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

ROALD KÅRE NILSEN, TERJE TORSVIK, AND TORLEIV LIEN
Department of Microbiology, University of Bergen, N-5020 Bergen, Norway

The organism described in this paper, strain ST90\textsuperscript{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: H\textsubscript{2}-CO\textsubscript{2}, lactate, pyruvate, ethanol, propanol, butanol, and C\textsubscript{2} to C\textsubscript{10} 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\textsuperscript{T} is a member of a new species, Desulfotomaculum thermosternum. D. thermosterum 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 nigricans, 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 geothermocold 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 thermopropidans, 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 H\textsubscript{2}S 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 H\textsubscript{2}S 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\textsuperscript{T} [T = type strain]) was isolated from a Staffjord 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\textsuperscript{T} and other thermophilic members of the genus Desulfotomaculum, we propose that strain ST90\textsuperscript{T} should be placed in a new species, Desulfotomaculum thermosternum.

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 Staffjord 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 Staffjord 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 Na\textsubscript{2}CO\textsubscript{3}. 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); axenic 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\textsuperscript{T} was also grown in coculture with Methanococcus thermolithotrophicus ST22 (= DSM 8766) at 60°C; the medium used was the medium described...
### Table 1. Properties of *Desulfotomaculum* species and strains T93B and ST90T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>D. nigricans</th>
<th>D. geothermicus</th>
<th>D. kaznetsovii</th>
<th>D. thermooxidans</th>
<th>D. thermoacetoxidans</th>
<th>D. thermostoaceticus</th>
<th>D. thermostoapovorans</th>
<th>D. australicum</th>
<th>Strain T93B</th>
<th>Strain ST90T</th>
</tr>
</thead>
<tbody>
<tr>
<td>c^ donors^s</td>
<td>H^+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Formate (10 mM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetate (20 mM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Propionate (10 mM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pyruvate (10 mM)</td>
<td>+</td>
<td>NT^a</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactate (20 mM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Butyrate (10 mM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Isobutyrate (10 mM)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>Pentanoate (10 mM)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Hexanoate (10 mM)</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Heptanoate (10 mM)</td>
<td>NT</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Octanoate (2.5 mM)</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nonanoate (0.5 mM)</td>
<td>NT</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Decanate (0.5 mM)</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dodecanoate (0.5 mM)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Octadecanoate (1 mM)</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Acetate (20 mM)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Methanol (30 mM)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Ethanol (20 mM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Propanol (20 mM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Butanol (20 mM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benzene (5 mM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenyacetate (10 mM)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Fructose (4 mM)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>c^- acceptors</td>
<td>SO_4^-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>S_2O_3^-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>SO_3^-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>NO_3^-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Temp range (°C)</td>
<td>30–70</td>
<td>30–57</td>
<td>50–85</td>
<td>45–65</td>
<td>40–70</td>
<td>35–60</td>
<td>40–74</td>
<td>43–78</td>
<td>41–75</td>
<td></td>
</tr>
<tr>
<td>Optimum temp (°C)</td>
<td>55</td>
<td>54</td>
<td>60–65</td>
<td>55–60</td>
<td>62</td>
<td>50</td>
<td>68</td>
<td>65</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>pH range</td>
<td>NT</td>
<td>6.0–8.0</td>
<td>NT</td>
<td>6.0–7.5</td>
<td>6.0–8.0</td>
<td>NT</td>
<td>5.5–8.5</td>
<td>6.7–7.5</td>
<td>6.2–8.9</td>
<td></td>
</tr>
<tr>
<td>Optimum pH</td>
<td>NT</td>
<td>7.2–7.4</td>
<td>NT</td>
<td>6.5</td>
<td>7.2</td>
<td>7.2–7.5</td>
<td>7.0–7.4</td>
<td>7.0</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Optimum NaCl concn (mM)</td>
<td>0–NT</td>
<td>34–60</td>
<td>0–1,200</td>
<td>0–260</td>
<td>0–NT</td>
<td>0–1,200</td>
<td>0–800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl concn (mM)</td>
<td>6.5</td>
<td>5.5–8.5</td>
<td>6.0–7.5</td>
<td>6.0–8.0</td>
<td>6.0–7.5</td>
<td>6.0–8.0</td>
<td>6.0–7.5</td>
<td>6.2–8.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>48–50</td>
<td>50</td>
<td>49</td>
<td>50</td>
<td>53</td>
<td>48</td>
<td>51</td>
<td>56</td>
<td>57</td>
<td></td>
</tr>
</tbody>
</table>

^a Data from references 7, 13, 16, 22, 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 ST90T.

^e NT, not tested.

