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# *Deferribacter desulfuricans* sp. nov., a novel sulfur-, nitrate- and arsenate-reducing thermophile isolated from a deep-sea hydrothermal vent

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A novel anaerobic, heterotrophic thermophile was isolated from a deep-sea hydrothermal vent chimney at the Suiyo Seamount in the Izu-Bonin Arc, Japan. The cells were bent, flexible rods, with a single polar flagellum. Growth was observed between 40 and 70 °C (optimum temperature: 60–65 °C; doubling time, 40 min) and between pH 5·0 and 7·5 (optimum pH 6·5). The isolate was a strictly anaerobic heterotroph capable of using complex organic compounds (yeast extract, tryptone, peptone, casein and Casamino acids), ethanol and various organic acids as energy and carbon sources. Hydrogen could serve as a supplementary energy source. Elemental sulfur ($S^0$), nitrate or arsenate was required for growth as an electron acceptor. The G+C content of the genomic DNA was 38·6 mol%. Phylogenetic analysis based on 16S rDNA sequences indicated that isolate SSM1$^T$ is closely related to *Deferribacter thermophilus* BMA$^T$ (98·1 %). However, the novel isolate could be clearly differentiated from *D. thermophilus* BMA$^T$ on the basis of its physiological and genetic properties. The name *Deferribacter desulfuricans* sp. nov. (type strain SSM1$^T$=JCM 11476$^T$=DSM 14783$^T$) is proposed.

Diverse sulfur-reducing hyperthermophiles and thermophiles have been isolated from a variety of microhabitats occurring in deep-sea hydrothermal vent systems. Members of the orders *Thermococcales* and *Thermotogales* are isolated most frequently and are widely distributed in global deep-sea hydrothermal systems (Pledger & Baross, 1989; 1991; Stetter *et al.*, 1990, 1993; Gonzalez *et al.*, 1995, 1998; Godfroy *et al.*, 1996, 1997; Antoine *et al.*, 1997; Takai & Horikoshi, 2000; Takai *et al.*, 2000; Takai & Fujiwara, 2002). Recently, several investigations using a combination of culture-dependent and -independent analyses have revealed that these sulfur-reducing, heterotrophic thermophiles dominate microbial populations in deep-sea hydrothermal vent chimney structures, specifically on the surfaces of the chimney structures (Harmsen *et al.*, 1997; Takai *et al.*, 2001). However, the community structures of sulfur-reducing, heterotrophic thermophiles other than members of *Thermococcales* and their ecological contribution remain poorly understood.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain SSM1$^T$ is AB086060.

Micrographs of cells of *Deferribacter desulfuricans* sp. nov. SSM1$^T$ and graphs showing the effects of temperature, pH and total salt concentration on growth are available as supplementary material in IJSEM Online.

From subsamples obtained from the East Pacific Rise (EPR) deep-sea hydrothermal vent site (13 °N), a population of dissimilatory Fe(III)-reducing thermophilic bacteria belonging to *Deferribacter* was detected by culture-dependent techniques in a deep-sea hydrothermal vent environment (Slobodkin *et al.*, 2001). The genus *Deferribacter* is represented by a single strain, *Deferribacter thermophilus* BMA$^T$, isolated from a subseafloor petroleum reservoir (Greene *et al.*, 1997). It is a strictly anaerobic, heterotrophic thermophile, using a variety of organic compounds and hydrogen as energy sources and nitrate, Fe(III) and Mn (IV) as electron acceptors. The versatile energy-generating metabolism and physiological features of members of *Deferribacter* may indicate that deep-sea hydrothermal vent environments are a potential niche other than subsurface oil reservoirs. In this study, we sought to find sulfur-reducing, heterotrophic thermophiles other than members of *Thermococcales* and *Thermotogales* in deep-sea hydrothermal vent environments.

