# *Desulfoconvexum algidum* gen. nov., sp. nov., a psychrophilic sulfate-reducing bacterium isolated from a permanently cold marine sediment

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A sulfate-reducing bacterium, designated JHA1$^T$, was isolated from a permanently cold marine sediment sampled in an Artic fjord on the north-west coast of Svalbard. The isolate was originally enriched at 4 °C in a highly diluted liquid culture amended with hydrogen and sulfate. Strain JHA1$^T$ was a psychrophile, growing fastest between 14 and 16 °C and not growing above 20 °C. Fastest growth was found at neutral pH (pH 7.2–7.4) and at marine concentrations of NaCl (20–30 g $l^{-1}$). Phylogenetic analysis of 16S rRNA gene sequences revealed that strain JHA1$^T$ was a member of the family *Desulfobacteraceae* in the *Deltaproteobacteria*. The isolate shared 99 % 16S rRNA gene sequence similarity with an environmental sequence obtained from permanently cold Antarctic sediment. The closest recognized relatives were *Desulfobacula phenolica* DSM 3384$^T$ and *Desulfobacula toluolica* DSM 7467$^T$ (both <95 % sequence similarity). In contrast to its closest phylogenetic relatives, strain JHA1$^T$ grew chemolithoautotrophically with hydrogen as an electron donor. CO dehydrogenase activity indicated the operation of the reductive acetyl-CoA pathway for inorganic carbon assimilation. Beside differences in physiology and morphology, strain JHA1$^T$ could be distinguished chemotaxonomically from the genus *Desulfobacula* by the absence of the cellular fatty acid $C_{16:0}$ 10-methyl. Phylogenetic differentiation from other genera was further supported by DsrAB and AprBA sequence analysis. Based on the described phylogenetic and phenotypic differences between strain JHA1$^T$ and its closest relatives, the establishment of a novel genus and a novel species, *Desulfoconvexum algidum* gen. nov., sp. nov. is proposed. The type strain is JHA1$^T$ (=DSM 21856$^T$ =JCM 16085$^T$).

Anaerobic degradation of organic matter in marine sediments generally proceeds via several steps of catabolic processes which are catalysed by a variety of micro-organisms. Since sulfate occurs at a high concentration in ocean water, the dissimilatory reduction of sulfate represents the major terminal remineralization process in anoxic marine sediments (Jørgensen, 1982). The major electron donors for sulfate reduction are end products of fermentation, such as short-chain organic acids and hydrogen (Rabus *et al.*, 2000). The concentrations of these compounds in marine sediments are generally low, indicating the close coupling of fermentation and the terminal oxidation step (Finke & Jørgensen, 2008).

Most of the ocean's seafloor, including the deep ocean and polar coastal areas, exhibits permanently cold conditions. Yet, only a limited number of psychrophilic or psychrotolerant sulfate-reducing bacteria (SRB) have been isolated so far. However, rates of *in situ* sulfate reduction in permanently cold sediments at the coast of the Svalbard archipelago have been determined to be as high as those of temperate marine sediments, pointing to the occurrence of cold-adapted SRB (Sagemann *et al.*, 1998). From the same location, novel psychrophilic SRB were selectively isolated at low temperatures with acetate, lactate or propionate as the electron donors (Knoblauch & Jørgensen, 1999, Knoblauch *et al.*, 1999) and most of the described isolates

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Abbreviation: SRB, Sulfate-reducing bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, *dsrAB* and *aprBA* sequences of strain JHA1$^T$ are EF442984, JQ901403 and EF442915, respectively.

Two supplementary figures are available with the online version of this paper.

