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Pathways of carbon oxidation in an Arctic fjord sediment (Svalbard) and isolation of psychrophilic and psychrotolerant Fe(III)-reducing bacteria

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ABSTRACT: The main mineralization pathways were determined in permanently cold fjord sediment on the west coast of Svalbard. In whole core incubations, the total oxygen uptake rate was 4.2 ± 0.4 mmol m^{-2} d^{-1} and the sulfate reduction rate 2.6 ± 0.6 mmol m^{-2} d^{-1} at 0 to 20 cm depth. Sulfate reduction was the most important anaerobic mineralization process, accounting for 57% of anaerobic organic carbon oxidation in anoxic bag incubations of the top 5 cm of the sediment. The remaining 43% oxidation was attributed to microbial Fe(III) reduction. Both processes occurred concurrently in the uppermost 2 cm, and the Fe-reducing community appeared to be limited mainly by the availability of Fe(III). Below 2 cm, sulfate reduction was the dominant electron-accepting process. Calculations for the uppermost 10 cm of the sediment yielded the following contribution of the different respiratory pathways to total carbon oxidation: aerobic respiration 53%, sulfate reduction 34%, Fe(III) reduction 13%. *In situ*, the importance of Fe(III) reduction may vary through competition for substrate with oxygen- and nitrate-reducing bacteria in the surface sediment. Fe(III)-reducing bacteria belonging to the genera *Desulfuromonas*, *Desulfuromusa*, *Shewanella* and *Desulfovibrio* were isolated from enrichment cultures of 2 fjord sediments from Svalbard. Strains related to *Desulfovibrio* reduced Fe(III) without energy generation for growth. All isolates were psychrophilic or psychrotolerant and grew at –2°C, the freezing point of sea water, indicating adaptation to permanently cold temperatures. Besides Fe(III), the strains reduced other electron acceptors such as oxygen, manganese, elemental sulfur and sulfate.

KEY WORDS: Fe reduction · Sulfate reduction · Psychrophiles · Fe(III)-reducing bacteria · Isolation · Svalbard · Sediment bag incubation

INTRODUCTION

The exploration of permanently low temperature environments is motivated by their quantitative importance, as 90% of the ocean floor permanently has temperatures below 5°C (Levitus & Boyer 1994). Benthic microbial processes are controlled, among other things, by temperature and by substrate availability. The annual primary production in southern fjords of Svalbard is estimated to be ~150 g C m^{-2} yr^{-1} and comparable to that of more southern latitudes where there is no significant ice coverage (Eilertsen et al. 1989). Thus, the organic carbon deposition to the sediment is correspondingly high. The organic carbon content in sediments along the west and south coasts of Svalbard is higher than on the north and east coasts, where the periods of ice coverage are longer, resulting in lower primary productivity (Hulth et al. 1996). The degradation of organic matter arriving at the sea floor involves a complex bacterial food web. Metabolic end-products of some bacteria can serve as substrates for others (e.g. fermentation products are substrates for Mn-, Fe- and sulfate-reducing bacteria). A sequence of respiratory processes with different inorganic electron acceptors — O_2, NO_3^-, Mn(IV), Fe(III) and SO_4^{2-} — is responsible for the complete degradation to CO_2. Studies on

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metabolic rates of different microbial processes in the sediment have shown that the rates of organic carbon turnover in Arctic coastal sediments are as high as in comparable temperate sediments (Arnosti et al. 1998, 2005, Sagemann et al. 1998, Thamdrup & Fleischer 1998, Kostka et al. 1999). Thus, the benthic microbial communities in Arctic sediments do not seem to be limited by low temperature but rather by organic carbon availability (Glud et al. 1998, Rysgaard et al. 1998).

In 3 permanently cold fjord sediments of Svalbard, sulfate reduction was found to be the dominant terminal electron accepting process (58 to 92% of total mineralization; Kostka et al. 1999). In 2 of the 3 sediments studied, microbial Fe reduction was the second most important anaerobic respiration pathway (10 and 26%), while it was below detection at the third station. In Arctic fjord sediments on the east coast of Greenland, Fe reduction accounted for 21 to 26% of total carbon oxidation (Rysgaard et al. 1998, Glud et al. 2000). These studies, together with studies in temperate coastal sediments, determined an average contribution of 17% of Fe reduction to total carbon mineralization for coastal marine sediments (reviewed by Thamdrup 2000).

Bacteria able to respire with Fe(III) are phylogenetically diverse (Lovley et al. 2004). Most species of the Geobacteraceae within the δ-Proteobacteria (including the genera *Desulfuromonas*, *Geobacter*, *Pelobacter*, *Malonomonas* and *Desulfuromusa*) are able to reduce both Fe(III) and elemental sulfur (Holmes et al. 2004b, Lovley et al. 2004). In a Svalbard fjord sediment 13% of clones in a bacterial 16S rDNA clone library were most closely related to *Desulfuromonas* species (Ravenschlag et al. 1999), indicating that this group might contribute to the iron- and sulfur-cycles in permanently cold sediment. Psychrophilic, Fe-reducing bacteria of the genus *Shewanella* within the γ-Proteobacteria have been isolated from Antarctic and Arctic sea ice as well as from Antarctic sediments (Bowman et al. 1997b, 2003, Bozal et al. 2002, Brinkmeyer et al. 2003). A contribution of sulfate-reducing bacteria of the genus *Desulfovibrio* to benthic Fe reduction was suggested from pure-culture studies and biomarker analyses of saltmarsh sediments (Coleman et al. 1993, Lovley et al. 1993, Li et al. 2004).

Fe-reducing species belonging to the Geobacteraceae, *Shewanella*, and *Desulfovibrio* can alternatively use other environmentally important electron acceptors such as oxygen, nitrate, Mn(IV) oxide, elemental sulfur and sulfate, or they may grow by fermentation. Therefore, the isolation of these bacteria from natural sediment samples does not imply Fe-reducing activity *in situ*. However, the isolation and characterization of bacteria in pure culture provides a good approach for identifying their potential activities and studying their adaptation to the ambient environmental conditions, e.g. temperature, pH or salinity. Bacteria isolated from permanently cold habitats such as sea ice or sediments have revealed adaptation to low temperatures (Bowman et al. 1997a, 2003, Knoblauch & Jørgensen 1999). Sulfate-reducing and sulfur-oxidizing bacteria with temperature optima ranging from 7 to 18°C and with the ability to grow at –2°C, the freezing point of sea water, have been isolated from fjord sediments of Svalbard (Knoblauch et al. 1999, Knittel et al. 2005).

The aim of the present study was to identify bacteria potentially contributing to the Fe- and sulfur cycles in fjord sediments on the west coast of Svalbard and to investigate the importance of Fe(III) reduction for the degradation of organic carbon in one of the fjord sediments.