## Sample collection, enrichment and purification

Samples from a black smoker vent were obtained from the hydrothermal field at the Suiyo Seamount in the Izu-Bonin Arc, Japan (28°34·287′N, 140°38·663′E), at a depth of 1385 m by the manned submersible *Shinkai 2000* in a

dive (dive #1237) performed in November 2000. A bulk chimney sample with a vent emission temperature of 310·8 °C was brought to the sea surface in a sample box, which is part of the equipment of the *Shinkai 2000*, and immediately divided into four different sections (top part of the chimney, surface layer of the chimney, inside structure and vent orifice surface) as described by Takai *et al.* (2001). The chimney was composed mainly of iron sulfide, and large pyrite or chalcopyrite crystals were observed where hot fluid was emitted (the vent orifice surface). Each of the sample sections (approx. 10 g each) was suspended in 20 ml sterilized MJ synthetic sea water (Sako *et al.*, 1996; Takai *et al.*, 1999) containing 0·05 % (w/v) sodium sulfide in a 100-ml glass bottle (Schott Glaswerke) tightly sealed with a butyl rubber cap under a gas phase of 100 % $N_2$ (100 kPa). These suspended portions of the samples were inoculated (0·1 % volume of the medium) with MJNS medium (described below) supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) tryptone under a gas phase of 80 % $H_2$ and 20 % $CO_2$ (300 kPa). The cultures were incubated onboard at 70 and 85 °C in dry ovens.

Growth of anaerobic, heterotrophic thermophiles was observed in MJNS medium supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) tryptone, which was inoculated only with the sample section from the chimney surface layer (1–3 mm), after 2 days of incubation at 70 and 85 °C. Based on direct cell counting of DAPI-stained samples, the chimney surface layer contained approximately $1{\cdot}0\times10^8$ cells $g^{-1}$, while the other sample sections had microbial population densities below the detection limit ($<10^3$ cells $g^{-1}$). Unlike previously reported results for a black smoker chimney structure from the Manus Basin deep-sea hydrothermal field (Takai *et al.*, 2001), a sizeable microbial community probably occurred only in the microhabitat along the chimney surface in the Suiyo Seamount deep-sea hydrothermal field.

The enrichment cultures grown at 70 °C contained predominantly bent, flexible rods, with a small population of highly motile irregular coccoids, while the cultures at 85 °C had a population of highly motile coccoids alone. Since the highly motile, irregular coccoids were probably members of the *Thermococcales*, a pure culture of bent, flexible rods grown at 70 °C was obtained by using the dilution-to-extinction technique at 65 °C (Takai & Horikoshi, 2000). The culture in the tube showing growth at the highest dilution was designated strain SSM1$^T$ (=JCM 11476$^T$ =DSM 14783$^T$). Purity was confirmed routinely by microscopic examination and by repeated partial sequencing of the 16S rRNA gene using several PCR primers.

## Morphology

Cells were routinely observed under a phase-contrast Olympus BX51 microscope with the Olympus Camedia C3030 digital camera system. Transmission electron microscopy of negatively stained cells was carried out as described by Zillig *et al.* (1990). Cells grown in MJNS medium supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) tryptone at 62 °C in the mid-exponential phase of growth were negatively stained with 2 % (w/v) uranyl acetate and observed under a JEOL JEM-1210 electron microscope at an accelerating voltage of 120 kV. Cells of strain SSM1$^T$ were Gram-negative and were bent, flexible rods, which were about 1·5–2·5 µm long and 0·3–0·7 µm wide in the exponential growth phase (see Fig. A, available as supplementary material in IJSEM Online). In the stationary growth phase, the cells tended to be long and spiral-shaped (Supplementary Fig. B). Each of the cells in the exponential growth phase appeared to have a polar flagellum, although no motility was evident in the laboratory cultures. These morphological features were quite similar to those of *D. thermophilus* BMA$^T$ (Greene *et al.*, 1997).

## Growth characteristics

Strain SSM1$^T$ was routinely cultivated in MJNS medium supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) tryptone. MJNS medium consisted of ($l^{-1}$ MJ synthetic sea water): 1 ml vitamin solution (Balch *et al.*, 1979), 50 mg sodium selenite, 30 mg sodium tungstate, 1 mg resazurin, 10 mM $NaNO_3$, 3 % (w/v) elemental sulfur, 20 g $NaHCO_3$ and 0·5 g $Na_2S.9H_2O$ (Sako *et al.*, 1996; Takai *et al.*, 1999). To prepare the supplemented medium, sodium selenite, sodium tungstate, resazurin, yeast extract (1 g) and tryptone (1 g) were dissolved in 1 l MJ synthetic sea water and the pH of the medium was adjusted to around 7·0 with NaOH before autoclaving. After autoclaving under an air atmosphere, a concentrated solution of vitamins (Balch *et al.*, 1979), $NaHCO_3$, $NaNO_3$, elemental sulfur and $Na_2S$ (pH adjusted to 7·0) were added to the medium under gas purging with 80 % $H_2$ and 20 % $CO_2$. These solutions were sterilized separately by autoclaving except for the vitamins and elemental sulfur, which were respectively filter-sterilized and steam-sterilized (three times at 95 °C for 3 h). The medium was dispensed at 20 % of the total bottle (Schott Glaswerke) or tube (Iwaki glass) volume and containers were tightly sealed with a butyl rubber stopper under a gas phase of 80 % $H_2$ and 20 % $CO_2$ at 300 kPa. All experiments described below were conducted in duplicate.

