Extremophiles (2003) 7:283–290
DOI 10.1007/s00792-003-0320-0

ORIGINAL PAPER

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A novel lineage of sulfate-reducing microorganisms: Thermodesulfobiaceae fam. nov., *Thermodesulfobium narugense*, gen. nov., sp. nov., a new thermophilic isolate from a hot spring

Received: 23 August 2002 / Accepted: 26 February 2003 / Published online: 28 March 2003

Abstract A novel type of a sulfate-reducing microorganism, represented by strain Na82^T, was isolated from a hot spring in Narugo, Japan. The isolate was a moderate thermophilic autotroph that was able to grow on H_2/CO_2 by sulfate respiration. The isolate could grow with nitrate in place of sulfate, and possessed menaquinone-7 and menaquinone-7(H_2) as respiratory quinones. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain Na82^T was a member of the domain Bacteria and distant from any known bacteria, as well as from other sulfate-reducing bacteria (sequence similarities less than 80%). The phylogenetic analysis of the *dsrAB* gene (alpha and beta subunits of dissimilatory sulfite reductase) sequence also suggested that strain Na82^T was not closely related to other sulfate reducers. On the basis of the phenotypic and phylogenetic data, a new taxon is established for the isolate. We proposed the name *Thermodesulfobium narugense* gen. nov., sp. nov. with strain Na82^T (= DSM 14796^T = JCM 11510^T) as the type strain. Furthermore, a new family, Thermodesulfobiaceae fam. nov., is proposed for the genus.

Keywords Anaerobe · Hot spring · Sulfate-reducing bacterium · Thermodesulfobiaceae fam. nov. · *Thermodesulfobium narugense* gen. nov., sp. nov. · Thermophile

Communicated by J. Wiegel

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Introduction

Energy conversion by dissimilatory sulfate reduction is widespread among prokaryotes. Sulfate-reducing microorganisms are phylogenetically divided into four Bacterial lineages and two Archaeal lineages. In the Archaea, several hyperthermophilic sulfate reducers belonging to the genera *Archaeoglobus* and *Caldivirga* have been isolated from anaerobic submarine hydrothermal systems (Stetter et al. 1987; Burggraf et al. 1990; Huber et al. 1997) and an acidic hot spring (Itoh et al. 1999). Within the domain Bacteria, most of the known sulfate-reducing bacteria are found in two phylogenetic clades, the δ-Proteobacteria and the Bacillus/Clostridium group (low GC Gram-positive bacteria). The sulfate-reducing bacteria in the δ-Proteobacteria are generally mesophiles, the only three exceptions being *Thermodesulforhabdus norvegica*, *Desulfacinum infernum*, and *Desulfacinum hydrothermale*, which are able to grow at 60°C (Beeder et al. 1995; Rees et al. 1995; Sievert and Kuever 2000). Three genera, *Desulfotomaculum*, *Desulfosporosinus*, and *Thermoacetogenium*, belonging to the Bacillus/Clostridium group, are mesophilic or moderate thermophilic sulfate-reducing bacteria with the capacity for endospore formation (Stackebrandt et al. 1997; Hattori et al. 2000). Two genera, *Thermodesulfobacterium* and *Thermodesulfovibrio*, constitute deeply branching lineages within the domain Bacteria and include thermophilic isolates from thermal environments including hot springs, hot oil reservoirs, and deep-sea hydrothermal vents (Zeikus et al. 1983; Henry et al. 1994; Sonne-Hansen and Ahring 1999; Jeanthon et al. 2002).

Molecular in situ analyses based on 16S rRNA gene sequence suggest that many so-far uncultivated sulfate-reducing microorganisms inhabit various environments such as hot springs (Hugenholtz et al. 1998), deep-sea hydrothermal vent systems (Takai and Horikoshi 1999), cold marine sediments (Ravenschlag et al. 2000), and subsurface aquifers (Fry et al. 1997). Further insights

into the diversity of sulfate reducers in natural environments were provided by sequence analyses of *dsr* genes (Cottrell and Cary 1999; Minz et al. 1999; Thomsen et al. 2001) that code for dissimilatory sulfite reductase.

Recently, we isolated a novel thermophilic sulfate-reducing bacterium from Narugo hot spring (Miyagi, Japan). The isolate grew chemoautotrophically on H_2/CO_2 with sulfate as an electron acceptor. Phylogenetic analyses using 16S rRNA, DsrAB (alpha and beta subunits of dissimilatory sulfite reductase) and ApsA (alpha subunit of adenosine-5′-phosphosulfate reductase) gene sequences indicated that the isolate was distant from any known sulfate reducers. In this paper, we propose a new family, genus and species, Thermodesulfobiaceae fam. nov., and *Thermodesulfobium narugense* gen. nov., sp. nov. for the isolate.

Materials and methods

Sampling site

Narugo hot spring is located in the prefecture of Miyagi in Japan. A vent of the hot spring was detected near the shore of an acidic lake, Katanuma. It harbored white microbial mats, which were mainly formed by sulfur-oxidizing bacteria, *Thiomonas thermosulfata* (confirmed by 16S rRNA gene library and quinone composition analyses). A mat sample was taken at a site where the temperature and pH of the water were 58°C and 6.9, respectively.

