Desulfotomaculum arcticum sp. nov., a novel spore-forming, moderately thermophilic, sulfate-reducing bacterium isolated from a permanently cold fjord sediment of Svalbard

Verona Vandieken,[1] Christian Knoblauch[2] and Bo Barker Jørgensen[1]

[1]Max-Planck-Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany

[2]University of Hamburg, Institute of Soil Science, Allende-Platz 2, 20146 Hamburg, Germany

Correspondence
Verona Vandieken
vvandiek@mpi-bremen.de

Strain 15^T is a novel spore-forming, sulfate-reducing bacterium isolated from a permanently cold fjord sediment of Svalbard. Sulfate could be replaced by sulfite or thiosulfate. Hydrogen, formate, lactate, propionate, butyrate, hexanoate, methanol, ethanol, propanol, butanol, pyruvate, malate, succinate, fumarate, proline, alanine and glycine were used as electron donors in the presence of sulfate. Growth occurred with pyruvate as sole substrate. Optimal growth was observed at pH 7·1–7·5 and concentrations of 1–1·5 % NaCl and 0·4 % $MgCl_2$. Strain 15^T grew between 26 and 46·5 °C and optimal growth occurred at 44 °C. Therefore, strain 15^T apparently cannot grow at *in situ* temperatures of Arctic sediments from where it was isolated, and it was proposed that it was present in the sediment in the form of spores. The DNA G+C content was 48·9 mol%. Strain 15^T was most closely related to *Desulfotomaculum thermosapovorans* MLF^T (93·5 % 16S rRNA gene sequence similarity). Strain 15^T represents a novel species, for which the name *Desulfotomaculum arcticum* sp. nov. is proposed. The type strain is strain 15^T (=DSM 17038^T=JCM 12923^T).

The genus *Desulfotomaculum* includes meso- and thermophilic species mainly isolated from thermal sites. Under unfavourable environmental conditions that do not permit the organisms to grow or metabolize substrates, they may survive in the form of spores. Spores are resistant to drying, oxygenation, starvation and extreme temperatures. Thus, *Desulfotomaculum*-related strains have been isolated from environments that do not support growth of the organisms, such as extreme temperature environments. *Desulfotomaculum halophilum* SEBR 3139^T grows between 30 and 40 °C and was isolated from 85 °C hot fluids (Tardy-Jacquenod *et al.*, 1998). Strains of the spore-forming genera *Desulfotomaculum* and *Desulfosporosinus* were isolated from cold sediments off the coast of Denmark (Isaksen *et al.*, 1994), from permanently cold deep-sea sediments (Barnes *et al.*, 1998) and from permafrost soil (Vainshtein *et al.*, 1995), although these bacteria are not able to grow at *in situ* temperatures of those localities.

Strain 15^T was isolated at 28 °C from sediment of Nordfjorden, Station BC (water depth 100 m, bottom water temperature 1·8 °C) on the west coast of Svalbard. The first enrichment culture was artificial sea-water medium (Widdel & Bak, 1992) with 28 mM sulfate and a suspension of lyophilized cyanobacteria (*Spirulina*) as carbon and energy source. For isolation in deep-agar dilution series, the *Spirulina* suspension was replaced by a fatty acid mixture of acetate, lactate, butyrate and propionate. The tests for physiological characterization were performed in duplicate in medium with a lower salt concentration (salt-water medium) (Widdel & Bak, 1992) at 37 °C. Cultures growing with alternative substrates were transferred into fresh test medium for verification. Temperature tolerance of the strains was determined in an aluminium temperature-gradient block at 13 different temperatures between 20 and 50 °C (Sagemann *et al.*, 1998). The salt requirement was determined in media with 12 different NaCl concentrations between 0·05 and 5 % (w/v) and 10 different $MgCl_2.6H_2O$ concentrations between 0·02 and 3·6 % (w/v). The pH optima of the strains were determined in media with 12 different pH values that covered a range from pH 5·5 to 8·8. For all tests, growth was monitored spectrophotometrically (UV 1202; Shimadzu) by measuring optical density at 580 nm.

