

University of Montana

ScholarWorks at University of Montana

Biological Sciences Faculty Publications

Biological Sciences

9-1-2009

Desulfovibrio idahonensis sp. nov., sulfatereducing bacteria isolated from a metal(loid)-contaminated freshwater sediment

H. Sass

Cardiff University

S. Ramamoorthy

Cardiff University

C. Yarwood

University of Montana

H. Langner

University of Montana

P. Schumann

Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

See next page for additional authors

Follow this and additional works at: https://scholarworks.umt.edu/biosci_pubs



Part of the [Biology Commons](#)

Let us know how access to this document benefits you.

Recommended Citation

Sass, H.; Ramamoorthy, S.; Yarwood, C.; Langner, H.; Schumann, P.; Kroppenstedt, R. M.; Spring, S.; and Rosenzweig, R. Frank, "Desulfovibrio idahonensis sp. nov., sulfatereducing bacteria isolated from a metal(loid)-contaminated freshwater sediment" (2009). *Biological Sciences Faculty Publications*. 439. https://scholarworks.umt.edu/biosci_pubs/439

This Article is brought to you for free and open access by the Biological Sciences at ScholarWorks at University of Montana. It has been accepted for inclusion in Biological Sciences Faculty Publications by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.

Authors

H. Sass, S. Ramamoorthy, C. Yarwood, H. Langner, P. Schumann, R. M. Kroppenstedt, S. Spring, and R. Frank Rosenzweig

Desulfovibrio idahonensis sp. nov., sulfate-reducing bacteria isolated from a metal(loid)-contaminated freshwater sediment

H. Sass,¹ S. Ramamoorthy,² C. Yarwood,² H. Langner,³ P. Schumann,⁴ R. M. Kroppenstedt,⁴ S. Spring⁴ and R. F. Rosenzweig²

Correspondence

R. F. Rosenzweig

Frank.Rosenzweig@mso.umt.edu

¹School of Earth, Ocean and Planetary Sciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3YE, UK

²Division of Biological Sciences, University of Montana, Missoula, MT 59812-4824, USA

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

⁴DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7B, D-38124 Braunschweig, Germany

Two novel sulfate-reducing bacteria, strains CY1^T and CY2, were isolated from heavy-metal-contaminated sediments of Lake Coeur d'Alene, Idaho, USA. Strains CY1^T and CY2 were found to contain c-type cytochromes and to reduce sulfate, sulfite, thiosulfate, elemental sulfur, DMSO, anthraquinone disulfonate and fumarate using lactate as an electron donor. In a comparison of 16S rRNA gene sequences, CY1^T and CY2 were found to be 100% identical, but only 97 and 92.4% similar, respectively, to the type strains of *Desulfovibrio mexicanus* and *Desulfovibrio aminophilus*. Unlike these species, however, CY1^T was neither able to disproportionate thiosulfate nor able to use yeast extract or amino acids as electron donors. These data, considered in conjunction with differences among strain CY1^T and the two related type strains in chemotaxonomy, riboprint patterns, temperature and pH optima, support recognition of a distinct and novel species within the genus *Desulfovibrio*, *Desulfovibrio idahonensis* sp. nov., with the type strain CY1^T (=DSM 15450^T =JCM 14124^T).

The genus *Desulfovibrio* ranks among the most speciose, phenotypically diverse genera in the *Proteobacteria*. Diagnostic traits include dissimilatory sulfate reduction, absence of sporulation, presence of polar flagella and the presence of desulfovibrin and cytochrome *c* (Postgate & Campbell, 1965). Ribosomal gene sequence analyses indicate that the group's origin lies deep in the *Deltaproteobacteria* (Devereux *et al.*, 1990). To date, names of some 57 *Desulfovibrio* species have been validly published (http://www.dsmz.de/microorganisms/bacterial_nomenclature_info.php?genus=Desulfovibrio; four of these species have since been reclassified), enriched from such diverse environments as sediments (Bale *et al.*, 1997; Sass *et al.*, 1998), wastewater sludge (Baena *et al.*, 1998; Hernandez-Eugenio *et al.*, 2000) and animal intestines

(Goldstein *et al.*, 2003). *Desulfovibrios* facilitate metal corrosion (Neria-Gonzalez *et al.*, 2006) and have been repeatedly isolated from oil and gas production facilities (Miranda-Tello *et al.*, 2003; Magot *et al.*, 2004). *Desulfovibrio* ribosomal gene sequences have been retrieved from submerged mine timbers (Labrenz & Banfield, 2004), gas hydrate mounds (Mills *et al.*, 2003) and floating macrophyte rhizospheres (Acha *et al.*, 2005), while *desulfovibrio*-specific dissimilatory sulfite reductase genes (*dsrAB*) have been recovered from temperate sulfide-rich streams (Elshahed *et al.*, 2003) and frozen Antarctic lakes (Karr *et al.*, 2005).

