Desulfovibrio frigidus sp. nov. and *Desulfovibrio ferrireducens* sp. nov., psychrotolerant bacteria isolated from Arctic fjord sediments (Svalbard) with the ability to reduce Fe(III)

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Strains 18^T, 61^T and 77 were isolated from two permanently cold fjord sediments on the west coast of Svalbard. The three psychrotolerant strains, with temperature optima at 20–23 °C, were able to grow at the freezing point of sea water, −2 °C. The strains oxidized important fermentation products such as hydrogen, formate and lactate with sulfate as the electron acceptor. Sulfate could be replaced by sulfite, thiosulfate or elemental sulfur. Poorly crystalline and soluble Fe(III) compounds were reduced in sulfate-free medium, but no growth occurred under these conditions. In the absence of electron acceptors, fermentative growth was possible. The pH optimum for the strains was around 7·1. The DNA G+C contents were 43·3 and 42·0 mol% for strains 18^T and 61^T, respectively. Strains 18^T, 61^T and 77 were most closely related to *Desulfovibrio hydrothermalis* (95·0–95·7 % 16S rRNA gene sequence similarity). Strains 18^T and 77, exhibiting 99·9 % sequence similarity, represent a novel species for which the name *Desulfovibrio frigidus* sp. nov. is proposed. The type strain is strain 18^T (=DSM 17176^T=JCM 12924^T). Strain 61^T was closely related to strains 18^T and 77 (97·6 and 97·5 % 16S rRNA gene sequence similarity), but on the basis of DNA–DNA hybridization strain 61^T represents a novel species for which the name *Desulfovibrio ferrireducens* sp. nov. is proposed. The type strain is strain 61^T (=DSM 16995^T=JCM 12925^T).

Dissimilatory sulfate reduction is the most important anaerobic mineralization pathway in many temperate and permanently cold marine sediments (e.g. Jørgensen, 1982; Canfield *et al.*, 1993; Thamdrup & Canfield, 1996; Rysgaard *et al.*, 1998; Kostka *et al.*, 1999; Glud *et al.*, 2000). Most sulfate-reducing bacteria are phylogenetically placed within the *Deltaproteobacteria*, including the genus *Desulfovibrio*, which comprises 42 described species. A special characteristic of some *Desulfovibrio* strains is the ability to reduce Fe(III) compounds without gaining energy for growth (Coleman *et al.*, 1993; Lovley *et al.*, 1993; Li *et al.*, 2004). Here, we report the isolation of three novel psychrotolerant *Desulfovibrio*-related strains with the ability to reduce Fe(III).

Strain 61^T was isolated from an enrichment culture of artificial sea-water medium (Widdel & Bak, 1992) with approximately 30 mM poorly crystalline iron oxide, 0·4 mM $MgSO_4.7H_2O$ and 20 mM lactate at 10 °C, which was inoculated with surface sediment of Tempelfjorden, Station CD (78° 25·267′ N 17° 08·277′ E; bottom water temperature 2·8 °C). Iron oxide was replaced by ferric citrate (approx. 30 mM) for isolation in deep-agar dilution series. Cells of strain 61^T were motile vibrios and the 16S rRNA gene sequence was 95·7 % similar to the sequence of *Desulfovibrio hydrothermalis* AM13^T. The ability of *Desulfovibrio desulfuricans* to reduce Fe(III) for several consecutive transfers has been shown previously (Lovley *et al.*, 1993); however, the authors suggested that the strains grew with 0·3 mM sulfate in the medium and not by Fe(III) reduction. Correspondingly, we could not determine unequivocally whether strain 61^T grew by Fe(III) reduction or with 0·4 mM sulfate in the medium. Strains 18^T and 77 were enriched and isolated under sulfate-reducing conditions with 28 mM sulfate, 20 mM lactate and 10 mM formate at 4 and 17 °C from sediment of Tempelfjorden, Station CC (78° 26·039′ N 17° 19·722′ E; bottom water temperature 3·1 °C) and Smeerenburgfjorden, Station J (79° 42·006′ N 11° 05·199′ E; bottom water temperature 2·3 °C), respectively. 16S rRNA gene sequencing showed that strains 18^T and 77 were closely

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Desulfovibrio frigidus* strains 18^T and 77 are DQ148943 and DQ148945, and that for *Desulfovibrio ferrireducens* strain 61^T is DQ148944.

related to *Desulfovibrio hydrothermalis* and the novel strain 61^T.