PCR amplification of 16S rRNA gene was performed with the primers 8F and 1492R, and the PCR product was amplified for sequence analysis with primers 8F, 341F, 518F, 534R, 1099F and 1492R (Buchholz-Cleven *et al.*, 1997). The

Published online ahead of print on 18 November 2005 as DOI 10.1099/ijs.0.64058-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Desulfotomaculum arcticum* strain 15^T is DQ148942.

phylogenetic position was evaluated by the ARB program package (Ludwig *et al.*, 2004) using the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms with different sets of filters. Positions 109–1387 (*Escherichia coli* numbering) were used for analyses, as regions that either exhibited alignment uncertainties or were not sequenced were excluded.

Cells of strain 15^T were rods, 2–3 × 1 μm in size and, when endospores were formed, cells appeared lemon-shaped (Fig. 1). Endospores were spherical and located in the centre of the cells. Motility was not observed under the culture conditions used. Gram staining was negative. A negative Gram stain has been repeatedly described for species of *Desulfotomaculum*, yet the ultrastructure of the cell wall of these species, examined by electron microscopy, is usually typical for Gram-positive bacteria (Stackebrandt *et al.*, 1997); this was not tested for strain 15^T.

Vitamins were not required for growth. Strain 15^T used sulfate (30 mM), sulfite (2 mM) and thiosulfate (10 mM) in the presence of lactate. As electron acceptors strain 15^T did not reduce ferric citrate (30 mM), poorly crystalline iron oxide (30 mM), manganese oxide (30 mM), malate (20 mM), fumarate (20 mM), nitrate (20 mM), nitrite (10 mM), oxygen (air) or elemental sulfur. With sulfate as the electron acceptor the strain oxidized the following substrates: hydrogen (H_2/CO_2; 80 : 20, v/v), formate (10 mM), lactate (20 mM), propionate (10 mM), butyrate (10 mM), hexanoate (3 mM), methanol (10 mM), ethanol (10 mM), propanol (10 mM), butanol (10 mM), pyruvate (10 mM), malate (10 mM), succinate (10 mM), fumarate (10 mM), proline (10 mM), alanine (10 mM) and glycine (10 mM). Compounds tested but not utilized with sulfate were acetate (20 mM), glycerol (10 mM), glucose (1 g l^{-1}), fructose (1 g l^{-1}), glutarate (10 mM), serine (10 mM), betaine (10 mM), choline (10 mM), sorbitol (5 mM), nicotinate (1 mM), casein (0·05 g l^{-1}) and yeast extract (0·05 g l^{-1}). Fermentative growth was observed with pyruvate (20 mM), but not with lactate (20 mM), malate (20 mM), fumarate (20 mM), glucose (1 g l^{-1}) or fructose (1 g l^{-1}).

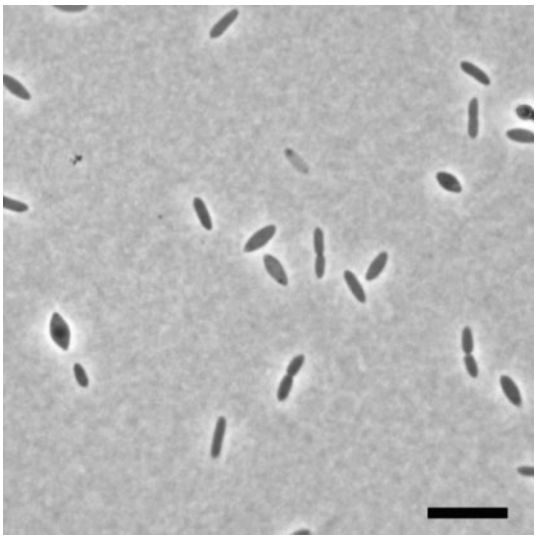

Fig. 1. Phase-contrast micrograph of *Desulfotomaculum arcticum* strain 15^T. Bar, 10 μm.