As a group, *desulfovibrios* tolerate extreme ranges of temperature (Bale *et al.*, 1997; Vandieken *et al.*, 2006), pH (Abildgaard *et al.*, 2006; Fröhlich *et al.*, 1999) and salinity (Sass & Cypionka, 2004; Ito *et al.*, 2002), and productively use a multitude of electron donors, from aromatic compounds (Reichenbecher & Schink, 1997) and halogenated hydrocarbons (Sun *et al.*, 2000) to amino acids (Baena *et al.*, 1998; Hernandez-Eugenio *et al.*, 2000). In addition to dissimilatory sulfate reduction, certain *Desulfovibrio* species use alternative terminal electron acceptors such as U(VI) (Payne *et al.*, 2002), Fe(III)

Abbreviation: AQDS, anthraquinone disulfonate.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CY1^T and CY2 are AJ582755 and AJ582758, respectively.

An electron micrograph of a cell of strain CY1^T and *PvuII* riboprints of the novel strains and *D. mexicanus* DSM 13116^T are available as supplementary material with the online version of this paper.

(Vandijken *et al.*, 2006) and Cr(VI) (Humphries & Macaskie, 2005), though these electron acceptors do not generally support growth. It has long been known that sulfidogenic microbes biomineralize aqueous-phase metals and metalloids (Tuttle *et al.*, 1969). Now, this activity is being harnessed to bioremediate soils and sediments contaminated by mining and radionuclide processing (Lovley, 2001). Because the genomes of several desulfovibrios have been fully sequenced (Heidelberg *et al.*, 2004; <http://cmr.tigr.org/tigr-scripts/CMR/GenomePage.cgi?org=ntds01>), autecological studies of *Desulfovibrio* species are now possible using genome-enabled technologies (e.g. Clark *et al.*, 2006).

To understand metal(loid) cycling better in mining-impacted freshwater sediments, our group has enriched for and studied iron-, arsenic- and sulfate-reducing bacteria, as the characteristic activities of such microbes are likely to influence contaminant fate and stability (Cummings *et al.*, 2000; Niggemyer *et al.*, 2001; Ramamoorthy *et al.*, 2009). Strains CY1^T and CY2 were isolated from the highest positive tubes of an MPN series inoculated with sediment (pH 6.8–7.2) from Lake Coeur d'Alene, Idaho, USA, a freshwater system historically impacted by lead, zinc and antimony mining (Ramamoorthy *et al.*, 2009). For enrichment and isolation, we used selective dithionite-reduced medium with sodium lactate (10 mM) and Na₂SO₄ (10 mM) (Widdel & Pfennig, 1977; Widdel, 1980). Bacteria were isolated by three passages in 1.5% agar shake tubes.

Gram staining, oxidase and catalase tests, electron microscopy and assessment of spore formation were performed as described previously (Magee *et al.*, 1975; Cappuccino & Sherman, 1998; Ramamoorthy *et al.*, 2006). Cells were tested for desulfovibrin as described by Postgate (1959); *Desulfovibrio desulfuricans* ATCC 27774 was used as a positive control and *Escherichia coli* K-12 as a negative control. Cytochromes were analysed by recording redox difference and CO difference spectra; separation and identification of *b*-type and *c*-type cytochromes were performed as described by Widdel (1980).

Cells of strains CY1^T and CY2 were Gram-negative, curved rods with a single polar flagellum (see Supplementary Fig. S1, available in IJSEM Online). Cells of strain CY1^T were 1.3–2.5 µm long and 0.5–0.65 µm wide, whereas cells of strain CY2 were 1.0–3.3 µm long and 0.4–0.6 µm wide (Table 1). Endospores were not formed. Strains CY1^T and CY2 produced black, round colonies in shake tubes amended with Fe(NH₄)₂(SO₄)₂. CY1^T and CY2 were catalase- and oxidase-negative and desulfovibrin-positive (λ_{max} 631 nm). They also possessed *c*-type cytochromes (λ_{max} 553 nm).