MATERIALS AND METHODS

Site and sampling. Marine sediments were sampled during 3 cruises in fjords along the west coast of Svalbard. Details of the stations are given in Table 1. Enrichment and isolation were started with marine sediment from a cruise in September 2001 to Stns CC, CD and J. Anoxic bag incubation experiments with Stn J sediment were performed on the second cruise in August 2003. The concentrations of DIC, Fe^{2+}, sulfate and sulfide in the pore water and the content of solid-phase Fe and Mn were measured in a sediment core from cruises to Stn J in 2003 and 2004, while concentrations of ammonium and elemental sulfur, total oxygen uptake rates, dissolved inorganic carbon (DIC) production rates and sulfate reduction rates were measured in sediment cores from Stn J in August 2004. In

Table 1. Sampling site and Fe content at 3 stations (CC, CD, J) along west coast of Svalbard. Temperature and Fe content for Stn J are means (±SE) for the 3 yr. nd: not determined

Description	CC Tempelfjorden	CD Tempelfjorden	J Smeerenburgfjorden
Latitude	78° 26.04′ N	78° 25.27′ N	79° 42.01′ N
Longitude	17° 19.72′ E	17° 08.27′ E	11° 05.20′ E
Water depth (m)	37	64	212
Bottom-water temperature (°C)	3.1	2.8	1.5 ± 0.9
Sampling year(s)	2001	2001	2001, 2003, 2004
Avg. total Fe content (μmol cm^{-3})	nd	91.9	78.0 ± 1.8

general, the concentrations and depth distributions were similar in 2003 and 2004. Therefore, we present only the complete data set from 2004.

Sediment cores of 14 cm diameter and up to 40 cm length were retrieved by a Haps corer (Kanneworff & Nicolaisen 1973), and subcores were stored on the ship at *in situ* temperature. In laboratories in Ny Ålesund or Longyearbyen, the sediments were stored at 0°C and handled outdoors at air temperatures between 0 and 5°C to prevent warming of the sediment.

The sediment of Stn J was characterized by extensive bioturbation, apparent from abundant polychaete tubes and polychaetes in the upper 20 cm of the sediment. On the surface of the sediment, brittle stars were observed. The total organic carbon content of the sediment was 2%.

Anoxic bag incubations. For the anoxic bag incubations in 2003, sediment from Stn J was sliced into 1 cm depth intervals and transferred into gastight plastic bags under a constant stream of N_2. The bags were incubated at 0°C in the dark inside a larger N_2-filled bag to ensure anoxia. Subsamples for pore water and solid phase analyses were withdrawn 10 times from each bag over a period of 7 d.

Sampling. Pore water was squeezed by a pore water press under N_2 through GF/F filters. Pore water was filtered directly into Ferrozine-solution to measure Fe^{2+} (see later subsection). We collected 1.8 ml aliquots for DIC analysis in glass vials without headspace, and either measured these within 2 d, or fixed the samples with $HgCl_2$ and stored them at 4°C until analysis. We froze 1 to 2 ml of pore water for NH_4^+, NO_3^- and NO_2^- analysis. For Mn^{2+} and Ca^{2+} determination, 0.5 to 2 ml were acidified with 6 M HCl and stored at 4°C. Pore water for sulfate and sulfide analyses was preserved with Zn acetate or $ZnCl_2$. Sediment for analysis of reactive Fe was extracted in HCl from separate samples. For extraction of Fe and Mn with dithionite, subsamples were stored frozen at –21°C. For analysis of elemental sulfur, a subsample of 0.5 to 2 g sediment was mixed in 2 ml 20% Zn acetate and stored frozen at –21°C. Bottom water from Stn J was frozen for determination of NO_3^- and NO_2^-.

Pore water analyses. DIC was analyzed by flow injection with conductivity detection (Hall & Aller 1992). Fe(II) was measured spectrophotometrically according to Stookey (1970) with Ferrozine (1 g l^{-1} in 50 mM HEPES buffer, pH 7) at 562 nm (Shimadzu UV 1202). NO_3^- and NO_2^- were measured using a NO_x-analyzer (Thermo Environmental Instruments) (Braman & Hendrix 1989). NH_4^+ samples were analyzed spectrophotometrically at 630 nm (Shimadzu UV 1202) (Grasshoff et al. 1999). Ca^{2+} and Mn^{2+} were measured by inductively-coupled plasma atomic emission spectrometry (Perkin Elmer Optima 3300 RL). Sulfate was analyzed by non-suppressed ion chromatography (Waters, Column IC-Pak^{TM}, 50 × 4.6 mm). Sulfide was determined by the methylene blue spectrophotometric method at 670 nm (Shimadzu, UV 1202) (Cline 1969).

Solid phase analyses. Particulate Mn and Fe were quantified after extraction with dithionite-citrate-acetic acid (Canfield 1989). The extract was analyzed by flame atomic absorption spectrometry for Mn concentrations (Perkin Elmer, Atomic Absorption Spectrometer 3110). Quantification of Fe from the dithionite-citrate-acetic acid extraction resulted in a lower Fe content compared to parallel HCl extractions (0.5 M HCl for 1 h), therefore only the results of the HCl extraction are presented. The HCl-extracts were analyzed for Fe(II) with Ferrozine and for total Fe (Fe(III) + Fe(II)) with Ferrozine + 1% (w/v) hydroxylamine hydrochloride. Freeze-dried samples for determination of total organic C content were pretreated with HCl, dried, and analyzed using a CNS analyzer (Fisons^{TM} Na1500 elemental analyzer). Elemental sulfur was analyzed according to Zopfi et al. (2004). With a Zorbax ODS column (125 × 4 mm, 5 µm; Knauer), using methanol as eluent, the sulfur was determined by HPLC from absorption at 265 nm (detection limit 1 µM).

Sulfate reduction rates. Sulfate reduction rates of whole core incubations were measured in 3 parallel cores of 3 cm diameter each using the $^{35}SO_4^{2-}$ core-injection technique (Jørgensen 1978). Sulfate reduction in the anoxic bags was determined at each sampling time-point in subsamples incubated with $^{35}SO_4^{2-}$ radiotracer in 5 ml glass tubes. The incubations were stopped with Zn acetate. Total reduced inorganic sulfur was analyzed by cold chromium distillation (Kallmeyer et al. 2004).

Oxygen consumption rates. Oxygen consumption rates were measured in 2004 in 3 sediment cores with an inner diameter of 54 mm closed (without gas phase) with rubber stoppers. The cores were incubated in the dark for 42 to 46 h at 0°C with continuous stirring of the water column by a magnetic stirring bar at the top of the water column (e.g. Canfield et al. 1993a, Glud et al. 1998). Oxygen consumption of the sediment was measured during the whole incubation with a microoptode (Holst et al. 1997). Samples for DIC were taken from the water column of the core before and after oxygen measurement and fixed with $HgCl_2$. The DIC samples were analyzed on a coulometer (CM5012, CO_2 coulometer; CM5130 Acidification module, UIC Coulometrics). The volume of the water column was determined by addition of a NaBr solution. Concentrations of NaBr were analyzed by anion chromatography (Dionex DX500; eluent: 9 mM $NaCO_3$; precolumn: AG9 HC; column: AS9 HC).

Calculation of Fe^{2+} flux. From the Fe^{2+} pore water profile, the flux (J) of Fe^{2+} at 0°C was calculated by

Fick's first law of diffusion: $J = \phi\ D_S\ dC/dz$, where ϕ is porosity, D_S is the diffusion coefficient in the sediment, and C is the solute concentration at a given depth z. D_S was calculated according to Iversen & Jørgensen (1993): $D_S = D_0/[1 + 3\ (1 - \phi)]$, where D_0, the diffusion coefficient in sea water, was taken from Schulz & Zabel (2000, p. 92): $D_0\ (Fe^{2+}) = 3.15\ 10^{-6}\ cm^2\ s^{-1}$ (at 0°C). The net Fe^{2+} production was calculated from the sum of the Fe^{2+} fluxes through the upper and lower boundary of each layer.