The pH optimum of strain 15^T was 7·1–7·5 and growth occurred over the range of pH 6·8–7·5. The strain grew at NaCl concentrations of 0·05–4·5 % and best at 1–1·5 %; the optimum concentration for $MgCl_2.6H_2O$ was 0·4 % and the growth range was 0·4–2·5 %. Optimum growth of strain 15^T occurred at 44 °C and growth was observed between 26 and 46·5 °C. Strain 15^T did not grow at 4, 10, 20 or 25 °C. The growth rate of strain 15^T with sulfate and lactate at 41 °C was 0·046 h^{-1}. Cells of strain 15^T contained MK-7 as sole menaquinone, as determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The DNA G + C content was 48·9 mol% (determined by the DSMZ).

Strain 15^T was closely related to *Desulfotomaculum thermosapovorans* (93·5 % 16S rRNA gene sequence similarity), *Desulfotomaculum sapomandens* (93·4 %), *Desulfotomaculum gibsoniae* (93·3 %) and *Desulfotomaculum geothermicum* (93·2 %) (Fig. 2), and shares important physiological characteristics with these species (Table 1). These include the usage of sulfate, sulfite and thiosulfate as electron acceptors and formate, hydrogen, butyrate, ethanol, butanol, propanol, malate, fumarate and pyruvate as electron donors (Cord-Ruwisch & Garcia, 1985; Daumas *et al.*, 1988; Fardeau *et al.*, 1995; Kuever *et al.*, 1999). The species are easily distinguished by their differences in temperature tolerance and usage of other electron donors and acceptors (Table 1).

Desulfotomaculum antarcticum was isolated from a pond sediment sample of the Antarctic (Iizuka *et al.*, 1969). This bacterium was described as having a significantly lower temperature optimum for growth (20–30 °C) than our novel strain 15^T (44 °C). However, the authors did not test for growth at *in situ* temperatures and today the strain is considered as lost (Stackebrandt *et al.*, 1997). Due to the moderately thermophilic growth range, 26–46·5 °C, strain 15^T apparently could not multiply at *in situ* temperatures of permanently cold Arctic sediments, where the temperature never exceeds 4 °C. During laboratory experiments with fjord sediments of Svalbard, sulfate reduction in the thermophilic temperature range (40–60 °C) has been observed (M. Nickel, personal communication). The experiments showed that sulfate reduction rates of fresh and pasteurized sediment exceed the *in situ* rates after a lag phase of 24 h (probably due to the germination of spores). This suggests that our isolate belongs to a significant number of thermophilic sulfate-reducing bacteria present as spores in the sediments. In cold sediments of Aarhus Bay (Denmark), thermophilic populations of sulfate-reducing and aerobic respiring bacteria have been found with optimum temperatures for thermophilic sulfate reduction of 60 °C and for aerobic