Medium, enrichment, and isolation

The medium was composed of the following salts and solutions (l^{-1}): KH_2PO_4, 0.75 g; K_2HPO_4, 0.78 g; NH_4Cl, 0.53 g; Na_3EDTA, 0.041 g; $FeSO_4{\cdot}7H_2O$, 0.011 g; $MgSO_4{\cdot}7H_2O$, 0.25 g; $CaCl_2{\cdot}2H_2O$, 0.029 g; NaCl, 0.23 g; Na_2SO_4, 2.8 g; sodium acetate, 0.16 g; trace element solution DSM 334 (DSMZ 1993), 10 ml; and vitamin solution DSM 141 (DSMZ 1993), 10 ml. The medium was autoclaved under an N_2/CO_2 (4:1, v/v) atmosphere in vials sealed with butyl-rubber stoppers and aluminum caps. Prior to inoculation, the medium was reduced with a sterile stock solution of cysteine HCl (final concentration 0.25 g l^{-1}), and the gas phase was replaced by H_2/CO_2 (4:1. v/v; 151.9 kPa). The pH of the medium was adjusted to 6.0. For enrichment of the sulfate reducers, a piece of the microbial mat was inoculated into the medium. As indicator for sulfide production a $FeSO_4$ solution (final concentration 0.2 g l^{-1}) was added to the medium. After a 4-day incubation at 55°C, pronounced sulfide production caused by growth of sulfate reducers was observed. The enrichment culture was transferred several times to new medium of the same composition. On the medium solidified with 2% agar in vials, single colonies were formed after 2 weeks of incubation. After a second purification step on agar, a uniformly shaped axenic culture, designated strain Na82^T, was obtained.

Morphological and physiological characteristics

Cell morphology was examined using phase-contrast microscopy. For transmission electron microscopy, a Hitachi model H-7000 was used (Hattori et al. 2000). The presence of lipopolysaccharides in the outer cell wall was determined by the polymyxin B-LPS test (Wiegel and Quandt 1982). The effect of temperature and initial pH on growth was determined by measurement of sulfate consumption. Tests were performed in a temperature range of 30°–75°C and a pH range of 3.5–7.8. The initial pH was adjusted by the addition of 10% (w/v) Na_2CO_3 or 0.6 N HCl. Electron donors were tested under N_2/CO_2 at 55°C. Electron acceptors were tested in medium without sulfate under H_2/CO_2 at 55°C. Utilization was recognized by increase in optical density and decrease of electron donor or acceptor.

Analytical methods

Optical density (A660) was measured with a spectrophotometer, the Beckman model DU640. Concentrations of anions and organic compounds were determined by HPLC, as described previously (Hattori et al. 2000). Quinones were extracted with chloroform–methanol (2:1, v/v). The extract was purified with a Sep-Pak Plus column (Waters), and analyzed by reverse-phase HPLC (Beckman System Gold with an Agilent Technologies Hypersil ODS column) for identification of quinones (Shintani et al. 2000). Cellular fatty acids were converted to methyl esters by the treatment with anhydrous methanolic HCl. The methyl esters were analyzed by a Hitachi M7200A GC/3DQMS system (Tokyo, Japan), equipped with a DB-5 ms capillary column (J&W Scientific, Folsom, CA, USA) coated with (5%-phenyl)-methylopolysiloxane (Hanada et al. 2002). Genomic DNA was extracted and purified according to the method of Mori et al. (2000). The G+C content was determined via enzymatic digestion of genomic DNA and HPLC separation using a Yamasa GC kit (Yamasa, Chiba, Japan).

Phylogenetic positions based on 16S rRNA, DsrAB, and ApsA gene sequence comparisons

The amplification and sequencing of the 16S rRNA gene were performed as described previously (Hattori et al. 2000). The *dsrAB* gene (coding for alpha and beta subunits of dissimilatory sulfite reductase) was amplified using the primers DSR1F and DSR4R (Wagner et al. 1998). An approximately 1.9-kb *dsrAB* gene was purified using a MicroSpin S-400 HR column (Amersham Pharmacia Biotech) and cloned directly into a pT7Blue T-Vector (Novagen) with a DNA ligation kit, version 2 (Takara Shuzo, Kyoto, Japan). For the determination of the sequence, a deletion kit for kilo-sequencing (Takara Shuzo) was used. PCR amplification of the fragment from the *apsA* gene (coding for alpha subunit of adenosine-5′-phosphosulfate reductase) was performed using primers APS7-F and APS8-R, and an annealing temperature of 45°C (Friedrich 2002). The PCR product of an approximately 900-b *apsA* gene fragment was directly sequenced using primers APS7-F, APS8-R (Friedrich 2002), ApsA405F (5′-CAT AAA AGC AAA GGC AAT CA-3′), ApsA405R (5′-TGA TTG CCT TTG CTT TTA TG-3′) and ApsA653R (5′-GCA GTA GTC CTC TCC TCT TG-3′). Sequences were compared with reference sequences using the BLAST program at the National Center for Biotechnology Information (http:// www.ncbi.nlm.nih.gov).

