Desulfotomaculum thermocisternum sp. nov., a Sulfate Reducer Isolated from a Hot North Sea Oil Reservoir

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The organism described in this paper, strain ST90^T (T = type strain), is a thermophilic, spore-forming, rod-shaped sulfate reducer that was isolated from North Sea oil reservoir formation water. In cultivation the following substances were used as electron donors and carbon sources: H₂-CO₂, lactate, pyruvate, ethanol, propanol, butanol, and C₃ to C₁₀ and C₁₄ to C₁₇ carboxylic acids. Sulfate was used as the electron acceptor in these reactions. Lactate was incompletely oxidized. Sulfite and thiosulfate were also used as electron acceptors. In the absence of an electron acceptor, the organism grew syntrophically on propionate together with a hydrogenotrophic methanogen. The optimum conditions for growth on lactate and sulfate were 62°C, pH 6.7, and 50 to 200 mM NaCl. The G+C content was 56 mol%, as determined by high-performance liquid chromatography and 57 mol% as determined by thermal denaturation. Spore formation was observed when the organism was grown on butyrate or propanol as a substrate and at low pH values. On the basis of differences in G+C content and phenotypic and immunological characteristics when the organism was compared with other thermophilic *Desulfotomaculum* species, we propose that strain ST90^T is a member of a new species, *Desulfotomaculum thermocisternum*. *D. thermocisternum* can be quickly identified and distinguished from closely related *Desulfotomaculum* species by immunoblotting.

The genus *Desulfotomaculum* comprises a heterogeneous group of gram-positive, spore-forming sulfate reducers that includes both mesophilic and thermophilic species. The following seven thermophilic species have been validly described previously: *Desulfotomaculum nigrificans*, which was isolated from canned food (52) and produced oil field water (1, 30); *Desulfotomaculum geothermicum*, *Desulfotomaculum australicum*, and *Desulfotomaculum kuznetsovii*, which were isolated from geothermal groundwater (13, 26, 29) (*D. kuznetsovii* has also been isolated from cold marine sediment [18]); *Desulfotomaculum thermoacetoxidans* and *Desulfotomaculum thermobenzoicum*, which were isolated from thermophilic fermentation reactors (28, 48); and *Desulfotomaculum thermosapovorans*, which was isolated from compost (16). Workers have also described several thermophilic *Desulfotomaculum* strains whose phylogenetic positions within the genus have not been determined (20, 39, 47).

North Sea oil field reservoirs are hot marine habitats that are 1.2 to 6 km below the seafloor; the pressures in these habitats range from 50 to 80 MPa, and the temperatures range from 60 to 200°C. The concentration of sulfate is usually between 0 and 0.6 mM and varies from one reservoir to another. Aliphatic carboxylic acids are the most abundant organic acids in petroleum reservoirs. In North Sea formation water, acetic acid is found at concentrations up to 20 mM, with decreasing concentrations of higher homologs up to octanoic acid (2, 5). These acids are potential electron donors for sulfate reduction. Formic acid is usually not detected (24). During offshore oil production, anaerobic seawater is injected into the reservoirs to enhance oil recovery.

A large number of thermophilic sulfate reducers have been isolated from produced oil field waters (1, 3, 4, 8, 9, 30, 37, 39, 40, 45). Biogenic production of H₂S causes corrosion of iron and steel alloys in oil wells and in oil-processing systems on the

platforms. Bacterial plugging (12, 38) and precipitation of sulfides in an oil reservoir may also reduce the permeability of oil formation (1, 11, 32). Exposure of oil field workers to H₂S represents a health hazard (21, 49).

In this paper we describe a new thermophilic, spore-forming sulfate reducer. The strain which we describe (strain ST90^T [T = type strain]) was isolated from a Statfjord oil field reservoir water sample. The sample was obtained before the breakthrough of injection water and consisted of pure formation water. Because of significant differences in G+C contents and physiological and immunological properties between strain ST90^T and other thermophilic members of the genus *Desulfotomaculum*, we propose that strain ST90^T should be placed in a new species, *Desulfotomaculum thermocisternum*.

MATERIALS AND METHODS

Isolation and cultivation. Thermophilic sulfate reducers were enriched from oil field water separated from crude oil. The sample which we used was collected from a wellhead on the Statfjord A platform in the Norwegian sector of the North Sea. The water originated from the Brent group formation 2.6 km below the sea floor, where the temperature was 90°C and the pressure was 30 MPa. The concentration of sulfate in the Brent Group formation water was less than 0.16 mM. The total organic acid concentration was about 20 mM, and acetic acid was the most abundant organic acid (the concentration of acetic acid was up to 18 mM) (2). The sample was obtained before seawater (injection water) breakthrough and consisted of 100% pure formation water. The temperature at the sampling point was 65°C. The in situ pH of the Statfjord reservoir has been estimated to be between 5.0 and 5.5. The pH of the sample after pressure release was 7.8.