**Table 1.** Selected characteristics for differentiation of strain JHA1$^T$ from its closest relatives

Strains: 1, *Desulfoconvexum algidum* gen. nov., sp. nov. JHA1$^T$; 2, *Desulfobacula toluolica* (DSM 7467$^T$) (data from Rabus *et al.*, 1993); 3, *Desulfobacula phenolica* (DSM 3384$^T$) (Bak & Widdel, 1986); 4, *Desulfospira joergensenii* (Finster *et al.*, 1997). All strains used butyrate (5 mM) and fumarate (10 mM) as electron donors, and sulfate (28 mM) and thiosulfate (10 mM) as electron acceptors. SRWG, Sulfate reduction without growth; +, positive; −, negative; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Cell morphology | Curved, vibrioid | Oval–coccoid | Oval to curved | Curved, vibrioid |
| Cell width (μm) | 1.5 | 1.2–1.4 | 1–1.5 | 0.7–0.8 |
| Cell length (μm) | 2.0–3.5 | 1.2–2.0 | 2–3 | 1–2 |
| Motility | + | + | + | − |
| G+C content (mol%) | 46 | 42 | 41 | 49 |
| Electron donors for sulfate reduction (mM) | | | | |
| Hydrogen | + | − | − | + |
| Formate (20) | + | − | + | + |
| Acetate (20) | − | − | + | − |
| Propionate (15) | − | − | − | |
| Valerate (5) | + | − | ND | ND |
| Caproate (5) | + | − | − | ND |
| Caprate (2) | + | ND | ND | ND |
| Lactate (10) | + | − | − | + |
| Malate (10) | + | + | + | − |
| Succinate (10) | − | + | + | + |
| Pyruvate (10) | − | + | + | + |
| Ethanol (10) | + | + | + | − |
| n-Propanol (10) | + | + | + | − |
| n-Butanol (10) | + | + | + | − |
| Methanol (10) | + | − | ND | SRWG |
| Glycerol (10) | + | ND | ND | + |
| Glycine (10) | + | ND | ND | SRWG |
| Alanine (10) | + | ND | ND | SRWG |
| Serine (10) | + | ND | ND | ND |
| Betaine (10) | + | ND | ND | + |
| Choline chloride (10) | + | ND | ND | + |
| Proline (10) | + | ND | ND | + |
| Sorbitol (10) | + | ND | ND | − |
| Mannitol (5) | + | ND | ND | − |
| Benzoate (3) | + | + | + | SRWG |
| Electron acceptors (mM) | | | | |
| Sulfite (5) | − | ND | − | + |
| Sulfur | + | ND | − | + |
| Nitrate | − | ND | − | − |
| Fermentable substrates (mM) | | | | |
| Pyruvate | − | ND | ND | − |
| Malate | + | ND | ND | − |
| Lactate | − | ND | ND | − |
| Fumarate | + | ND | ND | − |
| Autotrophy | + | − | − | + |
| Conditions for growth | | | | |
| Range (°C) | 0–20 | ND | ND | 8–30 |
| Optimum (°C) | 14–16 | 28 | 28 | 26–30 |
| Optimum pH | 7.2–7.4 | 7.0–7.1 | ND | 7.4 |
| Optimum NaCl (g $l^{-1}$) | 20–30 | 20 | 20 | 12–20 |

belonged to the order *Desulfobacterales*. Further studies using molecular methods verified the presence of active and abundant SRB in several coastal sediments sampled from different fjords of the Svalbard archipelago (Sahm *et al.*, 1999; Ravenschlag *et al.*, 1999, 2000, 2001). In order to culture and identify the potentially most abundant and active lithotrophic SRB from these fjords, liquid-dilution cultures were incubated at 4 °C with hydrogen or formate as the