The phylogenetic analyses were carried out using the 16S rRNA gene sequence and the deduced amino acid sequences of the *dsrAB* and *apsA* genes. The compiled 16S rRNA gene sequence was aligned against an ARB dataset using the ARB program (http:// www.arb-home.de/), and manually refined based on primary and secondary structural considerations. The data set of the *dsrAB* and *apsA* genes was aligned using program CLUSTAL W, version 1.6.1 (Thompson et al. 1994). A neighbor-joining (NJ) analysis was performed using program CLUSTAL W, version 1.6.1 (Thompson et al. 1994), and 1,000 replicate data sets were used for bootstrap analysis (Saitou and Nei 1987). Maximum-likelihood (ML) analysis was carried out using the MOLPHY software, version 2.3b3 (Adachi and Hasegawa 1995). A ML distance matrix was calculated using the NucML program, and a NJ topology as the starting tree for the ML tree was obtained using NucML with option R (local rearrangement search) based on the HKY model (Hasegawa et al. 1985). For analyses of the amino acid sequences, the ProtML program based on the JTT model (Jones et al. 1992) was used

(option R). Local bootstrap probabilities were estimated by the RELL (resampling of estimated log-likelihood) method (Kishino et al. 1990; Hasegawa and Kishino 1994). A maximum-parsimony (MP) tree reconstruction was performed using the PAUP software, version 4.0b8a (Swofford 1998). A heuristic search was used with a random stepwise addition sequence of 100 replicates, tree-bisection-reconnection branch swapping, and the MULTREES option. Further analyses were run with 100 bootstrap replicates, each consisting of 100 additional random replicates.

Results

Morphology

Cells of strain Na82^T were rod-shaped (0.5×2–4 μm; see Fig. 1a). The isolate showed no motility under the microscope. Spore formation was not observed. Gram-staining was negative. An ultrathin section of cells at the late exponential phase is shown in Fig. 1b. Strain Na82^T possessed a Gram-negative type of cell wall with an outer membrane. Neither storage compounds nor extensive internal membranes were observed. The polymyxin B-LPS test (Wiegel and Quandt 1982) also suggested that the isolate possessed the typical Gram-negative cell wall, since the fibrous structure and blebs of lipopolysaccharides were observed around the surface of polymyxin-B-treated cells.

Growth properties

Strain Na82^T was a strictly anaerobic bacterium able to grow under an H_2/CO_2 atmosphere, and which could not grow under aerobic conditions. Anaerobic growth was always coupled to sulfate reduction (Fig. 2a). Measurement of sulfate reduction rates at various temperatures revealed growth of strain Na82^T between 37° and 65°C, with an optimum at 50°–55°C (Fig. 2b). Strain Na82^T did not grow below pH 4.0 and above pH 6.5; the shortest doubling time was observed between pH values 5.5 and 6.0. The pH value increased during growth, and then the isolate stopped growing at pH 7.0. The isolate grew optimally in the absence of NaCl, and growth did not occur above 1% (w/v) NaCl. Growth was not affected by the removal of acetate from the medium and the replacement of cysteine hydrochloride with sodium sulfide; therefore the isolate was a chemoautotroph. The doubling time under the optimum growth conditions (55°C, pH 5.5, no addition of NaCl) was 14 h.

In the presence of sulfate, the isolate also used formate (20 mM) as electron donor, but growth on the formate was clearly lower than that on H_2. Strain Na82^T did not utilize glucose (10 mM), acetate (20 mM), lactate (20 mM), pyruvate (20 mM), malate (20 mM), propionate (20 mM), butyrate (20 mM), fumarate (20 mM), succinate (20 mM), citrate (20 mM), ethanol (20 mM), propanol (20 mM), or methanol (20 mM).

With H_2/CO_2 (80:20), strain Na82^T utilized thiosulfate (5 mM), nitrate (5 mM), or nitrite (2.5 mM) as a substitute for sulfate. The isolate, however, did not use

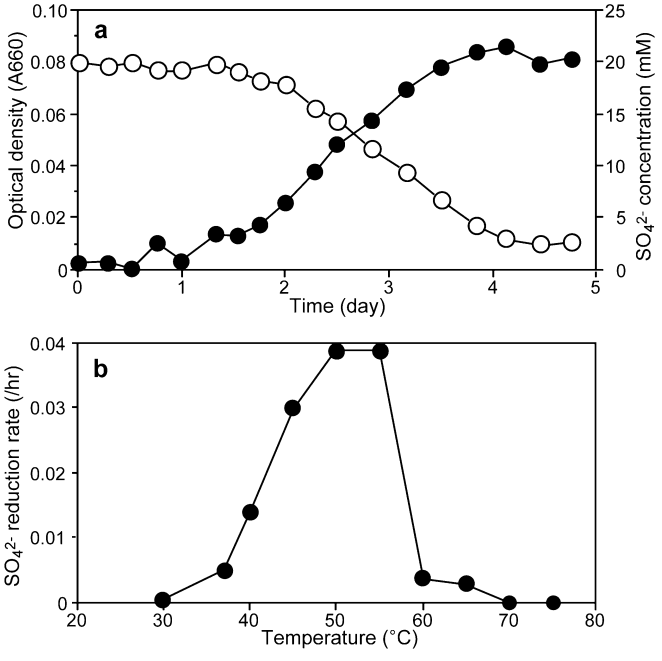

Fig. 2 Growth (*filled circles*) and decrease in sulfate (*open circles*) of strain Na82^T in the medium under H_2/CO_2 at 55°C (**a**). Effect of temperature on sulfate reduction by strain Na82^T in the medium under H_2/CO_2 (**b**). Sulfate reduction rates were mean values from duplicate cultures

