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EXPERIMENTAL ARTICLES

Description of "*Desulfotomaculum nigrificans* subsp. *salinus*" as a New Species, *Desulfotomaculum salinum* sp. nov.

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Abstract—This study focused on the physiological, chemotaxonomic, and genotypic characteristics of two thermophilic spore-forming sulfate-reducing bacterial strains, 435^T and 781, of which the former has previously been assigned to the subspecies *"Desulfotomaculum nigrificans* subsp. *salinus*". Both strains reduced sulfate with the resulting production of H_2S on media supplemented with $H_2 + CO_2$, formate, lactate, pyruvate, malate, fumarate, succinate, methanol, ethanol, propanol, butanol, butyrate, valerate, or palmitate. Lactate oxidation resulted in acetate accumulation; butyrate was oxidized completely, with acetate as an intermediate product. Growth on acetate was slow and weak. Sulfate, sulfite, thiosulfate, and elemental sulfur, but not nitrate, served as electron acceptors for growth with lactate. The bacteria performed dismutation of thiosulfate to sulfate and hydrogen sulfide. In the absence of sulfate, pyruvate but not lactate was fermented. Cytochromes of *b* and *c* types were present. The temperature and pH optima for both strains were 60–65°C and pH 7.0. Bacteria grew at 0 to 4.5–6.0% NaCl in the medium, with the optimum being at 0.5–1.0%. Phylogenetic analysis based on a comparison of incomplete 16S rRNA sequences revealed that both strains belonged to the C cluster of the genus *Desulfotomaculum*, exhibiting 95.5–98.3% homology with the previously described species. The level of DNA–DNA hybridization of strains 435^T and 781 with each other was 97%, while that with closely related species *D. kuznetsovii* 17^T was 51–52%. Based on the phenotypic and genotypic properties of strains 435^T and 781, it is suggested that they be assigned to a new species: *Desulfotomaculum salinum* sp. nov., comb. nov. (type strain 435^T = VKM B 1492^T).

Key words: sulfate-reducing bacteria, *Desulfotomaculum salinum* sp. nov., 16S rRNA, fatty acids, autotrophy, taxonomy, complete oxidation of organic compounds.

The genus *Desulfotomaculum* is a heterogeneous group of anaerobic spore-forming sulfate-reducing bacteria, which comprises thermophilic, mesophilic, and psychrophilic bacteria growing at neutral or alkaline pH values. In spite of gram-negative staining, the ultrastructure of their cell wall is characteristic of gram-positive bacteria [1, 2]. In the course of sulfate reduction, representatives of *Desulfotomaculum* oxidize a broad spectrum of organic compounds, either completely to CO_2 or incompletely to acetic acid; some species are capable of autotrophic growth with $H_2 + CO_2$. A number of species can also grow in the absence of sulfate; they obtain energy by fermentation, by homoacetogenic growth on $H_2 + CO_2$, or in the course of syntrophic growth with methanogens and other prokaryotes [1].

The species *Desulfotomaculum nigrificans* have, for a long time, been the only known thermophilic representative of the genus under consideration. Then, a number of novel thermophilic bacteria belonging to the genus *Desulfotomaculum* were isolated from a variety of natural and technogenic environments: *D. kuznetsovii, D. geothermicum, D. thermoacetoxidans, D. thermobenzoicum, D. australicum, D. thermosapovorans, D. thermocisternum, D. luciae, D. putei*, and *D. solfataricum* [3–11]. The methods of phenotypic, genotypic, and chemotaxonomic analyses, as well as molecular biological techniques, were used to determine the taxonomic position of these species. Analysis of 16S rRNA sequences revealed significant phylogenetic diversity within the genus *Desulfotomaculum* [12]. Of the clusters identified, the one containing *Desulfotomaculum orientis* was described as the new genus *Desulfosporosinus* and this bacterium was designated *Desulfosporosinus orientis* [12]. The continuing description of new thermophilic species permanently increases the phylogenetic heterogeneity of the genus *Desulfotomaculum*.

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The present study deals with reclassification of two thermophilic spore-forming sulfate-reducing strains, 435^T and 781, isolated in 1978 from western Siberian oil and gas fields. Based on their morphological and physiological characteristics, these strains were assigned to the species *Desulfotomaculum nigrificans*, which was, at that time, the only known thermophilic *Desulfotomaculum* species [13]. Since one of the strains, 435^T, is halotolerant, it was classified as a new subspecies, *"Desulfotomaculum nigrificans* subsp. *salinum"* [13]. This subspecies, although it has not been validated, has often been mentioned in publications [1]. A study of strains 435^T and 781 using modern methods revealed metabolic differences between these organisms and *D. nigrificans* [14]. These results necessitated a more thorough study of strains 435^T and 781.