The general physiological characteristics of strains 18^T, 61^T and 77 were evaluated under sulfate-reducing conditions with lactate as the electron donor in a medium with a lower salt concentration (salt-water medium) (Widdel & Bak, 1992) at their respective isolation temperature. 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 12 different temperatures between −2 and 32 °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 (in triplicate) 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). Phylogenetic positions of the three novel strains were evaluated by using the ARB program (Ludwig *et al.*, 2004) with the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms in combination with different sets of filters.

Strains 61^T and 77 showed a vibrioid or sigmoid morphology, 2·5–5·5 × 0·5–0·7 μm in size, whereas cells of strain 18^T were straight rods, 3·5–4·5 × 0·5–0·7 μm in size. Cells of all strains were motile by means of a single polar flagellum as indicated by electron microscopy (Fig. 1). Gram staining was negative for all strains.

Vitamins were not required for growth. The strains grew at sea-water concentrations of NaCl and $MgCl_2$. NaCl optima were 2–3 % for strains 18^T and 77 and 1–2·5 % for strain 61^T, and strains 18^T, 61^T and 77 grew with NaCl concentrations of 2–3·5, 0·7–4 and 1·5–4 %, respectively. $MgCl_2$ optima were 0·04–1·9, 0·02–2·5 and 0·4 % and $MgCl_2$ growth ranges were from 0·02 to 2·5, to 3·5 and to 1·9 % for strains 18^T, 61^T and 77, respectively. The pH optima were 6·9–7·2, 7·1–7·5 and 7·1 and growth was observed at pH 6·9–7·5, 6·3–7·5 and 6·7–7·5 for strains 18^T, 61^T and 77, respectively. Common end-products of fermentation such as lactate, formate and hydrogen served as electron donors (Table 1). The strains reduced sulfate and other sulfur compounds like sulfite, thiosulfate or elemental sulfur (Table 1). Reduction of ferric citrate or poorly crystalline iron oxide in sulfate-free medium was observed in two to four consecutive transfers for all three strains. Reduction of Fe(III) became slower with every transfer and we suggest that the strains did not conserve energy for growth. The ability for Fe(III) reduction was previously described for several species of *Desulfovibrio* (*Desulfovibrio desulfuricans*, *Desulfovibrio vulgaris*, *Desulfovibrio sulfodismutans*, *Desulfovibrio baarsii* and *Desulfovibrio* sp. strain G-11) as well as for *Desulfomicrobium baculatum* and *Desulfobacterium autotrophicum* (Coleman *et al.*, 1993; Lovley *et al.*, 1993; Li *et al.*, 2004). Growth by Fe(III) reduction has so far only been shown for the two sulfate-reducing bacteria *Desulfobulbus propionicus* and '*Desulfotomaculum reducens*' (Tebo & Obraztsova, 1998; Holmes *et al.*, 2004).