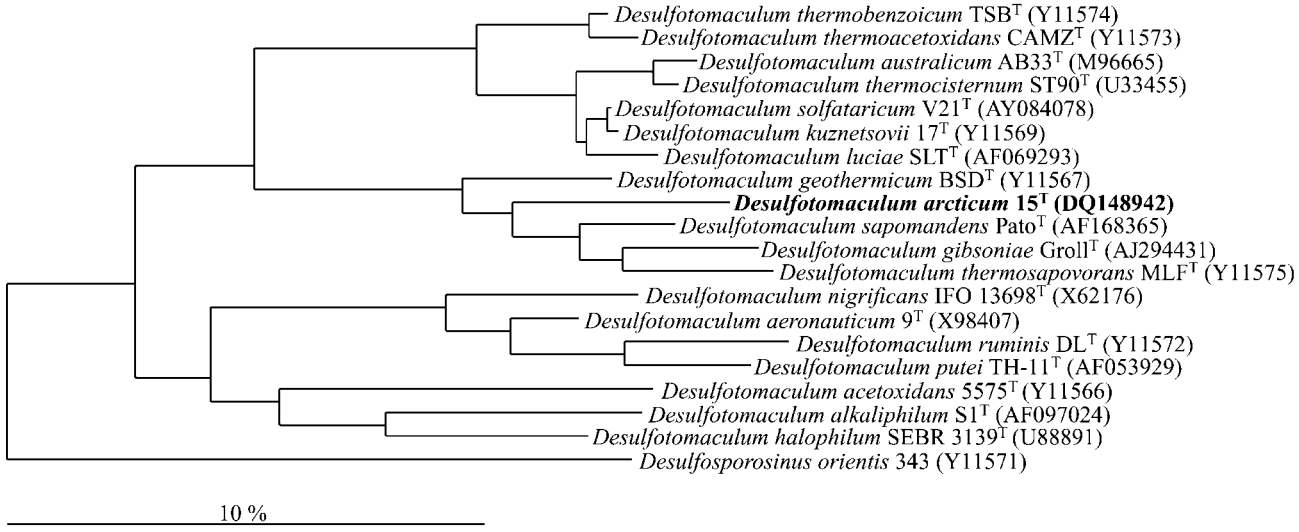

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain 15^T and its closest relatives. The tree was calculated by maximum-likelihood analysis. Bar, 10 % estimated sequence divergence.

respiration of 55 °C (Isaksen *et al.*, 1994; Thamdrup *et al.*, 1998). From most probable number enumeration at 60 °C with propionate, a population density of thermophilic sulfate reducers of $2{\cdot}8 \times 10^4$ cells cm^{-3} for the Aarhus Bay sediment was estimated and a thermophilic spore-forming bacterium with similarities to *Desulfotomaculum kuznetsovii* was isolated (Isaksen *et al.*, 1994). Aerobic and sulfate-reducing, spore-forming bacteria with minimum growth temperatures considerably above *in situ* temperatures have been isolated from permanently cold habitats, such as deep-sea sediments and permafrost soil (Bartholomew & Paik, 1966; Barnes *et al.*, 1998; Vainshtein *et al.*, 1995). The origin of the different thermophilic populations have been proposed to be geothermal environments or anthropogenic sources from which the spores were dispersed by wind or terrestrial river run-off (Bartholomew & Paik, 1966; Isaksen *et al.*, 1994; Thamdrup *et al.*, 1998). We do not know the growth habitat of our isolates.

Table 1. Comparison of the characteristics of *Desulfotomaculum arcticum* 15^T and closely related species

Strains/species: 1, *D. arcticum* sp. nov. 15^T; 2, *D. thermosapovorans*; 3, *D. sapomandens*; 4, *D. gibsoniae*; 5, *D. geothermicum*. Data from Fardeau *et al.* (1995), Cord-Ruwisch & Garcia (1985), Kuever *et al.* (1999) and Daumas *et al.* (1988). ND, Not determined; +, substrate used for growth, −, substrate not used for growth. Not all electron donors and acceptors used by the species are listed in this table.

Characteristic	1	2	3	4	5
Temperature optimum (°C)	44	50	38	35–37	54
Temperature range (°C)	26–46·5	35–60	20–43	20–42	37–57
Electron acceptors:					
Thiosulfate	+	+	+	+	ND
Elemental sulfur	−	−	+	−	−
Electron donors:					
Acetate	−	−	+	−	−
Lactate	+	+	−	−	+
Propionate	+	−	−	+	+
Methanol	+	+	ND	−	−
Succinate	+	−	+	+	ND
Fructose	−	−	−	−	+
DNA G+C content (mol%)	48·9	51·2	48·0	54·8	50·4

Description of *Desulfotomaculum arcticum* sp. nov.

Desulfotomaculum arcticum (arc′ti.cum. L. neut. adj. *arcticum* from the Arctic, referring to the place where the type strain was isolated).

Cells are rod-shaped, endospore-forming, $2–3 \times 1$ μm in size, strictly anaerobic. No vitamins are required for growth. Sulfate, thiosulfate and sulfite serve as electron acceptors. Oxidation of hydrogen, formate, lactate, propionate, butyrate, hexanoate, methanol, ethanol, propanol, butanol, pyruvate, malate, succinate, fumarate, proline, alanine and glycine occurs in the presence of sulfate. Ferments pyruvate. pH range of growth is 6·8–7·5, optimum pH 7·1–7·5. Temperature range for growth is 26–46·5 °C, optimum temperature 44 °C. The DNA G+C content is 48·9 mol%.

The type strain, strain 15^T (=DSM 17038^T=JCM 12923^T), was isolated from a permanently cold fjord sediment of Svalbard.