The task of the present study was to further investigate the physiological, chemotaxonomic, and genomic characteristics of strains 435^T and 781, as well as to determine the sequences of their 16S rRNA genes, with the aim of refining their phylogenetic and taxonomic positions.

MATERIALS AND METHODS

Bacterial strains. Strains 435^T (VKM B-1492), 781 (VKM B-1379), and type strain *D. kuznetsovii* 17^T (= VKM B-1805^T = DSM 6115^T) have been stored in the Laboratory of Oil Microbiology since their isolation [3, 13].

Media and cultivation conditions. Strains 435^T, 781, and *D. kuznetsovii* 17^T were maintained by culture transfers performed once every three months using Widdel slightly saline medium no. 1 [15], which contains (g/l) NaCl, 5.0; $MgCl_2$, 0.6; KCl, 0.3; $CaCl_2$, 0.1; NH_4Cl, 0.3; KH_2PO_4, 0.2; Na_2SO_4, 2.8; and $NaHCO_3$, 1.5. The medium was supplemented with $Na_2S \cdot 9\ H_2O$, 0.3 g/l, and $Na_2S_2O_4$, 0.05 g/l, which were used as reducing agents; sodium lactate, 3.5 g/l; and trace elements according to Pfennig and Lippert (cited in [3]). The bacteria were grown in 16-ml Hungate tubes containing 10 ml of the medium and argon or nitrogen as the gas phase. In order to determine the temperature, salinity, and pH ranges for the growth of strains 435^T and 781, Widdel medium was also used, with 1 g/l of fumarate as the substrate. Strain 17^T was grown on Widdel freshwater medium [15].

Analytical methods. Bacterial growth was assessed from light scattering at 420 nm, which was measured with a Specol 21 spectrophotometer. The sulfide concentration in the media was determined colorimetrically with *N,N*-dimethyl-*p*-phenylenediamine according to Trüper and Schlegel (1964) (cited in [3]). The possible electron acceptors for strains 435^T and 781 were tested using Widdel medium with sulfate substituted by thiosulfate (2.0 g/l), sulfite (1.0 g/l), elemental sulfur (1.5 g/l), or sodium nitrate (1 g/l). Thiosulfate dismutation was determined using Widdel mineral medium with thiosulfate substituting for sulfate. The formation of SO_4^{2-} was determined turbidimetrically [16].

Analysis of cellular fatty acids. Strains 435^T and 781 were grown on a medium containing lactate (3.5 g/l) at 60°C for five days. Cell suspensions were centrifuged at 5000 rpm for 30 min, and the lipid components were extracted from the pellet by acid methanolysis at 80°C for 3 h. Methyl esters of fatty acids and aldehydes were then extracted from the reaction mixture with two 200-μl portions of hexane. To obtain a mixture of trimethylsilyl esters of hydroxyacids, alcohols, and sterols, the extract was dried and treated with 20 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) for 15 min at 80°C. The reaction mixture was diluted with hexane to 100 μl, and 2 μl was taken for analysis on an HP-5973D gas chromatograph–mass spectrometer (Agillent Technologies, formerly Hewlett Packard, United States). A 25-m melted quartz capillary column (inner diameter, 0.25 mm) with a 0.2-μm-thick HP-5ms immobile phase (Agillent Technologies) was used for chromatographic separation of the sample. Chromatography was performed at a temperature programmed to rise from 130 to 320°C at a rate of 5°C/min. The injector temperature was 280°C, and the interface temperature was 250°C. As an internal standard, 3-deuteromethyl ester of tridecanoic acid was used. The NIST mass spectrum library software package was used to identify the compounds in the chromatographic peaks.

DNA analysis. DNA was extracted from the bacterial biomass using the Marmur method. The G+C content of the DNA was determined from its melting temperature, with *Escherichia coli* K-12 DNA used as the standard. The extent of DNA–DNA hybridization was determined using the DeLey *et al.* optical reassociation method (cited in [17]).

Determination of the 16S rRNA gene sequences and their phylogenetic analysis. Amplification and sequencing of the 16S rRNA genes of strains 435^T and 781 were performed with universal bacterial primers [18] using automatic DNA Sequencer 373A and the Applied Biosystems no. 402080 Ready Reaction Dye Terminator Sequencing kit with AmpliTaq DNA Polymerase (FS) as described in [19, 20].