Electron donors were tested using sulfate (5 mM) as the terminal electron acceptor, while electron acceptors were tested with sodium lactate (10 mM) as the electron donor. Organisms were considered positive for utilization of an electron donor or acceptor if 2.5 to 3 doublings occurred

after three successive passages. Negative controls consisted of (i) live cells in medium without lactate or sulfate, (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. Reduction of sulfate, sulfite, sulfur and thiosulfate to sulfide was tested by adding 0.5% Fe(NH₄)₂(SO₄)₂ to culture tubes. For accurate determination of sulfide (as hydrogen sulfide, H₂S) concentrations, the spectrophotometric methylene blue assay of Cline (1969) was used. Reduction of anthraquinone disulfonate (AQDS) was measured photometrically at 450 nm, as described by Lovley *et al.* (1996). As CY1^T and CY2 were isolated from metal(loid)-contaminated sediments, we tested for reduction of Mn(IV), As(V), Fe(III) and Se(VI) presented respectively as MnO₂, Na₂HAsO₄·7H₂O, Fe₄(P₂O₄)₃ and Na₂SeO₄. MnO₂ reduction was scored by the characteristic colour change from black MnO₂ to white MnCO₃, while formation of red elemental selenium precipitate was scored as indicating Se(VI) reduction. Reduced arsenic [as arsenite, As(III)] was quantified photometrically from the A₈₆₅ using the procedure originally described by Johnson & Pilson (1972) and later modified by Niggemyer *et al.* (2001). Sulfate, thiosulfate, lactate and acetate concentrations were determined using ion chromatography (Ramamoorthy *et al.*, 2006). Culture density was estimated photometrically as OD₄₂₀. Fluorimetry after staining with SybrGreenI (Martens-Habbena & Sass, 2006) or 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 (DAPI; Sigma) ml⁻¹, and cells were then 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).

Desulfovibrio strains CY1^T and CY2 were able to use hydrogen, formate, lactate, pyruvate, fumarate, succinate and malate as electron donors (Table 1) and carbon sources and grew fermentatively on pyruvate in the absence of sulfate. In contrast to their closest relative, *Desulfovibrio mexicanus* (Hernandez-Eugenio *et al.*, 2000), strains CY1^T and CY2 did not grow with yeast extract or amino acids as electron donors. Both strains oxidized lactate to acetate and CO₂, identifying them as incompletely oxidizing sulfate-reducers.

In addition to sulfate, CY1^T and CY2 could use fumarate, thiosulfate, elemental sulfur and sulfite as terminal electron acceptors for growth. Strains CY1^T and CY2 and *Desulfovibrio mexicanus* DSM 13116^T grown at 30 °C showed the highest growth yield with DMSO as electron acceptor, followed by thiosulfate and sulfate (Table 2). Manganese oxide as an electron acceptor supported growth of strain CY1^T only. Ferric iron was reduced by all three strains but supported little growth.

Strain CY1^T failed to disproportionate sulfur presented in the form of thiosulfate, bisulfite or elemental sulfur. Lack of growth and absence of sulfate and sulfide production

Table 1. Biochemical and chemotaxonomic features that distinguish strains CY1^T and CY2 from the most closely related type strain

D. mexicanus DSM 13116^T was isolated from a UASB reactor treating cheese wastewater; data for this strain were taken from Hernandez-Eugenio *et al.* (2000) unless indicated. In our study, all three strains used elemental sulfur (10 mM) as an electron acceptor in the presence of lactate and H₂+CO₂ and formate (10 mM) as an electron donor (with 2 mM sodium acetate as carbon source) in the presence of sulfate, and none of the strains tested used acetate (10 mM), propionate (5 mM), butyrate (5 mM), benzoate (1 mM), methanol (10 mM), propanol (5 mM), butanol (5 mM), ethylene glycol (10 mM), 1,2-propanediol (5 mM), glycerol (5 mM), glutamate (5 mM), isoleucine (5 mM), ethanolamine (10 mM) or choline (10 mM) when each was presented as an electron donor. None of the strains tested used As(III), Se(VI) or nitrate as terminal electron acceptor in our study. ND, Not determined; ++, strongly positive (OD₄₂₀>0.250; this study); +, positive (in this study, 0.1<OD₄₂₀≤0.250); -, negative (in this study, OD₄₂₀<0.1).

Characteristic	CY1 ^T	CY2	<i>D. mexicanus</i> DSM 13116 ^T
Morphology	Curved rods	Curved rods	Curved rods, sometimes in chains
Cell size (µm)	0.6 × 1.3–2.5	0.5 × 1–3.3	0.5 × 1.7–2.5
Flagella	Single, polar	Single, polar	Non-motile
Salinity range (% NaCl)	0–0.75	0–0.75	0–1.5
Temperature range (optimum) (°C)	10–37 (28)	10–37 (32)	20–40 (37)
pH range (optimum)	6.5–7.5 (6.5)	6.5–7.5 (7.0)	6.3–8.2 (7.2)
Growth rate at 30 °C (h ⁻¹)	0.05	0.05	0.23
Electron acceptors in the presence of lactate			
Sulfate (10 mM)	++	++	+
Sulfite (5 mM)	++	++	+
Thiosulfate (5 mM)	++	++	+
Fumarate (20 mM)	++	++	-
DMSO (10 mM)	++	++	+*
AQDS (4 mM)	+	+	-*†
Manganese oxide (20 mM)	+	-	-*
Electron donors in the presence of sulfate‡			
Fumarate (10 mM)	++	++	-
Lactate (10 mM)	++	++	+
Malate (1 mM)	+	+	-
Pyruvate (10 mM)	++	++	+
Succinate (1 mM)	+	+	-
Cysteine (5 mM)	-	-	+
Serine (10 mM)	-	-	++
Alanine (10 mM)	-	-	+*
Casamino acids (0.1 %)	-	-	+
Yeast extract (0.2 %)	-	-	+*
Disproportionation§	-	-	+ (Thiosulfate)
DNA G + C content (mol%)	63.5	ND	66.0