Enrichment and isolation of pure cultures. Enrichment of bacteria was started with sediments from Stns CC, CD and J collected in 2001. Isolates of Fe-reducing bacteria were obtained in artificial sea water medium (Widdel & Bak 1992) of low sulfate concentration (0.4 mM instead of 28 mM to prevent growth of sulfate reducing bacteria). As electron donors, acetate (20 mM) or lactate (20 mM) were used, and as electron acceptor for the enrichment we used poorly crystalline Fe oxide (~30 mM), which was later replaced by Fe citrate (~30 mM) for isolation in the agar dilution series. Sulfate-reducing bacteria were enriched and isolated similar to Fe-reducing bacteria, but using sulfate (28 mM) instead of Fe(III) as electron acceptor and with lactate (20 mM) or formate (10 mM) as electron donor. Cultures were enriched, isolated, and incubated at temperatures of 4, 10 and 17°C. Growth of bacteria was indicated by the production of Fe^{2+} for Fe-reducing bacteria or the production of sulfide for sulfate-reducing bacteria.

The nucleotid sequences have been deposited in the GenBank database as follows: Strain 112: AY835388; Strain 102: AY835392; Strain 18: DQ148943; Strain 61: DQ148944; Strain 77: DQ148945; Strain 62: DQ325517; Strain 86: DQ325520; Strain 104: DQ325518; Strain 109: DQ325519.

Some of the isolates have been deposited in culture collections: Strain 102 (*Desulfuromusa ferrireducens* DSM 16956, JCM 12926), Strain 112 (*Desulfuromusa svalbardensis* DSM 16958, JCM 12927), Strain 61 (*Desulfovibrio ferrireducens*, DSM 16995, JCM 12925), Strain 18 (*D. frigidus*, DSM 17176, JCM 12924).

Growth parameters and physiology. Temperature regulation of respiration of the strains was determined in a temperature-gradient block (Sagemann et al. 1998) at temperatures between –2 and 32°C. Growth was monitored in duplicate by measuring production of Fe^{2+} or sulfide. The ability of the strains to use alternative electron acceptors and donors was determined in duplicate tubes after growing cultures had been transferred to new medium for verification. The following electron donors were tested (in combination with Fe citrate or sulfate) (mM): acetate (20), lactate (20), formate (10), propionate (10), butyrate (10), H_2 (H_2/CO_2, 80/20, v/v), ethanol (10), propanol (10), butanol (10), fumarate (10) and succinate (10). As electron acceptors the following were tested (in combination with lactate or acetate) (mM): poorly crystalline Fe oxide (~30), Fe citrate (~30), Mn oxide (~30), elemental sulfur, sulfate (30), thiosulfate (10), sulfite (2), oxygen (air) and fumarate (20).

Fe(III) reduction by *Desulfovibrio*-related strains. Cells of Strains 18, 61 and 77 were transferred from sulfate-reducing cultures to sulfate-free medium. As substrates, lactate and Fe(III) citrate or poorly crystalline Fe oxide were added. If Fe(III) was reduced, 10% of the culture was transferred to new medium.

Fe reduction by Strain 61 was investigated in more detail. Cells of Strain 61 were grown by sulfate reduction (400 ml medium with 28 mM sulfate, 20 mM lactate). In the log phase of growth, the culture was centrifuged for 10 min and washed twice with 100 ml sulfate-free medium, for which the reducing agent sulfide was replaced by $Fe(II)Cl_2{\cdot}4H_2O$ (2 to 3 mM end concentration). The cells were resuspended in sulfate-free medium in an anoxic glove box and the suspension was filled into serum vials (50 ml each). Lactate (20 mM) and Fe citrate (~30 mM) or Fe oxide (~30 mM) were added to the vials. The incubation temperature was 0 or 20°C (triplicates).

Phylogenetic analysis. Universal bacterial primers 8F and 1492R (Buchholz-Cleven et al. 1997) were used to amplify 16S rDNA of the isolated strains. The PCR products were amplified with Primers 8F, 341F, 518F, 534R, 1099F and 1492R (Buchholz-Cleven et al. 1997) for sequence analysis (Applied Biosystems Model 3100 Genetic Analyzer DNA sequencer). The sequences were analyzed with the ARB program package (Ludwig et al. 2004). Phylogenetic trees were calculated with the ARB program applying neighbor-joining, maximum-parsimony and maximum-likelihood methods with different sets of filters to a subset of data that included only complete sequences of representative members of Proteobacteria.

RESULTS

Pore water and solid phase chemistry at Stn J

The nitrate and nitrite concentrations in the bottom water were 12 and 0.5 µM, respectively. The dissolved inorganic carbon (DIC) and ammonium concentrations in the pore water increased with increasing depth (Fig. 1A,B). The Fe^{2+} pore water profile showed a peak at 3 to 4 cm (59 µM) (Fig. 1C) from which we calculated the Fe^{2+} fluxes from the 1 to 4 cm depth interval. The fluxes were 4.9 and 2.8 nmol $cm^{-2}\ d^{-1}$ through the top and bottom layers of the depth interval, respectively, yielding a net Fe^{2+} production of 2.6 nmol $cm^{-3}\ d^{-1}$ for the 1 to 4 cm interval. The sulfate concentration in the pore water was constant in the uppermost 21 cm of the

