumarate, nitrate, sulfur, selenate and Mn(IV) are not used as electron acceptors. In the presence of sulfate, H_2/CO_2,

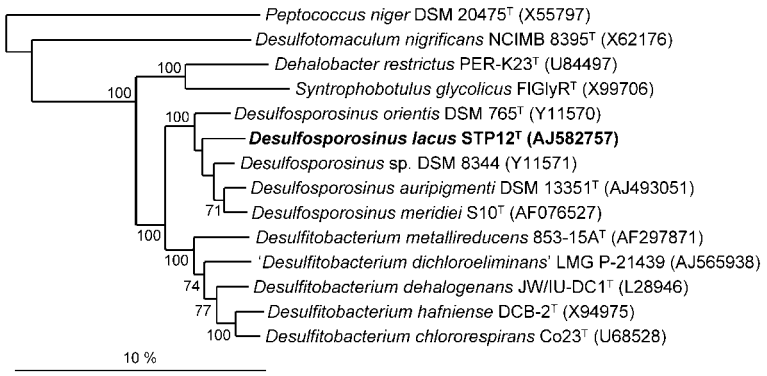

Fig. 1. Unrooted phylogenetic tree based on an alignment of almost-complete 16S rRNA gene sequences showing the affiliation of the novel strain STP12^T to the genus *Desulfosporosinus*. The neighbour-joining method of Saitou & Nei (1987) was used to reconstruct the matrix and the algorithm described by Jukes & Cantor (1969) was used to calculate phylogenetic distance values. Only bootstrap values above 70 % (1000 resamplings for each node) are shown at the respective branching points. Bar, 10 % estimated sequence divergence.

H_2/CO_2 plus acetate, formate, butyrate, pyruvate, lactate, ethanol, methanol, yeast extract and glycerol are utilized as electron donors and carbon sources. Cannot use acetate, propionate, benzoate, fumarate, succinate, malate or glucose as electron donors or carbon sources. Cells are capable of fermentative growth using pyruvate and lactate as energy sources in the absence of any electron acceptor. The temperature and pH ranges for growth are 4–32 °C and pH 6·5–7·5. Possesses a P_{582}-type sulfite reductase and *c*-type cytochromes with traces of *b*-type cytochromes. The diagnostic diamino acid of the peptidoglycan is LL-diaminopimelic acid. The predominant whole-cell fatty acids are 16 : 1*cis*9, 16 : 0, 18 : 1*cis*11, 16 : 1*cis*7. The major isoprenoid quinones are MK-7 and MK-5. The DNA G+C content of the type strain is 42·7 mol%.

The type strain, STP12^T (=DSM 15449^T=JCM 12239^T), was isolated from sediment of Lake Stechlin (northern Germany).

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References

Allison, J. D., Brown, D. S. & Novo-Gradac, K. J. (1991). *MINTEQA2/PRODEFA2, a Geochemical Assessment Model for Environmental Systems.* EPA/600/3-91/021. Cincinnati, OH: US Environmental Protection Agency.

Bade, K. (2000). *Survival of sulfate-reducing bacteria in oxic, oligotrophic environments related to drinking water.* Doctoral dissertation, Technical University of Berlin, Berlin, Germany. http://edocs.tu-berlin.de/diss/2000/bade_karen.htm

Barney, M., Volgyi, A., Navarro, A. & Ryder, D. (2001). Riboprinting and 16S rRNA gene sequencing for identification of brewery *Pediococcus* isolates. *Appl Environ Microbiol* **67**, 553–560.

Bozzola, J. J. & Russell, L. D. (1999). *Electron Microscopy – Principles and Techniques for Biologists*, 2nd edn. Boston: Jones and Bartlett.

Bruce, J. L. (1996). Automated system rapidly identifies and characterizes microorganisms in food. *Food Technol* **50**, 77–81.

Campbell, L. L. & Postgate, J. R. (1965). Classification of the spore-forming sulfate-reducing bacteria. *Bacteriol Rev* **29**, 359–363.

Cappuccino, J. G. & Sherman, N. (1999). *Microbiology – a Laboratory Manual*, 5th edn. Menlo Park, CA: Benjamin/Cummings.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Cline, J. D. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanogr* **14**, 454–458.

Coleman, M. L., Hedrick, D. B., Lovley, D. R., White, D. C. & Pye, K. (1993). Reduction of Fe(III) in sediments by sulphate-reducing bacteria. *Nature* **361**, 436–438.

Cypionka, H. & Pfennig, N. (1986). Growth yields of *Desulfotomaculum orientis* with hydrogen in chemostat culture. *Arch Microbiol* **143**, 366–369.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Detmers, J., Strauss, H., Schulte, U., Bergmann, A., Knittel, K. & Kuever, J. (2004). FISH shows that *Desulfotomaculum* spp. are the dominating sulfate-reducing bacteria in a pristine aquifer. *Microb Ecol* **47**, 236–242.

Franzmann, P. D., Robertson, W. J., Zappia, L. R. & Davis, G. B. (2002). The role of microbial populations in the containment of aromatic hydrocarbons in the subsurface. *Biodegradation* **13**, 65–78.

Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

Huß, 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.

Jahnke, K. D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from Gilford system 2600 spectrophotometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.