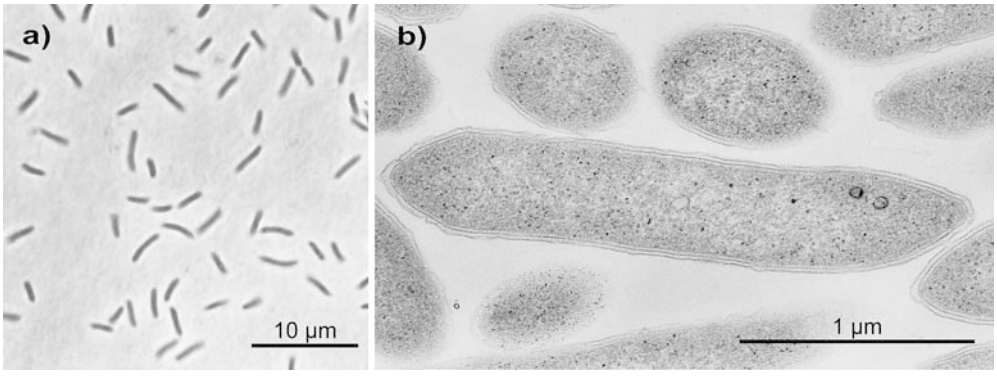

Fig. 1 Phase contrast micrograph (**a**) and ultrathin section (**b**) of strain Na82^T grown in the medium under H_2/CO_2 at a late-log phase

sulfite (2.5 mM), elemental sulfur (20 g l^{-1}), Fe (III) citrate (5 mM), fumarate (5 mM), dimethyl sulfoxide (5 mM), or O_2 (5%) as electron acceptor.

Chemotaxonomic characteristics

The G+C content of the genomic DNA from strain Na82^T was 35.1 mol%. The isolate contained menaquinone(MK)-7(H_2) and MK-7 (53.6% and 35.8%, respectively, of total quinones) as the major quinones. MK-7(H_4) (5.1%) and MK-8 (5.5%) were detected as minor fractions. Analysis of the cellular fatty acids revealed $C_{16:0}$ (45.7% of the total fatty acids) as major component. The following fatty acids were also detected: cyclo-$C_{19:0}$(9,10)*cis* (15.2%), $C_{18:0}$ (14.8%), $C_{18:1}$(△9)*cis* (13.9%), $C_{20:0}$ (3.8%), $C_{14:0}$ (3.2%), $C_{12:0}$-3OH (2.6%), and $C_{12:0}$ (0.7%).

Phylogenetic analysis

The nearly complete sequence of the 16S rRNA gene from strain Na82^T (1,363 bp, *E. coli* positions 16–1,399, AB077817) was analyzed. A bacterial domain reference sequence dataset (Hugenholtz 2002), including most of the whole recognized bacterial phyla, was used for the phylogenetic analysis based on 16S rRNA gene sequence (Fig. 3). The analysis used 350 sequences distributed over the bacterial division: the dataset contained 21 valid phyla with 15 putative phyla represented only by environmental clone sequences. The tree constructed by the ARB program revealed that the isolate was phylogenetically distant from any other bacteria. To make the phylogenetic relationship more clear, a dataset mainly including sulfate reducers was also used (Fig. 4). The NJ (neighbor-joining) method indicated that the isolate was phylogenetically distant from all known sulfate reducers. The closest relative of strain Na82^T was the environmental clone sequence OPB46, retrieved from Yellowstone hot spring and classified as a candidate division OP9 (Hugenholtz et al. 1998); however, the sequence similarity between strain Na82^T and OPB46 was only 81%, and the bootstrap value was low. The topology of the tree demonstrated by ML (maximum-likelihood) and MP (maximum-parsimony) methods was not different from that of NJ tree.

The *dsrAB* gene from strain Na82^T was also sequenced (AB077818, 1,754 bp, 585 aa). The ML tree (Fig. 5a) based on the deduced amino acid sequence was constructed with the sequence of *Thermodesulfovibrio islandicus* as an outgroup (Klein et al. 2001). Strain Na82^T was clearly separated from the other sulfate-reducing microorganisms. The NJ and MP analyses based on the *dsrAB* gene showed similar results to the tree based on ML analysis.