Preliminary analysis of the 16S rRNA gene sequences was performed using the data and software available at the Ribosomal Database Project site (http://rdp.cme.msu.edu). The sequences were edited with the BioEdit software package (http://jwbrown.mbio.ncsu.edu/ BioEdit/bioedit.html) and aligned with the corresponding sequences of the closest species with the help of the CLUSTALW v 1.75 software package. Unrooted phylogenetic trees were constructed using the methods implemented in the TREECONW software package (http://bioc-www.uia.ac.be/u/yvdp/treeconw.html).

The results of the 16S rRNA gene sequencing for strains 435^T and 781 were deposited with GenBank under the accession numbers AY918122 and AY918123, respectively.

RESULTS AND DISCUSSION

Cell morphology and physiological characteristics. The cells of strains 435^T and 781 were rod-shaped with rounded ends and measured 0.9–1.3 × 2–5 μm and 0.6–1.0 × 2–5 μm, respectively. Strain 435^T often exhibited the lemon-shaped or spindle-shaped cells characteristic of *Desulfotomaculum* representatives; the size of these cells was up to 2–2.5 × 4.5–8 μm. In young cultures, the cells of both strains were motile by means of peritrichous flagella. Oval spores 0.5–0.7 μm in diameter were located centrally or subterminally and slightly widened the cells. When the submicroscopic organization of the cells was earlier studied in detail, it was shown that, in spite of their negative Gram staining, the cell walls had a gram-positive structure [2].

Growth at different incubation temperatures was assessed from hydrogen sulfide production. Strain 435^T grew at 45–75°C, and strain 781, at 45–70°C. The optimal temperature for both strains was 60–65°C. An increase in the optical density of the bacterial suspensions, together with an increase in the sulfide content, occurred at NaCl concentrations ranging from 0 to 6.0% for strain 435^T and from 0 to 4.5% for strain 781, with the salinity optima at 1.0 and 0.5%, respectively. The strains under study can therefore be termed halotolerant bacteria. Both strains grew in a pH range of 6.0–8.5. The largest biomass was accumulated at pH 7.0, whereas the highest H_2S production occurred at a higher pH (8.5) (Figs. 1a, 1b). Different pH optima for biomass accumulation and sulfide production have already been reported for *D. sapomandens* [1].

The strains under study were obligate anaerobes. They did not require vitamins. Their spectra of electron donors and acceptors were identical (Table 1). Both strains could grow autotrophically with sulfate and $H_2 + CO_2$. Under these conditions, small amounts of methane were found in the gas phase (the so-called mini-methane) [14]. On media containing sulfate, growth occurred with sodium salts of lactic, pyruvic, malic, formic, fumaric, succinic, butyric, valeric, and palmitic acids, as well as with methanol, ethanol, propanol, and butanol. Glucose, fructose, lactose, L-alanine, L-serine, L-arginine, L-cysteine, benzoate, citrate, tartrate, glycerol, glutamate, threonine, tryptophan, asparagine, and phenylalanine were not utilized. Both strains exhibited very slow acetate consumption, which became detectable after 56 days of incubation [14]. Lactate was oxidized to acetate. A number of substrates, including butyrate, were oxidized completely to CO_2. Acetate was found in the medium as an intermediate product, but it disappeared in the course of further incubation. The growth of strains 435^T and 781 on lactate, butyrate, and acetate was described in detail in [4].

In addition to sulfate, SO_3^{2-}, $S_2O_3^{2-}$, and elemental sulfur, but not nitrate, could serve as electron acceptors. Both strains performed dismutation of thiosulfate to sulfate and hydrogen sulfide. In the absence of sulfate, the bacteria could grow by fermenting pyruvate. Weak fermentative growth occurred with fumarate. Lactate was not fermented.