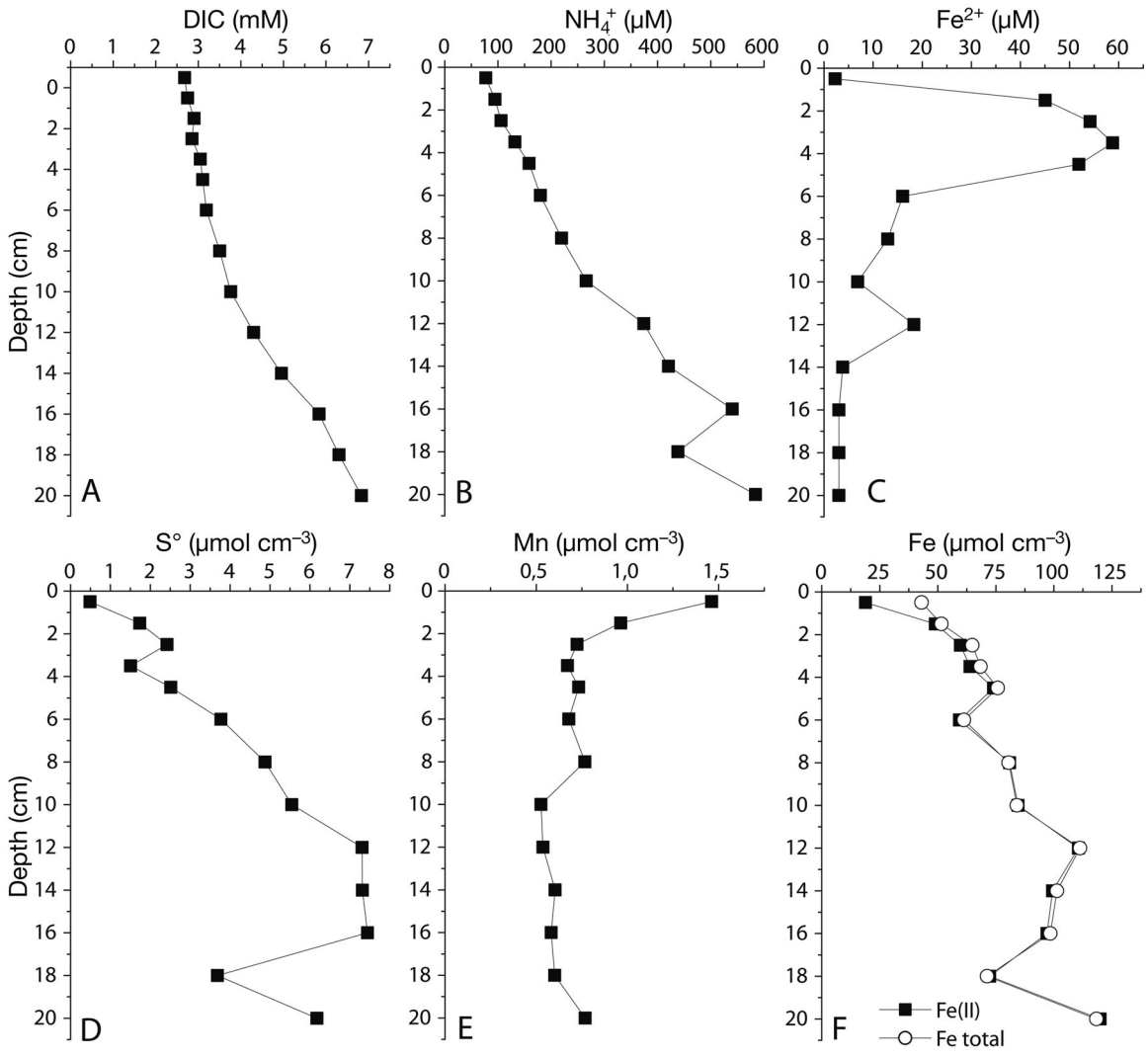

Fig. 1. Pore water dissolved inorganic carbon (DIC), NH_4^+, Fe^{2+} and solid phase S°, Mn, Fe constituents of sediment at Stn J (see Table 1 for station description)

sediment, and free sulfide did not exceed the detection limit of 1 µM. Extractable S° increased with increasing depth to 7.4 µmol cm^{-3} at 11 to 17 cm (Fig. 1D). Mn oxide was enriched near the surface (1.5 µmol cm^{-3}) and dropped to a stable background level of ≤0.8 µmol cm^{-3} below 2 cm (Fig. 1E). The HCl-extractable Fe(III) concentration decreased from the surface with increasing depth, while the Fe(II) concentration increased (Fig. 1F). The total Fe concentration was on average 78.0 µmol cm^{-3} (Table 1).

Oxygen consumption and sulfate reduction rates in whole core incubations

The mean total oxygen uptake rate was 4.2 ± 0.4 mmol m^{-2} d^{-1} and the DIC release rate 7.1 ± 1.6 mmol m^{-2} d^{-1}. Highest sulfate reduction rates were measured between 2 and 4 cm (Fig. 2), and the mean depth-integrated rate for 0 to 20 cm was 2.6 ± 0.6 mmol m^{-2} d^{-1}.

Anaerobic carbon mineralization in bag incubations

Anoxic bags of pooled sediment sections (1 cm depth interval each) from the uppermost 5 cm were incubated at 0°C. The Fe^{2+} concentration in the pore water increased in all bags throughout the whole incubation. The rate of Fe^{2+} accumulation was highest in the surface sediment (10.8 nmol cm^{-3} d^{-1}) and decreased with increasing depth to 0.5 nmol cm^{-3} d^{-1} at 4 to 5 cm (Fig. 3A). Mn^{2+} concentrations increased only in bags from the upper 2 cm, with rates of 2.2 and 0.3 nmol cm^{-3} d^{-1} (Fig. 3A), which scaled with the distribution of reactive Mn (Fig. 1E). In all 5 bags, the

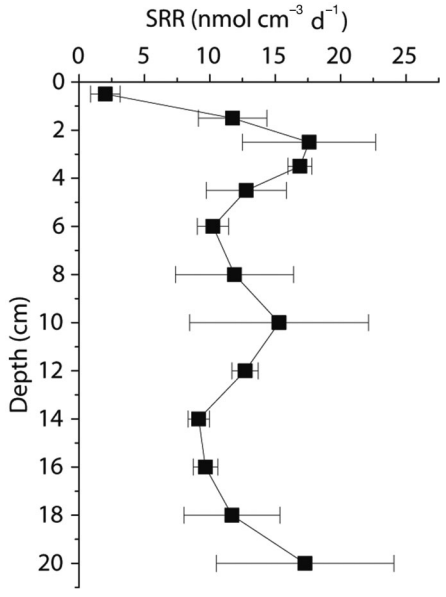

Fig. 2. Mean (±SE; n = 3) sulfate reduction rates at Stn J

pore water concentrations of DIC increased linearly throughout the 7 d incubation, indicating that the reaction rates remained constant and that no precipitation of carbonate occurred. This was supported by constant Ca^{2+} concentrations in the pore water during the incubation (data not shown). The DIC production rate was highest in the surface layer of the sediment and decreased with increasing depth (Fig. 3B), whereas sulfate reduction rates showed no change with depth (Fig. 3B) and were constant over time. DIC accumulation rates matched sulfate reduction rates in the bags from 2 to 5 cm (Fig. 3B), assuming an overall stoichiometry of 2:1 for DIC production to sulfate reduction as terminal electron acceptor (Thamdrup & Canfield 1996). In contrast, the 2 rates diverged significantly in the top 0 to 2 cm of the sediment, indicating that respiration pathways other than sulfate reduction were in operation.

Enrichment and isolation of Fe- and sulfate-reducing bacteria

For enrichment of bacteria from the 3 stations, we chose as electron donors acetate, lactate and formate, as these are important fermentation products in marine sediments. Using acetate and Fe(III), we isolated 2 strains (Strains 112 and 102) related to species of the