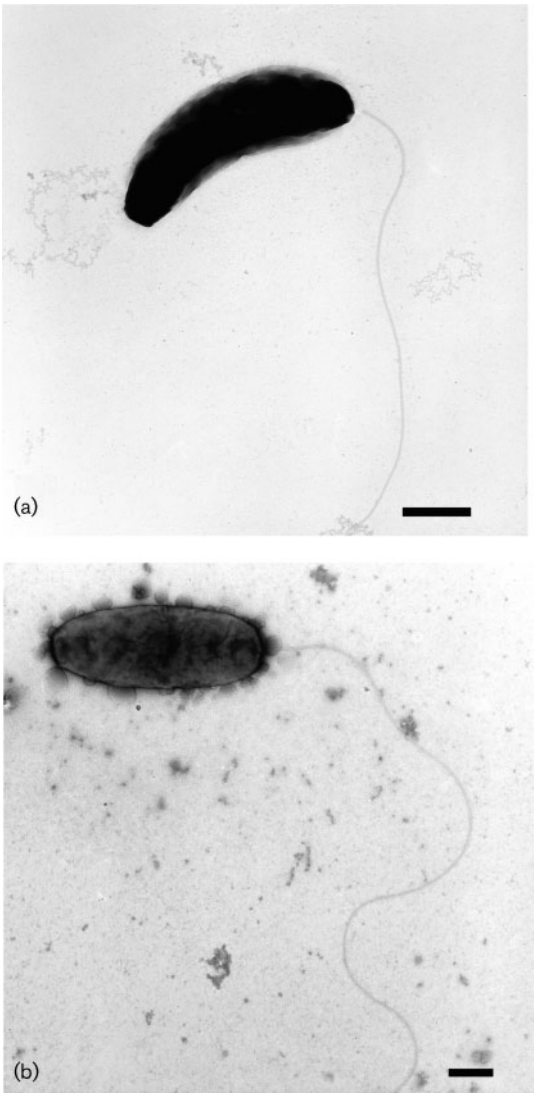

Fig. 1. Electron micrographs (negative stain with uranyl acetate) of *Desulfovibrio ferrireducens* sp. nov. 61^T (a) and *Desulfovibrio frigidus* sp. nov. 18^T (b), showing the sigmoid shape of strain 61^T and the straight rod shape of strain 18^T. Cells of both strains are motile by a single monopolar flagellum. Bars, 0·5 μm.

Although isolated at different temperatures (4, 10 and 17 °C), all three strains showed similar temperature optima for growth at 20–23 °C (Table 1) and were able to grow at the freezing point of sea water, −2 °C. According to their

Table 1. Comparison of the characteristics of *Desulfovibrio ferrireducens* strain 61^T, *Desulfovibrio frigidus* strains 18^T and 77 and closely related species

Strains/species: 1, *Desulfovibrio ferrireducens* sp. nov. 61^T; 2, *Desulfovibrio frigidus* sp. nov. 18^T; 3, *Desulfovibrio frigidus* sp. nov. 77; 4, *Desulfovibrio hydrothermalis*; 5, *Desulfovibrio zosterae*; 6, *Desulfovibrio salexigens*. Data from Postgate & Campbell (1966), Postgate (1984), Zellner *et al.* (1989), Nielsen *et al.* (1999) and Alazard *et al.* (2003). +, Substrate used for growth; −, substrate not used for growth; +/−, substrate reduced but no growth; (+), substrate poorly utilized; ND, not determined. Electron acceptors tested but not reduced by strains 18^T, 61^T and 77: nitrate (20 mM), nitrite (10 mM), oxygen (air), malate (20 mM), fumarate (20 mM) and manganese oxide (approx. 30 mM). Electron donors tested but not oxidized: acetate (20 mM), butyrate (10 mM), propionate (10 mM), hexanoate (3 mM), malate (10 mM), butanol (10 mM), pyruvate (10 mM), fructose (1 g l^{-1}), glucose (1 g l^{-1}), glycerol (10 mM), glycine (10 mM), glutarate (10 mM), serine (10 mM), proline (10 mM), betaine (10 mM), sorbitol (5 mM), nicotinate (1 mM), yeast extract (0·05 g l^{-1}), casein (0·05 g l^{-1}) and choline chloride (10 mM). Substrates tested for disproportionation but not used: lactate and fructose.