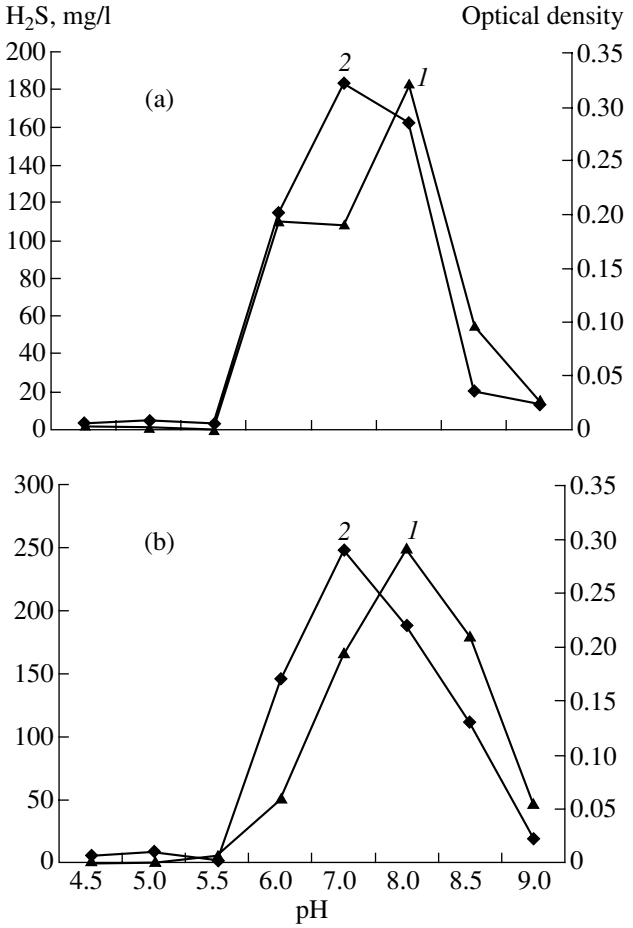

Fig. 1. (*1*) Production of hydrogen sulfide and (*2*) optical density of the culture after 8 days' growth of strains (a) 435^T and (b) 781 at 60°C and different pH values.

Genotypic characteristics and phylogenetic analysis. The G+C content of the DNA of strains 435^T and 781 was 50.8 and 50.6 mol %, respectively. As is shown by the 97% level of DNA–DNA hybridization between these strains, they belong to the same species. The levels of DNA–DNA hybridization with *D. kuznetsovii* 17^T were 52 and 51% respectively. Such values, above 30% and below 70%, suggest the affiliation of strains 435^T and 781 to a separate species within the genus *Desulfotomaculum* [21].

In order to determine the phylogenetic positions of strains 435^T and 781, almost complete sequences of their 16S rRNA genes were determined (ca. 1500 nucleotides approximately corresponding to *E. coli* positions 8–1520). The sequences had exact lengths of 1528 and 1457 nucleotides and exhibited 99.9% identity. The G+C content of the 16S rRNAs was 59.1–59.2 mol %. According to the performed screening, both sequences

Table 1. Differentiating features of strains 435^T and 781 and of closely related representatives of the genus *Desulfotomaculum*

Feature	Strains 435^T and 781	*D. nigrificans*	*D. kuznetsovii*	*D. australicum*	*D. thermocisternum*	*D. luciae*	*D. solfataricum*
Cell size, μm	1)* 0.9–1.3 × 2–5; 2)* 0.6–1 × 2–5	0.3–0.5 × 3–6	1–1.4 × 3.5–5	0.8–1 × 3–6	0.7–1 × 2–5.2	1 × 3	1.5 × 3.5–5
Oxidation of organic substrates	Complete	Incomplete	Complete	Complete	Incomplete[3*)]	ND	ND
Electron donors in the presence of SO_4^{2-}:							
$H_2 + CO_2$	+	+**	+	+	+	+	+
formate	+	+**	+	–	–	+	+
acetate	+***	–	+	+	–	–	+
propionate	+	–	+	–	+	ND	+
butyrate	+	–	+	–	+	ND	+
higher fatty acids (carbon chain length)	+ (C_5, C_6, C_{16})	ND	+ (C_5–C_7, C_{17}, C_{18})	+ (C_{16})	+ (C_3–C_{10}, C_{14}–C_{17})	ND	ND
methanol	+	–	+	–	–	–	+
propanol	+	+**	+	ND	+	ND	+
butanol	+	+**	+	ND	+	ND	+
malate	+	–	+	–	ND	–	ND
fumarate	+	–	+	–	ND	ND	+
succinate	+	–	+	–	ND	ND	+
benzoate	–	–	–	+	–	ND	–
alanine	ND	+	–	ND	ND	–	ND
glucose	–	–	–	–	ND	–	+
fructose	–	+	–	ND	–	–	+
Fermented in the absence of SO_4^{2-}	pyruvate; weakly, fumarate	pyruvate, fructose	pyruvate, fumarate	pyruvate	pyruvate	pyruvate, lactate	lactate
Electron acceptors:							
elemental sulfur	+***	–	–	ND	–	–	ND
nitrate	–	–	–	ND	–	–	–
Vitamin requirement	–	–	–	–	+ (biotin)	–	+
Temperature growth range (optimum), °C	1) 45–75 (60–65); 2) 45–70 (60–65)	30–70 (55)	50–85 (60–65)	40–74 (68)	41–75 (62)	50–<70, (60–65)	48–65 (60)
pH growth range (optimum)	1) 6.0–8.5 (7.0); 2) 6.5–8.5 (7.0)	ND	(7.0–7.2)	5.5–8.5 (7–7.4)	6.2–8.9 (6.7)	6.3–8.3 (7.0–7.9)	6.4–7.9 (7.3)
NaCl growth range (optimum), %	1) 0–6 (1); 2) 0–4.5 (0.5)	0–ND	0–3 (0–1)	NaCl required (1)	0–4.6 (0.3–1.1)	0–3 (<1)	0–1.5 (0)
DNA G+C content, mol %	1) 50.8; 2) 50.6	48.5–49.9	49.0	48.1	56–57	51.4	48.3–48.7
References	present paper, [13], [14]	[1]	[3]	[7]	[9]	[10]	[11]