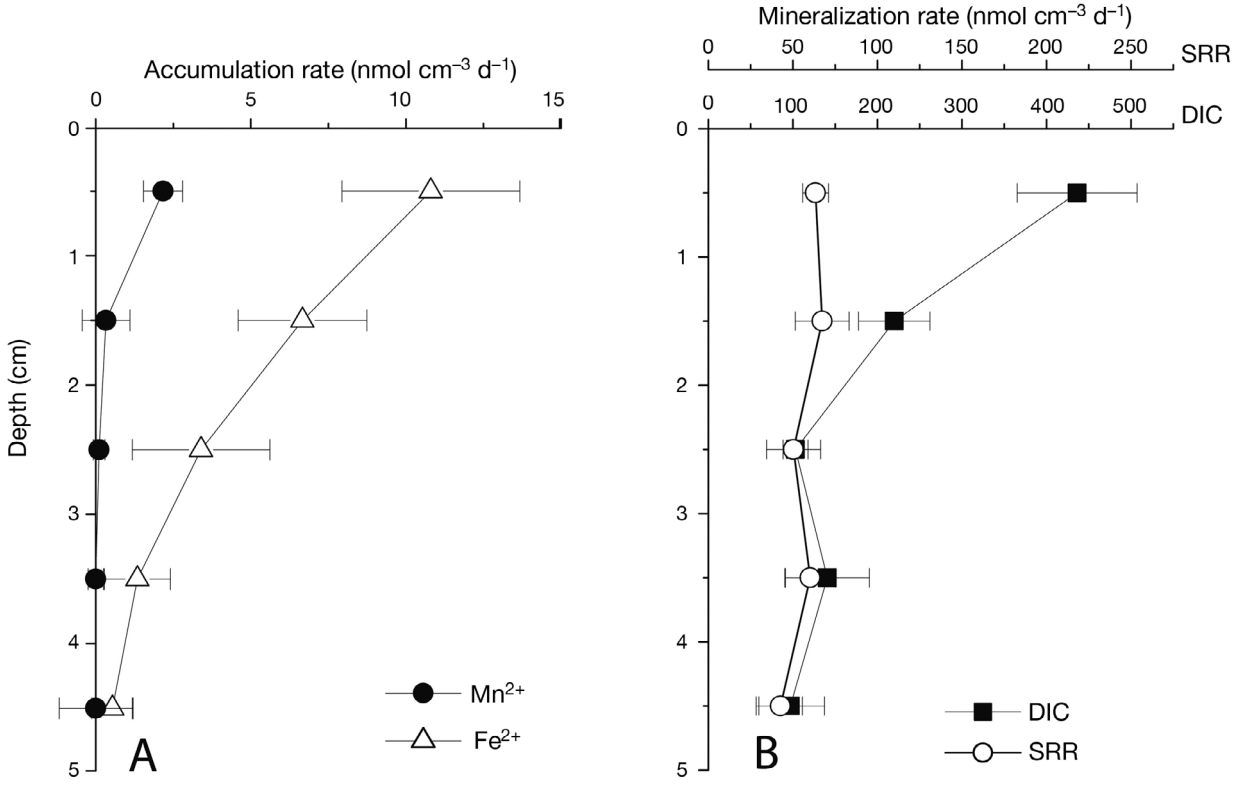

Fig. 3. Accumulation and mineralization rates measured during anoxic bag incubation. (A) Depth distribution of dissolved Fe^{2+} and Mn^{2+} accumulation rates during incubation; error bars: SE of linear regression. (B) Depth profiles of anaerobic mineralization of organic carbon; error bars: SE of linear regression of DIC production rates and standard deviation of sulfate reduction rates (SRR). Scales plotted at ratio of 2:1 for DIC production and SRR

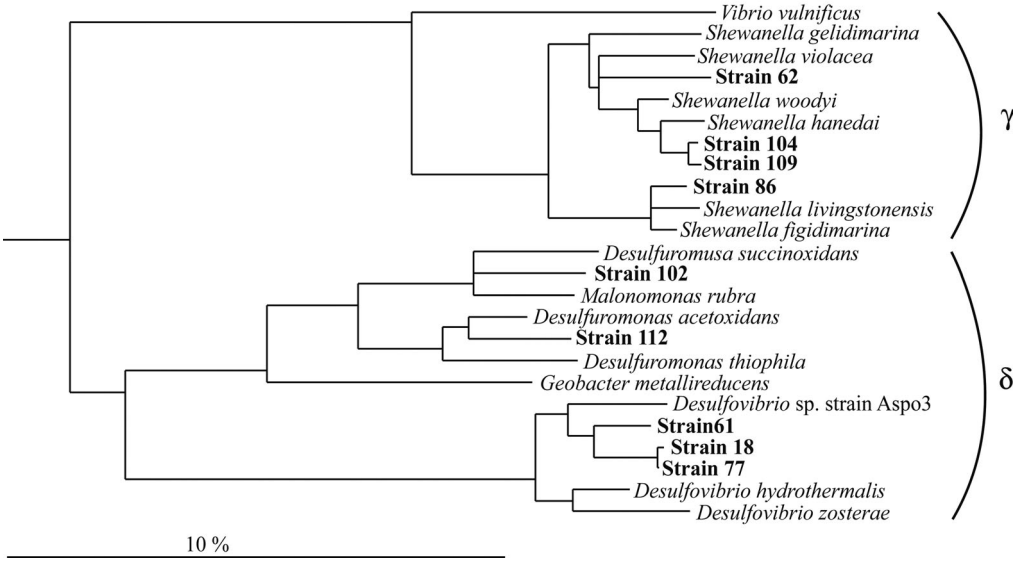

Fig. 4. Phylogenetic tree based on 16S rDNA sequences of isolated bacteria and reference sequences of γ- and δ-subclasses of Proteobacteria. Scale bar indicates 10 % sequence divergence. Consensus tree evaluated according to results of maximum-likelihood, neighbor-joining and maximum-parsimony analyses. Multifurcation indicates topologies that could not unambiguously be resolved

Geobacteraceae within the δ-Proteobacteria (Fig. 4) (Vandieken et al. 2006b). Within the γ-Proteobacteria, and closely related to species of the genus *Shewanella*, Strains 62, 86, 104, and 109 were isolated by lactate oxidation coupled with Fe reduction (Fig. 4).

Strain 61, related to species of the genus *Desulfovibrio* within the δ-Proteobacteria (Fig. 4), was isolated under Fe-reducing conditions with lactate as electron donor (Vandieken et. al 2006a). We isolated 2 strains closely related to Strain 61 (Strains 18 and 77; Fig. 4) by sulfate reduction (28 mM sulfate) with formate and lactate (Vandieken et al. 2006a).

Table 2. Characterization of isolated strains. Station: station where strain was originally isolated (details in Table 1). +: substrate used for growth; -: substrate not used for growth; (+): substrate used by some strains; +*: substrate reduced but no growth observed. Data for Strains 102 and 112 from Vandieken et al. (2006b), data for Strains 18, 61, 77 from Vandieken et al. (2006a)

Characteristic	102	112	62, 86, 104, 109	18, 61, 77
Related genus	*Desulfuro-musa*	*Desulfuro-monas*	*Shewanella*	*Desulfovibrio*
Station	CD	J	CC, CD, J	CC, CD, J
Optimum T (°C)	14–17	14	~15	20–23
T (°C) range for growth	–2–23	–2–20	–2–20	–2–30
Electron acceptors				
Fe oxide / Fe citrate	+	+	+	+*
Mn oxide	+	+	+	–
Elemental sulfur	+	+	–	(+)
Sulfate	–	–	–	+
Thiosulfate	–	–	–	(+)
Sulfite	–	–	–	+
Oxygen	–	–	+	–
Fumarate	+	+	(+)	–
Electron donors				
Acetate	+	+	(+)	–
Lactate	+	–	+	+
Formate	+	–	+	+
Propionate	–	+	–	–
Butyrate	–	–	–	–
Hydrogen	+	–	+	+
Ethanol	+	+	–	+
Propanol	+	+	–	(+)
Butanol	+	+	–	–
Fumarate	+	–	–	+
Succinate	+	–	–	(+)

Characterization of isolated strains

All isolates were able to grow at –2°C (Table 2). For the strains related to *Shewanella*, *Desulfuromonas*, and *Desulfuromusa/Malonomonas*, highest Fe reduction rates were measured at ~15°C (Table 2), and the bacteria are therefore true psychrophiles. The 3 strains related to *Desulfovibrio* grew best (by sulfate reduction) between 20 and 23°C, although they were isolated at different temperatures (4, 10 and 17°C) (Table 2), and can be characterized as psychrotolerants.