*Data from this study.

†AQDS reduction, but no growth.

‡Electron donor assays for *D. mexicanus* DSM 13116^T were conducted in the presence of 20 mM thiosulfate (Hernandez-Eugenio *et al.*, 2000) or 10 mM sulfate (this study).

§With 2 mM sodium acetate as carbon source.

Table 2. Growth yields of strains CY1^T and CY2 and *D. mexicanus* DSM 13116^T grown with lactate and different electron acceptors

Values are g dry weight per mol lactate.

Strain	Sulfate	Thiosulfate	DMSO	Fe(OH) ₃	MnO ₂
CY1 ^T	6.8 ± 5.8	12.5 ± 1.4	13.6 ± 10.4	1.8 ± 0.8	5.9 ± 0.9
CY2	5.5 ± 0.8	6.0 ± 0.5	6.5 ± 1.6	2.9 ± 1.4	0.6 ± 0.3
<i>D. mexicanus</i> DSM 13116 ^T	5.9 ± 3.8	5.3 ± 1.3	10.8 ± 7.0	1.3 ± 0.6	0

were inferred from the absence of change in OD₄₂₀ and the absence of black FeS precipitate following addition of 0.05 % Fe(NH₄)₂(SO₄)₂ at the end of each experiment.

Temperature ranges were estimated by culturing cells in basal medium amended with 10 mM sodium lactate and 5 mM sodium sulfate 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 and the initial medium pH was set using 1 M HCl or NaOH. Population growth at 30 °C and pH 6.0, 6.5, 7.0 and 7.5 was estimated by following the change in optical density; growth rates were calculated from the linear portion of a semi-logarithmic plot of OD against time. Oxygen sensitivity was tested by transferring late-exponential phase cells grown on lactate and sulfate into sterile flasks and shaking them under air at 30 °C for 24 h, whereafter they were reintroduced into anoxic growth medium.

While strains CY1^T and CY2 could both respire sulfate at 10–37 °C, only CY1^T could grow at 4 °C. Strains CY1^T and CY2 attained μ_{\max} of 0.05 h⁻¹ at 28 and 32 °C, respectively. Sulfidogenic growth of CY1^T and CY2 was confined to the range pH 6.5–7.5. Strain CY1^T grew fastest at the lowest pH tested, 6.5, compared with pH 7.0 for CY2. Strains CY1^T and CY2 were also incapable of growth after exposure to atmospheric oxygen for 24 h.

The bacterial strains were assayed for their tolerance to environmental contaminants that are relevant in Lake Coeur d'Alene. Specifically, they were inoculated into medium containing lactate (10 mM) and Na₂SO₄ (10 mM) and exposed to incremental levels of Na₂HAsO₄·7H₂O (up to 10 mM), CdSO₄ (up to 10 mM), K₂CrO₄ (up to 10 mM) or ZnSO₄ (up to 50 mM). The maximum metal(loid) concentrations were chosen near the saturation point calculated using the geochemical equilibrium-modelling program MINTEQA2 (Allison *et al.*, 1991), in order to avoid any precipitates other than the sulfides. Prior to inoculation in contaminant-amended media, cells were washed in order to remove all previously produced sulfide. Isolates were considered metal-tolerant if the medium turned black as a result of formation of FeS precipitate following addition of 0.05 % Fe(NH₄)₂(SO₄)₂ and the cell density increased more than threefold as determined by epifluorescence microscopy after staining with DAPI.

Both strains were incapable of growth in the presence of chromium, even at the lowest concentration tested (0.4 mM). On the other hand, strain CY1^T was able to grow in the presence of all concentrations of As, Cd and Zn tested (up to 10, 10 and 50 mM, respectively). Strain CY2 was capable of growth at lower concentrations of As, Cd and Zn, but its growth was inhibited by each metal at the highest concentrations tested (not shown).