Note: All the microorganisms listed in Table 1 formed spores; had rod-shaped cells with a gram-positive structure of the cell wall; were motile by means of peritrichous flagella; reduced sulfate, sulfite, and thiosulfate (however, the list of electron acceptors for *D. australicum* is not available); reduced sulfate on media containing lactate, ethanol, and pyruvate (however, no data are available on the growth of *D. solfataricum* on these substrates). "+" means positive result; "–", negative result; ND stands for "no data".

* (1) strain 435^T, (2) strain 781, (3) data on the decomposition of 20 mM lactate; ** in the presence of 1 mM acetate; *** very slow growth.

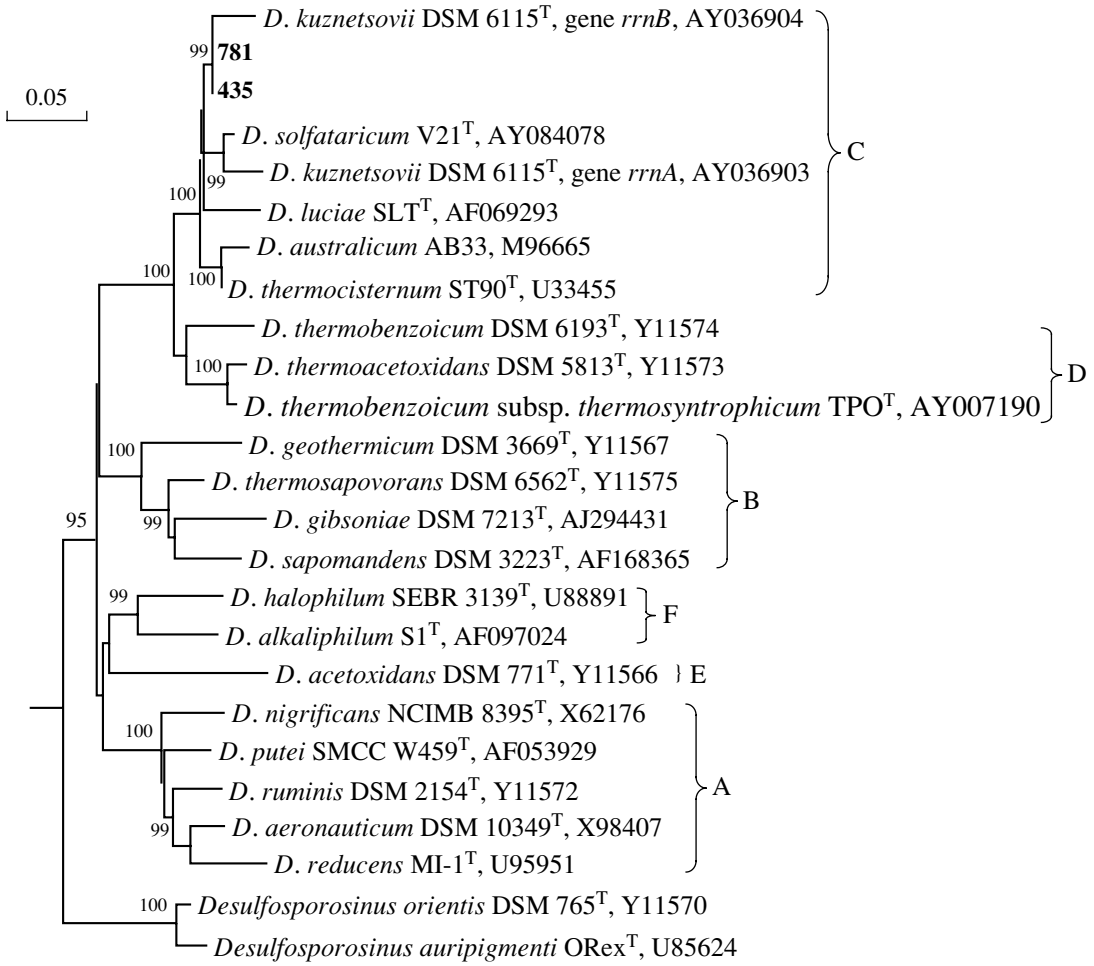

Fig. 2. Phylogenetic tree of sulfate-reducing bacteria of the genus *Desulfotomaculum* and representatives of closely related taxa. The tree was constructed on the basis of a matrix of normal evolutionary distances. The root was determined by including an *E. coli* sequence as an outgroup. The scale bar corresponds to 5 nucleotide substitutions per 100 nucleotides (evolutionary distances). The numerals at the branching points indicate the statistical significance of the branching order as determined by bootstrap analysis of 100 alternative trees (values above 90% were considered significant).

belonged to the genus *Desulfotomaculum* within the family *Peptococcaceae* of the clostridial line of gram-positive bacteria. A phylogenetic tree was constructed using the neighbor-joining method (Fig. 2); it had the same topology as the analogous trees previously reported in [11, 12, 19]. In the phylogenetic tree, the 16S rRNA sequences of both strains occurred on a branch representing the genus *Desulfotomaculum.* This genus is currently subdivided into five clusters (A–E) (Fig. 2) [12]. The 16S rRNA homology levels between these clusters range from 83.7 to 93%, while those within the clusters usually exceed 93.0%. Within the C cluster, which is made up of the closely related thermophilic species *D. kuznetsovii, D. australicum, D. thermocisternum, D. luciae*, and *D. solfataricum* [9–12, 21], strains 435^T and 781 formed a separate branch that clustered with *D. kuznetsovii.* The level of homology between the 16S rRNA sequences of strains 435^T and 781 and those of cluster C species was relatively high, specifically, 95.5–98.3% (without taking into account insertion and deletion sites).