A fragment of the *apsA* gene (AB080361, 892 bp, 297 aa) was analyzed and compared with reference sequences. The closest relatives were *Desulfonatronovibrio hydrogenovorans* and *T. islandicus* (the similarity of the deduced amino acid sequences were 61% and 60%, respectively). The ML tree based on the *apsA* gene sequence (Fig. 5b) showed that the isolate formed a cluster with *T. islandicus* with a high bootstrap value (96%). The analyses by the NL and MP methods recovered the same sort of phylogenetic relationships as the ML analysis.

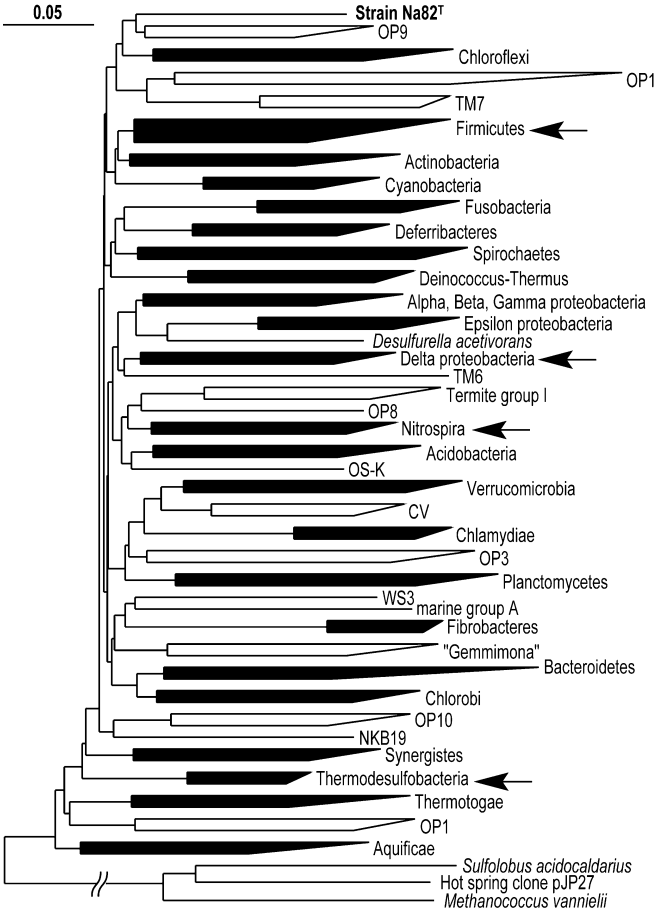

Fig. 3 Phylogenetic relationship based on 16S rRNA gene sequences of strain Na82^T and the major recognized bacterial phyla, inferred from the ARB program (http://arb-home.de/). In this analysis, 350 sequences distributed over the bacterial domain (21 validated phyla and 15 putative represented only by environmental sequences) were used. *Shaded wedges* indicate phyla with cultivated representatives and *unshaded wedges* indicate phyla currently represented only by environmental sequences. *Scale bar* shows substitutions per the compared nucleotides. The phyla *indicated by arrows* include sulfate reducers

Discussion

Phylogenetic analysis using the 16S rRNA gene sequence revealed that strain Na82^T is distant from any valid bacteria and environmental clone sequences, with sequence similarities of less than 81% (Fig. 3). The isolate, therefore, was phylogenetically different from any other known sulfate-reducing microorganisms such