Strain	1	2	3	4	5	6
Temperature range (°C)	−2 to 30	−2 to 25	−2 to 26	20–40	Up to 34·5	Up to 42–45
Temperature optimum (°C)	23	20–23	21–22	35	32·5–34·5	34–37
Electron acceptors:						
Thiosulfate (10 mM)	+	−	−	+	+	ND
Sulfite (2 mM)	+	+	+	+	+	ND
Elemental sulfur	−	+	−	−	+	ND
Fe(III) citrate or oxide (approx. 30 mM)	+/−	+/−	+/−	ND	ND	ND
Electron donors:						
Formate (10 mM)	+	+	+	+	−	+
Hydrogen (H_2/CO_2; 80 : 20, v/v)	+	+	+	+	−	+
Propanol (10 mM)	+	+	−	−	−	+
Succinate (10 mM)	+	−	−	−	−	+
Alanine (10 mM)	−	+	+	−	+	ND
Pyruvate (10 mM)	−	−	−	+	+	+
Malate (10 mM)	−	−	−	+	+	+
Choline (10 mM)	−	−	−	+	+	+
Glycerol (1 g l^{-1})	−	−	−	+	ND	+
Fructose (1 g l^{-1})	−	−	−	−	+	ND
Disproportionation:						
Malate (10 mM)	+	+	−	ND	−	−
Pyruvate (10 mM)	+	−	+	+	+	−
Fumarate (10 mM)	+	+	−	(+)	+	ND
Glucose (1 g l^{-1})	−	−	+	ND	−	ND
Fructose (1 g l^{-1})	−	−	−	−	+	ND
DNA G+C content (mol%)	42·0	43·3	ND	47	42·7	45·5

temperature range, the strains can be considered as psychrotolerant.

The DNA G+C contents were 42·0 and 43·3 mol% for strains 61^T and 18^T, respectively (Table 1), and were determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. Strains 18^T and 77 were closely related to each other (99·9 % 16S rRNA gene sequence similarity) (Fig. 2), therefore we suggest that these two strains belong to the same species. 16S rRNA gene sequence similarity was 97·6 % between strains 61^T and 18^T and 97·5 % between strains 61^T and 77 (Fig. 2). DNA–DNA hybridization was done by the DSMZ and DNA–DNA relatedness was 14·5 % between strains 61^T and 18^T and 18·3 % between strains 61^T and 77. Therefore, we propose the description of two novel species: *Desulfovibrio ferrireducens* (type strain 61^T) and *Desulfovibrio frigidus* (type strain 18^T). Both strains 61^T and 18^T are closely related to the undescribed *Desulfovibrio* sp. strain Aspo3 (respectively 97·4 and 95·4 % 16S rRNA gene sequence similarity) isolated from subterranean groundwater (Pedersen *et al.*, 1996), as well as to *Desulfovibrio hydrothermalis* (95·7 and 95·0 %) isolated from a deep-sea hydrothermal chimney (Alazard *et al.*, 2003), *Desulfovibrio zosterae* (94·8 and 94·3 %) isolated from marine seagrass roots (Nielsen *et al.*, 1999) and *Desulfovibrio salexigens* (94·6 and 95 %) (Fig. 2).

The isolated strains and their closest relatives, *Desulfovibrio hydrothermalis*, *Desulfovibrio zosterae* and *Desulfovibrio salexigens*, share the ability to reduce sulfate and use lactate, ethanol, fumarate, formate plus acetate and hydrogen plus acetate as electron donors. They can be distinguished by their substrate usage and additionally by their temperature ranges of growth (Table 1). *Desulfovibrio hydrothermalis*,