Cell walls and isoprenoid quinones were analysed as described previously (Ramamoorthy *et al.*, 2006). 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 and fatty acid methyl esters were analysed as described previously (Kämpfer & Kroppenstedt, 1996; Kroppenstedt, 1985; Miller, 1982).

Cellular fatty acid profiles for the novel strains and *D. mexicanus* DSM 13116^T are displayed in Table 3. In strains CY1^T and CY2, anteiso-15:0 (15.6 and 15.4 %, respectively) and 16:0 (15.3 and 15.6 %) are the predominant fatty acids. The diagnostic amino acid of CY1^T was meso-diaminopimelic acid and the predominant isoprenoid quinone was MK-6(H₂).

Following PCR using universal primers 8-27F and 1541R, purification of nearly full-length (1529 bp) bacterial 16S rRNA gene fragments and sequence analysis were performed as described previously (Rainey *et al.*, 1996; Ramamoorthy *et al.*, 2006). Almost-complete 16S rRNA gene sequence of strains CY1^T and CY2 were 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 visually inspected 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 via the Internet at <http://www.arb-home.de>. The G + C content was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989). 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).

Fig. 1 depicts phylogenetic relationships of CY1^T to other *Desulfovibrio* species. Similarity values based on almost-complete 16S rRNA gene sequences indicate that strains CY1^T and CY2 are 100 % identical to one another, but only 97.0 and 92.4 % similar to *Desulfovibrio mexicanus* DSM 13116^T and *Desulfovibrio aminophilus* ALA-3^T, respectively. Ribotype patterns 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) have been shown to discriminate among closely related bacterial strains (Scott *et al.*, 2003). The ribotype pattern for CY1^T using *Pvu*II was nearly identical to that of CY2, but distinct from that of the type strain of the closest related species with a validly published name, *D. mexicanus* (97.0 % 16S rRNA sequence similarity) (Supplementary Fig. S2). The G + C content for strain CY1^T was 63.5 mol%.

Considered together, the physiological, chemotaxonomic and genomic data strongly support recognition of strains

Table 3. Fatty acid composition of strains CY1^T and CY2 and *D. mexicanus* DSM 13116^T

Values are percentages of total fatty acids. Major components are highlighted in bold. DMA, Dimethylacetal. –, Not detected.

ECL	Fatty acid	CY1 ^T	CY2	<i>D. mexicanus</i> DSM 13116 ^T
13.62	iso-14:0	0.8	0.7	–
14.00	14:0	0.9	0.8	–
14.62	iso-15:0	9.1	7.5	5.3
14.71	anteiso-15:0	15.6	15.4	17.3
15.00	15:0	0.5	0.8	1.9
15.46	iso-16:1	7.8	9.1	16.1
15.63	iso-16:0	7.2	7.3	15.6
15.81	16:1 <i>cis</i> 9	7.8	7.9	2.7
16.00	16:0	15.3	15.6	4.2
16.13	iso-15:0 3-OH	0.8	0.7	–
16.41	iso-17:1 <i>cis</i> 7	15.2	12.9	10.3
16.52	anteiso-17:1	6.2	6.1	5.8
16.63	iso-17:0	3.5	2.9	3.4
16.72	anteiso-17:0	2.9	3.0	5.8
16.79	17:1 <i>cis</i> 9	–	0.6	1.1
16.86	17:1 <i>cis</i> 11	0.7	1.2	2.0
17.00	17:0	0.9	1.2	2.6
17.47	17:0 DMA	1.0	1.4	3.4
17.82	18:1 <i>cis</i> 11	2.4	2.5	1.7
18.00	18:0	1.6	2.3	1.1

CY1^T and CY2 as representatives of a novel species within the genus *Desulfovibrio*, for which the name *Desulfovibrio idahonensis* sp. nov. is proposed.

Description of *Desulfovibrio idahonensis* sp. nov.

Desulfovibrio idahonensis (i.da.ho.nen'sis. N.L. gen. n. *idahonensis* of Idaho, referring to the source of isolation of the first strains).

Cells are rod-shaped and Gram-negative with single polar flagella. Individual cells are 0.4–0.6 µm wide and 1.0–3.3 µm long. Capable of using sulfate, sulfite, thiosulfate, sulfur, DMSO and fumarate as terminal electron acceptors in the presence of lactate. Arsenate, nitrate and selenate cannot be used as electron acceptors; use of manganese oxide is strain dependent. Iron(III) is reduced but does not support growth. In the presence of sulfate, cells are capable of using H₂/CO₂ plus acetate, formate, pyruvate, lactate, fumarate, succinate or malate as electron donor and carbon source. Unable to use acetate, propionate, benzoate, butyrate, methanol, propanol, butanol, ethylene glycol, 1,2-propanediol, glycerol, ethanolamine, choline, yeast extract or amino acids as electron donor and carbon source. Also capable of fermentative growth using pyruvate as energy source in the absence of any electron acceptor, i.e. as sole source of carbon and energy. The temperature and pH ranges for growth are 10–37 °C and pH 6.5–7.5. The type strain is desulfovibrin-positive and possesses *c*-type cytochromes. The diagnostic amino acid of the peptidoglycan is *meso*-diaminopimelic acid. The predominant whole-cell fatty acids are anteiso-15:0, iso-16:0, 16:0, iso-15:0 and 16:1*cis*9. The predominant isoprenoid quinone is MK-6(H₂). The DNA G + C content of the type strain is 63.5 mol%.