electron donor in combination with sulfate as the sole electron acceptor (Könneke, 2001). From dilution series with formate, seven isolates were obtained that were identified by 16S rRNA gene sequence analysis as *Desulfotalea psychrophila* or *Desulfotalea arctica*. Three lithotrophic SRB were isolated in pure culture with hydrogen as the sole electron donor. According to their 16S rRNA genes, two strains were affiliated with the *Desulfobulbaceae* within the *Deltaproteobacteria*, sharing highest identity with the psychrophilic SRB strain LSv53 (99.9 %) and an environmental 16S rRNA gene sequence (Sva0999; 97.2 %). The third pure culture, strain JHA1$^T$, was isolated from Smeerenburgfjorden and was found to be a member of the *Desulfobacteraceae*. Strain JHA1$^T$ was subjected to a phylogenetic, physiological and chemotaxonomic characterization and comparison with its closest described relatives, *Desulfobacula phenolica* DSM 3384$^T$, *Desulfobacula toluolica* DSM 7467$^T$ (both <95 % 16S rRNA gene sequence similarity) and *Desulfospira joergensenii* DSM 10085$^T$ (<93 %).

Strain JHA1$^T$ was isolated from sediment at a depth of 5 cm from a fjord on the north-west coast of Svalbard (Smeerenburgfjorden; 79° 42′ 815″ N 11° 05′ 189″ E). The bottom water temperature during sampling (July 1998) was 0 °C. Initial enrichment was performed in a 10,000-times dilution in defined carbonate-buffered saltwater medium (Widdel & Bak, 1992) with hydrogen (headspace $H_2/CO_2$ 90 : 10) as the sole electron donor. Acetate (1 mM) was provided as an alternative carbon source to bicarbonate/$CO_2$. Significant sulfide production was observed after an incubation period of 18 months at 4 °C. Strain JHA1$^T$ was isolated by the deep agar dilution technique (Widdel & Bak, 1992) in three subsequent series in autotrophic medium with hydrogen. Purity was checked by microscopic observation and finally by transferring the strain into anoxic media containing yeast extract, peptone or glucose. Growth in these complex media was not observed. Further cultivation, growth experiments and strain maintenance were carried out with an inoculum volume of 5 % (v/v) at 15 °C in the dark. Gram-staining of heat-fixed cells was performed as described by Murray *et al.* (1994). Cells of strain JHA1$^T$ were Gram-negative, motile and slightly curved vibrios (1.5 μm wide and 2.0–3.5 μm long). Formation of endospores was not observed.

Growth experiments were performed in duplicates and monitored by sulfide formation (Cord-Ruwisch, 1985) and/or by determination of cellular protein concentration (Bradford, 1976). Growth rates were calculated from linear regression of protein production over time. The effect of NaCl concentration on growth was determined for the range 1–50 g NaCl $l^{-1}$. The highest growth rates were found with 20–30 g NaCl $l^{-1}$ and no growth was observed with <5 g NaCl $l^{-1}$. Growth was tested at pH 5.5–8.5. The highest growth rates were found at pH 7.2–7.4. Growth at 0–35 °C was determined in a temperature-gradient block as described elsewhere (Knoblauch & Jørgensen, 1999). Strain JHA1$^T$ grew at 0–20 °C, with the highest rates at 14–16 °C, and is thus, by definition (Wiegel, 1990), a psychrophilic bacterium.

A variety of organic compounds were tested as potential electron donors for sulfate reduction. Substrates were added from sterile stock solutions at a final concentration of 2–20 mM. Hydrogen was provided ($H_2/CO_2$, 90 : 10) with 1 bar overpressure in the headspace. Acetate and lactate oxidation were determined with an HPLC system equipped with an Aminex HPX-87H ion-exclusion column (Bio-Rad) and analysed at 60 °C using 5 mM $H_2SO_4$ as the mobile phase. Spectrophotometric detection and quantification were performed at 210 nm (UVIS 204; Linear Instruments).