The effects of temperature, pH and total salt concentration on growth were tested. With MJNS medium supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) tryptone, strain SSM1$^T$ grew over the temperature range of about 40–70 °C, showing optimal growth at 60–65 °C, and the generation time at 62 °C, pH 6·5, was about 40 min (see Supplementary Fig. C in IJSEM Online at http://ijs.sgmjournals.org). No growth was observed at 35 or 75 °C. To determine the effect of pH on growth, the pH of MJNS medium containing 0·1 % (w/v) yeast extract and tryptone was adjusted to various levels with 10 mM acetate/acetic acid buffer (pH 4–5), MES (pH 5–6), PIPES (pH 6–7), HEPES (pH 7–7·5) and Tris (pH 8–9·5). Growth of strain SSM1$^T$ at 62 °C occurred at pH 5·0–7·5, with optimum growth at about pH 6·5 (Supplementary Fig. D). The pH

was found to be stable during the cultivation period. The effect of total salt concentration on growth was determined using MJNS medium supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) tryptone, in which varying dilutions of 4 × MJ synthetic sea water were used (1 × MJ synthetic sea water contains 30 g NaCl $l^{-1}$). Isolate SSM1$^T$ grew over the range of total salt concentration between 18 and 96 g $l^{-1}$, with optimum growth at 36 g total salt $l^{-1}$ at 62 °C, pH 6·5 (Supplementary Fig. E). Compared with *D. thermophilus* BMA$^T$, strain SSM1$^T$ can grow at slightly higher temperatures.

The oxygen sensitivity of strain SSM1$^T$ was tested with MJNS medium supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) tryptone under a gas phase replaced with a mixture of 80 % $H_2$, 19 % $CO_2$ and 1 % $O_2$ (300 kPa) or a mixture of 80 % $H_2$, 19·9 % $CO_2$ and 0·1 % $O_2$ (300 kPa). Strain SSM1$^T$ grew only under strictly anaerobic culture conditions and was extremely sensitive to oxygen (Table 1). In contrast to the oxygen-resistant growth (at an oxygen concentration of up to 0·6 % in the gas phase) observed for *D. thermophilus* BMA$^T$, growth of strain SSM1$^T$ was completely inhibited in the presence of 0·1 % oxygen.

Autotrophic growth was examined in MJNS medium in the absence of yeast extract and tryptone, but strain SSM1$^T$ was not able to grow under autotrophic conditions using $H_2$ as a sole energy source and nitrate and elemental sulfur as electron acceptors (Table 1). In an attempt to determine potential energy sources for heterotrophic growth, yeast extract, peptone, tryptone, Casamino acids (each at 0·2 %, w/v), formate, acetate, glycerol, citrate, tartrate, fumarate, malate, succinate, propionate, maleate, lactate, oxalate, thioglycolate, pyruvate, each of 20 amino acids (each at 5 mM), 0·025 % (v/v) methanol, 0·05 % (v/v) ethanol, 0·1 % (v/v) 2-propanol, glucose, galactose, sucrose, fructose, lactose, maltose, arabinose, trehalose and starch (each at 0·1 %, w/v) were examined in MJNS medium under a gas phase of 80 % $H_2$ + 20 % $CO_2$ (300 kPa), 80 % $N_2$ + 20 % $CO_2$ (300 kPa) or 100 % $N_2$ (300 kPa). Strain SSM1$^T$ was able to grow on yeast extract, tryptone, peptone, casein, Casamino acids, ethanol, citrate, tartrate, fumarate, malate, succinate, propionate, lactate, oxalate, pyruvate, acetate or formate as sole energy and carbon sources. Yeast extract, tryptone, peptone, pyruvate and acetate yielded the highest level of growth. 2-Propanol, methanol, maleate, thioglycolate, glycerol, various amino acids and sugars did not support growth as sole energy and carbon sources. Strain SSM1$^T$ grew better in the presence of hydrogen than in the presence of nitrogen or argon (Table 1). This indicates that hydrogen serves as a supplementary energy source for growth of strain SSM1$^T$, similar to *D. thermophilus* BMA$^T$.