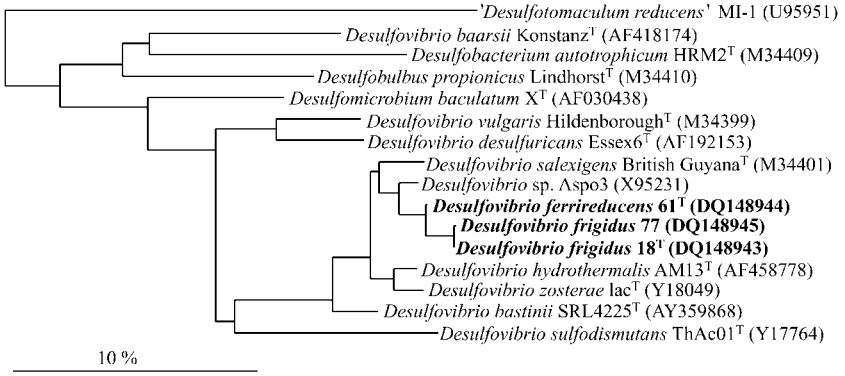

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the position of the isolated strains 18^T, 61^T and 77 within the genus *Desulfovibrio* and in relation to other sulfate-reducing bacteria with the ability to reduce Fe(III). The tree was calculated using maximum-likelihood algorithm with a 50 % filter for *Deltaproteobacteria*. Bar, 10 % estimated sequence divergence.

Desulfovibrio zosterae and *Desulfovibrio salexigens* are mesophiles with temperature optima at 33–37 °C (Postgate, 1984; Nielsen *et al.*, 1999; Alazard *et al.*, 2003), whereas strains 18^T, 61^T and 77 are psychrotolerant (temperature optima at 20–23 °C) and able to grow at −2 °C. As the three strains were isolated from fjord sediments with temperatures of 2–3 °C at the time of sampling, they are able to grow at the permanently low *in situ* temperature of Svalbard sediments.

Description of *Desulfovibrio ferrireducens* sp. nov.

Desulfovibrio ferrireducens [fer.ri.re.du′cens. L. n. *ferrum* iron; L. part. adj. *reducens* leading back, bringing back and in chemistry converting to a different oxidation state; N.L. part. adj. *ferrireducens* reducing Fe(III) to Fe(II)].

Cells are vibrioid or sigmoid, 2·5–5·5 × 0·7 μm in size, motile by a single polar flagellum. Gram-negative. No vitamins are required for growth. Lactate, formate, hydrogen, ethanol, propanol, fumarate and succinate are oxidized with sulfate reduction. Sulfate, thiosulfate and sulfite serve as electron acceptors. Iron compounds [Fe(III) oxide and Fe(III) citrate] are reduced without growth. Disproportionation of malate, pyruvate and fumarate. Optimum NaCl concentration is 1–2·5 %, and growth occurs between 0·7 and 4 % NaCl; for $MgCl_2$ the optimum concentration is between 0·02 and 2·5 % and growth occurs up to a concentration of 3·5 %. pH optimum is 7·1–7·5 and pH range is 6·3–7·5. Temperature optimum is 23 °C and growth range is between −2 and 30 °C. The DNA G+C content is 42·0 mol%.

The type strain, 61^T (=DSM 16995^T=JCM 12925^T), was isolated from a permanently cold sediment of the west coast of Svalbard.

Description of *Desulfovibrio frigidus* sp. nov.

Desulfovibrio frigidus (fri′gi.dus. L. masc. adj. *frigidus* cold, referring to growth in the permanently cold sediment of Svalbard).

Cells are rod-shaped or vibrioid, 2–5 × 0·7 μm in size, motile by a single polar flagellum. Gram-negative. No vitamins are required for growth. Lactate, formate, hydrogen, ethanol, fumarate and alanine are oxidized with sulfate reduction; one strain oxidizes propanol. Sulfate and sulfite serve as electron acceptors; one strain reduces elemental sulfur. Iron compounds [Fe(III) oxide and Fe(III) citrate] are reduced without growth. Disproportionation of malate, pyruvate, fumarate and glucose is possible for one or the other strain. Growth range for NaCl and $MgCl_2$ is different for the two strains, but the optimum NaCl concentration is 2–3 %, and growth occurs at 2–3·5 % NaCl; for $MgCl_2$ the optimum concentration is around 0·4 % and growth occurs up to a concentration of 1·9 %. pH optimum is 7·1 and pH range is 6·9–7·5. Temperature optimum is at 20–23 °C and growth range is from −2 to 25 °C. The DNA G+C content is 43·3 mol%.

Strain 18^T (=DSM 17176^T=JCM 12924^T) is the type strain. Strain 77 is a second strain of the species. Both strains were isolated from a permanently cold sediment at the west coast of Svalbard.

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