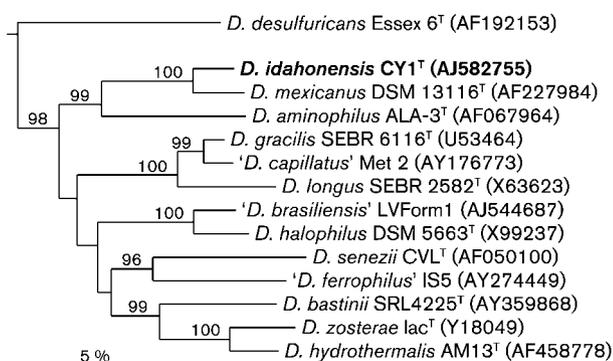


Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the position of the novel strains CY1^T and CY2 among members of the genus *Desulfovibrio*. The sequence of *Escherichia coli* K-12 MG1655 (GenBank accession no. L10328) was used to define the root (not shown). The neighbour-joining method of Saitou & Nei (1987) was used to reconstruct the matrix and the algorithm of Jukes & Cantor (1969) was used to calculate phylogenetic distances. Only bootstrap values above 70% (1000 resamplings for each node) are shown at branching points. Bar, 5% estimated sequence divergence.

The type strain CY1^T (=DSM 15450^T =JCM 14124^T) and reference strain CY2 were isolated from sediment of Lake Coeur d'Alene, Idaho, USA.

Acknowledgements

The authors gratefully acknowledge grant support from the US Geological Survey (99-HQGR-0218), the National Science Foundation (EPS-00-91995) and the US Department of Energy (DE-FG02-00ER63036) to R.F.R. The technical assistance of I. Kramer (DSMZ) is acknowledged. We thank Jean Euzéby for his assistance in correctly naming the species. The manuscript was significantly improved by the critical commentary of Heidi Kuehne, Evgeny Kroll, the Associate Editor and two anonymous reviewers.