The use of nitrate, nitrite, elemental sulfur, sulfite, thiosulfate, sulfate, soluble (ferric citrate) or insoluble (ferrihydrite) iron (III), manganese (IV) (vernadite), selenite, selenate, arsenate, arsenite or fumarate as potential sole electron acceptors in place of nitrate and elemental sulfur

**Table 1.** Electron donors and acceptors for growth of *Deferribacter desulfuricans* sp. nov. SSM1$^T$

These experiments were conducted at 62 °C and pH 6·5. Maximum cell yield (cells $ml^{-1}$) is scored as: + + +, $>8\times10^8$; + +, $<8\times10^8$, $>2\times10^8$; +, $<2\times10^8$; NG, no growth. Acetate (5 mM) was added as an electron donor instead of both yeast extract and tryptone to MJNS medium. Each of the potential electron acceptors replaced nitrate (5 mM) and elemental sulfur (3 %, w/v) in MJNS medium.

| Electron donor | Electron acceptor | Gas phase (300 kPa) | Maximum cell yield |
|---|---|---|---|
| Acetate (5 mM) + $H_2$ | $O_2$ (0·1 %) | $H_2:CO_2:O_2$, 80 : 20 : 0·1 | NG |
| Acetate (5 mM) + $H_2$ | $NO_3^-$ (5 mM) | $H_2:CO_2$, 80 : 20 | + + + |
| Acetate (5 mM) + $H_2$ | $NO_2^-$ (5 mM) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | $SO_3^{2-}$ (5 mM) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | $S_2O_3^{2-}$ (5 mM) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | $S^0$ (3 %, w/v) | $H_2:CO_2$, 80 : 20 | + + + |
| Acetate (5 mM) + $H_2$ | Ferric citrate (5 mM) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | $Fe(OH)_3$ (0·1 %, w/v) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | $MnO_2$ (0·1 %, w/v) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | $HAsO_4^{2-}$ (2 mM) | $H_2:CO_2$, 80 : 20 | + |
| Acetate (5 mM) + $H_2$ | $AsO_2^-$ (2 mM) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | $SeO_4^{2-}$ (2 mM) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | $SeO_3^{2-}$ (2 mM) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) + $H_2$ | Fumarate (5 mM) | $H_2:CO_2$, 80 : 20 | NG |
| Acetate (5 mM) | $NO_3^-$ (5 mM) | $Ar:CO_2$, 80 : 20 | + + |
| Acetate (5 mM) | $S^0$ (3 %, w/v) | $Ar:CO_2$, 80 : 20 | + + |
| Acetate (5 mM) | $HAsO_4^{2-}$ (2 mM) | $Ar:CO_2$, 80 : 20 | NG |
| $H_2$ | $NO_3^-$ (5 mM) | $H_2:CO_2$, 80 : 20 | NG |
| $H_2$ | $S^0$ (3 %, w/v) | $H_2:CO_2$, 80 : 20 | NG |
| $H_2$ | $HAsO_4^{2-}$ (2 mM) | $H_2:CO_2$, 80 : 20 | NG |

was examined in MJNS medium with acetate as an energy source (Table 1). Ferric citrate, ferrihydrite [$Fe(OH)_3$] and vernadite ($MnO_2$) were prepared as described by Kostka & Nealson (1998). These tests were performed under anaerobic conditions in the absence of $Na_2S.9H_2O$ to avoid chemical reduction of the potential electron acceptors by the reductant. To ensure that conditions were anaerobic, the media were purged with vigorous bubbling with 80 % $H_2$ + 20 % $CO_2$ or 80 % $N_2$ + 20 % $CO_2$ for 30 min and then compressed with 80 % $H_2$ + 20 % $CO_2$ (300 kPa) or 80 % $N_2$ + 20 % $CO_2$ (300 kPa). Nitrate, elemental sulfur or arsenate was required as a potential electron acceptor for growth of strain SSM1$^T$, while nitrite, sulfite, thiosulfate, sulfate, soluble or insoluble iron (III), manganese (IV), selenite, selenate and arsenite did not support growth (Table 1). The requirement for elemental sulfur as the primary electron acceptor and the inability to utilize iron (III) or manganese (IV) observed for strain SSM1$^T$ were distinct from the inorganic substrate requirements of strain BMA$^T$.