The psychrophilic isolate utilized a variety of organic compounds, including fatty acids, alcohols and sugars as well as amino acids (Table 1). Lactate as an electron donor was completely oxidized to $CO_2$. Strain JHA1$^T$ shared the capacity with *Desulfobacula toluolica* and *Desulfobacula phenolica* to grow with the aromatic compound benzoate as an electron donor for sulfate reduction. In contrast to both members of the genus *Desulfobacula*, strain JHA1$^T$ grew chemolithoautrophically with hydrogen as a sole electron donor and with $CO_2$/bicarbonate as a sole carbon source. Autotrophic growth was verified by transferring the culture repeatedly into medium free of any organic substrate. Activity of CO dehydrogenase in a cell-free extract of strain JHA1$^T$, measured as previously described at 20 °C (Galushko & Schink, 2000), indicated the operation of the

**Table 2.** Major cellular fatty acids of strain JHA1$^T$ and its closest relatives

Strains: 1, *Desulfoconvexum algidum* gen. nov., sp. nov. JHA1$^T$; 2, *Desulfobacula toluolica* (DSM 7467$^T$) (data from Kuever *et al.*, 2001); 3, *Desulfobacula phenolica* (DSM 3384$^T$) (Kuever *et al.*, 2001); 4, *Desulfospira joergensenii* (Finster *et al.*, 1997).

| Fatty acid (%) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| $C_{14:1}$ | – | – | 1.4 | – |
| $C_{14:0}$ | 12.7 | 8.3 | 8.7 | 13.9 |
| anteiso-$C_{15:0}$ | 0.4 | – | – | – |
| iso-$C_{15:0}$ | – | – | – | 1.8 |
| $C_{15:1}\omega 9c$ | – | – | – | – |
| $C_{15:0}$ | 0.5 | 1.6 | 2.0 | 1.3 |
| 3-OH $C_{14:0}$ | 3.3 | – | 1.3 | 1.6 |
| $C_{16:1}\omega 7c$ | 4.6 | – | – | – |
| $C_{16:1}\omega 9c$ | 40.3 | 19.7 | 16.5 | 38.9 |
| $C_{16:1}\omega 11c$ | 1.8 | – | – | – |
| $C_{16:0}$ | 27.8 | 31.2 | 20.4 | 28.4 |
| $C_{16:0}$ 10-methyl | – | 17.6 | 17.2 | – |
| $C_{17:1}\omega 11c$ | – | – | – | – |
| $C_{17:0}$ cyclo | 0.8 | 2.6 | 3.9 | 2.4 |
| $C_{17:0}$ | – | 0.7 | 0.7 | 0.6 |
| $C_{16:0}$ 3-OH | – | – | – | 2.3 |
| $C_{18:1}\omega 13c$ | 1.3 | – | – | – |
| $C_{18:1}\omega 11c$ | 1.7 | 6.0 | 3.9 | 5.3 |
| $C_{18:0}$ | 1.0 | 3.1 | 2.1 | 0.7 |
| iso-$C_{19:0}$ | 1.7 | 2.5 | – | – |

reductive acetyl-CoA pathway (Wood-Ljungdahl pathway) for inorganic carbon assimilation. No activity of 2-oxoglutarate dehydrogenase was found. Sulfidogenic growth with hydrogen was also observed when sulfur or thiosulfate were provided as an alternative electron acceptor in sulfate-free medium. Strain JHA1$^T$ grew by fermentation of malate and fumarate.

Cellular fatty acid analysis was performed as described previously (Könneke & Widdel, 2003). Cells were cultured with hydrogen and sulfate and harvested in the late exponential growth phase by centrifugation. The major fatty acids of strain JHA1$^T$ were $C_{14:0}$, $C_{16:1}\omega9c$ and $C_{16.0}$, which were most like those of *Desulfospira joergensenii* (Table 2). In contrast to the two members of the genus *Desulfobacula*, strain JHA1$^T$ did not synthesize the methyl-branched fatty acid $C_{16:0}$ 10-methyl.

The G+C content of strain JHA1$^T$ was determined by HPLC at the Identification Service of the DSMZ (Braunschweig, Germany). The G+C content was 46 mol%, which is within the range of those for the genus *Desulfobacula* (41–42 mol%) and *Desulfospira joergensenii* (49 mol%).