For enrichment, 5-ml water samples were added to 50-ml portions of the marine medium described by Widdel and Pfennig (54). Then 1 ml of trace element solution SL-10 (53) per liter of medium and 5 ml of vitamin solution (34) per liter of medium were added. The pH was adjusted to 7.1 with HCl or Na₂CO₃. Lactate (final concentration, 20 mM) was added to the medium from a sterile anoxic stock solution.

Pure cultures were isolated in a dilution series by using the shake tube culture method (55); anoxic Gelrite gellan gum (Kelco Div., Merck and Co., San Diego, Calif.) was used as the gelling agent, and the cultures were incubated at 60°C with lactate as the substrate.

The growth medium used in this study was the medium described by Beeder et al. (4) except that sodium acetate was omitted. All growth experiments were carried out at 60°C and atmospheric pressure, unless indicated otherwise. Strain ST90^T was also grown in coculture with *Methanococcus thermolithotrophicus* ST22 (= DSM 8766) at 60°C; the medium used was the medium described

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TABLE 1. Properties of *Desulfotomaculum* species and strains T93B and ST90^T^a

Characteristic	<i>D. nigrificans</i>	<i>D. geothermicum</i>	<i>D. kuznetsovii</i>	<i>D. thermoacetoxidans</i>	<i>D. thermobenzoicum</i>	<i>D. thermosapovorans</i>	<i>D. australicum</i>	Strain T93B ^b	Strain ST90 ^T ^c
e ⁻ donors ^d									
H ₂ ^e	+	+	+	+	+	+	+	+	+
Formate (10 mM)	+	+	+	+	+	+	—	+	—
Acetate (20 mM)	—	—	+	+	—	—	+	+	—
Propionate (10 mM)	—	+	+	+	+	—	—	+	+
Pyruvate (10 mM)	+	NT ^g	+	+	+	+	+	+	+
Lactate (20 mM)	+	+	+	+	+	+	+	+	+
Butyrate (10 mM)	—	+	+	+	+	+	—	+	+
Isobutyrate (10 mM)	NT	NT	NT	NT	NT	—	NT	NT	—
Pentanoate (10 mM)	NT	NT	+	+	+	+	NT	+	+
Hexanoate (10 mM)	NT	+	+	+	+	+	NT	+	+
Heptanoate (10 mM)	NT	—	NT	NT	NT	+	NT	NT	+
Octanoate (2.5 mM)	NT	+	+	+	NT	+	NT	+	+
Nonanoate (0.5 mM)	NT	+	NT	NT	NT	+	NT	NT	+
Decanoate (0.5 mM)	NT	—	NT	NT	NT	+	NT	NT	+
Dodecanoate (0.5 mM)	NT	NT	NT	NT	NT	+	NT	NT	—
Hexadecanoate (1 mM)	NT	+	+	—	NT	+	NT	NT	+
Octadecanoate (0.5 mM)	NT	+	NT	NT	NT	+	NT	NT	—
Methanol (30 mM)	—	—	+	NT	—	+	—	+	—
Ethanol (20 mM)	+	+	+	—	+	+	+	+	+
Propanol (20 mM)	+	NT	+	+	+	+	NT	+	+
Butanol (20 mM)	+	NT	+	+	+	+	NT	+	+
Benzoate (5 mM)	—	—	—	—	+	—	+	—	—
Phenylacetate (10 mM)	NT	NT	NT	NT	NT	NT	NT	NT	—
Fructose (4 mM)	+	+	—	NT	—	—	NT	—	—
e ⁻ acceptors									
SO ₃ ²⁻	+	+	+	—	+	+	NT	+	+
S ₂ O ₃ ²⁻	+	NT	+	+	+	+	NT	+	+
S ⁰	—	—	—	—	—	—	NT	NT	—
NO ₃ ⁻	—	—	—	—	+	NT	NT	—	—
Temp range (°C)	30–70	30–57	50–85	45–65	40–70	35–60	40–74	43–78	41–75
Optimum temp (°C)	55	54	60–65	55–60	62	50	68	65	62
pH range	NT	6.0–8.0	NT	6.0–7.5	6.0–8.0	NT	5.5–8.5	6.7–7.5	6.2–8.9
Optimum pH	NT	7.2–7.4	NT	6.5	7.2	7.2–7.5	7.0–7.4	7.0	6.7
NaCl concn range (mM)	0–NT	34–860	0–510	0–260	0–NT	0–600	NT	0–1,200	0–800
Optimum NaCl concn (mM)	NT	410–580	0	0	NT	260	NT	NT	50–200
G+C content (mol%)	48–50	50	49	50	53	51	48	51	56 (57)

^a Data from references 7, 13, 16, 22, 25, 27, 28, and 47 unless indicated otherwise.^b Data from reference 39.^c Data from this study.^d The concentrations in parentheses are the concentrations used in experiments with strain ST90^T.^e Strain ST90^T was grown in the presence of H₂-CO₂ (80:20, vol/vol; 0.2 MPa).^f In the presence of 1 mM acetate.^g NT, not tested.

previously (4), except that sodium acetate and Na₂SO₄ were omitted and propionate was the sole carbon and energy source.