Strains 435^T and 781 were significantly different in their 16S rRNA structure from *D. kuznetsovii*, which contains two 16S rRNA gene copies in its genome that differ by extra long insertions in variable 3' and 5' terminal portions of the 16S rRNA gene (positions 68–101 and 1445–1457 in *E. coli* numbering) [20]. The presence of extra long insertions in variable parts of the sequences was revealed in the 16S rRNA genes of all the known species of the C cluster (except *D. thermocisternum*, for which the terminal 5' fragment of the molecule has not been sequenced) [20]. However, in the sequences of the 16S rRNA genes of strains 435^T and 871, the extra long insertions were not found. Comparative analysis of 16S rRNA gene sequences that did not take into account variable regions revealed 94.0–96.6% homology between the two 16S rRNA genes of *D. kuznetsovii* 17^T and the 16S rRNA gene of strain 435^T. In the C cluster, this value is within the range of species differences. Strains 435^T and 781 were phylogenetically remote (88.6%) from the type species of the genus, *D. nigrificans*, which belongs to the A cluster.

Fatty acid composition. In the fatty acid spectra of strains 435^T and 781, the isoacids $C_{15:0}$ (29.6 and 14.9% of the total fatty acids of strains 435^T and 781, respectively) and $C_{17:0}$ (14.2 and 25.0%) and the normal fatty acids $C_{16:0}$ (26.6 and 24.1%) and $C_{18:0}$ (16.7 and 22.4%) predominated (Table 2). The strains were somewhat different in their fatty acid compositions, and the correlation coefficient did not permit them to be assigned to any of the *Desulfotomaculum* species with a known fatty acid composition. The unusual presence of both odd branched (*iso* $C_{15:0}$ and *iso* $C_{17:0}$) and even straight-chain ($C_{16:0}$ and $C_{18:0}$) fatty acids in these strains is an important differentiating chemotaxonomic feature.

The presence of *b* and *c* type cytochromes and the absence of desulfoviridin in the cells of strains 435^T and 781 were demonstrated previously[13].

Our results show that strains 435^T and 781 have similar morphologies and spectra of utilized electron donors and acceptors, as well as similar genotypic and phylogenetic characteristics (Tables 1, 2). Certain differences have been found in the temperature and salinity parameters of growth. Strain 435^T exhibits a higher maximum growth temperature and tolerates a higher maximum NaCl concentration than strain 781 (Table 1).