The time-course of consumption of acetate and reduction of elemental sulfur, nitrate or arsenate and concomitant bacterial growth of strain SSM1$^T$ were examined with MJNS medium supplemented with 5 mM acetate and each of 3 % (w/v) elemental sulfur, 5 mM nitrate or 2 mM arsenate under a gas phase of 80 % $H_2$ + 20 % $CO_2$ (300 kPa). The concentration of nitrogen in the gas phase during growth was measured using a Micro GC CP2002 gas chromatograph (GL Sciences). Anion samples (nitrate, nitrite and acetate) were analysed by ion chromatography using a Shim-pack IC column (Shimadzu). The diazotization method was employed to determine the concentrations of nitrate and nitrite (Matsunaga & Nishimura, 1969) independently from ion chromatography and Nessler's reagent was employed to measure the ammonium ion concentration in the medium (Allen *et al.*, 1974). Sulfide was analysed quantitatively using the methylene blue method (Fonselius, 1983). Determination of arsenate and arsenite was performed as described previously (Johnson & Pilson, 1972).

When any electron acceptor was provided, the concentration of acetate decreased as the number of cells increased (Fig. 1a), indicating that acetate was consumed during growth. Although the amount of elemental sulfur was not quantified, the concentrations of nitrate and arsenate decreased and the concentrations of sulfide, nitrite and arsenite increased during growth of strain SSM1$^T$ (Fig. 1). Since the control media without bacterial inocula did not demonstrate acetate consumption or the reduction of elemental sulfur, nitrate or arsenate to sulfide, nitrite or arsenite (data not shown), bacterial reduction of elemental sulfur, nitrate and arsenate occurred during growth. The accumulated concentrations of nitrite and arsenite were stoichiometrically equivalent to the concentrations of nitrate and arsenate reduced (Fig. 1b, c). In addition, strain SSM1$^T$ was unable to utilize nitrite or arsenite as potential

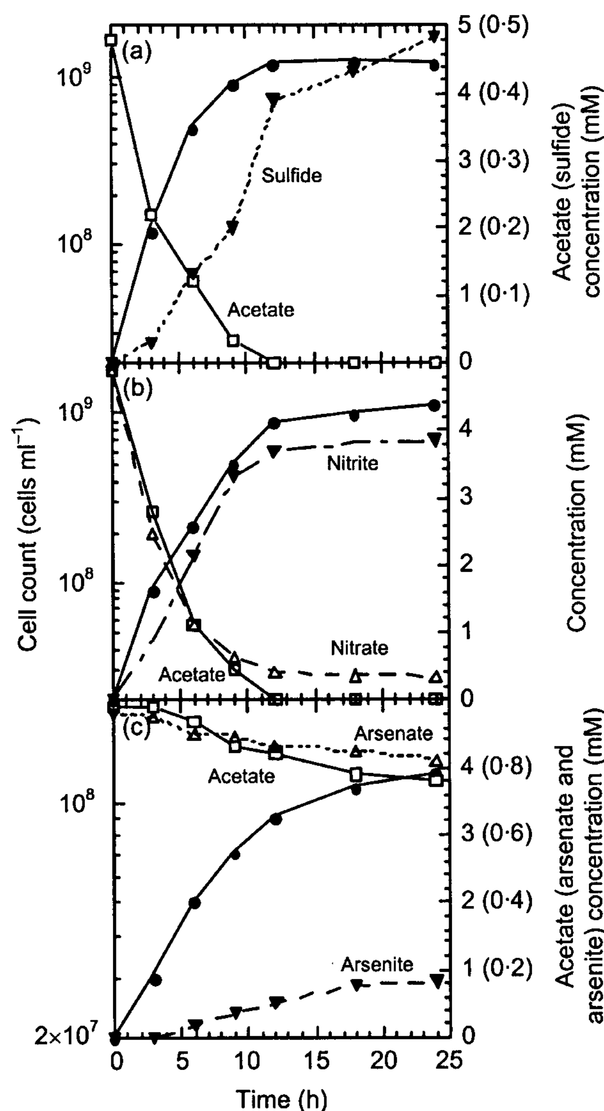


**Fig. 1.** Time-course of consumption of acetate and reduction of elemental sulfur (a), nitrate (b) or arsenate (c) and concomitant bacterial growth (●) of *D. desulfuricans* SSM1$^T$. Acetate (5 mM; □) and elemental sulfur (3 %, w/v), nitrate (5 mM) or arsenate (2 mM) were tested. Concentrations of supplied electron acceptor (△) and product (▼) are shown as appropriate.

electron acceptors (Table 1). Hence, nitrite and arsenite were likely the end products of nitrate and arsenate reduction. On the basis of these results, strain SSM1$^T$ was found to be a respiratory elemental sulfur-, nitrate- or arsenate-reducing heterotroph.