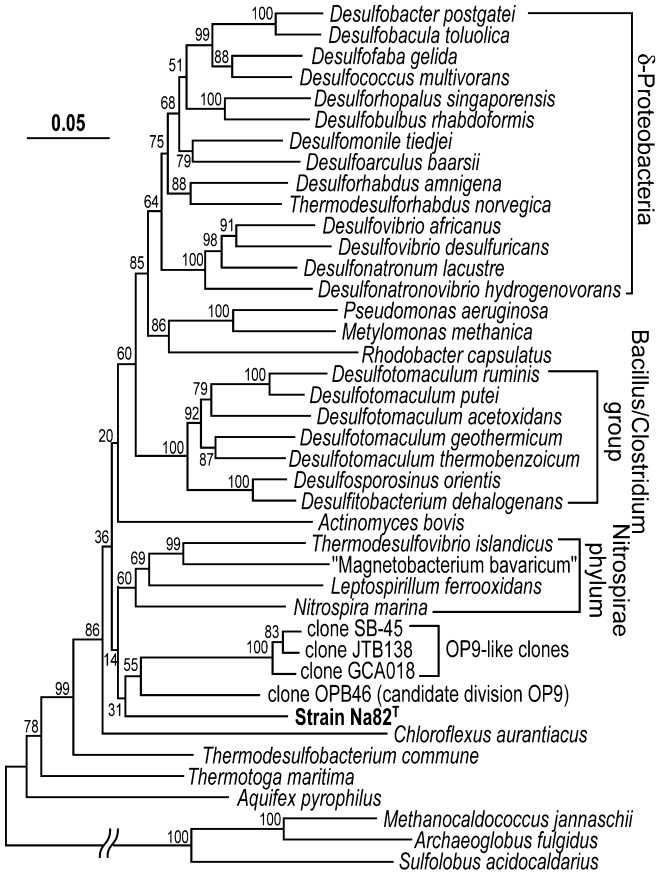

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Fig. 4 Phylogenetic relationships based on 16S rRNA gene sequences of strain Na82^T and relatives, inferred from the neighbor-joining (*NJ*) method. Bootstrap probabilities are indicated at branching points. *Scale bar* shows substitutions per the compared nucleotides. Clone OPB46 belongs to candidate division OP9 (Hugenholtz et al. 1998). Clones SB-45, JTB138 and GCA018 were OP9-like sequences that were reported as being retrieved from a benzene-mineralizing consortium (Phelps et al. 1998), deep-sea sediment (Li et al. 1999), and lake sediment in Antarctica (Bowman et al. 2000), respectively. The accession numbers (*in parentheses*) of the reference sequences used in the analyses are as follows: strain Na82^T (AB077817), *Actinomyces bovis* P1S^T (M33909), *Aquifex pyrophilus* Kol5a^T (M83548), *Archaeoglobus fulgidus* VC-16^T (Y00275), *Chloroflexus aurantiacus* J-10-fl^T (D38365), *Desulfitobacterium dehalogenans* JW/IU-DC1^T (L28946), *Desulfoarculus baarsii* 2st14^T (M34403), *Desulfobacter postgatei* 2ac9^T (AF418180), *Desulfobacula toluolica* Tol2^T (X70953), *Desulfobulbus rhabdoformis* M16^T (U12253), *Desulfococcus multivorans* 1be1^T (M34405), *Desulfofaba gelida* PSv29^T (AF099063), *Desulfomonile tiedjei* DCB-1^T (M26635), *Desulfonatronovibrio hydrogenovorans* Z-7935^T (X99234), *Desulfonatronum lacustre* Z-7951^T (AF418171), *Desulforhabdus amnigena* ASRB1^T (X83274), *Desulforhopalus singaporensis* Singapore T1^T (AF118453), *Desulfotomaculum acetoxidans* VKM B-1644^T (Y11566), *Desulfotomaculum geothermicum* BSD^T (X80789), *Desulfotomaculum putei* TH-11^T (AF053929), *Desulfotomaculum ruminis* DL^T (Y11572), *Desulfotomaculum thermobenzoicum* TSB^T (L15628), *Desulfosporosinus orientis* Singapore I^T (Y11570), *Desulfovibrio africanus* DSM 2603^T (X99236), *Desulfovibrio desulfuricans* Essex6^T (AF192153), *Leptospirillum ferrooxidans* L15^T (X86776), "*Candidatus* Magnetobacterium bavaricum" (X71838), *Methanocaldococcus jannaschii* JAL-1^T (M59126), *Methylomonas methanica* ATCC 35067^T (AF304196), *Nitrospira marina* 295 (X82559), *Pseudomonas aeruginosa* ATCC 10145^T (AF094713), *Rhodobacter capsulatus* ATH2.3.1^T (D16428), *Sulfolobus acidocaldarius* 98–3^T (D14053), *Thermotoga maritima* MSB8^T (M21774), *Thermodesulfobacterium commune* YSRA-1^T (AF418169), *Thermodesulforhabdus norvegica* A8444^T (U25627), *Thermodesulfovibrio islandicus* R1Ha3^T (X96726), clone GCA018 (AF154105), clone JT138 (AB015269), clone OPB46 (AF027081), and clone SB-45 (AF029050)

as *Thermodesulfobacteria*, *Thermodesulovibrio*, *Desulfotomaculum*, and *Desulfobacter* (sequence similarities less than 79%: Fig. 4). This suggests that the isolate represents a novel sulfate-reducing group. A phylogenetic analysis of DsrAB agreed with the separate lineage of strain Na82^T among the sulfate-reducing microorganisms (Fig. 5a). On the other hand, the ApsA sequence of the isolate was close to that of *T. islandicus* (Fig. 5b). *Thermodesulfovibrio* species are Gram-staining negative, motile, and non-sporulating chemoheterotrophs that are isolated from hot springs (Henry et al. 1994; Sonne-Hansen and Ahring 1999). However, strain Na82^T differed phenotypically from *Thermodesulfovibrio* species by the following properties (Table 1): (1) Strain Na82^T grew preferentially chemoautotrophically rather than chemoheterotrophically and could not ferment, whereas *Thermodesulfovibrio* species did not show autotrophic growth, and were able to grow well by fermentation; (2) strain Na82^T used nitrate and nitrite as a substitute for sulfate, which were not reduced by *Thermodesulfovibrio yellowstonii*; (3) growth of strain Na82^T was hardly observed at temperatures above 55°C, but the optimum growth of *Thermodesulfovibrio* species was at 65°C; (4) *Thermodesulfovibrio* species grew at neutral pH, whereas strain Na82^T preferred slightly acidic conditions (Henry et al. 1994; Sonne-Hansen and Ahring 1999). Based on these phylogenetic and phenotypic analyses, we propose a new family, genus, and species, Thermodesulfobiaceae fam. nov., and *Thermodesulfobium narugense* gen. nov., sp. nov.

