Desulfovibrio arcticus sp. nov., a psychrotolerant sulfate-reducing bacterium from a cryopeg

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A psychrotolerant sulfate-reducing bacterium, designated B15^T, was isolated from supercooled water brine from within permafrost of the Varandey Peninsula, on the southern coast of the Barents Sea. Cells were Gram-negative, motile vibrios (3.0–4.0×0.4–0.5 µm) with a single polar flagellum. The isolate was positive for desulfovibrin as a bisulfite reductase. Strain B15^T grew at –2 to 28 °C (optimum 24 °C) and with 0–2.0 % NaCl (optimum 0.2 %). The isolate used H₂ plus acetate, formate, ethanol, lactate, pyruvate and choline as electron donors and used sulfate, sulfite, thiosulfate, elemental sulfur, DMSO and Fe³⁺ as electron acceptors. Pyruvate and lactate were not fermented in the absence of sulfate. The G+C content of genomic DNA was 55.2 mol%. Analysis of the 16S rRNA gene sequence showed that the isolate belonged to the genus *Desulfovibrio*. Its closest relatives were *Desulfovibrio idahonensis* CY1^T (98.8 % 16S rRNA gene sequence similarity) and *Desulfovibrio mexicanus* Lup1^T (96.5 %). On the basis of genotypic, phenotypic and phylogenetic characteristics, the isolate represents a novel species, for which the name *Desulfovibrio arcticus* sp. nov. is proposed; the type strain is B15^T (=VKM B-2367^T=DSM 21064^T).

Cryopegs are perennially cryotic layers of unfrozen ground (a part of permafrost) in which freezing is prevented by freezing-point depression due to dissolved solids in the water (Gilichinsky *et al.*, 2005). Cryopegs are located within permafrost marine sediments of the High Arctic at different depths. Highly mineralized Arctic cryopegs have been shown to harbour diverse micro-organisms, including cold-adapted strains (Gilichinsky *et al.*, 2005; Shcherbakova *et al.*, 2005; Bakermans *et al.*, 2006). Viable bacteria of different phylogenetic and physiological groups have been found recently in cryopegs of the Varandey Peninsula, on the southern coast of the Barents Sea, Russia (Pecheritsyna *et al.*, 2007).

A cold-adapted, sulfate-reducing representative of the genus *Desulfovibrio*, designated B15^T, was isolated from the water of a cryopeg (mineralization 9.3 g l^{–1}), which was sampled at a depth of 9 m. The sample was taken during the field expedition in 2004 and kept frozen (at –20 °C)

for 2 months until analysis. The drilling system and the subsequent procedure of recovering uncontaminated material are described elsewhere (Shi *et al.*, 1997). Enrichment and isolation were performed using basal growth medium [(BGM) containing l^{–1}: 0.33 g KH₂PO₄, 0.33 g KCl, 5.0 g NaCl, 0.33 g NH₄Cl, 0.33 g MgCl₂·6H₂O, 0.33 g CaCl₂, 4.0 g Na₂SO₄, 0.002 g resazurin, 10 ml trace element solution SL-10 (medium 320; DSMZ) and 5 ml vitamin solution (Wolin *et al.*, 1963); supplemented prior to inoculation with 25 ml NaHCO₃ (10 %, w/v), 2.5 ml HCl (25 %, v/v) and 10 ml sodium lactate (20 %, w/v) per litre]. For enrichment and isolation, the tenfold serial dilution method was used, as described previously (Pecheritsyna *et al.*, 2007). Culture purity was assessed by observing uniform cell types under a phase-contrast microscope and the absence of growth in BGM with 1 g glucose and 1 g peptone l^{–1}. The pure culture was maintained at 24 °C in modified BGM (0.2 % NaCl). *Desulfovibrio idahonensis* CY1^T (=DSM 15450^T) and *Desulfovibrio mexicanus* Lup1^T (=DSM 13116^T), used as reference strains, were grown in DSMZ 63 and 860 media, respectively.

Cells were examined by phase-contrast and electron microscopy as described previously (Shcherbakova *et al.*, 2005). Cells of strain B15^T were motile vibrios (3.0–4.0×0.4–0.5 µm), each with a single polar flagellum (Supplementary

Abbreviation: AQDS, anthraquinone-2,6-disulfonate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B15^T is DQ296030.

Three supplementary figures and a supplementary table are available with the online version of this paper.