D. kuznetsovii VKM B-1805^T (= DSM 6115^T) was grown at 60°C in a medium containing (per liter) 4.00 g of NaCl, 12.50 g of MgSO₄·7H₂O, 0.50 g of CaCl₂·2H₂O, 2.00 g of (NH₄)₂SO₄, 1.40 g of K₂HPO₄·3H₂O, 0.40 g of KCl, 2 mg of Fe(NH₄)₂(SO₄)₂·3H₂O, 1 mg of resazurin, 1.00 g of yeast extract (Difco Laboratories), 5.00 g of NaHCO₃, 0.50 g of Na₂S·9H₂O, 10 ml of a trace element solution (56), 5 ml of a vitamin solution (34), and 5.0 g of lactate (sodium salt). *D. thermobenzoicum* TSB^T (= DSM 6193^T) and *D. australicum* AB33^T (= ACM 3917^T) were grown in medium G (35) containing lactate as the carbon and energy source at 50 and 60°C, respectively.

Chemical and other determinations. Grids coated with Formvar-carbon (Balzers AG) and rendered hydrophilic by glow discharge were used for negative staining with 1% (wt/vol) uranyl acetate (pH 4.2). For transmission electron micrographs cells were fixed with glutaraldehyde and embedded in Spurr low-viscosity resin (43) as described by Walther-Mauruschat et al. (51). Thin sections were contrasted with uranyl acetate (3%) and lead citrate. The preparations were examined with a JEOL model 100S electron microscope. The concentration of hydrogen sulfide was determined by using copper sulfate (10). Acetate was assayed as described previously (3). The amount of methane in the gas phase was determined as described previously (31).

Preparation of anti-ST90^T. Cells were preserved in 2% (vol/vol) formaldehyde and washed twice in phosphate-buffered saline (8.50 g of NaCl per liter, 1.44 g of Na₂HPO₄·2H₂O per liter, 0.25 g of KH₂PO₄ per liter; pH 7.2). Polyclonal

antisera against strain ST90^T (anti-ST90^T) was produced as described previously (8).

Serological test. Antigens were characterized by performing a Western blot (immunoblot) analysis of whole-cell extracts that had been solubilized with sodium dodecyl sulfate (SDS). Polyacrylamide gel electrophoresis (PAGE) of the whole-cell extracts was carried out as described by Laemmli (23). Electrophoresis was performed at 190 V by using a Mini Protean II dual-slab cell (Bio-Rad Laboratories, Richmond, Calif.). After electrophoresis the gels were stained with Coomassie brilliant blue R-250 (Serva). Unstained gels were immunoblotted as described by Burnette (6) by using a Mini Trans Blot cell (Bio-Rad).

16S rDNA analysis. DNA extraction, PCR-mediated amplification of the 16S ribosomal DNA (rDNA), and purification of PCR products were carried out as described previously (36). Purified PCR products were sequenced by using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) as recommended by the manufacturer. An Applied Biosystems model 373A DNA sequencer was used to electrophorese the sequence reaction products. The 16S rDNA sequences were aligned manually with the sequences of representative gram-positive bacteria. The sequence of *D. kuznetsovii* was not available. Pairwise evolutionary distances were computed by using the correction of Jukes and Cantor (19). The least-squares distance method of DeSoete (15) was used to construct a phylogenetic dendrogram from distance matrix data. The 16S rDNA analysis was performed by F. A. Rainey at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

DNA analysis. The G+C content was determined by both a thermal denatur-

ation method (14) and a chemical method in which high-performance liquid chromatography (HPLC) (27, 46) was used to separate the nucleotides. The DNA samples used for the thermal denaturation method and for the chemical method were extracted and purified as described by Silhavy et al. (41, 42) and Visuvanathan et al. (50), respectively. The determination of G+C content by the chemical method was performed at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

Storage. For long-term preservation 0.9 ml of a culture in the mid-exponential growth phase and 0.9 ml of anoxic glycerol medium (80% growth medium, 20% glycerol) were transferred to a screw-cap plastic vial (Cryo-tube). A crystal of dithionite was added, and the vial was capped and stored at -80°C . Strain ST90^T has been stored in this way for more than 4 years.

Nucleotide sequence accession number. The 16S rDNA sequences of strains ST90^T and T93B have been deposited in the GenBank database under accession numbers U33455 and U33456, respectively.

RESULTS

Enrichment and isolation. Pure formation water from the Statfjord oil field was used as the inoculum for enrichment of thermophilic sulfate reducers, and lactate was used as the substrate. Smooth, pale yellow, disc-shaped colonies were observed in Gelrite gellan gum after 7 days of incubation at 60°C . A pure culture, which was designated strain ST90^T, became dense after 2 days of incubation at 60°C in growth medium containing lactate as the substrate. This isolate has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as strain DSM 10259^T.

Cell morphology. The cells of strain ST90^T were straight rods that occurred singly and in chains. The cell diameter ranged from 0.7 to 1.0 μm , and the cell length ranged from 2.0 to 5.2 μm . Cells of different sizes were observed in the same chain. Single cells were motile and had peritrichous flagella. Spores were spherical and central and distended the cells to a diameter of 1.7 μm . No gas vacuoles were observed. Transmission electron micrographs revealed a cell wall structure characteristic of gram-positive bacteria (not shown).