Sulfate respiration is one of the primary metabolic functions (Cameron 1982) and characteristics of several Bacterial lineages and two extremely thermophilic genera of the Archaea. A recent study suggested that lateral gene transfer of the *dsrAB* genes has frequently occurred between major lineages of the Bacteria and probably between Bacteria and Archaea, in addition to vertical transmission (Klein et al. 2001). Phylogenetic analysis based on the *apsA* gene similarly suggested lateral gene transfer as a frequent event during evolution (Friedrich 2002). While strain Na82^T possessed distinctive *dsrAB* genes, its *apsA* gene sequence was closely related to *D. hydrogenovorans* (δ-Proteobacteria), which clearly differed from the isolate in other phylogenetic respects (16S rRNA and *dsrAB* genes). These findings suggest that the essential genes coding for enzymes involved in sulfate respiration have been transferred in an independent manner, even in strain Na82^T. The new isolate could represent one of the key organisms to resolve the evolutionary link in sulfate respiration.

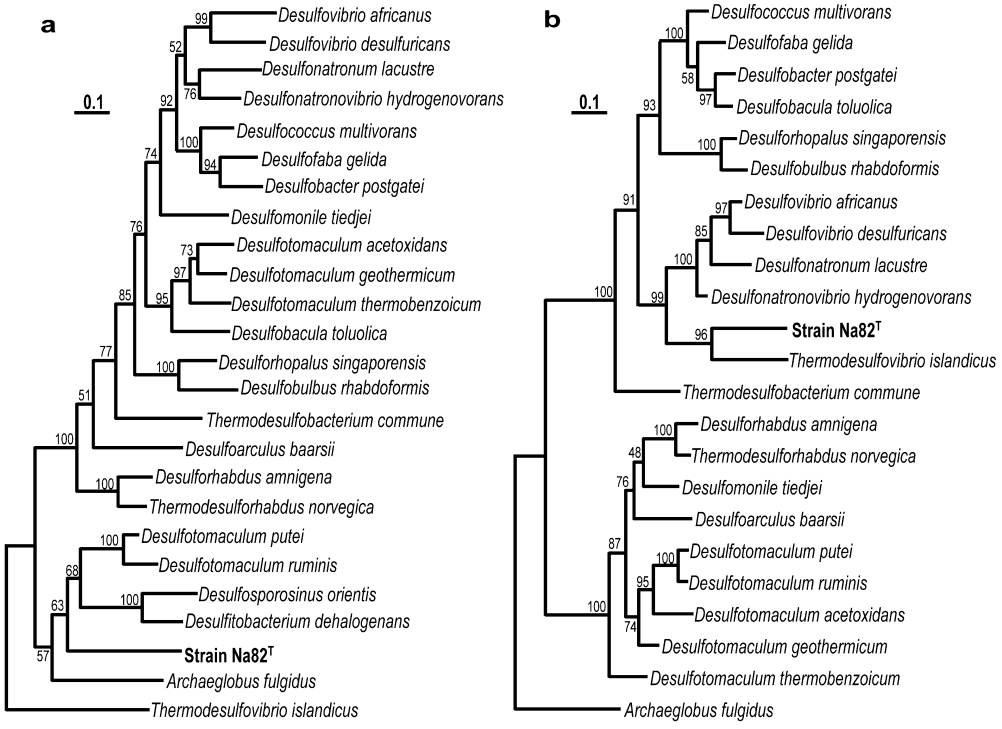

Fig. 5 Phylogenetic relationships based on DsrAB (**a**) and ApsA (**b**) deduced amino acid sequences of strain Na82^T and relatives inferred from the maximum-likelihood (*ML*) method. Bootstrap probabilities are indicated at branching points. *Scale bar* shows substitutions per compared amino acids. The accession numbers (*in parentheses*) of the reference sequences used in the analyses are as follows (*dsrAB*, *apsA*): strain Na82^T (AB077818, AB080361), *Archaeoglobus fulgidus* VC-16^T (M95624, AE000988), *Desulfitobacterium dehalogenans* JW/IU-DC1^T (AF337903), *Desulfoarculus baarsii* 2st14^T (AF334600, AF418149), *Desulfobacter postgatei* 2ac9^T (AF418198, AF418157), *Desulfobacula toluolica* Tol2^T (AF271773, AF418128), *Desulfobulbus rhabdoformis* M16^T (AJ250473, AF418110), *Desulfococcus multivorans* 1be1^T (U58126, AF418136), *Desulfofaba gelida* PSv29^T (AF334593, AF418118), *Desulfomonile tiedjei* DCB-1^T (AF334595, AF418162), *Desulfonatronovibrio hydrogenovorans* Z-7935^T (AF418197, AF418111), *Desulfonatronum lacustre* Z-7951^T (AF418189, AF418137), *Desulforhabdus amnigena* ASRB1^T (AF337901, AF418139), *Desulforhopalus singaporensis* Singapore T1^T (AF418196, AF418163), *Desulfotomaculum acetoxidans* VKM B-1644^T (AF271768, AF418153), *Desulfotomaculum geothermicum* BSD^T (AF273029, AF418115), *Desulfotomaculum putei* TH-11^T (AF273032, AF418147), *Desulfotomaculum ruminis* DL^T (U58118, AF418164), *Desulfotomaculum thermobenzoicum* TSB^T (AF273030, AF418161), *Desulfosporosinus orientis* Singapore I^T (AF271767), *Desulfovibrio africanus* DSM 2603^T (AF271772, AF418140), *Desulfovibrio desulfuricans* Essex6^T (AJ249777, AF226708), *Thermodesulfobacterium commune* YSRA-1^T (AF334596, AF418114), *Thermodesulforhabdus norvegica* A8444^T (AF334597, AF418159), and *Thermodesulfovibrio islandicus* R1Ha3^T (AF334599, AF418113)

Description of Thermodesulfobiaceae fam. nov.