With regard to their metabolic characteristics, the strains are closest to *D. kuznetsovii*, differing from it by a lower maximum growth temperature (70–75°C as compared to 85°C for *D. kuznetsovii*) and resistance to a higher salt content in the medium. When grown on $H_2 + CO_2$, strains 435^T and 781 produce minor amounts of methane [14], while *D. kuznetsovii* synthesizes acetate from $H_2 + CO_2$ (data provided by A.E. Ivanova). Production of mini-methane has previously been detected in a *D. acetoxidans* culture grown on an acetate-containing medium, whereas acetate production has been found in a culture of *D. thermoacetoxidans* grown on a medium with $H_2 + CO_2$ [1].

Strains 435^T and 781 differed from *D. solfataricum*, another phylogenetically close representative of the genus *Desulfotomaculum*, by a wider range of NaCl concentrations suitable for growth, inability to reduce sulfate on media with glucose and fructose, absence of vitamin requirements, and composition of fatty acids. Strains 435^T and 781 differed from *D. luciae* in that they had a higher resistance to NaCl and ability to utilize malate, methanol, hexanoate, and acetate, albeit very slowly.

The following features are characteristic of the thermophilic bacteria of *Desulfotomaculum* cluster C (Table 1): sulfate reduction during autotrophic growth at the expense of $H_2 + CO_2$ oxidation, organotrophic growth on lactate and salts of fatty acids with various chain lengths (an exception is *D. australicum* [12]), and oxidation of fatty acids salt without acetate accumulation (an exception is *D. thermocisternum* [13]). It is possible that, in *Desulfotomaculum* representatives that are currently considered to be incapable of acetate oxidation, this capacity can be revealed by using prolonged incubation and introduction of an additional substrate, as was the case with strain 781 [14]. *D. thermocisternum* has been reported to oxidize lactate to acetate stoichiometrically [9]. However, acetate formation could have been caused by the high lactate concentration used (20 mM). According to our data, acetate accumulation by the lactate-grown cultures of strains 435^T and 781 decreased as the lactate concentration was decreased; as a result, the lactate–acetate stoichiometry changed [14].

The thermophilic species *D. nigrificans*, which grows lithoheterotrophically on $H_2 + CO_2$ in the presence of yeast extract or acetate and is incapable of decomposition of fatty acids or complete oxidation of organic matter, belongs to the A cluster, together with mesophilic species also incapable of complete oxidation (Fig. 2).

According to the data from DNA–DNA hybridization and phylogenetic analysis of 16S rRNA, lipid fatty acid composition, and the spectrum of electron donors, strains 435^T and 781 can be assigned to a new species, for which we propose the name *Desulfotomaculum salinum* sp. nov.

Description of *Desulfotomaculum salinum* sp. nov.

Basonym, *"Desulfotomaculum nigrificans* subsp. *salinus"* (Nazina, Rozanova, 1978); *sal.in.um*: L. *sale*, salt; *in*, suffix; *um*, ending; saline.

Straight or slightly curved rods motile by means of peritrichous flagella. Spherical spores are located centrally or subterminally and slightly widen the cell to a lemonlike or spindlelike shape. The cells stain Gram negative, although the cell wall structure is typical of gram-positive bacteria. The temperature growth range is 45 to 70–75°C, with an optimum at 60–65°C. Grows within a pH range from 6.0 to 8.5, with an optimum for biomass accumulation at pH 7.0. NaCl is not required for growth. Growth occurs within the NaCl concentration range from 0 to 4.5–6.0% NaCl, with an optimum at 0.5–1.0%.

Strictly anaerobic. Reduces sulfates, which results in hydrogen sulfide production on media with $H_2 + CO_2$, formate, lactate, pyruvate, malate, fumarate, succinate, methanol, ethanol, propanol, butanol, butyrate, valerate, and palmitate. Lactate is oxidized with the production of acetate; butyrate is oxidized completely, with acetate as an intermediate product. Slow and weak growth occurs on acetate. No vitamins or other growth factors are required, although the addition of yeast extract stimulates growth. Mini-methane is produced on sulfate-containing media with $H_2 + CO_2$. L-alanine, L-serine, L-arginine, L-cysteine, glucose, fructose, lactose, benzoate, citrate, tartrate, glycerol, glutamate, threonine, tryptophan, asparagine, and phenylalanine are not utilized. Pyruvate and, weakly, fumarate are fermented; lactate is not fermented. Sulfate, sulfite, thiosulfate, and elemental sulfur but not nitrate can serve as electron acceptors. Performs thiosulfate dismutation to sulfate and hydrogen sulfate. Cytochromes of the *b* and *c* types are present. Desulfoviridin is absent. The dominant fatty acids are the isoacids *iso* $C_{15:0}$ (14.9–29.6% of total fatty acids) and *iso* $C_{17:0}$ (14.2–25.0%) and normal