Johnson, D. L. & Pilson, M. E. (1972). Spectrophotometric determination of arsenite, arsenate, and phosphate in natural waters. *Anal Chim Acta* **58**, 289–299.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

Klemps, R., Cypionka, H., Widdel, F. & Pfennig, N. (1985). Growth with hydrogen, and further physiological characteristics of *Desulfotomaculum* species. *Arch Microbiol* **143**, 203–208.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, SAB Technical Series, no. 20, pp. 173–179. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press

Kusel, K., Roth, U., Trinkwalter, T. & Peiffer, S. (2001). Effect of pH on the anaerobic microbial cycling of sulfur in mining-impacted freshwater lake sediments. *Environ Exp Bot* **46**, 213–223.

Liu, A., Garcia-Dominguez, E., Rhine, E. D. & Young, L. Y. (2004). A novel arsenate respiring isolate that can utilize aromatic substrates. *FEMS Microbiol Ecol* **48**, 323–332.

Madigan, M. T., Martinko, J. M. & Parker, J. (1997). *Brock: Biology of Microorganisms*, 8th edn. Upper Saddle River, NJ: Prentice Hall.

Magee, C. M., Rodeheaver, G., Edgerton, M. T. & Edlich, R. F. (1975). A more reliable gram staining technic for diagnosis of surgical infections. *Am J Surg* **130**, 341–346.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.

Nevin, K. P., Finneran, K. T. & Lovley, D. R. (2003). Microorganisms associated with uranium bioremediation in a high-salinity subsurface sediment. *Appl Environ Microbiol* **69**, 3672–3675.

Newman, D. K., Kennedy, E. K., Coates, J. D., Ahmann, D., Ellis, D. J., Lovley, D. R. & Morel, F. M. (1997). Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum* sp. nov. *Arch Microbiol* **168**, 380–388.

Niggemyer, A., Spring, S., Stackebrandt, E. & Rosenzweig, R. F. (2001). Isolation and characterization of a novel As(V)-reducing bacterium, implications for arsenic mobilization and the genus *Desulfitobacterium*. *Appl Environ Microbiol* **67**, 5568–5580.

Postgate, J. R. (1959). A diagnostic reaction of *Desulphovibrio desulphuricans*. *Nature* **183**, 481–482.

Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.

Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955). The behavior of the isomers of α,ε-diaminopimelic acid on paper chromatograms. *J Am Chem Soc* **77**, 4844–4846.

Robertson, W. J., Franzmann, P. D. & Mee, B. J. (2000). Spore-forming, *Desulfosporosinus*-like sulphate-reducing bacterium from a shallow aquifer contaminated with gasoline. *J Appl Microbiol* **88**, 248–259.

Robertson, W. J., Bowman, J. P., Franzmann, P. D. & Mee, B. J. (2001). *Desulfosporosinus meridiei* sp. nov., a spore-forming sulfate-reducing bacterium isolated from gasolene-contaminated groundwater. *Int J Syst Evol Microbiol* **51**, 133–140.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sass, H., Cypionka, H. & Babenzien, H.-D. (1997). Vertical distribution of sulfate-reducing bacteria at the oxic-anoxic interface in sediments of the oligotrophic Lake Stechlin. *FEMS Microbiol Ecol* **22**, 245–255.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 1–6.

Scheid, D., Stubner, S. & Conrad, R. (2004). Identification of rice root associated nitrate, sulfate and ferric iron reducing bacteria during root decomposition. *FEMS Microbiol Ecol* **50**, 101–110.

Scott, T. M., Parveen, S., Portier, K. M., Rose, J. B., Tamplin, M. L., Farrah, S. R., Koo, A. & Lukasik, J. (2003). Geographical variation in ribotype profiles of *Escherichia coli* isolates from humans, swine, poultry, beef, and dairy cattle in Florida. *Appl Environ Microbiol* **69**, 1089–1092.

Shelobolina, E. S., O'Neill, K., Finneran, K. T., Hayes, L. A. & Lovley, D. R. (2003). Potential for *in situ* bioremediation of a low-pH, high-nitrate uranium-contaminated groundwater. *Soil Sediment Contam* **12**, 865–884.

Stackebrandt, E., Sproer, C., Rainey, F. A., Burghardt, J., Pauker, 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.

Stackebrandt, E., Schumann, P., Schüler, E. & Hippe, H. (2003). Reclassification of *Desulfotomaculum auripigmentum* as *Desulfosporosinus auripigmenti* corrig., comb. nov. *Int J Syst Evol Microbiol* **53**, 1439–1443.

Suzuki, Y., Kelly, S. D., Kemner, K. M. & Banfield, J. F. (2002). Nanometre-sized products of uranium bioreduction. *Nature* **419**, 134.

Suzuki, Y., Kelly, S. D., Kemner, K. M. & Banfield, J. F. (2003). Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment. *Appl Environ Microbiol* **69**, 1337–1346.

Suzuki, Y., Kelly, S. D., Kemner, K. M. & Banfield, J. F. (2004). Enzymatic U(VI) reduction by *Desulfosporosinus* species. *Radiochim Acta* **92**, 11–16.

Vainshtein, M., Gogotova, G. & Hippe, H. (1994). A sulphate-reducing bacterium from the permafrost. In *Viable Microorganisms in Permafrost*, pp. 68–74. Edited by D. Gilichinsky. Pushchino: Russian Academy of Science Pushchino Research Centre.

Widdel, F. (1980). *Anaerober Abbau von Fettsauren und Benzoesaure durch neu isolierte Arten Sulfat-reduzierender Bakterian*. Doctoral thesis, University of Gottingen, Gottingen, West Germany.

Widdel, F. & Pfennig, N. (1977). A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*. *Arch Microbiol* **112**, 119–122.