Growth and nutritional properties. We examined the ability of strain ST90^T to use different electron donors and acceptors. In addition to the results shown in Table 1, the following results were obtained: tetradecanoate (10 mM), pentadecanoate (0.5 mM), and heptadecanoate (1 mM) were utilized as carbon and energy sources, while undecanoate (0.5 mM), tridecanoate (0.5 mM), *n*-dodecane, *n*-tetradecane, and crude oil were not utilized. The presence of crude oil did not inhibit growth on lactate and sulfate. Biotin was required as a growth factor.

When grown on lactate and sulfate, strain ST90^T produced stoichiometric amounts of acetate, showing that the isolate oxidized this substrate incompletely. When lactate, propanol, or heptadecanoate was used as the substrate, strain ST90^T grew in the presence of sulfide concentrations up to 9 mM. Spore formation was observed when butyrate and propanol were used as substrates. Fermentative growth was observed on pyruvate in the absence of any electron acceptor. In sulfate-free medium strain ST90^T grew on propionate in a coculture with a hydrogenotrophic methanogen that produced methane.

Strain ST90^T grew at temperatures between 41 and 75°C , and optimal growth occurred at 62°C ; no growth was observed at 37 or 77°C . Growth occurred at initial pH values between 6.2 and 8.9, and the optimum pH was 6.7. Spore formation was observed when the initial pH was 6.2 but not under more alkaline conditions. Growth was observed in medium containing 17 μM to 800 mM NaCl, and optimum growth occurred in the presence of 50 to 200 mM NaCl. NaCl concentrations lower than 17 μM were not tested. No growth occurred in the presence of 900 mM NaCl.

Whole-cell protein profile. SDS-PAGE revealed notable differences in the protein profiles of *D. thermobenzoicum*, *D.*

FIG. 1. Protein patterns (A) and immunoblots obtained with anti-ST90^T (B) after SDS-PAGE of SDS-soluble whole-cell extracts of *D. thermobenzoicum* (lanes 1), *D. australicum* (lanes 2), *D. kuznetsovii* (lanes 3), and strain ST90^T (lanes 4). The positions of molecular mass standards (in kilodaltons) are indicated on the left.

australicum, *D. kuznetsovii*, and strain ST90^T (Fig. 1A). Prominent bands at molecular weights of 64,000 and 59,000 were unique to strain ST90^T. *D. kuznetsovii* produced a protein profile that was quite different from the protein profiles of the other strains tested, especially in the molecular weight range from 45,000 to 100,000.

Serological characterization. Immunoblots of the whole-cell protein profiles of *D. thermobenzoicum*, *D. australicum*, and *D. kuznetsovii* were compared with an immunoblot of strain ST90^T obtained by using anti-ST90^T (Fig. 1B). A number of differences in the antigen profiles of these organisms were evident, and several antigens were unique to strain ST90^T. *D. thermobenzoicum* and *D. australicum* had antigens in common with strain ST90^T at molecular weights of 34,000 to 104,000 and 15,000 to 23,000, respectively. Anti-ST90^T also reacted with several proteins from *D. kuznetsovii*, but this organism had

TABLE 2. Sequence similarity matrix for members of the genus *Desulfotomaculum* and related taxa

Organism	% Sequence similarity													
	Strain ST90 ^T	Strain T93B	<i>Desulfotomaculum australicum</i>	<i>Desulfotomaculum thermobenzoicum</i>	<i>Desulfotomaculum geothermicum</i>	<i>Desulfotomaculum thermosapovorans</i>	<i>Desulfotomaculum nigrificans</i>	<i>Desulfotomaculum ruminis</i>	<i>Desulfotomaculum orientis</i>	<i>Desulfotomaculum dehalogenans</i>	<i>Syntrophospora bryantii</i>	<i>Thermoanaerobacter ethanolicus</i>	<i>Thermoanaerobacterium thermosulfurigenes</i>	<i>Moorella thermoacetica</i>
Strain T93B	99.9													
<i>Desulfotomaculum australicum</i>	98.6	98.5												
<i>Desulfotomaculum thermobenzoicum</i>	94.1	94.0	93.6											
<i>Desulfotomaculum geothermicum</i>	89.1	89.0	88.7	89.0										
<i>Desulfotomaculum thermosapovorans</i>	88.6	88.5	88.3	88.2	93.1									
<i>Desulfotomaculum nigrificans</i>	86.3	86.2	85.7	86.5	86.8	86.3								
<i>Desulfotomaculum ruminis</i>	85.3	85.2	84.7	84.5	86.6	86.2	91.1							
<i>Desulfotomaculum orientis</i>	86.5	86.4	85.9	84.8	87.1	86.2	84.4	84.9						
<i>Desulfotomaculum dehalogenans</i>	86.0	85.9	85.4	84.9	87.3	86.2	85.3	86.1	93.9					
<i>Syntrophospora bryantii</i>	85.2	85.1	84.8	84.1	82.0	81.4	81.9	82.0	84.9	83.9				
<i>Thermoanaerobacter ethanolicus</i>	86.6	86.5	86.1	85.6	85.1	84.2	83.1	82.8	85.0	84.1	85.2			
<i>Thermoanaerobacterium thermosulfurigenes</i>	83.7	83.6	83.3	83.9	83.6	82.7	81.3	80.7	82.7	82.6	83.6	86.0		
<i>Moorella thermoacetica</i>	88.1	88.0	87.8	88.2	87.0	87.0	85.2	83.3	86.5	85.6	85.6	87.5	86.7	
<i>Megasphaera elsdenii</i>	81.0	80.9	80.6	80.3	82.3	81.4	81.2	82.0	82.9	81.8	79.6	81.2	81.4	83.6