The substrate utilization of the isolated strains is shown in Table 2. In addition to Fe(III), the strains reduced electron acceptors such as oxygen, Mn oxide, elemental sulfur and sulfate. Beside acetate and lactate, the strains oxidized other important fermentation products such as formate, hydrogen and propionate.

For all *Desulfovibrio*-related strains (18, 61 and 77), the reduction of ferric citrate and poorly crystalline Fe oxide

was tested at their respective isolation temperature, and 2 to 4 transfers with significant Fe reduction in sulfate-free medium were possible (data not shown). Nevertheless, the cultures reduced the Fe slower with every transfer, and we suggest that this was due to dilution, as microscopic observations showed that no growth occurred. The ability of Strain 61 to reduce Fe was studied in more detail. Cells grown under sulfate-reducing conditions were washed with sulfate-free medium and incubated with ferric citrate or poorly crystalline Fe oxide as sole electron acceptor at 0 or 20°C. Besides being sulfate free, the medium was reduced with Fe(II) instead of sulfide to exclude an internal S-cycle with Fe(III) as oxidant for sulfide. The reduction of ferric citrate without sulfate was fast at temperatures of 0 and 20°C (Fig. 5). With poorly crystalline Fe oxide reduction of Fe(III) at 20°C was as fast as the reduction of ferric citrate at 0°C, but Fe oxide was not reduced at 0°C. We presume that the low temperature prevented the establishment of certain threshold conditions in the medium (e.g. redox potential) or of microniches necessary for Strain 61 to start reduction of Fe oxide. In all Fe-reducing cultures of Strain 61, these bacteria did not seem to gain energy for growth, as indicated by consistently low bacterial counts (data not shown).

DISCUSSION

Benthic carbon mineralization

The areal oxygen uptake rate for Smeerenburgfjorden sediment was 4.2 ± 0.4 mmol m^{-2} d^{-1}, which is similar to rates measured in other fjord sediments at Svalbard (3.6 to 8.1 mmol m^{-2} d^{-1}) (Glud et al. 1998). In another Arctic area along the northeast coast of Greenland, oxygen uptake rates were within the same range at water depths comparable to those at our study site (3.6 to 5 mmol m^{-2} d^{-1}) (Rysgaard et al. 1998, Glud et al. 2000).

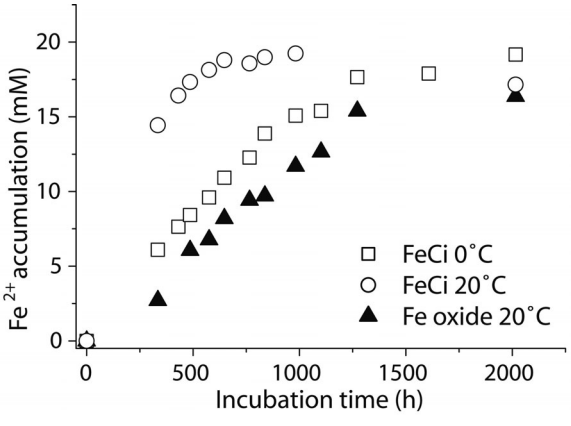

Fig. 5. Fe reduction of ferric citrate (FeCi) and Fe oxide by bacterial Strain 61 in absence of sulfate at 0 and 20°C (average of triplicate measurements). Initial Fe(II) concentration attributable to $FeCl_2$ used as reducing agent of the culture medium has been subtracted

The depth-integrated sulfate-reduction rate of whole core incubations at Stn J of 2.6 ± 0.6 mmol m^{-2} d^{-1} (0 to 20 cm) was within the range measured in other fjord sediments from the west coast of Svalbard (0.9 to 4.1 mmol m^{-2} d^{-1}) (Sagemann et al. 1998, Knoblauch et al. 1999, Finke 2003). The depth-integrated sulfate reduction rate of the bag incubations (2.8 mmol m^{-2} d^{-1}, 0 to 5 cm) was 5-fold higher than the rates of whole core incubations for the same sediment interval, indicating a stimulation that is probably due to sediment mixing inside the bags. A similar stimulation was reported previously for fjord sediments of Svalbard (Kostka et al. 1999, Arnosti et al. 2005). The stimulation might be due to a more homogeneous distribution of substrates or the death of benthic fauna which increased the amount of fresh organic material. We believe that the relative stimulation was similar for the different anaerobic pathways of respiration, since experimental addition in substrate to the sediment resulted in similar relative increases in sulfate and Fe reduction (data not shown).

The anaerobic carbon oxidation rate in sediment bag incubations was similar to rates for permanently cold sediments as well as comparable temperate sediments. The depth-integrated anaerobic DIC production rate was 10.0 mmol m^{-2} d^{-1} at 0 to 5 cm. In previous studies measuring depth intervals of 9 or 10 cm, rates were 11 to 24 mmol m^{-2} d^{-1} in fjord sediments of Svalbard (Kostka et al. 1999), 6 and 12 mmol m^{-2} d^{-1} in coastal sediments of Greenland (Rysgaard et al. 1998, Glud et al. 2000), 8 to 40 mmol m^{-2} d^{-1} in sediments of Denmark and Norway (Canfield et al. 1993a, Kostka et al. 1999, Jensen et al. 2003), and 9.2 and 12 mmol m^{-2} d^{-1} in sediments of the Chilean slope (Thamdrup & Canfield 1996). We conclude that rates of carbon mineralization in Smeerenburgfjorden are similar to those of comparable permanently cold and temperate sediments, and that the benthic microbial community is adapted to the permanently low temperature.

Fe and Mn reduction

In contrast to oxygen uptake and sulfate reduction rates, a method for direct quantification of Fe reduction rates in sediment cores is currently not available. The most commonly used approach for marine sediments is to perform anoxic sediment incubations, whereby the excess of anaerobic carbon oxidation

that is not coupled to sulfate reduction is attributed to other available electron acceptors such as Mn or Fe oxides (Thamdrup 2000).

Sulfate reduction rates matched DIC production rates (assuming a stoichiometry of 2:1 mol of carbon oxidized to sulfate reduced) in the anoxic bag incubation at 3 to 5 cm sediment depth (Fig. 3B). At 0 to 2 cm, the carbon oxidation rates clearly exceeded the carbon oxidation coupled to sulfate reduction (Fig. 3B). The excess DIC production is attributed to electron acceptors other than sulfate. We excluded oxygen and nitrate reduction because the maximum possible oxygen and nitrate contents would have been reduced within less than 3 h, i.e. before the first sampling timepoint (calculations based on oxygen [311 µM], nitrate [12 µM], and nitrite [0.5 µM] concentrations in the bottom water, an oxygen-penetration depth of 0.6 cm and an oxygen-consumption rate of 4.2 mmol m^{-2} d^{-1}). The maximum DIC produced by this oxygen and nitrate reduction in the bags would be <3% of the complete DIC concentration increase measured during bag incubations. Thus, the contribution of these processes to the carbon oxidation in the bags was negligible. Because of the low content of reactive Mn oxide in comparison to Fe, Mn reduction was probably mainly mediated by abiotic oxidation of Fe(II) and microbial Mn reduction was only of minor importance (Canfield et al. 1993b). Therefore, we attribute the carbon oxidation in the uppermost 2 cm that was independent of sulfate reduction to microbial Fe(III) reduction (Fig. 3B). This is supported by the Fe^{2+} accumulation rates in the pore water, which decreased with increasing depth (Fig. 3A). Sulfate reduction contributed 5.7 mmol m^{-2} d^{-1} to the 10.0 mmol m^{-2} d^{-1} depth-integrated DIC-production rate in the bags; the remaining 4.3 mmol m^{-2} d^{-1} (43% of the total anaerobic DIC production) were attributed to microbial Fe(III) reduction.