Acknowledgements

We thank the Svalbard team 2001, Stig Henningsen (Captain) and John Mortensen (first mate) for the nice trip to Svalbard. We thank the Alfred-Wegener-Institute for providing laboratory space at the Koldewey Station. This project was supported by the Max Planck Society.

References

Barnes, S. P., Brandbrook, S. D., Cragg, B. A., Marchesi, J. R., Weightman, A. J., Fry, J. C. & Parkes, R. J. (1998). Isolation of sulfate-reducing bacteria from deep sediment layers of the Pacific Ocean. *Geomicrobiol J* **15**, 67–83.

Bartholomew, J. W. & Paik, G. (1966). Isolation and identification of obligate thermophilic sporeforming bacilli from ocean basin cores. *J Bacteriol* **92**, 635–638.

Buchholz-Cleven, B. E. E., Rattunde, B. & Straub, K. L. (1997). Screening for genetic diversity of isolates of anaerobic Fe(II)-oxidizing bacteria using DGGE and whole-cell hybridization. *Syst Appl Microbiol* **20**, 301–309.

Cord-Ruwisch, R. & Garcia, J.-L. (1985). Isolation and characterization of an anaerobic benzoate-degrading spore-forming sulfate-reducing bacterium, *Desulfotomaculum sapomandens* sp. nov. *FEMS Microbiol Lett* **29**, 325–330.

Daumas, S., Cord-Ruwisch, R. & Garcia, J.-L. (1988). *Desulfotomaculum geothermicum* sp. nov., a thermophilic, fatty acid-degrading, sulfate-reducing bacterium isolated with H_2 from geothermal ground water. *Antonie van Leeuwenhoek* **54**, 165–178.

Fardeau, M.-L., Ollivier, B., Patel, B. K. C., Dwivedi, P., Ragot, M. & Garcia, J.-L. (1995). Isolation and characterization of a thermophilic sulfate-reducing bacterium, *Desulfotomaculum thermosapovorans* sp. nov. *Int J Syst Bacteriol* **45**, 218–221.

Iizuka, H., Okazaki, H. & Seto, N. (1969). A new sulfate-reducing bacterium isolated from Antarctica. *J Gen Appl Microbiol* **15**, 11–18.

Isaksen, M. F., Bak, F. & Jørgensen, B. B. (1994). Thermophilic sulfate-reducing bacteria in cold marine sediments. *FEMS Microbiol Ecol* **14**, 1–8.

Kuever, J., Rainey, F. A. & Hippe, H. (1999). Description of *Desulfotomaculum* sp. Groll as *Desulfotomaculum gibsoniae* sp. nov. *Int J Syst Bacteriol* **49**, 1801–1808.

Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Sagemann, J., Jørgensen, B. B. & Greef, O. (1998). Temperature dependence and rates of sulfate reduction in cold sediments of Svalbard, Arctic Ocean. *Geomicrobiol J* **15**, 85–100.

Stackebrandt, E., Sproer, C., Rainey, F. A., Burghardt, J., Päuker, O. & Hippe, H. (1997). Phylogenetic analysis of the genus *Desulfotomaculum*: evidence for the misclassification of *Desulfotomaculum guttoideum* and description of *Desulfotomaculum orientis* as *Desulfosporosinus orientis* gen. nov., comb. nov. *Int J Syst Bacteriol* **47**, 1134–1139.

Tardy-Jacquenod, C., Magot, M., Patel, B. K. C., Matheron, R. & Caumette, P. (1998). *Desulfotomaculum halophilum* sp. nov., a halophilic sulfate-reducing bacterium isolated from oil production facilities. *Int J Syst Bacteriol* **48**, 333–338.

Thamdrup, B., Hansen, J. W. & Jørgensen, B. B. (1998). Temperature dependence of aerobic respiration in a coastal sediment. *FEMS Microbiol Ecol* **25**, 189–200.

Vainshtein, M. B., Gogotova, G. I. & Hippe, H. (1995). A sulfate-reducing bacterium from permafrost. *Mikrobiologiia* **64**, 436–439.

Widdel, F. & Bak, F. (1992). Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.