only one antigen band (at a molecular weight of 41,000) in common with strain ST90^T. All of the *Desulfotomaculum* strains examined contained a strong antigen that had a molecular weight of 33,000.

Phylogenetic analysis and G+C content. On the basis of 16S rDNA similarity values (Table 2), strain ST90^T falls within the radiation of the genus *Desulfotomaculum* and is most closely related to *D. australicum* and *D. thermobenzoicum*. Strain T93B, a thermophilic *Desulfotomaculum* strain that also was isolated from North Sea oil field water (38), exhibited 99.9% homology with strain ST90^T. Figure 2 is a phylogenetic dendrogram generated from the matrix in Table 2 and shows the relationship of strains ST90^T and T93B to other *Desulfotomaculum* species.

The G+C content of the DNA of strain ST90^T was 56 mol% as determined by the chemical method and 57 mol% as determined by the thermal denaturation method.

DISCUSSION

On the basis of phenotypic characteristics (i.e., dissimilatory sulfate reduction to sulfide, gram-positive cell wall structure, and formation of endospores), strain ST90^T was identified as a member of the genus *Desulfotomaculum*. Furthermore, our 16S rDNA analysis revealed that strain ST90^T was most closely related to *D. australicum* (level of sequence similarity, 98.6%). However, 16S rDNA sequence analysis is not suitable for distinguishing closely related species (i.e., species that exhibit levels of 16S rDNA similarity of 97% or more) (44). According to Stackebrandt and Goebel (44), it is the differences in phenotypic properties among strains that should be the decisive factor when species are described.

Previously described guidelines suggest that 5 mol% is the maximum range for G+C contents that is permissible for a

species (25). Strain ST90^T has a G+C content of 57 mol% as determined by thermal denaturation, whereas *D. australicum* has a G+C content of 48 mol% (Table 1). This difference alone is enough to warrant classification of strain ST90^T as a member of a separate species. In addition, significant differ-

FIG. 2. Phylogenetic dendrogram based on 16S rDNA sequence comparison, showing the positions of strains ST90^T and T93B within the radiation of members of the genus *Desulfotomaculum* and related taxa. Bar = 10 nucleotide changes per 100 nucleotides.

ences in substrate utilization patterns, temperature optima, pH ranges (Table 1), and antigen patterns support this conclusion.

The 16S rDNA analysis revealed that the level of similarity between *D. thermobenzoicum* and strain ST90^T was 94.1%; this, together with phenotypic differences (Table 1), showed that strain ST90^T is not a member of *D. thermobenzoicum*.

The protein profile of strain ST90^T is quite different from that of *D. kuznetsovii*. Together with physiological and genetic differences (Table 1), this shows that strain ST90^T is not closely related to *D. kuznetsovii*. Strain ST90^T can quickly be identified and distinguished from *D. thermobenzoicum*, *D. australicum*, and *D. kuznetsovii* by using immunoblotting.

Rosnes et al. (39) isolated two thermophilic *Desulfotomaculum* strains (strains T93B and T90A) from formation water that originated from the Statfjord oil field in the North Sea. During some of the growth experiments, strain T90A cultures were contaminated by strain T93B. Therefore, some of the growth characteristics of strain T90A (39) are incorrect, and this strain is considered lost. Our 16S rDNA analysis revealed that the level of similarity between strains T93B and ST90^T was 99.9%. However, because of differences in G+C contents and phenotypic characteristics (Table 1), these strains do not belong to the same species. Strain T93B should be assigned to the taxon *D. australicum*. Differences in phenotypic characteristics can be used to distinguish strain T93B and the type strain of this species.

Strain ST90^T was isolated from produced oil reservoir water obtained before breakthrough of injected seawater. Therefore, we concluded that it did not originate from injected seawater. Introduction of microorganisms into the Statfjord reservoir during drilling of production wells has been discussed by Rosnes et al. (39). These authors concluded that drilling operations could not be a source of contamination of formation water from this reservoir. Consequently, the most probable origin of strain ST90^T is the formation water itself.