References

- Abildgaard, L., Nielsen, M. B., Kjeldsen, K. U. & Ingvorsen, K. (2006). *Desulfovibrio alkalitolerans* sp. nov., a novel alkalitolerant, sulphate-reducing bacterium isolated from district heating water. *Int J Syst Evol Microbiol* **56**, 1019–1024.
- Acha, D., Iniguez, V., Roulet, M., Guimaraes, J. R. D., Luna, R., Alanoca, L. & Sanchez, S. (2005). Sulfate-reducing bacteria in floating macrophyte rhizospheres from an Amazonian floodplain lake in Bolivia and their association with Hg methylation. *Appl Environ Microbiol* **71**, 7531–7535.
- 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.
- Baena, S., Fardeau, M.-L., Labat, M., Ollivier, B., Garcia, J.-L. & Patel, B. K. C. (1998). *Desulfovibrio aminophilus* sp. nov., a novel amino acid degrading and sulfate reducing bacterium from an anaerobic dairy wastewater lagoon. *Syst Appl Microbiol* **21**, 498–504.
- Bale, S. J., Goodman, K., Rochelle, P. A., Marchesi, J. R., Fry, J. C., Weightman, A. J. & Parkes, R. J. (1997). *Desulfovibrio profundus* sp. nov., a novel barophilic sulfate-reducing bacterium from deep sediment layers in the Japan Sea. *Int J Syst Bacteriol* **47**, 515–521.
- 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.
- Bruce, J. (1996). Automated system rapidly identifies and characterizes microorganisms in food. *Food Technol* **50**, 77–81.
- Cappuccino, J. G. & Sherman, N. (1998). *Microbiology: a Laboratory Manual*, 5th edn. Menlo Park, CA: Benjamin/Cummings.
- Clark, M. E., He, Q., He, Z., Huang, K. H., Alm, E. J., Wan, X.-F., Hazen, T. C., Arkin, A. P., Wall, J. D. & other authors (2006). Temporal transcriptomic analysis of *Desulfovibrio vulgaris* Hildenborough transitions into stationary phase during electron donor depletion. *Appl Environ Microbiol* **72**, 5578–5588.
- Cline, J. D. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanogr* **14**, 454–458.
- Cummings, D. E., March, A. W., Bostick, B., Spring, S., Caccavo, F., Fendorf, S. E. & Rosenzweig, R. F. (2000). Evidence for microbial Fe(III) reduction in anoxic, mining-impacted lake sediments (Lake Coeur d'Alene, USA). *Appl Environ Microbiol* **66**, 154–162.
- Cypionka, H. & Pfennig, N. (1986). Growth yields of *Desulfotomaculum orientis* with hydrogen in chemostat culture. *Arch Microbiol* **143**, 366–369.
- Devereux, R., He, S.-H., Doyle, C. L., Orkland, S., Stahl, D. A., LeGall, J. & Whitman, W. B. (1990). Diversity and origin of *Desulfovibrio* species: phylogenetic definition of a family. *J Bacteriol* **172**, 3609–3619.
- Eishahed, M. S., Senko, J. M., Najar, F. Z., Kenton, S. M., Roe, B. A., Dewers, T. A., Spear, J. R. & Krumholz, L. (2003). Bacterial diversity and sulfur cycling in a mesophilic sulfide-rich spring. *Appl Environ Microbiol* **69**, 5609–5621.
- Fröhlich, J., Sass, H., Babenzien, H. D., Kuhnigk, T., Varma, A., Saxena, S., Nalepa, C., Pfeiffer, P. & König, H. (1999). Isolation of *Desulfovibrio intestinalis* sp. nov. from the hindgut of the lower termite *Mastotermes darwiniensis*. *Can J Microbiol* **45**, 145–152.
- Goldstein, E. J. C., Citron, D. M., Peraino, V. A. & Cross, S. (2003). *Desulfovibrio desulfuricans* bacteremia and review of human *Desulfovibrio* infections. *J Clin Microbiol* **41**, 2752–2754.
- Heidelberg, J. F., Seshadri, R., Haveman, S. A., Hemme, C. L., Paulsen, I. T., Kolonay, J. F., Eisen, J. A., Ward, N., Methe, B. & other authors (2004). The genome sequence of the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. *Nat Biotechnol* **22**, 554–559.
- Hernandez-Eugenio, G., Fardeau, M.-L., Patel, B. K. C., Macarie, H., Garcia, J.-L. & Ollivier, B. (2000). *Desulfovibrio mexicanus* sp. nov., a sulfate-reducing bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor treating cheese wastewaters. *Anaerobe* **6**, 305–312.
- Humphries, A. C. & Macaskie, L. E. (2005). Reduction of Cr(VI) by palladized biomass of *Desulfovibrio vulgaris* NCIMB 8303. *J Chem Technol Biotechnol* **80**, 1378–1382.
- Ito, T., Okabe, S., Satoh, H. & Watanabe, Y. (2002). Successional development of sulfate-reducing bacterial populations and their activities in a wastewater biofilm growing under microaerophilic conditions. *Appl Environ Microbiol* **68**, 1392–1402.
- 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. O. (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*, vol. 3, 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.
- Karr, E. A., Sattley, W. M., Rice, M. R., Jung, D. O., Madigan, M. T. & Achenbach, L. A. (2005). Diversity and distribution of sulfate-reducing bacteria in permanently frozen Lake Fryxell, McMurdo Dry Valleys, Antarctica. *Appl Environ Microbiol* **71**, 6353–6359.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: Academic Press.
- Labrenz, M. & Banfield, J. F. (2004). Sulfate-reducing bacteria-dominated biofilms that precipitate ZnS in a subsurface circumneutral-pH mine drainage system. *Microb Ecol* **47**, 205–217.
- Lovley, D. R. (2001). Anaerobes to the rescue. *Science* **293**, 1444–1446.
- Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P. & Woodward, J. C. (1996). Humic substances as electron acceptors for microbial respiration. *Nature* **382**, 445–448.
- 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., Egerton, M. T. & Edlich, R. F. (1975). A more reliable Gram-staining technique for diagnosis of surgical infections. *Am J Surg* **130**, 341–346.
- Magot, M., Basso, O., Tardy-Jacquenod, C. & Caumette, P. (2004). *Desulfovibrio bastinii* sp. nov. and *Desulfovibrio gracilis* sp. nov., moderately halophilic, sulfate-reducing bacteria isolated from deep subsurface oilfield water. *Int J Syst Evol Microbiol* **54**, 1693–1697.
- Martens-Habbena, W. & Sass, H. (2006). Sensitive determination of microbial growth by nucleic acid staining in aqueous suspension. *Appl Environ Microbiol* **72**, 87–95.
- 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.
- Mills, H. J., Hodges, C., Wilson, K., MacDonald, I. R. & Sobbeck, P. A. (2003). Microbial diversity in sediments associated with surface-breaching gas hydrate mounds in the Gulf of Mexico. *FEMS Microbiol Ecol* **46**, 39–52.
- Miranda-Tello, E., Fardeau, M. L., Fernandez, L., Ramirez, F., Cayol, J. L., Thomas, P., Garcia, J. L. & Ollivier, B. (2003). *Desulfovibrio capillatus* sp. nov., a novel sulfate-reducing bacterium isolated from an oil field separator located in the Gulf of Mexico. *Anaerobe* **9**, 97–103.
- Neria-Gonzalez, I., Wang, E. T., Ramirez, F., Romero, J. M. & Hernandez-Rodriguez, C. (2006). Characterization of bacterial community associated to biofilms of corroded oil pipelines from the southeast of Mexico. *Anaerobe* **12**, 122–133.
- Niggemyer, A., Spring, S., Stackebrandt, E. & Rosenzweig, R. F. (2001). Isolation and characterization of a novel As(V)-reducing bacterium, implications for arsenic mobilizations and the genus *Desulfotobacterium*. *Appl Environ Microbiol* **67**, 5568–5580.
- Payne, R. B., Darren, M., Gentry, D. M., Rapp-Giles, B. J., Casalot, L. & Wall, J. D. (2002). Uranium reduction by *Desulfovibrio desulfuricans* strain G20 and a cytochrome *c*₃ mutant. *Appl Environ Microbiol* **68**, 3129–3132.
- Postgate, J. R. (1959). A diagnostic reaction of *Desulphovibrio desulphuricans*. *Nature* **183**, 481–482.
- Postgate, J. R. & Campbell, L. L. (1965). Classification of *Desulfovibrio* species, the non-sporulating sulfate-reducing bacteria. *Bacteriol Rev* **29**, 359–363.
- 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.
- Ramamoorthy, S., Sass, H., Langner, H., Schumann, P., Kroppenstedt, R. M., Spring, S., Overmann, J. & Rosenzweig, R. F. (2006). *Desulfosporosinus lacus* sp. nov., a sulfate-reducing bacterium isolated from pristine freshwater lake sediments. *Int J Syst Evol Microbiol* **56**, 2729–2736.
- Ramamoorthy, S., Piotrowski, J. S., Langner, H. W., Holben, W. E., Morra, M. J. & Rosenzweig, R. F. (2009). Ecology of sulfate-reducing bacteria in an iron-dominated, mining-impacted freshwater sediment. *J Environ Qual* **38**, 675–684.
- Reichenbecher, W. & Schink, B. (1997). *Desulfovibrio inopinatus*, sp. nov., a new sulfate-reducing bacterium that degrades hydroxyhydroquinone (1,2,4-trihydroxybenzene). *Arch Microbiol* **168**, 338–344.
- 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. (2004). Isolation of sulfate-reducing bacteria from the terrestrial deep subsurface and description of *Desulfovibrio cavernae* sp. nov. *Syst Appl Microbiol* **27**, 541–548.
- Sass, H., Berchtold, M., Branke, J., König, H., Cypionka, H. & Babenzien, H. D. (1998). Psychrotolerant sulfate-reducing bacteria from anoxic freshwater sediment, description of *Desulfovibrio cuneatus* sp. nov. and *Desulfovibrio litoralis* sp. nov. *Syst Appl Microbiol* **21**, 212–219.
- 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.
- Sun, B. L., Cole, J. R., Sanford, R. A. & Tiedje, J. M. (2000). Isolation and characterization of *Desulfovibrio dechloracetivorans* sp. nov., a marine dechlorinating bacterium growing by coupling the oxidation of acetate to the reductive dechlorination of 2-chlorophenol. *Appl Environ Microbiol* **66**, 2408–2413.
- Tuttle, J. H., Dugan, P. R. & Randles, C. I. (1969). Microbial sulfate reduction and its potential utility as an acid mine water pollution abatement procedure. *Appl Microbiol* **17**, 297–302.
- Vandieken, V., Knoblauch, C. & Jørgensen, B. B. (2006). *Desulfovibrio frigidus* sp. nov. and *Desulfovibrio ferrireducens* sp. nov., psychrotolerant bacteria isolated from Arctic fjord sediments (Svalbard) with the ability to reduce Fe(III). *Int J Syst Evol Microbiol* **56**, 681–685.
- Widdel, F. (1980). *Anaerobier Abbau von Fettsäuren und Benzoesäure durch neu isolierte Arten Sulfat-reduzierender Bakterien*. Doctoral thesis, University of Göttingen, Göttingen, Federal Republic of Germany (in German).
- Widdel, F. & Pfennig, N. (1977). A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*. *Arch Microbiol* **112**, 119–122.