Table 2. Fatty acid composition (%) of strains 435^T and 781 and other representatives of the genus *Desulfotomaculum*

Fatty acids	435^T	781	*D. kuznetsovii* 17^T	*D. australicum* ACM 3917^T	*D. putei* SMCC W459^T	*D. thermobenzoicum* subsp. *thermosyntrophicum* DSM 14055^T	*D. solfataricum* DSM 14956T	*D. geothermicum* DSM 3669$_T$	*D. thermosapovorans* DSM 6562^T	*D. nigrificans* ATCC 7946
iso $C_{14:0}$					0.5				1.4	
$C_{14:0}$	2.7		1.1	0.6	8.8	2.1		7.9	2.1	2.0
iso $C_{15:1}$								6.5	1.0	
iso $C_{15:1\omega7c}$					2.2					
iso $C_{15:0}$	29.6	14.9	9.0	48.6	15.4	55.3	68.3	32.8	23.1	8.0
anteiso $C_{15:0}$	0.9		0.4		1.6	0.2	0.2	1.4		4.0
$C_{15:0}$	1.3	0.7	3.2	0.8	3.3	6.6	1.0	1.5	1.4	
iso C_{15} aldehyde			1.0					2.2	1.2	
C_{15} aldehyde			1.7							
iso $C_{16:1}$						1.8				
iso $C_{16:1\omega8c}$					0.7					
iso $C_{16:1\omega6c}$					1.5					
iso $C_{16:0}$	1.4	1.7	0.4	2.0	1.8	1.2		1.6	2.6	1.0
$C_{16:1}$	1.0	1.1	0.9							
$C_{16:1\omega9c}$					4.4			4.0	3.6	
$C_{16:1\omega7c}$					13.3	1.6			0.7	5.0
$C_{16:1\omega5c}$					0.9	0.1				
$C_{16:0}$	26.6	24.1	27.6	7.4	20.7	15.7	2.5	10.1	15.7	28.0
C_{16} alcohol	1.1	2.0	1.2							
$C_{16:1\omega9c}$ aldehyde									1.1	
iso $C_{17:1}$						0.9				
iso $C_{17:1\omega9c}$					1.2					
iso $C_{17:1\omega7c}$					12.1			7.2	5.0	11.0
10Me$C_{16:0}$										3.0
anteiso $C_{17:1\omega7c}$					0.5					1.0
C_{16} aldehyde								5.0	3.9	
iso $C_{17:0}$	14.2	25.0	5.5	37.4	5.2	6.2	28.1	2.4	8.9	14.0
anteiso $C_{17:0}$	1.4	0.7	0.7		0.5	0.7				6.0
cyclo $C_{17:0}$						0.4		3.7	5.8	
$C_{17:0}$	1.1	0.8	1.7		0.3	1.1			1.4	1.0
iso C_{17} aldehyde		2.7	5.2						7.5	
C_{17} aldehyde			0.7							
$C_{18:1\omega9c}$	1.0	1.9	1.6		0.4	2.3				2.0
$C_{18:1\omega7c}$					0.8	1.5				3.0
iso $C_{18:0}$										
$C_{18:0}$	16.7	22.4	36.5	2.0	1.0	1.5			3.2	2.0
C_{18} alcohol	1.0	2.0	1.6							
cyclo $C_{19:0}$						0.6				
Others				1.2	2.9	0.2		13.7	10.4	
Total	100	100	100	100	100	100	100.1	100	100	91.0
Reference	present paper	present paper	present paper	[7]	[10]	[11]	[11]	[23]	[23]	[22]

fatty acids $C_{16:0}$ (24.1–26.6%) and $C_{18:0}$ (16.7–22.4%). The G+C content of DNA is 50.6–50.8 mol %. Phylogenetically belongs to the C cluster of the genus *Desulfotomaculum.* The type strain is 435^T (VKM B-1492). Isolated from a mixture of formation and condensation water extracted together with gas at the Igrim gas condensate field (western Siberia). Reference strain, 781 (VKM B-1379).

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