It has been shown that *Desulfotomaculum* sp. strain T93B grows at 80°C and 30 MPa (i.e., conditions that are representative of large parts of North Sea oil reservoirs) (38). The presence of heat-resistant endospores would allow *Desulfotomaculum* strains to survive for a long time in reservoirs with temperatures higher than the maximum growth temperatures of these organisms. In many unexplored North Sea oil reservoirs, such as the Statfjord reservoir, the concentration of sulfate is constantly low. In the absence of an electron acceptor, strain ST90^T is able to ferment pyruvate. However, pyruvate has not been found in formation waters from North Sea oil reservoirs. *M. thermolithotrophicus* was recently isolated from produced Statfjord reservoir water (31). The ability of strain ST90^T to grow syntrophically with this hydrogenotrophic methanogen indicates that this may be one way that *Desulfotomaculum* strains grow in unexplored North Sea oil field reservoirs. Syntrophic growth of mesophilic propionate-degrading sulfate reducers and H₂-utilizing methanogens has been described previously (17, 57), and such growth gives these organisms an ecological advantage in systems where sulfate is intermittently available (33). The ability to grow syntrophically could be a successful survival strategy used by sulfate reducers that inhabit ecological niches where the sulfate concentration is always low.

Description of *Desulfotomaculum thermocisternum* sp. nov. *Desulfotomaculum thermocisternum* (ther.mo.cis.ter'num. Gr. adj. *thermos*, hot; L. fem. n. *cisterna*, reservoir; N. L. adj. *thermocisternum*, hot reservoir, referring to the original habitat of the organism). Straight rods that occur singly and in chains and are 0.7 to 1.0 µm in diameter and 2.0 to 5.2 µm long. Cells of different sizes can occur in the same chain. Cells have peritri-

chous flagella. Spores are spherical and central and distend the cells. No gas vacuoles are observed. The following substrates are utilized as carbon and energy sources in the presence of sulfate: H₂-CO₂, lactate, pyruvate, propionate, butyrate, pentanoate, hexanoate, heptanoate, octanoate, nonanoate, decanoate, tetradecanoate, pentadecanoate, hexadecanoate, heptadecanoate, ethanol, propanol, and butanol. Lactate is incompletely oxidized to acetate. The organism grows fermentatively on pyruvate. In the absence of sulfate it also grows on propionate in coculture with a hydrogenotrophic methanogen. Sulfate, sulfite, and thiosulfate are utilized as electron acceptors. Biotin is required as a growth factor. The temperature range for growth is 41 to 75°C; the optimum temperature is 62°C. The pH range for growth is 6.2 to 8.9; the optimum pH is 6.7. The NaCl concentration range for growth is 17 µM to 800 mM; the NaCl optimum concentration is 50 to 200 mM. The G+C content of the DNA is 56 mol% as determined by HPLC and 57 mol% as determined by thermal denaturation.

Isolated from pure formation water that originated from the subterranean Brent Group oil formation 2.6 km below the sea floor in the Norwegian sector of the North Sea. The type strain is strain ST90 (= DSM 10259).

ACKNOWLEDGMENTS

Financial support from the Norwegian oil company Statoil is gratefully acknowledged.

We thank Statoil and especially Egil Sunde for contributing samples and information. We also thank Bente E. Thorbjørnsen for technical assistance and B. K. C. Patel for providing *D. thermobenzoicum* TSB and *D. australicum* AB33.

REFERENCES

1. Antloga, K. M., and W. M. Griffin. 1985. Characterization of sulfate-reducing bacteria isolated from oilfield waters. *Dev. Ind. Microbiol.* **26**:597-610.
2. Barth, T., and M. Riis. 1992. Interactions between organic acid anions in formation waters and reservoir mineral phases. *Org. Geochem.* **19**:455-482.
3. Beeder, J., R. K. Nilsen, J. T. Rosnes, T. Torsvik, and T. Lien. 1994. *Archaeoglobus fulgidus* isolated from hot North Sea oil field waters. *Appl. Environ. Microbiol.* **60**:1227-1231.
4. Beeder, J., T. Torsvik, and T. Lien. 1995. *Thermodesulfurhabdus norvegicus*, gen. nov., sp. nov., a novel thermophilic sulfate-reducing bacterium from oil field water. *Arch. Microbiol.* **164**:331-336.
5. Borgund, A. E., and T. Barth. 1994. Generation of short-chained organic acids from crude oil by hydrous pyrolysis. *Org. Geochem.* **21**:943-952.
6. Burnette, W. N. 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* **112**:195-203.
7. Campbell, L. L., and J. R. Postgate. 1965. Classification of the spore-forming sulfate-reducing bacteria. *Bacteriol. Rev.* **29**:359-363.
8. Christensen, B., T. Torsvik, and T. Lien. 1992. Immunomagnetic captured thermophilic sulfate-reducing bacteria from North Sea oil field waters. *Appl. Environ. Microbiol.* **58**:1244-1248.
9. Cochran, W. J., P. S. Jones, P. F. Sanders, D. M. Holt, and M. J. Mosley. 1988. Studies on the thermophilic sulfate-reducing bacteria from a souring North sea oil field. *Soc. Petrol. Eng. SPE* **18368**:301-316.
10. Cord-Ruwisch, R. 1985. A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate reducing bacteria. *J. Microbiol. Methods* **4**:33-36.
11. Cord-Ruwisch, R., W. Kleinitz, and F. Widdel. 1987. Sulfate-reducing bacteria and their activities in oil production. *J. Petrol. Technol.* **1**:97-106.
12. Cunningham, A. B., E. J. Bouwer, and W. G. Characklis. 1990. Biofilms in porous media, p. 697-732. In W. G. Characklis and K. C. Marshall (ed.), *Biofilms*. Wiley, New York.
13. Daumas, S., R. Cord-Ruwisch, and J. L. Garcia. 1988. *Desulfotomaculum geothermicum* sp. nov., a thermophilic, fatty acid-degrading, sulfate-reducing bacterium isolated with H₂ from geothermal ground water. *Antonie Leeuwenhoek* **54**:165-178.
14. De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J. Bacteriol.* **101**:738-754.
15. DeSoete, G. 1983. A least squares algorithm for fitting additive trees to proximity data. *Psychometrika* **48**:621-626.
16. Fardeau, M.-L., B. Ollivier, B. K. C. Patel, P. Dwivedi, M. Ragot, and J.-L.