Zones of Mn and Fe reduction are in general indicated by the accumulation of Mn^{2+} and Fe^{2+} in the pore water. Metal liberation rates are, however, often 1 or 2 orders of magnitude lower than the gross metal reduction rates due to adsorption and/or precipitation (Canfield et al. 1993b, Thamdrup & Canfield 1996, Glud et al. 2000, Thamdrup et al. 2000, Jensen et al. 2003). In our incubation experiment, Fe^{2+} accumulation rates were similar to rates measured in previous bag incubation experiments with a comparable relative contribution of Fe reduction to carbon oxidation (Canfield et al. 1993b, Glud et al. 2000). Additionally, the calculated net-production rate of Fe^{2+} from the pore water profile of 2.6 nmol cm^{-3} d^{-1} at 1 to 4 cm sediment depth was in good agreement with the net Fe^{2+} production rates in the bags from the same depth interval, with a mean of 3.4 nmol cm^{-3} d^{-1}.

With a pure Fe(III)-reducing culture growing on Fe-containing sands and soils, Roden (2004) showed that 28 to 78% of the total Fe(II) production was accounted for by solid phase Fe(II) accumulation. The Fe^{2+} accumulation rates in bags from 0 to 1 and 1 to 2 cm accounted for <2% of the calculated Fe reduction rates based on the DIC production rates that were independent of sulfate reduction. A potential sink for Fe(II) in sediments is precipitation with sulfide, (produced during sulfate reduction) to form FeS or pyrite. An indication for this was the black color of the sediment and the fact that H_2S was not detected in the pore water throughout the incubation despite high rates of sulfate reduction. Additionally, Fe^{2+} might adsorb to Fe or Mn oxides, be reoxidized by Mn(IV) oxide, or react through other unknown pathways (Van Cappellen & Wang 1996). On the other hand, we cannot exclude that Fe(III) oxide was abiotically reduced by sulfide and a quantification was not possible.

In the bag incubations, dissimilatory sulfate and Fe reduction were measured simultaneously in the uppermost 2 cm, whereas below 2 cm sulfate reduction was the sole detectable respiration process (Fig. 3B). The overlap of sulfate reduction and metal reduction has also been reported for other habitats, suggesting an incomplete competition for substrates (Canfield et al. 1993b, Kostka et al. 1999, Jensen et al. 2003). For Fe-reducing bacteria, the availability of reactive Fe(III) oxide is often important in limiting turnover rates (Thamdrup 2000), and competitive inhibition of sulfate reduction by Fe-reducing bacteria is usually not complete. Fe(III) was rapidly depleted in the uppermost 2 cm of Smeerenburgfjorden sediments (Fig. 1F). Thus, the bacterial Fe-reducing community in these sediments seemed to be mainly limited by Fe(III) rather than by organic carbon.

The relative contribution of microbial Fe reduction to carbon oxidation *in situ* depends on the faunal activity and sediment accumulation rate, with a high importance of Fe reduction if the faunal activity is high and the organic carbon deposition is moderate, which results in a deeper mixing zone (Kostka et

Table 3. Rates and contributions of different mineralization pathways to carbon oxidation calculated for 0 to 10 cm sediment depth interval. Percentages: % total organic carbon oxidation by each pathway; nd: not determined

Pathway	Rate (mmol C m^{-2} d^{-1})	%
O_2 respiration	3.8	53
Denitrification	nd	nd
Mn(IV) reduction	~0	~0
Fe(III) reduction	0.9	13
SO_4^{2-} reduction	2.4	34

al. 1999). The presence of fauna indicated a high bioturbation and a fast turnover of Fe, which pro bably favor Fe-reducing bacteria in Smeerenburg-fjorden sediment.

Pathways of carbon mineralization

To estimate the relative contribution of the different mineralization pathways for the sediment depth interval of 0 to 10 cm (Table 3), we used a respective contribution of 43 and 57% of Fe and sulfate reduction to anaerobic carbon mineralization in the uppermost 5 cm of the sediment, as determined in the bag incubations. We assumed that below 5 cm sulfate reduction was the sole terminal respiration pathway. We used whole core sulfate reduction rates (1.2 mmol m^{-2} d^{-1}, 0 to 10 cm) to calculate the absolute Fe reduction rates (Rysgaard et al. 1998), since the processes in the bag incubations were stimulated by the mixing of the sediment. The denitrification rate was not measured and we do not include this pathway in our calculations but assume that it contributed not more than 2 to 3% to carbon oxidation as described for other fjord sediments at Svalbard (Kostka et al. 1999). Bacterial oxygen consumption was determined from the difference between the DIC release of the whole-core incubations (7.1 mmol m^{-2} d^{-1}) and the calculated anaerobic DIC production from sulfate and Fe reduction (3.3 mmol m^{-2} d^{-1}, 0 to 10 cm). These calculations determined aerobic respiration to be the most important mineralization pathway in 0 to 10 cm sediment depth at Stn J (53%), and sulfate reduction as the second most important (34%). The contribution of Fe reduction to total carbon oxidation was 13%. *In situ*, the contribution of Fe reduction might vary due to competition with oxygen- and nitrate-respiring bacteria for substrate, depending on the supply of oxygen and nitrate from the water column to the sediment by diffusion and bioturbation. A similar contribution of Fe reduction to carbon oxidation in the uppermost 10 cm (10 and 26%) was shown for sediments of Van Mijenfjorden and Storfjorden on the southwest and southeast coasts of Svalbard (Kostka et al. 1999). In another fjord (Hornsund), however, no contribution of dissimilatory Fe reduction to carbon mineralization was detected; this was explained by a high sedimentation rate and a shallow mixing zone (Koska et al. 1999). In permanently cold sediments of Greenland, Fe reduction accounted for 21 to 26% of total mineralization, and in temperate sites such as the coasts of Denmark and Norway and the continental slope of Chile for 0 to 50% (Canfield et al. 1993a, Thamdrup & Canfield 1996, Rysgaard et al. 1998, Glud et al. 2000).

Fe-reducing bacteria

To investigate the bacteria responsible for Fe reduction in the permanently cold sediments of Svalbard, we isolated Fe-reducing strains from 3 fjord sediments with similar Fe contents (Table 1). The isolates belong to the δ- and γ-subclasses of Proteobacteria and were characterized with respect to temperature tolerance and substrate utilization (Table 2). As all isolates grew well at the freezing point of sea water at –2°C, the strains are adapted to the low *in situ* temperatures of their habitat. Isolations of bacteria from different physiological groups from sea ice and sediments of Antarctic and Arctic revealed a high abundance of psychrophilic strains, indicating adaptation to permanently low temperature (Knoblauch et al. 1999, Bowman et al. 2003, Brinkmeyer et al. 2003, Knittel et al. 2005).