Fig. S1, available in IJSEM Online). The Gram reaction was negative and electron microscopy of sectioned cells revealed a typical Gram-negative cell wall (Supplementary Fig. S1a). Spore formation was not observed.

Strain B15^T was tested for production of desulfoviridin (Postgate, 1959) with *Desulfovibrio desulfuricans* VKM B-1799^T and *Escherichia coli* K-12 as positive and negative controls, respectively. Cytochrome *c* was analysed by recording the redox difference and CO difference spectra as described by Widdel (1980). The spectra of the cell extracts exhibited the characteristic absorption bands of desulfoviridin and cytochrome *c* at 631 nm and 552 nm, respectively. Acetate was detected as a product of incomplete lactate oxidation by GC (Shcherbakova *et al.*, 2005).

Conditions for growth of strain B15^T and reference strains were tested in their respective cultivation media supplemented with 20 mM sodium lactate and 20 mM sulfate, unless otherwise stated. Electron donors were tested with 20 mM sulfate as a terminal electron acceptor and electron acceptors were tested with 20 mM sodium lactate as an electron donor. All tests were performed in triplicate and confirmed by two transfers. Growth was assessed by measuring the changes in OD₅₈₀ and hydrogen sulfide production (Cline, 1969). Fe³⁺ (90 mmol l⁻¹) was added as amorphous iron (III) oxide, prepared by titration of acidic FeCl₃ solution with 10% (w/v) NaOH to pH 7.0. Reduction of Fe³⁺ and anthraquinone-2,6-disulfonate (AQDS) was studied as described by Lovley & Phillips (1986) and Lovley *et al.* (1996). The effect of temperature was examined at -2, 5, 10, 18, 24, 28, 37, 42 and 48 °C. The incubation at -2 °C was carried out in a cryobath filled with ethylene glycol. The effect of pH was examined at pH 5.5–8.5 (at intervals of 0.5 pH units) with the pH adjusted with sterile anaerobic 1 M HCl, 10% (w/v) NaHCO₃ or 8% (w/v) Na₂CO₃. The effect of NaCl was examined in DSMZ 63 containing 0, 0.1, 0.2, 0.3, 0.5, 1.0, 2.0, 3.0 and 5.0% (w/v) NaCl.

Strain B15^T used (mM, unless otherwise stated) H₂ plus acetate (20), formate (10), lactate (20), ethanol (10), pyruvate (10) and choline (10) as electron donors in the presence of sulfate, but did not oxidize propionate (10), butyrate (10), valerate (10), methanol (10), acetate (20), propanol (10), butanol (10), glycerol (10), glucose (1 g l⁻¹), fructose (1 g l⁻¹), glutamate (10), serine (10), betaine (10), sorbitol (5), malate (10), succinate (10), fumarate (10), proline (10), alanine (10), cysteine (10), glycine (10), Casamino acids (2 g l⁻¹) or yeast extract (0.5 g l⁻¹). Fermentative growth was not observed with pyruvate (20), lactate (20), malate (20), fumarate (20), cysteine (10), Casamino acids (1 g l⁻¹), peptone (1 g l⁻¹) or glucose (1 g l⁻¹). Strain B15^T did not require any vitamins for growth. Yeast extract (0.1–0.5 g l⁻¹) greatly stimulated growth.

The characteristics of strain B15^T and the reference strains are given in Table 1. Strain B15^T and the reference strains used lactate, formate and H₂ plus acetate as electron donors in the presence of sulfate and used sulfate (20), sulfite (2),

thiosulfate (10), DMSO (10) and elemental sulfur as electron acceptors in the presence of lactate. Strain B15^T and *D. idahonensis* CY1^T reduced Fe³⁺ without visible growth. Strain B15^T and *D. mexicanus* Lup1^T did not use malate (20), fumarate (20) or AQDS (4) as electron acceptors. In contrast to *D. idahonensis* DSM 15450^T, strain B15^T did not

Table 1. Differential characteristics of strain B15^T and its closest phylogenetic relatives

Strains: 1, *Desulfovibrio arcticus* sp. nov. B15^T; 2, *D. idahonensis* CY1^T; 3, *D. mexicanus* Lup1^T. All data were taken from this study. All strains used H₂ plus acetate, formate, lactate and pyruvate as electron donors with sulfate and used sulfate, sulfite, thiosulfate, DMSO and elemental sulfur as electron acceptors with lactate. None of the strains used acetate, propionate, butyrate, valerate, benzoate, methanol, propanol, butanol, glycerol, ethylene glycol, sorbitol or glutamate as electron donors or used nitrate and nitrite as electron acceptors.