Phylogenetic analysis of the 16S rRNA gene and the alpha and beta subunits of dissimilatory adenosine-5′-phosphosulfate reductase (AprBA) was performed as described previously (Meyer & Kuever, 2007). A large fragment of the alpha and beta subunits of the dissimilatory sulfite reductase (DsrAB) was amplified and sequenced as described previously (Loy *et al.*, 2004). Based on the 16S rRNA gene sequence analysis, strain JHA1$^T$ affiliated with the deltaproteobacterial family *Desulfobacteraceae* (Fig. 1). It was almost identical (99 % sequence similarity) to an environmental sequence obtained from coastal Antarctic sediment (Purdy *et al.*, 2003). The closest relatives with validly described names were *Desulfobacula toluolica* DSM 7467$^T$ (94.8 % sequence similarity) and *Desulfobacula phenolica* DSM 3384$^T$ (94.6 % sequence similarity). For the phylogenetic analysis of *dsrAB* and *aprBA*, only amino acid sequences generated from nearly complete gene sequences were used. A major problem is here that the database for both genes is not identical. Nevertheless, solid trees using most members of the family *Desulfobacteraceae* were constructed using the maximum-likelihood method with the WAG model in MEGA 5 (Tamura *et al.* 2011). Figs. S1 and

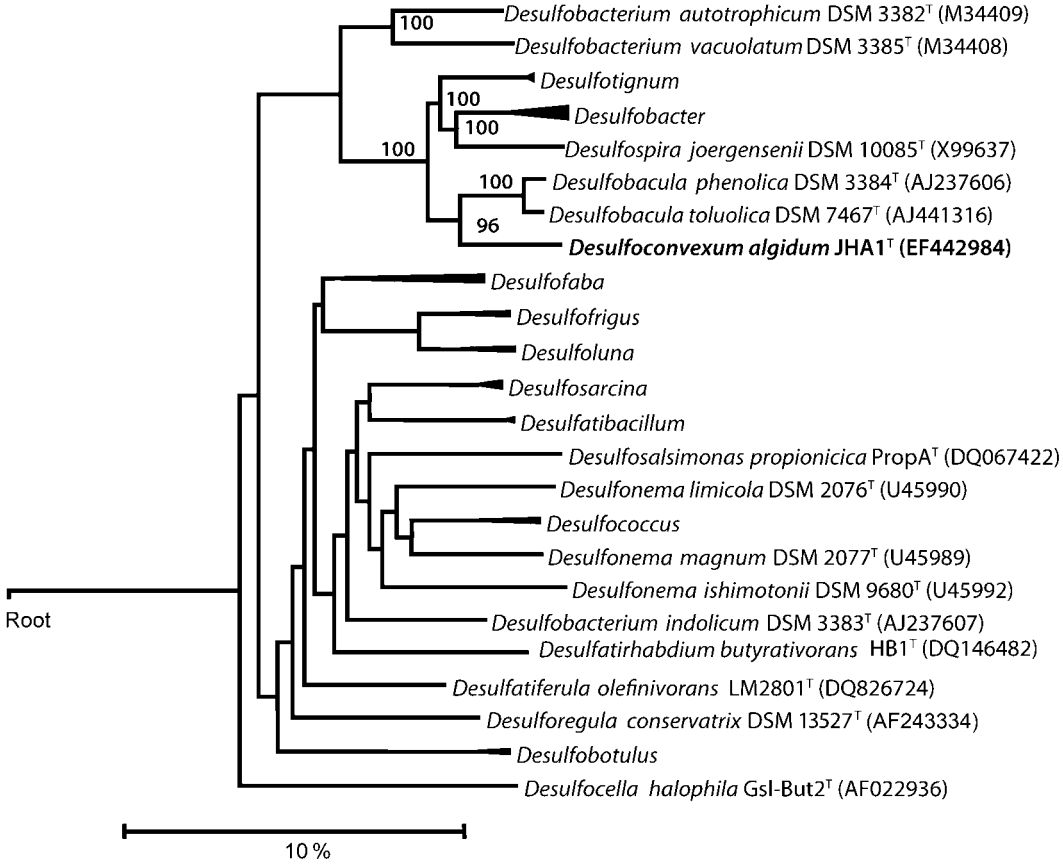


**Fig. 1.** Consensus neighbour-joining tree based on 16S rRNA gene sequences showing the affiliation of strain JHA1$^T$ to members of the family *Desulfobacteraceae* with validly published names. Bootstrap values (>70 %) based on 1000 replicates are shown at branch nodes. *Desulfobulbus propionicus* (DSM 2052$^T$; M34410) was used as an outgroup (not shown). Bar, 10 % sequence divergence.

S2 (available in IJSEM Online) show the phylogenetic trees for DsrAB and AprBA, respectively.

Both members of the genus *Desulfobacula* use various aromatic compounds and short-chain fatty acids as electron donors but not hydrogen. By using a common genus boundary of <95 % 16S rRNA gene sequence similarity (Konstantinidis & Tiedje, 2007), the 16S rRNA gene analysis of strain JHA1$^T$ suggested the establishment of a novel genus. *dsrAB* and *aprBA* sequence analysis as additional phylogenetic markers supported the separation of strain JHA1$^T$ from all other genera within the family *Desulfobacteraceae* (Figs. S1 and S2). Whereas the DsrAB analysis showed 92–93 % similarity between the isolate and the genera *Desulfospira*, *Desulfobacula* and *Desulfotignum*, the AprBA analysis showed that the isolate had a close affiliation with the genus *Desulfobacterium* (91 % sequence similarity).