Of the strains isolated, 2 (Strains 102 and 112) belong to the genera *Desulfuromusa/Malonomonas* and *Desulfuromonas* (Fig. 4). These genera, together with *Geobacter* and *Pelobacter*, form an important group of Fe-reducing bacteria: the Geobacteraceae within the δ-Proteobacteria (Holmes et al. 2004b). The presence of members of the Geobacteraceae has been shown for various habitats, including freshwater and marine sediments in temperate and permanently cold habitats by cultivation-independent as well as cultivation-dependent methods (e.g. Ravenschlag et al. 1999, Purdy et al. 2003, Lovley et al. 2004 and references therein, Mußmann et al. 2005). In the sediments of Smeerenburgfjorden (Stn J) FISH (fluorescence *in situ* hybridization) analysis showed the highest abundance of *Desulfuromonas– Pelobacter* (up to 2.2% of DAPI cell counts) between 0.5 and 3 cm depth (Ravenschlag et al. 2000), which was within the Fe reduction zone determined in the present study. For the major group of sulfate reducing bacteria, *Desulfosarcina–Desulfococcus*, highest cell numbers were found below the Fe reduction zone at 2.25 cm depth (Ravenschlag et al. 2000). However, recent studies indicate that the FISH probe for the *Desulfuromonas–Pelobacter* group might be unspecific. Nevertheless, 13% of clones in a bacterial 16S rDNA clone library of another Svalbard fjord sediment were closely related to *Desulfuromonas* species (Ravenschlag et al. 1999). Together with the isolation of the psychrophilic Strains 102 and 112, we suggest that the group Geobacteraceae is present in fjord sediments on the west coast of Svalbard. An important characteristic of the isolated Strains 112 and 102, and of other species of this group, is their ability to reduce Fe(III) and elemental sulfur (Table 2). The concentration of S° increased from 0.5 µmol cm^{-3} at the surface to 7.4 µmol cm^{-3} at 16 cm depth in the sediment of Stn J (Fig. 1D), providing an alternative electron acceptor for strains of the

Geobacteraceae. Most Geobacteraceae species are able to couple the reduction of Fe(III) and sulfur to the oxidation of acetate (Lovley et al. 2004) (Table 2), which is an important fermentation product in marine sediments including the sediments at Svalbard (e.g. Sørensen et al. 1981, Finke 2003). Thus, these bacteria might play an important role in the sulfur- and Fe-cycles of marine sediments.

Shewanella is a genus with known psychrophilic Fe-reducing species. All strains isolated with lactate (Strains 62, 86, 104, and 109) in the present study were closely related to the psychrophilic species *Shewanella gelidimarina*, *S. frigidimarina*, *S. hanedai*, *S. violacea* and *S. livingstonensis* isolated from Antarctic, Arctic or deep-sea sediments (Jensen et al. 1980, Bowman et al. 1997b, Nogi et al. 1998, Bozal et al. 2002) (Fig. 4). Further psychrophilic strains and clone-sequences related to *Shewanella* were identified in Antarctic and Arctic sea ice and Antarctic shelf sediments (Bowman et al. 1997b, 2003, Junge et al. 2002, Brinkmeyer et al. 2003). Besides permanently cold habitats, strains of this genus have been isolated from a variety of habitats including coastal, open and deep-sea environments and from invertebrates (Ivanova et al. 2004 and references therein), and a wide distribution of this genus is likely. However, their quantitative importance is not understood, since sequences related to *Shewanella* are generally not found in clone libraries of marine sediments, including the clone library of fjord sediment from Svalbard (Ravenschlag et al. 1999). Facultatively anaerobic strains of *Shewanella* are well adapted to conditions in surface sediments. Here, oxygen might be an important alternative electron acceptor. In coastal sediments of Svalbard oxygen was found to penetrate 3 to 11 mm into the sediment (Glud et al. 1998, Kostka et al. 1999), and oxygenated water can be introduced into deeper sediment layers by bioirrigating fauna (Jørgensen et al. 2005). Reactive Mn oxide is typically enriched in surface sediments (Fig. 1E), which can be reduced by the isolated strains related to *Shewanella* and the Geobacteraceae.

We isolated strains belonging to the genus *Desulfovibrio* under Fe(III)-reducing as well as sulfate-reducing conditions from sediments of all 3 stations, and demonstrated their ability to reduce poorly crystalline and soluble Fe(III) forms in sulfate-free medium. So far, nearly all species of this genus have been isolated by sulfate reduction, but the ability to reduce Fe(III) was demonstrated for some species (Coleman et al. 1993, Lovley et al. 1993, Li et al. 2004). Lovley et al. (1993) suggested, however that, in their experiments, *D. desulfuricans* obtained energy for growth from the 300 µM sulfate present in the medium and not from reduction of Fe(III). Correspondingly, in the present study, it could not be unequivocally determined whether Strain 61 grew during enrichment and isolation by Fe(III) reduction or by sulfate reduction using the low concentration of sulfate in the medium (400 µM). Growth of sulfate-reducing bacteria with Fe as electron acceptor has so far only been reported for *Desulfobulbus propionicus* and '*Desulfotomaculum reducens*' (Tebo & Obraztsova 1998, Holmes et al. 2004a). *In situ* reduction of Fe(III) by populations of *Desulfovibrio* was suggested based on the presence of biomarkers characteristic for *Desulfovibrio* in a salt-marsh sediment (Coleman et al. 1993). From our results, we conclude that our strains did not grow during Fe reduction. The Fe reduction rate of Strain 61 was 12 µM h^{-1} at *in situ* temperatures (Fig. 5) (0°C, Fe citrate) compared to e.g. 69 µM h^{-1} for the *Desulfuromonas*-related Strain 112 under the same conditions. Therefore, *Desulfovibrio* related strains are able to reduce Fe at modestly lower rates compared to other Fe-reducing bacteria. Yet, because of their concurrent growth by sulfate reduction they might also be involved in Fe reduction in their habitat.

In previous investigations of fjord sediments from Svalbard, *Desulfovibrio*-related strains were not isolated, nor were related sequences found in 16S rDNA libraries (Knoblauch et al. 1999, Ravenschlag et al. 1999). However, from sediments of all 3 stations of Svalbard, we isolated psychrotolerant strains of *Desulfovibrio* with the ability to reduce the quantitatively important electron acceptors sulfate and Fe.

CONCLUSIONS

Fe reduction is an important process for the mineralization of organic material in Smeerenburgfjorden (Stn J) sediment. The Strains 112, 109 and 77 isolated from Smeerenburgfjorden represent organisms of all 3 isolated phylogenetic groups (Geobacteraceae, *Shewanella* and *Desulfovibrio*). The relatively high Fe(III) content of the sediment provides supportive evidence for microbial Fe reduction in the uppermost 2 cm. The importance of Fe reduction at Stns CC and CD in Tempelfjorden, where the Strains 102, 18, 61, 62, 86 and 104 were isolated, was not determined, but the Fe content at this site was similar to that at Smeerenburgfjorden. However, not only a high Fe content is important for microbial Fe reduction but also high bioturbation and an intermediate sedimentation rate. The isolation of psychrophilic, dissimilatory, Fe-reducing bacteria from all 3 stations shows that this physiological group is present and adapted to low temperatures of permanently cold sediments on the west coast of Svalbard. In marine sediments, Fe(III) additionally might be reduced by sulfate-reducing bacteria.

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