### 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 microscopy, cells were fixed with glutaraldehyde and embedded in Spurr low-viscosity resin (43) as described by Walther-Maucher 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-ST90T.** 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 Na_2HPO_4·2H_2O per liter, 0.25 g of K_H_2PO_4 per liter; pH 7.2). Polyclonal antiserum against strain ST90T (anti-ST90T) 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 immunooblotted 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 *Tag 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. kaznetsovii* 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-
with a hydrogenotrophic methanogen that produced methane.

were used as substrates. Fermentative growth was observed on alkaline conditions. Growth was observed in medium containing pH 6.7. Spore formation was present of 900 mM NaCl.

free medium strain ST90T grew on propionate in a coculture with strain T93B. Growth was observed when butyrate and propanol or heptadecanoate were used as the substrate, strain ST90T oxidized this substrate incompletely. When lactate, propanol, or heptadecanoate was used as the substrate, strain ST90T 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.

We examined the ability of strain ST90T 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 ST90T produced stoichiometric amounts of acetate, showing that the isolate oxidized this substrate incompletely. When lactate, propanol, or heptadecanoate was used as the substrate, strain ST90T 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 ST90T grew on propionate in a coculture with a hydrogenotrophic methanogen that produced methane.

Strain ST90T 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. australicum, D. kuznetsovii, and strain ST90T (Fig. 1A). Prominent bands at molecular weights of 64,000 and 59,000 were unique to strain ST90T. 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 ST90T obtained by using anti-ST90T (Fig. 1B). A number of differences in the antigen profiles of these organisms were evident, and several antigens were unique to strain ST90T. D. thermobenzoicum and D. australicum had antigens in common with strain ST90T at molecular weights of 34,000 to 104,000 and 15,000 to 23,000, respectively. Anti-ST90T also reacted with several proteins from D. kuznetsovii, but this organism had
only one antigen band (at a molecular weight of 41,000) in common with strain ST90\textsuperscript{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\textsuperscript{T} falls within the radiation of the genus Desulfotomaculum and is most closely related to \textit{D. australicum} and \textit{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\textsuperscript{T}. Figure 2 is a phylogenetic dendrogram generated from the matrix in Table 2 and shows the relationship of strains ST90\textsuperscript{T} and T93B to other Desulfotomaculum species.

The G+C content of the DNA of strain ST90\textsuperscript{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\textsuperscript{T} was identified as a member of the genus Desulfotomaculum. Furthermore, our 16S rDNA analysis revealed that strain ST90\textsuperscript{T} was most closely related to \textit{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\textsuperscript{T} has a G+C content of 57 mol\% as determined by thermal denaturation, whereas \textit{D. australicum} has a G+C content of 48 mol\% (Table 1). This difference alone is enough to warrant classification of strain ST90\textsuperscript{T} as a member of a separate species. In addition, significant differ-
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 \textit{D. thermobenzoicum} and strain ST90\textsuperscript{T} was 94.1\%; this, together with phenotypic differences (Table 1), showed that strain ST90\textsuperscript{T} is not a member of \textit{D. thermobenzoicum}.

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

Rosnes et al. (39) isolated two thermophile \textit{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\textsuperscript{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 \textit{D. australicum}. Differences in phenotypic characteristics can be used to distinguish strain T93B and the type strain of this species. Strain ST90\textsuperscript{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\textsuperscript{T} is the formation water itself.

It has been shown that \textit{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 \textit{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\textsuperscript{T} is able to ferment pyruvate. However, pyruvate has not been found in formation waters from North Sea oil reservoirs. \textit{M. thermolithothrophicus} was recently isolated from produced Statfjord reservoir water (31). The ability of strain ST90\textsuperscript{T} to grow syntrophically with this hydrogenotrophic methanogen indicates that this may be one way that \textit{Desulfotomaculum} strains grow in unexplored North Sea oil field reservoirs. Syntrophic growth of mesophilic propionate-degrading sulfate reducers and H\textsubscript{2}-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.

\textbf{Description of \textit{Desulfotomaculum thermocisternum} sp. nov. \textit{Desulfotomaculum thermocisternum} (ther.mo.cis.ter.num. Gr. adj. thermos, hot; L. fem. n. cisterna, reservoir; N. L. adj. \textit{thermo-}, cisternum, 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 \textmu m in diameter and 2.0 to 5.2 \textmu m long. Cells of different sizes can occur in the same chain. Cells have peritrichous 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: H\textsubscript{2}-CO\textsubscript{3}, 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 \textmu M 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).

\textbf{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 \textit{D. thermobenzoicum} TSB and \textit{D. australicum} AB33.

\textbf{REFERENCES}

402
27. Mesbah, M.,
29. Nazina, T. N.,
40. Rozanova, E. P., and T. A. Pirovskaya. 1986. Reclassification of D. ther-
52. Werkmann, C. H., and H. J. Weaver. 1927. Studies in the bacteriology of