Considering the morphological, physiological and chemotaxonomic differences as well as the phylogenetic distance between strain JHA1$^T$ and members of genera of the family *Desulfobacteraceae*, we propose the establishment of a novel genus, *Desulfoconvexum* gen. nov., with *Desulfoconvexum algidum* sp. nov. as the type species. Members of this novel genus have been detected in the Arctic as well in the Antarctic area demonstrating their widespread distribution in permanently cold marine sediments.

### Description of *Desulfoconvexum* gen. nov.

*Desulfoconvexum* (De.sul.fo.con.vex′um L. pref. *de* from; L. n. *sulfur* sulfur; L. n. n. *convexum* bow, curve; *Desulfoconvexum* sulfate reducer shaped like a curve).

Psychrophilic, marine sulfate-reducing bacteria belonging to the family *Desulfobacteraceae* within the delta subclass of the Proteobacteria. Cells are Gram-negative and non-spore-forming. Chemo-organoheterotrophic and chemolithoautotrophic using the CO dehydrogenase-dependent oxidative pathway and the reductive acetyl-CoA pathway, respectively. The type species is *Desulfoconvexum algidum*.

### Description of *Desulfoconvexum algidum* sp. nov.

*Desulfoconvexum algidum* (al.gi′dum. L adj. n. *algidum* ice-cold, algid, living in ice-cold conditions).

Cells are curved rods or vibrioids (1.5 μm wide and 2.0–3.5 μm long). A strictly anaerobic, marine bacterium utilizing a variety of organic compounds, including fatty acids, alcohols, sugars and aromatic compounds as well as amino acids that are oxidized completely to $CO_2$. Sulfate, thiosulfate and sulfur are used as terminal electron acceptors and are reduced to sulfide. Maximal growth rate with 20–30 g NaCl $l^{-1}$, at pH 7.2–7.4 and at 14–16 °C. Chemolithoautotrophic growth occurs with hydrogen and $CO_2$/bicarbonate. Grows by malate and fumarate fermentation. The major cellular fatty acids are $C_{14:0}$, $C_{16:1}\omega 9c$ and $C_{16.0}$.

The type strain is JHA1$^T$ (=DSM 21856$^T$ =JCM 16085$^T$), isolated from a permanently cold marine sediment of an Artic fjord on the north-west coast of Svalbard. The DNA G+C content of the type strain is 46 mol% (HPLC).

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