The potential requirements for nutrients such as selenate, selenite, tungstate, vitamins and nitrogen sources (10 mM $NH_4Cl$, $NaNO_2$ or $NaNO_3$) for growth were also examined. Isolate SSM1$^T$ utilized ammonium ion and nitrate as nitrogen sources but could not utilize nitrite. Selenium, tungsten and vitamins were not required for growth.

## Fatty acid and DNA base compositions

The cellular fatty acid composition was analysed using cells grown in the standard medium at 62 °C in the late-exponential growth phase. Lyophilized cells (300 mg) were placed in a Teflon-lined, screw-capped tube containing 5 ml anhydrous methanolic HCl and heated at 100 °C for 3 h. The resulting fatty acid methyl esters (FAMEs) were extracted twice with *n*-hexane and concentrated under a stream of nitrogen gas. The FAMEs were analysed using a gas-liquid chromatograph (model GC-380; GL Science) or gas-liquid chromatography-mass spectrometer (GCMS-QP5050; Shimadzu). The FAMEs standard ($C_4$–$C_{24}$) was purchased from Supelco. The major cellular fatty acids of strain SSM1$^T$ were iso-$C_{14:0}$ (8·3 %), iso-$C_{15:0}$ (10·3 %), anteiso-$C_{15:0}$ (23·3 %), iso-$C_{16:0}$ (7·3 %), iso-$C_{17:0}$ (7·3 %), anteiso-$C_{17:0}$ (19·4 %), iso-$C_{18:0}$ (11·6 %), $C_{18:0}$ (4·3 %), iso-$C_{19:0}$ (4·4 %) and anteiso-$C_{19:0}$ (3·7 %).

Genomic DNA of strain SSM1$^T$ was prepared as described by Marmur & Doty (1962). The G+C content of DNA was determined by direct analysis of deoxyribonucleotides on HPLC (Tamaoka & Komagata, 1984). Non-methylated DNA from bacteriophage $\lambda$ (49·8 mol% G+C; TaKaRa) was used as a reference. The G+C content of the genomic DNA of strain SSM1$^T$ was 38·6 mol%, which is slightly higher than that of *D. thermophilus* BMA$^T$ (Table 2).

## Phylogenetic analyses and DNA–DNA hybridization

The 16S rRNA gene (rDNA) was amplified by PCR using Bac27F and 1492R primers (DeLong, 1992; Lane, 1985). The nearly complete sequence (1446 bp) of the 16S rRNA gene from strain SSM1$^T$ was sequenced directly on both strands using the dideoxynucleotide chain termination method with a DNA sequencer model 3100 (Perkin-Elmer Applied Biosystems). The rDNA sequence was analysed using the gapped-BLAST search algorithm (Altschul *et al.*, 1997; Benson *et al.*, 1998) and was found to be most closely related (98·1 %) to that of *D. thermophilus* BMA$^T$ (Greene *et al.*, 1997). This suggests that strain SSM1$^T$ belongs to the genus *Deferribacter*.

The nearly complete sequence was manually realigned to 16S rDNA data from the Ribosomal Data Project II (RDP-II) (Maidak *et al.*, 2000) based on alignments determined using the Sequence Aligner program of RDP-II. Phylogenetic analyses were restricted to nucleotide positions that could be unambiguously aligned. Evolutionary distance matrix analysis (using the Kimura two-parameter method, the least-squares distance method and a transition/transversion rate of 2·0) and neighbour-joining analysis were performed using the PHYLIP package (version 3.5; obtained from J. Felsenstein, University of Washington, Seattle, WA, USA). Bootstrap analysis was performed to provide confidence estimates for phylogenetic tree topologies. The phylogenetic tree indicated that strain SSM1$^T$ is closely related to *D. thermophilus* BMA$^T$, as determined by sequence similarity analysis (Fig. 2). Phylogenetically related bacterial species were the nitrate-, nitrite- or arsenate-reducing mesophile *Chrysiogenes arsenatis* BAL-1$^T$ (Macy *et al.*, 1996), the slightly thermophilic, fermentative bacterium *Flexistipes sinusarabici* MAS 10$^T$ isolated from deep-sea brine water (Fiala *et al.*, 1990) and the Fe(III)-, Co(III)- or sulfur-reducing mesophile *Geovibrio ferrireducens* PAL-1$^T$ (Caccavo *et al.*, 1996). These bacterial strains may