Characteristic	1	2	3
Cell dimensions (µm)			
Length	3.0–4.0	1.3–2.5	1.7–2.5
Width	0.4–0.5	0.6	0.5
Flagella*	SP	SP	–
Temperature for growth (°C)			
Range	–2 to 28	5–42	18–42
Optimum	24	28	37
NaCl for growth (%)			
Range	0–2.0	0–1.0	0–1.0
Optimum	0.2	0.1	0
Optimum pH for growth	6.7–7.0	6.5	7.2
Electron donors with sulphate:			
Fumarate	–	+	–
Malate	–	+	–
Succinate	–	+	–
Ethanol	+	–	–
Serine	–	–	+
Cysteine	–	–	+
Choline	+	–	+
Alanine	–	–	+
Casamino acids	–	–	+
Yeast extract	–	–	+
Substrates fermented:			
Pyruvate	–	+	+
Lactate	–	–	+
Electron acceptors with lactate:			
Fumarate	–	+	–
AQDS	–	+	–
Fe ³⁺	+	+	–
DNA G + C content (mol%) (T _m)	55.2	63.2 (63.5)†	64.0 (66)‡

*SP, Single polar.

†Data in parentheses from Sass *et al.* (2009) (HPLC method).

‡Data in parentheses from Hernandez-Eugenio *et al.* (2000) (HPLC method).

grow with fumarate, malate or succinate but grew with ethanol and choline.

The temperature range (-2 to 28 °C) and optimum (24 °C) for growth of strain B15^T differed from those of the reference strains (Supplementary Fig. S2, available in IJSEM Online). Strain B15^T grew at pH 6.0–8.0 (optimum pH 6.7–7.0) and with ≤ 2.0 % NaCl. The reference strains had lower ranges of NaCl concentration for growth than strain B15^T and were mesophilic (optima of 28 and 37 °C for strains CY1^T and Lup1^T, respectively), whereas strain B15^T was psychro-tolerant (growth at -2 °C; optimum 24 °C).

Cellular fatty acids and menaquinones were determined using cells grown at the optimal temperature in DSMZ 63 and harvested during the late exponential growth phase. Lipid extraction, esterification, purification and quantification by GC were performed as described previously (Shcherbakova *et al.*, 2005). Menaquinones were analysed following the procedure of Collins (1985). The fatty acid compositions of strain B15^T and the reference strains (Supplementary Table S1, available in IJSEM Online) showed the presence of iso- $C_{17:1}$, which is characteristic of the family *Desulfovibrionaceae*. The dominant fatty acids of strain B15^T and the reference strains were $C_{16:1\omega7C}$, $C_{16:0}$ and anteiso- $C_{15:0}$. The major menaquinone in the isolate and the reference strains was MK-6(H_2).

DNA was isolated from cell biomass according to Marmur (1961). DNA–DNA hybridization (four replications) was performed as described by De Ley *et al.* (1970) and modified by Huß *et al.* (1983) using a Pye Unicam SP 1800 spectrophotometer equipped with a thermoprogrammer and hermetically sealed thermocuvettes. The standard deviations of the hybridization experiments were between 5.5 and 9.0 %. DNA–DNA relatedness values of strain B15^T with *D. idahonensis* DSM 15450^T and *D. mexicanus* DSM 13116^T were 38 and 32 %, respectively, and that between *D.*

idahonensis DSM 15450^T and *D. mexicanus* DSM 13116^T was 29 %. The DNA G + C content (T_m method) of strain B15^T, *D. idahonensis* DSM 15450^T and *D. mexicanus* DSM 13116^T was 55.2 ± 0.5 , 63.2 ± 0.5 and 64.0 ± 0.6 mol%, respectively. The DNA G + C content measured for *D. idahonensis* DSM 15450^T was close to the value (63.5 mol%) determined by Sass *et al.* (2009), whereas that for *D. mexicanus* DSM 13116^T differed from the value (66 mol%) reported in the original species description (Hernandez-Eugenio *et al.*, 2000).