Ther.mo.de.sul.fo.bia.ce.ae. Gr. adj. *thermos* hot; L. pref. *de* from; L. n. *sulfur* sulfur; Gr. n. *bios* life; L. *aceae* denoting a family; L. neut. n. *Thermodesulfobiaceae* a family of thermophilic organisms that reduces a sulfur compound.

Rod-shaped, non-sporulating and Gram-staining negative cells. Moderately thermophilic. Chemoautotrophic and strictly anaerobic. Growth occurs by anaerobic respiration with sulfate and nitrate as electron acceptors. Represent a distinct phylogenetic lineage based on 16S rRNA gene sequence comparison.

The type genus is *Thermodesulfobium*.

Description of *Thermodesulfobium* gen. nov.

Ther.mo.de.sul.fo.bi.um. Gr. adj. *thermos* hot; L. pref. *de* from; L. n. *sulfur* sulfur; Gr. n. *bios* life; L. neut. n. *Thermodesulfobium* a thermophilic organism that reduces a sulfur compound.

Strictly anaerobic, moderate thermophilic, non-motile rods. Gram-staining negative. Non-sporulating. Chemoautotroph. Growth occurs on H_2/CO_2 by anaerobic respiration with sulfate, thiosulfate, nitrate, or nitrite as an electron acceptor.

The G + C content of genomic DNA is 35.1 mol% (as determined by HPLC).

The type species is *Thermodesulfobium narugense*.

Description of *Thermodesulfobium narugense* sp. nov.

Na.ru.gen′.se. L. adj. *narugense* from Narugo.

Cells are rod-shaped, about 0.5 μm in width and 2–4 μm in length. Motility and spore formation are not observed. Growth occurs between 37° and 65°C, with an optimum of 50°–55°C. The pH range for growth is 4.0–6.5. Growth does not occur above a NaCl concentration of 1% (w/v). The doubling time is 14 h under optimum

Table 1 Comparison of characteristics of sulfate-reducing microorganisms

	Archaeoglobus	*Caldivirga*	*Thermode sulfobacterium*	*Thermode sulfovibrio*	*Desulfotomaculum* and relatives	*Desulfovibrio* and relatives	Strain $Na82^T$
Phylogenetic position	*Euryarchaeota*	*Crenarchaeota*	*Thermodesulfobacteria*	*Nitrospirae*	*Firmicutes*	*δ-Proteobacteria*	
Optimum growth temperature (°C)	82–85	85	70–75	65	20–68	10–38[a]	50–55
Chemoautotrophic growth	±	–	±	–	±	±	+
Reduction of nitrate	±	+	–	±	–	±	+
Spore formation	–	–	–	–	+	–	–
Genomic G+C content (mol%)	41–46	28	28–40	30, 38	38–57	34–69	35

[a] Except for *Thermodesulforhabdus norvegica*, *Desulfacinum infernam*, and *Desulfacinum hydrothermale* (optimum temperature for growth is 60°C)

growth conditions. Sulfate, thiosulfate, nitrate, and nitrite are used as electron acceptors, but not sulfite, elemental sulfur, Fe(III), fumarate, dimetyl sulfoxide, and O_2. Electron donors utilized in the presence of sulfate are H_2 and formate. No growth occurs with glucose, acetate, lactate, pyruvate, malate, propionate, butyrate, fumarate, succinate, citrate, ethanol, propanol, or methanol.

The G+C content of genomic DNA is 35.1 ml%. MK-7(H_2) and MK-7 are the major quinones. MK-8 and MK-7(H_4) are found in trace amounts. The major cellular fatty acid is $C_{16:0}$. Minor components are cyclo-$C_{19:0}$(9,10)*cis*, $C_{18:0}$, $C_{18:1}$(△9)*cis*, $C_{20:0}$, $C_{14:0}$, $C_{12:0}$-3OH, and $C_{12:0}$.

The type strain is $Na82^T$ (=DSM 14796^T=JCM 11510^T). It has been isolated from Narugo hot spring (the prefecture of Miyagi, Japan).

Acknowledgments We thank Xian-Ying Meng (National Institute of Advanced Industrial Science and Technology) for electron microscopy. The research was supported by the Ministry of Education, Science and Technology (MEST), Japan, through Special Coordination Fund "Archaean Park Project" (International Research Project on Interaction Between Sub-Vent Biosphere and Geo-Environments).

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