**Table 2.** Comparison of properties between *D. desulfuricans* sp. nov. SSM1$^T$ and *D. thermophilus* BMA$^T$

Data for *D thermophilus* were taken from Greene *et al.* (1997). ND, Not determined. Both species use $H_2$ and complex organic compounds as electron donors and nitrate as an electron acceptor.

| Characteristic | *D. desulfuricans* SSM1$^T$ | *D. thermophilus* BMA$^T$ |
|---|---|---|
| Susceptibility of growth to oxygen | Sensitive | Slightly resistant (up to 0·6 %) |
| Temperature range (°C) | 40–70 | 50–65 |
| Temperature optimum (°C) | 60–65 | 60 |
| Doubling time under optimal conditions (min) | 40 | 70 |
| Electron donor: | | |
| Ethanol | + | − |
| Formate | + | − |
| Propionate | + | − |
| Oxalate | + | − |
| Fumarate | + | − |
| Electron acceptor: | | |
| Elemental sulfur | + | − |
| Fe(III) | − | + |
| Mn(IV) | − | + |
| Arsenate | + | ND |
| G+C content of genomic DNA (mol%) | 38·6 | 34 |

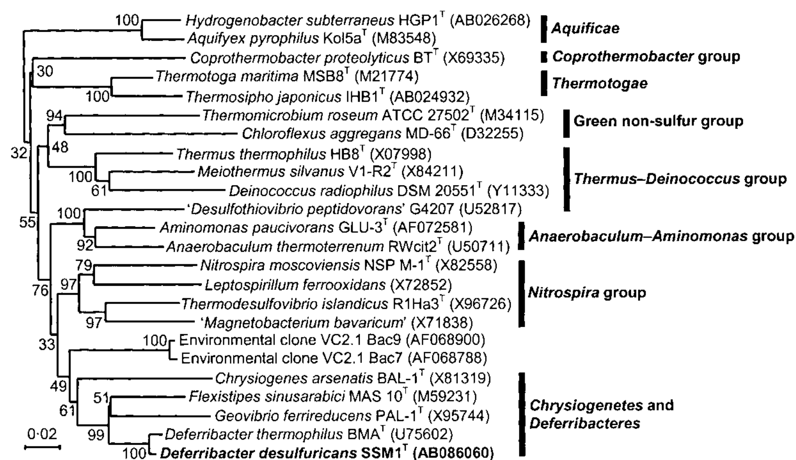


**Fig. 2.** Phylogenetic tree of representative bacterial strains potentially related to *D. desulfuricans* SSM1$^{T}$ inferred from 16S rDNA sequences using the neighbour-joining method on 1206 homologous sequence positions for each organism. Numbers at nodes represent bootstrap values (100 replicates). The scale bar indicates 2 substitutions per 100 nucleotides.

be classified into a phylogenetic group with the genus *Deferribacter* at the order or phylum level with a potential common ancestor, based on the phylogenetic tree (Fig. 2).

DNA–DNA hybridization was carried out at 42 °C for 3 h and was measured fluorometrically using photobiotin according to the method of Ezaki *et al.* (1989). *D. thermophilus* BMA$^{T}$ (=ACM 5093$^{T}$) was obtained from the Australian Collection of Microorganisms. It was cultivated under the optimal conditions as described previously (Greene *et al.*, 1997). The mean hybridization rate was 5·8 %, indicating that strain SSM1$^{T}$ could be genotypically differentiated from the previously described species of the genus *Deferribacter*.