For amplification and sequencing of the 16S rRNA gene, DNA was extracted by the method described by Sambrook *et al.* (1989). The 16S rRNA gene was amplified using the prokaryotic 16S rRNA primers 27F (5'-AGAGTTTGATCC-TGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTA-CGATT-3'). PCR products were purified using the Wizard PCR Preps DNA Purification system (Promega). Sequencing reactions were performed using a CEQ Dye Terminator Cycle Sequencing kit and analysed using a CEQ2000 XL automatic sequencer (Beckman Coulter), according to the manufacturer's instructions. A large fragment (1425 bp) of the 16S rRNA gene sequence was obtained from strain B15^T. The NCBI BLAST utility (Altschul *et al.*, 1997; Benson *et al.*, 1998) was used to identify the closest known relatives of strain B15^T. Nucleotide sequences were aligned in CLUSTAL X (Chenna *et al.*, 2003; Thompson *et al.*, 1997). TREECON (Van de Peer & De Wachter, 1994) and MEGA version 4 (Tamura *et al.*, 2007) were used for phylogenetic analysis with the neighbour-joining method (Saitou & Nei, 1987). The percentages of replicate trees where associated taxa formed the same clusters were calculated from 1000 replicates using the bootstrap test (Felsenstein, 1985). Evolutionary distances were computed using the Jukes–Cantor method (Juke & Cantor, 1969). The neighbour-joining tree (Fig. 1) and the maximum-likelihood tree (Supplementary Fig. S3, available in IJSEM Online) had similar topologies; the only difference

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain B15^T in the genus *Desulfovibrio*. Bootstrap values (>50 %) based on 1000 replicates are shown at branch nodes. *Escherichia coli* K-12 MG1655 (accession no. L10328) was used as the outgroup (not shown). Bar, 0.02 substitutions per nucleotide position.

was the position of *Desulfovibrio halophilus* SL 8903^T. The closest relatives of strain B15^T were *D. idahonensis* CY1^T (98.8% 16S rRNA gene sequence similarity) and *D. mexicanus* Lup1^T (96.5%).

Dissimilatory sulfate reduction is the most important anaerobic mineralization pathway in cold marine habitats (Jørgensen, 1982; Knoblauch *et al.*, 1999a). Recently, psychrophilic and psychrotolerant sulfate-reducing bacteria from permanently cold habitats have been described as representatives of new taxa (Knoblauch *et al.*, 1999b; Vandieken *et al.*, 2006a, b; Vatsurina *et al.*, 2008). Arctic cryopegs are characterized by a high sulfate content (0.62–3.84 g l⁻¹), but our previous attempts to isolate sulfate-reducing bacteria from this habitat have failed. Strain B15^T is the first-reported sulfate-reducing bacterium from a cryopeg within the Arctic permafrost and is able to grow and generate sulfide at -2 °C.

The chemotaxonomic, genotypic and phenotypic data strongly support the recognition of strain B15^T as a representative of a novel species within the genus *Desulfovibrio*. The name *Desulfovibrio arcticus* sp. nov. is proposed.

Description of *Desulfovibrio arcticus* sp. nov.

Desulfovibrio arcticus (arc'ti.cus. L. masc. adj. *arcticus* northern, Arctic, referring to the region of isolation).

Cells are Gram-negative vibrios (3.0–4.0 × 0.4–0.5 µm) and motile by a single polar flagellum. Cells contain desulfovibrin. Utilizes H₂ plus acetate, formate, pyruvate, lactate, choline and ethanol as electron donors and carbon sources in the presence of sulfate. Pyruvate and lactate are not fermented in the absence of sulfate. Utilizes sulfate, sulfite, thiosulfate, elemental sulfur, DMSO and Fe³⁺ as terminal electron acceptors in the presence of lactate. Grows at -2 to 28 °C (optimum 24 °C), at pH 6.0–8.0 (optimum pH 6.7–7.0), but not at pH 5.5 or 8.5, and with 0–2.0% NaCl (optimum 0.2%). Growth is stimulated by the addition of 0.1–0.5 g yeast extract l⁻¹. The predominant fatty acids (>10%) are C_{16:1}ω7c, C_{16:0} and anteiso-C_{15:0}. The predominant isoprenoid quinone is MK-6(H₂).

The type strain is B15^T (=VKM B-2367^T=DSM 21064^T), isolated from a cryopeg within permafrost on the Varandey Peninsula, on the southern coast of the Barents Sea, Russia. The DNA G+C content of the type strain is 55.2 mol%.

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