- Garcia. 1995. Isolation and characterization of a thermophilic sulfate-reducing bacterium, *Desulfotomaculum thermosapovorans* sp. nov. *Int. J. Syst. Bacteriol.* **45**:218–221.
17. Heppner, B., G. Zellner, and H. Diekmann. 1992. Start-up and operation of a propionate-degrading fluidized-bed reactor. *Appl. Microbiol. Biotechnol.* **36**:810–816.
  18. Isaksen, M. F., F. Bak, and B. B. Jørgensen. 1994. Thermophilic sulfate-reducing bacteria in cold marine sediment. *FEMS Microbiol. Ecol.* **14**:1–8.
  19. Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules, p. 21–132. *In* H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, New York.
  20. Karnachow, T. M., S. F. Koval, and K. F. Jarrell. 1992. Isolation and characterization of three thermophilic anaerobes from a St. Lucia hot spring. *Syst. Appl. Microbiol.* **15**:296–310.
  21. Kilburn, K. H. 1993. Case report: profound neurobehavioral deficits in an oil field worker overcome by hydrogen sulfide. *Am. J. Med. Sci.* **306**:301–305.
  22. Klemps, R., H. Cypionka, F. Widdel, and N. Pfennig. 1985. Growth with hydrogen, and further physiological characteristics of *Desulfotomaculum* species. *Arch. Microbiol.* **143**:203–208.
  23. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680–685.
  24. Lewan, M. D., and J. B. Fisher. 1994. Organic acids from petroleum source rocks, p. 70–114. *In* E. D. Pittman and M. D. Lewan (ed.), *Organic acids in geological processes*. Springer-Verlag, Berlin.
  25. Logan, N. A. 1994. *Bacterial systematics*. Blackwell Scientific Publication, Oxford.
  26. Love, C. A., B. K. C. Patel, P. D. Nichols, and E. Stackebrandt. 1993. *Desulfotomaculum australicum* sp. nov., a thermophilic sulfate-reducing bacterium isolated from the Great Artesian Basin of Australia. *Syst. Appl. Microbiol.* **16**:244–251.
  27. Mesbah, M., U. Premachandran, and W. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**:159–167.
  28. Min, H., and S. H. Zinder. 1990. Isolation and characterization of a thermophilic sulfate-reducing bacterium, *Desulfotomaculum thermoacetoxidans* sp. nov. *Arch. Microbiol.* **153**:399–404.
  29. Nazina, T. N., A. E. Ivanova, L. P. Kanchaveli, and E. P. Rozanova. 1987. A new sporeforming thermophilic methylotrophic bacterium, *Desulfotomaculum kuznetsovii* sp. nov. *Microbiology (Engl. Transl. Mikrobiologiya)* **57**: 659–663.
  30. Nazina, T. N., and E. P. Rozanova. 1977. Thermophilic sulfate reducing bacterium from oil strata. *Microbiology (Engl. Transl. Mikrobiologiya)* **47**: 773–778.
  31. Nilsen, R. K., and T. Torsvik. 1996. *Methanococcus thermolithotrophicus* isolated from North Sea oil field reservoir water. *Appl. Environ. Microbiol.* **62**:728–731.
  32. Odom, J. M. 1993. Industrial and environmental activities of sulfate-reducing bacteria, p. 189–210. *In* J. M. Odom and R. Singleton, Jr. (ed.), *The sulfate-reducing bacteria: contemporary perspectives*. Springer-Verlag, New York.
  33. Oude Elferink, S. J. W. H., A. Visser, L. W. Hulshoff Pol, and A. J. M. Stams. 1994. Sulfate reduction in methanogenic bioreactors. *FEMS Microbiol. Rev.* **15**:119–136.
  34. Pfennig, N. 1978. *Rhodocyceus purpureus* gen. nov. and sp. nov., a ring-shaped, vitamin B₁₂-requiring member of the family *Rhodospirillaceae*. *Int. J. Syst. Bacteriol.* **28**:283–288.
  35. Postgate, J. R. 1984. *The sulfate-reducing bacteria*, 2nd ed. Cambridge University Press, Cambridge.
  36. Rainey, F. A., M. Dorsch, H. W. Morgan, and E. Stackebrandt. 1992. 16S rDNA analysis of *Spirochaeta thermophila*: position and implications for the systematics of the order *Spirochaetales*. *Syst. Appl. Microbiol.* **16**:224–226.