## Comparison with related species

Phylogenetic analysis indicated that strain SSM1$^{T}$ is most closely related to *D. thermophilus* BMA$^{T}$, which was isolated from a subseafloor oil reservoir in the North Sea (Greene *et al.*, 1997), and is likely a member of the genus *Deferribacter*. Most of the morphological, physiological and molecular characteristics of strain SSM1$^{T}$ are similar to those of *D. thermophilus* BMA$^{T}$ (Table 2). However, strain SSM1$^{T}$ differs markedly from *D. thermophilus* BMA$^{T}$ with respect to several physiological features (Table 2). The most distinctive feature is the utilization of electron acceptors; strain SSM1$^{T}$ is able to use elemental sulfur as a primary electron acceptor but is unable to reduce iron (III) or manganese (IV), which are the primary electron acceptors for *D. thermophilus* BMA$^{T}$. In addition, the growth rate of strain SSM1$^{T}$ is higher than that of *D. thermophilus* BMA$^{T}$ under the optimal conditions for each of the strains and the temperature range and optimum of strain SSM1$^{T}$ are shifted to slightly higher values than those of *D. thermophilus* BMA$^{T}$ (Table 2). Growth of *D. thermophilus* BMA$^{T}$ is resistant to microaerobic conditions (0·6 % oxygen in the gas phase), whereas strain SSM1$^{T}$ is extremely sensitive to oxygen (Table 2). DNA hybridization analysis clearly showed that strain SSM1$^{T}$ can be differentiated genetically from *D. thermophilus* BMA$^{T}$ at the species level. On the basis of these physiological and genetic properties of strain SSM1$^{T}$ (=JCM 11476$^{T}$=DSM 14783$^{T}$), we propose that it be classified as the type strain of *Deferribacter desulfuricans* sp. nov.

In the report on the isolation of the first *Deferribacter* species, from a subsurface oil reservoir, Greene *et al.* (1997) suggested the occurrence of an ecological niche for iron- or manganese-reducing thermophiles in oil-bearing rocks commonly containing iron and manganese oxides and hydroxides. An ecological niche containing abundant iron, manganese oxides and hydroxides might be more likely to occur in relatively oxidative microhabitats such as chimney surface areas in deep-sea hydrothermal vent environments. Recent investigations on an iron-reducing microbial population in the EPR deep-sea hydrothermal vent environment suggested the abundant occurrence of a thermophilic iron-reducing microbial population, predominantly represented by members of *Thermococcus* and *Deferribacter*, in relatively oxidative microhabitats (Slobodkin *et al.*, 2001). A body of evidence has demonstrated that sulfidogenic, thermophilic bacteria and archaea, mainly consisting of sulfur-reducing members of *Thermotogales* and *Thermococcales*, are predominantly present in global subsurface oil-reservoir environments other than marine hydrothermal systems (L'Haridon *et al.*, 1995; Orphan *et al.*, 2000; Slobodkin *et al.*, 1999; Stetter *et al.*, 1993; Takahata *et al.*, 2000). In addition to members of *Thermotogales* and *Thermococcales*, sulfur-reducing and/or metal-reducing members of *Deferribacter* such as *D. desulfuricans* SSM1$^{T}$ and *D. thermophilus* BMA$^{T}$ may be widely distributed in global deep-sea hydrothermal systems and subsurface oil-reservoir environments and may represent significant microbial components.

## Description of *Deferribacter desulfuricans* sp. nov.

*Deferribacter desulfuricans* (de.sul.fu′ri.cans. L. pref. *de* from; L. n. *sulfur* sulfur; N.L. part. adj. *desulfuricans* reducing sulfur).

Bent, flexible rods, with a mean length of 1·5–2·5 μm and

mean width of 0·3–0·7 μm. Cells stain Gram-negative and occur singly in the exponential growth phase and as long, spiral forms in the stationary phase. Exhibits no evident motility with a polar flagellum. Strictly anaerobic and obligately heterotrophic. The temperature range for growth is 40–70 °C, with the optimum being 60–65 °C. The pH range for growth is 5·0–7·5, with optimum growth at pH 6·5. Sea salts are required for growth and the concentration range is 18–96 g $l^{-1}$, with optimum growth at 36 g $l^{-1}$. Growth uses organic compounds such as yeast extract, tryptone, peptone, casein, Casamino acids, ethanol, citrate, tartrate, fumarate, malate, succinate, propionate, lactate, oxalate, pyruvate, acetate and formate as sole energy and carbon sources. Elemental sulfur, nitrate or arsenate are absolutely required for growth as electron acceptors, being respectively reduced to sulfide, nitrite and arsenite. Ammonium and nitrate serve as nitrogen sources. The G+C content of genomic DNA is 38·6 mol% (by HPLC). The 16S rDNA sequence exhibits 98·5 % similarity to that of *D. thermophilus* $BMA^T$. The DNA–DNA relatedness to *D. thermophilus* $BMA^T$ is low (5·8 %).

The type strain, strain $SSM1^T$ (=JCM $11476^T$=DSM $14783^T$), was isolated from a deep-sea hydrothermal vent chimney at the Suiyo Seamount in the Izu-Bonin Arc, Japan.

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