  37. Rees, G. N., G. S. Grassia, A. J. Sheeny, P. P. Dwivedi, and B. K. C. Patel. 1995. *Desulfacinum inferum* gen. nov., sp. nov., a thermophilic sulfate-reducing bacterium from a petroleum reservoir. *Int. J. Syst. Bacteriol.* **45**:85–89.
  38. Rosnes, J. T., A. Graue, and T. Lien. 1991. Activity of sulfate-reducing bacteria under simulated reservoir conditions. *Soc. Petrol. Eng. SPE* **19429**: 217–220.
  39. Rosnes, J. T., T. Torsvik, and T. Lien. 1991. Spore-forming thermophilic sulfate-reducing bacteria isolated from North Sea oil field waters. *Appl. Environ. Microbiol.* **57**:2302–2307.
  40. Rozanova, E. P., and T. A. Pirovanova. 1986. Reclassification of *D. thermophilus* (Rozanova and Khudyakova 1974). *Microbiology (Engl. Transl. Mikrobiologiya)* **57**:85–89.
  41. Silhavy, T. J., M. L. Berman, and L. W. Enquist. 1984. Procedure 25. DNA extraction from bacterial cells, p. 137–139. *In* *Experiments with gene fusions*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
  42. Silhavy, T. J., M. L. Berman, and L. W. Enquist. 1984. Procedure 40. Phenol/chloroform extraction of DNA samples, p. 177–179. *In* *Experiments with gene fusions*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
  43. Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**:31–43.
  44. Stackebrandt, E., and B. M. Goebel. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**:846–849.
  45. Stetter, K. O., R. Huber, E. Blöchl, M. Kurr, R. D. Eden, M. Fielder, H. Cash, and I. Vance. 1993. Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. *Nature (London)* **365**:743–745.
  46. Tamaoka, J., and K. Komagata. 1984. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25**:125–128.
  47. Tanimoto, Y., and F. Bak. 1994. Anaerobic degradation of methylmercaptan and dimethyl sulfide by newly isolated thermophilic sulfate-reducing bacteria. *Appl. Environ. Microbiol.* **60**:2450–2455.
  48. Tasaki, M., Y. Kamagata, K. Nakamura, and E. Mikami. 1991. Isolation and characterization of a thermophilic benzoate-degrading, sulfate-reducing bacterium, *Desulfotomaculum thermobenzoicum* sp. nov. *Arch. Microbiol.* **155**: 348–352.
  49. Tvedt, B., K. Skyberg, O. Aaserud, Å. Hobbesland, and T. Mathisen. 1991. Brain damage caused by hydrogen sulfide: a follow-up study of six patients. *Am. J. Ind. Med.* **20**:91–101.
  50. Visuvanathan, S., M. T. Moss, J. L. Stanford, J. Hermon-Taylor, and J. J. McFadden. 1989. Simple enzymatic method for the isolation of DNA from diverse bacteria. *J. Microbiol. Methods* **10**:59–64.
  51. Walther-Mauruschat, A., M. Aragno, F. Mayer, and H. G. Schlegel. 1977. Micromorphology of Gram-negative hydrogen bacteria. II. Cell envelope, membranes, and cytoplasmic inclusions. *Arch. Microbiol.* **114**:101–110.
  52. Werkmann, C. H., and H. J. Weaver. 1927. Studies in the bacteriology of sulphur stinkers spoilage of canned sweet corn. *Iowa State Coll. J. Sci.* **2**: 57–67.
  53. Widdel, F., G. W. Kohring, and F. Mayer. 1983. Studies of dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. and sp. nov. and *Desulfonema magnum* sp. nov. *Arch. Microbiol.* **134**:286–294.
  54. Widdel, F., and N. Pfennig. 1981. Studies of dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov. and sp. nov. *Arch. Microbiol.* **129**:395–400.
  55. Widdel, F., and N. Pfennig. 1984. Dissimilatory sulfate- and sulfur-reducing bacteria, p. 663–679. *In* N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
  56. Wolin, E. A., M. J. Wolin, and R. S. Wolfe. 1963. Formation of methane by bacterial extracts. *J. Biol. Chem.* **238**:2882–2886.
  57. Wu, W. M., M. K. Jain, E. Conway de Macario, J. H. Thiele, and J. G. Zeikus. 1992. Microbial composition and characterization of prevalent methanogens and acetogens isolated from syntrophic methanogenic granules. *Appl. Microbiol. Biotechnol.* **38**:282–290.